Proton Magnetic Resonance Spectroscopic Imaging with Neuropathological and Neurophysiological Analyses Defines the Extent of Neuronal Impairments in Murine HIVE-1 Encephalitis

J. A. Nelson[1,2], H. Dou[2,3], M. D. Boska[1,2], B. Ellison[2,3], M. Uberti[1,2], H. Xiong[2,3], E. Anderson[2,3], M. Mellon[1], H. A. Gelbard[4,5], H. E. Gendelman[2,3]

[1]Radiology, University of Nebraska Medical Center, Omaha, NE, United States, [2]Center for Neurovirology and Neurodegenerative Disorders, University of Nebraska Medical Center, Omaha, NE, United States, [3]Pharmacology, University of Nebraska Medical Center, Omaha, NE, United States, [4]Center for Aging and Developmental Biology and the Departments of Neurology, Pediatrics, University of Rochester Medical Center, Rochester, NY, United States, [5]Microbiology & Immunology, University of Rochester Medical Center, Rochester, NY, United States

INTRODUCTION: Relatively few immune activated and virus-infected mononuclear phagocytes may affect widespread neuronal dysfunction during human immunodeficiency virus type one (HIV-1)-associated dementia (HAD). However, histopathological evidence of neuronal dropout often belies the extent of cognitive impairment. To define relationships between neuronal function and histopathology, proton magnetic resonance spectroscopic imaging (^{1}H MRSI) and hippocampal long term potentiation (LTP) were compared directly to neuronal and glial immunohistology in a murine model of HIV-1 encephalitis (HIVE).

METHODS: Three groups, 7 mice per group, of severe combined immunodeficient (SCID) mice were compared: 1) an HIVE infected group where HIV-1_{ADA} infected human monocyte derived macrophages (MDM, $3x10^5$ in 10µl) were injected into the subcortex, 2) a sham-injected group and 3) an unmanipulated (normal) group. Data were collected 7 days post-injection. Four 1.0 microliter voxels were analyzed from each animal, two along the injection line and two from the contralateral hemisphere. Spectra were fit using AMARES in jMRUI 1.3 package (1,2) and obtained on a Bruker Biospec 7T/21 magnet operating at 300.41MHz. ^{1}H MRSI parameters: FOV=20mm, TE=33ms, TR=4 s, Averages=3, matrix size=24x24 encoding. LTP was generated by high frequency stimulation (HFS, 100Hz, 500ms x2) of Schaeffer-collateral-commissural axons in transverse hippocampal slices.

RESULTS: HIV-1 was confirmed in HIVE group with vimentin and HIV-1p24. Average HIV-1 infection in MDM=88.22%. Glial fibrillary acidic protein (GFAP) staining showed hypertrophic and reactive astrocytes with significantly (p<0.01) increased staining in the injected hemispheres of HIVE compared to sham-injected mice (Fig.1a,1e). Antibodies to Iba1 demonstrated activated microglia in the area of neurodegeneration (Fig. 1b,1f). In HIVE animals, neuronal loss, as measured with neuron-specific Nuclear protein (NeuN) and microtubule-associated protein-1 (MAP-2) extended beyond the areas of MDM, and significant neurite loss (p<0.01) was present throughout the injection site when compared to sham-injected and unmanipulated controls (Figure 1c, 1g). Synaptophysin was only significantly decreased (p<0.01) in the injection hemisphere of HIVE mice (Fig. 1e, 1h). LTP magnitude recorded in the CA1 region of hippocampal slices was significantly reduced (p<0.05) in HIVE mice compared to unmanipulated mice (Fig 2). However, no significant differences were found between the LTP magnitudes recorded from the injected and non-injected brain hemispheres of HIVE mice. Significantly lower (p<0.05) N-acetyl aspartate concentrations ([NAA]) were found in HIVE mice compared to sham-injected and unmanipulated controls, regardless of hemisphere (Fig 3). These results demonstrate that an HIV-1 infection in one brain hemisphere affects neuronal function in the contralateral hemisphere, suggesting diffusible neurotoxins.

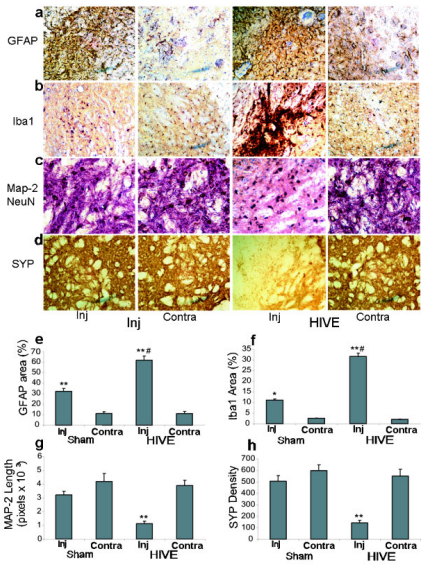

Figure 1 (left)**:** Serial 25µm frozen coronal brain sections from MDM injection site of sham-injected (left column) and HIVE (right column) animals. Immunostaining (200x magnification) for GFAP ***(a)*** (astrocyte activation), Iba1 ***(b)*** (activated microglia and macrophages), MAP-2 and NeuN ***(c)*** (neuritis and neuronal nuclei) and Synaptophysin ***(d)*** (SYP, a pre-synaptic protein) are shown for the injected site (Inj) and the corresponding contralateral (Contra) area for a sham-injected and HIVE mouse. Graphical representation of % GFAP ***(e)*** staining, % Iba1 ***(f)*** positive staining, MAP-2 ***(g)***, and SYP ***(h)*** positive staining showing significant differences in the area of the injection compared to the contralateral hemisphere. (* p< 0.05, ** p< 0.01, and **# p<0.0001).

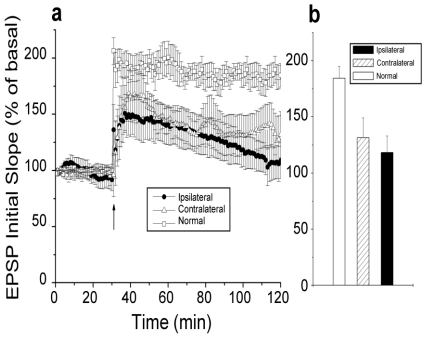

Figure 2 (left)**:** ***a:*** Time courses and magnitudes of LTP recorded from injection/ipsilateral and contralateral hippocampal brain slices taken from HIVE mice and from unmanipulated controls. ***b:*** LTP magnitudes measured 70 min after high frequency stimulation.

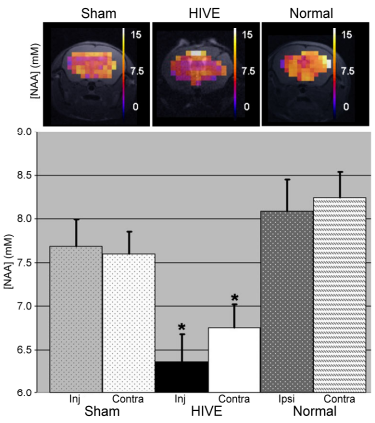

Figure 3 (right)**:** ***Top:*** Overlay of [NAA] normalized to the scale (between 0 and 15 mM). ***Bottom:*** Graph depicting [NAA] for each hemisphere.

References:

1. Vanhamme L, et al. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson 1997;129(1):35-43.
2. Naressi A, Couturier et al. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. Comput Biol Med 2001;31(4): 269-86.

NIH Grants: 1 T32 NS07488-01, 5 R37 NS36126-07, 5 R01 NS034239-10, 1 P01 NS43985-01A1, 5 P01NS31492-11, 5 R01 MH64570-03, P01 NS11766-28 and 20 RR15635 supported this work.