31P Magnetization Transfer Magnetic Resonance Spectroscopy: Assessing the Activation Induced Change in Cerebral ATP Metabolic Rates at 3 T

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**Purpose:** In vivo 31P magnetic resonance spectroscopy (MRS) magnetization transfer (MT) provides a direct measure of neuronal activity at the metabolic level. This work aims to use functional 31P MRS-MT to investigate the change in cerebral adenosine triphosphate (ATP) metabolic rates in healthy adults upon repeated visual stimuli.

**Methods:** A magnetization saturation transfer sequence with narrowband selective saturation of γ-ATP was developed for 31P MT experiments at 3 T.

**Results:** Using progressive saturation of γ-ATP, the intrinsic T1 relaxation times of phosphocreatine (PCr) and inorganic phosphate (Pi) at 3 T were measured to be 5.1 ± 0.8 s and 3.0 ± 1.4 s, respectively. Using steady-state saturation of γ-ATP, a significant 24% ± 14% and 11% ± 7% increase in the forward creatine kinase (CK) pseudo-first-order reaction rate constant, k1, was observed upon visual stimulation in the first and second cycles, respectively, of a paradigm consisting of 10-minute rest followed by 10-minute stimulation, with the measured baseline k1 being 0.35 ± 0.04 s⁻¹. No significant changes in forward ATP synthase reaction rate, PCr/γ-ATP, Pi/γ-ATP, and nicotinamide adenine dinucleotide/γ-ATP ratios, or intracellular pH were detected upon stimulation.

**Conclusion:** This work demonstrates the potential of studying cerebral bioenergetics using functional 31P MRS-MT to determine the change in the forward CK reaction rate at 3 T. Magn Reson Med 79:22–30, 2018. © 2017 The Authors Magnetic Resonance in Medicine published by Wiley Periodicals, Inc. on behalf of International Society for Magnetic Resonance in Medicine. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

**Key words:** adenosine triphosphate metabolism; magnetization transfer; in vivo 31P magnetic resonance spectroscopy; visual stimulation; bioenergetics; brain

**INTRODUCTION**

In vivo 31P magnetic resonance spectroscopy (MRS) has proven to be a useful technique for the study of bioenergetics associated with different levels of brain activity (1–4). It not only allows the noninvasive detection of a number of essential phosphate compounds involved in cerebral adenosine triphosphate (ATP) metabolism, which plays a central role in cerebral bioenergetics, but also permits the measurement of cerebral pH and key cerebral ATP metabolic rates and fluxes. The forward ATP synthase (ATPase) reaction rate, measured using 31P MRS in resting human and rat brains, has been shown to be consistent with the rate of oxidative phosphorylation (4,5). A study in rats showed that the forward creatine kinase (CK) and ATPase reaction rates gradually decreased with increasing depth of anesthesia, suggesting a close coupling between forward ATPase reaction rates and the level of brain activity (4). In the human brain, a significant decrease in the ratio of phosphocreatine (PCr) to inorganic phosphate (Pi) and slight increase in pH was observed during ~13 minutes of photic stimulation (6), whereas a 34% increase in forward CK reaction rate, without significant change in PCr concentration, was reported in response to an 8-Hz flashing visual stimulation (7). More recently, Barreto et al. (8) reported activation-induced increases in the Pi/α-ATP ratio and decreases in the ratio of nicotinamide adenine dinucleotide (NAD) to α-ATP during both short (1.5 minutes) and long (5 minutes) periods of visual stimulation. No prior studies have assessed the changes in cerebral ATP metabolic rates induced by repeated, prolonged visual stimuli using functional 31P MRS.

Cerebral ATP metabolic rates and fluxes can be measured in vivo using 31P MRS in combination with magnetization transfer (MT) techniques, such as saturation transfer (ST) (2–4), inversion transfer (9,10), and two-dimensional chemical exchange spectroscopy (11). The ST method is the most commonly used in vivo, due to its high efficiency and methodological simplicity (12). The tightly-coupled ATPase and CK reactions can be modeled as a three-pool 31P-spin chemical exchange kinetic network involving ATP, PCr, and Pi.
Changes in ATP Metabolic Rates

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\begin{align*}
\text{PCr} + \text{ADP}^- &+ \text{H}^+ \xrightarrow{\text{ATPase}} \text{Cr} + \text{Pi} + \text{PPi} \\
\text{ATP} + \text{Cr} &\xrightarrow{\text{T}_{\text{ATPase}}} \text{PCr} + \text{Pi} + \text{ADP}
\end{align*}
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where \(k_1, k_2\) are the forward and reverse reaction rates; \(\gamma\) is the pseudo-forward rate constant; \(\Delta G^0\) is the standard free energy change; \(T_{\gamma}\) is the effective saturation time; \(\Delta T_{\gamma}\) is the effective saturation time; \(\gamma\) is the pseudo-forward rate constant; \(\Delta G^0\) is the standard free energy change; \(T_{\gamma}\) is the effective saturation time; \(\Delta T_{\gamma}\) is the effective saturation time.

In this study, we use highly selective saturation pulses in the MT-ISIS sequence, with the aim of effectively suppressing only the \(\gamma\)-ATP resonance while remaining within SAR constraints.

Conventional in vivo \(^{31}\)P spectra from the human brain with no saturation contain a broad, intense phospholipid baseline due to the chemical shift anisotropy of phospholipids, whose resonant frequency depends on their orientation in the field (20,21). This baseline is significantly reduced by MT saturation at the frequency of \(\gamma\)-ATP, but this confounds the comparison of \(^{31}\)P spectra with and without saturation. To compensate for this, the baseline in the \(^{31}\)P spectrum with no MT saturation is normally removed before quantification using a cubic spline or Gaussian fit, or by removing the first few points of data in the time domain (12). However, this procedure can induce extra postprocessing errors or affect signal-to-noise ratio (SNR). In this study, we suppressed the phospholipid baseline by applying irradiation in a region of the \(^{31}\)P spectrum with no peaks of interest. Because the rotation of the phospholipids is fast compared to the saturation time, phospholipid nuclei that contribute to the broad baseline pass through the orientation corresponding to saturation frequency at some point, and are thus saturated (22).

The principal aim of this study was to measure the changes in ATP metabolic rates in response to repeated, prolonged visual stimulation using \(^{31}\)P MRS-MT. First, the performance of the highly selective saturation sequence for \(\gamma\)-ATP irradiation and baseline suppression was assessed. Second, longitudinal relaxation times (\(T_1\)) of PCr and Pi at 3 T were determined using the progressive saturation of \(\gamma\)-ATP. Finally, using the steady-state saturation of \(\gamma\)-ATP, the pseudo-forward rate constants of CK and ATPase reactions (\(k_1\) and \(k_2\)), concentrations of PCr and Pi, and intracellular pH (\(pHi\)) were quantified during alternating rest and visual stimulation periods.

METHODS

This study was approved by the medical school ethics committee of the University of Nottingham, and each subject gave informed consent. The study was performed on a 3T Philips Achieva system (Philips Medical Systems, Best, the Netherlands) using a \(^{31}\)P transmit–receive 14-cm diameter loop coil (P-140). A \(^1\)H image localizer was acquired at the start of each experiment, and the \(^{31}\)P coil was tuned and matched before \(^{31}\)P MRS acquisition.

Shimming was performed using the Philips pencil beam method, which is based on the FASTMAP method (23).

Saturation Sequence With High Selectivity

Figure 1 shows the MT-ISIS sequence used in the \(^{31}\)P MRS experiments. Prior to the ISIS localization sequence, a highly selective MT sequence was applied comprising multiple amplitude modulated pulses with a
The saturation sequence could be adjusted by varying the energy deposition to allow the use of the pulse train resonance, to have negligible sidebands and to limit acquisition (baseline alone. Baseline) or at 15 ppm to suppress the phospholipid ATP resonance (but also unavoidably the phospholipid resonance at 0 ppm) to irradiate the /C0 resonance saturation was applied at either 2.5 ppm and 15 ppm of the PCr peak.

Progressive Saturation of g-ATP: Intrinsic T1 Measurement

Six subjects (age range: 22–27 years, four males) were recruited to participate in the g-ATP progressive saturation experiment. 31P spectra were acquired at resting state from a localized volume (5 x 9 x 7 cm) chosen to cover the occipital lobe using the MT-ISIS sequence (echo time/repetition time [TR] = 0.1/12,000 ms, samples = 4096, spectral bandwidth = 3000 Hz, number of signal averages (NA) = 24, phase cycles = 8, scan time = 5 minutes). Seven 31P spectra were obtained at varying MT saturation times (tsat = 0, 565, 1058, 2045, 3032, 5005, 8295 ms) using a varying number of saturation pulses (np = 0, 3, 6, 12, 18, 30, 50) at a fixed pulse gap, \( \tau \) of 50 ms.

The first scan had no saturation at the g-ATP resonance yielding the Boltzmann equilibrium PCr and Pi magnetizations (\( M_{PCr}^0 \) and \( M_{Pi}^0 \)), with the broad baseline attenuated by applying a train of 32 pulses off-resonance at +15 ppm. The remaining six g-ATP saturation scans were acquired in a random order. The total scan time was approximately 45 minutes. The intrinsic T1 of PCr and Pi were determined by fitting \( M_{PCr}^0 \) and \( M_{Pi}^0 \) measured for different values of tsat in this progressive g-ATP saturation experiment, to Eq. [1].

Functional MRS With Steady-State Saturation of g-ATP: Measurement of Changes in \( k_1 \) and \( k_2 \)

Nine subjects (age range: 22–27 years, six males) undertook a visual stimulus paradigm which applied two cycles of a 10-minute rest block followed by a 10-minute visual stimulation block. The visual stimulation comprised contrast-defined wedges moving toward or away from a fixation cross of randomized color (24,25), which was presented through MR-compatible goggles (VisualSystem; NordicNeuroLab, Bergen, Norway). A median grey background was presented during rest periods. The built-in dioptr correction system and fine-tuning of pupil distance were adjusted for each subject. Subjects were asked to focus on the fixation point, a small cross, in the center of the field of view. To keep subjects focused during the experiment, they were also asked to press the fiber-optic response grips (NordicNeuroLab) with their index fingers immediately when the fixation cross turned green.

During each block, two 31P spectra (NA = 24) were acquired: one without MT saturation at the g-ATP resonance (tsat = 0 s) to measure Boltzmann equilibrium magnetizations (\( M_{PCr}^0 \) and \( M_{Pi}^0 \)), and one with sufficiently long MT saturation time (tsat = 9820 ms) to measure steady-state magnetization (\( M_{PCr}^0 \), \( M_{Pi}^0 \)). The total scan time of this session was approximately 50 minutes. The forward rate constants of CK and ATPase reactions (\( k_1 \) and \( k_2 \)) were calculated based on Eq. [2].

31P MRS Analysis

The 31P data were analyzed in jMRUI version 4 (http://www.jmrui.eu/) (26). The quantification of 31P metabolites was performed with the AMARES (Advanced Method for Accurate, Robust and Efficient Spectral fitting) algorithm (27). With soft constraints on signal linewidths and resonant frequencies, the in vivo 31P spectra were fitted to 12 individual components for 10 metabolite resonances belonging to phosphoethanolamine, phosphocholine, intracellular Pi, glycerophosphoethanolamine, glycerophosphocholine, membrane phospholipids, PCr, g-ATP, \( \alpha \)-ATP, and NAD(H). The doublets of g-ATP (–2.5 ppm) and \( \alpha \)-ATP (–7.5 ppm) were both fitted as two peak components with fixed separation of 16 Hz (J-coupling constant) (28) and with the same linewidth and amplitude. Apodization (line broadening = 15 Hz) was applied to improve the SNR, assisting the visual inspection (in the frequency domain) of the quality of the fit.
FIG. 2. $^{31}$P spectra acquired with saturation at −2.5 ppm and a saturation time ($t_{sat}$) of 3 s (green), at +2.5 ppm and a $t_{sat}$ of 10 s (red), and at +15 ppm and a $t_{sat}$ of 5 s as used in the main study (blue). These spectra were acquired from the human occipital region (voxel size = 6 × 6 × 6 cm$^3$). They demonstrate that saturation at 2.5 ppm (even for 10 s) has a negligible RF bleed-over effect on the PCr peak, that off-resonance saturation of 5 s duration provides adequate baseline suppression, and that saturation at −2.5 ppm (for just 3 s) is effective for complete γ-ATP saturation.

Next, the quantified signals (peak areas) of PCr and Pi from the γ-ATP progressive saturation experiment were least squares fitted to the model in Eq. [1] using MATLAB (The MathWorks, Inc., Natick, MA) to estimate the intrinsic $T_1$ of PCr and Pi. The goodness of fit was assessed from the coefficient of determination, $R^2$. The forward rate constants of CK and ATPase reactions were calculated from Eq. [2] based on the PCr and Pi signals ($S_{PCr}$, $S_{Pi}$) measured in the steady-state γ-ATP saturation experiment. For each block in the visual stimulation paradigm, the concentrations of PCr ([PCr]) = ($S_{PCr}/S_{-ATP}$) × [ATP]) and Pi ([Pi]) = ($S_{Pi}/S_{-ATP}$) × [ATP]) were calculated based on the fully relaxed signals from the $^{31}$P spectra in the absence of γ-ATP saturation (TR = 12 s), assuming the relatively strong ATP concentration in the human brain to be 3 mM (2,29). From these, the forward fluxes (in mM/s) were calculated as $F_{\text{CK}}^{\text{CK}} = k_1 \times [\text{PCr}]$ and $F_{\text{ATPase}}^{\text{ATPase}} = k_2 \times [\text{Pi}]$, and converted into units of μmol/g/min, assuming a brain tissue density of 1.1 g/mL (2). The pH$_i$ values for each block were determined based on the chemical shift difference ($\delta_{\text{Pi}-\text{Pi}}$) between the PCr and intracellular Pi signals (10,30,31). Lastly, paired t-tests were performed to test for significant differences in forward reaction rate constants, metabolite concentrations, and pH$_i$ between stimulation and rest blocks.

Error Analysis

To assess the error in the estimate of the intrinsic longitudinal relaxation time ($T_1^{\text{inst}}$) via the progressive saturation of γ-ATP, a Monte Carlo (MC) simulation study was performed, based on the time courses of PCr and Pi signals simulated using the model given in Eq. [1], and the assumed $T_1^{\text{inst}}$ s and forward reaction rates ($T_1^{\text{inst}}_{\text{PCR}} = 5$ s, $T_1^{\text{inst}}_{\text{Pi}} = 3$ s, $k_1 = 0.3$ s$^{-1}$, $k_2 = 0.2$ s$^{-1}$) [2]. Random normally distributed noise of similar variance as found in the in vivo $T_1$ measurements was added to the simulated signal curves, producing 1000 datasets, which were then individually least squares fitted, to predict the average uncertainty (standard deviation) and mean value of the estimated $T_1^{\text{inst}}$ of PCr and Pi.

A second MC simulation was performed to assess the effect of this uncertainty in the estimated $T_1^{\text{inst}}$ values on the resulting measurement of the reaction rate constants ($k_1$ and $k_2$) via the steady-state saturation of γ-ATP. For different assumed relative errors in $T_1^{\text{inst}}$ (0%, 20%, 40%), and simulated signals of PCr and Pi (Eq. [1]) at two saturation times ($t_{sat} = 0$ and 10 s) with added noise (1000 datasets), $k_1$ and $k_2$ were calculated using Eq. [2], and the coefficients of variation of the estimated values were determined.

RESULTS

Figure 2 shows that the fully relaxed PCr signals were similar for saturation at +2.5 ppm or +15 ppm, indicating that the bandwidth of the γ-ATP saturation profile was sufficiently narrow to prevent RF bleed-over to the PCr peak. Thus, the suppression of the PCr peak resulting from saturation of γ-ATP at −2.5 ppm can be considered to be due to saturation transfer only. In addition, it shows that saturation applied at +15 ppm reduced the phospholipid baseline to a similar level to that produced by γ-ATP saturation at −2.5 ppm. This suggests that more consistent baselines can be achieved between scans with and without γ-ATP saturation, by applying saturation at +15 ppm (a region without peaks of interest) for the scans acquired without γ-ATP saturation. Results of spectral peak fitting are shown in Figure 3a for a representative subject. The $^{31}$P spectra acquired were of excellent spectral quality across all subjects (PCr linewidth, FHWM ~6–8 Hz, before apodization).

Figure 3b illustrates that in the progressive saturation experiment, as expected, the magnitude of PCr signals decreased gradually with increased γ-ATP saturation time ($t_{sat}$). The Pi signal decreased more slowly and to a lesser extent with increasing $t_{sat}$, due to the slower ATPase reaction rate compared to the CK reaction rate [3] and the lower concentration of Pi compared to PCr. Figure 4 shows the normalized PCr and Pi magnetizations (N = 6) plotted against γ-ATP saturation time, together with the least-square regression fitting (Eq. [1]) for the group-averaged data. From this, the intrinsic longitudinal relaxation times ($T_1^{\text{inst}}$) at 3T were measured to be $5.1 \pm 0.8$ s for PCr ($R^2 = 0.99$) and $3.0 \pm 1.4$ s for Pi ($R^2 = 0.7$). The uncertainties in the estimated intrinsic $T_1$ values were determined from the MC simulation (Table 1), and indicated as expected from Figure 4, that estimated $T_1^{\text{inst}}$ is more reliable than $T_1^{\text{PCR}}$.

Using the estimated $T_1^{\text{inst}}$ of PCr, the forward rate constant of the CK reaction ($k_1$) during the functional MRS (fMRS) experiment was determined for each block, and found to be $0.35 \pm 0.04$ s$^{-1}$ (block1: visual stimulation OFF = baseline), $0.43 \pm 0.02$ s$^{-1}$ (block2: visual stimulation ON), $0.37 \pm 0.03$ s$^{-1}$ (block3: visual stimulation OFF), and $0.41 \pm 0.04$ s$^{-1}$ (block4: visual stimulation ON). Figure 5a shows that the change in the forward rate constant of the CK reaction, $k_1$, between stimulus states was quite consistent across subjects. On average, a $24 \% \pm 14 \%$ increase in $k_1$ from baseline was observed.
during the first visual stimulation ON block ($P = 2 \times 10^{-6}$). There was no significant change in $k_1$ between the first and second rest (OFF) blocks ($P = 0.36$), indicating that $k_1$ returned to baseline level during the second rest block after visual stimulation. In addition, an 11% increase was observed during the second visual stimulation block ($P = 0.001$). There was no significant difference in $k_1$ between the first and second visual stimulation ($P = 0.08$), with $k_1$ elevated by 18% when averaged over both visual stimulation periods ($P = 2 \times 10^{-6}$). The forward exchange fluxes for the CK reaction ($F_{CK} = k_1 [PCr]$) were found to increase from 1.32 ± 0.15 mM/s (or 72.2 ± 8.2 μmol/g/min) at rest to 1.56 ± 0.11 mM/s (or 85.5 ± 6.0 μmol/g/min) on visual stimulation (an 18% increase).

Using the estimated $T_{1,p}$ of Pi, the forward rate constant of the ATPase reaction ($k_2$) in each simulation block was found to be 0.15 ± 0.05 s$^{-1}$ (stim-OFF), 0.18 ± 0.09 s$^{-1}$ (stim-ON), 0.15 ± 0.08 s$^{-1}$ (stim-OFF), and 0.17 ± 0.10 s$^{-1}$ (stim-ON). The relative change in $k_2$ showed a similar pattern across blocks to $k_1$ (Fig. 5b), with a trend for an increase in $k_2$ between the first visual stimulation OFF and ON blocks of 21% ($P = 0.35$), and between the second OFF and ON blocks of 12% ($P = 0.72$). Results from the MC simulation (Table 2) show that the coefficient of variation in estimated $k_1$ (CV$_{k1}$% ≈ 7%) is smaller than for $k_2$ (CV$_{k2}$% ≈ 23%), largely independent of the relative errors in the estimated intrinsic $T_1$. This indicates that a change less than 7% in $k_1$ or 23% in $k_2$ is unlikely to be detected. The baseline forward exchange flux for the ATPase reaction

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F_{ATP} = k_2
\]

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F_{ATP} = k_2
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Changes in ATP Metabolic Rates

ATP ratios, and the pH measured in each block. The no significant difference observed in the average pH at between rest and visual stimulation (P to be 1.6 k1 during each ON block.

This enabled the use of a TR of 12 s (SAR max = 3 W/kg) compared to 30 s or longer required by a conventional BISTRO saturation sequence with ISIS (16). The resulting scan time made it feasible to assess changes in response to repeated visual stimulation in humans. In addition, this study showed that saturation could be applied at +15 ppm to suppress the broad phospholipid baseline in human brain 31P spectra, and therefore produce a more consistent baseline across acquisitions with and without MT saturation at the γ-ATP resonance.

A measurement of T1 PCr (5.1 ± 0.8 s) was obtained at 3T in this study, which can be compared to 4.9 ± 0.5 s measured in the human brain at 7T [2] and 3.8 ± 0.6 s in the rat brain at 9.4 T [4]. The decrease in T1 PCr with the increasing B0 field strength suggests that the relaxation of 31P spins in PCr is dominated by the chemical shift anisotropy mechanism (32). In contrast, the T1 PCr measured at 3T in this study (3.0 ± 1.4 s) was shorter than the values of 3.6 ± 0.7 s, 3.8 ± 0.4 s, and 4.0 ± 0.6 s reported at 4T, 7T and 9.4 T (2,4,33), respectively, indicating that the relaxation of 31P spins in Pi may be dominated by dipolar interactions.

The baseline forward rate constant of the CK reaction (k1) measured using the steady-state saturation of γ-ATP (0.35 ± 0.04 s−1), is consistent with k1 at resting state obtained from the progressive saturation experiment (0.37 ± 0.07 s−1). Early 31P studies reported k1 values of 0.3 ± 0.04 s−1 and 0.16 ± 0.02 s−1 in the human brain regions of predominantly gray matter and white matter, respectively (34), and an average value of 0.42 s−1 over the entire human brain (35). Our k1 values are more comparable with the more recently reported values of 0.33 ± 0.04 s−1 [2] and 0.32 ± 0.08 s−1 (16) measured from the human occipital lobe at rest. In addition, the forward rate constants of ATPase reaction (k2) at rest obtained in this study from the steady-state and progressive MT experiments (0.15 ± 0.05 s−1, 0.19 ± 0.07 s−1, respectively) are comparable with each other, and with the previously reported values of 0.17 ± 0.04 s−1 [3], 0.18 ± 0.05 s−1 [2], and 0.21 ± 0.04 s−1 (10).

The cerebral ATP synthesis flux through oxidative phosphorylation estimated from PET measurements of the cerebral metabolic rate of oxygen (CMRO2) utilization (CMRO2 = 1.71 ± 0.18 μmol/g/min) in the human occipital lobe (36) is 10.3 ± 1.1 μmol/g/min, based on a P:O2 ratio of 6 [4]. Our estimate for the forward exchange flux for the ATPase reaction, F ATPase = 13.2 ± 4.4 μmol/g/min, derived from the 31P steady-state MT measurement of k2 and [Pi] at rest (baseline), is in good agreement with this, as well as values reported in previous 31P MT studies (12.1 ± 2.8 μmol/min [3] and 8.8 ± 1.9 μmol/min [2]), which did not use a localization sequence, but relied on

DISCUSSION AND CONCLUSIONS

The magnetization saturation transfer sequence used in this study was designed to provide high frequency-selectivity for effective saturation of γ-ATP while keeping the RF power deposition low enough to allow it to be used with ISIS localization with a reasonable TR. The magnetization saturation transfer sequence used in this study was designed to provide high frequency-selectivity for effective saturation of γ-ATP while keeping the RF power deposition low enough to allow it to be used with ISIS localization with a reasonable TR. The magnetization saturation transfer sequence used in this study was designed to provide high frequency-selectivity for effective saturation of γ-ATP while keeping the RF power deposition low enough to allow it to be used with ISIS localization with a reasonable TR.
the spatial selectivity of the surface coils used. This suggests that the forward exchange flux of the ATPase reaction measured by the $^{31}$P MT method reflects the net oxidative ATP phosphorylation rate in the human brain. The possible reasons for this are discussed at length in Lei et al. (3), and the present findings, obtained from a well-localized volume in the occipital lobe, lend weight to their arguments.

In this study, increases of 24% ± 14% and 11% ± 7% in the pseudo-forward rate constant of the CK reaction, $k_1$, were observed in the first and second 10-minute blocks of visual stimulation, respectively, giving an average increase of 18% ± 12%. This was less than the 34% increase reported previously for a light flashing at 8 Hz (7), and greater than the 8% increase induced by repeated short (20 s) visual stimulation (37). In addition, there was a trend for an increase in the forward exchange rate constant of the ATPase reaction during visual stimulation, although it did not reach statistically significance due to the high coefficient of variation associated with the measurement (Table 2) and the lower SNR. With the benefit of greater SNR, a recent study at 7 T measured a 21% increase in the forward chemical exchange flux of ATPase reaction during repeated short periods of visual stimulation (37).

The baseline PCr/γ-ATP ratio obtained in this study was 1.22 ± 0.09 (at rest), which is comparable to the previously reported value of 1.3 ± 0.1 measured from the human occipital lobe (2). In addition, despite the possibility of quantification error due to the limited SNR of the Pi resonance, the baseline concentration of Pi ([Pi] = 1.6 ± 0.1 mM) measured is within the range of previously reported values (0.85–1.7 mM) (3,10,30,38). Some early $^{31}$P MRS studies in animal (39) and human brains (6) have indicated a decline in PCr concentration and PCr/Pi ratio during visual stimulation. However, no significant difference in the PCr concentration between rest and activation was measured in this study, supporting similar findings reported more recently in the human brain during visual stimulation under normal and mild hypoxic conditions (7,40,41) as well as in the rat brain (14). The unchanged PCr concentration and increased pseudo-forward CK reaction rate ($k_1$) during stimulation can be possibly explained by a slightly elevated ADP concentration (7,40), which shifts the CK reaction in the direction of ATP synthesis, as well as stimulating glycolysis and lactate production (7,42). Increased local lactate concentration during functional activation has been found in a few $^3$H MRS studies at 7 T (25,43–45). With the same stimulus paradigm, an attenuated response was observed in the second period of 10-minute visual stimulation for lactate by Lin et al. (25). A similar trend was observed for the forward CK reaction rate in this study. This may relate to neuronal adaptation upon repeated stimulation and requires further investigation.

The pH change during brain functional activation is unclear (46). A slight rise in cerebral pH$_i$ was previously reported during photic stimulation (6,47), whereas a small decrease during checkerboard visual stimulation has also been reported (48). Murashita et al. (41) suggested the change in pH$_i$ due to stimulation is age dependent, as the pH$_i$ increase found in a middle-aged group (54.4 ± 10.5 years) was not observed in a younger group (28.9 ± 10.5 years). No significant change in pH$_i$ was detected in response to visual stimulation (moving contrast-defined wedges, 10 minutes) in this study in young healthy volunteers. This agrees with other recent $^{31}$P human studies (6,40), in which no change was observed during visual stimulation even in mild hypoxic hypoxia. The limited temporal resolution, sensitivity, and the possible pH heterogeneity in the cortical tissues within the large $^{31}$P MRS voxel (40,49) make it difficult to capture possible transient changes in cerebral pH$_i$ during the prolonged visual stimulation. Significant decreases of ~5% and ~2% in NAD(H)/ATP have been recently reported during short (1.5 minutes) and long (5 minutes) photic stimulations (8). In our study, an insignificant decrease was detected in response to a longer period of visual stimulation (10 minutes).

There are a few possible sources of error leading to potential bias in our results. Firstly, poor or incomplete saturation of γ-ATP, especially at short saturation time ($t_{sat}$), would tend to cause an underestimation of $k_1$ and $k_2$ (16) in the progressive saturation experiments. In general, the highly selective saturation used in this study was effective for γ-ATP irradiation over a large voxel of interest. The residual γ-ATP signals in the $^{31}$P spectra acquired at the shortest $t_{sat}$ (<7% of the unsuppressed γ-ATP signals) were not much higher than the noise level (~4%). Secondly, using a short TR could cause partial saturation of the acquired $^{31}$P signals, especially for scans with long $t_{sat}$, which would then lead to an underestimation of $k_1$ and $k_2$ in the steady-state saturation experiments. Based on a model that considers partial saturation [3], the partial saturation effect on $M(t_{sat})/M^0$ when using a TR of 12 s (as in this study), was found to be small (~3% for PCr and ~1% for Pi at the longest $t_{sat}$ = 10 s). Thirdly, the $^{31}$P signals from Pi in the intracellular and extracellular compartments were not well resolved at 3 T (this study) or 4 T (33), despite good smoothing. Recent studies (50,51) at ultra–high field have found that the extracellular Pi resonance is insensitive to selective saturation or inversion of γ-ATP, implying lack of chemical exchange between the extracellular pool of

| Blocks | PCr/γ-ATP | Pi/γ-ATP | NAD(H)/γ-ATP | pH$_i$ |
|--------|-----------|-----------|--------------|-------|
| [1] Stim-OFF | 1.22 ± 0.09 | 0.540 ± 0.061 | 0.157 ± 0.017 | 6.99 ± 0.04 |
| [2] Stim-ON | 1.25 ± 0.08 | 0.540 ± 0.052 | 0.150 ± 0.020 | 7.01 ± 0.03 |
| [3] Stim-OFF | 1.22 ± 0.07 | 0.535 ± 0.049 | 0.157 ± 0.021 | 7.00 ± 0.03 |
| [4] Stim-ON | 1.23 ± 0.13 | 0.535 ± 0.069 | 0.153 ± 0.025 | 7.00 ± 0.02 |
Pi and γ-ATP. The calculation of $k_2$ based on the total Pi signal may therefore lead to the underestimation of $k_2$. The fact that our measured pH (6.99 ± 0.04) was closer to the previously reported intracellular pH values of 7.06 (50) and 7.04 (8) than to the extracellular pH value of 7.39 (50), indicates that the concentration of Pi and $k_2$ reported in this study are primarily determined by the dominant intracellular Pi signal. In future studies, the design of the $^{31}$P MR metabolite measurement could be improved by interleafing the two types of scan (t_{1sd} = 0 and 10 s), as this would enable an increase in the temporal resolution for the $k_1$ and $k_2$ measurements, thus potentially allowing the observation of progressive changes within each stimulation block (assuming that the SNR was sufficient).

This work has demonstrated the effectiveness of a highly selective saturation sequence for selective γ-ATP irradiation and phospholipid baseline suppression. Our findings suggest that the forward creatine kinase reaction rate is sensitive to increased brain functional activity. Results show the feasibility of using $^{31}$P MRS-MT techniques at 3T for future noninvasive studies of bioenergetics in response to stimulation, and in a variety of neurological and neuropsychiatric disorders.

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