—Review—

# Magnetic Resonance Imaging of Neurotransmitter-Related Molecules

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Molecular imaging implies the method capable of pictorially displaying distribution of target molecules and their relative concentration in space. In clinical medicine, where non-invasiveness is mandatory, diagnostic molecular imaging has been considered virtually identical to positron emission tomography (PET). However, there is another powerful, apparently underutilized molecular imaging, namely, proton magnetic resonance spectroscopic imaging ($^1$H-MRSI). The technique can detect target molecules endogenous in brain in virtue of their own specific resonance frequencies (chemical shift) and can create quantitative images of each molecule. $^1$H-MRSI is conventionally utilized for imaging relatively easily detectable molecules such as N-acetyl-aspartate or lactate. More recently, however, the method is extended into imaging of more challenging molecules such as glutamate or γ-aminobutyric acid (GABA). In this small review, we summarize basic concept of $^1$H-MRSI and introduce an advanced technique, i.e. chemical exchange saturation transfer magnetic resonance imaging (CEST MRI), which made realistic glutamate imaging *in vivo* possible. (J Nippon Med Sch 2017; 84: 160–164)

**Key words:** Brain, MRI, MRS, glutamate, GABA

## Introduction

Recent advances in molecular imaging, which implies the method capable of pictorially displaying distribution of target molecules and their relative concentration in space, provide opportunity to assess the physical and pathological basis of live tissue. In clinical field, PET plays a main role for most of the field of molecular imaging. However, elimination of all invasive aspects from diagnostic imaging, including intravenous infusion or use of ionizing radiation, is one of the final goals for the clinical imaging. Proton magnetic resonance spectroscopy ($^1$H-MRS) and its related modern imaging techniques represent such idealistic methodology. In this small review, we have focused on the advanced magnetic resonance (MR) based molecular imaging technique, chemical exchange saturation transfer magnetic resonance imaging (CEST MRI) and its application for glutamate *in vivo* mapping with respect to cerebral ischemia and other central nervous system (CNS) diseases.

## $^1$H-MRS

Magnetic resonance spectroscopy can non-invasively detect molecules in live tissues (**Fig. 1-a**) in virtue of their own specific resonance frequencies (chemical shift). The use of a spatial encoding technique would allow each peak to map tissue distributions (e.g., $^1$H-magnetic resonance spectroscopic imaging, $^1$H-MRSI; **Fig. 1-b**).

The findings of the first proton magnetic resonance spectroscopy ($^1$H-MRS) from a living rat brain were reported in 1983[1]. Since then, $^1$H-MRS has been extensively applied to clinical diagnosis and scientific research studies[2]. It had been conventionally utilized for assessing relatively easily detectable molecules such as N-acetyl-aspartate[3] or lactate[4]. With the recent developments in high-field (3–7 T) human[5] and animal MR systems, the use of MR for measuring neurotransmitter-related molecules, namely glutamate[6], γ-aminobutyric acid (GABA)[7], and glycine[8], has become the primary focus of research.

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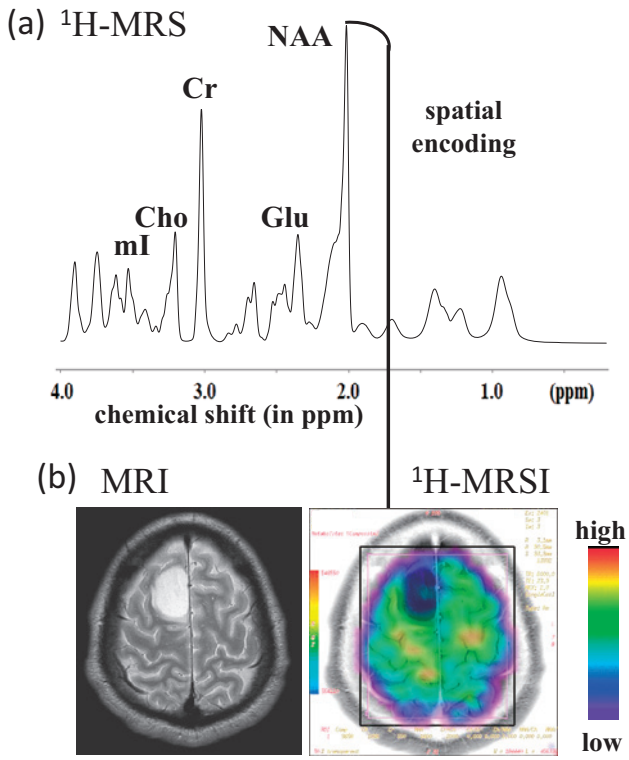


Fig. 1 Detection of small molecules using $^{1}$H-MRS and $^{1}$H-MRSI
$^{1}$H-MRS reveals many small molecules in live brain (a, mI:myo-inositol, Cho:choline compunds, Cr: creatine and phosphor-creatine, Glu:glutamate, NAA:N-acetyl aspartate). The spatial encoding of each peak reveals the distribution map of the molecule ($^{1}$H-MRSI, b).

## MRS-visible Intrinsic Neurotransmitters and Their Interpretations

In vivo, MRS-visible intrinsic neurotransmitters and related molecules should fulfill at least three fundamental conditions to be detected as an MR signal: the molecules should be hydrophilic, should have a relatively low molecular weight (approximately several hundred grams per mole), and should be present in a sufficient concentration (more than several mM in wet tissue). The main targets fulfilling these conditions are glutamate, GABA, and glycine. Among these, glutamate is the most popular MRS measurement target because of its abundance in the brain and its distinct peak at approximately 2.35 ppm (**Fig. 1-a**) in high-field MR systems.

Glutamate is the most abundant excitatory neurotransmitter in the mammalian brain. A unique two-compartment metabolic pathway between neurons (mainly synapse) and astrocytes, the glutamate-glutamine cycle, plays a primary role in excitatory neurotransmission[9]. As a metabolite, glucose is the primary source for de novo glutamate synthesis, and transamination between several amino acids and glutamate forms intermediate cellular metabolites[10]. Because visible glutamate in MRS represents the sum of these glutamates, changes in the glutamate peak in a pathological condition should be carefully interpreted according to the suspected mechanism. In brief, the mechanism of these changes can be interpreted as "static" or "dynamic" changes in a pathological condition. Static changes affecting the glutamate peak mainly involve chronic morphological changes such as synaptic losses in Alzheimer's disease[11]. Dynamic changes affecting the glutamate peak mainly involve acute metabolic changes due to a pathological condition. **Figure 2** shows metabolic changes in a rat model of focal cerebral ischemia. The ischemic lesion (left middle cerebral artery area) exhibited a prominent increase in glutamate because of a transient increase in glutamate synthesis following the massive release of glutamate into the extracellular space, as well as hindered transamination from glutamate[5]. In most pathological conditions, changes in the glutamate peak can be attributed to both these mechanisms.

## CEST MRI

$^{1}$H-MRS assessments of the distributions of neurotransmitter-related molecules have several disadvantages. Because the concentrations of protons in these molecules are quite lower than those of water, $^{1}$H-MRS measurements are time-consuming (e.g., 45 min; **Fig. 2**) and have a low resolution, which reduces the spatial specificity. However, an enhanced measurement sensitivity would allow the assessment of focal changes in the molecules during an appropriate measurement period. CEST MRI may therefore be useful for enhancing the measurement sensitivity.

During MRS, visible molecular peaks mainly represent unexchangeable upfield protons (lower ppm than water peak protons at 4.7 ppm). These molecules also consist of downfield (higher ppm) chemical shift protons (e.g., hydroxyl, amino, or amide residues) that are perpetually exchanged between the molecules and water. These peaks are difficult to be detected by in vivo $^{1}$H-MRS because of the proton exchange. However, the saturation of exchanged protons in the suspected chemical shift position leads to a decrease in the water peak because of the continuous exchange of saturated protons with water (**Fig. 3**)[12]. In 2003, Zhou reported the pH mapping of brain using changes in the pH-dependent exchange rates of amide proton signals of intracellular proteins and pep-

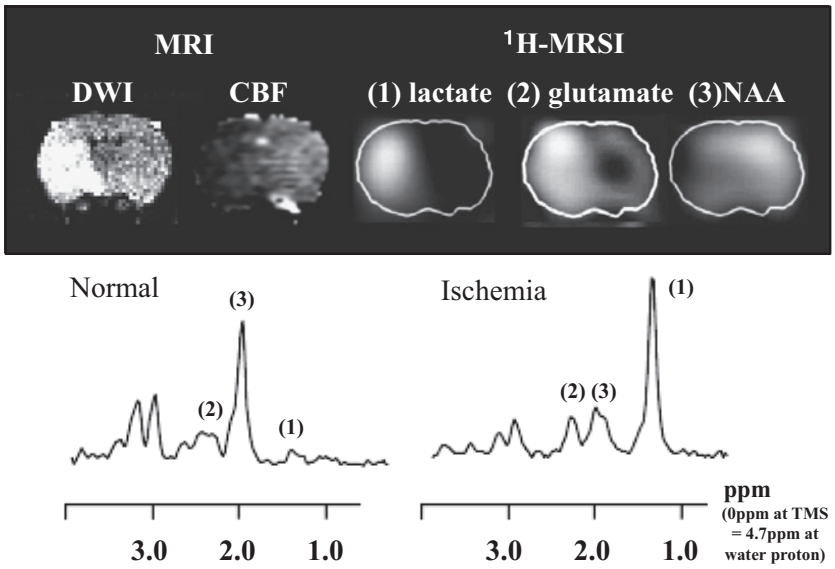


Fig. 2 $^{1}$H-MRS and $^{1}$H-MRSI of a rat model of acute focal ischemia
The ischemic lesion appeared as an area of high signal intensity on diffusion-weighted images (DWI) and as an area of severe low perfusion on cerebral blood flow (CBF) images. Compared with the normal area, the ischemic area exhibited prominent increases in the lactate and glutamate peaks on $^{1}$H-MRS. Decreases were observed in all other peaks. The distributions of lactate, glutamate, and N-acetyl aspartate (NAA) were clearly detected using $^{1}$H-MRSI.

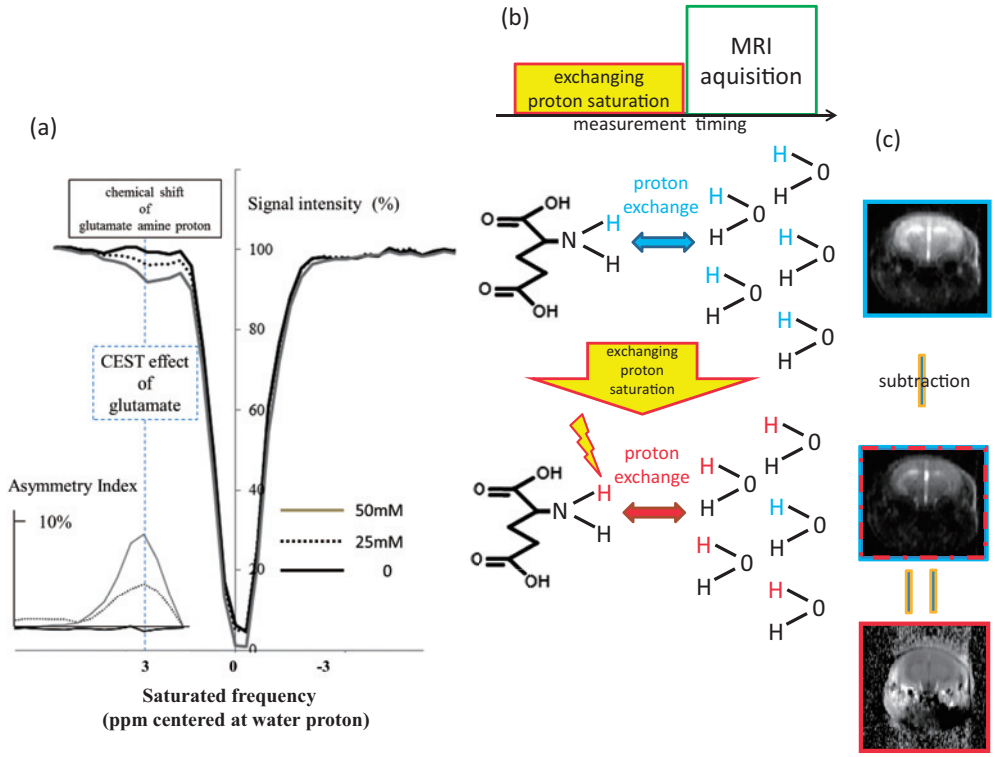


Fig. 3 Simplified schematic illustration of CEST effect and image acquisition procedure of glutamate
The saturation of amine protons of glutamate, exhibiting a specific chemical shift 3 ppm downfield of water protons (a), by preparation before MRI acquisition led to the exchange of these saturated protons (which means that these protons become "inactive" as the MR signal source) with water protons (b). This procedure decreased the MRI signal intensity according to the glutamate concentration and exchange rate. Subtraction of saturated image from unsaturated image reveals distribution of CEST effect (asymmetry index versus mirror position of water frequency; c).

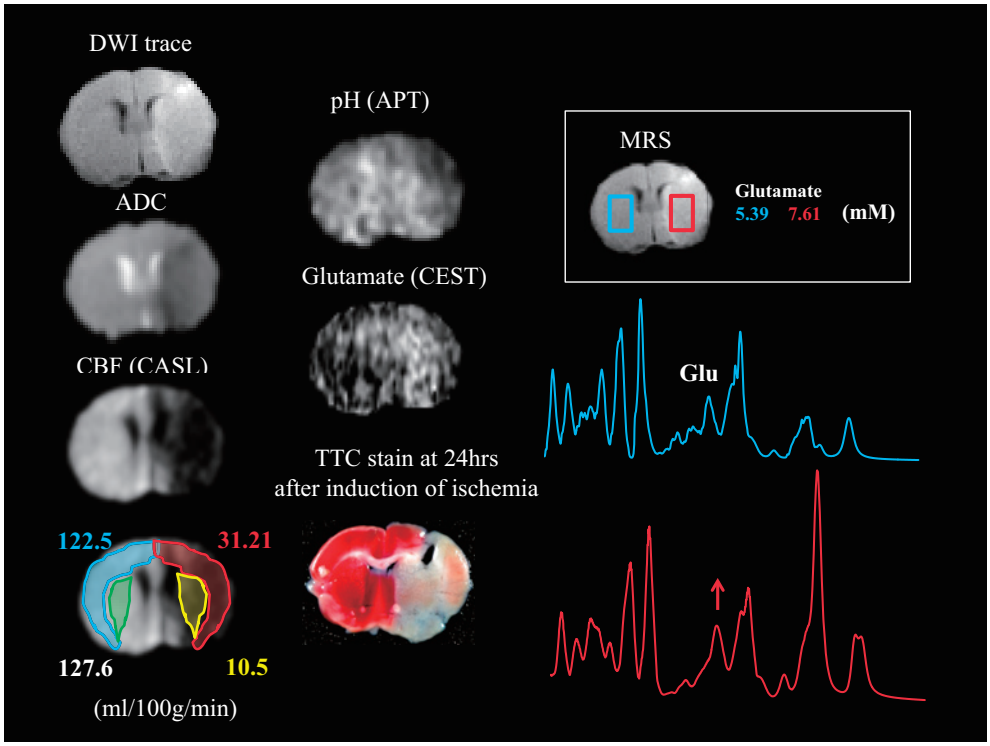


Fig. 4 Multiparametric MR of a mouse model of acute focal ischemia
Reduced pH of the ischemic lesion in APT MRI as well as increase in glutamate in the ischemic lesion, particularly at the border zone, were clearly shown in CEST MRI.
This change in glutamate in the ischemic lesion was also visible using $^{1}$H-MRS, which could quantify the concentration of glutamate. ADC: apparent diffusion coefficient, CASL: continuous arterial spin labeling, TTC: 2,3,5-triphenyl tetrazolium chloride, Glu: glutamate, Lac: lactate.

tides, namely amide proton transfer (APT) MRI[13]. Theoretically, this technique can be applied to specific target molecules such as glutamate[14], GABA[15], or myo-inositol[16] and can be used to enhance sensitivity by several thousand-fold.

**Figure 4** shows multiparametric MRI and $^{1}$H-MRS assessments in an acute-stage experimental mouse model of focal cerebral ischemia. Hypoxia accelerated the rate of anaerobic metabolism, resulting in a prominent lactate (Lac) peak on $^{1}$H-MRS and reducing the pH of the ischemic lesion on APT MRI. An increase in glutamate concentration in the ischemic lesion, particularly at the border zone, was clearly demonstrated by CEST MRI in the consort of MRS. Because CEST MRI is basically noninvasive, its application in humans is now in progress. However, some issues such as a high specific absorption rate (SAR)[17] and susceptibility to magnetic field heterogeneity[18] should be resolved before applying this technique to routine clinical settings.

## Conclusion

Recent MR techniques, particularly CEST MRI, allow the noninvasive measurement of neurotransmitter-related molecules in the brain and therefore provide new research and diagnostic strategies. MRI of neurotransmitter-related molecules can be a potential diagnostic tool in clinical settings in the future.

**Conflict of Interest:** We declare that we have no conflicts of interest in the authorship or publication of this contribution.

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