Establishing Upper Limits on Neuronal Activity–Evoked pH Changes With APT-CEST MRI at 7 T

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Purpose: To detect neuronal activity–evoked pH changes by amide proton transfer–chemical exchange saturation transfer (APT-CEST) MRI at 7 T.
Methods: Three healthy subjects participated in the study. A low-power 3-dimensional APT-CEST sequence was optimized through the Bloch-McConnell equations. pH sensitivity of the sequence was estimated both in phantoms and in vivo. The feasibility of pH–functional MRI was tested in Bloch-McConnell-simulated data using the optimized sequence. In healthy subjects, the visual stimuli were used to evoke transient pH changes in the visual cortex, and a 3-dimensional APT-CEST volume was acquired at the pH-sensitive frequency offset of 3.5 ppm every 12.5 s.
Results: In theory, a three-component general linear model was capable of separating the effects of blood oxygenation level–dependent contrast and pH. The Bloch-McConnell equations indicated that a change in pH of 0.03 should be measurable at the experimentally determined temporal signal-to-noise ratio of 108. However, only a blood oxygenation level–dependent effect in the visual cortex could be discerned during the visual stimuli experiments performed in the healthy subjects.
Conclusions: The results of this study suggest that if indeed there are any transient brain pH changes in response to visual stimuli, those are under 0.03 units pH change, which is extremely difficult to detect using the existing techniques.

Key words: CEST; APT-CEST; pH-fMRI; hypercapnia; Bloch-McConnell equations

INTRODUCTION

Traditional methods of mapping brain neuronal activity rely on blood oxygenation level–dependent (BOLD) contrast. Blood oxygenation level–dependent contrast is sensitive to T₂ contrast and originates from the inhomogeneity in the magnetic field as a result of variation in blood oxygen level, cerebral blood flow, and cerebral blood volume (1–3). A whole cascade of physiological processes take place between brain neuronal activity and changes in the hemodynamic properties of the surrounding vasculature, measured by BOLD functional MRI (fMRI). The BOLD-fMRI measures neuronal activity indirectly; therefore, it suffers from the low specificity, as the mechanisms underlying the neurovascular coupling may be altered (such as in pathology and disorder) (4,5).

Normal functioning of all cellular processes critically depends on pH homeostasis. There is a great body of literature suggesting that neuronal activity gives rise to pH shifts in both the intra- and extracellular milieu (6–9). Sustained neuronal activity triggers acidification of the cytosol through metabolic production of CO₂, lactic acid, internal H⁺ ions released in response to elevated Ca²⁺ levels, and acid influx through ligand- or voltage-gated channels (11–14). Interestingly, neuronal behavior itself can be modulated by small shifts in intracellular pH (pHi), as pH-dependent activity of ion channels may influence neuronal excitability (15). Noninvasive means of mapping these local and global transient pHi changes present a unique way of looking at brain activation, which may shed light on the mechanisms underlying many neurological and psychological disorders. The challenge, however, is to separate the pHi effect from concurrent T₂-dependent BOLD effects.

A variety of methods to measure pH noninvasively are described in the literature. Phosphorous-31 (³¹P) magnetic resonance spectroscopy (MRS) is a current gold standard (16–18). The pH calculations of ³¹P MRS are based on changes in chemical shift between a pH-sensitive probe (i.e. inorganic phosphate [Pi] and a pH-insensitive reference, such as phosphocreatine). However, ³¹P MRS suffers from inherently low resolution (typical acquisition volume = 3 cm isotropic), low signal-to-noise ratio, and long scan times (upwards of 5 min). Despite being highly specific to pH of the cytosol (19,20), these current limitations make it very challenging for ³¹P MRS to map neuronal activity–elicited transient pH changes.
Other approaches used to measure pH have been based on spin locking (21) and chemical exchange saturation transfer (CEST) (22) methods, both of which probe the inherently pH-dependent chemical-exchange process between the selectively saturated labile proton pool and the abundant water proton pool. For instance, Magnotta et al. used on-resonance spin locking to measure spin-lattice relaxation time (T1) in the rotating frame (T1p) to map brain neuronal activity by measuring pH changes in the human brain elicited by the visual stimulus (23). The authors noticed a similar activation pattern between the human brain elicited by the visual stimulus (23). The chemical-exchange contrast depends on a number of factors, was applied to the in vivo experiments to separate BOLD and T1p, with the latter being independent of blood oxygenation. In response to the visual stimulation in the healthy subject, a change in pH of approximately 0.1 was measured by 31P MRS, so the authors concluded that the observed T1p changes were the result, at least in part, of pH, thereby presenting pH-fMRI as a new way of looking at brain activity. However, many mechanisms may contribute to T1p relaxation in biological tissue, depending on the sequence parameters (e.g., macromolecules (24), pH (25), diffusion (26-28), cerebral blood volume (29), inflow (30), BOLD (28)). In addition, on-resonance T1p does not differentiate among the different metabolites present in vivo, many of which may exhibit direct and inverse relationships of their corresponding chemical-exchange processes with pH (31). As a result, the origin of T1p contrast in vivo should be treated with caution, as the effects of BOLD and inflow can be readily mistaken for the desired pH effect.

Recently, amide proton transfer (APT) CEST, the contrast of which is believed to predominantly originate from intracellular amides (32–35), was applied to quantitative pH mapping in hyperacute stroke patients (36). The technique showed potential in identifying the ischemic penumbra following the stroke (36–40), and a pH drop of 0.1 in the ischemic tissue was quantified (36). In addition, CEST is an image-based technique that allows for an inherently high spatial resolution. The high pH sensitivity and specificity at high resolution turn APT-CEST into an attractive tool to study neuronal activity–elicited fast transient pH changes in the brain.

In this work, we present our initial attempts at detecting neuronal activity–evoked pH changes using APT-CEST at 7 T. First, we used the Block-McConnell simulations to optimize a 3-dimensional (3D) steady-state, low-power, pulsed APT-CEST sequence in terms of sequence timing and sensitivity to the pH-dependent APT-CEST effect with the subsequent pH-sensitivity estimations in phantoms. Second, we manipulated arterial carbon dioxide level in the healthy human subjects to modulate brain pH and estimated ΔpH limit of detection for the optimized APT-CEST sequence in vivo. Then, a three-component general linear model was designed to separate BOLD and pH effects in the Block-McConnell simulated data. Finally, the model, tested in the simulations, was applied to the in vivo experiments to separate BOLD and pH effects evoked by the visual stimulus during the flashing checkboard in the healthy human subjects.

**THEORY**

The chemical-exchange contrast depends on a number of parameters including the rate of chemical exchange between the labile metabolite protons and the abundant exchange-mediating water proton pool (kex) and the Larmor frequency separation between them (Δω0). In a CEST experiment, a so-called Z-spectrum is acquired (22), which is a plot of the normalized attenuated water-signal intensity versus off-resonance saturation frequency (Δω). The water is assigned a chemical shift of 0 (Δω= 0, i.e., on resonance), and the labile protons of the endogenous metabolites contribute to the water-signal attenuation downfield (Δω in the range of 0–5 ppm) from the water resonance. Most of those labile protons have distinct off-resonance frequencies, such as hydroxyproline protons (0–1.5 ppm), amino protons (1.8–3.3 ppm), and amide protons (3.3–3.8 ppm). The CEST effect for each type of labile proton depends on the metabolite concentration and pH-dependent exchange rate (kex). The exchange rate of amide protons is base-catalyzed in the physiological pH range (i.e., it depends on the hydroxyl ions concentration), and therefore reflects changes in pH. This specificity, present in CEST but absent in on-resonance spin locking, allows the labile protons of a certain type to be probed independently of the others, making CEST contrast more pH-specific.

**METHODS**

**Numerical Simulations: Sequence Optimization**

A 3D steady-state pulsed CEST sequence (41) was optimized for maximum sensitivity to APT-CEST effect through the Bloch-McConnell equation simulations (42). The following sequence parameters were investigated: the number of saturation pulses (saturation time), transmit field amplitude of CEST saturation prepulse (B1 is used as the peak amplitude throughout the paper), and radiofrequency (RF) duty cycle. All other sequence parameters are the same as for data acquisition (see subsequently).

Four-pool (free water in tissue, APT-CEST, nuclear Overhauser Enhancement and magnetization transfer) Bloch-McConnell equations were solved numerically (43), assuming the following gray-matter pool parameters: free-water pool (T2 = 1.9 s/55 ms), APT pool (T2 = 10 ms, Δω = 3.5 ppm, M0 = 0.13%, R = 22.2 Hz), nuclear Overhauser Enhancement pool (T2 = 0.3 ms, Δω = −3.5 ppm, M0 = 3%, R = 10 Hz), and magnetization-transfer pool (T2 = 10 μs, Δω = −2.4 ppm, M0 = 3%, R = 50 Hz) (44). The T1 values of other than water pools (i.e., APT-CEST, nuclear Overhauser Enhancement, and magnetization transfer) were fixed to 1 s (45).

An assumption was made that there are only four pools in the system and that the only interactions are with water. The APT-CEST effect size (contribution to the z-spectrum) was quantified by the pool difference method as follows:

\[ \text{Amide-CEST} = \frac{M_z(3.5\, \text{ppm}, M_A = 0)}{M_0 - M_z(3.5\, \text{ppm}, M_A = 1)/M_0} \]

where APT-CEST is the effect size of cytosolic amides, \( M_z(\Delta \omega, M_A) \) is the signal in the z-spectrum at \( \Delta \omega \), \( M_0 \) is the steady-state signal at 300 ppm, and \( M_A \) is the amplitude of the APT-CEST compartment (\( M_A = 0 \) and \( M_A = 1 \))
Numerical Simulations: fMRI Data and Separation of BOLD and pH Effects

The pH-fMRI data were simulated using four-pool Bloch-McConnell equations (42,43) by changing an exchange rate \( k_{ex} \) of APT-CEST pool (from 22.2 Hz at pH = 7.0 to 17.6 Hz at pH = 6.9, using the following relationship: \( pH = \log_{10} k_{ex} + 5.654 \) derived in (35)) during the visual stimulation, and assuming a BOLD signal change of 1.1% (APT-CEST signal change at 3.5 ppm due to BOLD effect) and a \( \Delta pHi \) of 0.1 (or 0.52% APT-CEST signal change at 3.5 ppm due to pH effect). A BOLD effect of 1.1% was assumed based on a short echo time of the CEST sequence used, whereas a \( \Delta pHi \) of approximately 0.1 was previously measured by \( ^{31} \)P MRS (23) during the visual stimulation. Because the only varied CEST parameter was pH, APT-CEST signal change reflects \( \Delta pHi \). A Gaussian noise of 1.5% (of the steady-state signal at 300 ppm) was added to the simulated data for a final temporal SNR of 108, to match that of the experimental data.

To separate the pH effect from BOLD signal changes, a general linear model was composed of three regressors: (i) BOLD, (ii) pH, and (iii) APT-CEST signal \( M_z \) (3.5 ppm) variation, as a result of switching the RF CEST prepulse “on” and “off.” The BOLD effect was assumed to be activated when visual stimulus was “on” (regardless of RF CEST prepulse being “on” or “off”). The pH effect was assumed to be activated when both the visual stimulus and RF CEST prepulse were “on.” A visual explanation of the three-regressor model is provided in the “Results” section. All other sequence parameters and the fMRI paradigm are the same as for data acquisition (see subsequently).

Data Acquisition

This study was approved by the Medical Research Ethics Committee of University Medical Center Utrecht, and all of the volunteers gave informed consent. All experiments were done according to the guidelines and regulations of the Wet Medisch Wetenschappelijk Onderzoek. Three subjects were scanned on a 7T Achieva MR system (Philips, Best, Netherlands) using a quadrature transmit coil with a 32-channel receive head coil (NOVA Medical, Houston, Texas, USA).

Three-Dimensional Steady-State CEST Protocol

A 3D steady-state pulsed CEST sequence at 7 T was first introduced in (41) because of its power efficiency, low specific absorption rate, and low system burden compared with continuous-saturation CEST schemes. The steady-state sequence consists of a short RF pulse for presaturation (applied at the off-resonance frequency of the metabolite of interest), a gradient spoiler to destroy the residual magnetization in the x-y plane, and a segmented echo-planar-imaging readout. All of these components add up to a relatively short repetition time, which is repeated multiple times to reach the steady state. During the steady-state signal build-up, the k-space is acquired from the edge to the center of the k-space center, and the k-space center is sampled after the steady state has been achieved. The temporal resolution of a steady-state sequence is limited by the amount of time it takes to reach the steady state.

In this work, a low-power 3D steady-state CEST sequence was used with the following scan parameters: 1.5-mT (peak amplitude) saturation prepulse (a single RF-spoiled 8-ms (267-Hz bandwidth) sinc-Gaussian pulse followed by a 50-mT/m spoiler of 10 ms) interleaved with a sagittal, segmented echo-planar imaging readout (echo-planar imaging factor 7 with a binomial RF pulse for water-only excitation, repetition time/echo time/flip angle = 25 ms/4.2 ms/12°, field of view = 150 × 225 × 190, matrix size 128), voxel size = 2 mm isotropic, sensitivity-encoding factor 1.9 (anterior–posterior) and 2.4 (right–left), center of k-space weighted acquisition with the k-space center measured at 6.9 s, time per volume = 12.6 s. The data were acquired at two frequencies, 3.5 and 300 ppm, for the breathing experiments, and at 3.5 ppm (alternating saturation RF CEST prepulse between “on” and “off”) for the pH-fMRI experiments. Both alternating schemes (3.5 versus 300 ppm and RF CEST prepulse “on” versus “off”) were used to determine the remaining BOLD effect in the data. The data at 300 ppm and with RF switched “off” serve the same purpose and can be acquired interchangeably. For fMRI experiments, however, data acquisition with RF CEST prepulse switched “of” (instead of data at 300 ppm) is preferred, because of the RF amplifiers’ duty cycle limitations. A T1-weighted anatomical scan was used to create masks of white matter and gray matter, which were subsequently used to calculate white-matter and gray-matter averaged CEST signals. Third-order shims were applied to improve the homogeneity of the magnetic field across the whole brain.

Demonstration of pH Sensitivity

To validate the sensitivity of the sequence to changes in pH, dual phantoms were created with 10% (wt/vol) bovine serum albumin (BSA, ~66 kD) and 10 mM phosphocreatine in 10-mM phosphate-buffered solution as a pH buffering system containing 0.1% sodium azide for phantom preservation. Overall, six phantoms were made in 50-mL Falcon centrifuge tubes (Fisher Scientific, Hampton, New Hampshire, USA), and pH was adjusted to 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 by NaOH and HCl. All chemicals were purchased from Sigma-Aldrich (Zwijndrecht, Netherlands) and used as received without further purification. The phantom measurements were performed at room temperature.

Carbon-Dioxide Breathing Experiments

The CO2 breathing experiments were done to estimate the \( \Delta pHi \) detection limit of the optimized CEST sequence. The brain pH was modulated by carbon dioxide delivered using a computer-controlled gas delivery system (RespirAct, Thornhill Research Inc, Toronto, Ontario, Canada). During the breathing experiments, the volunteers were scanned throughout a hypercapnic breathing challenge (6 min) in which end-tidal CO2...
(PetCO₂) was targeted at 10 mmHg above individual subject baseline levels. CO₂ was used as a stimulus to change the brain pH (24). These changes were then investigated using APT-CEST MRI and 31P MRS.

31P MRS Protocol
For quantitative pH measurements, 3D 31P chemical shift imaging (echo time/repetition time = 0.5 ms/250 ms, field of view = 480 × 180 × 320 mm³, resolution = 20 × 20 × 20 mm³ isotropic, 2 min 25 s per scan, N = 2) was performed using inorganic phosphate and phosphocreatine as a probe and a reference, respectively (46).

Functional MRI Protocol
Functional imaging was performed using the APT-CEST sequence. The visual paradigm consisted of 10 blocks. Four equal-duration cycles of flashing checkerboard were interleaved with a 126-s period of visual fixation, followed by two extra cycles of visual fixation. A total of 100 3D volumes were acquired at the off-resonance frequency offset of 3.5 ppm (pH sensitive APT-CEST effect) by alternating RF CEST prepulse “on” (with CEST prepulse) and “off” (no CEST prepulse). The APT-CEST signal (M_z (3.5 ppm)) variation due to the RF CEST prepulse switching was used as a third regressor, in addition to the regressors describing the time variation of BOLD and pH. The total acquisition time was 21 min 14 s.

The stimulus was an 8-Hz flickering black and white checkerboard with a red fixating dot in the middle. The rest condition was a uniform gray stimulus with the same red fixating dot in the middle. The subjects participating in the fMRI experiments were experienced volunteers. After the experiments, confirmation was obtained from all subjects that the subjects were on task.

Data Processing
A general linear model (FSL, FMRI Expert Analysis Tool) consisting of three regressors (BOLD, pH, and APT-CEST signal (M_z (3.5 ppm)) variation as a result of RF CEST prepulse switching “on” and “off” was used to generate individual statistical maps and to calculate signal change. The last regressor was used to separate the effects of BOLD and pH. Coregistration and segmentation were done in FSL (FMRIB version 6.0, FLIRT, Oxford, United Kingdom) (47,48). To compensate for B₀ inhomogeneity, whole-brain white-matter and gray-matter masks were further intersected by the corresponding B₀ maps thresholded in between ±0.1 ppm.
Temporal SNR in the visual cortex was calculated as 

$$tSNR = \frac{S}{SD}$$

where $S$ and $SD$ are the mean signal and the standard deviation, respectively, in the visual cortex across 30 dynamic images acquired at 3.5 ppm without visual stimulation.

Simulations and further image processing and analysis were done using MATLAB (The MathWorks Inc, Natick, Massachusetts, USA).

RESULTS

In Figure 1, using the Bloch-McConnell simulations, the CEST sequence was optimized in terms of the temporal resolution (Fig. 1a), and the sensitivity to APT-CEST effect (Fig. 1b). Figure 1a shows the APT-CEST signal ($M_z/M_0$, %) during the approach to the steady state as a function of the number of saturation pulses (or repetition-time repeats), for various water $T_1$ ($T_{1w}$) relaxation times. The signal scales with $T_{1w}$ also govern the rate at which the steady state is achieved. The longer the $T_{1w}$, the more saturation pulses are required to reach the steady state, and hence the lower the temporal resolution. Assuming a maximum tissue $T_{1w}$ of 2.5 s (49,50) for a normal brain, the steady state (signal change within 0.5%) is reached in 6.9 s. This translates to 504 saturation pulse, which corresponds to the temporal resolution of 12.6 s.

The optimum saturation time parameters determined in Figure 1a were used to find the optimum saturation pulse ($B_1$ amplitude and duty cycle) parameters in Figure 1b. Both $B_1$ amplitude and duty cycle were varied to cover all possible ranges, and their influence on APT-CEST effect size is depicted. Taking into account RF
amplifiers-imposed duty cycle limitations, the optimum $B_1$ and duty cycle were chosen to be 1.5 $\mu$T and 32%, respectively. These parameters are close to those for the originally proposed steady-state 3D CEST sequence (41).

The optimized CEST sequence was evaluated in dual BSA/phosphocreatine phantoms (Fig. 2). The strong inverse relationship ($R = -0.94$, $P < 0.05$) of the normalized signal ($S@3.5$ ppm)/$S_0$ with pH (Fig. 2b) suggests that the new CEST sequence is sensitive to pH in the physiological range, which was further corroborated by $^{31}$P pH measurements in the same phantoms (Fig. 2a). The APT-CEST sequence $\Delta pH$ sensitivity per acquisition was estimated to be 0.8 using the following formula:

$$\Delta pH_{detectable} = 2 \times \text{sigma} \times \frac{\Delta pH}{\Delta S}$$  \[3\]

where sigma is the noise estimate (the average standard deviation across all samples), $\Delta pH$ (9–5) and $\Delta S$ (0.955–
lated amide-CEST signal (%) for each particular of partial volume effects. The right axis shows a change in iso-
vated pixels in the pHFMRI ROI to the number of pixels in the
Figure 5. Normalized pixels (left) axis represents a ratio of acti-
3.5 ppm) (Fig. 3b). After normalization of the APT-CEST
in phantoms (Fig. 2) translates to 0.08 for fMRI
experiments (Fig. 5) increases the sensitivity by a factor
of 2 weighting for the used APT-CEST sequence.
Nonetheless, the BOLD effect can be effectively removed
using the RF “off” interleaved dynamic acquisition
scheme, allowing isolation of potential pH signal varia-
tions after visual stimulation. In Figures 6e and 6f, the
Mixture ROI is also activated, because the underlying
signal in this ROI contains both pH and BOLD effects.
The input and output of the model were similar in mag-
nitude within the error introduced by the added noise.

The Bloch-McConnell equations (43) were used to fur-
ther study the limitations of the model for separating
pHi effect from a BOLD contaminated signal. At the
experimental temporal SNR, the ΔpH detection limit of
the APT-CEST sequence for the visual stimulus para-
digm in Figure 5 was estimated to be 0.03 (Fig. 7). Simi-
larly, the temporal encoding of 100 repetitions in fMRI
experiments (Fig. 5) increases the sensitivity by a factor
of 100; thus, a pH sensitivity of 0.8 (per acquisition point)
found in phantoms (Fig. 2) translates to 0.08 for fMRI
experiments. These estimates of pH sensitivity obtained
from the simulations and phantom experiments explain
why no detectable APT-CEST changes were measured
during the mild hypercapnia stimulus experiments.

The B₀-field inhomogeneity map and the temporal
SNR map are show in Figures 8a and 8b, respectively.
The inhomogeneity across the visual cortex was calcu-
lated to be under 10 Hz, whereas the temporal SNR was
found to be 108. The experimental fMRI data of a
healthy control is presented in Figure 9. The visual stim-
ulus during the flashing checkboard induced a BOLD
effect of approximately 1% in the visual cortex (Fig. 9a).
There is some residual activation in the pHi activation
map (Fig. 9b); however, no statistically significant pHi
effect could be extracted in the visual cortex. These
results suggest that pH changes evoked by the visual
stimulus during the flashing checkboard are under 0.03
units pH change.
DISCUSSION

The APT-CEST sequence is believed to originate from intracellular compartment (i.e., pH-sensitive contrast). In this work, we present our initial efforts at detecting pH changes during visual brain activation using APT-CEST at 7 T. To this end, a 3D steady-state pulsed CEST sequence was optimized by means of Bloch-McConnell simulations in terms of temporal resolution and sensitivity to the pH-dependent APT-CEST effect. In Bloch-McConnell simulated data, a three-component general linear model was capable of separating the pH and confounding BOLD effects. In vivo, however, only the BOLD effect could be clearly identified in the visual cortex, and no significant pH changes could be detected.

The pH changes in the brain evoked by visual stimuli are expected to be transient; thus, the method to track the related signal variations should have a high temporal resolution. For the steady-state CEST sequence used in this work, there is a trade-off between the steady-state signal and the temporal resolution (Fig. 1a). The highest allowable temporal resolution was determined to be 12.6 s for a signal change within 0.5% of the steady-state signal. To be within RF amplifiers duty cycle limitations, the optimum B1 amplitude and duty cycle were chosen to be 1.5 μT and 32%, respectively (Fig. 1b). These optimized parameters are in line with the fact that amide protons are slowly exchanging protons (35) and require a low power level to reach the maximum saturation efficiency (41). The sequence timing (Fig. 1a) and CEST pre-pulse (Fig. 1b) parameters are largely uncorrelated and can therefore be optimized independently. The B0-inhomogeneity correction of CEST data requires acquisition of densely sampled CEST spectra (water saturation shift referencing method) (51), which is not possible when high temporal resolution is desired. To make our CEST sequence relatively insensitive to small B0-inhomogeneity effects, we chose to use a short CEST pre-pulse of only 8 ms (267-Hz bandwidth). This also has the advantage of increasing SNR (both image and temporal) by sensitizing the sequence to the whole APT effect (3–4 ppm or 298 Hz at 7 T). The optimized APT-CEST sequence was validated to be pH sensitive in BSA phantoms that are rich in amide protons. The APT-CEST sequence sensitivity to pH changes in BSA phantoms was estimated to be 0.8 (per acquisition point), which translates to 0.08 for fMRI experiments (due to averaging the data from 100 repeated measurements, i.e., 0.8/100).

Fitting regressors for fMRI data is expected to further increase this pH sensitivity, but the precise estimation is difficult. Although BSA is a good model protein, care has to be taken when directly translating the phantom results to in vivo studies, because of differences in water T1 relaxation times (52–55), protein concentration, protein conformation, and catalytic microenvironment (56), among others.

The exact magnitude of visual stimuli-evoked pH changes in the brain is unknown, but it is expected to be relatively small as a result of acid-base homeostasis. Therefore, it is important to estimate the ΔpH detection limit of the sequence used to detect those changes. From the literature, the short (6 min) mild hypercapnic exposure (Fig. 3a) used in this work is expected to cause a pH drop of approximately 0.02 ± 0.01 (24). As expected, all points (pH-sensitive APT-CEST and controls) have
an additional contribution of BOLD effect during the breathing challenge as a result of $T_2^*$ weighting of the echo-planar imaging readout and a finite echo time (Fig. 3b). However, only pHi-sensitive APT-CEST points are expected to be influenced by a pHi effect. This means that normalization of the APT-CEST points by the control data should effectively remove the BOLD contamination, revealing a small $\Delta pHi$ effect. As expected, after the normalization, we did not see any BOLD effect. Unfortunately, no visible pHi effect could be discerned either. With these results, we concluded that the $\Delta pHi$ detection limit of the APT-CEST scheme is above 0.02. In similar breathing experiments, we tried to detect those pHi changes by using the gold standard (i.e., $^{31}$P MRS) (Fig. 4). No pHi changes could be detected even during two consecutive hypercapnic exposures. However, this is not surprising, considering the low pH precision of $^{31}$P MRS itself, which is estimated to be approximately 0.05 (20,57,58). In this work, we chose to do hypercapnic exposures, as those are easy to control. A pHi change of approximately 0.1 with increased lactate detection was reported earlier in hyperventilation experiments (59).

With the knowledge of the optimized sequence parameters and the limitations determined in the previous steps (Figs. 1–4), the APT-CEST sequence was used in the Bloch-McConnell simulations to test the feasibility of pHi-IMRI (Fig. 5). The effects of pHi (0.1 $\Delta pHi$ encoded in the pHFMRI ROI in Fig. 6a) and BOLD (1.1% signal change encoded in the boldFMRI ROI in Fig. 6a) were simulated separately and in combination (the Mixture ROI in Fig. 6a), as would be expected in the in vivo fMRI experiments. The simulated time series were corrupted with a Gaussian noise, resulting in a temporal SNR of 108 (matching that of the experimental data). The general linear model was successful in separating the effects of both pH and BOLD from the mixture of two, and the isolated contrast resembles that of the control contrast (the pHFMRI and boldFMRI ROIs were used as controls for pH and BOLD effects in Figs. 6e and 6f, respectively). These results serve as a proof of principle of the feasibility of pHi-IMRI. However, despite the fact that the Bloch-McConnell equations are known to describe chemical exchange processes precisely, it is important to realize that many in vivo parameters used in the simulations are unknown beforehand. As a result, we had to make an assumption on a few parameters, taking the available literature into account. In addition, the magnitude of pH changes evoked by the neuronal activity in vivo is also unknown, and was assumed in the simulations to be 0.1 (23), which may not correspond with the reality. This level of $\Delta pHi$ could be successfully isolated from a strongly BOLD-contaminated MRI signal. The breathing experiments reported in this work appear to be the most practical way to measure the $\Delta pHi$ detection limit in the healthy human brain noninvasively. However, those experiments would require extremely high intolerable levels of carbon dioxide to cause a measurable pHi change (Figs. 3 and 4). The advantage of the Bloch-McConnell equations comes from the fact that the influence of many parameters can be studied without actually doing the experiments. By varying the $\Delta pHi$ effect, the $\Delta pHi$ detection limit of the sequence coupled with the visual stimulus paradigm was found to be 0.03. This seemingly high pH sensitivity comes from fitting the repetitive temporal pattern of the fMRI experiments with a model with three regressors.

In the in vivo experiments, B$_0$ inhomogeneity may be an issue, especially for the dynamic measurements used in this study. The B$_0$ field was optimized across the whole brain using third-order shims (Fig. 8a). Small inhomogeneity (in our case under 10 Hz) in the visual cortex can be neglected, as the CEST prepulse was 8 ms (267 Hz bandwidth), and the APT-CEST effect covers a range of 298 Hz (from 3 to 4 ppm).

The same general linear model, the performance of which was tested in these simulations, was applied to the in vivo fMRI data (Fig. 9) to separate the pHi and BOLD effects evoked by the visual stimulus in healthy human brain. A small BOLD effect (Fig. 9a) of approximately 1% (on average) could be isolated in the visual cortex, but there was no statistically significant pHi effect in the same location (Fig. 9b). The seemingly spurious pHi activation below the ventricles, we attribute to an accidental residual activation, which we observed in a similar location in all three subjects. The fact that a pHi change of 0.1 units could be isolated in the Bloch-McConnell simulations, and the $\Delta pHi$ limit of detection for APT-CEST was estimated to be 0.03, suggests that activity-evoked pH changes in vivo are below 0.03 units. Despite the fact that APT-CEST was unable to detect visual stimuli–evoked pHi changes in this study, it is a powerful tool for quantifying larger pH effects, which has shown potential in identifying ischemic penumbra in multiple studies (36–40).

In this study, we chose a pHi-sensitive APT-CEST technique to detect activity-evoked pHi changes. The results of this study suggest that the pHi changes are extremely small and easily hidden by the larger BOLD effects, which can be mistaken for the desired pHi effects. Thus, care must be taken when interpreting fMRI data using a pHi-sensitive sequence. Therefore, further investigation is required to determine whether the activity-evoked changes in $T_2^*$ contrast, such as reported recently in (23), originated from pH effects or can be attributed to the confounding effects of diffusion (25,27,28), cerebral blood volume (29), inflow (30), and/or BOLD (28).

**Limitations**

The CEST-MRI of amides, which is sensitive to changes in pH as small as 0.1 (36), has been used in multiple studies to detect the pH-weighted APT effect (36–40). Recently, it has been shown that CEST-MRI of amines may offer higher pH sensitivity compared with APT-CEST (60). However, sufficient labeling of amine protons requires much higher power levels because of their faster exchange rates. The duration of our fMRI experiments was 21 min 14s, which limited our choice of the pH-sensitive CEST-MRI technique to APT-CEST as a result of RF amplifiers limitations. Because of a low $B_1^*$, no fast exchanging protons have been included in the simulations. The currently available $B_1^*$ correction methods (61,62) would have increased the scan time dramatically.
and therefore have not been used. Although there could be a spatial B$_2$ pattern, its temporal pattern remains unchanged. Even though both BOLD (63) and pH (phantom data not shown) can influence water T$_1$, their effects are negligibly small and have not been included in the model. The exchange rate was assumed to be only a function of pH (35).

**CONCLUSIONS**

In this report, we present our initial attempts at detecting neuronal activity-evoked pH changes with APT-CEST MRI at 7T. In theory, it was successfully shown that as small as 0.03 units pH change can be separated from a 1.1% BOLD effect at the experimentally determined temporal SNR of 108. During the visual stimuli experiments in vivo, however, only the BOLD effect could be detected in the visual cortex with no significant pH changes. The results of this study suggest that neuronal activity-evoked pH changes are under 0.03, which is extremely difficult to detect using the existing techniques.

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