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Vitamin A deficiency and behavioral and motor deficits in the human immunodeficiency virus type 1 transgenic rat

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The human immunodeficiency virus type 1 (HIV-1) transgenic (Tg) rat model incorporates a noninfectious viral genome that is under similar regulatory control mechanisms *in vivo* as those that exist with natural infection in humans. Vitamin A (VA) deficiency in humans has been associated with progressive systemic HIV disease and with impaired cognition in rodent models. The effects on of VA deficiency on the development of behavioral abnormalities with HIV infection have not been previously described. In these studies, wild-type (Wt) and Tg rats maintained on either a normal (VA+) or a VA-deficient (VA−) diet were examined for activity in an open field (horizontal activity, total distance, vertical activity, and rearing) and on rotarod testing. On both open field and rotarod testing, the Tg rats performed worse than the Wt rats, with the most severe deficits noted in the TgVA− animals. Analysis of the specific effects of the presence of the HIV transgene and the diet on the performance on the open field tests showed a dominant effect from the transgene on all of the tests, with an effect from the diet on only the number of rearings. On rotarod testing, effects form both the diet and the transgene were observed at lower speeds, at the highest speeds, and on the accelerating rotarod. These studies therefore demonstrate that behavioral and motor abnormalities can be detected in this model and are likely due to similar mechanisms by which humans infected with HIV might develop cognitive-motor impairment in association with VA deficiency. *Journal of NeuroVirology* (2010) **15**, 380–389.

Keywords: HIV-1; transgenic rat; vitamin A; retinoids; rotarod; open field

Introduction

The human immunodeficiency virus (HIV) transgenic (Tg) rat model incorporates a noninfectious viral genome that is under similar regulatory control mechanisms *in vivo* that exist with natural infection (Reid et al. 2001). Over time, the rats develop immune abnormalities and clinical manifestations in the presence of the transgene that are similar to what occurs with infection in humans (Reid et al. 2001a, 2004a). Retinoids have been demonstrated to either enhance or suppress replication of HIV in infected cultures (Kitano et al. 1990; Lee et al. 1994; Maciaszek et al. 1998; Poli et al. 1992; Semmel et al. 1994; Towers et al. 1995; Yamaguchi et al. 1994). Clinically, HIV-infected patients with vitamin A deficiency have been demonstrated to be more at risk for developing HIV-related complications (Semba et al. 1995; Tang et al. 1993, 1997). On the other hand, in one study low levels have been also associated with a decreased risk of HIV seroconversion (MacDonald et al. 2001).

HIV infection has been associated with an increased risk of developing neurocognitive impairment, characterized by defective memory, abnormal behavior, and psychomotor slowing (Antinori et al. 2007; Janssen et al. 1989). In studies of rats administered a vitamin A–deficient diet, the animals developed impaired spatial learning memory that

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was reversed by the administration of vitamin A (Cocco et al. 2002). Similarly, in mice, vitamin A deficiency was shown to contribute to age-related decline in cognitive function, as demonstrated by assessments of short-term, long-term, and relational memory in deficient animals (Etchamendy et al. 2003; Krezel et al. 1996). The combined effects of HIV infection and vitamin A deficiency have not been previously examined. In order to model these possible effects, HIV-1 transgenic rats were rendered vitamin A (VA) deficient and assessed for the development of behavioral and motor abnormalities on open field and rotarod testing. These studies showed that the rats developed impairment in behavior and motor learning, and that the impairment is more severe in the Tg animals and can be enhanced by VA deficiency. These studies therefore identify vitamin A deficiency as a potential important etiologic factor in the development of cognitive-motor abnormalities in HIV infection.

Results

Open field activity

Thirty rats (10 TgVA+, 9 TgVA−, 5 WtVA−, and 6 WtVA+) were tested for open field activity. On the testing, measures of horizontal activity and total distance were significantly decreased for the TgVA + and TgVA− rats as compared to both the WtVA+ and the WtVA− rats (Figure 1). Similarly, vertical activity and the number of rearings were lower for the Tg versus the corresponding Wt rats (Figure 2). In addition, Wt (wild-type) rats on the vitamin A–deficient diet had fewer rearings than the Wt rats on the VA+ diet.

Analysis of the F statistics from the analysis of variance (ANOVA) showed that the transgenic state and diet were independent variables and that the presence of the transgene contributed significantly to the variance that was observed for all portions of the open field testing (Table 1). The VA-deficient diet, however, was a significant factor only with respect to the number of rearings.

Rotarod test

For the rotarod testing, a total of 26 rats in the four groups (9 TgVA+, 8 TgVA−, 5 WtVA−, and 4 WtVA+) were used. During the initial training period, at speeds ranging from 4 to 8 rpm, all rats on the VA-deficient diet had greater difficulty staying on the rotarod (Figure 3). Notably, the Tg rats on the VA+ diet performed similarly to the Wt rats on the same diet, suggesting that the transgenic state, per se,

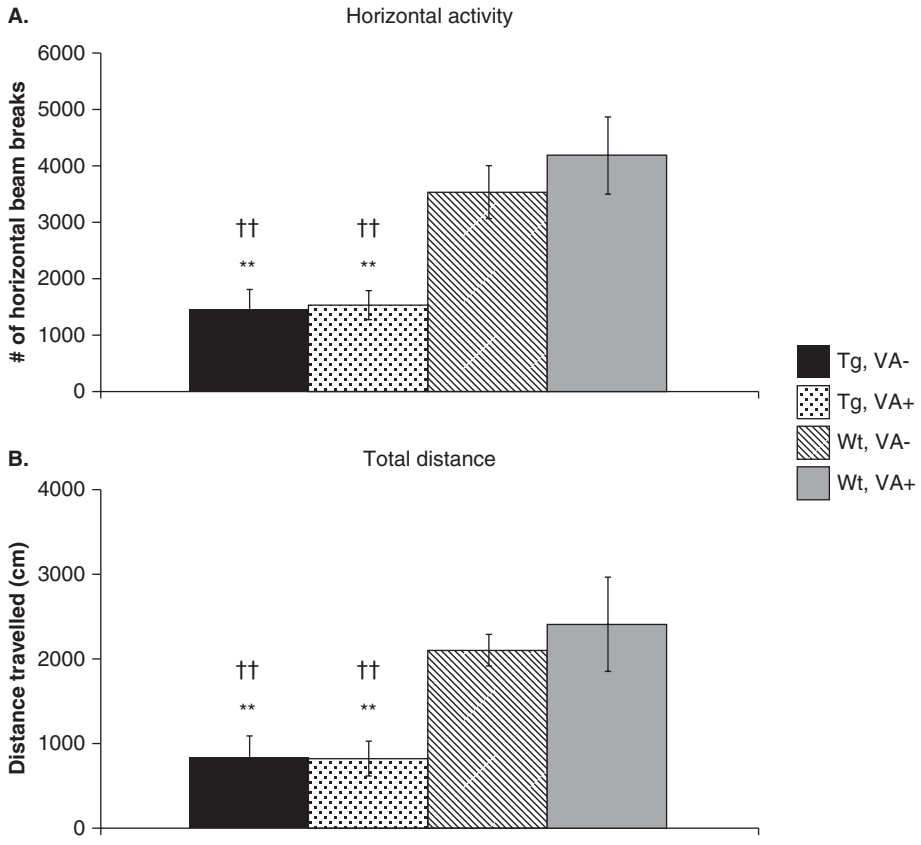

Figure 1 Horizontal activity and total distance measurements for the HIV-1 transgenic (Tg) and the wild-type (Wt) rats on the normal (VA +) or vitamin A–deficient (VA−) diet. $^{**}P < .01$, respectively, when compared against wild-type animals with normal diet; $\dagger\dagger P < .01$, respectively, when compared against wild-type animals with vitamin A–deficient diet.

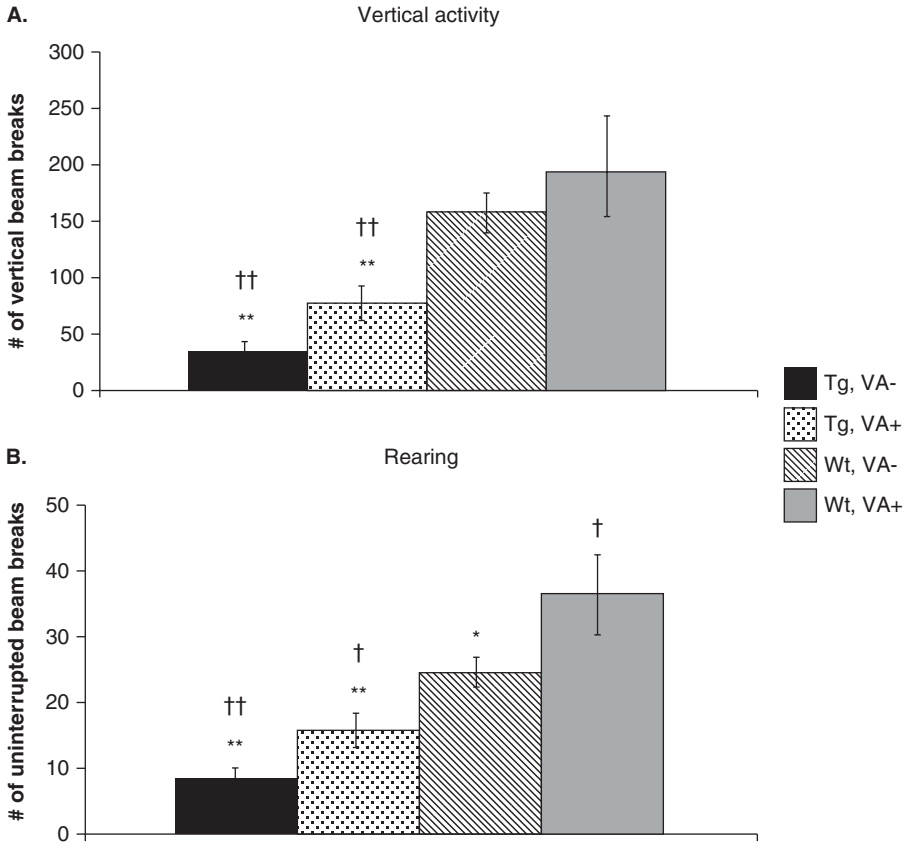

Figure 2 Vertical and rearing activity for the HIV-1 transgenic (Tg) and wild-type (Wt) rats on the normal (VA+) or vitamin A–deficient (VA−) diet. *, **$P < .05$ and.01, respectively, when compared against wild-type animals with normal diet; †, ††$P < .05$ and.01, respectively, when compared against wild-type animals with vitamin A–deficient diet.

Table 1 Locomotor activity *F* statistics

Horizontal Activity		
Rat Line	$F(1, 30) = 29.04$	$P < .0001$
Diet	$F(1, 1) = 0.9206$	$P = .345$
Interaction	$F(1, 30) = 0.6369$	$P = .4311$
Total Distance		
Rat Line	$F(1, 30) = 24.32$	$P < .0001$
Diet	$F(1, 1) = 0.2532$	$P = .6185$
Interaction	$F(1, 30) = 0.2638$	$P = .6113$
Vertical Activity		
Rat Line	$F(1, 30) = 19.43$	$P = .0001$
Diet	$F(1, 1) = 2.115$	$P = .1562$
Interaction	$F(1, 30) = 0.0197$	$P = .8893$
Rearing		
Rat Line	$F(1, 30) = 18.58$	$P = .0002$
Diet	$F(1, 1) = 5.103$	$P = .0313$
Interaction	$F(1, 30) = 0.2423$	$P = .6261$

had little impact on learning. At higher speeds, the effect of the VA− diet became less apparent, with the retention times on the rod becoming similar for all four groups of animals by the time that the speed was increased to 20 rpm. However, when tested beyond the initial period of learning and with the rotarod speed at the highest level (40 rpm), impairment was noted for TgVA+ and TgVA− rats versus the Wt rats, with the TgVA− rats showing more severe impairment than the Tg rats on the normal diet. Similarly, on the accelerated rotarod (Figure 4), impairment was noted for both Tg groups versus the Wt rats, with the TgVA− rats also showing the most impairment (Figure 4).

As noted for the open field testing, analysis of the *F* statistics for the rotarod data again demonstrated independent effects from the presence of the transgene and from the diet (Table 2). A significant percentage of the total variance was accounted for by the transgene at speeds of 4 and 40 rpm and on the accelerating rotarod. For the diet, a significant effect was seen at 4, 8, 12, and 20 rpm, with a diminishing effect observed from the low to high speeds. In addition, a significant effect from the diet was seen on the accelerating rotarod.

Discussion

Cognitive and behavioral abnormalities are common among individuals with HIV infection and can be the cause of significant morbidity and an increased mortality. Similarly, it has been shown that vitamin A deficiency can increase the risk of HIV-associated complications, particularly in infants

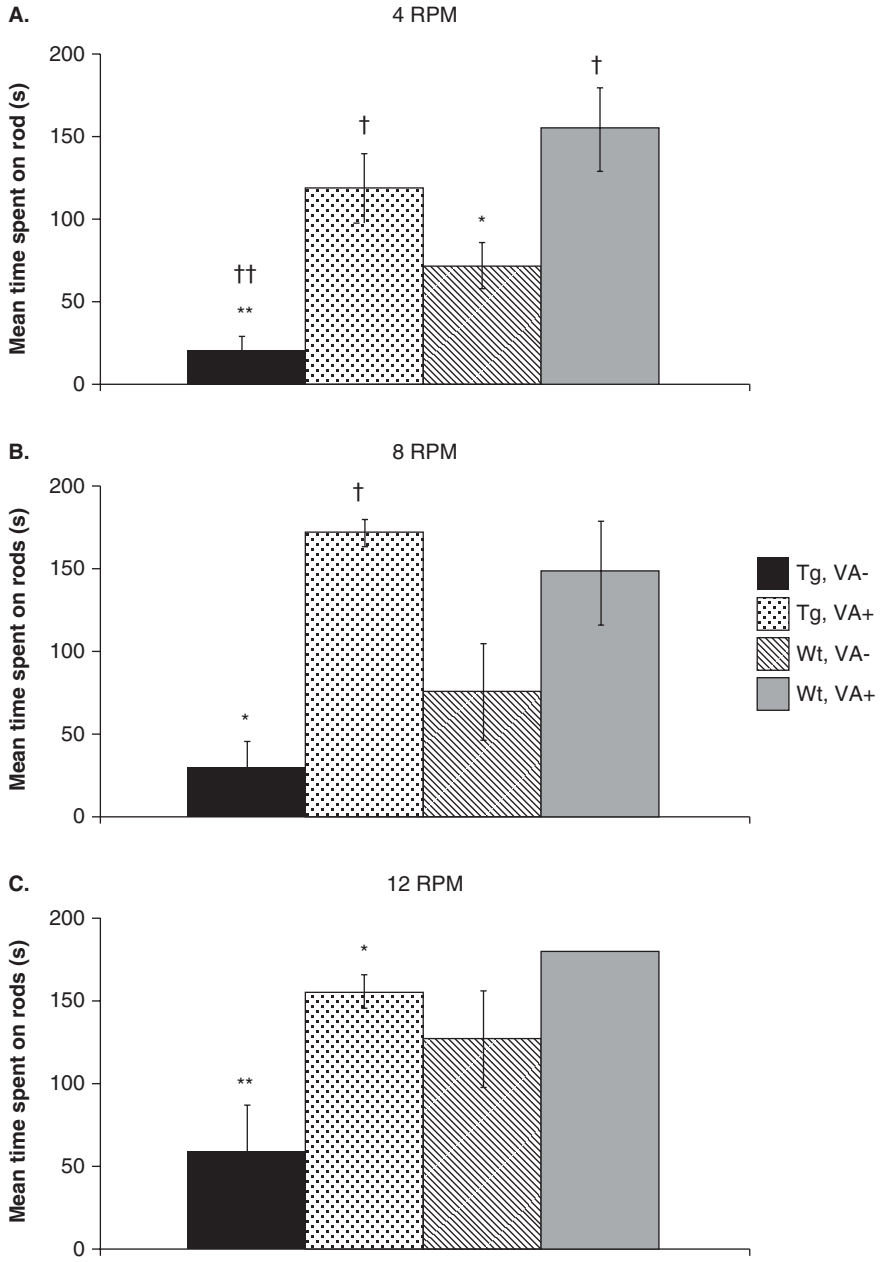

Figure 3 Rotarod performance for the Wt and Tg rats on the VA+ and VA− diets during training and at increasing rod speeds. At 12 rpm, all of the Wt rats on the normal diet remained on the rotarod for the maximum time allowed (180 s). *, **$P < .05$ and.01, respectively, when compared against wild-type animals with normal diet; †, ††$P < .05$ and.01, respectively, when compared against wild-type animals with vitamin A–deficient diet.

and children, which may be reversed by supplementation (Fawzi *et al*, 2000; Greenberg *et al*, 1997; Humphrey *et al*, 2006; Semba *et al*, 1993, 1994). In previous studies, it has been well documented that the Tg rat develops a number of manifestations of HIV-1 infection, such as immune abnormalities (Reid *et al*, 2001, 2004b; Royal *et al*, 2007), skin disease (Cedeno-Laurent *et al*, 2009), and neurological disease (Lashomb *et al*, 2008; Reid *et al*, 2001; Vigorito *et al*, 2007). Also, in studies of cognitive function in the Tg rat, it has been demonstrated that the animals develop abnormalities in spatial learning and memory (Lashomb *et al*, 2008; Vigorito *et al*, 2007).

Rotarod testing is used for assessing motor coordination and balance and also for examining motor learning. In the studies reported here, the data for both the Tg and Wt rats suggest that diet was important during the initial learning phase for these animals. The diet, however, did not impair the ability of the Wt rats on the diet to ultimately learn to remain on the rod with increasing rotarod speeds. In contrast,

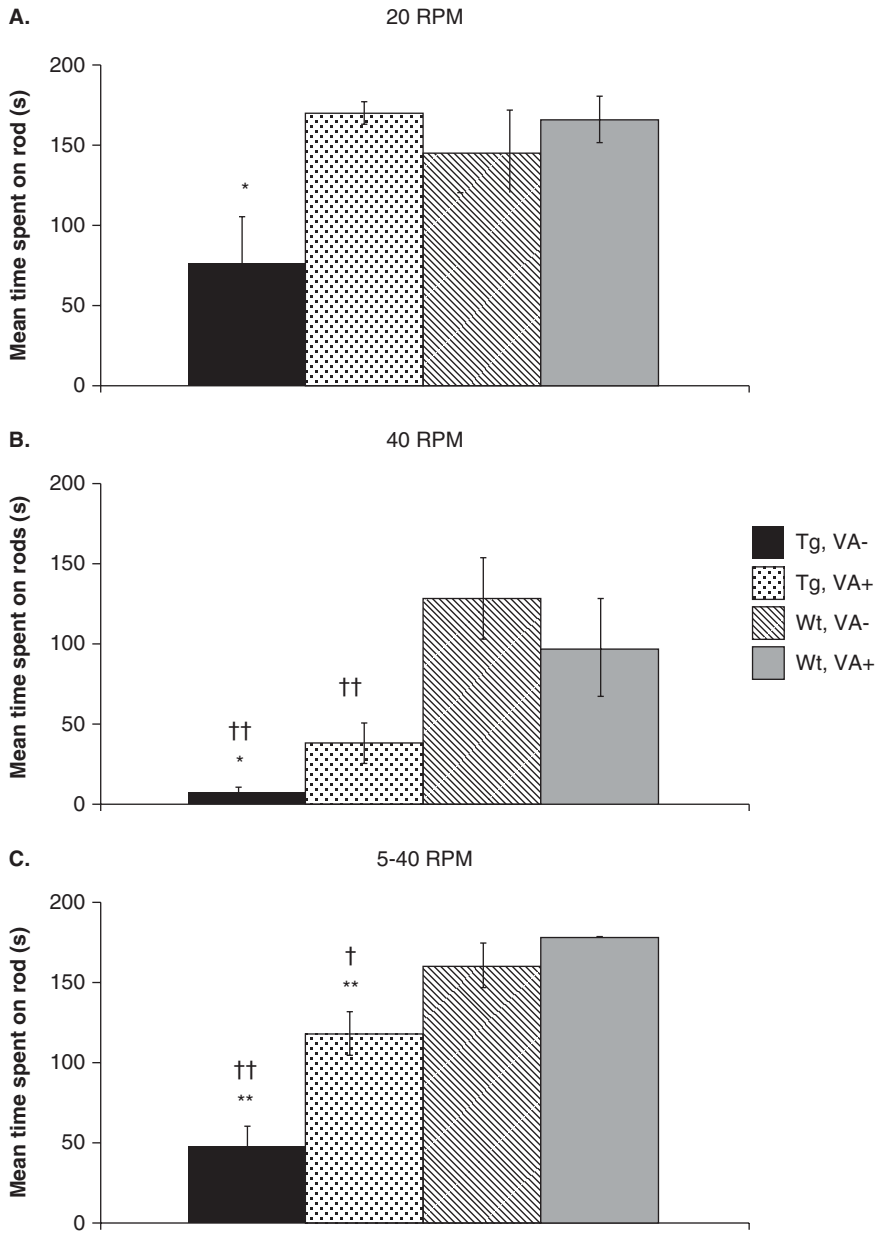

Figure 4 Performance by the Wt and Tg rats on the accelerating rotarod. *, **$P < .05$ and.01, respectively, when compared against wild-type animals with normal diet; †, ††$P < .05$ and.01, respectively, when compared against wild-type animals with vitamin A–deficient diet.

rats on the vitamin A–deficient diet were not able to overcome the challenge of remaining on the accelerating rotarod, likely due to the greater complexity of the task. For the Tg rats, the vitamin A–deficient diet was associated with impaired initial learning, which did not improve in later trials, suggesting that the presence of the transgene induced additional detrimental effects on motor learning. In addition, the testing at the highest speed following the learning phase showed a detrimental effect of the diet in the Tg rats relative to the Wt groups, whereas the accelerating rod testing revealed additional effects from the deficient diet that resulted in the TgVA− rats performing worse than TgVA+ rats. Therefore, the transgene and deficiency can have both independent and additive effects that result in impaired motor performance on these tests. It is likely that these findings are associated with effects on specific brain regions or neural pathways in these animals, as suggested by rotarod testing in mice, which showed specific induction of gene expression in the cerebellum, hippocampus, olfactory bulb, and frontal cortex (Nadler *et al*, 2006). In these studies, the level of expression for 10% of the genes that were expressed in specifically the cerebellum correlated with performance on the test.

Table 2 Rotarod *F* statistics

4rpm		
Rat Line	$F(1, 22) = 4.983$	$P = .0361$
Diet	$F(1, 1) = 21.4$	$P = .0001$
Interaction	$F(1, 22) = 0.1503$	$P = .702$
8rpm		
Rat Line	$F(1, 22) = 0.3361$	$P = .568$
Diet	$F(1, 1) = 30.93$	$P < .0001$
Interaction	$F(1, 22) = 3.241$	$P = .0855$
12rpm		
Rat Line	$F(1, 22) = 4.077$	$P = .0558$
Diet	$F(1, 1) = 10.67$	$P = .0035$
Interaction	$F(1, 22) = 0.8991$	$P = .3533$
20rpm		
Rat Line	$F(1, 22) = 1.954$	$P = .1761$
Diet	$F(1, 1) = 5.91$	$P = .0237$
Interaction	$F(1, 22) = 2.445$	$P = .1322$
40rpm		
Rat Line	$F(1, 22) = 28.58$	$P < .0001$
Diet	$F(1, 1) = 1.47E\text{-}05$	$P = .997$
Interaction	$F(1, 22) = 3.456$	$P = .0764$
5–40rpm		
Rat Line	$F(1, 22) = 35.49$	$P < .0001$
Diet	$F(1, 1) = 9.243$	$P = .006$
Interaction	$F(1, 22) = 3.384$	$P = .0794$

Open field testing, on the other hand, examines spontaneous motor behavior. The rationale for evaluating both ambulatory and rearing behaviors stems from the fact that the two parameters of open field behaviors have been suggested to be regulated via different neuroanatomical brain substrates (Caldecott-Hazard *et al*, 1988; Kehne *et al*, 1981; Summavielle *et al*, 2002). On all phases of this testing, it appeared that vitamin A deficiency and the presence of the transgene both resulted in less activity. Of note is the fact that vertical activity and rearing, which are sensitive measures of the activational effects of various influences in rats (Frye and Breese, 1981; Lister, 1987), were decreased for both Tg rat groups on the vitamin A–deficient diet as compared to the Wt groups. Examination of the data by analysis of variance, however, showed effects mainly due to the HIV transgene. Effects from the vitamin A–deficient diet were noted only on rearing and the noted abnormalities were worse for the Tg than for the Wt rats. These findings altogether provide further evidence for the fact that, as shown in previous studies, behavioral deficits can be induced in the presence of the transgene in this model (Lashomb *et al*, 2008; Vigorito *et al*, 2007). In addition, we now show that abnormalities can result specifically as a result of vitamin A deficiency in the Tg rats.

In our studies, pregnant rats were placed on the vitamin A–deficient diet at 2 weeks of gestation, which is approximately when the developing animal goes from the embryonic to the fetal stages. At this point in gestation, the development of the rodent spinal cord, brainstem, basal ganglia, and thalamus are nearly complete. However, the other forebrain structures and the cerebellum continue to develop for significant periods beyond the second week. In the case of the hippocampus, formation does not take place until the third week of gestation and it continues past weeks 2 to 3 of the postnatal period. In studies of the nervous system effects of vitamin A deficiency in Wistar rats, it was found that exposing these animals to a deficient diet from the time of weaning through age 6 months resulted in the development of features of motor neuron disease with hind limb spasticity and decreased numbers of motor neurons in the cervical and lumbar spinal cord (Corcoran *et al*, 2002). Histological analysis of spinal cord tissue from these rats showed the presence of astrocytosis and decreased expression of the retinoid receptor RAR (retinoic acid receptor)-alpha, retinaldehyde dehydrogenase, and isl-1, a homeodomain gene that appears to be important for motor neuron development (Thor S et al. 1991). In our studies, neither the Wt nor the vitamin A–deficient rats exhibited gross phenotypic features of abnormal morphogenesis. In addition, previous investigators have shown that spasticity has little effect on performance on the rotarod test (Poggi *et al*, 2005; Than *et al*, 2007). The observed effect from the diet was most prominent at the lower speeds, subsequently decreased then disappeared with increasing rpm, then reappeared on the accelerating rod. Similarly, an effect from the transgene was present at the lowest speed and at the highest speed and on the accelerating rod. These findings are not consistent with the rats being weak or spastic and suggest that the findings that we observed are likely due to other factors.

Changes in several brain neuronal populations have been noted in rodents with vitamin A deficiency that could impact on performance of the animals in behavioral tests. For example, the mouse striatum expresses high levels of retinoid receptors and retinoic acid–binding proteins (Zetterstrom *et al*, 1999) and Sprague-Dawley rats maintained on a vitamin A–deficient diet for 6 months develop locomotor deficits and impaired motor coordination related to D1 receptor hypersensitivity and decreased striatal acetylcholine (Carta *et al*, 2006). RAR-β, RARγ, RXR-β, and RXR-γ are all normally expressed in the mouse striatum. In studies of double knock-out of both murine RXR genes or of either RAR-β/RXR-β or RAR-β/RXR-γ gene combinations, and not single-gene mutations, impairment was seen on rotarod and open field testing (Krezel *et al*, 1998). In hippocampus, the importance of retinoids was demonstrated in studies in which low concentrations 13-*cis* retinoic acid increased rat hippocampal neuronal dendritic arborization in slice cultures, an effect that could be blocked by selective RXR and RAR antagonists (Liu *et al*, 2008).

Previous studies have shown that behavioral deficits can be induced in the presence of the transgene in this model (Lashomb *et al*, 2008; Vigorito *et al*, 2007). We now show that abnormalities in

motor learning can occur specifically as a result of vitamin A deficiency in the rats and can be enhanced by HIV-1. The mechanisms that underlie these apparent HIV-related effects are also unclear at this time. Neurotoxicity from HIV-1 gp120, tat, and nef has been demonstrated in both *in vivo* and *in vitro* models (Bansal *et al*, 2000; Hudson *et al*, 2000). In humans, current criteria for the diagnosis of HIV-related neurocognitive impairment require the occurrence of cognitive deficits in association with either motor or behavioral abnormalities or both. The identification and detailed characterization of the neural substrates that are impacted by these factors may prove useful for the development of therapeutic approaches for the treatment of patients with neurocognitive disorders resulting from HIV-1 infection.

Materials and methods

Animals

All experiments were performed using 3- to 6-month-old male and female specific pathogen free Tg and age-matched wild-type (Wt) Fisher 344/NHsd control rats. The details on the construction of the HIV-1 Tg rat have been previously described (Reid *et al*, 2001). The Tg and Wt rats were administered either a normal diet comprised of the Bio-Serv AIN-93M rodent maintenance diet (Bio-Serv; Frenchtown, NJ), which contains 400,000 IU/kg of retinyl palmitate, the major dietary form of vitamin A, or a diet based on that used to induce vitamin A deficiency in mice (Carman and Hayes, 1991), which is the Bio-Serv AIN-93M rodent diet mix formulated minus retinyl palmitate. Female rats maintained on the normal maintenance diet were impregnated then randomly divided into two groups at 2 weeks' gestation. One group of pregnant females was subsequently fed a vitamin A–deficient diet and the other was fed the vitamin A–sufficient diet. Weanlings were maintained on the same diets as their dams.

Control groups of Tg and Wt animals receiving the vitamin normal (A-sufficient) diet were studied parallel to the vitamin A–deficient rats. A total of 34 aged-matched rats were used in the behavioral assays: 9 WtVA+, 5 WtVA-, 10 TgVA+, and 10 TgVA− rats. The Tg rats weighed between 340 and 390 g and the Wt rats weighed between 387 and 442 g at the beginning of the experiment and were individually housed. Vivarium conditions were 21°C, and a normal 12-h light/dark cycle was used. All rats were provided with *ad libitum* access to their respective diets and water. The training and experimental sessions for all subjects took place between 8 AM and 6 PM. All procedures were conducted in adherence with the National Institutes of Health Guide for the Care and Use of Laboratory Animals at the University of Maryland School of Medicine.

Open Field Activity

Apparatus

Locomotor activity parameters (i.e., total distance, horizontal activity, and rearing behaviors [e.g., discreet rearing, vertical activity]) were recorded individually in a Plexiglas chamber (42 cm × 42 cm × 30 cm) using a 16-beam infrared Digiscan Activity Monitoring System (Accuscan Electronics, Columbus, OH, USA). Total distance measured as the total number of centimeters (cm) traveled, and horizontal activity measured as the total number of beams broken in the horizontal plane were evaluated as the ambulatory behavioral parameters (Kralic *et al*, 2003; Markel *et al*, 1989; Walsh and Cummins, 1976). In addition to ambulatory behaviors, the present study also evaluated rearing behaviors (Markel *et al*, 1989; Whimbey and Denenberg, 1967). To accomplish this, an additional set of photo beams were placed approximately 15 cm above the base of the activity apparatus. These vertical beams quantified rearing behavior as the (1) number of discrete rears (i.e., number of uninterrupted beam breaks) and (2) the number of infrared beam breakage in the vertical plane (i.e., number of interrupted beam breaks, or vertical activity) (Caldecott-Hazard *et al*, 1988; Markel *et al*, 1989). All experiments were conducted under dim lighting (25 Watts). The activity chamber was cleaned prior to testing of each animal to eliminate odors and related stimuli and to prevent the next subject from following the path of the prior rat (McKay *et al*, 2004).

Procedures

To evaluate locomotor performance, all rats received two consecutive 10-min sessions in the open field. Specifically, following the conclusion of the initial 10-min session, the analyzer was programmed such that the second 10-min session began. Thus, the duration of behavioral observation in the open field for each of the four locomotor activity parameters (e.g., total distance, horizontal activity, discreet rearing, and vertical activity) was 20 min.

Rotarod

To evaluate balance, motor coordination, as well as motor learning, we employed the Rotamex 8 rotarod (Columbus Instruments, Columbus, OH, USA) (Kralic *et al*, 2003; Taylor *et al*, 2003). The rotarod consisted of a textured rod (3.0 cm in diameter) suspended (29.1 cm) above an electrified grid floor. The rod was subdivided into lanes (9.3 cm wide) to allow for the simultaneous testing of four animals. It was powered by a variable-speed motor capable of running at a fixed speed, or accelerating at a constant rate. Rats received a mild shock of 0.2 mA, which served as a motivator to remain on the rotarod.

During week 1, rats received 5 days of training on the rotarod beginning at a constant-speed of 4 rounds per minute (rpm). This consisted of five 3-min (i.e., 180 s) trials over 5 days. During the second, third, fourth, and fifth weeks, the rats were evaluated under a similar protocol, except that the rpm were increased to 8, 12, 20, and 40 rpm, respectively. Our preliminary data revealed that over 80% of the Wt A+ rats successfully met criterion of >120 s at constant speeds between 4 and 40 rpm. In contrast, as predicted, some of the transgenic rats had difficulty remaining on the rod, even at the low rpm. During the sixth week, an accelerating assay was employed (Kralic *et al*, 2003; Taylor *et al*, 2003). During the initial day of the accelerated assay, the initial speed began at 5 rpm, accelerating up to 40 rpm over the duration of the 180 s, or until the rat fell off the rod. The rate of acceleration was 0.2 rpm/s. Rats were trained under the accelerating assay for 2 weeks.

Statistical analyses

Data are reported as the mean ± SEM value. The data were analyzed using the Stat-Most Statistical Program Package (San Diego, CA) using a between-group analysis of variance (ANOVA) to evaluate the difference between the TgVA+, TgVA−, WtVA−, and WtVA+ groups. Post hoc statistical comparisons between groups were conducted using Student's *t* test in all experiments. Data collected in the open field for the two 10-min sessions were summed and evaluated for a 20-min observation period. Data collected in the rotarod assays (e.g., nonaccelerated and accelerated) were collected for the five 3-min (i.e., 180 s) trials over days 1 to 5. However, only the data for the final three trials of day 5 were subjected to statistical analyses. The data for these three trials were averaged and a mean calculated for each of the four groups at the 4, 8, 12, 20, 40 (nonaccelerated assay) and 5–40 (accelerated assay) rpm. By evaluating the last three sessions of the final training day, optimal stabilization of behaviors can be quantified across each of the six different rotarod assays.

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