Journal of Neuroimmune Pharmacology (2019) 14:391–400
https://doi.org/10.1007/s11481-019-09859-w

BRIEF REPORT

Glutamine Antagonist JHU083 Normalizes Aberrant Glutamate Production and Cognitive Deficits in the EcoHIV Murine Model of HIV-Associated Neurocognitive Disorders

Michael T. Nedelcovych[1,2,3] · Boe-Hyun Kim[4] · Xiaolei Zhu[3,5] · Lyndah E. Lovell[1,2,3] · Arena A. Manning[3,6] · Jennifer Kelschenbach[3,4] · Eran Hadas[4] · Wei Chao[4] · Eva Prchalová[1,2,3] · Ranjeet P. Dash[1,2,3] · Ying Wu[1,2,3] · Jesse Alt[1,2,3] · Ajit G. Thomas[1,2,3] · Rana Rais[1,2,3] · Atsushi Kamiya[3,5] · David J. Volsky[4] · Barbara S. Slusher[1,2,3,5,7,8]

Received: 27 February 2019 / Accepted: 28 May 2019 / Published online: 17 June 2019

Abstract

HIV-associated neurocognitive disorders (HAND) have been linked to dysregulation of glutamate metabolism in the central nervous system (CNS) culminating in elevated extracellular glutamate and disrupted glutamatergic neurotransmission. Increased glutamate synthesis via upregulation of glutaminase (GLS) activity in brain immune cells has been identified as one potential source of excess glutamate in HAND. However, direct evidence for this hypothesis in an animal model is lacking, and the viability of GLS as a drug target has not been explored. In this brief report, we demonstrate that GLS inhibition with the glutamine analogue 6-diazo-5-oxo-L-norleucine (DON) can reverse cognitive impairment in the EcoHIV-infected mouse model of HAND. However, due to peripheral toxicity DON is not amenable to clinical use in a chronic disease such as HAND. We thus tested JHU083, a novel, brain penetrant DON prodrug predicted to exhibit improved tolerability. Systemic administration of JHU083 reversed cognitive impairment in EcoHIV-infected mice similarly to DON, and simultaneously normalized EcoHIV-induced increases in cerebrospinal fluid (CSF) glutamate and GLS activity in microglia-enriched brain CD11b + cells without observed toxicity. These studies support the mechanistic involvement of elevated microglial GLS activity in HAND pathogenesis, and identify JHU083 as a potential treatment option.

Keywords HIV-associated neurocognitive disorders (HAND) · EcoHIV · Glutaminase · 6-diazo-5-oxo-L-norleucine (DON) · Microglia

Michael T. Nedelcovych and Boe-Hyun Kim contributed equally to this work.

✉ David J. Volsky
david.volsky@mssm.edu

✉ Barbara S. Slusher
bslusher@jhmi.edu

[1] Johns Hopkins Drug Discovery, 855 North Wolfe Street, Baltimore, MD 21205, USA

[2] Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[3] Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[4] Department of Medicine, Icahn School of Medicine at Mount Sinai, Annenberg Building Floor 21, Room 42, 1468 Madison Ave, New York, NY 10029, USA

[5] Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[6] Graduate Program in Neuroscience, University of Washington, Seattle, WA, USA

[7] Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[8] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction

HIV-associated neurocognitive disorders (HAND) characterized by mild to moderate impairments in multiple domains of cognition and psychomotor performance occur in nearly half of chronically HIV-infected individuals despite viremic control with combined antiretroviral therapy (cART) (Antinori et al. 2007; Harezlak et al. 2011; Heaton et al. 2008; Heaton et al. 2011; Kaul et al. 2005; McArthur et al. 2010; Robertson et al. 2007). HAND symptoms are associated with structural and functional alterations in cortical, subcortical, and limbic brain regions known to be important for cognition including prefrontal cortex (PFC) and hippocampus (Castelo et al. 2006; Ernst et al. 2002; Fennema-Notestine et al. 2013; Heaps et al. 2012; Jernigan et al. 2011; Melrose et al. 2008; Ragin et al. 2004; Steinbrink et al. 2013; Thomas et al. 2013b; Thompson et al. 2005). However, the underlying pathophysiological mechanisms of cART-insensitive HAND are not well understood and there are no effective treatments or validated biomarkers of disease prognosis (Potter et al. 2013; Saylor et al. 2016).

Evidence is mounting to suggest that HAND is associated with dysregulation of glutamate metabolism in the central nervous system (CNS) culminating in elevated extracellular glutamate and disrupted glutamatergic neurotransmission (Bairwa et al. 2016; Cassol et al. 2014; Ernst et al. 2010; Ferrarese et al. 2001; Mohamed et al. 2010; Potter et al. 2013; Sailasuta et al. 2009; Saylor et al. 2016; Schifitto et al. 2007; Stankoff et al. 2001; Vazquez-Santiago et al. 2014). The primary source of glutamate overproduction may be persistently activated resident microglia and invading macrophages which have been shown to increase synthesis and secretion of glutamate upon immune challenge or HIV infection (N. Erdmann et al. 2009; N. Erdmann et al. 2007; N. B. Erdmann et al. 2006; Huang et al. 2011; Potter et al. 2013; Tian et al. 2008; Tian et al. 2012; Vazquez-Santiago et al. 2014; Wu et al. 2015; Ye et al. 2013; J. Zhao et al. 2004; L. Zhao et al. 2012; L. Zhao et al. 2013). These cells have also been shown to upregulate and secrete glutaminase (GLS) (N. Erdmann et al. 2009; N. Erdmann et al. 2007; N. B. Erdmann et al. 2006; Huang et al. 2011; Potter et al. 2013; Tian et al. 2008; Tian et al. 2012; Vazquez-Santiago et al. 2014; Wu et al. 2015; Ye et al. 2013; J. Zhao et al. 2004; L. Zhao et al. 2012; L. Zhao et al. 2013), the enzyme responsible for de novo glutamate synthesis in the brain (Cooper and Jeitner 2016; Potter et al. 2013; Vazquez-Santiago et al. 2014). Clinical studies have supported these observations; elevated glutamate concentrations have been detected in the cerebrospinal fluid (CSF) of HAND patients and correlated with the presence of cognitive impairment (Cassol et al. 2014; Ferrarese et al. 2001). Microarray analyses in post-mortem brain samples from HAND patients have also shown frontal cortical alterations in the expression of glutaminase and other genes important for the regulation of glutamate-dependent synaptic plasticity (Gelman et al. 2012a, b; L. Zhao et al. 2012).

Our laboratories recently reported that the GLS inhibitor 6-diazo-5-oxo-L-norleucine (DON) could prevent the development of cognitive impairment in EcoHIV-infected mice (Nedelcovych et al. 2017), a murine model of cART-insensitive HAND (Gu et al. 2018; Kim et al. 2019; Potash et al. 2005). Specifically, mice that began DON treatment 1 day prior to EcoHIV infection were found to be completely protected from the emergence of cognitive impairment 30 days later as measured by performance in a radial arm water maze (RAWM) (Nedelcovych et al. 2017). Because DON's clinical viability is hampered by significant gastrointestinal (GI) toxicities, our laboratory synthesized novel prodrugs of DON with enhanced brain-to-plasma ratios. The brain targeted prodrugs were designed to circulate inert in plasma and be taken up and bioactivated to DON selectively in the brain. When evaluated in swine and primates, the prodrugs led to a 10–15-fold increase in the CSF-to-plasma ratio, and a 9-fold enhanced brain-to-plasma ratio, respectively, relative to equimolar doses of DON (Nedelcovych et al. 2017; Rais et al. 2016). While our initial studies with DON in EcoHIV mice provided proof-of-principle data that targeting glutamate metabolism could have positive behavioral effects in HAND, addressing HAND prophylactically with DON is not clinically feasible.

In the current study, we more closely modeled the clinical scenario by initiating DON treatment weeks after established EcoHIV infection and emergence of a HAND-like behavioral phenotype, and report that DON could reverse existing cognitive impairment in the EcoHIV model. Given that DON's clinical development has been hampered by its GI toxicities, we next evaluated the ability of our novel brain penetrant DON prodrug, JHU083, to rescue EcoHIV-induced behavioral and physiological changes. We first conducted systemic JHU083 pharmacokinetic studies to confirm relevant concentrations of DON were delivered to the CNS. Studies were then conducted to examine the effect of in vivo JHU083 treatment on EcoHIV-induced changes in CSF glutamate concentrations, ex vivo GLS activity in microglia-enriched CD11b + cells, and hippocampal-dependent cognitive function as measured by contextual conditioned fear (CF) and RAWM.

Methods

Animals All mouse studies were conducted in compliance with NIH guidelines and with the approval of the Institutional Animal Care and Use Committee at Johns Hopkins University and Icahn School of Medicine at Mount Sinai. For animal studies conducted at Johns Hopkins (pharmacokinetics, CSF glutamate, CD11b + GLS activity, and contextual CF), male C57BL/6 mice at 6 weeks old were

obtained from Envigo (Frederick, MD) and maintained on a 12 h light–dark cycle with ad libitum access to food and water throughout all studies. For animal studies conducted at Mount Sinai (including RAWM), male C57BL/6 J mice at 6 weeks old were obtained from The Jackson Laboratory (Farmington, CT) and maintained on a 12 h light–dark cycle with ad libitum access to food and water throughout all studies.

EcoHIV Infection and Drug Treatment EcoHIV chimeric virus was generated as previously reported (Gu et al. 2018; Kim et al. 2019). Briefly, HEK293T cells were transfected with plasmid DNA containing a previously described construct of EcoNDK harboring the V5C5 fragment of gp120 (Gu et al. 2018; Kim et al. 2019; Potash et al. 2005). EcoHIV was then isolated from culture media by centrifugation and concentrations measured by p24 ELISA (Advanced Biosciences Laboratory, Rockville, MD). For all experimental endpoints, mice were inoculated with EcoHIV (2-4 × 10^6 pg p24, i.p.) or a sham injection of saline. For behavioral studies with DON, every other day treatment of 1 mg/kg, i.p. was initiated on day 25 post-inoculation and continued through RAWM testing which began on day 30. For the pharmacokinetic study, JHU083 treatment and tissue collection was performed on day 15 post-inoculation. For all other experiments, every other day JHU083 (1.83 mg/kg, i.p.) or vehicle administration was initiated on day 15 post-inoculation and continued through behavioral analysis or tissue collection conducted on day 30 or 35, respectively. For all tissue collection endpoints, mice were euthanized 30 min after the last dose of JHU083 or vehicle. An every other day dose regimen for DON and JHU083 was chosen based on the observation that DON is an irreversible GLS inhibitor (Chen and Cui 2015; Conti and Minelli 1994; Crosby et al. 2015; Crosby and Miller 2016; Lemberg et al. 2018; Nedelcovych et al. 2017; Song et al. 2018; A. G. Thomas et al. 2014; J. Zhao et al. 2004; Zhu et al. 2018), and that a similar dose and schedule yielded positive results in another model of CNS disease dependent on glutamatergic dysfunction and GLS hyperactivity (Zhu et al. 2018).

Compounds JHU083 was synthesized as previously described (Rais et al. 2016) and dissolved in HEPES (50 mM) buffered saline for experimental use. DON was obtained from Sigma Aldrich (Cat# D2141) and used for preparing the calibration curve for LC-MS/MS bioanalysis of the pharmacokinetic study samples.

Mouse Pharmacokinetics and DON Bioanalysis EcoHIV-infected mice were administered JHU083 (1.83 mg/kg, i.p.) and euthanized 0.25, 0.5, 1, 3, or 6 h later by rapid decapitation after brief isoflurane anesthesia. Trunk blood was collected in heparin-coated tubes from which plasma was isolated by centrifugation at 4000 g at 4 °C. Whole brain were dissected and quickly frozen on dry ice and stored at −80 °C prior to further processing. Tissues were prepared, and DON bioanalysis and quantification was then conducted by LC-MS/MS as we have previously described (Nedelcovych et al. 2017; Rais et al. 2016).

CSF Glutamate CSF was terminally obtained from the cisterna magna of deeply anesthetized mice by puncturing the dura mater with a pulled glass pipette after incision and separation of subcutaneous muscles as previously described (Liu and Duff 2008). After the procedure, all mice were immediately euthanized by rapid decapitation under isoflurane anesthesia. CSF samples were quickly frozen on dry ice and stored at −80 °C prior to further preparation and analysis. Due to large differences in basal glutamate concentrations between CSF and blood (Abbott et al. 2010; Coccaro et al. 2013), any blood-contaminated samples as determined by visual inspection were a priori discarded from analysis. Glutamate bioanalysis was then conducted by LC-MS/MS as previously described (Nedelcovych et al. 2017; Rais et al. 2016).

CD11b + Cell Isolation Thirty minutes after the last dose of JHU083 or vehicle, CD11b + and non-CD11b + cells were obtained from the whole brains of mice as previously described (Zhu et al. 2018). Briefly, brain tissue was minced in HBSS (cat # 55021C, Sigma-Aldrich, St. Louis, MO, USA) and dissociated using the Neural Tissue Dissociation Kit (MACS Militenyi Biotec, Auburn, CA) according to manufacturer instructions. Resulting homogenates were pushed through a 70 μm cell strainer and centrifuged at 300×g for 10 min prior to removal of supernatants. Cell pellets were then resuspended and subjected to Myelin Removal Beads II (MACS Militenyi Biotec, Auburn, CA) according to manufacturer instructions. Cell pellets were again resuspended, incubated with CD11b MicroBeads (MACS Militenyi Biotec, Auburn, CA) for 15 min, loaded on LS columns and separated on a quadroMACS magnet. Non-CD11b + cells were flushed through the column then washed and resuspended in sterile HBSS (cat # 55037C, Sigma-Aldrich, St. Louis, MO) prior to centrifugation and storage of pellets at −80 °C. CD11b + cells were eluted from the column then washed and resuspended in sterile HBSS (cat # 55037C, Sigma-Aldrich, St. Louis, MO) prior to centrifugation and storage of pellets at −80 °C. CD11b + cells isolated from brain homogenates through this method have been shown to consist of >95% microglia (Zhu et al. 2018).

Fluorescence-Activated Cell Sorting (FACS). For immunostaining experiments to verify the identity of CD11b positive cells collected with this strategy, the bilateral cerebrum of wild-type mouse is removed using our established method (Ballinger, M. D. et al. Adolescent cannabis exposure interacts with mutant DISC1 to produce impaired adult emotional memory. Neurobiology of disease 82, 176–184). Microglia (CD11b+/CD45+/CX3CR1+/P2RY12+ cells) are stained

from dissociated brain tissue by a FACS Aria Flow Cytometer using published method (De Biase, L. M. et al. Local Cues Establish and Maintain Region-Specific Phenotypes of Basal Ganglia Microglia. Neuron 95, 341–356 e346). Briefly, dissociated cells were resuspended in FACS staining buffer (1× PBS with 0.5% BSA) and incubated for 30 min on ice with the following antibodies: PE conjugated Rat anti-CD11b (1:100, BioLegend), PE-Cy7 conjugated Rat anti-CD45 (1:400, BD Pharmingen), Brilliant Violet-421 conjugated mouse anti-CX3CR1 (1:400, BioLegend), and APC conjugated Rat anti-P2RY12 (1:100, BioLegend). Cells were then washed once, resuspended in 300 μL FACS staining buffer and filtered, the population of cells containing microglia could be readily identified based on forward scattering (FSC) and side scattering (SSC) properties. A gating strategy based on FSC and SSC width and height were used to select only single cells. CD11b + cells within this population were then gated, cell suspensions from same wild-type brain tissue were used as a CD11b negative control for establishment of CD11b negative gates. Further, CD45 + CX3CR1 + P2RY12+ microglia were identified within this population.

GLS Activity GLS activity from isolated CD11b + and non-CD11b + cells was measured as previously described (Zhu et al. 2018).

Contextual Conditioned Fear (CF) Test Contextual CF consisted of a conditioning day followed by testing 1 day later. Conditioning was conducted for all mice prior to JHU083 or vehicle administration. For conditioning, mice were placed into a plexiglass chamber with a steel grid floor (Coulborn/Harvard Apparatus) through which a mild footshock was applied. The conditioning session consisted of 200 s habituation followed by 10s of 1000 Hz 80 dB tone co-terminating in a 1 s 0.4 mA footshock. Mice remained in chamber for an additional 30s of rest before removal to their home cage. Chambers were cleaned in between each trial with 200 ppm chlorine dioxide use-solution. One day later, all mice were returned to the same conditioning chamber as during training and movement was recorded for 10 min in the absence of footshock. Time spent freezing was analyzed from video recordings using automated Freezescan software from Cleversys, Inc., Reston, VA and calculated as a percent of total time in the chamber.

Radial Arm Water Maze (RAWM) Test RAWM was conducted in a radial six-arm water maze as previously described (Gu et al. 2018). Briefly, an escape platform was placed in one arm, submerged in opaque water, and a set of visual cues was fixed at the end of each arm. The location of the hidden escape platform and the lane in which mice were placed to initiate the trial was randomly changed on each day to compel mice to use working memory during spatial learning (Diamond et al. 1999). Each day, mice underwent 4 learning trials (LT) of 60 s with 20 s rest after each LT and 1 post-training 60 s retention trial (RT) administered after 30 min rest, to evaluate working memory. Testing was considered complete when control mice reached criteria performance defined as one error or fewer in finding the hidden platform on both trials LT4 and RT. Errors for the final 3 days of testing were then averaged and used for statistical analysis. Errors were manually scored and defined as entering an arm without the platform and/or 20 s of immobility. To control for possible changes in vision, motivation, or motor function, the hidden platform test was followed by a visible visual platform test in which latency to reach a platform submerged in clear water was assessed.

Statistical Analysis Statistical analysis was conducted using GraphPad Prism (MathWorks, Natick, MA). For all experiments, values greater than two standard deviations from the mean were excluded from statistical analysis. Analysis of group effects was conducted by two-way ANOVA. If significant, post-hoc Fisher's LSD was applied to identify individual differences between groups. Significance was defined as $p < 0.05$.

Results

DON Reverses Cognitive Impairment in EcoHIV-Infected Mice as Measured by RAWM The ability of DON to normalize impaired cognitive functions in EcoHIV-infected mice was first tested in RAWM. EcoHIV infected mice made significantly higher number of errors while navigating the maze compared to vehicle treated but not DON treated groups (main effect of group [$F(3,140) = 392.3$, $p < 0.0001$], trial [$F(4,140) = 107.0$, $p < 0.0001$], and interaction [$F(12,140) = 8.561$, $p < 0.0001$]) (Fig. 1a), suggesting reversal of disease by DON. In contrast, there was no difference between the groups in finding a visible platform, indicating that neither EcoHIV infection nor DON treatment had deleterious effects on mouse vision, motor performance, or motivation. (Fig. 1b). Having confirmed the ability of DON to reverse established cognitive impairment in this HAND model, we next tested a brain penetrant DON prodrug, JHU083.

JHU083 Administration Results in Micromolar Brain Delivery of DON in EcoHIV Mice The pharmacokinetics of DON were assessed after JHU083 (1.83 mg/kg, i.p.) administration in EcoHIV-infected mice. JHU083 administered at this dose delivered DON brain exposure ($AUC_{0-\infty}$) of 3.7 nmol*h/g and a peak brain concentration (C_{max}) of 1.19 nmol/g at 30 min post-injection (t_{max}) (Fig. 2a), which is similar to the GLS K_i that our laboratory previously reported (A. G. Thomas et al. 2013a, b). Plasma DON C_{max} was 2.64 nmol/mL and $AUC_{0-\infty}$ was 3.40 nmol*h/mL yielding a brain/plasma ratio of 0.68 (Fig. 2a).

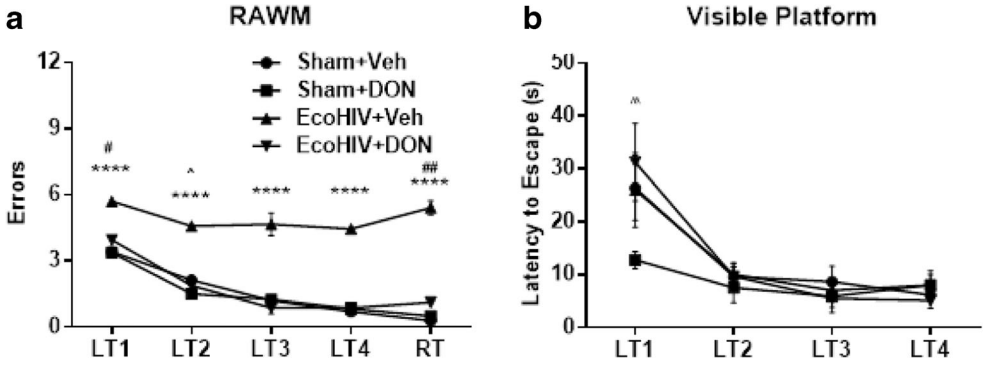

Fig. 1 DON Reverses EcoHIV-Induced Cognitive Impairment. **a** EcoHIV infection resulted in significantly impaired performance on RAWM as measured by errors made in the maze during learning trials (LT) and a retention trial (RT). DON treatment normalized RAWM performance with no effect on baseline performance in Sham mice. **b** No increase in latency to escape to a visible platform was detected in any group relative to Sham+Veh mice. $n = 8$/group; ****$p < 0.0001$ EcoHIV+Veh vs. Sham+Veh; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ EcoHIV+DON vs. Sham+Veh, $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$ Sham+DON vs. Sham+Veh

JHU083 Normalizes EcoHIV-Induced Deficits in Recall of Contextual CF and Spatial Learning and Working Memory In both contextual CF and RAWM behavioral assays, EcoHIV induced a cognitive deficit that was sensitive to JHU083 treatment. EcoHIV infection significantly impaired contextual CF recall as measured by a reduction in % time spent freezing that was restored by JHU083 treatment (main effect of interaction [$F(1,69) = 5.13$, $p = 0.0267$]) (Fig. 2b). In RAWM, EcoHIV infection caused a significant increase in the number of errors made while navigating the maze in vehicle treated but not JHU083 treated cohorts (main effect of group [$F(3,140) = 53.82$, $p < 0.0001$] and trial [$F(4,140) = 56.65$, $p < 0.0001$]) (Fig. 2c). There was a significant effect of group [$F(3,112) = 2.97$, $p = 0.0351$] and trial [$F(3,112) = 28.03$, $p < 0.0001$] observed on the latency to escape to a visible platform in the RAWM, but no post-hoc significant difference attributable to infection status or treatment, indicating intact visual and motor performance (Fig. 2d).

JHU083 Normalizes EcoHIV-Induced Increases in CSF Glutamate CSF obtained from EcoHIV-infected mice exhibited elevated glutamate concentrations relative to non-infected controls and was normalized by JHU083 treatment (main effect of infection [$F(1,14) = 5.53$, $p = 0.0339$] and treatment [$F(1,14) = 17.64$, $p = 0.0009$]; no significant interaction [$F(1,14) = 3.48$, $p = 0.0833$]) (Fig. 3a). Mean CSF glutamate

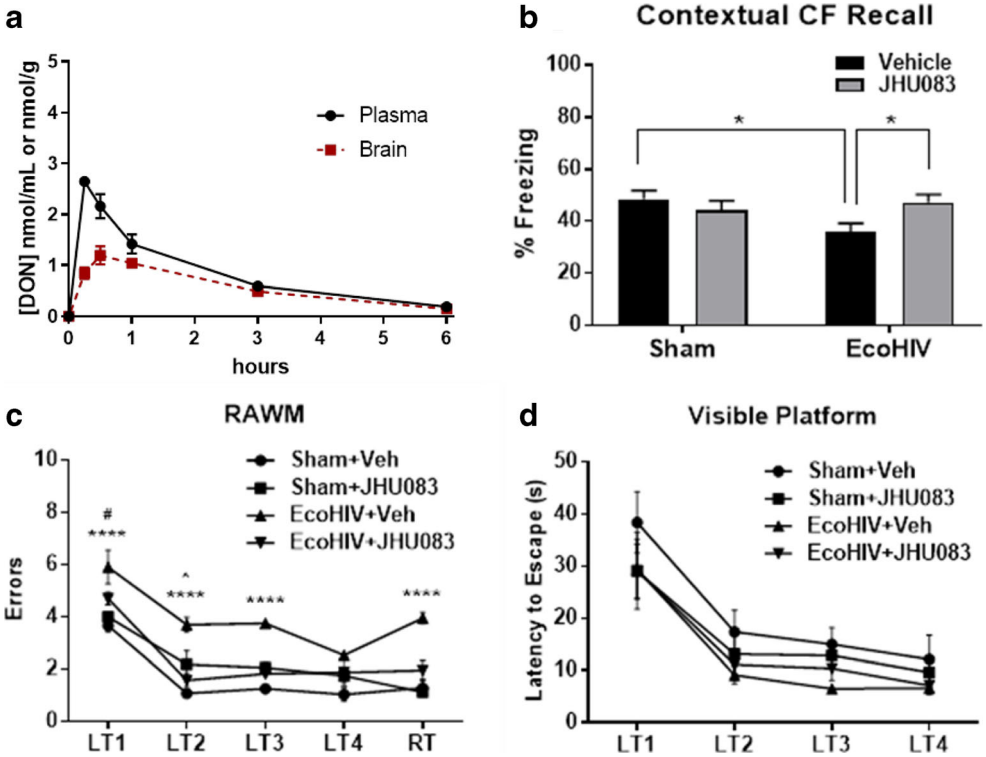

Fig. 2 JHU083 Delivers μM DON to the Brain and Reverses EcoHIV-Induced Cognitive Deficits. **a** JHU083 (1.83 mg/kg, i.p) delivered μM concentrations of DON to the brains of EcoHIV-infected mice with a C_{max} of 1.19 nmol/g and $AUC_{0-\infty}$ of 3.7 nmol*h/g ($n = 3$/time point). **b** JHU083 administration reversed EcoHIV-induced cognitive deficits in contextual CF recall ($n = 17$–19/group) and **c** normalized RAWM performance as measured by errors made during learning trials (LT) and retention trial (RT) ($n = 8$/group). **d** No significant effect was found for EcoHIV infection or JHU083 treatment on ability to swim to a visible platform ($n = 8$/group). *$p < 0.05$, ****$p < 0.0001$ EcoHIV+Veh vs. Sham+Veh, $^{\#}p < 0.05$ EcoHIV+JHU083 vs. Sham+Veh, $^{\wedge}p < 0.05$ Sham+JHU083 vs. Sham+Veh

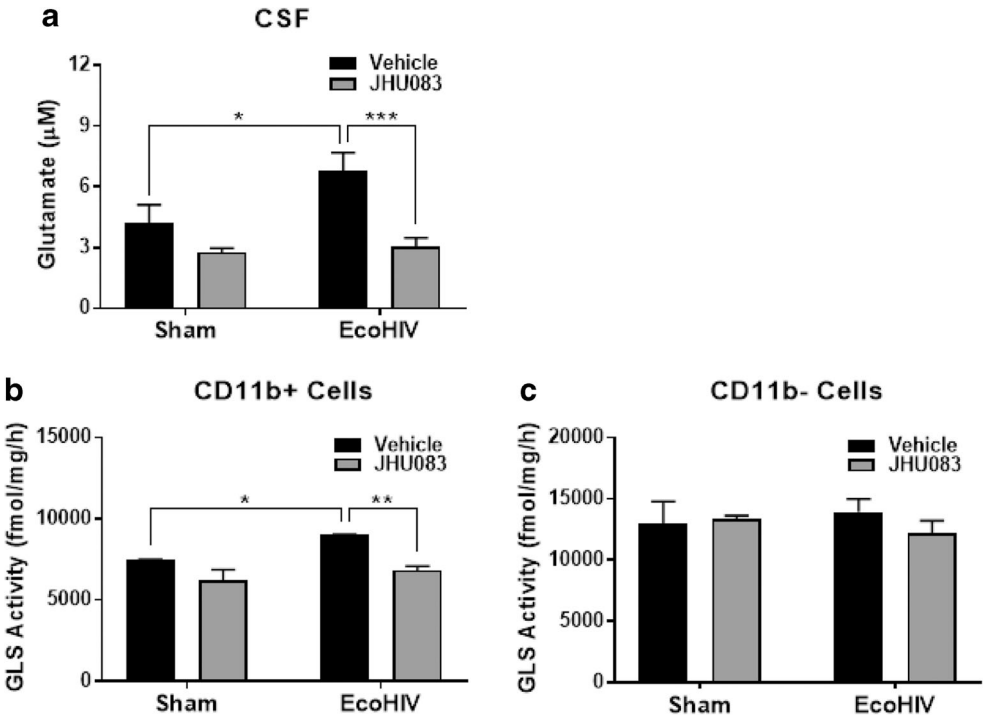

Fig. 3 JHU083 Normalizes EcoHIV-Induced Increases in CSF Glutamate and GLS Activity in Microglia-Enriched CD11b + Cells. a EcoHIV infection significantly increased CSF glutamate concentrations which were normalized by JHU083 treatment ($n = 4$–5/group). **b** JHU083 treatment also normalized GLS activity in CD11b + cells derived from the whole brains of EcoHIV-infected mice ($n = 4$/group). **c** Neither EcoHIV infection nor JHU083 treatment affected GLS activity in CD11b- cells ($n = 4$/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

concentrations in non-infected mice were 4.2 μM for the vehicle treated cohort and 2.8 μM for the JHU083 treated cohort, both values falling into the range of previously reported basal values (Danbolt 2001; Featherstone and Shippy 2008; Moussawi et al. 2011). EcoHIV infection resulted in a 62% increase in CSF glutamate that was restored to basal levels by JHU083 treatment.

JHU083 Normalizes EcoHIV-Induced Increases in Glutaminase Activity Derived from Microglia-Enriched CD11b + Cells

CD11b-expressing cells previously shown to be composed of >95% microglia (Sharma et al. 2015; Zhu et al. 2018) (confirmed by FACS to be >99% *$CD11b^{+}CD45^{+}CX3CR1^{+}P2RY12^{+}$; data not shown)* were isolated from the whole brains of mice on day 33 post-inoculation. GLS derived from EcoHIV-infected CD11b + cells exhibited significantly increased activity relative to non-infected cells, an effect that was normalized by JHU083 treatment (main effect of infection [$F(1,12) = 9.36$, $p = 0.0099$] and treatment [$F(1,12) = 23.29$, $p = 0.0004$]; no significant interaction [$F(1,12) = 1.71$, $p = 0.2158$]) (Fig. 3b). Cell fractions not expressing CD11b for which the source of GLS activity is primarily neuronal (Kvamme et al. 2001; Schousboe et al. 2014) showed no significant effect of EcoHIV infection or JHU083 treatment (Fig. 3c).

Discussion

Markers of glutamatergic dysfunction such as elevated CSF glutamate have been found in HAND patients and may subserve cognitive impairment associated with chronic HIV infection (Kim et al. 2019; Potter et al. 2013; Saylor et al. 2016; Vazquez-Santiago et al. 2014). The studies presented herein demonstrate that EcoHIV infection in mice recapitulates this phenotype and enables probing of the glutamatergic pathophysiology that contributes to HAND symptoms. We find that cognitive impairment in EcoHIV-infected mice is at least partially mediated by overproduction of glutamate in the CNS that can be normalized in concert with inhibition of GLS activity in CD11b + cells via systemic administration of the glutamine antagonist DON or its prodrug JHU083. This effect is notable given that there are no currently available treatments for HAND (Potter et al. 2013; Saylor et al. 2016).

Even in the absence of antiretroviral treatment, the EcoHIV model has previously been shown to recapitulate a disease phenotype similar to HAND patients on cART (Gu et al. 2018; Nedelcovych et al. Nedelcovych et al. 2017; Potash et al. 2005) and thus is suitable for testing mechanistic hypotheses related to mild HAND pathogenesis in virally suppressed patients. The finding that EcoHIV infection also causes increases in CSF glutamate similar to those reported in HAND patients (Cassol et al. 2014; Ferrarese et al. 2001) supports this approach and opens new avenues for exploration of mechanism and potential therapeutics. Early efforts to target the glutamatergic system for HAND treatment focused on N-methyl-D-aspartate (NMDA) receptor antagonism, in particular the use of memantine, a non-competitive low-affinity antagonist approved for Alzheimer's Disease. Initial clinical trials in HAND showed no benefit (Schifitto et al. 2006), suggesting that selective blockade of postsynaptic NMDA receptors may

be insufficient to address the various deleterious effects of diffuse, excessive extracellular glutamate likely present in the brains of HAND patients.

DON offers a therapeutic alternative to selective receptor blockade by exerting more global effects on glutamate homeostasis through inhibition of GLS, the enzyme responsible for the production of glutamate in both neuronal and glial cell populations in the brain (Cooper and Jeitner 2016; Kvamme et al. 2001; Potter et al. 2013; Schousboe et al. 2014; Vazquez-Santiago et al. 2014; Zhu et al. 2018). GLS appears to be mechanistically important for HAND and may be one of the sources of elevated CSF glutamate. In the current study, GLS in microglia-enriched CD11b + cells exhibited increased activity upon EcoHIV infection similar to other monocyte models of HIV infection (N. Erdmann et al. 2009; N. Erdmann et al. 2007; N. B. Erdmann et al. 2006; Huang et al. 2011; Potter et al. 2013; Tian et al. 2008; Tian et al. 2012; Vazquez-Santiago et al. 2014; Wu et al. 2015; Ye et al. 2013; J. Zhao et al. 2004; L. Zhao et al. 2012; L. Zhao et al. 2013). DON delivery to the brain via systemic JHU083 administration normalized EcoHIV-induced increases in both CD11b + GLS activity and CSF glutamate concentrations coincident with cognitive improvements. In addition to supporting GLS as a therapeutic target, this result suggests the possibility that CSF glutamate changes could be used as a translational biomarker for drug effects and possibly HAND patient selection. It is important to note, however, that while CSF represents a clinically available tissue compartment and may be representative to a certain extent of the neurotransmitter milieu present in parenchymal interstitial fluid (Hladky and Barrand 2014; Redzic 2011; Rodan et al. 2015), it is not a direct measure of synaptic or extraneuronal glutamate. Studies aimed at directly assessing glutamate concentrations in the hippocampus and cortex by in vivo microdialysis or amperometry would be informative.

Given that HAND is a chronic condition, potential HAND drugs targeting the glutamatergic system would likely need to be administered long term for continued efficacy. This treatment paradigm would require a drug with a very safe adverse effect profile. DON has been found to cause GI toxicity that would preclude its use as a chronic treatment, but newly developed DON prodrugs such as JHU083 with enhanced brain delivery (Nedelcovych et al. 2017; Rais et al. 2016) may improve tolerability by enabling peripheral dose reduction. Thus, DON prodrugs may offer a clinically viable strategy for targeting glutamate overproduction in HAND. The present results also suggest that repurposing of approved drugs which broadly modulate glutamate availability such as riluzole (Cheah et al. 2010; Hunsberger et al. 2015; Lener et al. 2017) also merit investigation in HAND and could have the potential for more immediate clinical impact.

Although the current study provides compelling evidence for altered glutamate metabolism as a final common pathway and therapeutic target in HAND, it leaves open an important question. In the absence of viremia, what is the upstream cause of glutamatergic dysfunction? Chronic neuroinflammation due to persistent viral protein synthesis (Saylor et al. 2016), microbial translocation in the gut (Marchetti et al. 2013), or low level HIV production in the CNS (Clements et al. 2011; Fois and Brew 2015; Saylor et al. 2016) have been posited as root causes of HAND. Evidence for some of these changes has been uncovered in EcoHIV-infected mice (Gu et al. 2018; Sindberg et al. 2015). Resulting inflammation could cause downstream GLS hyperactivity and glutamate overproduction (N. Erdmann et al. 2009; N. Erdmann et al. 2007; N. B. Erdmann et al. 2006; Huang et al. 2011; Potter et al. 2013; Tian et al. 2008; Tian et al. 2012; Vazquez-Santiago et al. 2014; Wu et al. 2015; Ye et al. 2013; J. Zhao et al. 2004; L. Zhao et al. 2012; L. Zhao et al. 2013), but more direct examination of the link between neuroinflammation and glutamate metabolism in the EcoHIV model is warranted.

Together, the results of this study point to aberrant glutamate metabolism as an important component of HAND pathophysiology and identify GLS as a promising therapeutic target. DON prodrugs with improved brain delivery such as JHU083 may offer a clinically viable means of targeting GLS for the treatment of HAND and other CNS disorders characterized by excess glutamate production.

Acknowledgements This research was supported by multiple grants including P30MH075673 (B.S.S.), R01CA193895 (B.S.S.), R01DA037611 (D.J.V.), R01MH105145 (D.J.V.), R01DA041208 (A.K.), R25MH080661 Pilot award (X.Z.), a Postdoctoral Fellowship in Pharmacology/Toxicology from the PhRMA Foundation (M.T.N.), a TEDCO Maryland Innovation Initiative award (B.S.S.), and R25GM109441 (a PREP grant supporting A.A.M.).

Compliance with Ethical Standards

Disclosures Under a license agreement between Dracen Pharmaceuticals, Inc. and the Johns Hopkins University, B.S.S., R.R., and J.A. are entitled to royalty distributions related to technology used in the research described in this publication. B.S.S. and R.R. are also co-founders of and hold equity in Dracen Pharmaceuticals, Inc. which is clinically developing glutamine antagonist prodrugs. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policies. B.S.S., D.J.V., M.T.N. and A.K. are also inventors on a patent application relevant to the research in this publication.

References

Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ (2010) Structure and function of the blood-brain barrier. Neurobiol Dis 37(1):13–25. https://doi.org/10.1016/j.nbd.2009.07.030

Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, ... Wojna VE (2007) Updated research nosology for HIV-associated neurocognitive disorders. Neurology 69(18): 1789–1799. https://doi.org/10.1212/01.WNL.0000287431.88658.8b

Bairwa D, Kumar V, Vyas S Das BK, Srivastava AK, Pandey RM ... Sinha S (2016) Case control study: magnetic resonance spectroscopy of brain in HIV infected patients. BMC Neurol 16(1): 99. https://doi.org/10.1186/s12883-016-0628-x

Cassol E, Misra V, Dutta A, Morgello S, Gabuzda D (2014) Cerebrospinal fluid metabolomics reveals altered waste clearance and accelerated aging in HIV patients with neurocognitive impairment. AIDS 28(11):1579–1591. https://doi.org/10.1097/qad.0000000000000303

Castelo JM, Sherman SJ, Courtney MG, Melrose RJ, Stern CE (2006) Altered hippocampal-prefrontal activation in HIV patients during episodic memory encoding. Neurology 66(11):1688–1695. https://doi.org/10.1212/01.wnl.0000218305.09183.70

Cheah BC, Vucic S, Krishnan AV, Kiernan MC (2010) Riluzole, neuroprotection and amyotrophic lateral sclerosis. Curr Med Chem 17(18):1942–1199

Chen L, Cui H (2015) Targeting glutamine induces apoptosis: A Cancer therapy approach. Int J Mol Sci 16(9):22830–22855. https://doi.org/10.3390/ijms160922830

Clements JE, Gama L, Graham DR, Mankowski JL, Zink MC (2011) A simian immunodeficiency virus macaque model of highly active antiretroviral treatment: viral latency in the periphery and the central nervous system. Curr Opin HIV AIDS 6(1):37–42. https://doi.org/10.1097/COH.0b013e3283412413

Coccaro EF, Lee R, Vezina P (2013) Cerebrospinal fluid glutamate concentration correlates with impulsive aggression in human subjects. J Psychiatr Res 47(9):1247–1253. https://doi.org/10.1016/j.jpsychires.2013.05.001

Conti F, Minelli A (1994) Glutamate immunoreactivity in rat cerebral cortex is reversibly abolished by 6-diazo-5-oxo-L-norleucine (DON), an inhibitor of phosphate-activated glutaminase. J Histochem Cytochem 42(6):717–726. https://doi.org/10.1177/42.6.7910617

Cooper AJ, Jeitner TM (2016) Central role of glutamate metabolism in the maintenance of nitrogen homeostasis in Normal and Hyperammonemic brain. Biomolecules 6(2):E16. https://doi.org/10.3390/biom6020016

Crosby HA, Miller KE (2016) Evaluating the analgesic effect of the GLS inhibitor 6-Diazo-5-Oxo-L-Norleucine in vivo. Pharm Pharmacol Int J 3(3):279–286. https://doi.org/10.15406/ppij.2015.03.00055

Crosby HA, Ihnat M, Miller KE (2015) Evaluating the toxicity of the analgesic Glutaminase inhibitor 6-Diazo-5-Oxo-L-Norleucine in vitro and on rat dermal skin fibroblasts. MOJ Toxicol 1(1). https://doi.org/10.15406/mojt.2015.01.00005

Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65(1):1–105

Diamond DM, Park CR, Heman KL, Rose GM (1999) Exposing rats to a predator impairs spatial working memory in the radial arm water maze. Hippocampus 9(5):542–552. https://doi.org/10.1002/(sici)1098-1063(1999)9:5<542::aid-hipo8>3.0.co;2-n

Erdmann NB, Whitney NP, Zheng J (2006) Potentiation of excitotoxicity in HIV-1 associated dementia and the significance of Glutaminase. Clin Neurosci Res 6(5):315–328. https://doi.org/10.1016/j.cnr.2006.09.009

Erdmann N, Zhao J, Lopez AL, Herek S, Curthoys N, Hexum TD, ... Zheng J (2007) Glutamate production by HIV-1 infected human macrophage is blocked by the inhibition of glutaminase. J Neurochem 102(2): 539–549. https://doi.org/10.1111/j.1471-4159.2007.04594.x

Erdmann N, Tian C, Huang Y, Zhao J, Herek S, Curthoys N, Zheng J (2009) In vitro glutaminase regulation and mechanisms of glutamate generation in HIV-1-infected macrophage. J Neurochem 109(2): 551–561. https://doi.org/10.1111/j.1471-4159.2009.05989.x

Ernst T, Chang L, Jovicich J, Ames N, Arnold S (2002) Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients. Neurology 59(9):1343–1349

Ernst T, Jiang CS, Nakama H, Buchthal S, Chang L (2010) Lower brain glutamate is associated with cognitive deficits in HIV patients: a new mechanism for HIV-associated neurocognitive disorder. J Magn Reson Imaging 32(5):1045–1053. https://doi.org/10.1002/jmri.22366

Featherstone DE, Shippy SA (2008) Regulation of synaptic transmission by ambient extracellular glutamate. Neuroscientist 14(2):171–181. https://doi.org/10.1177/1073858407308518

Fennema-Notestine C, Ellis RJ, Archibald SL, Jernigan TL, Letendre SL, Notestine RJ et al (2013) Increases in brain white matter abnormalities and subcortical gray matter are linked to CD4 recovery in HIV infection. J Neuro-Oncol 19(4):393–401. https://doi.org/10.1007/s13365-013-0185-7

Ferrarese C, Aliprandi A, Tremolizzo L, Stanzani L, De Micheli A, Dolara A, Frattola L (2001) Increased glutamate in CSF and plasma of patients with HIV dementia. Neurology 57(4):671–675

Fois AF, Brew BJ (2015) The potential of the CNS as a reservoir for HIV-1 infection: implications for HIV eradication. Curr HIV/AIDS Rep 12(2):299–303. https://doi.org/10.1007/s11904-015-0257-9

Gelman BB, Chen T, Lisinicchia JG, Soukup VM, Carmical JR, Starkey JM, ... Morgello S (2012a) The national NeuroAIDS tissue consortium brain gene array: two types of HIV-associated neurocognitive impairment. PLoS One 7(9): e46178. https://doi.org/10.1371/journal.pone.0046178

Gelman BB, Lisinicchia JG, Chen T, Johnson KM, Jennings K, Freeman DH Jr, Soukup VM (2012b) Prefrontal dopaminergic and enkephalinergic synaptic accommodation in HIV-associated neurocognitive disorders and encephalitis. J NeuroImmune Pharmacol 7(3):686–700. https://doi.org/10.1007/s11481-012-9345-4

Gu CJ, Borjabad A, Hadas E, Kelschenbach J, Kim BH, Chao W, ... Volsky DJ (2018) EcoHIV infection of mice establishes latent viral reservoirs in T cells and active viral reservoirs in macrophages that are sufficient for induction of neurocognitive impairment. PLoS Pathog 14(6): e1007061. https://doi.org/10.1371/journal.ppat.1007061

Harezlak J, Buchthal S, Taylor M, Schifitto G, Zhong J, Daar E, ... Navia B (2011) Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment. AIDS 25(5): 625–633. https://doi.org/10.1097/QAD.0b013e3283427da7

Heaps JM, Joska J, Hoare J, Ortega M, Agrawal A, Seedat S, ... Paul R (2012) Neuroimaging markers of human immunodeficiency virus infection in South Africa. J Neuro-Oncol 18(3): 151–156. https://doi.org/10.1007/s13365-012-0090-5

Heaton RK, Clifford DB, Franklin DR, Jr, Woods SP, Ake C, Vaida F, ... Grant I (2008) HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER study. Neurology 75(23): 2087–2096. https://doi.org/10.1212/WNL.0b013e318200d727

Heaton RK, Franklin DR, Ellis RJ, McCutchan JA, Letendre SL, Leblanc S, ... Grant I (2011) HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. J Neuro-Oncol 17(1): 3–16. https://doi.org/10.1007/s13365-010-0006-1

Hladky SB, Barrand MA (2014) Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. Fluids Barriers CNS 11(1):26. https://doi.org/10.1186/2045-8118-11-26

Huang Y, Zhao L, Jia B, Wu L, Li Y, Curthoys N, Zheng JC (2011) Glutaminase dysregulation in HIV-1-infected human microglia mediates neurotoxicity: relevant to HIV-1-associated neurocognitive disorders. J Neurosci 31(42):15195–15204. https://doi.org/10.1523/jneurosci.2051-11.2011

Hunsberger HC, Weitzner DS, Rudy CC, Hickman JE, Libell EM, Speer RR, ... Reed MN (2015) Riluzole rescues glutamate alterations, cognitive deficits, and tau pathology associated with P301L tau

expression. J Neurochem 135(2): 381–394. https://doi.org/10.1111/jnc.13230
Jernigan TL, Archibald SL, Fennema-Notestine C, Taylor MJ, Theilmann RJ, Julaton MD, ... Grant I (2011) Clinical factors related to brain structure in HIV: the CHARTER study. J Neuro-Oncol 17(3): 248–257. https://doi.org/10.1007/s13365-011-0032-7
Kaul M, Zheng J, Okamoto S, Gendelman HE, Lipton SA (2005) HIV-1 infection and AIDS: consequences for the central nervous system. Cell Death Differ 12(Suppl 1):878–892. https://doi.org/10.1038/sj.cdd.4401623
Kim BH, Kelschenbach J, Borjabad A, Hadas E, He H, Potash MJ, ... Volsky DJ (2019) Intranasal insulin therapy reverses hippocampal dendritic injury and cognitive impairment in a model of HAND in EcoHIV-infected mice. AIDS. https://doi.org/10.1097/qad.0000000000002150
Kvamme E, Torgner IA, Roberg B (2001) Kinetics and localization of brain phosphate activated glutaminase. J Neurosci Res 66(5):951–958. https://doi.org/10.1002/jnr.10041
Lemberg KM, Vornov JJ, Rais R, Slusher BS (2018) We're not "DON" yet: optimal dosing and prodrug delivery of 6-Diazo-5-oxo-L-norleucine. Mol Cancer Ther 17(9):1824–1832. https://doi.org/10.1158/1535-7163.Mct-17-1148
Lener MS, Kadriu B, Zarate CA Jr (2017) Ketamine and beyond: investigations into the potential of glutamatergic agents to treat depression. Drugs 77(4):381–401. https://doi.org/10.1007/s40265-017-0702-8
Liu L, Duff K (2008) A technique for serial collection of cerebrospinal fluid from the cisterna magna in mouse. J Vis Exp 21. https://doi.org/10.3791/960
Marchetti G, Tincati C, Silvestri G (2013) Microbial translocation in the pathogenesis of HIV infection and AIDS. Clin Microbiol Rev 26(1): 2–18. https://doi.org/10.1128/cmr.00050-12
McArthur JC, Steiner J, Sacktor N, Nath A (2010) Human immunodeficiency virus-associated neurocognitive disorders: mind the gap. Ann Neurol 67(6):699–714. https://doi.org/10.1002/ana.22053
Melrose RJ, Tinaz S, Castelo JM, Courtney MG, Stern CE (2008) Compromised fronto-striatal functioning in HIV: an fMRI investigation of semantic event sequencing. Behav Brain Res 188(2):337–347. https://doi.org/10.1016/j.bbr.2007.11.021
Mohamed MA, Barker PB, Skolasky RL, Selnes OA, Moxley RT, Pomper MG, Sacktor NC (2010) Brain metabolism and cognitive impairment in HIV infection: a 3-T magnetic resonance spectroscopy study. Magn Reson Imaging 28(9):1251–1257. https://doi.org/10.1016/j.mri.2010.06.007
Moussawi K, Riegel A, Nair S, Kalivas PW (2011) Extracellular glutamate: functional compartments operate in different concentration ranges. Front Syst Neurosci 5:94. https://doi.org/10.3389/fnsys.2011.00094
Nedelcovych MT, Tenora L, Kim BH, Kelschenbach J, Chao W, Hadas E, ... Slusher BS (2017) N-(Pivaloyloxy)alkoxy-carbonyl prodrugs of the glutamine antagonist 6-Diazo-5-oxo-l-norleucine (DON) as a potential treatment for HIV associated neurocognitive disorders. J Med Chem 60(16): 7186–7198. https://doi.org/10.1021/acs.jmedchem.7b00966
Potash MJ, Chao W, Bentsman G, Paris N, Saini M, Nitkiewicz J, ... Volsky DJ (2005) A mouse model for study of systemic HIV-1 infection, antiviral immune responses, and neuroinvasiveness. Proc Natl Acad Sci U S A 102(10): 3760–3765. https://doi.org/10.1073/pnas.0500649102
Potter MC, Figuera-Losada M, Rojas C, Slusher BS (2013) Targeting the glutamatergic system for the treatment of HIV-associated neurocognitive disorders. J NeuroImmune Pharmacol 8(3):594–607. https://doi.org/10.1007/s11481-013-9442-z
Ragin AB, Storey P, Cohen BA, Edelman RR, Epstein LG (2004) Disease burden in HIV-associated cognitive impairment: a study of whole-brain imaging measures. Neurology 63(12):2293–2297
Rais R, Jancarik A, Tenora L, Nedelcovych M, Alt J, Englert J, ... Slusher BS (2016) Discovery of 6-Diazo-5-oxo-l-norleucine (DON) prodrugs with enhanced CSF delivery in monkeys: A potential treatment for glioblastoma. J Med Chem 59(18): 8621–8633. https://doi.org/10.1021/acs.jmedchem.6b01069
Redzic Z (2011) Molecular biology of the blood-brain and the blood-cerebrospinal fluid barriers: similarities and differences. Fluids Barriers CNS 8(1):3. https://doi.org/10.1186/2045-8118-8-3
Robertson KR, Smurzynski M, Parsons TD, Wu K, Bosch RJ, Wu J, ... Ellis RJ (2007) The prevalence and incidence of neurocognitive impairment in the HAART era. AIDS 21(14): 1915–1921. https://doi.org/10.1097/QAD.0b013e32828e4e27
Rodan LH, Gibson KM, Pearl PL (2015) Clinical use of CSF neurotransmitters. Pediatr Neurol 53(4):277–286. https://doi.org/10.1016/j.pediatrneurol.2015.04.016
Sailasuta N, Shriner K, Ross B (2009) Evidence of reduced glutamate in the frontal lobe of HIV-seropositive patients. NMR Biomed 22(3): 326–331. https://doi.org/10.1002/nbm.1329
Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M, ... McArthur JC (2016) HIV-associated neurocognitive disorder–pathogenesis and prospects for treatment. Nat Rev Neurol 12(4): 234–248. https://doi.org/10.1038/nrneurol.2016.27
Schifitto G, Yiannoutsos CT, Simpson DM, Marra CM, Singer EJ, Kolson DL, ... Adult A C T G.t (2006) A placebo-controlled study of memantine for the treatment of human immunodeficiency virus-associated sensory neuropathy. J Neuro-Oncol 12(4): 328–331. https://doi.org/10.1080/13550280600873835
Schifitto G, Navia BA, Yiannoutsos CT, Marra CM, Chang L, Ernst T, ... Lipton SA (2007) Memantine and HIV-associated cognitive impairment: a neuropsychological and proton magnetic resonance spectroscopy study. AIDS 21(14): 1877–1886. https://doi.org/10.1097/QAD.0b013e32813384e8
Schousboe A, Scafidi S, Bak LK, Waagepetersen HS, McKenna MC (2014) Glutamate metabolism in the brain focusing on astrocytes. Adv Neurobiol 11:13–30. https://doi.org/10.1007/978-3-319-08894-5_2
Sharma K, Schmitt S, Bergner CG, Tyanova S, Kannaiyan N, Manrique-Hoyos N, ... Simons M (2015) Cell type- and brain region-resolved mouse brain proteome. Nat Neurosci 18(12): 1819–1831. https://doi.org/10.1038/nn.4160
Sindberg GM, Sharma U, Banerjee S, Anand V, Dutta R, Gu CJ, ... Roy S (2015) An infectious murine model for studying the systemic effects of opioids on early HIV pathogenesis in the gut. J NeuroImmune Pharmacol 10(1): 74–87. https://doi.org/10.1007/s11481-014-9574-9
Song M, Kim SH, Im CY, Hwang HJ (2018) Recent development of small molecule Glutaminase inhibitors. Curr Top Med Chem 18(6):432–443. https://doi.org/10.2174/1568026618666180525100830
Stankoff B, Tourbah A, Suarez S, Turell E, Stievenart JL, Payan C, ... Lubetzki C (2001) Clinical and spectroscopic improvement in HIV-associated cognitive impairment. Neurology, 56(1), 112–115
Steinbrink F, Evers S, Buerke B, Young P, Arendt G, Koutsilieri E, ... Husstedt IW (2013) Cognitive impairment in HIV infection is associated with MRI and CSF pattern of neurodegeneration. Eur J Neurol 20(3): 420–428. https://doi.org/10.1111/ene.12006
Thomas AG, Rojas C, Tanega C, Shen M, Simeonov A, Boxer MB, ... Slusher BS (2013a) Kinetic characterization of ebselen, chelerythrine and apomorphine as glutaminase inhibitors. Biochem Biophys Res Commun 438(2): 243–248. https://doi.org/10.1016/j.bbrc.2013.06.110
Thomas JB, Brier MR, Snyder AZ, Vaida FF, Ances BM (2013b) Pathways to neurodegeneration: effects of HIV and aging on resting-state functional connectivity. Neurology 80(13):1186–1193. https://doi.org/10.1212/WNL.0b013e318288792b

Thomas AG, O'Driscoll CM, Bressler J, Kaufmann W, Rojas CJ, Slusher BS (2014) Small molecule glutaminase inhibitors block glutamate release from stimulated microglia. Biochem Biophys Res Commun 443(1):32–36. https://doi.org/10.1016/j.bbrc.2013.11.043

Thompson PM, Dutton RA, Hayashi KM, Toga AW, Lopez OL, Aizenstein HJ, Becker JT (2005) Thinning of the cerebral cortex visualized in HIV/AIDS reflects CD4+ T lymphocyte decline. Proc Natl Acad Sci U S A 102(43):15647–15652. https://doi.org/10.1073/pnas.0502548102

Tian C, Erdmann N, Zhao J, Cao Z, Peng H, Zheng J (2008) HIV-infected macrophages mediate neuronal apoptosis through mitochondrial glutaminase. J Neurochem 105(3):994–1005. https://doi.org/10.1111/j.1471-4159.2007.05197.x

Tian C, Sun L, Jia B, Ma K, Curthoys N, Ding J, Zheng J (2012) Mitochondrial glutaminase release contributes to glutamate-mediated neurotoxicity during human immunodeficiency virus-1 infection. J NeuroImmune Pharmacol 7(3):619–628. https://doi.org/10.1007/s11481-012-9364-1

Vazquez-Santiago FJ, Noel RJ Jr, Porter JT, Rivera-Amill V (2014) Glutamate metabolism and HIV-associated neurocognitive disorders. J Neuro-Oncol 20(4):315–331. https://doi.org/10.1007/s13365-014-0258-2

Wu B, Huang Y, Braun AL, Tong Z, Zhao R, Li Y et al (2015) Glutaminase-containing microvesicles from HIV-1-infected macrophages and immune-activated microglia induce neurotoxicity. Mol Neurodegener 10:61. https://doi.org/10.1186/s13024-015-0058-z

Ye L, Huang Y, Zhao L, Li Y, Sun L, Zhou Y, ... Zheng JC (2013) IL-1beta and TNF-alpha induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. J Neurochem 125(6): 897–908. https://doi.org/10.1111/jnc.12263

Zhao J, Lopez AL, Erichsen D, Herek S, Cotter RL, Curthoys NP, Zheng J (2004) Mitochondrial glutaminase enhances extracellular glutamate production in HIV-1-infected macrophages: linkage to HIV-1 associated dementia. J Neurochem 88(1):169–180

Zhao L, Huang Y, Tian C, Taylor L, Curthoys N, Wang Y, ... Zheng J (2012) Interferon-alpha regulates glutaminase 1 promoter through STAT1 phosphorylation: relevance to HIV-1 associated neurocognitive disorders. PLoS One 7(3): e32995. https://doi.org/10.1371/journal.pone.0032995

Zhao L, Huang Y, Zheng J (2013) STAT1 regulates human glutaminase 1 promoter activity through multiple binding sites in HIV-1 infected macrophages. PLoS One 8(9):e76581. https://doi.org/10.1371/journal.pone.0076581

Zhu X, Nedelcovych MT, Thomas AG, Hasegawa Y, Moreno-Megui A, Coomer W, ... Kamiya A (2018) JHU-083 selectively blocks glutaminase activity in brain CD11b(+) cells and prevents depression-associated behaviors induced by chronic social defeat stress. Neuropsychopharmacology. https://doi.org/10.1038/s41386-018-0177-7