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Measurement of intravascular Na^+ during increased CBF using ^{23}Na NMR with a shift reagent

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ABSTRACT: Sodium ions are intimately involved with neural activity. Thus, it is highly desirable to devise a way of mapping brain activity via sodium imaging. Sodium ions exist in the extravascular and intravascular spaces. To separate the two components, the shift reagent $Tm(DOTP)^{5-}$ was intravenously introduced into rats. Intravascular sodium changes in the rat brain were measured during increased blood flow induced by hypercapnia using volume-localized ^{23}Na-NMR. The intravascular sodium changes, equivalent to cerebral blood volume changes, are significant during hypercapnia conditions and correlate well with the increase in arterial pCO_2. This suggests that the intravascular sodium change is dominant in total ^{23}Na spectroscopy or imaging of the brain during blood flow increase induced by external perturbation. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: ^{23}Na-NMR; shift reagent; brain activation; localized spectroscopy; hypercapnia; functional MRI

INTRODUCTION

Existing functional MRI methods based on the BOLD effect[2,3] provide indirect information on neuronal activity using the hemodynamic response to the neuronal activation. The spatial specificity of current functional MRI methods is closely dependent on vascular structures and intrinsic coupling between neural activity and hemodynamic response. To improve spatial specificity, ^{23}Na-MRI and MRS methods have been proposed, since Na^+ ions are intimately involved with neuronal activity through cross-membrane currents. For example, Goodyear *et al.* obtained ^{23}Na functional imaging at 4T using a standard gradient-echo imaging technique during visual stimulation.[1]

Na^+ ions in the brain exist in the extravascular compartment, in which Na^+ resides in the intracellular and interstitial space, and in the intravascular compartment, in which most Na^+ ions are present in the blood plasma. During neuronal stimulation, sodium flux within the extravascular compartment increases due to cross-membrane currents, and cerebral blood volume will concomitantly increase, thus enhancing intravascular sodium signals. If the sodium signal change during increased neural activity occurs only at the extravascular space, sodium imaging will potentially provide high spatial specificity to neuronal activity, since the Na^+ ion dynamics occur at the actual polarization/depolarization loci. However, if the intravascular signal change is significant, sodium functional imaging based on simple excitation techniques provides information mostly about cerebral blood volume (CBV) changes. In that case, the separation of neuronal-based and hemodynamic-based phenomena using ^{23}Na-MRI is essential.

The most simple and dependable approach for obtaining compartment-specific information from ^{23}Na-NMR involves the use of ^{23}Na shift reagents, such as $Dy(PPP)_2^{7-}$ and $Dy(TTHA)^{3-}$,[4] and more recently $Tm(DOTP)^{5-}$.[5] The mechanism through which the ^{23}Na resonance is shifted has been broadly investigated and the reagents have been introduced in several *in vivo* ^{23}Na-NMR studies.[6–12] Although the use of shift reagents *in vivo* is made complicated primarily because of their moderate toxicity through their high affinity to Ca^{2+} and Mg^{2+} ions,[10] it allows the simultaneous detection of Na^+ in more than one compartment with simple detection schemes and the highest possible SNR.

In this study we intravenously administered the shift reagent $HNa_4TmDOTP$, allowing discrimination between the intravascular and extravascular sodium NMR

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Abbreviations used: BOLD, blood oxygenation level dependent; CBV, cerebral blood volume; CBF, cerebral blood flow; TQF, triple quantum filtered; TQC, triple quantum coherence.

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signals in the rat brain. Then we directly measured the fractional intravascular sodium content upon increase in cerebral blood flow (CBF), caused by increase in inhaled CO_2 (hypercapnia). This provides a direct measurement of the relative cerebral blood volume (CBV). We used a modified version of the STEAM sequence, tailored for using extremely short *TE* and TM periods[13] in order to obtain relatively high SNR ^{23}Na-NMR spectra that were not contaminated by signal from surrounding muscles. Ultimately, we show the feasibility of directly measuring cerebral blood volume (CBV) changes in the rat brain *in vivo*, following regulation of the rat cerebral blood flow (CBF) by using the well-established hypercapnia model.

MATERIALS AND METHODS

Animal preparation

Six rats (250–300 g) were initially anesthetized with 2% isoflurane in a 3:7 O_2:N_2 mixture, and orally intubated. Then, both femoral veins and one femoral artery were catheterized with PE-50 tubing for drug administration and blood-gas sampling, respectively. End-tidal CO_2 level was controlled via a capnometer (Capnocheck, Pryon Co., WI) and body temperature was monitored through a rectal probe. The animal body temperature was kept constant using a feedback controlled water bath. Following the surgery, the anesthesia level was brought down to 1.5% and the animal was secured in a home-built head-holder with ear bars and a bite bar. A 350 mM solution of the shift reagent Na_4HTmDOTP (Macrocyclics, TX) was infused into the femoral vein using an infusion pump (Harvard Apparatus, South Natick, MA). During the first half-hour of infusion, the infusion rate was kept about 0.5 ml/h, and then increased to about 0.8 ml/h until the desired shift (~150 Hz) was achieved, typically after a total infusion time of about 2 h. This infusion rate results in a delivered dose of Na_4HTmDOTP of ~1.6 mmol/kg for a 300 g animal. Although addition of Ca^{2+} helps reduce the toxicity of the shift reagent, the Na_4HTmDOTP solution was not added with Ca^{2+} since adding Ca^{2+} reduces the observed shift, as observed by Bansal *et al.*[10] The infusion rate was kept slow enough so that the physiology of the animals was not compromised.

MR experiments

Experiments were performed on a 9.4 T/31 cm horizontal MRI scanner (Magnex Scientific, UK) equipped with 30 G/cm gradients (11 cm I.D., 300 μs risetime, Magnex Scientific) and driven by a ^{Unity}INOVA console (Varian, CA). A home-built ^{1}H/^{23}Na double-surface coil (^{1}H figure-8 coil and ^{23}Na 1.5 cm diameter double loop coil, $\pi/2$ degree pulse at the ^{23}Na frequency 35 μs at 10 W and 140 μs at the ^{1}H frequency at the same power) was placed above the animal head. Shimming and positioning were performed using the ^{1}H coil. Shimming was performed using the automatic shimming procedure FASTMAP[14] on an $11 \times 8 \times 8$ mm^3 voxel, until a typical linewidth of ~20 Hz for the water signal was achieved. Then a smaller voxel of $8 \times 4 \times 8$ mm^3 located at the top of the rat brain was selected for the acquisition of the ^{23}Na-NMR spectra. Localization of the ^{23}Na-NMR signal was achieved using a modified STEAM sequence that allows very short *TE* and *TM* periods.[13] *TE* was set to 2 ms and *TM* was 0.9 ms.

The animal was then infused i.v. with an initial dose of 0.3 ml pancuronium bromide, and the isoflurane level was lowered to 1%. For the rest of the experiment the animal was infused *i.v.* with pancuronium bromide at 0.1–0.2 ml/h and the shift reagent infusion rate was regulated in real time so that the shift was kept roughly constant and the physiology of the animal was not compromised. Graded hypercapnia conditions were induced by setting the CO_2 concentration in the gas mixture at six different levels between 0 and 10%, so pCO_2 levels ranged between 25 and about 65 mmHg. At each CO_2 level, Three ^{23}Na NMR spectra were acquired (2048 scans, $TR = 0.1$ s).

RESULTS

Figure 1 shows a ^{1}H-MRI axial and coronal views of the rat brain, on which the voxel used for the localized ^{23}Na spectra is superimposed. Figure 2 shows three typical ^{23}Na NMR spectra taken before and after the shift reagent was infused. The bottom spectrum was taken before the infusion of the shift agent. As expected, only a single ^{23}Na NMR peak was observed, which contains extravascular and intravascular Na^+ ions. The spectrum in the middle was taken without any localization after infusion of the shift agent. The non-shifted peak (right) is generated by the Na^+ pools that are not in contact with the shift reagent, i.e. the extravascular (extra- and intracellular) Na^+ in the brain and the intracellular Na^+ in muscle tissue. The shift reagent does not cross the blood–brain barrier,[9] hence the brain tissue ^{23}Na peak remains unshifted. The large shifted peak is assigned to Na^+ present in the head muscles, where the shift reagent permeates through the vessel walls into the interstitial space. To eliminate muscle contribution to sodium NMR, localized spectrum (top) was acquired. The non-shifted peak is attributed to extravascular Na^+ ion pool, whether the small shifted peak (approximately 10–12% of the large peak) is attributed to the intravascular-plasma Na^+. Since the hematocrit Na^+ concentration, less than 10 mM, is fairly low in comparison to the Na^+ concentration in the blood plasma, about 150 mM, the contribution of the hematocrit Na^+ to the non-shifted Na peak is negligible.

Based on the changes in the relative area of the shifted peak and on the formula:

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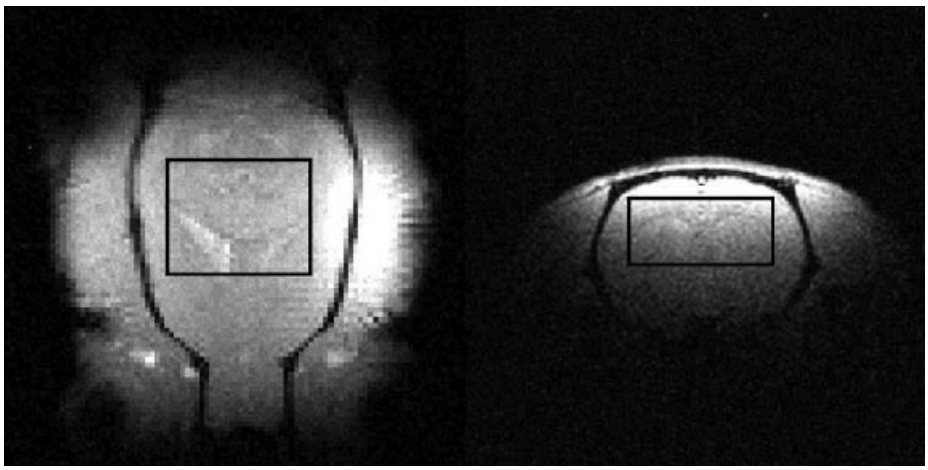

Figure 1. An axial (left) and coronal (right) FLASH images of the rat brain, on which the volume of interest (VOI) used for the acquisition of the localized spectra is marked. The VOI dimensions are $8 \times 4 \times 8$ mm^3

$$A_{\mathrm{iv}} \propto \rho_{\mathrm{iv}} = \frac{C_{\mathrm{iv}} \cdot V_{\mathrm{iv}}}{C_{\mathrm{iv}} \cdot V_{\mathrm{iv}} + C_{\mathrm{is}} \cdot V_{\mathrm{is}} + C_{\mathrm{ic}} \cdot V_{\mathrm{ic}}}$$

the changes in the intravascular volume are then calculated. In the formula above, A_{iv} is the relative area under the shifted peak, C_{iv}, C_{is} and C_{ic} are the Na^+ concentrations at the intravascular, interstitial and intracellular spaces, and V_{iv}, V_{is} and V_{ic} are the respective volumes. It is assumed that the intracellular volume is 0.75 of the tissue volume, and that the initial Na^+ concentration ration between the intracellular and extracellular space is 1:10. The increase in the extracellular and intravascular Na^+ concentration due to the infusion of the shift reagent solution alone, in which the Na^+ concentration is 1.65 M, is calculated in a similar way from the increase in the area of the non-shifted peak.

Figure 3 shows the relative intravascular volume calculated from the data acquired during the graded hypercapnia conditions, as a function of the arterial blood

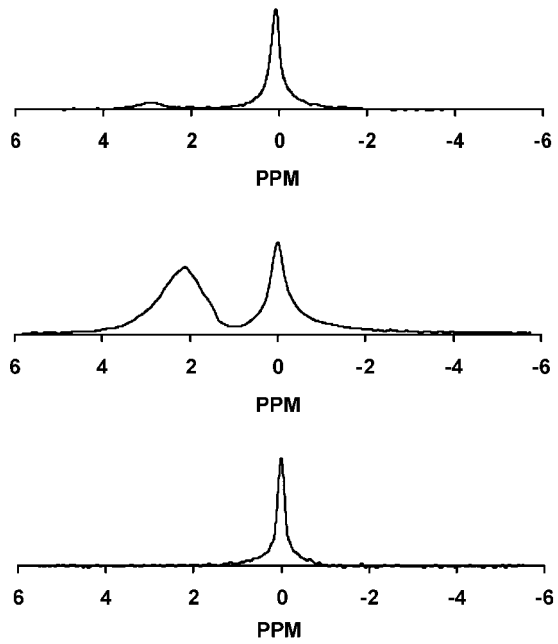

Figure 2. Three typical ^{23}Na spectra taken from the rat brain. Bottom, localized spectrum taken before the i.v. infusion of the shift reagent; middle, non-localized spectrum of the rat head. The shifted peak includes large contribution from muscle areas. Top, localized spectrum using the modified STEAM sequence, taken from the voxel seen in Fig. 1. The area of the shifted peak is much smaller and includes only contributions from intravascular Na^+ (about 10–12% of the total Na^+ signal)

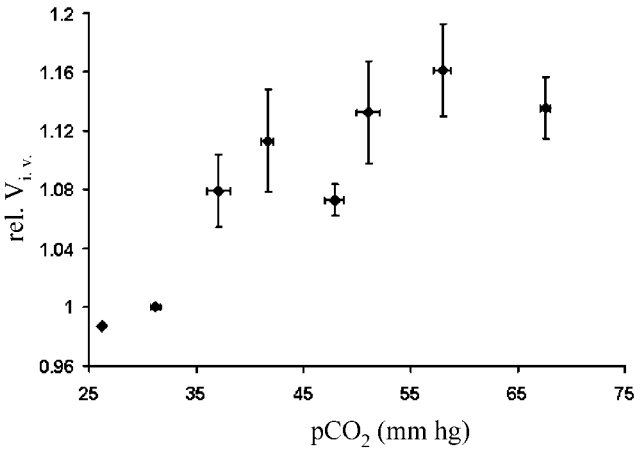

Figure 3. A plot of the relative area of the shifted peak, proportional to the relative intravascular Na^+ content, *vs* the arterial pCO_2. The error bars represent standard error. The solid line represents the calculated increase in the relative intravascular Na^+ content due to the increase in intravascular and extracellular Na^+ concentration as a result of the infusion only

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pCO_2. The pCO_2 was binned in 5 mmHg intervals, and the average and standard error were taken in each data bin for both the pCO_2 and the relative i.v. volume values. Because of the variability in the baseline values for the relative area of the intravascular ^{23}Na peak used in the calculation of the i.v. volume, those values were arbitrarily normalized to be 1 at normal pCO_2 level (pCO_2 = 30–35 mmHg).

The permeability of the vessel wall to Na^+ ions does not significantly increase, or at least the exchange rate does not become comparable with the time scale of the ^{23}Na-NMR, so no significant changes in the extravascular ^{23}Na-NMR lineshape and shift were detected. The intravascular CBV monotonically increases with arterial pCO_2. This result is consistent with previous observation of the relation between CBV and arterial pCO_2.[16]

DISCUSSION AND CONCLUSIONS

We have shown that the intravascular sodium signal, which is indicative of regional CBV, monotonously increases with the blood pCO_2. Hence it can be inferred that, during increased CBF induced by external perturbations, including neural stimulation, the intravascular sodium signal is significantly modulated. Thus, sodium spectroscopy and/or imaging of total sodium ions in the brain contain information about hemodynamic changes. This finding makes a strong case for a ^{23}Na-NMR method that yields compartment-specific information about Na^+ ions, and in particular, the intra- and extracellular Na^+ pools.

We used a shift agent to separate different compartments. Similar approaches were used by other investigators. Lee *et al.*[9] and Bansal *et al.*[10] showed that the shift reagent $Na_4HTmDOTP$, which does not cross the blood–brain barrier, can be intravenously infused into a live rat, thus allowing the use of ^{23}Na-NMR to discriminate between the intravascular and extravascular Na^+ ion pools in the rat brain. Alternatively, Eleff *et al.* have shown that the shift reagent $Dy(TTHA)^{3-}$ can be delivered to the parenchima of a dog brain through temporarily breaking the blood–brain barrier.[11] In that case, the shifted peak is that of the extracellular Na^+, giving rise to the possibility of measuring the dynamics between intra- and extracellular Na^+ ions during various activation conditions. In that case, the change in cross-membrane ion currents following neuronal activation will be expressed in changes in the lineshape or shift of either of the ^{23}Na-NMR resonances. Similarly, direct infusion of the shift reagent into the extracellular space allows separation between the intracellular and extracellular Na^+ pools, and measure compartment-specific Na^+ dynamics during brain activation.

One approach to distinguishing between the intracellular and extracellular sodium signals is to utilize ^{23}Na triple quantum filtered (TQF) NMR. Triple quantum coherence (TQC) in ^{23}Na-NMR can be generated when the motional correlation time of the Na^+ ions falls much below the extreme narrowing limit ($\omega_0\tau_c \ll 1$), thus only slow-tumbling Na^+ ions, i.e. those bound to macromolecules, can generate TQC that would pass the filter.[17,18] In earlier works it has been shown that because the concentration of macromolecules in the intracellular space is significantly larger than that in the extracellular space, the triple-quantum-filtered ^{23}Na signal is primarily originated by intracellular Na^+ ions, and that extracellular ^{23}Na-NMR signal is practically eliminated.[19] TQF ^{23}Na MR imaging has already been applied on animal[20] and human[21] models. TQF-^{23}Na-NMR, possibly with diffusion enhancement, would be the method of choice for human studies, although the low SNR might dictate prohibitively long experiments. In addition, contamination of the TQC with single quantum coherence and small TQC signal generated by i.v. and extracellular Na may considerably complicate the analysis of TQF data.

Another approach to analyzing ^{23}Na-NMR data in living tissue is that of relaxation analysis, and in particular T_2 analysis. It is known that fast tumbling Na^+ ions give rise to a monoexponential decay of the transverse magnetization, whether slow tumbling Na^+ ions give rise to a biexponential relaxation with two distinct relaxation times, commonly denoted as T_{2s} and T_{2f}, with a population ratio of 2:3 accordingly.[20] Analysis of the relaxation behavior yields information about the nature of the bonding of Na^+ ions to macromolecules and the exchange process between 'free' and 'bound' Na^+ ions.[23–25] This particular approach is not very suitable for investigating neural activity for several reasons: it is substantially time consuming—the collection of data from a reasonably small voxel for relaxation analysis is prohibitively long. Then, it is unlikely that the possible changes in exchange rate between intra- and extracellular Na^+ following neuronal activation would have a significant impact on the exchange process between free and bound Na^+ in the intracellular space. In addition to these, analysis of multiexponential behavior in a heterogeneous system is not accurate enough to yield numbers that would be statistically significant in the case where such differences are expected to be very small.

There are several problems associated with ^{23}Na-NMR of living tissue. One is the limited SNR inherent to ^{23}Na-NMR, which is a combined result of low concentration (particularly in the intracellular space), broad lines and a gyromagnetic ratio that is considerably lower than that of protons. This implies that temporally resolved ^{23}Na-NMR studies of neural activation are practically not feasible. The other significant problem is the short relaxation times of ^{23}Na, which not only result in low SNR, but also limit the range of the dynamic effects that can be investigates using ^{23}Na-NMR. In particular, very slow chemical exchange processes that occur between two Na^+ ion pools may become very hard to detect. An additional and very serious problem is the moderate

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toxicity of $Tm(DOTP)^{5-}$, which in spite of being very efficient in terms of shift/mol, tends to compromise the physiology of the animal. In particular, the administration of the $Tm(DOTP)^{5-}$ into the interstitial space may result in seizure-like effects, due to sequestration of Ca^{2+} and Mg^{2+} ions. This might dictate in future studies an administration of smaller doses, resulting in smaller shifts, some compensation of Ca^{2+} ions, or using a less toxic, albeit less efficient shift reagent, such as $Dy(TTHA)^{3-}$ which appears to be less toxic.

In conclusion, it has been shown that increase in CBV is a major source of signal increase in ^{23}Na-NMR of the brain during global activation, and that the use of a shift reagent provides an accurate and simple method for measuring these changes. ^{23}Na-NMR will be useful to provide information on cross-membrane ion currents, when the shift reagent is in contact with the Na^+ ions in the interstitial space, rather than with the intravascular Na^+ ions.

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REFERENCES

1. Goodyear BG, Gati JS, Menon RS. ^{23}Na functional MR imaging at 4 T using gradient-recalled echo FLASH. (Abstract.) In *Proceedings of the International Society of Magnetic Resonance Medicine* 6th Scientific Meeting and Exhibition, 1998; 1439.
2. Ogawa S, Lee TM, Nayak AS, Glynn P. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magn. Reson. Med.* 1990; **14**: 68–78.
3. Ogawa S, Tank DW, Menon RS, Ellermann JM, Kim S-G, Merkle H, Ugurbil K. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc. Natl Acad. Sci. USA* 1992; **89**: 5951–5955.
4. Chu SC, Pike MM, Fossel ET, Smith TW, Balschi JA, Springer CS Jr. Aqueous shift reagents for high-resolution cationic nuclear magnetic resonance. III. $Dy(TTHA)^{3-}$, $Tm(TTHA)^{3-}$ and $Tm(PPP)_2^{7-}$. *J. Magn. Reson.* 1984; **56**: 33–47.
5. Sherry AD, Malloy CR, Jeffrey FMH, Cacheris WP, Geraldes CFGC. $Dy(DOTP)^{5-}$: a new, stable ^{23}Na shift reagent. *J. Magn. Reson.* 1988; **76**: 528–533.
6. Naritomi H, Kanashiro M, Sasaki M, Kuribayashi Y, Sawada T. *In vivo* measurements of intra- and extracellular Na^+ and water in the brain and muscle by nuclear magnetic resonance spectroscopy with shift reagent. *Biophys. J.* 1987; **52**: 611–616.
7. Albert MS, Lee J-H, Springer CS Jr. The use of $Tm(DOTP)^{5-}$ as a ^{23}Na shift reagent in living rat Studies at 9.4 T. (Abstract.) In *Society of Magnetic Resonance Medicine, 9th Annual Scientific Meeting and Exhibition*, Book of Abstracts, 1990; 1269.
8. Balschi JA, Bittl JA, Springer CS Jr, Ingwall JS. ^{31}P and ^{23}Na NMR spectroscopy of normal and ischemic rat skeletal muscle. Use of a shift reagent *in vivo*. *NMR Biomed.* 1990; **3**: 47–58.
9. Lee JH, Albert MS, Huang W, Springer CS Jr. ^{23}Na CSI of the rat brain *in vivo* at 9.4 T: on the impermeability of the blood–brain barrier to shift reagents. (Abstract.) In *Society of Magnetic Resonance Medicine, 10th Annual Scientific Meeting and Exhibition*, Book of Abstracts, 1991; 440.
10. Bansal N, Germann MJ, Lazar I, Malloy CR, Sherry AD. *In vivo* ^{23}Na MR imaging and spectroscopy of rat brain during $TmDOTP^{5-}$ infusion. *J. Magn. Reson. Imag.* 1992; **2**: 385–391.
11. Eleff SM, Mclennan IJ, Hart GK, Maruki Y, Traystman RJ, Koehler RC. Shift reagent enhanced concurrent ^{23}Na and ^{1}H magnetic resonance spectroscopic studies of transcellular sodium distribution in the dog brain *in vivo*. *Magn. Reson. Med.* 1993; **30**: 11–17.
12. Winter PM, Seshan V, Makos JD, Sherry AD, Malloy CR, Bansal N. Quantitation of intracellular $[Na^+]$ *in vivo* by using $TmDOTP^{5-}$ as an NMR shift reagent and extracellular marker. *J. Appl. Physiol.* 1998; **85**: 1806–1812.
13. Tkáč I, Starčuk Z, Choi I-Y, Gruetter R. *In vivo* 1H NMR spectroscopy of rat brain at 1 ms echo time. *Magn. Reson. Med.* 1999; **41**: 649–656.
14. Gruetter R. Automatic, localized *in vivo* adjustment of all first- and second-order shim coils. *Magn. Reson. Med.* 1993; **29**: 804–811.
15. Preston EP, Foster DO. Diffusion into rat brain of contrast and shift reagents for magnetic resonance imaging and spectroscopy. *NMR Biomed.* 1993; **6**: 339–344.
16. Grubb RL, Raichle ME, Eichling JO, Ter-Pogossian MM. The effects of changes in $PaCO_2$ on cerebral blood volume, blood flow, and vascular mean transit time. *Stroke* 1974; **5**: 630–639.
17. Jaccard G, Wimperis S, Bodenhausen G. Multiple-quantum NMR spectroscopy of $S = 3/2$ spins in isotropic phase: a new probe for multiexponential relaxation. *J. Chem. Phys.* 1986; **85**: 6282–6293.
18. Chung C-W, Wimperis S. Optimum detection of spin-3/2 biexponential relaxation using multiple-quantum filtration techniques. *J. Magn. Reson.* 1990; **88**: 440–447.
19. Navon G. Complete elimination of the extracellular ^{23}Na NMR signal in triple quantum filtered spectra of rat hearts in the presence of shift reagents. *Magn. Reson. Med.* 1993; **30**: 503–506.
20. Kalyanapuram R, Seshan V, Bansal N. Three-dimensional triple-quantum-filtered ^{23}Na imaging of the dog head *in vivo*. *J. Magn. Reson. Imag.* 1998; **8**: 1182–1189.
21. Hancu I, Boada FE, Shen GX. Three-dimensional triple-quantum-filtered ^{23}Na imaging of *in vivo* human brain. *Magn. Reson. Med.* 1999; **42**: 1146–1154.
22. Hubbard PS. Nonexponential nuclear magnetic relaxation by quadrupole interactions. *J. Chem. Phys.* 1970; **53**: 985–987.
23. Shinar H, Navon G. NMR relaxation studies of intracellular Na^+ in red blood cells. *Biophys. Chem.* 1984; **20**: 275–283.
24. Shinar H, Navon G. ^{23}Na-NMR relaxation times in body fluids. *Magn. Reson. Med.* 1986; **3**: 927–934.
25. Lee J-H, Labadie, Springer CS Jr. Relaxographic analysis of ^{23}Na resonances. (Abstract.) In *Society of Magnetic Resonance Medicine, 11th Annual Scientific Meeting*, Book of Abstracts, Vol. 2, 1992; 2214.

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