In vivo high-resolution localized ^{1}H spectroscopy in the unanesthetized rat brain at 7 Tesla

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Introduction

In vivo high resolution ^{1}H MRS has been employed extensively to simultaneously measure the concentration of a number of neuro-metabolites in normal or diseased brains of humans and animals. Unlike human MRS studies which can be readily performed in awake subjects, *in vivo* animal MRS is normally performed under anesthesia in order to immobilize the animal. It is well documented that neuronal activity, basal cerebral blood flow, hemodynamic coupling, and the blood-oxygenation-level-dependent signal can be altered by the anesthetic or paralytic agents and several unanesthetized fMRI protocols have been developed to circumvent these problems in primates[1,2] and rodents[3-7]. However, only one study investigated neuro-metabolic profiles using high resolution localized ^{1}H MRS in unanesthetized animals and it was performed in non human primates[8]. An unanesthetized MRS preparation that utilizes rodents is still lacking despite their extensive use in basic and translational neuroscience research. The goals of this study were (1) To develop a protocol to perform MRS experiments in unanesthetized rats; (2) To determine whether metabolic profiles in various regions of the rat brain can be measured reliably at 7T under this preparation using high resolution ^{1}H MRS. To the best of our knowledge, this is the first report of high resolution ^{1}H MRS in unanesthetized rats.

Materials and Methods

Animal training and acclimation

Female Sprague-Dawley rats (22 days old) were trained and acclimated to the MR environment using a custom made loose fitting restraining cloth and placed in a custom made plastic replica of the animal bed/holder used in the scanner. The animals were restrained for a period of 5 minutes initially and the restraining period was increased gradually to 30 minutes. The animals were also exposed to tape recordings of the sound bursts generated by gradient switching in the magnet during MRS experiments. By the end of the training period (2 months), the rats learned to remain calm in the replica of the scanner holder.

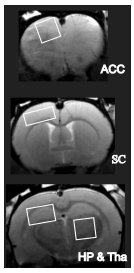

Fig. 1. The transverse MRI images obtained using RARE sequence (TR/TE_{eff} = 4500/28 ms) of the brain of awake rats. The voxel locations for MRS experiment are highlighted. ACC = anterior cingulated cortex, SC = somatosensory cortex, HP = hippocampus (left), Tha = thalamus (right).

Holder implantation surgery

The animals were anesthetized with 2% isofluorane and attached to a stereotaxic frame. Incision sites were injected with local anesthetic (0.5% marcaine) to further reduce the possible pain during the surgery. A midline incision overlying the skull extending from the inion to lambda (10 mm) was made. The scalp was reflected laterally and six predetermined atraumatic openings were prepared. Six polyetheretherketone screws were attached to a custom made acrylic resin plastic head holder which fits the calvarium of the rat intimately. At the end of surgery, the skin edges were approximated and sutured. One week after surgery the animals were reintroduced to the replica of the scanner holder. The rats cooperated and there was no difficulty positioning them in the restrainer.

MRS experiment

In vivo MRS experiments were performed on a Bruker BioSpec 7T MR scanner equipped with a BGA12S gradient system. A Bruker four-element ^{1}H surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. The animal was restrained in the scanner bed in a similar fashion as when it was undergoing training. A MR-compatible small-animal monitoring system was used to monitor animal respiration rate. Adjustments of all first- and second-order shims over the voxel of interest were accomplished with the Fastmap procedure. The PRESS pulse sequence was customized by using a *sinc7H* pulse with excitation band of 10 kHz. Two asymmetric pulses with a bandwidth of 2823 Hz were used for refocusing. The TE was shortened to 10 ms in order to alleviate signal attenuations caused by J modulation and T_2 relaxation. The center frequencies of the above three localization pulses were placed at 3.0 ppm to reduce the lipid contamination caused by the chemical shift displacement. The custom made PRESS sequence (TR/TE = 2500/10 ms, number of average = 356) was used for MRS data acquisition from various regions including the anterior cingulate cortex, somatosensory cortex, hippocampus, and thalamus. The acquisition time for each voxel was 15 minutes. LCModel package was used for quantification of the MRS data. The reliability of the major metabolites was estimated in the Cramér-Rao lower bounds (CRLB) from the LCModel analysis. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Maryland.

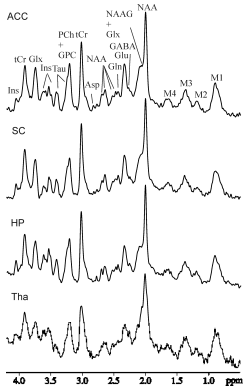

Fig. 2. *In vivo* ^{1}H short-TE MR spectra from 4 brain regions of unanesthetized head restrained rats acquired at 7 T with a localized PRESS sequence (TR/TE = 2500/10 ms). Asp = aspartate, tCr = total creatine, GABA = γ-aminobutyric acid, Gln = glutamine, GPC = glycerophosphocholine, Glu = glutamate, Glx = glutamine + glutamate, Ins = *myo*-Inositol, NAA = *N*-acetylaspartate, NAAG = *N*-acetylaspartylglutamate, PCh = phosphocholine, Tau = taurine, M1 – M4 = macromolecule , ACC = anterior cingulate cortex, SC = somatosensory cortex, HP = hippocampus, and Tha = thalamus.

Results

Axial anatomic images along with the 4 spectroscopic voxel locations are shown in Fig. 1. There were no obvious movement artifacts in the anatomic images suggesting that the animals remained still throughout the course of the MR experiments. The voxel sizes of the four interested regions varied in size from 27 to 32.4 mm^3. High resolution localized ^{1}H MR spectra from these regions are shown in Fig. 2. A number of metabolites were reliability detected (Table 1). In general, the CRLB values of Glu, Ins, Tau, GPC+PCh, NAA+NAAG, Cr+PCr and Glx were lower than 9 %. For the low concentration metabolites GABA and Gln, the CRLB value increased to 13 %. More variability was observed for GABA (23 %), Gln (26 %) and Tau (24 %) levels in the thalamus.

Table 1 Summary of the reliability of the major metabolites was estimated in the Cramér-Rao lower bounds (CRLB) from the LCModel analysis in the rat brain from the localized MRS. ACC = anterior cingulate cortex, SC = somatosensory cortex, HP = hippocampus, and Tha = thalamus.

Metabolites	CRLB (%)			
	SC	ACC	HP	Tha
GABA	11	9	12	23
Gln	13	13	12	26
Glu	4	3	4	7
Ins	7	7	6	9
NAA	3	3	3	7
Tau	8	6	6	24
GPC+PCh	6	5	6	8
NAA+NAAG	3	3	3	5
Cr+PCr	3	3	3	6
Glx	5	4	5	9

Discussions and Conclusions

A major concern of performing experiments in restrained animals is the stress produced and their effect on MRS results. To minimize this concern, we adopted similar methods to those described in King *et al.*[3] and acclimated and handled the animals from a young age for months before performing the experiments. These acclimation and training procedures proved effective in improving image quality and reducing alterations in heart rate, respiration, serum cortisone levels and ultimately stress in immobilized and anaesthetized animals[3].

We conclude that high-resolution high-quality ^{1}H MRS can be obtained from unanesthetized rat brains at 7 T. With proper training and restraint apparatus, several proton metabolites can be reliably measured even without any post processing correction scheme. The current development offers a novel approach to study major brain metabolites and possibly function in unanesthetized rodents. This method circumvents the effects of anesthesia, and allows us to perform longitudinal experiments for prolonged periods of time to study progression of disease especially in the areas of chronic pain research. It will also open the door for translational research that bridges the gap between animal and human studies.

References

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