Published in final edited form as:

J Neurovirol. 2011 August ; 17(4): 341–352. doi:10.1007/s13365-011-0040-7.

Early detection of neuropathophysiology using diffusion-weighted magnetic resonance imaging in asymptomatic cats with feline immunodeficiency viral infection

Daniel S Bucy¹, Mark S Brown², Helle Bielefeldt-Ohmann³, Jesse Thompson⁴, Annette M Bachand¹, Michelle Morges¹, John H Elder⁵, Sue VandeWoude⁴, and Susan L Kraft¹

¹Department of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

²Department of Radiology, University of Colorado Health Sciences Center, Aurora, CO, USA

³School of Veterinary Science, University of Queensland, Queensland, Australia

⁴Department of Microbiology Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

⁵Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA, USA

Abstract

HIV infection results in a highly prevalent syndrome of cognitive and motor disorders designated as HIV-associated dementia (HAD). Neurologic dysfunction resembling HAD has been documented in cats infected with strain PPR of the feline immunodeficiency virus (FIV), whereas another highly pathogenic strain (C36) has not been known to cause neurologic signs. Animals experimentally infected with equivalent doses of FIV-C36 or FIV-PPR, and uninfected controls were evaluated by magnetic resonance diffusion-weighted imaging (DW-MRI) and spectroscopy (MRS) at 17.5 – 18 weeks post-infection, as part of a study of viral clade pathogenesis in FIV infected cats. Goals of the MR imaging portion of the project were to determine whether this methodology was capable of detecting early neuropathophysiology in the absence of outward manifestation of neurological signs, and to compare MR imaging results for the two viral strains expected to have differing degrees of neurologic effects. We hypothesized that there would be increased diffusion, evidenced by the apparent diffusion coefficient as measured by DW-MRI, and altered metabolite ratios measured by MRS, in the brains of FIV-PPR infected cats relative to C36-infected cats and uninfected controls. Increased apparent diffusion coefficients were seen in the white matter, gray matter, and basal ganglia of both the PPR and C36 infected (asymptomatic) cats. Thalamic MRS metabolite ratios did not differ between groups. The equivalently increased diffusion by DW-MRI suggests similar indirect neurotoxicity mechanisms for the two viral genotypes. DW-MRI is a sensitive tool to detect neuropathophysiological changes *in vivo* that could be useful during longitudinal studies of FIV.

Corresponding Author: Susan L. Kraft, DVM, PhD, James L. Voss Veterinary Teaching Hospital, 300 West Drake Road, Fort Collins, CO 80523, Phone: (970) 297-0350, Fax: (970) 297-1254, Susan.Kraft@colostate.edu.

Preliminary portions of this data were presented at the following:

17th International Society of Magnetic Resonance in Medicine, Honolulu HI, April 2009;

9th International Feline Retrovirus Research meeting, Vienna Austria, Aug 2008

Keywords

Lentiviral neuropathology; HIV-associated dementia (HAD); Magnetic Resonance Spectroscopy (MRS); Diffusion-Weighted Imaging (DW-MRI); Apparent Diffusion Coefficient (ADC); FIV-PPR; FIV-C36

Introduction

HIV-associated dementia (HAD) is a poorly understood syndrome of cognitive and motor disorders that occurs in up to twenty percent of human immunodeficiency virus type I (HIV) infections (McArthur *et al*, 1993). Direct viral effects including virus-induced neurotoxicity, and indirect effects such as cytokine signaling and inflammatory-mediated neuroexcitatory dysfunction, have been implicated (Bell, 1998; Dow *et al*, 1992; Gray *et al*, 1996). HAD is only partially controlled by current antiretroviral therapies, thus mild forms of the neurologic disease predominate in developed countries (Gray *et al*, 2003; McArthur *et al*, 2004). There is also concern that as AIDS patients are living longer with more effective therapies, neurologic disorders will become more common. Sensitive indicators of HAD pathogenesis are needed for early diagnosis, staging and development of new therapies that better control HAD progression (Pomper and Sacktor, 2007).

Animal models are essential to developing a better understanding of lentiviral pathogenesis and for testing effective therapeutics for HAD. Feline immunodeficiency virus (FIV) is a particularly good model for the study of HAD pathogenesis. HIV and FIV are both lentiviruses in the same subfamily that share remarkably similar genetic makeup, structure, and pathogenesis. In addition, FIV is a well-characterized, naturally occurring, reproducible disease considered to have consistent, strain-dependent neuropathogenesis (Meeker, 2007; Phillips *et al*, 2000; Podell *et al*, 1997; Podell *et al*, 1993; Power *et al*, 1998a). Previous studies of FIV have shown it to infect the central nervous system early in infection and cause a neurologic syndrome characterized by specific behavioral abnormalities (Meeker, 2007; Phillips *et al*, 1996; Podell *et al*, 1993; Steigerwald *et al*, 1999). Furthermore, the feline model is logistically and economically easier to manage and manipulate in a controlled environment than human clinical studies or non-human primate animal models.

Evidence suggests that viral genotype may play a role in the severity of neuropathogenesis of HIV infection (Bratanich *et al*, 1998; Power *et al*, 1998b). As with HIV, FIV subtypes have been characterized that vary in their pathogenesis and neurologic effects (de Rozieres *et al*, 2004a; de Rozieres *et al*, 2008; Hein *et al*, 2003; Power *et al*, 1998a). FIV-C36 is a highly pathogenic viral clone of the clade C that causes high disease incidence and severity with acute immunodeficiency without documented neurologic effects (de Rozieres *et al*, 2004a). In contrast, FIV-PPR of the clade A is a strain that has demonstrated neurotropism and virulence but has less associated systemic pathogenicity than the FIV-C36 isolate (Phipps *et al*, 2000).

The use of advanced imaging methodology is potentially a sensitive method for the noninvasive longitudinal study of evolving disease processes such as FIV lentiviral neuropathogenesis with the potential for detecting physiological, functional and metabolic abnormalities prior to clinical manifestation and/or histopathology. There is a need for continued development of preclinical models for testing novel therapeutics for HAD (Crews *et al*, 2008), and the use of advanced imaging methodology for these animal models could be a powerful adjunct to evaluate preventative or early interventional therapy. Although the use of advanced imaging technology can be relatively expensive and usually requires general anesthesia for animal studies, its noninvasive nature allows the use of fewer animals while

reaching meaningful results, thereby sparing cost and optimizing the humane use of animals in preclinical trials.

Magnetic resonance imaging (MRI) has been used to demonstrate the gross anatomic changes associated with late HAD progression, including atrophy of the white matter and basal ganglia (Aylward *et al*, 1995; Aylward *et al*, 1993; Ge *et al*, 2003; Handelsman *et al*, 1993). MR also has several advanced capabilities that allow the detection of physiologic and metabolic processes, including diffusion-weighted imaging (DW-MRI), diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS). Although DW-MRI and DTI have been used to evaluate HIV patients in a number of human studies, to our knowledge there are few reports on brain diffusion abnormalities in preclinical animal models of HAD (He *et al*, 2003). Furthermore, the understanding of metabolic abnormalities as determined by MRS in preclinical models remains limited to only a few time points (Cloak *et al*, 2004b; Fuller *et al*, 2004; Gonzalez *et al*, 2006; Greco *et al*, 2002; Greco *et al*, 2004; Lentz *et al*, 2008a; Lentz *et al*, 2008b; Podell *et al*, 1999; Power *et al*, 1998a; Ratai *et al*, 2009). We performed MRI to investigate its usefulness for this application, at a single 4 month post-inoculation time point on a subset of FIV infected cats from an ongoing study of viral clade pathogenesis (de Rozieres *et al*, 2008). The goals of the MRI portion of the project were to determine whether clinical MRI methodology was capable of detecting early neuropathophysiology in these cats prior to outward manifestation of neurological signs, and to compare MR imaging results for two viral strains expected to have differing degrees of neurologic effects. The MRI results were evaluated in light of evidence for systemic pathogenicity including plasma viremia and proviral loads and degree of immunodeficiency. We anticipated that physiological methods of MRI such as DW-MRI or MRS would be more sensitive than anatomic MR imaging to neurological abnormalities in these cats. We hypothesized that there would be increased diffusion measured by DW-MRI measured as the apparent diffusion coefficient (ADC), and altered metabolite ratios measured by MRS in the brains of FIV-PPR infected cats, relative to C36 infected cats and uninfected controls. These data would provide preliminary evidence for the role of viral genotype on neurological effects of FIV.

Results

All cats remained clinically and neurologically normal throughout the study and had no visible gross lesions on the anatomic MR and the DW-MRI images. No clinical signs were observed in these cats other than mild lymphadenopathy and gingivitis. No treatments were instituted relative to FIV-induced disease. The ADC's derived from the DW-MRI of the FIV-PPR and FIV-C36 infected cat brains were significantly greater than the mean corresponding values from the control cats for the white matter, gray matter and basal ganglia. The increase in ADC values, therefore diffusion, was small at approximately 3–6% (Figure 1). The ADC values did not differ between infected cat brains and the control cats for the other brain regions including midbrain, brainstem and cerebellum. The ADC's of the infected cats were equivalently increased and did not statistically differ between the two viral clades, FIV-PPR and FIV-C36, for any brain region. For all cat groups, the ADC values differed consistently by brain region, being generally highest in the grey matter. The magnitude of ADC values decreased slightly with increasing *b* factor for all tissues.

Data reproducibility was assessed by scanning control cats twice, and paired t-tests showed no significant differences between the ADC values of the two scans for any brain region (data not shown). For *b* factors of 1000 and 1500, the CV for ADC fell between 1% and 6% for 5 of the 8 brain regions and was 8% for the basal ganglia and 9% for the brainstem. For *b* factor of 2000, the coefficient of variation was less than 5% for 7 of the 8 brain regions. A considerably greater coefficient of variation was found for the cerebellum at all *b* factors,

and ranged from 15 – 22%. For most analyses, the ADC values from the two scans were averaged for each brain region including the cerebellum for consistency. However, for the cerebellum, analyses were repeated using scan 1 ADC values only due to its high variability.

The NAA/Cr and Cho/Cr ratios derived from MR spectroscopy of the thalamus did not differ from control groups (Table 1).

Plasma viremia and proviral load for FIV-C36 cats were approximately two orders of magnitude greater than FIV-PPR infected cats throughout the course of the infection. Plasma viremia for the FIV-PPR infected cats peaked at 7.57×10^4 RNA copies / mL peripheral blood at 10 days post inoculation and FIV-C36 infected cats peaked at 6.17×10^7 RNA copies / mL peripheral blood at 14 days post inoculation (Figure 2a) (de Rozieres *et al*, 2008). Proviral load peaked at day 35 for both FIV-PPR and FIV-C36 infected cats with values of 3.47×10^3 and 1.35×10^5 per 10^6 peripheral blood mononuclear cells respectively (Figure 2b) (de Rozieres *et al*, 2008). The CD4:CD8 lymphocyte ratios increased initially, 7–14 days post inoculation, and then declined throughout the remainder of the study for all groups, including controls (de Rozieres *et al*, 2008). The CD4:CD8 ratios for FIV-PPR infected cats and controls did not differ, with values of approximately 1.5 at the time of MR imaging. The FIV-C36 infected cats had significantly decreased CD4:CD8 ratios in the later time points (>100 days PI) compared to controls, with values near 1.0 at the time of the MR imaging (111–127 days PI) (Figure 2c).

At the end of study (6 months PI) proviral loads in the brainstem, globus pallidus and putamen, caudate nucleus, and cortex varied by brain region and were greater in the FIV-C36 infected cats than FIV-PPR infected cats in all sections. FIV-PPR proviral loads were below detection limits (10 copies/million cell equivalents) in more than half of the tissues assayed (Figure 3). The significantly lower level of provirus in FIV-PPR infected cats is similar to findings in peripheral cells (Figure 2b).

Histopathology revealed only mild changes in the brains of infected cats. Most FIV-PPR and FIV-C36 infected cats had equivalent minimal to mild dispersed or more vasculocentric infiltration of lymphocytes, plasma cells, and monocyte/macrophages in the meninges and in the arachnoid of small penetrating vessels. In addition, in two of the FIV-PPR infected cats there was mild microgliosis and multifocal neuron degeneration with satellitosis in the basal ganglia and caudate nucleus. Many of the infiltrating leukocytes, most notably in the meninges, displayed signs of activation, including up-regulated expression of phospho-p38 α -MAPK (Figure 4).

Discussion

The first of our two objectives for this initial investigation in FIV infected cats was to determine whether anatomical or physiological indicators of neuropathology could be detected by MRI. No anatomical gross lesions were observed with routine MR imaging, which was not unexpected at this early PI time period. However, increased brain diffusion was detected in multiple brain regions using DW-MRI, at 17.5 – 18 wk PI in these asymptomatic cats. The increased diffusion in these infected cat brains was of similar magnitude and in similar brain regions as in a previous report on HIV patients with mild dementia (Cloak *et al*, 2004a; Usiskin *et al*, 2007; Yoon *et al*, 2007). This illustrates the highly sensitive ability of DW-MRI to detect small fluid shifts from neuropathophysiology not yet sufficient to cause visible signal nor anatomic changes on conventional MR images. These findings are in agreement with the previously proposed mechanism of neuronal injury during HIV and FIV infection due to indirect effects from cytokines, chemokines and

glutamate released by activated leukocytes and microglial cells (Bell, 1998; Eggert *et al*, 2009; Gray *et al*, 1996; Meeker, 2007; Poli *et al*, 1999).

Magnetic resonance diffusion-weighted imaging (DW-MRI) and diffusion tensor imaging (DTI) detect physiological changes in the random motion of water molecules, or diffusion. Altered diffusion can be semi-quantified using DW-MRI as the apparent diffusion coefficient, or ADC, which in inflammatory processes is thought to be secondary to the breakdown of barriers to the flow of water during microscopic brain injury (Gass *et al*, 2001; Le Bihan *et al*, 1986). Tissue changes can be detected by ADC quantification during region of interest analysis even when signal changes on diffusion images are imperceptible, and can provide better characterization of infectious brain processes (Mascalchi *et al*, 2005). Increased frontal white matter diffusion has been demonstrated in the brains of HIV infected patients using DW-MRI, reflecting inflammation and cellular injury (Cloak *et al*, 2004a; Usiskin *et al*, 2007; Yoon *et al*, 2007). DW-MRI is performed by applying a series of MR diffusion gradients during imaging, allowing the random movement of free water to be semi-quantified. The degree of diffusion weighting, expressed as the *b* factor, is based on the strength and timing of the diffusion gradients; the greater the degree of diffusion weighting, the greater the *b* factor (Cercignani and Horsfield, 2001). Certain tissues such as cerebral white matter have fiber bundles with non-random orientation and diffusion of fluid in this tissue is anisotropic in nature. DTI would be preferable for detecting diffusion differences because it accounts for directional water diffusion which is particularly important for white matter fiber tracts, but it was not available on our MRI equipment for this study (Filippi *et al*, 2001; Pomara *et al*, 2001; Ragin *et al*, 2005; Stebbins *et al*, 2007). DTI has sensitively detected altered diffusion and fractional anisotropy in a variety of scenarios with HIV patients (Filippi *et al*, 2001; Pomara *et al*, 2001; Stebbins *et al*, 2007) (Chang *et al*, 2008; Chen *et al*, 2009; Ragin *et al*, 2004; Ragin *et al*, 2005; Wu *et al*, 2006). (Gongvatana *et al*, 2009; Muller-Oehring *et al*, 2010; Pfefferbaum *et al*, 2009).

The optimal DW-MRI *b* factor varies by disease process, species and tissue type and was unknown for this application so we performed DW-MRI using three different *b* factors. Although differences were seen with all *b* values, overall the *b* factor of 2000 most consistently showed significant ADC differences and was most reliable when two scans were performed on control cats. The ADC values decreased with increasing *b* factor which was unexpected because theoretically, diffusion sensitivity should go up with higher *b* factor. This has been found with other studies evaluating a range of *b* values and is likely related to the complex biexponential nature of the diffusion decay process which transitions over a range of *b* values because of shifts in dominance of rapid extracellular and slower intracellular diffusion components (DeLano *et al*, 2000). Development of an advanced mathematical model to estimate diffusion decay in FIV infected cat brains was not in the scope of this initial study but could be considered in the future by designing more intensive DW-MRI experiments.

Although DW-MRI was capable of detecting early neuropathogenesis in FIV cats, the relatively small size of the feline brain led to sampling challenges due to the proximity to the frontal bone and large frontal sinuses, the osseous tentorium, and large tympanic bullae. The resulting susceptibility artifact and distortion near those structures limited the ROI sampling locations on the ADC maps for the feline frontal and cerebellar regions and led to increased sampling variability particularly for the cerebellum (Figure 1). Also, for this study we used a 1.5 T MRI instrument and DW-MRI and MRS pulse sequences that are typical of clinical examinations. This provided a realistic picture of achievable results in a translational clinical setting. However, the inherent signal-to-noise obtained from this scenario is lower than might be obtained using a high field strength MRI unit such as that which might be found in an experimental laboratory animal setting. The higher signal-to-noise associated with a

higher field MR instrument would have improved the diffusion and spectroscopic measurements. Finally, another limitation of our study is that the number of animals studied was small, which affected the power of our statistical analyses. However, our small sample size was similar to that of comparable studies and despite the low number of subjects, statistical differences were detected.

Magnetic resonance spectroscopy (MRS) is another MR technique potentially capable of detecting brain abnormalities prior to neurological signs in HIV infection. Metabolic alterations have been found in HIV-1 patients including increased myoinositol-creatine ratios (MI/Cr), increased creatine (Cr) and decreased N-acetyl-aspartate (NAA) indicating glial injury and inflammation, and neuronal injury or loss respectively in HIV-1 patients exhibiting dementia. These alterations have been mild or minimal in asymptomatic patients and severe in patients with dementia or other neurological impairment (Barker *et al*, 1995; Chang *et al*, 2002; Chang *et al*, 2003; Chang *et al*, 2004; Chong *et al*, 1993; Cloak *et al*, 2004a; Laubenberger *et al*, 1996; Marcus *et al*, 1998; Sacktor *et al*, 2005; Tarasow *et al*, 2003; Wenserski *et al*, 2003; Wilkinson *et al*, 1997). MRS abnormalities in preclinical animal models have been used to infer underlying mechanisms of HAD, to predict its eventual development and to evaluate various drug treatments. Decreased NAA/Cr ratios have been found in FIV-infected cats within the frontal white matter *in vivo* and also *ex vivo* by high resolution nuclear magnetic resonance 12 months PI, but not at 6 months PI (Podell *et al*, 1999). In macaques with SIV infection, transient decreases in NAA/Cr and increased MI/Cr and Cho/Cr have been shown to occur acutely at the time of peak viremia at two weeks PI (Greco *et al*, 2004; Ratai *et al*, 2009; Williams *et al*, 2005). Elevated basal ganglia Cho/Cr found at four weeks PI have preceded eventual development of SIV encephalitis (Fuller *et al*, 2004). Other studies using the macaque SIV model have shown that the acute transient decrease in NAA/Cr is correlated with decreased synaptic integrity rather than neuronal loss (Lentz *et al*, 2005; Williams *et al*, 2005). MRS of macaques with an accelerated form of SIV infection has recently been shown to be a good indicator of effective neuroprotection by therapy with minocycline or CNS improvement from combination antiretroviral therapy (CART) (Ratai *et al*, 2010; Williams *et al*, 2005).

In our study, MRS did not detect significant changes in metabolite ratios at four months post-inoculation (PI) time. This may have been related to the four month PI timing since metabolic changes can be normalized during this time period after the acute viremic phase. Also, sampling location may have played a role because we sampled the thalamus (rather than frontal lobes) to avoid the frontal sinuses which had associated magnetic inhomogeneity. Although NAA/Cho ratios were decreased in the thalamus of neurologically affected HIV positive individuals (Meyerhoff *et al*, 1996), more consistent spectral abnormalities have since been reported for frontal white matter, deep white matter and basal ganglia locations. The relatively long TE in our MRS protocol was used to optimize detection of NAA and Cr but is inaccurate for MI and would have prevented our detecting altered MI ratios. An additional limitation is that these data were measured as metabolite ratios based on creatine as a reference standard, rather than as absolute concentrations. The use of ratios could prevent detection of absolute changes in creatine concentration, assuming that NAA or choline concentrations had also changed.

Our second goal was to compare MR imaging results for two viral strains that reportedly have differing degrees of neurologic effects. Our initial hypothesis of viral strain-dependent neuropathophysiology by DW-MRI was not supported since nearly equivalent increases in diffusion were seen in both FIV-PPR and FIV-C36 infected cats. This may reflect a relatively low threshold effect from indirect cytokine effects, regardless of degree of viremia or proviral loads, systemic pathogenicity or viral strain. The MRI results obtained at 17.5 – 18 wk PI may reflect the initial phase of CNS inflammation.

Although longitudinal assessment of ADC and a direct comparison with histopathology would have been useful, this was not possible because the MRI examinations were added to an existing viral pathogenesis FIV project with a predetermined timeline. Only a single time point for MRI was possible and there was an unavoidable significant 33–37 d time interval between the MRI scans and necropsy. A future longitudinal study would be needed with serial MRIs and direct histopathological correlation to determine the exact nature and correspondence of the temporal changes through the acute and chronic PI time periods.

It is interesting to note, however, that at the time point of euthanasia, only minimal histological differences were seen between the two viral strains in the degree of brain inflammatory cell infiltration. The cats infected with FIV-C36 had low but detectable brain provirus which is likely a reflection of the higher plasma proviral and viremic levels for the cats infected with that viral strain. In comparison, the very low proviral and viral loads in FIV-PPR infected animals are likely due to both viral inoculum and host immune characteristics. Although FIV-C36 infection has been shown to cause acute immunodeficiency without documented neurologic effects, in this study these cats had similar diffusion changes during the inflammatory phase and similar brain histopathology as the FIV-PPR cats, suggesting comparable degree of neurotoxicity effects at those time periods. Therefore, it remains possible that cats with FIV-C36 have lacked clinical neurological problems because they succumb to their disease before neurological signs typically begin, which is at the 14–15 week time period for FIV-PPR infected cats (de Rozieres *et al*, 2004a; de Rozieres *et al*, 2004b; de Rozieres *et al*, 2008; Hokanson *et al*, 2000; Phillips *et al*, 1996). The low level of viral infection detected in brain tissues in this study would also suggest that neuropathogenicity is not directly related to viral load or active viral replication. This implies that early anti-retroviral therapy to decrease viral loads in the CNS are only likely to have a demonstrable effect on HAD development if CNS viral load is proportionate to viral load in the periphery.

In conclusion, DW-MRI detected increased brain diffusion at 17.5–18 wk PI in neurologically asymptomatic cats with FIV infection from two different viral clades with historically-reported differences in neurological manifestation. Both viral strains led to similarly increased brain diffusion despite differences in peripheral viremia and proviral load, implicating indirect threshold-associated effects from secondary cytokines. Advanced MR imaging techniques such as DW-MRI, DTI and MRS, have a promising role in furthering our understanding of the mechanisms of pathogenesis of HAD and for preclinical testing of new therapies.

Methods

Animals and inoculation

Fifteen 14–16 week old specific pathogen free (SPF) cats obtained from Cedar River Laboratories, Ames IA, and an SPF cat colony at Colorado State University, Fort Collins, CO were kept in barrier rooms in facilities accredited by AAALAC International. All procedures were approved by the CSU Institutional Animal Care and Use Committee following standards established in the *Guide for the Care and Use of Laboratory Animals* (National Academy of Science, National Academy Press, Washington D.C.). The cats were randomized by age and sex and each cat was inoculated both orally (0.5ml) and intravenously (0.5ml) with $10^{3.5}$ TCID₅₀ particles/ml of either FIV-PPR (n = 5) or FIV-C36 (n = 5) as described previously (de Rozieres *et al*, 2008). Uninfected controls (n = 5) were administered only media. Animals were examined daily for evidence of clinical disease and physical exams were performed during blood collections. Blood samples were collected at day –7, 7, 10, 14, 21, 30, 35, 42, 62, 77, 102, 132, and 156 post inoculation (p.i.) on un-anesthetized animals. Samples were collected and measured for CD4+ and CD8+

lymphocytes, proviral DNA and plasma RNA as described previously (de Rozieres *et al*, 2008).

MRI, DW-MRI and MRS

MRI was done with a General Electric Signa LX 1.5 Tesla MR HiSpeed Plus System (Milwaukee, USA). Imaging was performed with cats under general anesthesia maintained with isoflurane gas via a non-rebreathing circuit and positive pressure ventilation. Each of the FIV-infected cats was scanned once between 123–127 days (17.5 – 18 wk) post inoculation. All five control cats also underwent MRI scans. Four of the 5 control cats were scanned twice in order to assess baseline variability; the double control animal scans took place within a 3–5 day time period, between 111–127 days post sham inoculation. Cats were positioned in sternal recumbency with the head placed on foam sponges to center it within the quadrature head coil, using the 3 plane localizer series to verify location of the pituitary fossa in the center of the coil and laser light localization to standardize consistent longitudinal orientation along the z axis. Animals were in a deep plane of general anesthesia with their heads well-secured in the imaging coil, therefore there was no head movement during image acquisition. A complete anatomic brain MRI scan was performed using multiple pulse sequences. These included transverse dual fast spin echo proton density/T2 weighted scan (TR = 4000 ms, TE = 17/115 ms, echo train length = 16, slice thickness = 3mm, image matrix = 256 × 256), FLAIR (TR = 8125 ms, TE = 120, TI 2200 ms) and transverse T1 weighted scans (TR = 550 ms, TE = minimal) before and post-contrast T1 transverse, dorsal and sagittal images after intravenous gadolinium DTPA, Magnevist, at 0.5 mmol/kg (Bayer Pharmaceuticals, Wayne, NJ). Diffusion weighted imaging was performed at *b* factors of *b* = 1000, *b* = 1500, and *b* = 2000 using TE = 90–110 ms, TR = 8000 ms, FOV = 24 mm, slice thickness = 5mm, image matrix = 124 × 124, using NEX of 1, 2, or 4 (respectively for the three different *b* factors) in order to determine the most appropriate *b* factor value for this application. Multivoxel proton magnetic resonance spectroscopy (MRS) centered at the level of the thalamus was acquired using the point-resolved spectroscopy (PRESS) sequence, with a TR = 1500 ms, TE = 135 ms, field of view 16 cm and slice thickness = 10 mm, 256 avgs (Figure 5).

Euthanasia, Histology, Brain Proviral Load

Full necropsies were performed at 160–161 d PI. This represented a time interval between MRI and necropsy of 43 – 49 d for control cats, and 33 –37 d for FIV infected cats. Euthanasia was performed with an overdose of pentobarbital while cats were under ketamine and acepromazine sedation, then tissues were examined, collected and either fixed in 10 % neutral-buffered formaldehyde or snap-frozen for later RNA- and DNA-isolation. Samples from brain (left cerebral hemisphere, left sides of the cerebellum and brainstem), lymphoid tissues (thymus, spleen, tonsil, submandibular lymph node, several mesenteric lymph nodes, prescapular and popliteal lymph nodes) and gastrointestinal tract (jejunum, ileum with Peyer's patch and colon) were fixed for 72 hours, followed by storage in 70% ethanol until routine processing for paraffin-embedding. Five µm tissue sections were stained with hematoxylin and eosin (H&E) and examined on an Olympus BX41 microscope equipped with a Q-Color3 camera (Olympus) and corresponding computer software. Brain proviral load determinations were made by polymerase chain reaction (PCR) from the brainstem, globus pallidus and putamen, caudate nucleus, and cerebral cortex.

Viral RNA was extracted from plasma collected from EDTA-treated whole blood following centrifugation. Plasma was frozen at –70°C until processing. RNA was purified from 140 µl of plasma using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). SuperscriptII (Invitrogen, Carlsbad, CA) was implemented in reactions with random hexamers (Invitrogen) added, and treated with RNase Out (Invitrogen) for preparation of cDNA from

viral RNA. Genomic DNA was extracted from peripheral blood mononuclear cells purified on a Histopaque-1077 (Sigma, St. Louis, MO) gradient. Cells were washed and pellets frozen at -20°C overnight. DNA was isolated using a DNeasy Tissue Kit (Qiagen, Valencia, CA). Real-time PCR was performed on an iCycler thermocycler (Bio-Rad, Hercules, CA) to detect both proviral DNA and plasma viremia using the AmpliTaq Gold DNA polymerase-containing TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA). Derivation of FIV-A and FIV-C primer/probe sets and sequences have been previously described (Pedersen *et al*, 2001). PCR reactions in a total volume of 25 μl consisted of 12.5 μl master mix, 0.5 μl each of 20 μM forward and reverse primers, 0.2 μl of 10 μM probe, and 5 μl template. After 2 min at 50°C , the AmpliTaq Gold DNA polymerase was activated at 95°C for 10 min, followed by 45 cycles of 95°C for 15s and 60°C for 1 min. Threshold cycle values (CT) were defined as the point at which the fluorescence passed a threshold limit. Calculation of copy number for FIV provirus was done using a standard curve generated from dilutions of a sub-cloned *gag* PCR product. To calculate copy number of viral RNA in plasma, a standard curve was generated by diluting FIV-PPR virus stock in naïve cat plasma, preparing cDNA as described above, and comparing CT values to those of the sub-cloned *gag* standard.

To characterize inflammatory reactions in the brain, immunohistochemistry was carried out as described previously in detail (Bielefeldt-Ohmann *et al*, 2008; Tolnay *et al*, 2010). Briefly, paraffin-sections collected on charged slides were deparaffinized, subjected to antigen-retrieval and blocking steps and then incubated with primary antibodies specific for hemeoxygenase-1 (HO-1), inducible nitric oxide synthase (NOS2), vascular endothelial growth factor-A (VEGF-A), phospho-p38 α MAPK, and the apoptosis marker activated caspase-3. Additional sections were also labeled for glial fibrillar acidic protein (GFAP) to assess for gliosis or loss of astrocytes, for microglial cells using the Iba-1 marker and for oligodendrocytes using the transcription factor OLIG2 as cell marker. All H&E and immunolabeled sections were examined by a single veterinary pathologist (HBO) blinded to the grouping of the animals.

Blood was processed on the days noted above and CD4:CD8 lymphocyte ratios were calculated using standard flow cytometric analysis as reported. Proviral DNA and plasma RNA calculation and statistical analysis was also performed as described previously (de Rozieres *et al*, 2008).

DW-MRI and MRS Data Analysis

All pulse sequences from the MRI examinations were reviewed by a single veterinary radiologist (SLK) for gross lesions. Diffusion weighted images were first assessed subjectively and ADC maps were then generated using regions of interest (ROI) analysis using commercial software (Functool, General Electric Medical Systems, Milwaukee WI). A total of fifty-eight standardized ROI's were placed by a single research assistant (DSB) and all ROI's were verified by the radiologist (SLK) on the ADC maps on specific areas throughout each cat brain (Figure 6). The higher image quality proton density images were used to first locate ROI's at specific anatomic locations (Figure 6). The x, y coordinates of those ROI's were noted from the proton density images, and then those same image coordinates were used to place identically sized ROI's on the ADC maps in order to sample the same brain anatomy. Areas of interest included multiple white and gray matter points, the region of the basal ganglia (including the areas of the caudate nuclei, globus pallidus, and putamen), thalamus, pyriform lobes, midbrain/brainstem, and cerebellum. Data were collected and grouped and averaged by brain region for each treatment group.

Reproducibility of the DWI-MR scans was assessed by conducting two scans on the control cats, which was possible for only 4 of the 5 cats. We conducted paired t-tests and calculated

the coefficient of variation (CV) for each brain region (between-trial variability relative to the mean) (Hopkins, 2000).

To test for differences in ADC values between brain regions and between groups (control, FIV-PPR and FIV-C36) and to determine if differences between groups depended on the brain region we used repeated measures Analysis of Variance (ANOVA). Pair wise comparisons between groups were conducted using Tukey's test (which corrects for multiple comparisons). Assumptions for all statistical methods were tested and found to hold.

Magnetic resonance (^1H) proton spectra were analyzed offline by a single MR physicist (MSB) using LC Model software (Stephen Provencher Inc., Oakville, Ontario, Canada) to derive ratios of choline (Cho) and N-acetyl aspartate (NAA) to creatine (Cr) as an internal reference, for those voxels of the multivoxel complex that were centered within the thalamus. NAA/Cr and Cho/Cr ratios were then averaged, and analyzed with Tukey's tests for pair wise comparisons between FIV-PPR and FIV-C36 infected cats and controls.

Acknowledgments

This work was supported by NIAID AI048411, the Merck-Merial Summer Research Fellowship Program, and the Flint Animal Cancer Center of Colorado State University. Special thanks to Ms. Lisbeth Sestina and Elisa French for excellent technical assistance in MRI and animal care respectively, and to Craig Miller for assistance with immunohistochemistry.

Sources of support: NIAID AI048411 and the Merck-Merial Summer Research Fellowship Program.

References

- Aylward EH, Brettschneider PD, McArthur JC, Harris GJ, Schlaepfer TE, Henderer JD, Barta PE, Tien AY, Pearlson GD. Magnetic resonance imaging measurement of gray matter volume reductions in HIV dementia. *Am J Psychiatry*. 1995; 152:987–994. [PubMed: 7793469]
- Aylward EH, Henderer JD, McArthur JC, Brettschneider PD, Harris GJ, Barta PE, Pearlson GD. Reduced basal ganglia volume in HIV-1-associated dementia: results from quantitative neuroimaging. *Neurology*. 1993; 43:2099–2104. [PubMed: 8413973]
- Barker PB, Lee RR, McArthur JC. AIDS dementia complex: evaluation with proton MR spectroscopic imaging. *Radiology*. 1995; 195:58–64. [PubMed: 7892496]
- Bell JE. The neuropathology of adult HIV infection. *Rev Neurol (Paris)*. 1998; 154:816–829. [PubMed: 9932303]
- Bielefeldt-Ohmann H, Tolnay AE, Reisenhauer CE, Hansen TR, Smirnova N, Van Campen H. Transplacental infection with non-cytopathic bovine viral diarrhoea virus types 1b and 2: viral spread and molecular neuropathology. *J Comp Pathol*. 2008; 138:72–85. [PubMed: 18295788]
- Bratanich AC, Liu C, McArthur JC, Fudyk T, Glass JD, Mittoo S, Klassen GA, Power C. Brain-derived HIV-1 tat sequences from AIDS patients with dementia show increased molecular heterogeneity. *J Neurovirol*. 1998; 4:387–393. [PubMed: 9718130]
- Cercignani M, Horsfield MA. The physical basis of diffusion-weighted MRI. *J Neurol Sci*. 2001; 186(Suppl 1):S11–S14. [PubMed: 11334985]
- Chang L, Ernst T, Witt MD, Ames N, Gaiefsky M, Miller E. Relationships among brain metabolites, cognitive function, and viral loads in antiretroviral-naïve HIV patients. *Neuroimage*. 2002; 17:1638–1648. [PubMed: 12414302]
- Chang L, Ernst T, Witt MD, Ames N, Walot I, Jovicich J, DeSilva M, Trivedi N, Speck O, Miller EN. Persistent brain abnormalities in antiretroviral-naïve HIV patients 3 months after HAART. *Antivir Ther*. 2003; 8:17–26. [PubMed: 12713060]
- Chang L, Lee PL, Yiannoutsos CT, Ernst T, Marra CM, Richards T, Kolson D, Schifitto G, Jarvik JG, Miller EN, Lenkinski R, Gonzalez G, Navia BA. A multicenter in vivo proton-MRS study of HIV-associated dementia and its relationship to age. *Neuroimage*. 2004; 23:1336–1347. [PubMed: 15589098]

- Chang LD, Wong V, Nakama H, Watters M, Ramones D, Miller EN, Cloak C, Ernst T. Greater Than Age-Related Changes in Brain Diffusion of HIV Patients After 1 Year. *Journal of Neuroimmune Pharmacology*. 2008; 3:265–274. [PubMed: 18709469]
- Chen Y, An H, Zhu H, Stone T, Smith JK, Hall C, Bullitt E, Shen D, Lin W. White matter abnormalities revealed by diffusion tensor imaging in non-demented and demented HIV+ patients. *Neuroimage*. 2009; 47:1154–1162. [PubMed: 19376246]
- Chong WK, Sweeney B, Wilkinson ID, Paley M, Hall-Craggs MA, Kendall BE, Shepard JK, Beecham M, Miller RF, Weller IV, et al. Proton spectroscopy of the brain in HIV infection: correlation with clinical, immunologic, and MR imaging findings. *Radiology*. 1993; 188:119–124. [PubMed: 8099750]
- Cloak CC, Chang L, Ernst T. Increased frontal white matter diffusion is associated with glial metabolites and psychomotor slowing in HIV. *Journal of Neuroimmunology*. 2004a; 157:147–152. [PubMed: 15579292]
- Cloak CC, Chang L, Ernst T, Barr MC, Huitron-Resendiz S, Sanchez-Alavez M, Phillips TR, Henriksen S. Methamphetamine and AIDS: 1H MRS studies in a feline model of human disease. *J Neuroimmunol*. 2004b; 147:16–20. [PubMed: 14741420]
- Crews L, Lentz MR, Gonzalez RG, Fox HS, Masliah E. Neuronal injury in simian immunodeficiency virus and other animal models of neuroAIDS. *J Neurovirol*. 2008; 14:327–339. [PubMed: 18780234]
- de Rozieres S, Mathiason CK, Rolston MR, Chatterji U, Hoover EA, Elder JH. Characterization of a highly pathogenic molecular clone of feline immunodeficiency virus clade C. *J Virol*. 2004a; 78:8971–8982. [PubMed: 15308694]
- de Rozieres S, Swan CH, Sheeter DA, Clingerman KJ, Lin YC, Huitron-Resendiz S, Henriksen S, Torbett BE, Elder JH. Assessment of FIV-C infection of cats as a function of treatment with the protease inhibitor, TL-3. *Retrovirology*. 2004b; 1:38. [PubMed: 15555065]
- de Rozieres S, Thompson J, Sundstrom M, Gruber J, Stump DS, de Parseval AP, VandeWoude S, Elder JH. Replication properties of clade A/C chimeric feline immunodeficiency viruses and evaluation of infection kinetics in the domestic cat. *J Virol*. 2008; 82:7953–7963. [PubMed: 18550665]
- DeLano MC, Cooper TG, Siebert JE, Potchen MJ, Kuppusamy K. High-b-value diffusion-weighted MR imaging of adult brain: image contrast and apparent diffusion coefficient map features. *AJNR Am J Neuroradiol*. 2000; 21:1830–1836. [PubMed: 11110534]
- Dow SW, Dreitz MJ, Hoover EA. Feline immunodeficiency virus neurotropism: evidence that astrocytes and microglia are the primary target cells. *Vet Immunol Immunopathol*. 1992; 35:23–35. [PubMed: 1337399]
- Eggert D, Dash PK, Serradji N, Dong CZ, Clayette P, Heymans F, Dou H, Gorantla S, Gelbard HA, Poluektova L, Gendelman HE. Development of a platelet-activating factor antagonist for HIV-1 associated neurocognitive disorders. *J Neuroimmunol*. 2009; 213:47–59. [PubMed: 19541372]
- Filippi CG, Ulug AM, Ryan E, Ferrando SJ, van Gorp W. Diffusion tensor imaging of patients with HIV and normal-appearing white matter on MR images of the brain. *American Journal of Neuroradiology*. 2001; 22:277–283. [PubMed: 11156769]
- Fuller RA, Westmoreland SV, Ratai E, Greco JB, Kim JP, Lentz MR, He J, Sehgal PK, Masliah E, Halpern E, Lackner AA, Gonzalez RG. A prospective longitudinal in vivo 1H MR spectroscopy study of the SIV/macaque model of neuroAIDS. *BMC Neurosci*. 2004; 5:10. [PubMed: 15070430]
- Gass A, Niendorf T, Hirsch JG. Acute and chronic changes of the apparent diffusion coefficient in neurological disorders--biophysical mechanisms and possible underlying histopathology. *J Neurol Sci*. 2001; 186(Suppl 1):S15–S23. [PubMed: 11334986]
- Ge Y, Kolson DL, Babb JS, Mannon LJ, Grossman RI. Whole brain imaging of HIV-infected patients: quantitative analysis of magnetization transfer ratio histogram and fractional brain volume. *AJNR Am J Neuroradiol*. 2003; 24:82–87. [PubMed: 12533331]
- Gongvatana A, Schweinsburg BC, Taylor MJ, Theilmann RJ, Letendre SL, Alhassoon OM, Jacobus J, Woods SP, Jernigan TL, Ellis RJ, Frank L, Grant I. White matter tract injury and cognitive impairment in human immunodeficiency virus-infected individuals. *Journal of Neurovirology*. 2009; 15:187–195. [PubMed: 19306228]

- Gonzalez RG, Greco JB, He J, Lentz MR, O'Neil S, Pilkenton SJ, Ratai EM, Westmoreland S. New insights into the neuroimmunity of SIV infection by magnetic resonance spectroscopy. *J Neuroimmune Pharmacol.* 2006; 1:152–159. [PubMed: 18040781]
- Gray F, Chretien F, Vallat-Decouvelaere AV, Scaravilli F. The changing pattern of HIV neuropathology in the HAART era. *J Neuropathol Exp Neurol.* 2003; 62:429–440. [PubMed: 12769183]
- Gray F, Scaravilli F, Everall I, Chretien F, An S, Boche D, Adle-Biasette H, Wingertsman L, Durigon M, Hurtrel B, Chiodi F, Bell J, Lantos P. Neuropathology of early HIV-1 infection. *Brain Pathol.* 1996; 6:1–15. [PubMed: 8866743]
- Greco JB, Sakaie KE, Aminipour S, Lee PL, Chang LL, He J, Westmoreland S, Lackner AA, Gonzalez RG. Magnetic resonance spectroscopy: an in vivo tool for monitoring cerebral injury in SIV-infected macaques. *J Med Primatol.* 2002; 31:228–236. [PubMed: 12390545]
- Greco JB, Westmoreland SV, Ratai EM, Lentz MR, Sakaie K, He J, Sehgal PK, Masliah E, Lackner AA, Gonzalez RG. In vivo 1H MRS of brain injury and repair during acute SIV infection in the macaque model of neuroAIDS. *Magn Reson Med.* 2004; 51:1108–1114. [PubMed: 15170829]
- Handelsman L, Song IS, Losonczy M, Park S, Jacobson J, Wiener J, Aronson M. Magnetic resonance abnormalities in HIV infection: a study in the drug-user risk group. *Psychiatry Res.* 1993; 47:175–186. [PubMed: 8341770]
- He J, Greco J, Mui K, Aminipour S, Kim J, Fuller R, Ratai E, Lentz M, Sehgal P, Westmoreland S, de Crespigny A, Gonzalez R. Diffusion MR detection of early white matter changes in the SIV primate model of neuroAIDS. *International Society of Magnetic Resonance in Medicine.* 2003:2536.
- Hein A, Schuh H, Thiel S, Martin JP, Dorries R. Ramified feline microglia selects for distinct variants of feline immunodeficiency virus during early central nervous system infection. *J Neurovirol.* 2003; 9:465–476. [PubMed: 12907391]
- Hokanson RM, TerWee J, Choi IS, Coates J, Dean H, Reddy DN, Wolf AM, Collisson EW. Dose response studies of acute feline immunodeficiency virus PPR strain infection in cats. *Vet Microbiol.* 2000; 76:311–327. [PubMed: 11000529]
- Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med.* 2000; 30:1–15. [PubMed: 10907753]
- Laubenberger J, Haussinger D, Bayer S, Thielemann S, Schneider B, Mundinger A, Hennig J, Langer M. HIV-related metabolic abnormalities in the brain: depiction with proton MR spectroscopy with short echo times. *Radiology.* 1996; 199:805–810. [PubMed: 8638009]
- Le Bihan D, Breton E, Lallemand D, Grenier P, Cabanis E, Laval-Jeantet M. MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. *Radiology.* 1986; 161:401–407. [PubMed: 3763909]
- Lentz MR, Kim JP, Westmoreland SV, Greco JB, Fuller RA, Ratai EM, He J, Sehgal PK, Halpern EF, Lackner AA, Masliah E, Gonzalez RG. Quantitative neuropathologic correlates of changes in ratio of N-acetylaspartate to creatine in macaque brain. *Radiology.* 2005; 235:461–468. [PubMed: 15798152]
- Lentz MR, Lee V, Westmoreland SV, Ratai EM, Halpern EF, Gonzalez RG. Factor analysis reveals differences in brain metabolism in macaques with SIV/AIDS and those with SIV-induced encephalitis. *NMR Biomed.* 2008a; 21:878–887. [PubMed: 18574793]
- Lentz MR, Westmoreland SV, Lee V, Ratai EM, Halpern EF, Gonzalez RG. Metabolic markers of neuronal injury correlate with SIV CNS disease severity and inoculum in the macaque model of neuroAIDS. *Magn Reson Med.* 2008b; 59:475–484. [PubMed: 18306400]
- Marcus CD, Taylor-Robinson SD, Sargentoni J, Ainsworth JG, Frize G, Easterbrook PJ, Shaunak S, Bryant DJ. 1H MR spectroscopy of the brain in HIV-1-seropositive subjects: evidence for diffuse metabolic abnormalities. *Metab Brain Dis.* 1998; 13:123–136. [PubMed: 9699920]
- Mascalchi M, Filippi M, Floris R, Fonda C, Gasparotti R, Villari N. Diffusion-weighted MR of the brain: methodology and clinical application. *Radiol Med.* 2005; 109:155–197. [PubMed: 15775887]
- McArthur JC, Hoover DR, Bacellar H, Miller EN, Cohen BA, Becker JT, Graham NM, McArthur JH, Selnes OA, Jacobson LP, et al. Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology.* 1993; 43:2245–2252. [PubMed: 8232937]

- McArthur JC, McDermott MP, McClernon D, St Hillaire C, Conant K, Marder K, Schifitto G, Selnes OA, Sacktor N, Stern Y, Albert SM, Kieburtz K, deMarcaida JA, Cohen B, Epstein LG. Attenuated central nervous system infection in advanced HIV/AIDS with combination antiretroviral therapy. *Arch Neurol*. 2004; 61:1687–1696. [PubMed: 15534180]
- Meeker RB. Feline immunodeficiency virus neuropathogenesis: from cats to calcium. *J Neuroimmune Pharmacol*. 2007; 2:154–170. [PubMed: 18040840]
- Meyerhoff DJ, Weiner MW, Fein G. Deep gray matter structures in HIV infection: a proton MR spectroscopic study. *AJNR Am J Neuroradiol*. 1996; 17:973–978. [PubMed: 8733976]
- Muller-Oehring EM, Schulte T, Rosenbloom MJ, Pfefferbaum A, Sullivan EV. Callosal degradation in HIV-1 infection predicts hierarchical perception: a DTI study. *Neuropsychologia*. 2010; 48:1133–1143. [PubMed: 20018201]
- Pedersen NC, Leutenegger CM, Woo J, Higgins J. Virulence differences between two field isolates of feline immunodeficiency virus (FIV-APetaluma and FIV-CPGammar) in young adult specific pathogen free cats. *Vet Immunol Immunopathol*. 2001; 79:53–67. [PubMed: 11356250]
- Pfefferbaum A, Rosenbloom MJ, Rohlfing T, Kemper CA, Deresinski S, Sullivan EV. Frontostriatal fiber bundle compromise in HIV infection without dementia. *Aids*. 2009; 23:1977–1985. [PubMed: 19730350]
- Phillips TR, Billaud JN, Henriksen SJ. Methamphetamine and HIV-1: potential interactions and the use of the FIV/cat model. *J Psychopharmacol*. 2000; 14:244–250. [PubMed: 11106303]
- Phillips TR, Prospero-Garcia O, Wheeler DW, Wagaman PC, Lerner DL, Fox HS, Whalen LR, Bloom FE, Elder JH, Henriksen SJ. Neurologic dysfunctions caused by a molecular clone of feline immunodeficiency virus, FIV-PPR. *J Neurovirol*. 1996; 2:388–396. [PubMed: 8972420]
- Phipps AJ, Hayes KA, Buck WR, Podell M, Mathes LE. Neurophysiologic and immunologic abnormalities associated with feline immunodeficiency virus molecular clone FIV-PPR DNA inoculation. *J Acquir Immune Defic Syndr*. 2000; 23:8–16. [PubMed: 10708051]
- Podell M, Hayes K, Oglesbee M, Mathes L. Progressive encephalopathy associated with CD4/CD8 inversion in adult FIV-infected cats. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997; 15:332–340. [PubMed: 9342252]
- Podell M, Maruyama K, Smith M, Hayes KA, Buck WR, Ruehlmann DS, Mathes LE. Frontal lobe neuronal injury correlates to altered function in FIV-infected cats. *J Acquir Immune Defic Syndr*. 1999; 22:10–18. [PubMed: 10534142]
- Podell M, Oglesbee M, Mathes L, Krakowka S, Olmstead R, Lafrado L. AIDS-associated encephalopathy with experimental feline immunodeficiency virus infection. *J Acquir Immune Defic Syndr*. 1993; 6:758–771. [PubMed: 8389849]
- Poli A, Pistello M, Carli MA, Abramo F, Mancuso G, Nicoletti E, Bendinelli M. Tumor necrosis factor-alpha and virus expression in the central nervous system of cats infected with feline immunodeficiency virus. *J Neurovirol*. 1999; 5:465–473. [PubMed: 10568883]
- Pomara N, Crandall DT, Choi SJ, Johnson G, Lim KO. White matter abnormalities in HIV-1 infection: A diffusion tensor imaging study. *Psychiatry Research-Neuroimaging*. 2001; 106:15–24.
- Pomper MG, Sacktor N. New techniques for imaging Human Immunodeficiency Virus associated cognitive impairment in the era of highly active antiretroviral therapy. *Arch Neurol*. 2007; 64:1233–1235. [PubMed: 17846261]
- Power C, Buist R, Johnston JB, Del Bigio MR, Ni W, Dawood MR, Peeling J. Neurovirulence in feline immunodeficiency virus-infected neonatal cats is viral strain specific and dependent on systemic immune suppression. *J Virol*. 1998a; 72:9109–9115. [PubMed: 9765456]
- Power C, McArthur JC, Nath A, Wehrly K, Mayne M, Nishio J, Langelier T, Johnson RT, Chesebro B. Neuronal death induced by brain-derived human immunodeficiency virus type 1 envelope genes differs between demented and nondemented AIDS patients. *J Virol*. 1998b; 72:9045–9053. [PubMed: 9765449]
- Ragin AB, Storey P, Cohen BA, Epstein LG, Edelman RR. Whole brain diffusion tensor Imaging in HIV-Associated cognitive impairment. *American Journal of Neuroradiology*. 2004; 25:195–200. [PubMed: 14970017]

- Ragin AB, Wu Y, Storey P, Cohen BA, Edelman RR, Epstein LG. Diffusion tensor imaging of subcortical brain injury in patients infected with human immunodeficiency virus. *Journal of Neurovirology*. 2005; 11:292–298. [PubMed: 16036809]
- Ratai EM, Bombardier JP, Joo CG, Annamalai L, Burdo TH, Campbell J, Fell R, Hakimelahi R, He J, Autissier P, Lentz MR, Halpern EF, Masliah E, Williams KC, Westmoreland SV, Gonzalez RG. Proton magnetic resonance spectroscopy reveals neuroprotection by oral minocycline in a nonhuman primate model of accelerated NeuroAIDS. *PLoS One*. 2010; 5:e10523. [PubMed: 20479889]
- Ratai EM, Pilkenton SJ, Greco JB, Lentz MR, Bombardier JP, Turk KW, He J, Joo CG, Lee V, Westmoreland S, Halpern E, Lackner AA, Gonzalez RG. In vivo proton magnetic resonance spectroscopy reveals region specific metabolic responses to SIV infection in the macaque brain. *BMC Neurosci*. 2009; 10:63. [PubMed: 19545432]
- Sacktor N, Skolasky RL, Ernst T, Mao X, Selnes O, Pomper MG, Chang L, Zhong K, Shungu DC, Marder K, Shibata D, Schifitto G, Bobo L, Barker PB. A multicenter study of two magnetic resonance spectroscopy techniques in individuals with HIV dementia. *J Magn Reson Imaging*. 2005; 21:325–333. [PubMed: 15779034]
- Stebbins GT, Smith CA, Bartt RE, Kessler HA, Adeyemi OM, Martin E, Cox JL, Bammer R, Moseley ME. HIV-associated alterations in normal-appearing white matter: a voxel-wise diffusion tensor imaging study. *J Acquir Immune Defic Syndr*. 2007; 46:564–573. [PubMed: 18193498]
- Steigerwald ES, Sarter M, March P, Podell M. Effects of feline immunodeficiency virus on cognition and behavioral function in cats. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1999; 20:411–419. [PubMed: 10225221]
- Tarasow E, Wiercinska-Drapalo A, Kubas B, Dzienis W, Orzechowska-Bobkiewicz A, Prokopowicz D, Walecki J. Cerebral MR spectroscopy in neurologically asymptomatic HIV-infected patients. *Acta Radiol*. 2003; 44:206–212. [PubMed: 12694109]
- Tolnay AE, Baskin CR, Tumpey TM, Sabourin PJ, Sabourin CL, Long JP, Pyles JA, Albrecht RA, Garcia-Sastre A, Katze MG, Bielefeldt-Ohmann H. Extrapulmonary tissue responses in cynomolgus macaques (*Macaca fascicularis*) infected with highly pathogenic avian influenza A (H5N1) virus. *Arch Virol*. 2010 [E-pub ahead of print].
- Usiskin SI, Bainbridge A, Miller RF, Jager HR. Progressive multifocal leukoencephalopathy: serial high-b-value diffusion-weighted MR imaging and apparent diffusion coefficient measurements to assess response to highly active antiretroviral therapy. *AJNR Am J Neuroradiol*. 2007; 28:285–286. [PubMed: 17296996]
- Wenserski F, von Giesen HJ, Wittsack HJ, Aulich A, Arendt G. Human immunodeficiency virus 1-associated minor motor disorders: Perfusion-weighted MR imaging and H-1 MR spectroscopy. *Radiology*. 2003; 228:185–192. [PubMed: 12759468]
- Wilkinson ID, Miller RF, Miskiel KA, Paley MN, Hall-Craggs MA, Baldeweg T, Williams IG, Carter S, Newman SP, Kendall BE, Catalan J, Chinn RJ, Harrison MJ. Cerebral proton magnetic resonance spectroscopy in asymptomatic HIV infection. *Aids*. 1997; 11:289–295. [PubMed: 9147419]
- Williams K, Westmoreland S, Greco J, Ratai E, Lentz M, Kim WK, Fuller RA, Kim JP, Autissier P, Sehgal PK, Schinazi RF, Bischofberger N, Piatak M, Lifson JD, Masliah E, Gonzalez RG. Magnetic resonance spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV neuroAIDS. *J Clin Invest*. 2005; 115:2534–2545. [PubMed: 16110325]
- Wu Y, Storey P, Cohen BA, Epstein LG, Edelman RR, Ragin AB. Diffusion alterations in corpus callosum patients with HIV. *American Journal of Neuroradiology*. 2006; 27:656–660. [PubMed: 16552012]
- Yoon JH, Bang OY, Kim HS. Progressive multifocal leukoencephalopathy in AIDS: Proton MR spectroscopy patterns of asynchronous lesions confirmed by serial diffusion-weighted imaging and apparent diffusion coefficient mapping. *Journal of Clinical Neurology*. 2007; 3:200–203. [PubMed: 19513133]

Figure 1.

Apparent diffusion coefficients across multiple brain regions in cats infected with FIV-PPR, FIV-C36, and age-matched controls with b factor = 2000. Error bars represent one standard deviation of the mean (* = Tukey's test: $p < 0.05$).

Figure 2.

Plasma viremia (2a), proviral load (2b) and CD4:CD8 lymphocyte ratios (2c) in FIV-PPR and -C36 infected cats. Control cats had undetectable plasma viremia (data not shown). Error bars represent one standard deviation of the mean. MRI imaging and spectroscopy, indicated by a vertical line, was performed between 111–127 days PI for all cats. These data demonstrate the context of the timing of the MR studies (de Rozieres *et al*, 2008). For ease of viewing the CD4:CD8 lymphocyte ratios, error bars (standard deviation) are shown for the control group only. Statistically significant values ($p < 0.05$) relative to controls are signified by asterisks.

Figure 3.

Brain proviral load at six months PI in FIV-PPR and FIV-C36 infected cats. Each sample from each individual animal was run in triplicate and average viral loads calculated. FIV-C36 infected cats had higher proviral loads than PPR infected cats in all regions, and provirus was undetectable in the majority of samples from FIV-PPR animals. Fraction above each bar indicates fraction of animals with detectable provirus. 5/5 samples were positive in animals where a fraction is not indicated.

Figure 4.
Photomicrograph of the meninges of an FIV infected cat with infiltrating lymphocytes. The lymphocytes expressed phospho-p38 MAPK, indicating activation.

Figure 5. Transverse T2 weighted MR feline brain image showing placement of the excitation voxel (white rectangle) and the overlaid multivoxel phase encoding grid (in grey). Voxels 5–7 correspond to the feline thalamus.

Figure 6.

Transverse proton density MR feline brain images illustrating the locations of the ROI's used for the analysis; images such as these were used to locate anatomic structures and their coordinates were used to accurately place the ROI's on the ADC maps generated from diffusion images, (w = white matter, g = gray matter, cn = caudate nucleus, * = approximate region of putamen and globus pallidus, T = thalamus, P = pyriform lobe, B = brainstem/midbrain, C = cerebellum).

Table 1

Average metabolite ratios from the thalamus of FIV-infected cats and age-matched controls

	NAA/Cr	St Dev	P-value	Cho/Cr	St Dev	P-value
Control	1.60	0.19	-	2.65	0.17	-
FIV-PPR	1.43	0.17	0.38	2.53	0.35	0.95
FIV-C36	1.56	0.03	0.93	2.71	0.64	0.99

NAA/Cr and Cho/Cr metabolite ratios measured with multi-voxel MR spectroscopy of the thalamus of infected cats and controls. P-values are the results of Tukey's tests for pair wise comparisons between FIV-infected groups and controls. No significant differences were seen groups for either NAA/Cr or Cho/Cr.