Geriatric Rhesus monkeys have in vivo proton MRS signatures of human Alzheimer's Disease

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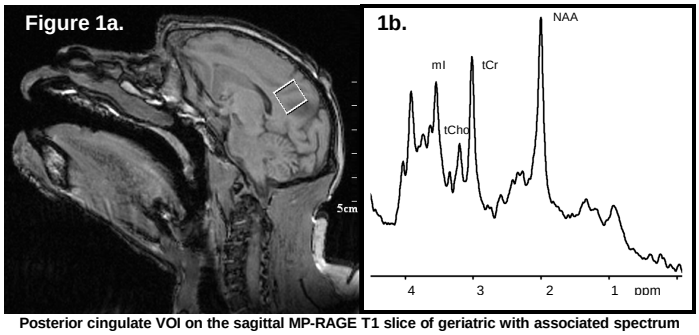
INTRODUCTION

Geriatric non-human primates have long been proposed as a model for age-associated neurodegenerative diseases. These monkeys develop behavioral (declines in cognitive and memory function) and brain anomalies similar to those that occur in aged humans and more commonly in persons with Alzheimer's disease (1-2). Alzheimer's disease is characterized by deposits of neurofibrillary tangles and amyloid plaques, and loss of neuronal connectivity with reduced synaptic density in the mesiotemporal structures, parietal and frontal association cortex. All geriatric (>24 years of age) monkeys exhibit diffuse amyloid deposits, congophilic angiopathy, and neuronal degeneration (3-6). Historical histological data (unpublished) confirm that all monkeys >24 years of age from the in-house colony have amyloid deposits. These animals provide the unique opportunity to examine processes that lead to the deposition of amyloid, subsequent neuronal death, and age-related changes in neuropathology/neurochemistry. MRS is able to non-invasively measure neurobiochemical information that might help elucidate mechanisms of disease, and potentially serve as a biomarker of disease progression. Since changes in AD brain have been consistently observed in human in vivo MRS studies, i.e. ↓ NAA (N-acetylaspartate) and ↑ mI (Myoinositol) (7-8), in vivo proton MRS was proposed in a study to [i] examine the feasibility of localized in vivo proton MRS in the brain of the Rhesus monkeys, [ii] quantify major brain metabolites and those implicated in neurodegenerative disease, and [iii] measure any differences in metabolite concentrations in young adult versus geriatric Rhesus monkeys.

MATERIALS & METHODS

All experiments were conducted on a Siemens Trio 3T scanner using a vendor-supplied ^{1}H extremity coil. To evaluate within-subject variability, three adult Rhesus monkeys were scanned repeatedly. The measured with-in subject variability (≈8%) was used to determine the sample size (n=7) required to reliably detect a 15% change in brain metabolites with 90% statistical power. In vivo water-suppressed MRS spectra were acquired for 8 geriatric (≥ 24 yrs) and 8 young adult (≤ 15 yrs) Rhesus monkeys. The animals were anesthetized with 1.0-1.5% isoflurane and physiologically monitored for temperature, respiration and blood O_2 levels. All animal handling procedures were approved by the Institutional Animal Care and Use Committee. After fast ^{1}H MR 3-plane localizers were obtained for ROI placement and shimming, all first- and second- order shim currents were adjusted on tissue water with an automated shim protocol. Linewidths of the H_2O resonance observed from the 1 cm^3 PRESS-localized voxel were approximately 20Hz. Voxels were consistently placed posterior and superior to the splenium of the corpus callosum in the posterior cingulate (Figure 1a). The spectrum in Figure 1b is representative of the quality consistently achieved in this study. Pulse sequence parameters were as follows: TR=5s, TE=30ms, NEX=128 scans, N=2048 and SW = 1200Hz. The data were reconstructed and fitted off-line. Peak quantification was obtained by using tCr (total creatine) as an internal standard. Statistical analysis was performed using JMP 6.0 (SAS Institute, Cary, NC). The ratios of NAA, tCho (total choline) and mI to tCr and NAA to mI were compared between young adults and geriatrics with two-tailed two-sample t-tests ($\alpha = 0.05$). One-tailed tests were also adopted for NAA/tCr, mI/tCr and NAA/mI on the basis of prior MRS findings in human AD. Metabolite ratios were plotted versus the age of the scanned animals, and associations between age and metabolite ratios (as well as interrelationships among ratios) were measured with Pearson correlation statistics.



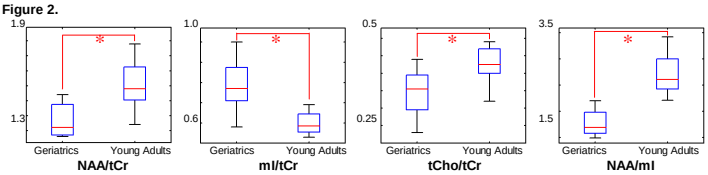
Posterior cingulate VOI on the sagittal MP-RAGE T1 slice of geriatric with associated spectrum

Table 1: Rhesus monkey brain metabolite ratios (mean ± SD) and corresponding human brain ratios (mean ± SD)

Metabolite	Young Adult Rhesus	Control Human	Geriatric Rhesus	AD Human
mI/tCr	**0.60 ± 0.06**	0.62 ± 0.09	**0.79 ± 0.13**	0.74 ± 0.08
NAA/tCr	**1.61 ± 0.17**	1.51 ± 0.09	**1.37 ± 0.11**	1.41 ± 0.11
tCho/tCr	**0.43±0.05**	0.60 ± 0.07	**0.35 ± 0.07**	0.71 ± 0.12
NAA/mI	**2.71 ± 0.40**		**1.77 ± 0.27**	

Note: Human data from Reference 9

Figure 2.



t Ratio	**3.275**	**t Ratio**	**-3.624**	**t Ratio**	**2.557**	**t Ratio**	**5.46**
Prob > |t|	**0.006**	**Prob > |t|**	**0.003**	**Prob > |t|**	**0.023**	**Prob > |t|**	**< 0.0001**
Prob > t	**0.003**	**Prob < t**	**0.001**	**Prob > t**	**0.011**		

Table 2: Pearson correlations between/among Rhesus monkey brain ratios and age

	NAA/tCr		mI/tCr		NAA/mI		tCho/tCr	
	Correlation	(p-value)	Correlation	(p-value)	Correlation	(p-value)	Correlation	(p-value)
mI/tCr	-0.315	(0.235)						
NAA/mI	**0.765**	**(0.0006)***	**-0.837**	**(<0.0001)***				
tCho/tCr	**0.660**	**(0.005)***	-0.264	(0.324)	**0.519**	**(0.039)***		
Age	**0.766**	**(0.0005)***	**0.656**	**(0.006)***	**0.864**	**(<0.0001)***	**0.683**	**(0.004)***

RESULTS

Table 1 presents a comparison of the measured MRS metabolite ratios of the Rhesus monkey young and geriatric cohorts, combined with human data found in Ref. 9. Rhesus geriatrics have significantly decreased NAA/tCr (1.37±0.11 vs. 1.61±0.17, p=0.003)*, increased mI/tCr (0.79±0.13 vs. 0.60±0.06, p=0.001)*, decreased tCho/tCr (0.35±0.07 vs. 0.43±0.05, p=0.01)* and decreased NAA/mI (1.77±0.27 vs. 2.71±0.40, p<0.0001)*. Group data and statistics are shown in Figure 2. Pearson correlations show that NAA/tCr, tCho/tCr and NAA/mI correlated negatively with age (p<0.001*, p<0.01* and p<0.05* respectively), mI/tCr correlated positively with age (p<0.01)*, and tCho/tCr correlated positively with NAA/tCr (p<0.01)* and NAA/mI (p<0.05)*.

DISCUSSION

This study shows the feasibility of localized in vivo quantitative proton MRS in Rhesus monkeys and reports statistically significant differences in the major metabolites of geriatrics versus young adult Rhesus monkeys. Though the geriatric Rhesus monkey model for neurodegenerative illness has not been characterized as extensively as transgenic mouse models of AD, historical data show evidence of behavioral and brain abnormalities characteristic of human AD. As with humans, these animals have been consistently shown to develop amyloid plaques as a normal part of the aging process. Since global brain atrophy associated with normal aging is not expected to alter metabolite ratios, their altered biochemical profiles suggest neuronal loss (NAA, ≈15%↓) and gliosis (mI, ≈30%↑) in the posterior cingulate. These changes correspond with neuropathological MRS biomarkers of human AD. Decreased (≈20%↓) tCho suggest abnormal membrane turnover in the posterior cingulate. Proton MRS in Rhesus monkey brains could prove to be very valuable in elucidating AD mechanisms as well as in assessing potential therapeutic strategies.

REFERENCES

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