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Alkaline brain pH shift in rodent lithium-pilocarpine model of epilepsy with chronic seizures

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Abstract

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Author contributions

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Potential Conflicts of Interests
The authors report no competing interests.
Brain pH is thought to be important in epilepsy. The regulation of brain pH is, however, still poorly understood in animal models of chronic seizures (SZ) as well as in patients with intractable epilepsy. We used chemical exchange saturation transfer (CEST) MRI to noninvasively determine if the pH is alkaline shifted in a rodent model of the mesial temporal lobe (MTL) epilepsy with chronic SZ. Taking advantage of its high spatial resolution, we determined the pH values in specific brain regions believed to be important in this model produced by lithium-pilocarpine injection. All animals developed status epilepticus within 90 min after the lithium-pilocarpine administration, but one animal died within 24 hrs. All the surviving animals developed chronic SZ during the first 2 months. After SZ developed, brain pH was determined in the pilocarpine and control groups (n = 8 each). Epileptiform activity was documented in six pilocarpine rats with scalp EEG. The brain pH was estimated using two methods based on magnetization transfer asymmetry and amide proton transfer ratio. The pH was alkaline shifted in the pilocarpine rats (one outlier excluded) compared to the controls in the hippocampus (7.29 vs 7.17, t-test, p<0.03) and the piriform cortex (7.34 vs. 7.06, p<0.005), marginally more alkaline in the thalamus (7.13 vs. 7.01, p<0.05), but not in the cerebral cortex (7.18 vs. 7.08, p>0.05). Normalizing the brain pH may lead to an effective non-surgical method for treating intractable epilepsy as it is known that SZ can be eliminated by lowering the pH.

**Keywords**
Medically refractory epilepsy; chronic seizures; brain pH; chemical exchange saturation transfer (CEST); amide proton transfer (APT); ketogenic diet

1. **Introduction**

Pediatric epilepsy affects 0.04–0.2% of the population (Camfield and Camfield, 2015). Of these 30–40% of the patients have medically refractory epilepsy (Laxer et al., 2014). Although neurosurgical intervention is effective in many of these patients, a large proportion of the families choose to treat their children with this disorder without surgery. Thus there are unmet urgent needs for developing alternative non-surgical strategies for controlling pharmacologically intractable epilepsy.

This report considers the manipulation of brain pH as a possible non-surgical strategy for controlling this class of epilepsy. It has been known that alkalosis in the brain enhances the neuronal activity and may produce epileptic seizures (Chesler 1990; Chesler and Kaila, 1992; Kaila and Ransom 1998; Chesler, 2003; Schuchmann et al., 2006; Ruffin et al., 2014; Traub et al., 2020). Lowering brain pH can eliminate or reduce SZ. Acidification of brain pH caused by CO₂ inhalation is known to be a potent, fast-acting anticonvulsant (Tolner et al., 2011; Yang et al., 2014).

The brain of patients with medically refractory epilepsy appears to be alkalotic. Laxer et al (1992) used 31-phosphorus magnetic resonance spectroscopy (³¹P-MRS) to measure the intracellular pH (pHᵢ) in the anterior temporal lobe of patients with mesial temporal lobe (MTL) epilepsy. ³¹P-MRS can measure the pHᵢ based on the chemical shift of inorganic phosphate (Pi) relative to phosphocreatine (PCr) (Moon and Richards 1973; Petroff et al., 1985). They found pHᵢ of 7.25 in the affected lobe compared to the contralateral control lobe.
However, the regional shift in brain pH is still not well understood in part because $^{31}$P-MRS requires a large volume for the assessment (84–105 ml or approximately $5 \times 5 \times 4 \text{ cm}^3$ in Laxer et al., 1992; 20–30 ml in more recent MRS – cf. Zhou et al., 2003) due to its relatively low sensitivity.

We studied the regional pH variation in the brain of the rats with chronic seizures (SZ) using chemical exchange saturation transfer (CEST) MRI (Ward and Balaban, 2000; Van Zijl and Yadav, 2011; Zhou et al., 2003; Sun et al., 2007, 2012, Wang et al., 2019). A particular form of this technique, called amide proton transfer (APT) imaging or APT MRI, can be used to measure the brain pH, believed to mostly reflect pH$_i$ (Zhou et al., 2003). Regional brain pH can be determined with CEST MRI because of its high spatial resolution (<0.5 × 0.5 × 1 mm$^3$) even in animals with a small brain such as rats.

We tested our hypothesis that brain pH is regionally alkaline shifted in the brain of animals and patients with chronic SZ by measuring the pH in a lithium-pilocarpine model of epilepsy in rats. Pilocarpine is a muscarinic agonist that produces status epilepticus (SE) after an injection, which is followed by a latent quiet period that subsequently transforms into chronic SZ (Turski et al., 1983, 1989; Curia et al., 2008). This procedure has been developed and used as a model of MTL epilepsy.

The hypothesis was evaluated by estimating the brain pH noninvasively during the chronic SZ stage in rats using CEST MRI. We found an alkaline shift in brain pH in the hippocampus and piriform cortex with a marginally significant increase in pH in the thalamus, but not in the amygdala and cerebral cortex. We discuss the implications of these results on developing non-surgical methods for controlling refractory epilepsy.

2. Estimation of brain pH

We describe the basics of CEST MRI before describing our results to facilitate the evaluation of our results. Since this technique is relatively new, the methods we have used for estimating brain pH may be unfamiliar to many readers.

2.1 CEST MRI

Brain pH was estimated in each region of interest (ROI) in the brain of the two groups of rats using CEST MRI. In CEST MRI, exchangeable solute protons that resonate at a frequency different from the bulk water protons are selectively saturated using RF irradiation. In a particular form of CEST MRI, called amide proton transfer (APT) MRI, amide protons are saturated and transferred to bulk water at a characteristic exchange rate. The exchange rate $k_{sw}$ from solute (s) to water (w) depends on the pH: the logarithm of $k_{sw}$ is proportional to pH: $k_{sw} = k_{base} \times k^{pH-pK_w}$, where $k_{base}$ is the base-catalyzed exchange rate and pK$_w$ = 15.4 is the potential for the self-ionization constant of water (Zhou et al., 2003). Brain pH can be, therefore, computed from the value of $k_{sw}$. To compute pH, the magnetization transfer ratio (MTR) is first calculated: $MTR = 1 - S_{sat}/S_o$, where $S_{sat}$ is bulk water signal with CEST RF saturation, and $S_o$ is the control signal without saturation. MTR is has a mixture of contributions from the semisolid macromolecular MT and direct water saturation in addition to the effect of amide proton transfer ratio (APTR). Thus, the magnetization transfer ratio
asymmetry ($MTR_{asym}$), which reasonably removes the effect of direct water saturation and direct RF saturation contributions, was calculated from MTR where

$$MTR_{asym} = \frac{S_{sat}(-\Delta \omega)}{S_o} - \frac{S_{sat}(\Delta \omega)}{S_o}$$  \hspace{1cm} (1)

where $\Delta \omega$ is the resonance frequency difference between amide and water protons (3.5 ppm) (van Zijl and Yadav, 2011). APTR was estimated from $MTR_{asym}$ by removing the contribution of the solid-phase magnetization transfer effect ($MTR'_{asym}$) (Zhou et al, 2003):

$$APTR = MTR_{asym} - MTR'_{asym}$$  \hspace{1cm} (2)

APTR is related to the amide proton exchange rate $k_{sw}$ by assuming that the exchange occurs from the solute to bulk water protons, which is predominantly base-catalyzed. These assumptions give the expression for relating APTR to pH as (Zhou et al 2003):

$$APTR = \frac{[\text{amide proton}]}{2[H_2O] R_{1w}} k_{sw} \left(1 - e^{-R_{1w} t_{sat}}\right)$$  \hspace{1cm} (3)

where

$$k_{sw} = 5.57 \times 10^{0.4 - 6.4}$$  \hspace{1cm} (4)

### 2.2 Method 1 for computing pH

We calculated pH from Eqs. 2, 3 and 4 based on the measured values of $APTR$ and $k_{sw}$, using the values of the parameters in Eq. 3 given by Zhou et al (2003): [amide proton] = 71.9 mM, $[H_2O] = 55M \times 0.84$ mL water per mL brain tissue, saturation time $t_{sat} = 4$ s. For the value of the longitudinal relaxation rate $R_{1w}$ (i.e., $R_{1w} = 1/T_{1w}$), Zhou et al. (2003) used an average value of 0.714 s$^{-1}$. In this paper, we used the value of $R_{1w}$ measured in each ROI of each animal in our study. $APTR$ was determined using Eq. 2 with $MTR_{asym}$ = 7.44%, taken from Sun et al (2012).

### 2.3 Method 2 for computing pH

We used an alternative method taken from Sun et al (2012) for computing pH. According to their method,

$$APTR = \frac{f_r k_{sw}}{R_{1w} + f_r k_{sw}} (1 - \sigma),$$  \hspace{1cm} (5)

where $\sigma$ is the labelling coefficient (0.97 for 0.75 μT irradiation Rf pulse), $\sigma$ is the spillover factor (0.1 for 0.75 μT RF), $f_r$ = labile amide proton fraction ratio (1/867) and $R_{1w}$ is the bulk water longitudinal relaxation rate. The values of $\alpha$, $1-\sigma$, $f_r$ were taken from Sun et al (2012). $APTR$ was calculated using Eq. 2 with $MTR_{asym}$ = 7.44% given by Sun et al (2012) as for Method 1. Equation 5 was used to compute $k_{sw}$, which was then in turn used to calculate pH based on Eq. 4.
3. Results

Figure 1 shows an example of interictal activity over 10 s measured with scalp EEG in one of the rats with chronic SZ. The inset indicates the position of the signal, reference and ground electrodes. The animal exhibited frequent spike-wave activity in this record and throughout the 15 min recording period.

Figures 2A and B show two coronal RARE images indicating the ROIs used in the APTR and brain pH analyses. Based on the prior lithium-pilocarpine studies, we selected five regions that may show a pH shift due to altered neural activity: neocortex (A), hippocampus (B), thalamus (C), amygdala (D) and piriform cortex (E). Figure 2C is the $MTR_{asym}$ image from a pilocarpine-treated rat. The value of $MTR_{asym}$ is higher in the hippocampus (ROI B) and piriform cortex (ROI E) compared to the rest of the brain. In the control rat (Fig. 2D), none of the ROIs show consistent elevation of $MTR_{asym}$.

Figure 3 shows the mean and standard error of the mean (SEM) of $MTR_{asym}$ in the five ROIs for the two groups of animals ($n = 7$ pilocarpine, one outlier was excluded, and $n = 8$ control). The value of $MTR_{asym}$ is generally higher in the pilocarpine group compared to the control group. The mean ± SEM for the pilocarpine and control groups were $-0.003 ± 0.005$ vs. $-0.024±0.004$ for the hippocampus and $+0.015 ± 0.008$ vs. $-0.031±0.005$ for the piriform cortex. The difference was statistically significant at $p < 0.004$ for the hippocampus (Between-group, 1-tailed Student t-test with equal variance) and at $p < 0.0002$ for the piriform cortex. For the amygdala, the mean ± SEM for the pilocarpine and control groups were $-0.013 ± 0.006$ vs. $-0.027 ± 0.005$; the difference was marginally significant ($p < 0.05$). The mean ± SEM for the pilocarpine and control groups were $-0.032 ± 0.005$ vs. $-0.041 ± 0.004$ for the thalamus and $-0.019 ± 0.005$ vs. $-0.030 ± 0.005$ for the cerebral cortex. The difference was not statistically significant at $p < 0.05$ for both the thalamus and the cerebral cortex.

Figure 4 shows the pH in the five ROIs based on Method 1 (A) and Method 2 (B). Method 1 generally gives higher values of pH than Method 2: The mean is 0.14 pH units higher across all ROIs in the pilocarpine group compared to the control.

According to Method 1, pH = 7.43 ± 0.05 vs. 7.30 ± 0.04 (1-tailed Student t-test significant at $p < 0.02$) in the hippocampus for the pilocarpine and control groups, and pH = 7.48 ± 0.05 vs. 7.20 ± 0.07 in the piriform cortex (t-test, $p < 0.002$) for the two groups, respectively. The pH was elevated in the thalamus (7.26 ± 0.05 vs. 7.15 ± 0.05); the difference was only marginally significant (t-test, $p < 0.05$). The pH was elevated in the amygdala (7.34 ± 0.04 vs. 7.26 ± 0.05) and the cerebral cortex (7.32 ± 0.04 vs. 7.22 ± 0.05), but the differences not statistically significant for both the amygdala and the cerebral cortex at $p = 0.05$.

According to Method 2, pH = 7.29 ± 0.05 vs. 7.17 ± 0.04 for the pilocarpine and control groups in the hippocampus, significant at $p < 0.03$, and pH = 7.34 ± 0.06 vs. 7.06 ± 0.07 in the piriform cortex (t-test, $p < 0.005$). The pH was elevated in the thalamus (7.13 ± 0.05 vs. 7.01 ± 0.05), but the difference was marginally significant (t-test $p < 0.05$). The differences in the amygdala and cerebral cortex (7.19 ± 0.05 vs. 7.12 ± 0.05 and 7.18 ± 0.05 vs. 7.08 ± 0.05, respectively) were not significant at $p$ of 0.05.

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4. Discussion

The key finding in this study is the alkaline pH shift in specific regions of the brain of rats with chronic SZ in the pilocarpine group compared to the control. We found that the pH values are significantly higher in the hippocampus and piriform cortex of the rats in the pilocarpine group compared to the control. The pH values were elevated in the other regions of the brain examined, but the elevation was only marginally significant in the thalamus and not significant in the amygdala and cerebral cortex.

The brain pH estimated with CEST MRI is believed to mostly reflect the pH$_i$ (Zhou et al., 2003). This assumption is supported by the close similarity of the pH values estimated with CEST MRI in this study and those by others. Method 1 of Zhou et al. (2003) we have used for the pH estimation was calibrated using the pH$_i$ values estimated with $^{31}$P-MRS. Method 2 based on the work of Sun et al. (2012) was calibrated against the pH$_i$ values calculated from the lactate concentration determined with MRS (Sun et al., 2012). Zhou et al (2003) found pH$_i$ values of 7.11 ± 0.13 (mean ± standard deviation [SD]) using $^{31}$P-MRS at normocapnia in rats, which is consistent with the values of 7.15 in the thalamus in the control group we found using their Method 1. Sun et al. (2012) found pH$_i$ of 7.03 ± 0.05 in the brain region contralateral to focal ischemia in rats, which primarily included the thalamus. These values are close to pH = 7.01 ± 0.13 (mean ± SD) that we found for the thalamus in our control group using their method. Our results are thus consistent with these earlier calibrated results based on CEST MRI for rats. The pH$_i$ values based on Methods 1 and 2 are very close to the values (7.02–7.17) found with well-established techniques in rat brain (Table 5, Chesler, 1990). Our pH values are also quite close to the pH$_i$ values found using $^{31}$P-MRS in human patients with medically refractory complex partial seizures ((7.25 vs. 7.08, p < 0.05) (Laxer et al., 1992). In our study the pH averaged over the limbic region (hippocampus, piriform cortex, and amygdala) was 7.27 and 7.11, respectively, in the pilocarpine and control groups based on Method 2. Thus, the pH$_i$ may be alkaline shifted in this model of MTL epilepsy with chronic SZ.

It is well known that neuronal activity produces changes in both extracellular and intracellular pH (Chesler, 1990, 2003; Ruffin et al., 2014). These changes may be either acidic or alkaline. The pH$_i$ can become temporarily acidic due to influx of H$^+$ into the cell, mediated for example by the plasma membrane Ca$^{2+}$-ATPase (PMCA) (Makani and Chesler 2010). Depolarization in neurons can lead to alkalization via the Na$^+/H^+$ exchanger, the electrogenic Na$^+/HCO_3^-$ cotransporter, Cl$^-$ dependent mechanisms (Svichar et al., 2011). When the neuronal activity is severe and persistent, as it would be the case during some forms of seizure, the cell can become acidotic in large part due to metabolic acids such as lactic acid (Siesjo et al., 1985; Chesler, 1990). This intracellular acidosis may become persistent and may lead to cell death. Protective mechanisms have been developed to keep the pH$_i$ within a viable range; the mechanisms for restoring the normal pH$_i$ include the Na$^+/H^+$ exchanger and, which extrudes H$^+$ from the cell, the Na$^+/HCO_3^-$ cotransporter, which provides influx of HCO$_3^-$ into the cell, and Na$^+$-driven HCO$_3^-$/Cl$^-$exchanger, which moves H$^+$ out of the cell and moves HCO$_3^-$ into the cell.
Although the activity-dependent change in pH may be acidic or alkaline, our results show that the net pH is alkaline during the period of chronic SZ. In the pilocarpine model of MTL epilepsy, the animal enters into SE soon after the injection of this drug. The SE is terminated by diazepam. The animal then enters into a latent state for a period determined by the particular protocol. This is followed by the period of chronic SZ. In this study, all the animals that exhibited SE entered into the chronic SZ period within two months after the SE induction. The epileptiform activity during the chronic SZ stage was mostly interictal. Seizures were observed at a mean frequency of 3.2 per week. The EEG records (e.g. Fig. 1) contained only interictal activity during the entire 15-min recording period. Thus, our results show that the pH was predominantly alkaline during the interictal period which was dominant during the stage of chronic SZ.

Changes in brain pH can in turn modulate neuronal activity. Neuronal activity can be reduced by lowering extra- and/or intracellular pH. Alkaline extracellular pH can enhance neuronal excitability, whereas acidic pH diminishes the excitability; the effects may be mediated by NMDA receptors, GABA \(_A\) receptors, voltage-gated Ca\(^{2+}\) channels and protonated channels (Chesler, 2003). Acidic intracellular pH can reduce several types of voltage-sensitive conductance (Na\(^+\), Ca\(^{2+}\) and K\(^+\)) (Moody, 1984; Takahashi and Copenhagen, 1996).

Reducing brain pH is useful for controlling epilepsy. Acidification of brain pH caused by CO\(_2\) inhalation, which reduces both the extra- and intracellular pH, is known to be a potent, fast-acting anticonvulsant (Tolner et al., 2011; Yang et al., 2014). Ketogenic diet (KD) can reduce seizure frequency by more than 50% in half of the patients and by more than 90% in a third of those who are treated (Lefevre and Aronson, 2000). There is some evidence that this diet may be effective in controlling epilepsy by lowering brain pH. It produces ketone bodies including acetone. Based on \(^1\)H-MRS, acetone is known to be elevated in concentration in the brain of patients with intractable epilepsy who are under a KD treatment (Seymour et al., 1999). Thus, KD could lower the brain pH via acetone, which is an effective anti-convulsant at physiological concentrations (Likhodii et al., 2008; Zarnowska et al., 2009).

In developing an effective non-surgical treatment for controlling pharmacologically intractable epilepsy, it is important to consider the role of the gap junctions since there is evidence that electrical coupling contributes to the initiation of seizures, at least in some conditions (Traub et al., 2001; 2020a). In certain experimental paradigms, alkalization enhances collective neuronal excitability apparently through effects on electrical coupling (Draguhn et al., 1998; Roopun et al., 2010; Traub et al., 2001, 2010, 2020a). Such actions could be mediated by pH effects on Cx45-containing gap junctions, which open at alkaline intracellular pH (Palacios-Prado et al., 2010), and for which there is evidence of localization on pyramidal cell proximal axons in the CA1 hippocampal region (Traub et al., 2020b). However, it should be noted that there is also evidence for Cx36 in the axons of at least some neocortical neurons (but not hippocampal neurons) (Thomas et al., in press); and Cx36-containing gap junctions are modulated by pH in an opposite direction to Cx45 – that is, they open with intracellular acidification (Gonzalez-Nieto et al., 2008). The opposing effects
of pH on these two types of connexin point to the need for determining their specific roles in controlling intractable epileptiform activity.

Overall, our study shows that the intracellular pH in specific regions of the brain is predominantly alkaline during the chronic SZ period in the pilocarpine-model of MTL epilepsy. These results suggest manipulations of brain pH as a potentially useful and powerful non-surgical strategy for controlling pharmacologically intractable epilepsy.

5. Experimental Procedure

5.1 Protocol and animals

All experiments were approved by the Institutional Animal Care and Use Committee of the Massachusetts General Hospital. Male Sprague–Dawley rats (170–210 g) were divided into a lithium pilocarpine group (n = 9) and a control group (n = 8).

5.2 Lithium-pilocarpine model of epilepsy

The pilocarpine procedure is a widely used method for creating a model of TLE in rats (Turski et al., 1983, 1989). Pilocarpine produces SE through its action as an agonist of the M1 muscarinic receptor (Cole and Nicoll, 1984; Hamilton et al., 1997). Although it has become an essential method for producing a chronic model of TLE, it has some drawbacks, depending on the specific procedures, such as relatively low induction rate of SE, high mortality rate after an SE induction and low rate of success in inducing chronic seizures (Curia et al., 2008). The use of lithium chloride with pilocarpine has improved these problems, but it can still produce variable results.

We were able to develop a procedure with 100% SE induction, a high survival rate (~90%) and 100% success rate in inducing chronic spontaneously occurring SZ. Lithium chloride (3 meq/kg, i.p.) was injected approximately 13 hrs before pilocarpine injection. This particular interval turned out to be the key for increasing the SE rate since it improved substantially after we shortened this period from 24 hrs to 13 hrs. We achieved the survival rate of 100% using Sprague-Dawley rats. Rats were pre-treated with methylscopolamine (1 mg/kg, i.p.) to reduce the peripheral effects of pilocarpine. Then, 30 min later, pilocarpine hydrochloride (30 mg/kg, i.p.) was administered to induce SE. Saline was injected in control animals instead of pilocarpine. After the injection of pilocarpine, all rats were continuously observed for the occurrence of behavioral SZ using the Racine scale (Racine, 1972). SE was defined as stage 4 or 5 on the Racine scale. Diazepam (5 mg/kg, i.p.) was given 90 minutes after SE onset to terminate the seizure. If the first injection of diazepam did not terminate the convulsive activity, additional injections of this drug were administered to prevent further SZ. All experimental rats developed SE, but one of them died within 24 hrs after the injection of pilocarpine. Two weeks after SE, rats were monitored using video recorders (8 hrs/day, 5 days/week) to assess the occurrence of spontaneous recurrent SZ. We defined the chronic period as a period in which one or more stage 3 or above spontaneous recurrent SZ occurred according to the Racine scale. Two months after SE, all pilocarpine treated rats (n = 8) entered into the chronic period. The mean seizure frequency during the chronic period...
was 3.2 per week. No SZ was observed in the control animals (n = 8). EEG and CEST MRI studies were performed 2 months after SE.

5.3 Scalp EEG

A single-channel bipolar scalp EEG was performed in six pilocarpine rats and one control rat for 10–15 min during the MRI measurements. The recording was performed with a 32-channel EEG amplifier (Brain Products, GmbH, Munich, Germany) modified to record single differential EEG inputs. All the recordings were performed outside the MRI environment. The recording bandwidth was 1 kHz and the sampling rate was 5000 Hz.

5.4 MRI data acquisition

Animals were imaged using a 4.7 T Bruker Biospec Scanner. All rats were anesthetized with 1.5–2.0% isoflurane in air during the MRI experiment and placed on a cradle with bite and ear bars, securing the head to minimize movement artifacts. Rectal temperature and respiratory rate were monitored by a physiological monitoring system (SA Instruments, Stony Brook, NY). Body temperature was maintained using a circulating warming water jacket system (Stryker Temperature Therapy Pad, Kalamazoo, MI).

Multiparametric MRI scans were performed, including T1, T2, and CEST MRI (field of view 20×20 mm², matrix 48×48, 6 slices, slice thickness 1 mm, bandwidth 200 kHz). T1-weighted images were acquired using inversion recovery EPI, with 7 inversion delays ranging from 250 ms to 3000 ms (TR/TE = 6500/15 ms, 4 averages, scan time 3 min), and T2-weighted images were obtained with two separate EPI scans (TR = 3250 ms, TE=30/100 ms, NA = 16, scan time= 1 min 40 s). The CEST Z-spectrum was acquired from −6 ppm to 6 ppm with intervals of 0.05 ppm (B1 = 0.75 μT, relaxation delay = 2500 ms, primary saturation time = 3000 ms, secondary saturation time = 500 ms, TE = 27 ms, NA = 2, scan time =44 min 43s). A water saturation shift referencing map was collected with an RF irradiation power level of 0.5 μT for TR/saturation time (TS) = 1500/500 ms for frequency offsets ranging between ±0.5 ppm with intervals of 0.05 ppm. Subsequently, MTR was calculated at +3.5 and −3.5 ppm from the Z-spectrum. Also, high-resolution rapid acquisition with relaxation enhancement (RARE) images were acquired (FOV= 20×20 mm², matrix = 128×128, TE=35 ms).

All data processing was performed using MATLAB (The MathWorks, Natick, Massachusetts). ROIs were manually drawn on high-resolution images. We outlined ROIs in five specific regions, including the bilateral hippocampus, amygdala, cortex, thalamus and piriform based on an atlas of the rat brain (Watson and Paxinos, 2013).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>SZ</td>
<td>seizures</td>
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<tr>
<td>SE</td>
<td>status epilepticus</td>
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<td>TLE</td>
<td>Temporal lobe epilepsy</td>
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<tr>
<td>CEST MRI</td>
<td>chemical exchange saturation transfer MRI</td>
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<tr>
<td>MTRasym</td>
<td>magnetization transfer ratio asymmetry</td>
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<td>APTR</td>
<td>amide proton transfer ratio</td>
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<td>APTR MRI</td>
<td>amide proton transfer MRI</td>
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<td>KD</td>
<td>Ketogenic diet</td>
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References


Sun PZ, Wang E, Cheung JS Imaging acute ischemic tissue acidosis with pHsensitive endogenous amide proton transfer (APT) MRI–correction of tissue relaxation and concomitant RF irradiation effects toward mapping quantitative cerebral tissue pH. Neuroimage 2012, 60:1–6. [PubMed: 22178815]


• Brain pH was determined with CEST MRI in lithium-pilocarpine rats with seizures.
• pH was alkaline in the hippocampus and piriform cortex compared to controls.
• The alkaline shift was marginal in the thalamus.
• The alkaline shift was not significant in the amygdala and cerebral cortex.
• Brain pH in patients with chronic seizures might be similarly alkaline shifted.
Fig. 1.
Scalp EEG from one animal with chronic seizure showing frequent interictal spike-wave complexes (a 10-s epoch out of a 15 min recording). The inset shows the electrode montage. Frequent inter-ictal spikes were seen throughout the entire 15 min period.
Fig. 2.
(A) and (B) Coronal RARE images indicating the Regions of Interest (ROIs) selected for the analysis of amide proton transfer ratio ($APTR$) and brain pH. A - cerebral cortex, B – hippocampus, C – thalamus, D – amygdala and E – piriform cortex. (C) $MTR_{asym}$ image in a pilocarpine rat exhibiting chronic seizure. $MTR_{asym}$ is higher in the hippocampus (ROI B) and piriform cortex (ROI E) compared to the rest of the brain. (D) The $MTR_{asym}$ image for a control rat that received saline instead of pilocarpine at the time corresponding to the pilocarpine injection in the experimental group. There is no clear elevation of $MTR_{asym}$ in the ROIs in the control rat. The color scale shows +/-10% in $MTR_{asym}$. 

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Fig. 3.
MTRasym in 5 ROIs in the pilocarpine group (n = 7) and control group (n = 8). Between-group, 1-tailed Student t-test results: # - p < 0.005, * - p < 0.05.
Fig. 4.
pH in 5 ROIs in the pilocarpine group (n = 7) and control group (n = 8) based on Method 1 (A) and 2 (B). Between-group, 1-tailed Student t-test results: # - p < 0.005, & - p < 0.03, * - p < 0.05.