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Reduced intrinsic excitability of CA1 pyramidal neurons in HIV transgenic rats

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Abstract

The hippocampus is involved in key neuronal circuits for cognition, memory and anxiety, and it is increasingly recognized as a vulnerable structure in neuroHIV contributing to the pathogenesis of HAND. However, the mechanisms contributing to hippocampal dysfunction in neuroHIV remain unknown. Here we used HIV transgenic (Tg) rats and patch-clamp electrophysiological techniques to study the effect of chronic low-level expression of HIV viral proteins on hippocampal CA1 pyramidal neurons. Since the dorsal and ventral areas of the hippocampus are involved in different neural circuits, they were evaluated separately. We found a significantly decreased intrinsic excitability in CA1 neurons in the dorsal hippocampus of HIV Tg rats by comparison of threshold neuronal spiking by current step injection and by dynamic clamp to simulate neuronal spiking activity. Decreased excitability in the dorsal hippocampus was accompanied by elevated rate of excitatory postsynaptic currents (EPSCs), while CA1 pyramidal neurons in the ventral hippocampus of HIV Tg rats showed increased EPSC amplitudes. We also observed reduced hyperpolarization-activated non-specific cationic current (I_h) in both dorsal and ventral hippocampus. As neurotoxic HIV proteins have been shown to increase neuronal excitation, reduced excitability of CA1 pyramidal neurons reported here may represent a maladaptive homeostatic plasticity aimed at stabilizing baseline neuronal firing activity but that may disrupt neural network functioning contributing to HIV-associated neuropsychological disorders, such as HAND and depression.

Introduction.

Despite the success of combined anti-retroviral therapy (cART) in suppressing viral replication in HIV patients, the incidence of HIV-associated neurocognitive disorder (HAND) has increased and its prevalence remains stable (Robertson, Smurzynski et al. 2007, Lescure, Omland et al. 2011). HIV-associated cognitive impairment and brain injury persist in the setting of chronic and stable disease in the cART setting (Harezlak, Buchthal et al. 2011, Yilmaz, Yiannoutsos et al. 2013). The mild form of HAND is expressed as

deficiency in memory, concentration, attention, motor skills and other symptoms leading to a decline in the patient's everyday functioning and overall quality of life (Woods, Moore et al. 2009, Doyle, Morgan et al. 2013). Neuroinflammatory changes in HIV-infected individuals persist despite the widespread use of cART (Harezlak, Buchthal et al. 2011, Yilmaz, Yiannoutsos et al. 2013). In addition, evidence indicates that cART itself can have detrimental effects on learning efficiency and complex attention (Cysique, Maruff et al. 2004).

Multiple HIV proteins are neurotoxic, including gp120 and Tat, and evidence indicates that most HIV proteins, such as gp41, Nef and *Vpr*, among others, also possess neurotoxic potential (Adamson, Kopnisky et al. 1999, Trillo-Pazos, McFarlane-Abdulla et al. 2000, Patel, Mukhtar et al. 2002, van de Bovenkamp, Nottet et al. 2002, Jones, Barsby et al. 2007). In particular, neurotoxic proteins such as gp120 (Lannuzel, Lledo et al. 1995, Wang and White 2000, Marchionni, Beaumont et al. 2012, Potter, Figuera-Losada et al. 2013) and Tat (Magnuson, Knudsen et al. 1995, Nath, Psooy et al. 1996, Song, Nath et al. 2003) have been shown to increase neuronal excitation. HIV-1 gp120 has been shown to potentiate N-methyl-D-aspartate (NMDA)-induced Ca^{2+} responses (Lannuzel, Lledo et al. 1995). Tat has been shown to increase both NMDA and non-NMDA receptor function (Magnuson, Knudsen et al. 1995, Nath, Psooy et al. 1996, Song, Nath et al. 2003).

The hippocampus is a key brain structure involved in cognition, episodic memory, spatial orientation and other cognitive functions. In humans, pathologic changes in the hippocampus are associated with mild cognitive impairment and dementia (Barber, Ballard et al. 2000, Pennanen, Kivipelto et al. 2004). There is growing evidence that pathologic changes of the hippocampus contribute to HAND (Wade, Valcour et al. 2015, Levine, Soontornniyomkij et al. 2016). For example, postmortem examination of HIV-positive brains revealed that levels of neuroinflammatory markers were highest in the hippocampus and comparable to those found in pre-HAART neuropathologic studies (Anthony, Ramage et al. 2005). A functional MRI study found that hippocampal function in HIV-positive, well-educated men was reduced compared to that in their control counterparts (Castelo, Sherman et al. 2006).

The detrimental effect of HIV on the hippocampus has been demonstrated in experiments using animal models. HIV proteins Tat or gp120 injected into the mouse hippocampus or expressed in transgenic mice or rats cause synaptic dysfunction, upregulate expression of inflammatory mediators, impair LTP and negatively affect animal performance in various behavioral paradigms involving hippocampus function (Pu, Tian et al. 2003, Behnisch, Francesconi et al. 2004, Li, Matsushita et al. 2004, Okamoto, Kang et al. 2007, Fitting, Booze et al. 2008, Li, Li et al. 2009, Fitting, Ignatowska-Jankowska et al. 2013, Repunte-Canonigo, Lefebvre et al. 2014) and low level expression of these proteins continue to be present in the human brain despite the antiretroviral therapy (Canestri, Lescure et al. 2010, Peluso, Ferretti et al. 2012).

Here we used HIV-1 Tg rats that express all but two of the genes contained in the HIV-1 virus, allowing for expression of functional viral proteins without active viral replication (Reid, Sadowska et al. 2001). This non-replicating HIV-1 Tg line has been characterized as a

model of cART-controlled HIV-1 infection due to the lack of viral replication but continued presence of low level expression of HIV-1 proteins (Peng, Vigorito et al. 2010). Similar to HIV-1 infected humans, HIV-1 Tg rats express multiple HIV products in microglia and astrocytes, but not in neurons (Reid, Sadowska et al. 2001, Royal, Zhang et al. 2012, Repunte-Canonigo, Lefebvre et al. 2014). Previously, we demonstrated broad dysregulation of genes involved in inflammation, neuronal trophism, synaptodendritic injury, and neurodegeneration in the hippocampus of HIV Tg rats (Repunte-Canonigo, Lefebvre et al. 2014). Importantly, we observed that the gene expression changes in this animal model (Repunte-Canonigo, Lefebvre et al. 2014) show strong functional similarities with gene expression changes in humans with HIV without HIV-encephalitis (HIVE) from the National NeuroAIDS Tissue Consortium (NNTC) microarray dataset (Sanna, Repunte-Canonigo et al. 2017).

Despite the growing interest in the role of the hippocampus in pathological processes during HAND, cellular and molecular mechanisms leading to the HIV-induced damage of the hippocampus remain largely unknown. In the present study, we used the HIV-1 Tg rats and the patch-clamp technique to compare synaptic and intrinsic membrane properties of CA1 pyramidal neurons of the hippocampus from diseased rats with those from control, non-transgenic, rats. We found changes in synaptic, and passive and active membrane properties of CA1 pyramidal neurons characterized by increased excitatory drive onto CA1 pyramidal neurons and reduced excitability, which are suggestive of adaptations aimed at maintaining stability in neuronal output via changes in intrinsic properties. The adaptive maintenance of functional stability in response to pathologic challenges or allostasis (Koob and Le Moal 2001) produces a cost to the systems - allostatic load - that can ultimately lead to a disease state. Since CA1 pyramidal neurons are required to fire at specific time scales and synchrony in the course of memory encoding and recall (Stella and Treves 2011, Rolls 2018, Sanders, Ji et al. 2019), the decreased intrinsic excitability of CA1 pyramidal in response to pathologically increased excitatory drive, may underlie memory deficits in the setting of neuroHIV.

Methods.

Animals.

HIV Tg and wild-type rats backcrossed to Wistar for 4–5 generations were used for the study. Female rats were used due to evidence that women with HIV may be more vulnerable to develop cognitive impairments than men (Fogel, Rubin et al. 2017, Keutmann, Gonzalez et al. 2017). *In vitro* electrophysiological experiments were performed on rats 6 – 8 months of age. All protocols and procedures were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. Rats were housed on a 12:12 h light-dark schedule at constant temperature and humidity. Prior to decapitation, rats were anesthetized with isoflurane (Webster Veterinary Supply Inc., Sterling, MA) and perfused transcardially with the dissection ACSF (see below). After decapitation, the head was placed in an ice-cold artificial cerebrospinal fluid (ACSF) and the brain was then quickly isolated, submerged in oxygenated ice-cold ACSF and glued on a Leica 1200 S vibratome (Leica Microsystems Inc., Buffalo Grove, IL) stage.

Electrophysiology.

For experiments in the dorsal or ventral areas of the hippocampus we used coronal or horizontal sections of the hippocampus, respectively. 300 μ m thick slices were prepared in ice-cold, dissection ACSF containing (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 0.5 CaCl₂, 4 MgCl₂ and 10 glucose (95% O₂/5% CO₂). Immediately after the slicing procedure, the slices were incubated at 32°C in a low-sodium solution for 12 min using the method developed by Ting et al. (Ting, Daigle et al. 2014). Then slices were placed in normal ACSF (in mM: 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 2 CaCl₂, 2 MgCl₂ and 10 glucose (95% O₂/5% CO₂)) and incubated at room temperature for the next 6 h, until the end of experiments. Recordings were performed in drug-free normal ACSF at 32°C. Cells were visualized with infrared differential interference contrast (IR-DIC) utilizing a water immersion objective (Nikon Instruments Inc., Melville, NY).

Patch pipettes were pulled from borosilicate glass tubing (1.5 mm outer diameter, 0.3 mm wall thickness) on a horizontal puller (P-97; Sutter Instrument, Novato, CA) and used without further modifications. Pipettes, with the resistance in the bath ranging from 4.0 to 7.0 M Ω , were filled with internal solution of the following composition (in mM): 155 potassium gluconate, 5 NaCl, 0.5 EGTA, 0.1 CaCl₂, 2 MgATP, 5 HEPES, 0.3 NaGTP, 2 phosphocreatine; pH adjusted to 7.2 with KOH. Somatic whole-cell recordings in CA1 pyramidal cells were made with a MultiClamp 700B Amplifier (Molecular Devices, Sunnyvale, CA). Data were filtered at 2 kHz and sampled at 20 kHz with National Instruments PCI-MIO-16-E4 multifunctional board controlled by the data acquisition software DASyLab 6.0 (Dasytec, Amherst, NH).

Data on EPSCs, neuronal excitability, voltage sag and voltage-gated currents were obtained from each individual neuron used in experiments performed in drug-free ACSF. Avoiding ion channel blockers helped in reproducing in vivo conditions which is particularly important while analyzing neuronal excitability. It has been previously established that EPSCs in hippocampal neurons recorded at –65 mV in the absence of blockers of NMDA, metabotropic or GABA receptors are specific to AMPA receptors and are not contaminated by currents through other ion channels (Spruston and Johnston 1992, Sokolova, Lester et al. 2006, Sokolova and Mody 2008, Sokolova, Schneider et al. 2015).

Recordings of EPSCs were performed at –65 mV holding voltage immediately after obtaining the whole-cell configuration of the patch clamp. The cells were then current-clamped at 0 pA and depolarized by applying steps of positive currents at 10–20 pA increments, to record action potentials (APs). Parameters of the APs and the repolarization phases were analyzed at minimal suprathreshold currents. Current-clamp recordings were followed by recordings of the voltage-gated currents. Voltage-gated currents were induced by 400-ms long voltage steps from the initial holding potential of –65 mV to voltages ranging from –105 mV to –45 mV in 10 mV increments. Recordings were not adjusted for the liquid junction potential prior to the analysis.

EPSCs were analyzed using a custom-made program. Asymmetric events of negative polarity with the rise time shorter than the decay time and amplitudes >4 pA (the threshold

for event detection) were chosen for the analysis. EPSC frequency and amplitudes were established by analyzing 90- to 240-s-long records.

Dynamic clamp protocol has been described previously (Nowotny, Szucs et al. 2006, Szucs, Berton et al. 2010, Szucs, Berton et al. 2012). Briefly, StpC dynamic clamp software was used to generate stimulus waveforms. Firing activity in the CA1 neurons were elicited by stimulating them with either regularly spaced simulated excitatory stimuli or a barrage of excitatory and inhibitory synaptic inputs resembling naturally occurring synaptic activity in the hippocampus (for details, see (Szucs, Berton et al. 2010)). The excitatory and inhibitory voltage waveforms were coupled to the analog inputs of the dynamic clamp computer. Neuronal response was recorded by the DASyLab 6.0 program (Dasytec, Amherst, NH). Dynamic clamp data were analyzed with a custom-made program. Experiments with unstable resting membrane potential (RMP) or with RMP changing more than 2 mV during the dynamic clamp recordings were not considered for analysis.

Results.

Reduced neuronal excitability in the hippocampus of HIV Tg rats with a conventional stimulation protocol.

CA1 pyramidal neurons are devoid of endogenous pacemaking activity and fire APs primarily in response to depolarizing action of their excitatory afferents. The propensity of a neuron to respond to depolarizing stimuli by firing APs, operationally referred to as neuronal excitability, is determined by the intrinsic membrane properties of the neuron. We assessed excitability of CA1 neurons of the hippocampus in HIV Tg rats and control wild-type rats first using a conventional protocol of neuronal stimulation by injection of current steps of rectangular shape and progressively increasing amplitudes. The amplitudes of the minimal injected currents triggering AP firing (threshold stimulation current, $I_{\min}$) in CA1 pyramidal neurons from HIV Tg rats were higher than those in control neurons. In the dorsal hippocampus the $I_{\min}$ in HIV Tg rats was significantly higher than that in the control (dorsal hippocampus: 254.3 ± 28.7 vs. 362.5 ± 50.1 (pA), $p < 0.03$, t-test, $n = 13/8$ [3/2 rats]). This trend toward elevated $I_{\min}$ in HIV Tg rat neurons was also observed in the ventral areas of the hippocampus, however it did not reach significance (ventral hippocampus: 110.1 ± 16.1 vs. 144.5 ± 18.0 (pA), $n = 9/11$ [3/2 rats], Figure 1 A). In addition to the elevated $I_{\min}$, HIV Tg rat neurons tended to fire APs at a reduced firing rate. This difference in firing rate frequency, however, did not reach statistical significance (repeated measures two-way Anova, Figure 1 B).

Reduced excitability observed in HIV Tg rat neurons are reflective of alterations in their passive and/or active membrane properties. Altered membrane properties, such as altered characteristics of transmembrane currents, can potentially change kinetics of APs. Indeed, analysis of the kinetics of APs in experiments with direct current injection revealed slower rise time and longer 1/2 width of the APs in HIV Tg rat neurons, compared to those in control. We observed these changes in the AP rise time in both the dorsal (0.78 ± 0.03 ms vs. 0.68 ± 0.02 ms, $n = 8-13$, $p < 0.002$, t-test, in HIV Tg and control rats, respectively) and the ventral (0.78 ± 0.02 vs. 0.66 ± 0.03 , $n = 8-11$, $p < 0.004$, t-test, in HIV Tg and control rats, respectively, Figure 2 A) areas of hippocampus. Similarly, the AP 1/2 width was also altered

in the HIV Tg rats in both the dorsal (1.1 ± 0.02 vs. 1.02 ± 0.02 , $n=11-13$, $p<0.02$, t-test, in HIV and control rats, respectively) and the ventral (1.11 ± 0.03078 vs. 0.99 ± 0.03 , $n=9-11$, $p<0.001$, $n=9-11$, $p<0.02$, t-test, in HIV Tg and control rats, respectively, Figure 2 B) areas of hippocampus.

Other characteristics of the APs in neurons from the two experimental groups were not significantly different. The threshold for spike initiation (V_{Na}) was similar in the neurons from both groups in both the dorsal (-42.9 ± 1.51 mV vs. -41.0 ± 0.95 mV, $n=8-13$, in control and HIV Tg rats, respectively, data not shown) and the ventral (-40.8 ± 1.0 mV vs. -42.4 ± 0.97 mV, $n=12-16$, in control and HIV Tg rats, respectively, data not shown) areas of the hippocampus. Similar were also the AP amplitudes in the neurons from the dorsal (97.46 ± 1.26 mV vs. 94.28 ± 1.409 mV, $n=8-13$, in control and HIV Tg rats, respectively, data not shown) and the ventral (90.6 ± 1.87 pA vs. 88.1 ± 1.18 pA, $n=12-16$, in control and HIV Tg rats, respectively, data not shown) areas of the hippocampus.

Importantly, HIV Tg rats showed altered resting membrane potential (E_{RMP}) of CA1 neurons, in both the dorsal and the ventral hippocampus. Neurons in HIV Tg rats displayed significantly more negative E_{RMP} than their counterparts in the control group (dorsal hippocampus: -73.1 ± 1.49 mV vs. -70.1 ± 0.99 mV, $n=8-13$, in the HIV Tg and control rats, respectively, $p<0.05$, t-test; ventral hippocampus: -70.2 ± 1.39 mV vs. -62.3 ± 1.23 mV, $n=9-11$, in the HIV Tg and control rats, respectively, $p<0.0004$, t-test). Other passive membrane properties of the CA1 neurons of the hippocampus were not altered by HIV. The membrane resistance (R_m) in the dorsal hippocampus was: 136.0 ± 9.19 M Ω and 138.8 ± 8.61 M Ω , $n=9-14$, in the control and HIV Tg rat neurons, respectively; in the ventral hippocampus the R_m was: 168.2 ± 18.06 M Ω and 162.1 ± 14.44 M Ω , $n=13-15$, in the control and HIV Tg rat neurons, respectively. The corresponding values of the membrane capacitance (C_m) in the dorsal hippocampus were: 157.2 ± 5.85 pF vs. 167 ± 8.3 pF, $n=9-14$, in control and HIV Tg rat neurons, respectively. In the ventral hippocampus the C_m were 140.1 ± 16.22 pF vs. 150.8 ± 18.25 pF, $n=13-15$, in control and HIV Tg rat neurons, respectively.

Reduced neuronal excitability in the hippocampus of HIV Tg rats with a dynamic clamp protocol.

The dynamic clamp method allows for the dynamic control of the injected current with the purpose of better reproducing natural synaptic inputs in neurons (Robinson and Kawai 1993, Sharp, O'Neil et al. 1993, Chance and Abbott 2009, Economo, Fernandez et al. 2010). The current injected into the cell membrane using the dynamic clamp is calculated using computation methods and then readjusted with high frequency (>10 kHz) to take into account continuously changing membrane potential of the neuron (Chance and Abbott 2009, Economo, Fernandez et al. 2010). Using the dynamic clamp, we injected a barrage of regularly spaced or stochastic, computer-generated synaptic inputs consisting of excitatory and inhibitory presynaptic waveforms of various frequencies. The dynamic clamp allows for changing the conductance of artificial synapses in a way that produce vigorous and reproducible neuronal spiking activity. The minimal synaptic conductance which triggered

neuronal spiking during each of stimulation patterns were compared between the control and HIV Tg rat neurons.

In the dorsal hippocampus, the minimal conductance that triggered spiking activity during all 4 stimulation patterns was significantly higher in HIV Tg rat neurons than that in control neurons ($n=8-14$, $p < 0.02$, $p < 0.03$, $p < 0.03$, $p < 0.04$, for stimulations EPSCs, Burst, 30 Hz, and 60 Hz, respectively, t-test, Figure 3 A). In the ventral hippocampus, the minimal strength of artificial stimulation to trigger neuronal spiking was also elevated in HIV Tg rat neurons but the difference between the groups was below the significance level (Figure 3 A).

Reduced hyperpolarization-activated non-specific cationic current (I_h) in the CA1 of HIV Tg rats.

Depolarization-activated steady-state membrane currents, contributed mostly by the non-inactivating delayed rectifier K^+ currents, were not affected by HIV (data not shown). However, the hyperpolarization-activated current contributed by the non-specific cationic current I_h was significantly reduced in the CA1 neurons of the hippocampus from HIV Tg rats, both in the dorsal and the ventral areas of the hippocampus. In the dorsal hippocampus, the difference in the I_h between the two groups of neurons reached statistical significance at membrane voltages more negative than -95 mV ($n = 9-13$, $p < 0.004$, repeated measures two-way Anova, Figure 4 A). In the ventral hippocampus, the I_h in CA1 neurons from HIV Tg rats was significantly lower than that in the control group at -115 mV membrane depolarization ($n = 11$, $p < 0.04$, repeated measures two-way Anova, Figure 4 A).

Consistent with the results on hyperpolarization-activated currents, the voltage sag (V_h) values in the current-clamp recordings at hyperpolarized potentials were also significantly different. The V_h is observed in the whole-cell recordings using the current-clamp mode during injection of hyperpolarizing current steps. Voltage sag during these recordings originate as a result of partial membrane depolarization following activation of the I_h . In the dorsal hippocampus, the V_h was significantly reduced in HIV Tg rat neurons hyperpolarized to voltages more negative than -105 mV ($n = 9-13$, $p < 0.05$, repeated measures two-way Anova, Figure 4 B). In the ventral hippocampus, the difference in the V_h between the control and HIV Tg rat neurons was even more pronounced than that in the dorsal hippocampus. The V_h in this area was significantly smaller in HIV Tg rat neurons than in the control at voltages more negative than -90 mV ($n = 11$, $p < 0.05$, repeated measures two-way Anova, Figure 4 B).

Increased excitatory inputs to CA1 neurons of HIV Tg rats.

The level of excitatory inputs, along with the intrinsic membrane properties of the postsynaptic neuron, has a profound role in stimulating neuronal activity and information coding in the hippocampus. We evaluated excitatory postsynaptic currents (EPSCs) in CA1 neurons in terms of the amplitude of individual EPSCs and their frequency. In both the dorsal and the ventral hippocampus, HIV increased excitatory inputs to CA1 neurons, albeit in two distinct ways. In the dorsal hippocampus, CA1 neurons displayed elevated of EPSC frequency while the EPSC amplitudes remained similar in the control and HIV Tg rats

(Figure 5 A, B). In the ventral hippocampus, to the contrary, CA1 neurons had enlarged EPSC amplitudes and the unchanged rate of EPSC events (Figure 5 A, B).

Discussion.

The main findings of the present study are that the intrinsic excitability of pyramidal neurons in the CA1 of the dorsal hippocampus was decreased in HIV Tg rats as compared to wild-type control rats. In particular, the magnitudes of the threshold stimulation currents were significantly greater in CA1 pyramidal neurons of the dorsal hippocampus of HIV Tg rats as measured both with a conventional stimulation protocol consisting of injection of rectangular direct current (DC) steps and by dynamic clamp. In the ventral hippocampus of HIV Tg rats there was a similar trend towards decreased excitability, which did not reach statistical significance. Concomitantly with reduced excitability, we observed that excitatory synaptic inputs assessed as mean frequency of mEPSCs, were significantly increased in dorsal CA1 neurons of the hippocampus from HIV Tg rats as compared with wild-type rats. We also observed a decrease of the I_h both in the dorsal and the ventral areas of the hippocampus in HIV Tg rats as compared to wild-type control rats.

The hippocampus is involved in key neuronal circuits for cognition, memory and anxiety, and it is increasingly recognized as a vulnerable structure in neuroHIV contributing to the pathogenesis of HAND (Anthony, Ramage et al. 2005, Castelo, Sherman et al. 2006, Wade, Valcour et al. 2015, Levine, Soontornniyomkij et al. 2016). In the present study we conducted separate sets of experiments in the dorsal and ventral hippocampal regions because a growing body of evidence indicates that the dorsal and ventral hippocampus are characterized by specific circuit connectivities and functions (Fanselow and Dong 2010, Strange, Witter et al. 2014, Tannenholz, Jimenez et al. 2014). In particular, the dorsal hippocampus is involved in spatial memory via projections to the retrosplenial area of the anterior cingulate cortex (Moser, Moser et al. 1995, Kjelstrup, Tuvnes et al. 2002, Vann and Aggleton 2004, Cenquizca and Swanson 2007, Kubik, Miyashita et al. 2012). Conversely, the ventral hippocampus is involved in anxiety and social behaviors (Kjelstrup, Tuvnes et al. 2002, Bannerman, Grubb et al. 2003, Maggio and Segal 2009, Felix-Ortiz, Beyeler et al. 2013, Felix-Ortiz and Tye 2014, Tannenholz, Jimenez et al. 2014, Huff, Emmons et al. 2016). HIV Tg female and wild-type rats were used for this study due to emerging evidence that women with HIV may be more vulnerable to develop cognitive impairments than men (Fogel, Rubin et al. 2017, Keutmann, Gonzalez et al. 2017).

The intrinsic excitability of neurons is the key determinant of the translation of synaptic input to the particular output function of the neuron. Since the main neurotoxic HIV proteins, including gp120 and Tat, increase neuronal excitability (Lannuzel, Lledo et al. 1995, Magnuson, Knudsen et al. 1995, Nath, Psooy et al. 1996, Song, Nath et al. 2003), the present reduction of the intrinsic excitability of pyramidal neurons in the CA1 of the dorsal hippocampus of HIV Tg rats appears to be a homeostatic mechanism aimed at stabilizing neuronal output. Additionally, neurons will adjust their excitability in order to maintain a fixed level of firing output in response to changes in their synaptic drive (Turrigiano 1999). Therefore, the opposite changes of the strength of the presynaptic input and intrinsic excitability in the dorsal CA1 of HIV Tg rats are also likely part of a set of homeostatic

changes within the circuits in which these neurons operate in the setting of increased excitatory challenge brought about by HIV products and possibly host immune mediators. In fact, the changes in synaptic properties and intrinsic excitability of dorsal CA1 pyramidal neurons have opposing effects on the potential of these neurons to develop spiking activity. Elevated rate of EPSCs, reflecting either the increase of the probability of quantal release or the increase of the number of excitatory synapses, is expected to promote postsynaptic neuron spiking activity. Conversely, reduced excitability of postsynaptic neurons is expected to counteract a stimulating effect of upregulated EPSCs. Such counteracting changes in HIV hippocampal neurons suggest the possible involvement of mechanisms of homeostatic plasticity in regulation of the baseline level of neuronal activity. Homeostatic plasticity is activated in response to changes in the environment and is aimed at counteracting the disturbance produced by these changes and at maintaining the baseline level of neuronal activity (Turrigiano 1999). Mechanisms aimed at decreasing neuronal excitability in response to increased excitatory synaptic drive have been described in other systems including opposite changes in neuronal excitability and excitatory synaptic drive (Turrigiano 1999, van Welie, van Hooft et al. 2004), changes in the inhibitory/excitatory balance (Brickley, Revilla et al. 2001), and compensatory expressions of currents (Etheredge, Murchison et al. 2007), among others.

Reduced I_h is a likely contributor to the decreased excitability of CA1 neurons from HIV Tg rats. As an inward rectifying current, the I_h has a depolarizing effect on neuronal membrane potential (Santoro and Tibbs 1999). Thus, reduction of I_h in CA1 neurons from HIV Tg rats will result in hyperpolarization of membrane potential as observed in both the dorsal and the ventral areas of the hippocampus. While the I_h and the E_{RMP} were similarly altered in both dorsal and ventral hippocampus in neurons from HIV Tg rats, neuronal excitability was not significantly decreased in the ventral hippocampus. This can be explained by the difference in the passive and, possibly, active membrane properties of CA1 neurons along the longitudinal hippocampal axis. Indeed, CA1 neurons from the ventral hippocampus had significantly higher R_{in} which would make them inherently more excitable compared their dorsal counterparts (data not shown). CA1 neurons from the ventral hippocampus were also significantly more depolarized compared to the dorsal neurons. Similar differences in the membrane properties of CA1 neurons along the longitudinal axis were reported in other studies (Dougherty, Islam et al. 2012, Marcelin, Liu et al. 2012, Honigspurger, Marosi et al. 2015, Miliot, Di Castro et al. 2016). In extended amygdala neurons that express the I_h , we found that decreased I_h accompanied reduced excitability in alcohol dependent rats (Szucs, Berton et al. 2012). Homeostatic regulation of the I_h has been shown in other systems, for instance it was increased concomitantly with an increase in I_A , in an invertebrate system, resulting in opposing actions on firing (MacLean, Zhang et al. 2005).

Neurocognitive impairment in HIV-infected patients is correlated with the amount of dendritic simplification, synaptodendritic degeneration, as well as atrophy in cortical and subcortical areas of the brain, including the hippocampus (Masliah, Heaton et al. 1997, Moore, Masliah et al. 2006, Wade, Valcour et al. 2015, Levine, Soontornniyomkij et al. 2016). Such neurodegenerative changes are believed to result at least in part from excitotoxic effects of certain HIV proteins and molecules of neuroimmune response. While alterations associated with homeostatic plasticity have stabilizing effects on baseline

neuronal activity, they can also be detrimental for the function of neuronal networks, such as in the case of the dorsal hippocampus, those that subserve spatial orientation or episodic memory formation. Both the reduced intrinsic excitability of CA1 neurons from HIV Tg rats and the upregulated excitatory inputs at CA3-CA1 synapses are expected to contribute to the reduced capacity of the long-term potentiation (LTP) which may lead to altered performance of HIV Tg rats in hippocampus-dependent behavioral tasks (Vigorito, LaShomb et al. 2007, Lashomb, Vigorito et al. 2009, Repunte-Canonigo, Lefebvre et al. 2014). Reduced intrinsic excitability is expected to contribute to impaired LTP as a stronger excitatory input to CA1 neurons would be required for its induction. Additionally, the upregulated baseline level of EPSCs brings evoked responses closer to their saturation level, thus reducing the high-frequency stimulation-induced potentiation at CA3-CA1 synapses (Barnes, Jung et al. 1994, Moser, Krobert et al. 1998, Moser and Moser 1999).

Ultimately, since CA1 pyramidal neurons are required to fire at specific time scales and synchrony in the course of memory encoding and recall (Stella and Treves 2011, Rolls 2018, Sanders, Ji et al. 2019), the decreased intrinsic excitability of CA1 pyramidal neurons in response to pathologically increased excitatory drive, may underlie memory deficits in the setting of neuroHIV. Thus, the maladaptive regulation of intrinsic neuronal excitability in response to the greater excitation by HIV products may result in reduced efficacy of the circuits involving the dorsal hippocampus that subserves memory encoding and recall (Stella and Treves 2011, Rolls 2018, Sanders, Ji et al. 2019), contributing to cognitive impairments.

In conclusion, here we show a set of synaptic and intrinsic plastic changes in the dorsal hippocampus of HIV Tg rats characterized by reduced excitability and elevated level of excitatory inputs to CA1 pyramidal neurons in HIV Tg rats as well as reduction in the hyperpolarization-activated inward current (I_h) both in the dorsal and the ventral CA1. As the dorsal hippocampus has been shown to be involved in the formation of episodic memory and spatial orientation, functional changes to the dorsal hippocampus in HIV Tg rats are likely to contribute to the memory and performance deficits of HIV Tg rats as well as neurocognitive impairments in patients with HAND.

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Figure 1.

Results of experiments using conventional protocol of neuronal stimulation by step current injection. **A)** The minimal stimulation currents (minimal injected currents eliciting the AP, I_{min}) was significantly higher in neurons from HIV Tg rats as compared to those in the wild-type rats. **B)** Frequencies of spiking in HIV neurons were reduced compared to the frequencies in control neurons, in both the dorsal and the ventral hippocampus. This reduction in firing frequencies, however, did not reach the significance levels (repeated measures two-way Anova, $p > 0.05$). **C)** Representative voltage recordings in HIV and control rats.

Figure 2.

Passive membrane properties and characteristics of the action potentials in CA1 pyramidal neurons from control and HIV rats. **A)** AP rise time was significantly longer in neurons from the HIV group than that in neurons from the control group in both the dorsal and the ventral hippocampus. **B)** AP 1/2 width was significantly larger in neurons from the HIV group compared to that in neurons from the control group, in both the dorsal and the ventral hippocampus. **C)** In both the dorsal and the ventral hippocampus the neurons from the HIV rats were significantly more hyperpolarized than those in the control rats. **D)** Representative traces of membrane potentials recorded in neurons during conventional stimulation protocol.

Figure 3.

A) Dynamic conductance threshold (g_{AMPA}) for firing of HIV and control neurons. g_{AMPA} was significantly increased in neurons from the dorsal hippocampus of the HIV rats. This was observed in experiments using all four patterns of synaptic stimulation. g_{AMPA} was also increased in neurons from the ventral hippocampus of the HIV rats. This increase, however, was below the significance level. **B)** Representative recordings of responses to the four patterns of synaptic stimulation.

Figure 4.

Hyperpolarization-activated non-specific cation current (I_h) and the V_{sag} in neurons from the dorsal and the ventral areas of the hippocampus from control and HIV Tg rats. **A)** Amplitudes of the I_h in pyramidal neurons of the dorsal hippocampus from HIV rats was significantly reduced at voltages < -90 mV. The I_h in the ventral hippocampus was significantly reduced only at voltages < -110 mV. **B)** The V_{sag} in HIV neurons was significantly reduced compared to the control neurons, in both the dorsal and the ventral areas of the hippocampus. Despite the relatively small change of the I_h in the ventral hippocampus, the decrease of the V_{sag} in the ventral hippocampal neurons was more pronounced than that in the dorsal hippocampal neurons. **C)** Representative traces of membrane voltage demonstrating the voltage sag in recordings using steps of negative currents in neurons from control and HIV groups of the dorsal and the ventral areas of the hippocampus.

Figure 5.

Excitatory postsynaptic currents (EPSCs) in neurons from the dorsal and the ventral areas of the hippocampus from control and HIV rats. EPSCs in the dorsal and ventral areas of the hippocampus were differentially affected by HIV. **A)** HIV had no effect on EPSC amplitudes in the dorsal hippocampus, but significantly enhanced EPSC amplitudes in the ventral hippocampus. **B)** Cumulative histogram of EPSC inter-event intervals showing that HIV significantly enhanced rates of EPSCs in neurons from the dorsal hippocampus but did not affect rates of EPSCs in neurons from the ventral hippocampus. **C)** Representative current traces in neurons from the dorsal and ventral areas of the hippocampus of control and HIV rats.