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MR brain volumetric measurements are predictive of neurobehavioral impairment in the HIV-1 transgenic rat

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ABSTRACT

Introduction: HIV infection is known to be associated with brain volume loss, even in optimally treated patients. In this study, we assessed whether dynamic brain volume changes over time are predictive of neurobehavorial performance in the HIV-1 transgenic (Tg) rat, a model of treated HIV-positive patients.

Materials and methods: Cross-sectional brain MRI imaging was first performed comparing Tg and wild type (WT) rats at 3 and 19 months of age. Longitudinal MRI and neurobehavioral testing of another group of Tg and WT rats was then performed from 5 to 23 weeks of age. Whole brain and subregional image segmentation was used to assess the rate of brain growth over time. We used repeated-measures mixed models to assess differences in brain volumes and to establish how predictive the volume differences are of specific neurobehavioral deficits.

Results: Cross-sectional imaging showed smaller whole brain volumes in Tg compared to WT rats at 3 and at 19 months of age. Longitudinally, Tg brain volumes were smaller than age-matched WT rats at all time points, starting as early as 5 weeks of age. The Tg striatal growth rate delay between 5 and 9 weeks of age was greater than that of the whole brain. Striatal volume in combination with genotype was the most predictive of rota-rod scores and in combination with genotype and age was the most predictive of total exploratory activity scores in the Tg rats.

Conclusion: The disproportionately delayed striatal growth compared to whole brain between 5 and 9 weeks of age and the role of striatal volume in predicting neurobehavioral deficits suggest an important role of the dopaminergic system in HIV associated neuropathology. This might explain problems with motor coordination and executive decisions in this animal model. Smaller brain and subregional volumes and neurobehavioral deficits were seen as early as 5 weeks of age, suggesting an early brain insult in the Tg rat. Neuroprotective therapy testing in this model should thus target this early stage of development, before brain damage becomes irreversible.

1. Introduction

The central nervous system is an early target of HIV infection (An et al., 1999). Along those lines, a multitude of studies have used MR volumetry in the assessment of HIV-positive (HIV +) patients, showing various levels of volume loss (Aylward et al., 1993; Cardenas et al., 2009; Hua et al., 2013; Kallianpur et al., 2013), reflective of HIV-associated neurodegeneration. Even in the post-antiretroviral therapy (ART) era, brain volume loss remains prevalent and progressive, suggesting ongoing brain injury (Cardenas et al., 2009; Becker et al., 2012).

As in humans, brain morphology is often an important biomarker of neuropathology in animal models of neurodegeneration (Zhang et al., 2010). The HIV-1 transgenic (Tg) rat model is a popular small animal model in NeuroHIV studies, is known to develop neurological abnormalities with age and has been proposed as a model of chronic HIV infection in the post-ART era (Vigorito et al., 2015; Vigorito et al., 2013; Moran et al., 2013a; Webb et al., 2010; Reid et al., 2001a). In this model, the transgene expresses 7 of the 9 HIV genes (including gp120, nef, and tat) and neurologic damage is assumed to result from chronic exposure to neurotoxic effects of viral proteins (Vigorito et al., 2015; Peng et al., 2010; Reid et al., 2001b). We have previously described structural and functional abnormalities in the Tg rat in comparison to age-matched wild type (WT) rats (Lee et al., 2014; Lee et al., 2015; Lentz et al., 2014) as well as defective exploratory and motor behavior (Reid et al., 2016a). The latter (neurobehavioral deficits) were assessed

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using a combination of open field box (measuring total exploratory activity, distances traveled in the internal and external zones, number of line crossings, rearing time and rearing time in the external zone) reflective of general locomotor activity, unconditioned motivated behavior, and behavioral plasticity (Walsh & Cummins, 1976) and accelerating rotarod apparatus (measuring the latency to fall detected by a photo-beam under the rotating shaft) reflective of motor learning/planning and locomotor activity (Shiotsuki et al., 2010).

Besides cross-sectional detection of brain volume discrepancies in the young and old Tg rats compared to WT rats, a main aim in this study was to longitudinally evaluate brain growth rates in both groups to better understand the dynamics of brain injury in the Tg rat and to document the earliest time point at which brain injury starts. Towards this goal, we used volumetric MR as a biomarker of neuropathology. We then correlated with concurrently obtained neurobehavioral testing measures that were previously used and reported in correlation with FDG PET imaging (Reid et al., 2016a). We also wanted to assess the growth rates of various brain subregions compared to the whole brain, since we suspected differential regional vulnerabilities to viral protein toxicity.

2. Materials and methods

2.1. Animals

Male HIV-1 Tg rats (F344/Hsd) and age-matched WT rats (F344) were purchased from Envigo (Indianapolis, IN) and used in various experiments. All rats were housed in a temperature-controlled environment with a 12-h light/dark cycle and free access to food and water. All procedures were conducted during the light cycle and all neurobehavioral tests were performed between 10 AM and 2 PM. All studies were approved by the Animal Care and Use Committee of the National Institutes of Health, Clinical Center.

2.2. In vivo MRI acquisition

MR images were acquired using a PharmaScan 7T/16 Scanner (Bruker, Germany). Rats were anesthetized with 1–2.5% isoflurane using a vaporizer and a facial mask. A heating pad was wrapped around the rat cradle for maintenance of physiological body temperature. For the cross-sectional study (n = 20), rats were divided into four cohorts: young Tg (n = 6, age = 3.6 ± 0.13 months), young WT (n = 4, age = 3.6 ± 0.12 months), old Tg (n = 5, age = 19.2 ± 0.10 months), and old WT (n = 5, age = 18.4 ± 0.05 months). A cross-coil setup was used which included a 72 mm transmission coil and a 20 mm surface coil placed on the animal's head. A series of sagittal T2-weighted images (rapid acquisition with refocused echoes, RARE) were initially obtained at the midline to allow for consistent positioning of the coronal images with respect to the corpus callosum. Parameters included echo time (TE) = 49 ms, repetition time (TR) = 1000 ms, slice thickness = 0.5 mm, matrix size = 256 × 256, field of view (FOV) = 35 × 35 mm (spatial resolution = 0.14 × 0.14 mm/pixel) and 8 averages. T2-weighted images for the segmentation portion of this study were acquired in the coronal plane. Parameters included: TE/TR = 12.3/3379 ms, slice thickness = 1 mm and RARE factor of 8 (image acquisition time ~15 min).

For the longitudinal study, MRI scans were obtained on 5 male Tg and 4 age-matched WT rats. The first MR acquisition was performed at 5 weeks of age, then every 4 weeks with the last time point acquired at 23 weeks of age. A 9 cm RF birdcage volumetric coil was used for image acquisition. Parameters for 3-dimensional (3D) T2-weighted fast spin echo included: TE/TR = 84/1000 ms, slice thickness = 0.312 mm, FOV = 40 × 40 × 40 mm, and matrix size: 128 × 128 × 128 (spatial resolution = 0.312 × 0.312 × 0.312 mm/pixel), flip angle = 180°, echo train length = 16 and 4 averages with image acquisition time of ~68 min. Images were post processed to matrix of 256 × 256 × 256 resulting in isotropic resolution of 0.156 mm. The animals' weights were recorded at each time point.

2.3. Image analysis

For the cross-sectional images, we used manual segmentation of whole brain volumes using the “Levelset VOI” and “Paint brush” tools in MIPAV (Medical Image Processing, Analysis, and Visualization), a free software developed at NIH.

For the longitudinal images, MIPAV was used for rigid alignment of all images (9 animals × 5 timepoints = 45 scans) to the same target space. Each image was segmented by registration of multiple atlases followed by manual refinement. An image template and atlas were created for each time point (5, 9, 13, 17, 23 weeks) and for each genotype (WT/Tg) (total of ten templates). Templates were created by averaging the MRI images for each subgroup. Nine volumes of interest were then delineated to create an atlas using ITK-Snap: whole brain, which includes CSF and parenchyma, striatum, corpus callosum, hippocampus, cortex, cerebellum, olfactory bulbs, brainstem, and ventricles. Segmentations were based on Paxinos and Watson rat brain atlas (Paxinos & Watson, 2007). Whole brain label propagation was used for skull stripping before subregional segmentation.

The multi-atlas image segmentation method was performed using affine registration of multiple atlases to each individual target (Artaechevarria et al., 2009; Cabezas et al., 2011). Afterwards, higher probability rules/majority voting and manual input determined pixel classification for each subregion (Lorenzo-Valdés et al., 2004). For ventricular segmentation, we used a combination of multi-atlas based segmentation and intensity thresholding.

2.4. Neurobehavioral testing

The neurobehavioral data included in this paper has been previously reported in correlation with FDG PET imaging in the same group of rats (Reid et al., 2016a). As previously described, neurobehavioral testing was performed longitudinally. Data on changes in motor function from Tg and WT rats was collected using a Rota-rod apparatus, operated in an accelerated mode (Med Associates, Inc., St. Albans, VT). Rats were tested starting at 5 weeks of age, with each time point consisting of 2 trials (total of thirteen time points, over a period of 16 weeks). The rota-rod has a default speed of 4 rpm and accelerates to 40 rpm over the 5 min testing period. Once a rat was removed from its cage, it was placed on the rod in the direction of rotation. A photo-beam under the rotating shaft detected when the animal had fallen and data was collected on the latency to fall.

Exploratory activity in the same group of animals was assessed using open field testing. The apparatus (ANY-maze, Stoelting Co., Wood Dale, IL) has a square floor with side lengths of 40 cm, surrounded by 35 cm high opaque Plexiglas walls, an overhead recording camera, photo-beam array (to detect rearing behavior) and tracking software (used for tracking and analyzing data in pre-defined zones). All trials were conducted at the same time of the day (10 AM–2 PM) at 30 min intervals. Under low illumination, tracking was initiated once the animal was placed in the center of the field. Tests were started when the animals were 11 week-old and seven time points were collected over 14 weeks. Total exploratory activity scores were collected for analysis and correlation with brain volumetry. We chose the total exploratory activity score since it mirrors the 6 min walk test, a clinical outcome measure to assess locomotive activity and coordination in small animals (Gould et al., 2009; Tatem et al., 2014).

2.5. Statistical analysis

2.5.1. Brain volume differences

For cross-sectional images, differences between Tg and WT animals and between young and old rats were assessed by analysis of variance