Factor Analysis Reveals Metabolic Differences in Macaques with SIV/AIDS and Encephalitis

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Introduction Magnetic resonance spectroscopy (MRS) has often been used to study metabolic processes in the HIV-infected brain [1]. However, it remains unclear how changes in individual metabolites are related to one another in this context of virus-induced central nervous system dysfunction. As more metabolites become quantifiable from high field in vivo spectra, the statistical method of factor analysis (FA) could be effective in reducing error from individual tests as well as uncovering broader patterns in the metabolic processes underlying different stages in the pathogenesis of neuroAIDS. We used FA to evaluate and identify patterns in a distribution of metabolites from an MRS study of healthy macaques and those infected with simian immunodeficiency virus (SIV), which were moribund with AIDS [2]. The purpose of this analysis is to identify metabolite patterns associated with SIV/AIDS and severity of virus-induced encephalitis. By moving beyond analysis of individual metabolites, this method can help enhance our understanding of how MRS metabolic markers, both glial and neuronal, are related in this disease.

Methods Twenty-three SIV-infected (SIVmac251 or SIVmac239) rhesus macaques were sacrificed when moribund with AIDS, along with six uninfected controls. Brain sections were formalin-fixed, paraffin-embedded, and sectioned for routine histopathology, which was used to divide macaques into two groups: those with giant-cell encephalitis and those without. Animals diagnosed with encephalitis were further classified as mild, moderate, or severe based on the size and frequency of the perivascular lesions/multinucleated giant cells in the brain sections evaluated. Sections included cerebral cortex (frontal, parietal, temporal, occipital), basal ganglia, thalamus, hippocampus, brain stem, and cerebellum. Frontal cortex tissues were snap frozen upon necropsy, and 50-90 mg portions were separated for methanol/chloroform extraction [3]. Extracted metabolites underwent high resolution ^{1}H MRS experiments on a 14.1 T Bruker AVANCE^{TM} spectrometer (recycle time 20 s; spectral width 7.2 kHz; 32,000 points; 64 scans). PERCH NMR software was used to determine the quantities of N-acetylaspartate (NAA), myo-inositol (MI), total choline-containing compounds (Cho; 3.19-3.22 ppm), creatine (Cr), γ-aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), N-acetylaspartylglutamate (NAAG), and glycine (Gly) in these frontal cortex samples. The relative level of each metabolite was calculated by dividing its area by the total area of all metabolites of interest, and patterns of metabolite levels were examined by FA on a correlation matrix. Each animal was assigned a score for each factor based on the respective loadings of its nine metabolite levels. The association between the resulting components and the severity of encephalitis was assessed by analysis of variance (ANOVA). If the ANOVA was significant, specific differences between disease classifications were isolated using two-tailed least squares means (LSM) t tests. Statistical tests were performed using JMP 5 software.

Results FA identified three main factors, which accounted for 67% of the variance in the data (Table). The scores for Factors 2 and 3 were significantly different across cohorts (ANOVA: $p = 0.005$, $p < 0.05$, respectively), while scores for Factor 1 were not (ANOVA: $p = 0.82$).

	Factor 1	Factor 2	Factor 3
Variance Explained			
Eigenvalue	1.615	2.304	2.093
Percent	17.949	25.598	23.250
Cum. Percent	17.949	43.548	66.798
Loadings			
Cr	-0.285	**-0.479**	**-0.637**
NAA	-0.130	**0.884**	0.013
Glu	0.246	**0.689**	**0.564**
Gln	-0.195	0.209	**-0.697**
GABA	**-0.412**	0.229	**0.679**
Gly	-0.240	-0.030	**0.633**
NAAG	**-0.879**	-0.062	0.078
Cho	**0.596**	-0.248	0.021
MI	0.252	**-0.810**	0.118

Analysis of Macaques with and without AIDS Scores for Factor 3 were able to discern between animals that had SIV/AIDS and those that did not. Lower scores were associated with SIV infection (Figure 1, top). Factor 3 was able to distinguish uninfected animals from infected animals with SIV-induced encephalitis ($p < 0.03$), as well as from infected animals without encephalitis ($p = 0.02$). However, no distinction between the two SIV-infected groups could be found using this factor ($p = 0.55$).

Analysis of Encephalitis and SIVE Severity among Macaques Factor 2 was able to discriminate animals with SIV-induced encephalitis from uninfected controls ($p = 0.002$), and from animals moribund with AIDS without SIVE ($p = 0.04$). This factor was, however, unable to discern between uninfected animals and infected animals that lack encephalitis ($p = 0.32$). Further analysis indicated that that Factor 2 could distinguish between disease classifications (ANOVA: $p < 0.002$), with lower scores associated with worse degrees of encephalitis (Figure 1, bottom). Factor 2 was able to differentiate animals with moderate or severe encephalitis from control animals ($p = 0.004$, $p < 0.0003$, respectively), from animals found moribund with AIDS but lacking encephalitis ($p = 0.04$, $p < 0.005$, respectively), and from mildly encephalitic animals ($p < 0.08$, $p = 0.01$, respectively).

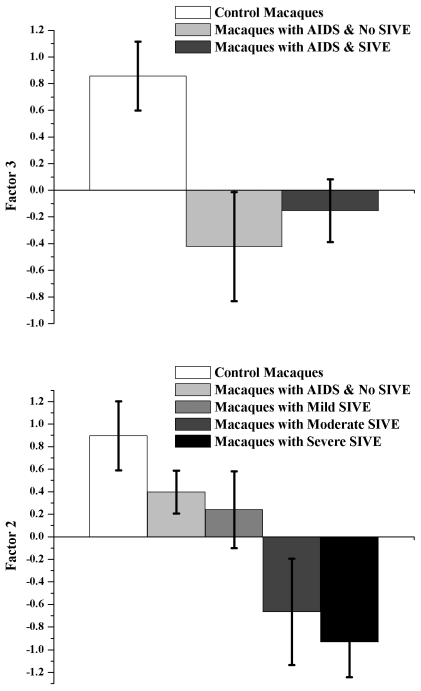

Figure 1

Conclusions Loadings from Factor 2 represent a linear combination that summarizes major metabolic changes in both neuronal (NAA, Glu) and glial (Cr, MI) cells during SIV infection. Lower levels of NAA and Glu and upregulation of Cr and MI were associated with more severe encephalitis, as reported in many MRS HIV studies. MI/Cr increases have not been reported in our chronically infected rhesus macaque model, most likely due to the masking of MI elevations by the accompanying higher levels of Cr. The possibility of Cr changes has often been raised as a note of caution against the use of metabolite ratios in favor of absolute concentrations in MRS studies. However, the use of ratios over Cr need not be discarded altogether. Previous work has reported that NAA/Cr alone is sensitive to distinguish between severities of encephalitis [2]. Individually NAA, Glu, Cr and MI are changing, but only marginally so, with the exception of NAA. The analysis of changes in brain metabolism of animals infected with SIV and animals with encephalitis is much more powerful when all four metabolites are examined together. Our factor analysis suggests that the divergence of lower NAA and higher Cr accompanying animals with worse encephalitis actually allows NAA/Cr to be a more sensitive marker of encephalitis. Therefore, while the quantification of absolute concentrations is valid, the analysis of ratios like NAA/Cr may still be beneficial in the examination of human subjects.

Factor 3 summarizes changes in metabolites that differ between infected animals and controls. It is interesting to note that the main contributions to Factor 3 include many metabolites that have resonances under the NAA peak at lower field strengths, while NAA itself is not included among them. NAA and NAA/Cr are typically found to be decreased in infected individuals without neurologic symptoms. While this does not disprove the possibility of NAA changes in these subjects, the data suggest the potential that other changes (such as those in glutamate, the most concentrated brain metabolite) may interfere with the accurate quantification of the NAA resonance, especially in subjects that are currently at an AIDS status, but neurologically asymptomatic.

References

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