Published in final edited form as:

J Neurovirol. 2018 February ; 24(1): 113–118. doi:10.1007/s13365-017-0588-y.

Aging alters voltage-gated calcium channels in prefrontal cortex pyramidal neurons in the HIV brain

Christina E. Khodr, Ph.D., Lihua Chen, Ph.D., Lena Al-Harthi, Ph.D., and Xiu-Ti Hu, M.D./Ph.D.

Department of Immunology and Microbiology, Rush University Medical Center, Chicago, IL 60612

Abstract

We assessed firing and voltage-gated Ca^{2+} influx in medial prefrontal cortex (mPFC) pyramidal neurons from older (12 month-old) HIV-1 transgenic (Tg) rats. We found that neurons from older Tg rats showed increased firing compared to non-Tg rats, but Ca^{2+} spikes were unchanged. However, stronger excitatory stimulation was needed to evoke Ca^{2+} spikes, which was associated with reduced mPFC $\text{Ca}_v1.2$ L-type Ca^{2+} channel (L-channel) protein. In contrast, L-channel protein was unaltered in younger (6-7 week-old) Tg rats, which we previously found had enhanced neuronal Ca^{2+} influx. These studies demonstrate that aging alters HIV-induced Ca^{2+} channel dysfunction that affects mPFC activity.

Keywords

HIV; HAND; medial prefrontal cortex; calcium; L-channel; HIV-1 Tg rat

Introduction

HIV-Associated Neurocognitive Disorders (HAND) affect ~50% of HIV⁺ adults and are expected to increase as the HIV⁺ population ages (Gill and Kolson 2014; Heaton et al. 2010). The HIV-vulnerable medial prefrontal cortex (mPFC) regulates neurocognition, and is majorly composed of glutamatergic pyramidal neurons that project to the striatum and midbrain (Ferris et al. 2008). HIV-1 proteins and HIV-induced inflammation disturb neuronal function by abnormally increasing intracellular Ca^{2+} (Hu 2015) through effects on the NMDAR (Mattson et al. 2005), voltage-gated Ca^{2+} channels (VGCCs), and intracellular Ca^{2+} release (Brini et al. 2014; Hu 2015).

L-type VGCCs (L-channels), which modulate neuronal signaling and gene expression (Brini et al. 2014), include a low voltage-activated (LVA- $\text{Ca}_v1.3$), and high voltage-activated (HVA- $\text{Ca}_v1.2$) subtype in the brain (Lipscombe 2002). We recently demonstrated that HIV-1 alters L-channel activity and expression in the mPFC using HIV-1 transgenic (Tg) rats (Khodr et al. 2016; Wayman et al. 2016), which emphasizes the importance of L-channel dysfunction in HIV-induced neuropathogenesis.

Corresponding author: Dr. Xiu-Ti Hu, Dept. of Pharmacology, Rush University Medical Center, Cohn Research Building, Rm.414, 1735 W. Harrison Street, Chicago, IL 60612, Phone: (312) 563-4560, Fax: (312) 563-3552, xiu-ti_hu@rush.edu.

Conflict of interest: The authors declare that they have no conflict of interest.

Understanding how low level HIV-1 expression in the CNS, particularly during aging, contributes to HAND persistence/progression will facilitate the development of effective therapeutics for HAND. Thus, we assessed the impact of comorbid aging/HIV-1 on the activity and expression of VGCCs in mPFC pyramidal neurons, using the Tg rat: a non-infectious, *gag/pol*-deleted HIV-1 animal model that expresses 7/9 HIV-1 genes, and exhibits HAND characteristics (Vigorito et al. 2015). The Tg rat model mimics the HIV reservoir in the CNS, associated with maximum suppression of HIV replication due to combined antiretroviral therapy (Peng et al. 2010). Here, we specifically examined the effects of aging on evoked firing and Ca^{2+} spikes mediated by VGCCs in pyramidal neurons, and on L-channel expression in the mPFC of older (12m-old) Tg rats.

Material and Methods

Animals

Male Tg and non-Tg rats (Envigo, Indianapolis, IN) were housed at the Rush University Comparative Research Center with food and water available *ad libitum*. Institutional Animal Care and Use Committee-approved animal care and use procedures were conducted in accordance with NIH, USDA and institutional guidelines.

Tissue Preparation

At 6-7wks or 12m of age, rats were transcardially perfused with cutting solution (in mM: 248 sucrose, 2.9 KCl, 2 MgSO_4 , 1.25 NaH_2PO_4 , 26 NaHCO_3 , 0.1 CaCl_2 , 10 glucose, 3 kynurenic acid, 1 ascorbic acid; pH=7.4-7.45) for electrophysiology, or 0.9% saline for biochemistry. For electrophysiology, 300 μm brain slices were prepared and incubated in oxygenated artificial cerebrospinal fluid (aCSF; in mM: 125 NaCl, 2.5 KCl, 25 NaHCO_3 , 1.25 NaH_2PO_4 , 1 MgCl_2 , 2 CaCl_2 , 15 glucose; pH=7.35-7.4, 305-315 mOsm) for 1hr before recordings. For biochemistry, the mPFC was dissected; RNA and protein were measured from left and right side, respectively.

Whole-cell patch-clamp recording from ex vivo brain slices

Electrophysiology was performed as previously described (Khodr et al. 2016; Wayman et al. 2016). Briefly, layer V-VI mPFC pyramidal neurons were patched using internal solution-filled recoding electrodes [4-6 M Ω ; pH=7.3-7.35 and 280-285 mOsm; for spiking (in mM): 120 K-gluconate, 10 HEPES, 0.1 EGTA, 20 KCl, 2 MgCl_2 , 3 Na_2ATP , and 0.3 NaGTP ; for Ca^{2+} potentials (in mM): 140 Cs-gluconate, 10 HEPES, 2 MgCl_2 , 3 Na_2ATP and 0.3 NaGTP]. To evoke spiking, depolarizing current pulses (0-300pA) were applied for 500ms at 25pA intervals. Data were analyzed if (1) resting membrane potential (RMP) was stable and more hyperpolarized than -60mV (for non-Tg), and (2) initial action potential amplitude (evoked by rheobase, the minimal depolarizing current) was >60mV and 40mV, respectively, for non-Tg and Tg. VGCC activity was isolated with blockade of Na^+ channels (0.5 μM tetrodotoxin), K^+ channels (20mM extracellular tetraethylammonium, 140mM intracellular Cs-gluconate), ionotropic glutamate receptors (2.5mM kynurenic acid), and GABA_A receptors (100 μM picrotoxin). V_m was held around average RMP (~-70mV) and 40ms rheobase currents were used to elicit Ca^{2+} potentials.

Quantitative real-time PCR (qRT-PCR)

RNA was measured using a Power SYBR® Green RNA-to-C_T™ 1-Step kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems ViiA7 real-time PCR system. Target-specific primers included: β -actin (forward primer: ccgcgagtacaaccttcttgc, reverse primer: atatcgctcatccatggcgaaactgg); *CACNA1C* (forward primer: ggcatcaccaacttcgaca, reverse primer: tacaccagggaactcata); *CACNA1D* (forward primer: gagaggagggaacgaaaca, reverse primer: tctttccaccagcaccagaga). Data, expressed as fold change (2^{-C_t}), are normalized to β -actin Ct and to non-Tg mPFC.

Western blotting

SDS-PAGE-separated protein (10 μ g) was transferred to PVDF membranes (Millipore, Billerica, MA), which were blocked, incubated in primary antibody (4°C, overnight; 1:1,000 anti-Ca_v1.2, 1:1000 anti-Ca_v1.3, Alomone Labs, Jerusalem, Israel; 1:7500 anti-actin, Santa Cruz, CA), followed by secondary antibody (RT, 1 hr; 1:5000 anti-rabbit, Millipore, Billerica, MA), and developed using Supersignal™ West Pico Chemiluminescent Substrate (Thermo Fisher Scientific). ImageJ software (NIH, Washington D.C.) was used for densitometry.

Statistical Analysis

Data were analyzed with two-way ANOVA or two-way repeated measures (rm) ANOVA, followed by Newman-Keuls *post-hoc* tests, and *t*-tests. Statistical significance was set at $p < 0.05$.

Results

mPFC pyramidal neurons in older Tg rats display increased firing.

Evoked firing in mPFC pyramidal neurons was assessed. We found that the neurons from Tg rats displayed significantly-increased spike numbers compared to those from non-Tg rats (two-way rmANOVA: genotype, $F_{(1,264)}=2.532$, $p=0.125$; current, $F_{(11,264)}=62.980$, $p < 0.001$; interaction, $F_{(11,264)}=3.952$, $p=0.019$; Fig.1a-b).

Pyramidal neurons from older Tg and non-Tg rats exhibit similar voltage-sensitive Ca²⁺ spikes, but greater stimulation is needed to generate Ca²⁺ spikes in older Tg rats.

To evaluate the involvement of VGCC-mediated Ca²⁺ dysregulation in the increased pyramidal neuron excitability in older Tg rats (Fig.1c-f), glutamate/GABA inputs, Na⁺ and K⁺ channels were selectively blocked. We found that Ca²⁺ spikes (duration and amplitude) in mPFC neurons from older Tg rats were not significantly affected compared to non-Tg rats (Fig.1d-e). However, VGCCs in mPFC neurons from Tg rats were less excitable than those from non-Tg rats because greater excitatory stimulation was needed to evoke Ca²⁺ potentials in Tg rats ($t_{(17)}=4.838$, $p=0.0002$) (Fig.1f). These findings indicate that VGCC-mediated Ca²⁺ influx does not contribute to the increased firing of mPFC pyramidal neurons in older Tg rats.

L-channel protein expression is significantly reduced in the mPFC of older Tg rats, but is not affected in the mPFC of adolescent Tg rats.

We previously demonstrated that L-channels mediate abnormally-enhanced voltage-sensitive Ca^{2+} influx and hyper-excitability of mPFC neurons from adolescent (6-7wk-old) Tg rats (Khodr et al. 2016). L-channel subtypes in the cortex include an LVA ($\text{Ca}_v1.3$, *Cacna1d*) and an HVA ($\text{Ca}_v1.2$, *Cacna1c*) form (Lipscombe 2002). Here, we determined mRNA and protein levels of both L-channel subtypes in the mPFC (Fig.2). We found that *Cacna1d* mRNA levels were altered by HIV in adolescent rats and by age in non-Tg rats (two-way ANOVA: genotype, $F_{(1,27)}=3.156$, $p=0.087$; age, $F_{(1,27)}=1.550$, $p=0.224$; interaction, $F_{(1,27)}=7.087$, $p=0.013$; Fig.2a), but that *Cacna1c* mRNA levels were unchanged (Fig.2b). Specifically, *Cacna1d* mRNA levels were increased in adolescent Tg rats compared to non-Tg rats ($t_{(13)}=3.957$, $p=0.002$; Fig.2a), and in older non-Tg rats compared to adolescent non-Tg rats ($t_{(13)}=3.345$, $p=0.005$; Fig.2a). In contrast, $\text{Ca}_v1.3$ protein levels in the mPFC were not changed (Fig.2c), but $\text{Ca}_v1.2$ protein levels were altered by HIV in older rats and by age (two-way ANOVA: genotype, $F_{(1,39)}=4.466$, $p=0.041$; age, $F_{(1,39)}=23.549$, $p<0.001$; interaction, $F_{(1,39)}=0.001$, $p=0.982$; Fig.2d). Specifically, $\text{Ca}_v1.2$ protein levels were significantly reduced in the mPFC of older Tg rats compared to age-matched non-Tg rats ($t_{(21)}=3.929$, $p=0.001$; Fig.2d), and in the mPFC of older rats compared to adolescent rats, whether Tg or non-Tg (non-Tg: $t_{(20)}=3.134$, $p=0.005$; Tg: $t_{(19)}=3.873$, $p=0.001$; Fig.2d). These findings indicate that aging and chronic HIV-1 *in vivo* differentially affect the post-transcriptional regulation of L-channel expression, and ultimately L-channel protein expression.

Aging does not alter total voltage-sensitive Ca^{2+} influx, but affects some Ca^{2+} spike properties in mPFC neurons.

To determine age effects on VGCCs, we compared the Ca^{2+} spike properties in mPFC neurons between adolescent and older, Tg and non-Tg rats (Table 1). In Tg rats, aging significantly increased the rheobase required to evoke Ca^{2+} spikes ($p<0.001$; two-way rmANOVA: genotype, $F_{(1,91)}=15.127$, $p<0.001$; age, $F_{(1,91)}=20.594$, $p<0.001$; interaction, $F_{(1,91)}=6.600$, $p=0.012$), reduced the Ca^{2+} spike half-amplitude duration ($p=0.017$; two-way rmANOVA: genotype, $F_{(1,77)}=2.716$, $p=0.103$; age, $F_{(1,77)}=4.635$, $p=0.034$; interaction, $F_{(1,77)}=1.223$, $p=0.272$). Intriguingly, whole-cell Ca^{2+} influx was not significantly affected by aging because there was no difference in the entire duration/area of evoked Ca^{2+} spikes between older and adolescent rats. Aging also significantly decreased the Ca^{2+} spike amplitude ($p=0.035$) in mPFC neurons from non-Tg rats (two-way rmANOVA: genotype, $F_{(1,78)}=15.127$, $p=0.059$; age, $F_{(1,78)}=20.594$, $p=0.009$; interaction, $F_{(1,78)}=6.600$, $p=0.615$). These data suggest that aging does not significantly alter total Ca^{2+} influx through VGCCs, but alters the gating dynamics of the remaining, functional VGCCs in mPFC pyramidal neurons.

Discussion

The current study determined that HIV-1-induced alterations in VGCC function (reflected by evoked Ca^{2+} spikes) and L-channel expression in the mPFC differs with age. We found that mPFC pyramidal neurons in older Tg rats were hyper-excitabile (with increased firing)

compared to those in non-Tg rats. However, the total Ca^{2+} influx through VGCCs was unchanged in mPFC neurons from older Tg rats, but was accompanied by a significantly-decreased VGCC sensitivity to V_m depolarization, and reduced protein expression of $\text{Ca}_v1.2$ L-channels. This contrasts strikingly with $\text{Ca}_v1.2$ L-channel protein levels in adolescent Tg rats, which were unaltered compared to age-matched non-Tg rats. Together, our current and previous studies (Khodr et al. 2016; Wayman et al. 2016) demonstrate that HIV-1 differentially affects the functional activity and expression of VGCCs in the mPFC in an age-dependent manner, and suggest that ion channel dysfunction underlying HIV-induced mPFC hyper-excitability evolves with age/HIV duration.

The present study indicates that mPFC pyramidal neurons in older Tg rats remain hyper-excitable, but the underlying mechanisms differ from those in younger Tg rats. Previously, we reported abnormally-increased firing in mPFC neurons from adolescent (6-7wk) (Khodr et al. 2016) and young adult (6-7m-old) (Wayman et al. 2016) Tg rats. This increase in younger Tg rats is associated with L-channel over-activation. However, the increased excitability in older (12m) Tg rats was not accompanied by changes in Ca^{2+} influx. Importantly, these findings reveal that the abnormally-increased excitability of mPFC neurons in aging Tg rats does not result from VGCC over-activation, but likely is mediated by dysfunction of other membrane ion channels, including K^+ channels and those regulating synaptic activity. Indeed, our parallel studies suggest that reduced voltage-gated K^+ channel activity contributes to the abnormally-increased mPFC neuronal excitability in older Tg rats (Chen et al. 2016).

Alterations in excitatory/inhibitory neurotransmission could also affect HIV-induced mPFC hyperactivity. For example, HIV-1 increases activity of ionotropic glutamate receptors (Epstein 1998; Mattson et al. 2005), and reduces GABAergic neurotransmission (Buzhdygan et al. 2016). Such changes could facilitate V_m depolarization and promote firing. Although changes in synaptic activity were not reflected in the current assessment of Ca^{2+} influx due to blockade of ionotropic glutamate receptors and GABA_A receptors, potential HIV-1-induced alterations in glutamate/GABA-mediated synaptic activity could also underlie the increased firing, where tonic glutamatergic and GABAergic neurotransmission was not blocked.

The present study also indicates that HIV-1 and age differentially affect VGCC activity and expression in the mPFC. We show that, in mPFC neurons from adolescent (6-7wk-old) Tg rats, the total L-channel protein levels are unaltered (Fig.2c-d), but L-channel-mediated Ca^{2+} influx is enhanced (Khodr et al. 2016), indicating HIV-1-induced L-channel over-activation in these rats. Similarly, L-channel over-activation is found in young adult Tg rats (6-7m-old) because selective L-channel blockade abolishes the increased spiking in mPFC neurons of these rats (Wayman et al. 2016). In contrast, L-channel hyperactivity in the mPFC of older (12m) Tg rats is also suggested, although it differs dramatically from those seen at younger ages due to unaltered VGCC-mediated Ca^{2+} influx, associated with a significant decrease in $\text{Ca}_v1.2$ L-channel protein levels. Furthermore, aging in general (whether in non-Tg or Tg rats) reduces $\text{Ca}_v1.2$ L-channel protein levels (reflecting a reduced number of channels) in the mPFC without producing significant alterations in the total Ca^{2+} influx through VGCCs. Together, these findings suggest that L-channel activity remains abnormally increased in the

HIV-infected mPFC during aging (from 6-7wks, 6m, and likely 12m), even with reduced L-channel number. Future studies examining more advanced ages (e.g., 24m) would provide a better understanding of how and to what extent HIV and age affect Ca^{2+} channel function.

The exact mechanism(s) by which HIV-1 and age differentially affect Ca^{2+} influx *via* VGCCs in mPFC pyramidal neurons is unknown, but likely results from compensatory neuronal responses to the enhanced Ca^{2+} influx at younger ages. Because significant Ca^{2+} alterations cause neuronal dysregulation, injury, and death (Gleichmann and Mattson 2011; Hu 2015), VGCC activity, which couples membrane depolarization with Ca^{2+} influx, must be properly regulated. Cell surface expression and functional activity of VGCCs are regulated by various mechanisms that operate over a range of time frames, including but not limited to rapid calmodulin-based/ Ca^{2+} -dependent inactivation, and long-term activity-dependent Ca^{2+} channel internalization, and channel cleavage (Brini et al. 2014; Groth et al. 2014). Therefore, the unaltered total Ca^{2+} influx *via* VGCCs found in mPFC neurons of older Tg rats could result from long-term, activity-dependent negative-feedback regulation (which may include, but is not limited to, channel internalization/proteolytic processing) that is triggered by earlier HIV-induced Ca^{2+} dysregulation in younger Tg rats.

An important consideration is how our current findings relate to the disease progression of HAND. Although behavioral deficits of Tg rats associated with the observed mPFC dysfunction were not assessed in the present study, the HIV-1 Tg rat is reported to display neurocognitive deficits that resemble those seen in HAND at various ages (Vigorito et al. 2015). These behavioral impairments include alterations in spatial memory, motivation, behavioral plasticity, habituation, and executive function (Vigorito et al. 2015). Of the few published longitudinal assessments of neurocognitive function in Tg rats, alterations in temporal processing (measured by prepulse inhibition of a startle response) have been thoroughly studied at varying ages and have been found to persist into advanced ages (from 1-10m of age) (McLaurin et al. 2016; McLaurin et al. 2017; Moran et al. 2013a; Moran et al. 2013b; Vigorito et al. 2015). Of specific interest to our current findings, Tg rats display differences in the development of temporal processing (which plateaus at 3m of age for Tg) compared to non-Tg rats (McLaurin et al. 2016). It is possible that the differing age-related mechanisms of mPFC hyper-excitability we have found in the present study could contribute to the mPFC dysfunction underlying this progression of deficits in temporal processing. Further longitudinal studies have reported a consistent increase in emotionality and anxiety in Tg rats at ages 2m to 11m, as determined by greater time spent in the periphery in the open field test (Moran et al. 2013b; Reid et al. 2016). Our observations of differing mPFC dysfunction could contribute to these behavioral deficits at different ages.

Collectively, the present study demonstrates that HIV-1 alters the nature of VGCC and likely other ion channel dysregulation that underlies mPFC hyper-excitability through disease progression and during aging. Although further studies are needed to identify the channels that contribute to the HIV-induced mPFC hyper-excitability in aging, our novel findings contribute to a better understanding of how aging affects HIV-1 neuropathogenesis, and therefore are informative and clinically relevant for the aging HIV^{+} population.

Acknowledgements:

This study was supported by the National Institutes of Health (USPHSGs NS084817 to X-TH, and DA033966 & NS060632 to LA).

Funding Information: National Institute of Neurological Disorders and Stroke (NS084817), Dr. Xiu-Ti Hu, National Institute on Drug Abuse (DA033966), Dr. Lena Al-Harhi, National Institute of Neurological Disorders and Stroke (NS060632), Dr. Lena Al-Harhi

Reference List

- Brini M, Cali T, Ottolini D, Carafoli E (2014) Neuronal calcium signaling: function and dysfunction. *Cell Mol. Life Sci* 71: 2787–2814. [PubMed: 24442513]
- Buzhdygan T, Lisinicchia J, Patel V, Johnson K, Neugebauer V, Paessler S, Jennings K, Gelman B (2016) Neuropsychological, Neurovirological and Neuroimmune Aspects of Abnormal GABAergic Transmission in HIV Infection. *J. Neuroimmune. Pharmacol* 11: 279–293. [PubMed: 26829944]
- Chen L, Khodr CE, Al-Harhi L, and Hu X-T 2016 Excitability of mPFC pyramidal neurons is increased in older HIV-1 transgenic rats through alteration in K⁺ channel activity.
- Epstein LG (1998) HIV neuropathogenesis and therapeutic strategies. *Acta Paediatr Jpn* 40: 107–111. [PubMed: 9581298]
- Ferris MJ, Mactutus CF, Booze RM (2008) Neurotoxic profiles of HIV, psychostimulant drugs of abuse, and their concerted effect on the brain: Current status of dopamine system vulnerability in NeuroAIDS. *Neurosci. Biobehav. Rev* 32: 883–909. [PubMed: 18430470]
- Gill AJ, Kolson DL (2014) Chronic inflammation and the role for cofactors (hepatitis C, drug abuse, antiretroviral drug toxicity, aging) in HAND persistence. *Curr. HIV. /AIDS Rep* 11: 325–335. [PubMed: 24929842]
- Gleichmann M, Mattson MP (2011) Neuronal calcium homeostasis and dysregulation. *Antioxid. Redox. Signal* 14: 1261–1273. [PubMed: 20626318]
- Groth RD, Tirko NN, Tsien RW (2014) CaV1.2 calcium channels: just cut out to be regulated? *Neuron* 82: 939–940. [PubMed: 24908477]
- Heaton RK, Clifford DB, Franklin DR Jr., Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, Marcotte TD, Atkinson JH, Rivera-Mindt M, Vigil OR, Taylor MJ, Collier AC, Marra CM, Gelman BB, McArthur JC, Morgello S, Simpson DM, McCutchan JA, Abramson I, Gamst A, Fennema-Notestine C, Jernigan TL, Wong J, Grant I (2010) HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* 75: 2087–2096. [PubMed: 21135382]
- Hu X-T (2015) HIV-1 Tat-induced calcium dysregulation and neuronal dysfunction in vulnerable brain regions. *Current Drug Targets* in press.
- Khodr CE, Chen L, Dave S, Al-Harhi L, Hu XT (2016) Combined chronic blockade of hyperactive L-type calcium channels and NMDA receptors ameliorates HIV-1 associated hyper-excitability of mPFC pyramidal neurons. *Neurobiol. Dis* 94: 85–94. [PubMed: 27326669]
- Lipscombe D (2002) L-type calcium channels - Highs and new lows. *Circ. Res* 90: 933–935. [PubMed: 12016257]
- Mattson MP, Haughey NJ, Nath A (2005) Cell death in HIV dementia. *Cell Death. Differ* 12 Suppl 1: 893–904. [PubMed: 15761472]
- McLaurin KA, Booze RM, Mactutus CF (2016) Progression of temporal processing deficits in the HIV-1 transgenic rat. *Sci. Rep* 6: 32831. [PubMed: 27596023]
- McLaurin KA, Moran LM, Li H, Booze RM, Mactutus CF (2017) A Gap in Time: Extending our Knowledge of Temporal Processing Deficits in the HIV-1 Transgenic Rat. *J. Neuroimmune. Pharmacol* 12: 171–179. [PubMed: 27699630]
- Moran LM, Booze RM, Mactutus CF (2013a) Time and time again: temporal processing demands implicate perceptual and gating deficits in the HIV-1 transgenic rat. *J. Neuroimmune. Pharmacol* 8: 988–997. [PubMed: 23690140]

- Moran LM, Booze RM, Webb KM, Mactutus CF (2013b) Neurobehavioral alterations in HIV-1 transgenic rats: evidence for dopaminergic dysfunction. *Exp. Neurol* 239: 139–147. [PubMed: 23063600]
- Peng J, Vigorito M, Liu X, Zhou D, Wu X, Chang SL (2010) The HIV-1 transgenic rat as a model for HIV-1 infected individuals on HAART. *J. Neuroimmunol* 218: 94–101. [PubMed: 19913921]
- Reid WC, Casas R, Papadakis GZ, Muthusamy S, Lee DE, Ibrahim WG, Nair A, Koziol D, Maric D, Hammoud DA (2016) Neurobehavioral Abnormalities in the HIV-1 Transgenic Rat Do Not Correspond to Neuronal Hypometabolism on 18F-FDG-PET. *PLoS. ONE* 11: e0152265. [PubMed: 27010205]
- Vigorito M, Connaghan KP, Chang SL (2015) The HIV-1 transgenic rat model of neuroHIV. *Brain Behav. Immun*
- Wayman WN, Chen L, Hu XT, Napier TC (2016) HIV-1 transgenic rat prefrontal cortex hyperexcitability is enhanced by cocaine self-administration. *Neuropsychopharmacology*: 1–9. [PubMed: 26657948]

Fig. 1. mPFC pyramidal neurons from older Tg rats display an increase in evoked firing, accompanied by reduced VGCC responses.

(a) Sample traces from a mPFC pyramidal neuron from a non-Tg (left) or Tg (right) rat showing evoked firing (at 75, 150 and 225pA). (b) The evoked spike frequency (current-response) is graphed, showing the number of firings at each current step in mPFC neurons from non-Tg (open circles, n=7 in 6 rats) or Tg rats (filled circles, n=18 in 8 rats). Spike numbers in neurons from Tg rats were significantly greater than those from non-Tg rats, (c) Sample traces from a mPFC neuron in a non-Tg or Tg rat showing Ca²⁺ spikes, which reflect voltage-gated Ca²⁺ influx, (d, e) The duration (d) and area (e) of the Ca²⁺ potentials are graphed, showing that Ca²⁺ influx through VGCCs in neurons from Tg rats (hatched bars, n=11 in 5 rats) was not significantly different from that in non-Tg rats (open bars, n=10 in 6 rats), (f) The bar graph shows the rheobase (the minimal current stimulation required to generate a Ca²⁺ spike; Tg: n=10 neurons in 4 rats; non- Tg: n=9 neurons in 6 rats). Note that greater excitatory stimulation was needed to evoke a Ca²⁺ potential in neurons from Tg rats, although Ca²⁺ influx was not significantly affected. Significance is denoted as *p 0.05, ***p 0.001.

Fig.2. L-channel protein expression is significantly reduced in the mPFC of older Tg rats, but is unaltered in the mPFC of adolescent Tg rats.

L-channel mRNA and protein levels were measured in mPFC dissected from 6-7wk-old and 12m-old, Tg and non-Tg rats, (a-b) The bar graphs show relative levels of L-channel mRNA normalized to β -actin mRNA and 12m non-Tg for the LVA-Ca_v1.3 (Cacna1d mRNA, n=7-8, a) and the HVA-Ca_v1.2 (Cacna1c mRNA, n=7-8, b) L-channels. (c-d) Sample western blots and bar graphs show relative levels of L-channel protein normalized to β -actin protein and to 12m non-Tg for the LVA-Ca_v1.3 L-channel (n=7-8, c) and the HVA-Ca_v1.2 L-channel (n=10-12, d). Asterisks represent significance based on Newman-Keuls *post-hoc* tests (following two-way ANOVA), and ϕ represents significance based on *t*-tests. Significance is denoted as *p 0.05; $\phi\phi$,** *p* 0.01; $\phi\phi\phi$,*** *p* 0.001.

Aging does not alter total voltage-sensitive Ca^{2+} influx, but affects some Ca^{2+} spike properties in mPFC neurons.

Table 1:

	12m Non-Tg		12m HIV-1 Tg		6-7wk Non-Tg		6-7wk HIV-1 Tg	
	Mean±SEM	N	Mean±SEM	N	Mean±SEM	N	Mean±SEM	N
Rheobase (pA)	192.2 ± 11.6	9	256.0 ± 6.9 ^{***}	10	172.8 ± 7.5	27	185.8 ± 5.8 ^{ΔΔΔ}	49
Threshold (mV)	-21.3 ± 2.0	10	-20.6 ± 1.2	11	-22.9 ± 0.8	23	-22.2 ± 0.7	40
Amplitude (mV)	56.8 ± 2.2	10	60.8 ± 1.8	11	62.0 ± 1.4 ^Δ	23	64.4 ± 1.0	38
1/2 amplitude duration (s)	1.1 ± 0.1	9	1.3 ± 0.2	10	1.5 ± 0.2	22	2.6 ± 0.3 ^{**Δ}	40
Entire duration (s)	8.9 ± 1.3	10	9.3 ± 1.3	11	7.9 ± 0.9	26	11.9 ± 0.9 ^{**}	49
Entire area (KmV [*] ms)	584.4 ± 80.9	10	650.1 ± 96.7	11	529.7 ± 64.2	26	791.2 ± 66.3 ^{**}	48

* differences between genotypes

Δ differences between ages

*Δ, $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$.