A method for estimating intracellular sodium concentration and extracellular volume fraction in brain in vivo using sodium magnetic resonance imaging

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In this feasibility study we propose a method based on sodium magnetic resonance imaging (MRI) for estimating simultaneously the intracellular sodium concentration \( (C_1, \text{ in mM}) \) and the extracellular volume fraction \( (\alpha) \) in grey and white matters (GM, WM) in brain in vivo. Mean \( C_1 \) over five healthy volunteers was measured, \( 11 \text{ mM} \) in both GM and WM, mean \( \alpha \) was measured, \( 0.22 \) in GM and \( 0.18 \) in WM, which are in close agreement with standard values for healthy brain tissue \((C_1 \sim 10–15 \text{ mM}, \alpha \sim 0.2)\). Simulation of ‘fluid’ and ‘solid’ inclusions were accurately detected on both the \( C_1 \) and \( \alpha \) 3D maps and in the \( C_1 \) and \( \alpha \) distributions over whole GM and WM. This non-invasive and quantitative method could provide new biochemical information for assessing ion homeostasis and cell integrity in brain and help the diagnosis of early signs of neuropathologies such as multiple sclerosis, Alzheimer’s disease, brain tumors or stroke.

Magnetic resonance imaging (MRI) is a powerful tool for imaging the body in vivo and diagnosing a large range of diseases in practically all parts of the human body. Standard MRI is based on the detection of protons \((^1H)\) present in water, lipids and macromolecules in the body. It can generate a wide range of different contrasts (such as proton density, T1-weighted, T2-weighted, diffusion, perfusion, blood-oxygen level dependent, etc.) giving different anatomical, structural, or sometimes functional, information on the tissues under investigation. However, standard MRI is generally not quantitative and cannot provide direct biochemical information on tissue viability (such as homeostasis or cell membrane integrity). This kind of information could be of upmost importance for diagnosing and prognosing diseases, or for assessing the effect of treatments and new drug tests. In the present feasibility/proof of concept study, we propose using a simple method based on sodium MRI for measuring simultaneously two tissue parameters, intracellular sodium concentration (called \( C_1 \) in this study) and extracellular volume fraction \( (\alpha) \), in brain in vivo in a quantitative and non-invasive manner on a clinical 3 T scanner. These two parameters are very sensitive to cell viability and ion homeostasis\(^2\)\(^\text{-}^4\) and could therefore be used as biomarkers of neurodegenerative diseases for measuring early impairment in energy production or membrane integrity before any sign can be detected with standard imaging techniques. Measuring changes in extracellular volume fraction would give information on effusion or disruption of cell packing\(^8\), dehydration\(^6\), changes in vascularization or tumor edema angiogenesis\(^8\)\(^\text{-}^9\) or even metabolite clearance in the brain\(^9\). Measuring changes in intracellular sodium concentration would help assess the degree of cell hypometabolism or injury\(^6\), tumor malignancy\(^10\)\(^,^1\) or resistance to therapy\(^12\).

Sodium is a vital component of the human brain, and sodium ion \((^23Na^+)\) homeostasis is a major process in cells through coupled exchange with potassium ions \((^K^+)\) between intra- and extracellular spaces using the \( Na^+/-K^+\)-ATPase (sodium-potassium pump)\(^13\). This pumping process maintains a constant gradient of concentrations across the membrane \((10–15 \text{ mM} \text{ intracellular versus } 140 \text{ mM extracellular})\) which is used to control cell volume, pH balance, glucose and neurotransmitter transport, calcium regulation, membrane electrical potential (and therefore nerve pulse transmission) and protect the cell from bursting as a result of osmotic swelling. This process is very energy-consuming and is therefore very dependent on adenosine triphosphate (ATP) production and hydrolysis in cells. Regulation of \( Na^+/-K^+\)-ATPase therefore plays an essential role in the etiology of brain pathologies. Dysregulation of the \( Na^+/-K^+\)-ATPase or impairment of ATP-dependent processes will provoke a loss of \( Na^+ \) homeostasis and therefore increase of intracellular sodium concentration.
(as the gradient cannot be maintained anymore) leading ultimately to cell death (and concomitant increase of extracellular volume fraction). Being able to estimate quantitatively both C1 and γ in brain in vivo could therefore provide fundamental new metabolic information for detecting early processes of loss of cell integrity in both acute diseases such as stroke14, and chronic diseases such as brain tumors15, multiple sclerosis17 or Alzheimer’s disease18. This biochemical information could be combined with anatomical proton MRI (for measuring structural changes in brain) or positron emission tomography (PET, for measuring glucose consumption for example), in order to assess the prognosis of pathologies, and their response to new treatments.

Sodium MRI is based on the detection of Na+ ions present in different concentrations in tissues19–21. Sodium ion has a quadrupolar nucleus of spin 3/2 that yields the second strongest nuclear magnetic resonance (NMR) signal among all nuclei present in biological tissues, after proton 1H. The average sodium concentration in brain is around 40 mM if we take into account the average extra- and intracellular volume fractions (about 0.2 and 0.6 respectively, with solid volume fraction −0.2) in brain. The NMR sensitivity of sodium is 9.27% of the proton sensitivity, which combined with low concentrations compared to water protons (110 M, for 2 protons/water molecule), leads to an average sodium NMR signal about 30,000 times lower than proton signal in brain. Moreover, due to their quadrupolar moment, sodium spins interact very strongly with the electric field gradients of their surroundings, leading to very short relaxation times in tissues (with monoexponential T1 ~ 30–40 ms, and biexponential T2_short ~ 1–5 ms and T2_long ~ 15–30 ms in brain) compared to protons. Because of all these parameters, sodium MRI can be accomplished using high magnetic fields (≥3 T), ultrashort echo time (UTE) acquisition sequences and with low resolution (≥4 mm). Many averages are also necessary for increasing the signal-to-noise ratio (SNR) of the images, but this leads to long acquisition times (10–20 min). Thanks to recent advances in hardware capabilities such as high fields and strong stable magnetic gradients, sodium MRI can now be implemented on many clinical scanners provided some specific hardware and software is installed (amplifiers, dual-tuned 1H/23Na radiofrequency coils, non-Cartesian UTE sequences and reconstruction algorithms). Previous studies have suggested the use of sodium MRI for assessing intracellular sodium and extracellular or intracellular volume but did not implement it completely22, or used the more complicated triple quantum filters (TQF)21. TQF acquisitions necessitate higher magnetic fields (7 T), are more sensitive to radiofrequency (RF) and static magnetic field inhomogeneities22–25, and previous studies did not separate grey and white matters (GM, WM) in brain.

We propose here a simple sodium MRI method that can be applicable on a clinical 3 T scanner, which can generate simultaneously C1 and γ 3D maps and their respective distributions in GM and WM separately. Data quantification can be performed by using a combination of five calibration phantoms with known sodium concentrations and relaxation times placed within the field-of-view (FOV) of the images, and two major steps. The first step consists of calculating the apparent total sodium concentration (aTSC) and apparent intracellular sodium concentrations (aISC) maps from two sodium MRI acquisitions: one simple full sodium acquisition, and one with fluid suppression by inversion recovery (IR)26–28, respectively. Using masks of grey matter and white matter from double inversion recovery (DIR) proton MRI with turbo spin echo acquisition29–32, we can obtain aTSC and aISC maps of GM and WM separately. See Figure 1 for a description of this process. The second step consist of calculating the C1 and γ maps in GM and WM from aTSC and aISC in combination with a simple three-compartment model (intracellular, extracellular, and solid compartments) as shown in Figure 2. The full data processing is described in more details in the Methods section.

**Results**

**Extracellular volume fraction and intracellular sodium concentration quantification in vivo.** Examples of extracellular volume fraction (γ) maps and intracellular sodium concentration (C1) maps from one volunteer are shown in Figure 3. Examples of the distributions of C1 and γ values from the same volunteer, over the whole 3D data for WM, GM and full brain (GM + WM) are shown in Figure 4. Table 1 summarizes the mean and standard deviation of different statistical parameters of the C1 and γ distributions over all volunteers (n = 5). The mean value of the mean, median and mode of C1 measured with this method is around 11 ± 2 mM in both GM and WM, with mean standard deviation about 6 ± 0.2 mM. These values match closely the usual values in healthy brain found in the literature, which are generally in the range 10–15 mM20,28. The mean value of mean γ was measured around 0.22 ± 0.04 in GM and 0.18 ± 0.05 in WM, which is also in close agreement with its standard average value in brain (γ ~ 0.2) measured in different mammal brains with diffusion techniques. Moreover, standard error (or uncertainty) propagation29 was calculated for typical and extreme variations of the parameters (aTSC, aISC, water fraction w, and extracellular/CSF sodium concentration C2) used to calculate C1 and γ. See Methods, and Supplementary Information, for details. Main results are shown in Table 2. In summary, an uncertainty of around 5 mM (36%) and 0.06 (32%) can be expected when measuring C1 and γ, for typical variations in water content, extracellular (or CSF) sodium concentration, and errors in data processing of aTSC and aISC due to noise or incomplete fluid suppression by inversion recovery.

Interestingly, both C1 and γ distributions exhibit non-zero skewness (which quantifies how asymmetrical the distribution is) and kurtosis (which quantifies how the shape of a distribution matches the Gaussian distribution)22,28. All volunteers exhibit similar skewness and kurtosis. An average skewness of ~0.4 was measured for C1 in WM and GM, ~0.9 for γ in WM, and ~1.4 for γ in GM: both distributions from normal brains are skewed towards higher values (right side). An average kurtosis of ~4 for C1 in WM and GM, and ~4.5 for γ in GM, and ~8 in WM, can be interpreted as a more ‘peaked’ distribution compared to a Gaussian.

**Simulations.** Simulation of artificial ‘fluid’ or ‘solid’ inclusions in the brain were investigated for testing the effectiveness of the method for detecting abnormalities in the brain. The ‘fluid’ inclusion (cystic fluid-type) was simulated by adding a 10 × 10 × 10 voxels inclusion in the brain region (mostly GM) of the aTSC and aISC maps, with aTSC = 120 mM (very high total sodium content compared to normal 30–40 mM) and aISC = 5 mM (low apparent intracellular sodium compared to normal 10–15 mM), prior to C1 and γ quantification. For the ‘solid’ inclusion (tumour-type), aTSC = 55 mM (high total sodium content) and aISC = 25 mM (high intracellular sodium content). Noise in the range ~[2,2] mM was also added to the aISC and aTSC values of the inclusion for a more realistic simulation of noisy sodium data. These inclusions represent about 1.15% of the total brain volume (1000 voxels over 86571 voxels in whole GM + WM). The corresponding C1 and γ maps and distributions are shown in the supplementary figures S1–S2 (‘fluid’) and S3–S4 (‘solid’). The ‘fluid’ inclusion is very distinct on the γ map with a mean value ~0.8 compared to normal brain with γ ~ 0.2, and appears dark on the C1 map with a mean value = 0. This ‘fluid’ inclusion can also be easily detected on the γ distributions in full brain, WM and GM as an additive peak around 0.8. Note that the mean, median and mode of γ remain practically unchanged compared to average values from normal brain, but that the skewness and kurtosis are greatly increased by factors ~3 and ~4 respectively. The ‘solid’ inclusion appears very distinctively on the C1 map with a mean value ~45 mM compared to normal brain with C1 ~ 10–15 mM, and is undetectable on the γ map with a mean value = 0.2. This ‘solid’ inclusion can also
be easily detected on the $C_1$ distributions in full brain, WM and GM as an additive peak around 40–50 mM. Note that the mean, median and mode of $C_1$ remain practically unchanged compared to average values from normal brain, but that the skewness and kurtosis are increased by factors $2–4$ and $2–3$ respectively.

**Discussion**

Mean values for both $C_1$ and $\alpha$ estimated with this simple sodium MRI method (2 acquisitions) and simple model (three-compartment) are in close agreement with standard values measured in healthy brain tissue. In all five volunteers, we found that the mean $\alpha$ in WM ($\sim 0.18$) was lower than in GM ($\sim 0.22$), but no conclusion can be drawn for the moment as our sample size is too small. Most of literature gives an average $\alpha$ 0.2 in brain, without distinction between GM and WM. More healthy subjects will be scanned for assessing the repeatability, reproducibility and robustness in estimating $C_1$ and $\alpha$ of the method prior to application to patients with neuropathologies. The robustness of the method is closely dependent on the sodium quantification using calibration phantoms, as a slight change in the slope of the linear regression function can induce large variations in the $a$ISC and $a$TSC maps. In our model, we therefore calculated the $a$ISC and $a$TSC maps only when the signal from the calibration phantoms was fitted by linear regression with the condition that both coefficients of determination $R^2 > 0.99$ and adjusted $R^2_{adj} > 0.98$ (which takes into account the number of variables and sample size). This condition held every time and is the norm. Only on one subject the values of $R^2$ and $R^2_{adj}$ were slightly below the thresholds (0.98 and 0.97 respectively), due to malposition of the gels next to the head.
From the uncertainties calculation (error propagation), variations of $C_1$ and $\alpha$ due to uncertainties in $aTSC$ and $aISC$ calculation (due to noise or imperfect inversion), and in estimation of $w$ and $C_2$, are within the range of the mean standard deviations (about 6 mM and 0.08, respectively) that we measured on the volunteers (see Table 1). This indicates that this method would be able to detect changes in $C_1$ and $\alpha$ of above 40% ($C_1 > 20$ mM and $\alpha > 0.28$) in pathologies, which are of the order of changes expected from the literature\textsuperscript{5–12}.

Both $C_1$ and $\alpha$ distributions had positive skewness and kurtosis in WM and GM, which can be interpreted from both (1) a methodological and (2) a biophysical point-of-view. (1) The sodium images were acquired with low resolution (5 and 6.7 mm isotropic), which generates large partial volume effect, mainly in the regions close to the ventricles and subarachnoid space (filled with cerebrospinal fluid - CSF). Sodium images were then reconstructed with a nominal resolution of 2.5 mm matching DIR MRI and then multiplied by...
Table 1 | Statistics of intracellular sodium concentration (C1) and extracellular volume fraction (\(\alpha\)) over all volunteers (n = 5). The mean, median, mode, standard deviation (std), skewness and kurtosis of C1 and \(\alpha\) (column data) are measured over all voxels of the full brain, GM or WM for each volunteer. The mean and std of these six parameters are then calculated over 5 volunteers (row data).

| Variation type | FULL BRAIN | GM | WM |
|----------------|------------|----|----|
| Mean (mM)     |            |    |    |
| C1            | Mean       | 11.4| 11.2| 11.1|
|               | Median     | 11.2| 11.2| 11.1|
|               | Mode       | 10.6| 12.2| 11.2|
|               | Std        | 2.2 | 2.5 | 3.8 |
| Skewness      |            | 0.5 | 0.4 | 0.5 |
| Kurtosis      |            | 3.9 | 3.9 | 3.9 |
| C1 (mM)       |            | 11.7| 11.4| 10.9|
|               | Mean       | 11.7| 11.4| 10.9|
|               | Median     | 11.4| 11.4| 10.9|
|               | Mode       | 10.6| 12.2| 11.2|
|               | Std        | 2.2 | 2.5 | 3.8 |
| Skewness      |            | 0.5 | 0.4 | 0.5 |
| Kurtosis      |            | 3.9 | 3.9 | 3.9 |
| \(\alpha\)    | Mean       | 0.20| 0.18| 0.08|
|               | Median     | 0.22| 0.22| 0.20|
|               | Mode       | 0.22| 0.22| 0.20|
|               | Std        | 0.04| 0.04| 0.01|
| Skewness      |            | 0.86| 0.86| 0.86|
| Kurtosis      |            | 4.45| 4.45| 4.45|

Table 2 | Results from error propagation calculation for C1 and \(\alpha\). Column 1 shows the mean values of \(S_1\) (aTSC) and \(S_2\) (aISC) over 5 volunteers and for typical mean values of \(C_2\) (extracellular/CSF sodium concentration) and \(w\) (water fraction) in healthy brain tissue. Uncertainties (standard deviations - std) in the estimation of C1 and \(\alpha\) were calculated for typical and extreme variations of \(C_2\) and \(w\), and for std of \(S_1\) and \(S_2\) over 5 volunteers (considered as typical values). Columns 2 and 3 show the resulting std of C1 and \(\alpha\) when the variations of all 4 parameters are taken into account, for typical and extreme std of \(C_2\) and \(w\), respectively. Columns 4 to 9 show the effect of individual (typical and extreme) variations of \(S_1\), \(S_2\), \(C_2\) or \(w\) on the uncertainties of C1 and \(\alpha\). All std results for C1 and \(\alpha\) are given in absolute value (and percentage).

| Variation type | 1        | 2        | 3        | 4        | 5        | 6        | 7        | 8        | 9        |
|----------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|                | Units    | Mean     | Std      | Mean     | Std      | Mean     | Std      | Mean     | Std      |
| \(S_1\)        | mM       | 35       | 8        | 8        | 8        | 3        | 3        | 5        | 10       |
| \(S_2\)        | mM       | 8        | 3        | 3        | 3        |          |          |          |          |
| \(C_2\)        | mM       | 140      | 5        | 10       |          |          |          |          |          |
| \(w\)          |          | 0.8      | 0.05     | 0.10     |          |          |          |          |          |
| \(C_1\)        | mM       | 13       | 4.8      | 5.1      | 1.2      | 4.5      | 0.15     | 0.3      | 1.1      |
| \(\alpha\)     |          | 0.2      | 0.061    | 0.063    | 0.060    | 0.021    | 0.007    | 0.014    | 0.014    |
Methods

MRI human subjects. The brains of five healthy subjects (3 males, 2 females, mean age = 33 ± 7 years) were scanned after approval from the Institutional Review Board of the New York University Langone Medical Center and signed informed consent. The methods were carried out in accordance with Food and Drugs Administration (FDA) guidelines.

MRI hardware. The MRI experiments were performed on a 3T Tim Trio system (Siemens, Erlangen, Germany) using a dual-tuned 1H/23Na birdcage radiofrequency coil tuned at 128/33 MHz (Stark Contrast, Erlangen, Germany).

Proton MRI acquisitions. Two double inversion recovery (DIR) 1H MRI acquisitions were performed. The first DIR image was acquired in order to suppress both CSF and WM using the DIR Turbo Spin Echo SPACE sequence34,35 with the following parameters: TR = 7500 ms, TE = 300 ms, field-of-view (FOV) = 220 × 320 × 320 mm³, resolution = 5.5 mm isotropic, acquisition times T1 = 2650 ms and T1 = 550 ms, time of acquisition (TA) = 4:00 min. The second DIR image was acquired in order to suppress both CSF and GM with the same parameters as the first DIR except: T1 = 2800 ms and T2 = 800 ms.

Sodium MRI acquisitions. Sodium acquisitions were performed using the 3D UTE non-Cartesian FLORET sequence36,37 with the following parameters:

1. Sequence 1 - without fluid suppression: TR = 80 ms, TE = 0.2 ms, flip angle (FA) = 80°/0.5 ms, 3 hubs at 45°, 200 interleaves/hub, 14 averages, FOV = 320 mm isotropic, acquisition resolution = 5 mm isotropic, acquisition time TA = 11:00 min.

2. Sequence 2 - with fluid suppression by inversion recovery (IR): a ‘soft’ rectangular inversion pulse of 80/6 ms was added to the FLORET sequence with an inversion time TI = 24 ms (calculated from the centers of the pulses), TR = 100 ms, TE = 0.2 ms, FA = 90°/0.5 ms, 3 hubs at 45°, 85 interleaves/hub, 6 averages, FOV = 320 mm isotropic, acquisition resolution = 6.7 mm isotropic, TA = 17:00 min. A spoiler gradient of 4 ms was also included during TI for removing any transverse magnetization generated by imperfections of the inversion pulse.

A chronogram and k-space trajectory of the FLORET acquisition is shown in Supplementary Figure S5. Two fast complementary sodium acquisitions were performed for calculating the transmit B1+ map of the coil, which will be used in the data processing for RF inhomogeneities correction using the double angle method38; TR = 220 ms, TE = 0.2 ms, 3 hubs at 45°, 30 interleaves/hub, 6 averages, FOV = 320 mm isotropic, acquisition resolution = 10 mm isotropic, with FA = 60°/0.5 ms (1° acquisition) and FA = 120°/0.5 ms (2° acquisition), TA = 2:00 min each. All sodium images were reconstructed offline in Matlab (MathWorks, Natick, MA, USA) with standard 3D gridding and density compensation39 with a nominal resolution of 2.5 mm isotropic (128 × 128 × 128 voxels).

Data processing. The 1H/23Na data processing is described in Figure 1:

1. All 1H and 23Na 3D data were acquired with the same axial FOV (320 mm) and all isocenter. All images were reconstructed with 2.5 mm isotropic nominal resolution. (1H data sets were completed with zero filling on both sides of the sagittal plane for matching the size of the 3D sodium data, which is 128 × 128 × 128 voxels) and were therefore already co-registered. Sodium images were corrected for B1+ inhomogeneities using the double angle method38.

2. The signal from five calibration phantoms placed within the FOV on the right side of the brain was measured and averaged over 4 consecutive slices (10 voxels/phantom/slice). These phantoms are made of 3% agar gel with known sodium concentration: 10, 30, 50, 70 and 100 mM (from NaCl dilution). Their relaxation times were also measured as T1 = 38 ms and T2 = 7 ms at 3 T. A full density operator simulation for spin 3/2 dynamics40,41 during the RF pulse sequence was implemented in Matlab in order to estimate the loss of signal of the sodium phantoms due to relaxation during RF pulses and delays. From this simulation, phantom signals were therefore corrected by a factor λ1,2,λ = 1.10 and λ1,2,μ = 1.60 for sequences 1 and 2 respectively, prior to linear regression. Moreover, the linear regression was considered as valid only when the coefficients of determination R² > 0.99 and adjusted R² > 0.98, in order to improve the robustness of the method against noise and signal variations in the phantoms. The parameters a and b corresponding to sequence i = 1,2 in the following equation (1) were calculated from simple linear regression in Matlab, with cph,i the vector of phantom sodium concentrations and Sph,i the vectors of corresponding sodium signals:

\[
\begin{align*}
\lambda_1,2,\mu S_{\text{ph}} = a_{\text{ph}} + b_{\text{ph}} + \epsilon, \quad (i = 1, 2)
\end{align*}
\]

3. The apparent total sodium concentration (aTSC) and apparent intracellular sodium concentration (aISC) maps are calculated from sequences 1 and 2 respectively from the coefficients a and b (i = 1, 2) obtained from the linear regression using equations (2) and (3), for each voxel:

\[
\begin{align*}
a_{\text{TSC}} &= \frac{a_{\text{ph}} - b_{\text{ph}}}{a_1} \\
a_{\text{ISC}} &= \frac{a_{\text{ph}} - b_{\text{ph}}}{a_2}
\end{align*}
\]

with Sph,max the signal in the brain from sequence i, λ1,2,μ, = 0.85 and λ1,2,μ, = 0.50 the correction factors for aTSC and aISC. These two factors were calculated from full density operator simulation of the sodium spin dynamics during the RF pulse sequences, with relaxation times T1 = 35 ms, and T2 = 5 ms, T2 = 25 ms, based on average values in parenchyma from the literature38,42,43,44.

4. 3D GM and WM and full brain (WM + GM) masks were calculated from the ‘1H DIR acquisitions using SPM845 in Matlab.

5. The aTSC and aISC maps were multiplied by the GM, WM and GM + WM masks. These masked aTSC and aISC maps can therefore be used in the following quantification section for measuring the distributions of intracellular sodium concentrations and extracellular volume fractions separately in WM and GM.

Intracellular sodium concentration (C1) and extracellular volume fraction (w) quantification. C1 and w quantification was based on a simple three-compartment model shown in Figure 2. In this model, the extracellular compartment (including interstitial volume, CSF, plasma and blood) has a constant average sodium concentration C2 = 140 mM46. We also considered in this study that the water (fluid) volume fraction is constant and take averages values wWM = 0.7, wWM = 0.85 and wWM = 0.775 (mean value from WM and GM)47. We also assumed that fluid sodium signals are completely suppressed by inversion recovery in sequence 2. We will use the notation SaTSC and SaISC in the following equations. The value of each voxel of the map SaTSC is by definition equal to the total sodium concentration within each voxel, that is SaTSC = (C1 + C2) × V1 / V (V = total volume of the voxel). The value of each voxel of the map SaISC is by definition equal to the intracellular sodium concentration only within each voxel, that is SaISC = (C1) × V1 / V. From these assumptions and equations, we can calculate the unknown parameters C1 and w of interest, using the relationships given in Figure 2:

\[\begin{align*}
2 &= \alpha - \beta, \quad C_2 = \frac{C_1 \alpha}{C_1 \alpha - C_2} \quad \text{(4)}
\end{align*}\n
with w taking the values wWM, wWM, and wWM depending on the masked aTSC and aISC maps used. This calculation is performed for each voxel. All voxels are then recomputed in 3D maps of C1 and w in WM, GM and full brain, as shown in Figure 3.

Error propagation. Uncertainties on C1 and w for typical and extreme variations (measured as standard deviations) of SaTSC, SaTSC, SaTSC, extracellular (CSF concentration) and w (water fraction) can be calculated using the standard variance (or error) propagation method48. See the "Error propagation" section in "Supplementary Information for detailed. Typical values of C1, C1, C1, extracellular sodium concentration (and CSF), are generally taken around 140 mM (range 135–150 mM)2,20,45,49,50, variations (std) were therefore estimated at 5 mM (typical) and 10 mM (extreme case). The effect of these variations uncertainties on C1 and w are shown in Table 2, for mean and std of SaTSC and SaTSC measured over full brain over 5 volunteers. In the two first std columns, all standard deviations of SaTSC, SaTSC, SaTSC, w (typical values in column 2 and extreme values in column 3) are taken into account. The last 6 std columns (columns 4–9 of the table) show the effect of individual (typical and extreme) variations from SaTSC, SaTSC, SaTSC, w.

See the caption of Table 2 for more details.

Simulations. Two artificial inclusions were also added to the aTSC and aISC maps of one volunteer prior to C1 and w quantification processing, for assessing the efficiency of the method in detecting fluid-type (such as fluid cysts or other effusions, with sodium concentrations around 100–140 mM) and solid-type (such as tumors or dying cells) inclusions in the brain. The fluid inclusion is expected to be characteristic of increase of extracellular volume fraction and probably loss of cells (and therefore loss of intracellular sodium). The ‘solid’ inclusion should be linked to increase of intracellular sodium concentration with constant extracellular volume fraction. Both inclusions were added in the brain as 10 × 10 × 10 voxels inclusions (see Supplementary Figures S1 and S3). These inclusions represent about 1.15% of the total brain volume (8000 voxels over 86571 voxels in whole GM + WM). For ‘fluid’ inclusion, aTSC = 120 mM and aISC = 5 mM (due to potential residual presence of cells, noise in data and/or imperfect fluid suppression). For ‘solid’ inclusion, aTSC = 55 mM and aISC = 25 mM. Uniform noise in the range [−2.2] (in mM) was also added to the aISC and aTSC values of the inclusion for a more realistic simulation.

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Author contributions
G.M. and R.K. designed the experiments. G.M. performed the experiments, reconstructed and processed the data, and performed the simulations. R.W. and G.M. wrote and implemented the FLORET sequence in the Siemens scanner. G.M., R.K., and R.W.R. interpreted the results and contributed to the final manuscript.

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