A PILOT STUDY ON MEASUREMENT OF METABOLITES IN THE HIPPOCAMPAL SUBFIELDS: BASED ON MULTIVOXEL 1HMRS AND SEGMENTATION FROM HIGH RESOLUTION VOLUMETRIC MRI

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TARGET AUDIENCE: neurologists and neuroradiologists.

Purpose: Atrophy of hippocampus is widely recognized as a bio-marker of AD. Recent researches indicated a higher sensitivity in atrophy of hippocampal subfields to early AD pathology compared with the whole hippocampus atrophy[1]. Further more, metabolite abnormalities detected by proton Magnetic Resonance Spectroscopy(^{1}HMRS), specifically those of N-Acetyl Aspartate (NAA) and Myo-Inositol (mI), were believed to occur earlier than structural changes [2]. Therefore, we ***hypotheses*** that the metabolite concentrations in subfields could obtain by co-registering multi voxel ^{1}HMRS and segmentation from high resolution volumetric MRI. Then metabolite abnormalities of individual hippocampal subfield might change in AD compared.

Methods: ***Subject:*** A total of 31 subjects (14 AD, and 17 Normal Aging Control(NC)) were included. AD: 9 female and 5 male, aged 61-85 yr (74.1 ± 9.4 yr); NC: 7 female, 11male, aged 44-82 yr (64.3±13.0 yr). ***Sequence:*** Each of subjects underwent 3D T_1W scan (TR=2000ms, TE=32ms, thickness 1mm), and 2D MRS with Volume of Interest(VOI) covering the left and right hippocampus, respectively, at a 3T MRI scanner (Achieva 3.0T TX, Philips Medical Systems, the Netherlands). 2D MRS sequence: PRESS, TR=2000ms, TE=32ms, NSA 4, VOI=64×32×8mm^3, voxel=4×4mm^2.

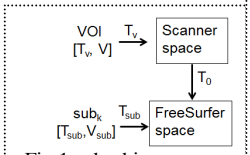

Fig.1 sub$_k$: hippo subfields

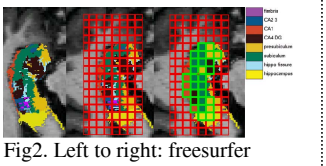

Fig2. Left to right: freesurfer segmentation, VOI co-registration, subfield labeling. Red frame:VOI; green frame: voxels with reliable MRS results; voxels colored correspondingly with the subfield it represents.

Hippocampal subfield segmentation and VOI coregistration: **(1)** All spatial registrations related were affine transforms with given transform matrixes (Fig.1). The transform matrix of VOI to FreeSurfer Software (version 5.3.0) space was then $T_{vfs}=T_vT_0$. **(2)** For each subject, 3D T_1W images were processed by FreeSurfer in native space to get labeled out the hippocampus as well as its individual subfields, as is shown in Fig. 2 (middle panel. 8 subfields were labeled for each hippocampus, namely CA1, CA2_3, etc., the label hippocampus denotes the remaining indivisible part). **(3)** Generating different VOIs for different metabolites due to chemical shift displacement. The PRESS sequence achieves frequency-dependent localization of VOI, and therefore generates different VOI locations for metabolites with different Raman-frequency, as illustrated in Fig. 3. Different VOIs were used further for different metabolites.

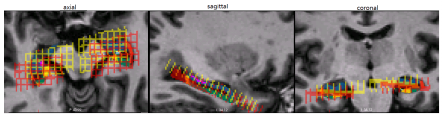

Fig.3 different VOIs for NAA(red) and mI(yellow) co-registered in FreeSurfer native space.

Table. 1 The detail mean ± SD and statistical result of metabolic parameters of individual subfields between each two of the three groups.

subfield	metabolite	AD	NC	F	P
left_CA4_DG	NAA/Cr	0.873±0.200	1.087±0.291	1.299	1.859
	mI/Cr	1.351±0.372	1.346±0.211	0.632	**0.028***
right_CA4_DG	NAA/Cr	0.891±0.175	1.205±0.268	0.979	**0.004***
	mI/Cr	1.099±0.229	1.005±0.095	3.884	0.298
right_presubiculum	NAA/Cr	0.924±0.228	1.128±0.275	0.112	**0.037***
	mI/Cr	1.050±0.150	1.012±0.240	1.064	0.670
right_subiculum	NAA/Cr	1.046±0.172	1.367±0.285	6.621	**0.001***
	mI/Cr	1.090±0.223	1.005±0.230	0.014	0.443

* = Significantly different between AD and NC. Statistically significant difference at P < 0.05.

Representative voxels for each subfield: **(1)** Each of the subfields was scrutinized to find out the voxels that could represent its metabolite profile. We used a rough equivalence that the subfield taking up the majority of the volume in a VOI voxel also contributes the majority of the metabolite signal in that voxel, and thus could to some extent represent the voxel metabolite profile. Let $P_k=\{p_{k,i}, i=1,...,N_k, k=1,...,16\}$ be the total of N_k points in the probability volume of subfield k(16 subfields includ 8 in the left and 8 in the right hippocampus), and $L_k=\{l_{k,i}\in[0,1], i=1,...,N_k, k=1,...,16\}$ be the corresponding probability of each point $p_{k,i}$. **(2)** Each of the subfields sub$_k$ were transformed to the VOI coordinates with the cascaded affine transform matrix: $T_v^{-1}T_0^{-1}T_{sub}$. Each point $p_{k,i}$ in set P_k is transformed correspondingly to $p_{k,i}$' in the set P_k' in VOI coordinates: $p_{k,i}'=(T_v^{-1}T_0^{-1}T_{sub})p_{k,i}$. Let $V=\{v_{m,k}, m=1,...,128, k=1,...,16\}$, $v_{m,k}=\sum_{p_{k,i}\in VOX_m} l_{k,i}$ was the volumetric percentage of subfield k covered by VOI voxel m(VOXm is the set of all points covered by voxel m). For each subfield k, when $v_{m,k}$ was larger than 40%, the voxel m was labeled as representative for subfield k. In case two subfields both take up more than 40% in the same voxel, say $v_{m,k1}$ and $v_{m,k2}$ are both larger than 40%, the voxel m was labeled as the subfield with the larger percent.

Metabolite profile of individual subfield: 2D ^{1}HMRS of both hippocampi were processed by LCModel software (version 6.3-1H) to obtain metabolite concentrations in all VOI voxels. Firstly we excluded those voxels with unreliable MRS results (with criteria recommended by LCModel). After labeling each voxel with the subfield it represents, the metabolite concentrations from voxels with the same label were averaged to obtain the metabolite profile of that subfield, particularly NAA/Cr and mI/Cr (Fig. 2 right panel).

Statistical method: we performed independent-samples T test of metabolite concentrations in individual subfield, between AD and NC group.

Results: Voxels that passed the threshold was 50.49%±9.45% for AD, and 49.65%± 8.91% for NC. 91.1%±5.97% of all subjects have representative voxels in one of the subfields CA1, CA4_DG, presubiculum, subiculum, none or very few subjects have representative voxels in fimbria, hippo_fissure. As is shown in table 1, subfields were differently affected(as in the case CA4_DG compared with other subfields), and statistical difference between AD and NC is highly significant(P value was 0.004 for right_CA4_DG and 0.001 for right_subiculum).

Discussion: The 40% threshold of determining voxels' representativeness for certain subfield, was empirical, while the statistical result indicates reasonable representativeness in practice. Due to the shapes of hippocampus and VOI location, not all subfields could be assigned with representative voxels. Hippocampal subfields CA1, CA4_DG, presubiculum, subiculum could be better studied with this method than fimbria, hippo_fissure etc.

Conclusion: The presented method presents a measurement with considerable reliability for metabolite concentration in some of the hippocampal subfields, particularly in CA1, CA4_DG, presubiculum, subiculum. And could probably serve as sensitive bio-marker from hippocampal subfield for AD with high significance.

Reference:

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2. Tumati S1, et al. Neurosci Biobehav Rev. 2013 Dec;37(10 Pt 2):2571-86.