Metabolic flux maps from 3D MRSI of rat brain *in situ*

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Introduction

Focused beam microwave irradiation (FBMI) has been shown to rapidly (<1s) halt cerebral metabolism in rodents, thereby preserving the *in vivo* metabolic profile (as measured by ^{1}H MRS) for up to 30 hours *in situ* [1]. Combining extensive signal averaging with improved coil design for the isolated rat skull/brain leads to greatly increased sensitivity, which in turn provides the possibility for high-resolution 3D MRSI. When FBMI and 3D MRSI are combined with ^{13}C-labeled substrate infusion *in vivo*, the resulting *ex vivo* data can be used to generate high-resolution metabolic maps of cerebral pathways, like the TCA cycle. In this study the tools and routines are described that are necessary to generate metabolic maps from 3D MRSI data acquired on different animals with variable duration ^{13}C-label infusions.

Materials and Methods

Sample preparation Male Sprague-Dawley rats (180 ~ 220 g) were secured with tail vein catheters under light anesthesia and allowed to recover for 30 min. After infusing [1,6-$^{13}C_2$]glucose for 8, 15 or 30 min or [1-^{13}C]glucose for 60 min, rats were euthanized by FBMI (5kW, 1.25s). Extracranial tissues were removed and the skull was immersed in a vial containing Fluorinert FC-43 (3M, St. Paul, MN) for magnetic susceptibility matching.

MR experiment Measurements were carried out on a horizontal 11.7 T magnet interfaced to an Agilent DirectDrive MR spectrometer (Palo Alto, CA, USA), using high-performance gradient coils (maximum strength 395 mT/m, rise time 180 µs). RF transmission and reception was performed using a custom-built dual-tuned coil. A double spin echo sequence was applied to acquire T1-weighted 3D MRI (TR 500 ms, TE 25 ms), 3D MRSI water spectra (TR 1500 ms, TE 15.2 ms) and 3D MRSI metabolite spectra (TR 4000 ms, TE 15.2 ms). For the metabolite MRSI measurement, a ^{13}C inversion pulse placing after the second refocusing pulse was on and off alternatively to accomplish heteronuclear editing.

Image segmentation To establish a template image, one 3D MR image was selected as a reference image to which the images from all other animals were spatially registered. All images were summed together to generate a high resolution image necessary for performing segmentation. In the template image 13 regions-of-interest (ROIs) were manually segmented (anterior commissure, caudate putamen, cerebellum, corpus callosum, cortex, fimbria, hippocampus, internal capsule, mid-brain, olfactory bulb, septum, thalamus, ventricles). The transformation matrix obtained from the non-linear registration was applied to the segmentation image to obtain tissue information for each animal. Finally the registered segmentation images were convoluted with the 3D MRSI point spread function to calculate tissue contributions in each voxel. A sequential order of registration, segmentation and calculation of tissue contributions was shown in Fig. 1a, 1b and 1c.

MRS processing 3D water MRSI datasets were used as means of assessing spectral quality. Water spectra passing three criteria (minimum amplitude: 7% of maximum, maximum frequency shift: 30 Hz, maximum linewidth: 30 Hz) were recorded and corresponding metabolite spectra from the 3D metabolite MRSI datasets were included for analysis. Eddy current correction was performed based on the phase information derived from the water signal. For each ROI a combined FID was calculated as the sum of individual MRSI pixels. A pixel was assigned to a ROI if its contribution was at least 75%. The spectral basis sets, necessary for quantifying total and difference spectra, were generated by numerical simulation based on density matrix formalism. The Linear Combination model fitting algorithm, developed for analyzing high-resolution NMR spectra [2], was extended for quantifying 3D MRSI spectra. A spectral range, between 1.2 ppm and 3.15 ppm, was selected for quantifying both total and difference spectra. In-house Matlab (MathWorks, Natick, MA, USA) routines were used for data processing with exception of registration, which used Bioimage Suite [3].

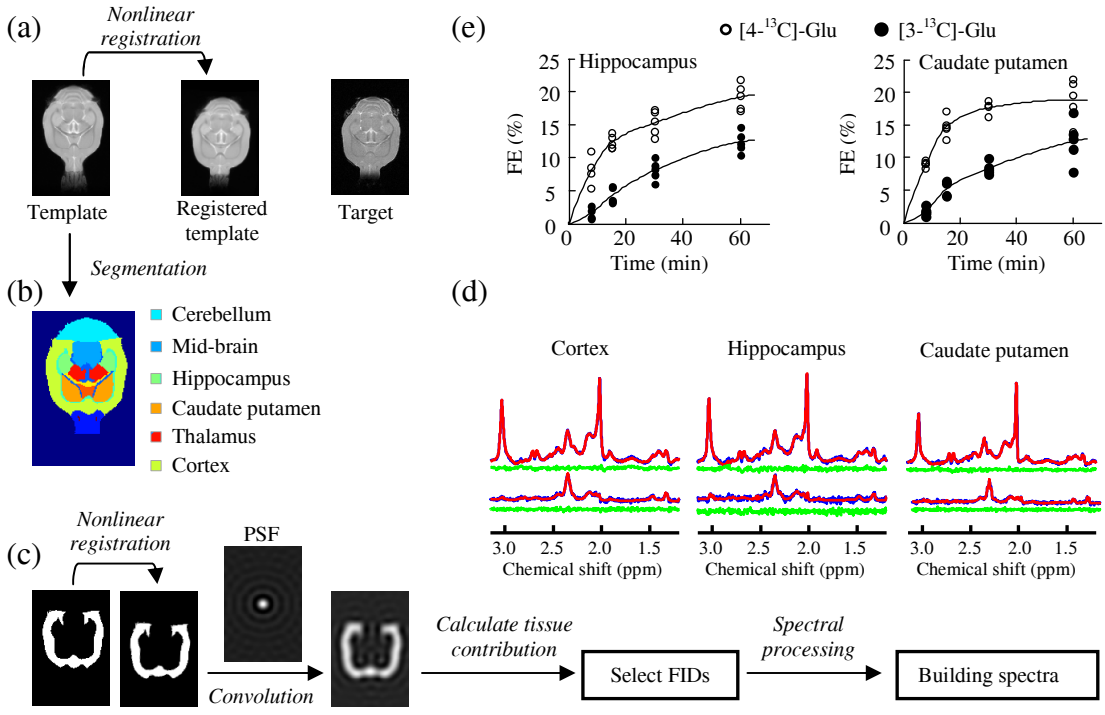

Figure 1. Schematic flow of processing procedures for 3D MRSI data. Template MR image before and after nonlinear registration with respect to a target image (a) and segmentation image of the template image (b). Example of registering cortex and being convoluted with PSF to calculate tissue contributions (c). Fitting results of total spectra and difference spectra (d) and the time courses of ^{13}C labeling in hippocampus and caudate putamen (e).

Results and Discussion

Fig. 1d shows fitting results (blue: raw, red: fitted, green: residuals) of total (^{13}C+^{12}C) and difference spectra (2x^{13}C) for three representative brain regions. Timed-infusions of [1,6-$^{13}C_2$]glucose followed by FBMI in different animals was used to generate metabolic turnover curves for the brain regions, as shown for [4-^{13}C]-Glu and [3-^{13}C]-Glu (Fig. 1e). The presented acquisition and processing routines allow for potential automation of processing of dynamic metabolic turnover maps depicting multiple brain regions simultaneously.

References [1] de Graaf RA et al. J. Neurochem. 2009; 109: 494-501. [2] de Graaf RA et al. Anal. Chem. 2011; 83: 216-224. [3] Papademetris X. http://www.bioimagesuite.org

Acknowledgements
This work was supported by NIH NIMH grant R01 MH095104.