Intranasal insulin therapy reverses hippocampal dendritic injury and cognitive impairment in a model of HIV-associated neurocognitive disorders in EcoHIV-infected mice

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Objective: Almost half of HIV-positive people on antiretroviral therapy have demonstrable mild neurocognitive impairment (HIV-NCI), even when virologically suppressed. Intranasal insulin therapy improves cognition in Alzheimer’s disease and diabetes. Here we tested intranasal insulin therapy in a model of HIV-NCI in Eco-HIV-infected conventional mice.

Design and methods: Insulin pharmacokinetics following intranasal administration to mice was determined by ELISA. Mice were inoculated with EcoHIV to cause NCI; 23 days or 3 months after infection they were treated daily for 9 days with intranasal insulin (2.4 IU/mouse) and examined for NCI in behavioral tests and HIV burdens by quantitative PCR. Some animals were tested for hippocampal neuronal integrity by immunostaining and expression of neuronal function-related genes by real time-quantitative PCR. The effect of insulin treatment discontinuation on cognition and neuropathology was also examined.

Results: Intranasal insulin administration to mice resulted in μIU/ml levels of insulin in cerebrospinal fluid with a half-life of about 2 h, resembling pharmacokinetic parameters of patients receiving 40 IU. Intranasal insulin treatment starting 23 days or 3 months after infection completely reversed NCI in mice. Murine NCI correlated with reductions in hippocampal dendritic arbors and downregulation of neuronal function genes; intranasal insulin reversed these changes coincident with restoration of cognitive acuity, but they returned within 24 h of treatment cessation. Intranasal insulin treatment reduced brain HIV DNA when started 23 but not 90 days after infection.

Conclusion: Our preclinical studies support the use of intranasal insulin administration for treatment of HIV-NCI and suggest that some dendritic injury in this condition is reversible.

Keywords: EcoHIV, fear conditioning, HIV-associated neurocognitive impairment, insulin, intranasal insulin therapy, mouse models, quantitative PCR, radial arm water maze, synaptodendritic injury, working memory

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Received: 17 August 2018; revised: 25 December 2018; accepted: 8 January 2019.

DOI:10.1097/QAD.0000000000002150

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Introduction

Neurocognitive impairments (NCI) in HIV-infected people are collectively known as HIV-associated neurocognitive disorders (HAND) of increasing severity from asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), to HIV-associated dementia (HAD) [1]. Chronically HIV-infected people who are virologically suppressed on long-term antiretroviral therapy (ART) rarely progress to HAD but about 50% of them will develop ANI or MND (here referred to as HIV-NCI) [2–4]. Some HIV-NCI patients report difficulties performing daily tasks, but the growing majority have ‘silent’ deficits only apparent in cognitive tests [5,6]. This largely asymptomatic NCI increases a patient’s risk of progression to symptomatic disease [7,8] and the severity of NCI manifestations increases with age [3,9,10] suggesting substantial long-term health concerns associated with NCI. There are currently no therapies to prevent NCI progression in virologically suppressed individuals, nor any to address the effects of NCI on long-term management of chronic HIV infection.

HIV-NCI is diagnosed primarily in neuropsychological tests [1,11]. In contrast to HAD, HIV-NCI under ART shows minimal overt neuropathology and the disease is thought to arise from neuronal dysfunction rather than frank neuronal loss [6,12,13]. HIV-NCI includes mild dysfunctions in attention, learning/memory, working memory, executive function, and fine motor skills, in one or multiple domains [1,14], resembling the mild cognitive impairment accompanying aging, type 2 diabetes mellitus (T2DM), and the early prodromal stages of Alzheimer’s disease [15–17]. One common determinant in all these conditions is a metabolic dysregulation in the brain, which for aging, obesity, and Alzheimer’s disease was at least in part linked in animal models to deficient insulin signaling (reviewed in [6,18]). Intranasal insulin treatment may improve cognitive function in both T2DM and early Alzheimer’s disease patients [19–22]. The efficacy of this treatment in HIV-NCI is unknown, but intranasal insulin administration was recently shown to inhibit HIV infection in culture and mitigate feline immunodeficiency virus (FIV)-induced encephalitis in cats [23]. The goal of the present work was to evaluate intranasal insulin therapy in a model of HIV-NCI in conventional mice infected with chimeric HIV, EcoHIV [24,25]. Short-term intranasal insulin therapy reverses hippocampal dendritic injury, gene dysregulation in the brain, and NCI in EcoHIV-infected mice, but these benefits diminish within 24 h of insulin treatment cessation.

Materials and methods

Mice and EcoHIV infection

Animal studies were conducted with the approval of Mount Sinai School of Medicine Institutional Animal Care and Use Committee (protocol IACUC-2014-0124) and were compliant with NIH guidelines. 6–8 week-old C57BL/6J and 129x1/SvJmice were from the Jackson Laboratory (Farmington, Connecticut, USA). EcoHIV (clone EcoNDK-V5) was prepared and inoculated intraperitoneally at a dose of $2.0 \times 10^5$ pg HIV p24 per mouse as described [24,25].

Intranasal insulin delivery and insulin pharmacokinetics

Human insulin (HumulinR, 100 IU/ml; Eli Lilly, Toronto, Ontario, Canada) was administered intranasal at 2.4 IU/mouse as described [26]. For pharmacokinetics measurements, insulin was administered to three to eight mice per group, animals were sacrificed at the indicated time points (Fig. 1) for isolation of plasma, cerebrospinal fluid (CSF), and cortex. Insulin concentrations were determined by human insulin ELISA kit (Alpco, Salem, New Hampshire, USA) [26].

Tissue collection and EcoHIV burden analysis

Plasma was obtained from blood drawn from retro-orbital sinus of anesthetized mice [27]. CSF (10–15 μl) was collected from cisterna magna essentially as described [28]. Spleens and brains were dissected from animals euthanized by 95% CO2 asphyxiation and processed for HIV burden analysis as described [24].

Behavioral tests

Mice were tested for spatial learning and working memory in radial arm water maze (RAWM) [29] and for contextual associative fear memory by fear conditioning, fold change [30], as previously described [24]. RAWM test consisted of four daily learning trials and a 30 min-delayed retention trial for assessment of working memory [17]. Error averages of the last 3 days of testing were used for statistical analysis. Contextual fold change responses were measured using a SDI Freeze Monitor (San Diego Instruments, San Diego, California, USA) following manufacturer’s protocol [24]. Results are presented as mean percentage of total time spent freezing on the training day (baseline) and the contextual fear readout day (context).

Evaluation of hippocampal neuronal integrity by immunofluorescence staining

Mouse perfusion, preparation of frozen brain sections, and immunofluorescence staining of sections were previously described [31]. Antibodies used were rabbit anti-microtubule-associated protein 2 (MAP2) for detection of dendrites (EMD Millipore, Burlington, Massachusetts, USA, 1:150) and mouse anti-neuronal nuclear antigen (NeuN) for detection of neuronal nuclei (EMD Millipore, Burlington, Massachusetts, USA) for statistical analysis. Contextual fold change responses were measured using a SDI Freeze Monitor (San Diego Instruments, San Diego, California, USA) following manufacturer’s protocol [24]. Results are presented as mean percentage of total time spent freezing on the training day (baseline) and the contextual fear readout day (context).
Effect of insulin treatment on expression of neuronal function and metabolism-related genes in the brain

Brain RNA was isolated and reverse-transcribed as described [24,31]. Its complementary DNA (cDNA) was tested for gene expression changes using custom-designed TaqMan Gene Expression Array Plates from Thermo-Fisher Scientific. Reactions were run in a ThermoFisher Scientific (Applied Biosystems, Waltham, Massachusetts, USA) 7900 instrument according to the manufacturer’s protocol.

Pharmacokinetic analysis of intranasal insulin

The pharmacokinetic parameters were calculated using noncompartmental method of software program Phoenix WinNonlin version 6.4 (Certara USA, Inc., Princeton, New Jersey, USA). The maximum plasma concentration ($C_{\text{max}}$) and time to $C_{\text{max}}$ ($T_{\text{max}}$) were the observed values. The area under the plasma concentration time curve (AUC) was calculated to the last quantifiable sample (AUC$_{\text{last}}$) by use of the log-linear trapezoidal rule. The brain to plasma partition coefficients were calculated as a ratio of mean AUCs (AUC$_{0–t,\text{ brain}}$/AUC$_{0–t,\text{ plasma}}$).

Statistical analysis

Differences in EcoHIV burdens and other parameters between control vs. infected and nontreated vs. treated mice were tested by paired Student’s $t$ test. Changes in cellular gene expression in brain tissues of infected or drug-treated mice were first normalized to respective uninfected or nondrug-treated controls then compared in $t$ test between infected and drug-treated-infected groups.

Results

Intranasal insulin pharmacokinetics in mice

Figure 1 shows pharmacokinetics evaluation of intranasal insulin delivery (2.4IU/mouse) in plasma, CSF, and cortex of mock-infected C57BL/6 mice. The insulin pharmacokinetics profiles for plasma and brain were similar, insulin concentrations reached their respective peaks at 30 min and the peptide cleared to baseline by 6 h. Cortex insulin levels remained more than 10-fold above baseline for 3 h following insulin administration. The CSF insulin profile was irregular with an early peak at 15 min, a $C_{\text{max}}$ of 17 μIU/ml (2.38 pmol/l) at 1 h, and clearance by 3 h. Insulin $C_{\text{max}}$ in cortex was substantially lower than that in plasma and brain.
Fig. 2. Intranasal insulin therapy restores deficient learning/memory in chronically EcoHIV-infected mice. (a–d) Effect of intranasal insulin treatment on EcoHIV-induced spatial learning/memory 1 month after infection. Mice were EcoHIV or mock (PBS) infected for 21 days, the animals were then acclimated to intranasal delivery with daily intranasal PBS administration (24 µl/mouse) for 5 days followed by daily intranasal insulin or intranasal vehicle (PBS) treatment for 9 days. The treatment groups (10 animals each) were PBS + PBS, PBS + insulin, EcoHIV + PBS, and EcoHIV + insulin. Mice were tested in radial arm water maze during days 2–9 of treatment and sacrificed on the last day of treatment (34 days after infection) for intranasal insulin pharmacokinetics test, tissue collection, and analysis. (a) Results of radial arm water maze behavioral test showing number of errors made (left panel) and time spent ...
higher than in plasma at 53.0 μIU/ml (7.42 pmol/l) vs. 11.7 μIU/ml (1.64 pmol/l), respectively. In addition, overall cortex insulin exposures (measured by area under the curve AUC₀₋₉₅) were also higher with AUC₉₅brain/AUC₉₅plasma ratio of 3 (table in Fig. 1). These results illustrate that intranasal insulin administration in mice at 2.4 IU produces a rapid intracerebral peptide accumulation with therapeutic concentrations lasting 2–3 h.

**Intranasal insulin treatment reverses murine HIV-neurocognitive impairment independent of HIV brain burdens**

EcoHIV-infected mice manifested cognitive impairment (murine HIV-NCI) 1 month after infection as shown by their failure to learn the hidden platform location in RAWM compared with uninfected mice (Fig. 2a). When treated daily with intranasal insulin for 9 days, infected mice performed similarly in RAWM to untreated or insulin-treated-uninfected mice (Fig. 2a), suggesting that their NCI was reversed. All mouse groups performed equally well in finding the visible platform (right panel in Fig. 2a), indicating that neither EcoHIV infection nor intranasal insulin–affected animal mobility, vision, or motivation. Intranasal insulin treatment had no effect on HIV levels in spleen but significantly reduced HIV brain burdens (Fig. 2b). EcoHIV infection of mice had no effect on brain insulin concentrations compared with mock–infected mice (Fig. 2c). Pharmacokinetics assessment of intranasal insulin delivery after RAWM evaluation indicated preferential peptide accumulation in the brain in both EcoHIV and mock–infected mice (Fig. 2c).

To confirm insulin effects on cognition, groups of mice were subjected to intranasal insulin treatment and evaluated for a contextual associative fear responses [30] by fold change (Fig. 2d). Infected conditioned mice spent less time exhibiting the threat-induced freezing response compared with control mice when placed in the fear conditioning chamber the next day (context panel in Fig. 2d), indicating impairment in recall of the conditioned response. Intranasal insulin treatment returned the freezing response time of infected mice to that of uninfected animals (Fig. 2d). Finally, we inquired whether EcoHIV-infected mice remain sensitive to the beneficial effects of insulin 3 months after infection (Fig. 2e). The RAWM measures of murine NCI were similar 1 and 3 months after HIV infection, confirming the chronic nature of HIV-NCI in mice [24]. This chronic NCI was also fully reversible with 9 days of intranasal insulin treatment (Fig. 2a and e). However, in contrast to early HIV-NCI in mice, HIV DNA brain levels were not significantly affected by insulin treatment 3 months after infection (Fig. 2f). Direct evaluation of insulin effects on EcoHIV replication in primary mouse macrophages in culture (see Figure in Supplemental Digital Content, http://links.lww.com/QAD/B435) indicated that insulin can inhibit HIV replication but only at IU/ml concentrations, which were about 100-fold higher than those attained in the brain after intranasal insulin (Fig. 1). These results suggest that intranasal insulin therapy is equally effective in reversing early and chronic HIV-NCI in EcoHIV-infected mice but that the effect of insulin on cognition in long-term–infected animals is independent of HIV DNA levels in brain.

**Intranasal insulin treatment reverses hippocampal dendritic injury and normalizes expression of selected brain function genes in murine HIV-neurocognitive impairment**

The spatial memory deficit in EcoHIV-infected mice (Fig. 2a) points to the hippocampus as the primary anatomical substrate of this dysfunction [17,29]. We tested hippocampal neuronal integrity in HIV-NCI mice by staining brain sections for the neuronal dendrite marker MAP2 [32,33] and neuronal cell body marker NeuN (Fig. 3a). We found a significant reduction in MAP2 staining in the CA1 and CA3 regions of the hippocampus of infected mice compared with controls (Fig. 3a and b), coinciding in real time with NCI in these animals shown in Fig. 2a. Insulin treatment of infected mice restored MAP2 stained dendrites to control levels (Fig. 3a and b), coinciding with restoration of spatial memory (Fig. 2a). In contrast, neuronal cell bodies visualized by NeuN staining remained unaltered under all conditions tested, suggesting that EcoHIV infection does not cause hippocampal neuronal loss (Fig. 3a and b), similar to the normal HIV neuropathology in HIV–infected patients on ART [13]. These results suggest that EcoHIV infection disrupts neuronal dendritic integrity in the hippocampus and that this process is reversible by intranasal insulin treatment.

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*P < 0.04, **P < 0.01. (b) EcoHIV burdens in spleen cells (SPC) and brain measured by quantitative PCR. **P < 0.0003. (c) Insulin pharmacokinetics measured after completion of radial arm water maze testing (n = 3/pharmacokinetics group). *P < 0.01, **P < 0.001. (d) Effect of intranasal insulin treatment on EcoHIV-induced fear-associative memory 1 month after infection. The experiment was repeated as described above using 129x1/SvJ mice and the animals were subjected to contextual fold change test during intranasal insulin treatment. The results are expressed as percentage mean time spent freezing and shown for mice tested prior to fear conditioning with paired sound-shock stimuli (baseline) and for conditioned mice placed 24 h later in chamber used for conditioning (context); *P < 0.05. (e) and (f) Effects of insulin upon neurocognitive impairment and virus burden in mice in long-term chronically EcoHIV-infected mice. The experiment described under part (a) was repeated except that intranasal-insulin treatment and radial arm water maze test were conducted 3 months after EcoHIV infection. The figure shows averaged results of last training trial and retention trial. *P < 0.05, **P < 0.05. (f) SPC and brain EcoHIV burdens measured by quantitative PCR. The differences between EcoHIV + PBS and EcoHIV + insulin were not significant: (P = 0.11 for spleen cells and P = 0.28 for brain).
Fig. 3. Intranasal insulin treatment reverses hippocampal dendritic injury and normalizes expression of selected brain function genes in EcoHIV-infected mice with neurocognitive impairment. Brain specimens were from mice (three per variable) that completed behavioral testing in experiment shown in Fig. 2a–c. (a) Representative confocal microscope images from the CA1 region of the hippocampus showing detection of neuronal dendritic [microtubule-associated protein 2 (MAP2), in green] and nuclear [neuronal nuclear antigen (NeuN), in red] proteins; scale bar = 24 µm. (b) Quantification of MAP2 and NeuN staining in the CA1 and CA3 regions of the hippocampus; *P < 0.01, **P < 0.005. (c) Expression of selected brain function-related genes in brain tissues. Total brain RNA was isolated
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To confirm these observations, mouse brain tissues were evaluated by custom quantitative PCR arrays for expression of 88 genes selected for their roles in synaptic plasticity, neuronal function, and brain metabolism (some of these genes were previously identified by us and others in the genome-wide microarray analyses of brain tissues from HAND patients [34,35]). Fourteen genes were significantly downmodulated ($P < 0.05$) by a factor of 1.5–3.8 in EcoHIV-infected mice compared with uninfected controls, and their downmodulation was fully reversed by intranasal insulin treatment (Fig. 3c and Table in Supplemental Digital Content, http://links.lww.com/QAD/B435). Ten significantly downregulated genes, CAMK2A, calcium/calmodulin-dependent protein kinase II, NRGN, neurogranin, DLG4, NR4A1, GPRIN1, KALRN, NGFR, BDNF, brain-derived neurotrophic factor, MAPK14, and GRIA2 are implicated in synaptic plasticity, dendrite biology, and neuronal signal transmission, two in neurogenesis (DCX, doublecortin, and NAV3, neuron navigator 3), and two (PPAT, phosphoribosyl pyrophosphate amidotransferase, and TMG5, transglutaminase 5) in glutamine and energy metabolism (Fig. 3c). Most of the other genes on the array showed a trend to below-normal expression in EcoHIV-infected mice and to normalization by insulin treatment. For complete array results and full names and functions of the significantly altered genes, see Table in Supplemental Digital Content, http://links.lww.com/QAD/B435.

**The benefits of intranasal insulin treatment in murine HIV-neurocognitive impairment diminish rapidly upon treatment discontinuation**

The contrast between the short half-life of intranasal-delivered insulin in mouse brain (Fig. 1) and the effectiveness of intranasal insulin in ameliorating HIV-NCI (Figs. 2 and 3), prompted us to investigate the durability of insulin effects on cognition. To this end, we discontinued intranasal insulin treatment in parallel groups of uninfected and EcoHIV-infected mice on the third day of cognitive evaluation in RAWM (Fig. 4a and b and blue columns in Fig. 4c). RAWM evaluation proceeded until completion, mice were tested for virus burden (Fig. 4c) and hippocampal dendrite integrity (Fig. 4d and e). The standard last 3-day average representation of RAWM results (Fig. 4a) shows that treatment cessation in infected animals dramatically worsened their performance in the maze compared with infected, continually treated animals. A plot of daily retention trial (working memory) data for all animal groups (Fig. 4b) revealed that intranasal insulin began to restore working memory in infected mice on the first day of testing, reached statistical significance on the second day, and maximum effect by day 5. However, discontinuation of insulin treatment in infected mice resulted in a complete return of working memory impairment within 24 h (Fig. 4b, blue columns). Reproducing data shown in Fig. 2, untreated infected mice tested 1 month after infection had detectable HIV DNA in the brain that was reduced following intranasal insulin by more than 90%; insulin had no effect on levels of splenic virus (Fig. 4c). Discontinuation of insulin treatment restored HIV brain burdens to close to pretreatment levels and significantly increased peripheral virus burdens compared with untreated mice (Fig. 4c). Analysis of hippocampal dendrite integrity revealed a similar pattern of efficacy (Fig. 4d and e), with insulin restoring dendrite MAP2 integrity in the CA1 and CA3 regions of the hippocampus to levels similar to uninfected mice. Cessation of insulin treatment resulted in a return of dendritic damage with MAP2 staining at levels seen in infected untreated mice. These results confirm the beneficial effects of intranasal insulin on cognition and hippocampal dendrite integrity in EcoHIV-infected mice (Figs. 2 and 3) and show that treatment discontinuation rapidly reverses these benefits.

**Discussion**

Our findings demonstrate that intranasal insulin treatment reverses cognitive impairment in conventional EcoHIV-infected mice. The behavioral defects and physiological changes in brains of these mice may form a useful model of HIV-NCI in HIV-positive people on ART. Chronically EcoHIV-infected mice have low lymphoid and brain HIV burdens and remain immunocompetent, in part due to control of HIV replication by the host immune system [24,31,36,37]. Like chronically HIV-infected patients on ART [38], despite virus control, infected mice develop mild abnormalities in gut, lung, and nervous system including chronic, ART-independent HIV-NCI [24,25,31,36,39–42]. Human HIV-NCI is a life-long condition that cannot be prevented even when ART is initiated several months after primary HIV infection [43]. Although animal models cannot fully replicate human disease, our results in EcoHIV-infected mice suggest that intranasal insulin delivery could be an effective treatment for HIV-NCI. This conclusion is based on several findings in the present work.

Starting with intranasal insulin administration, both peak central nervous system (CNS) concentrations and half-life of 2.4 IU intranasal-delivered insulin in mice resembled the intranasal-insulin pharmacokinetics profile in humans receiving 40 IU [44]. Separately, we have shown that intranasal insulin at this dose increases energy metabolism in mouse brain but not plasma glucose levels [26], affirming the

...and reverse-transcribed to complementary DNA (cDNA) as previously described [34]. The cDNAs were evaluated on custom TaqMan Array 96-Well Plates (ThermoFisher Scientific) according to manufacturer’s instructions. The 88 genes represented in the arrays (Table S1, http://links.lww.com/QAD/B435) were chosen according to their published functions in synaptic plasticity, neuronal function, and brain metabolism. Fold change in gene expression relative to control (PBS intraperitoneally + PBS intranasal); $P < 0.05$. 

References

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Fig. 4. Discontinuation of intranasal insulin therapy in EcoHIV-infected mice with neurocognitive impairment eliminates beneficial effects of insulin on hippocampal dendritic injury and neurocognitive impairment. Mice were infected and treated with intranasal insulin as described in Fig. 2(a–c) except that the groups (PBS + insulin) and (EcoHIV + insulin) contained 16 mice each. Insulin administration was discontinued in eight mice each in these groups from day 3 of radial arm water maze testing until experiment completion (blue bars in Fig. 2b) and the new groups were labeled (PBS + insulin/disc) and (EcoHIV + insulin/disc). (a) Standard presentation of radial arm water maze results in this experiment showing errors (left panel), latency (middle panel), and visible platform latency (right panel) averaged for the last 3 days of the maze testing. *P < 0.001, #P < 0.002. (b) Daily radial arm water maze retention trial results showing errors in finding hidden platform; red arrows indicate when insulin treatment was discontinued; blue-hued columns represent insulin-discontinued mouse groups. *P < 0.001, #P < 0.002. (c) Lymphoid and brain HIV burdens. *P < 0.001, #P < 0.002. (d) Representative confocal microscope images from the CA1 region of the hippocampus showing detection of neuronal dendritic (MAP2, in green) and nuclear (NeuN, in red) markers; scale bar = 45 μm. (e) Quantification of MAP2 staining in the CA1 and CA3 regions of the hippocampus; *P < 0.01, **P < 0.005.
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reported safety profile of short-term intranasal insulin administration in people [45]. Because 40 IU insulin shows reproducible therapeutic efficacy in clinical trials of Alzheimer’s disease and T2DM patients [19–22], we have achieved therapeutic doses of insulin in mouse CNS. As in animal models of nonviral cognitive diseases [46–48], short-term intranasal insulin treatment here was effective in reversing murine HIV-NCI. However, as in the SAMP8 mouse model of aging/Alzheimer’s disease [48], the beneficial effect of a single intranasal insulin administration on cognition in infected mice lasted only about 24 h, possibly due to combined limitations of short half-life of intranasal-delivered insulin (Fig. 1 and [44]) and transient nature of insulin signaling [49]. This suggests that long-term intranasal insulin administration will be essential for the preservation of cognitive function [50] and implies prospect of life-long adjunct intranasal insulin therapy to mitigate progression of NCI with aging [3,10]. Importantly, this is feasible, however, further studies are imperative to determine potential side effects of such therapy [45,51] and complications that may arise from patient noncompliance and other treatments.

Second, we show that intranasal insulin treatment reversed impairment in all three cognitive abilities tested in EcoHIV-infected mice, visuospatial learning, working memory, and contextual (explicit) memory. Working memory is a prefrontal cortex (PFC) executive function [52,53] required for spatial learning in RAWM [17,29], whereas both visuospatial and explicit memory require functional integrity of the hippocampus and entorhinal cortex in the medial temporal lobe [54,55]. Thus, mouse HIV-NCI resembles human HIV-NCI in the putative diffuse injury to the anatomical structures and neuronal networks spanning the PFC, stratum, and medial temporal lobe, a feature distinguishing mild NCI from the characteristic severe white matter injury and motor dysfunction of HIV dementia [3,12,56]. The cognitive abilities measured in EcoHIV-infected mice are among seven cognitive domains evaluated in standardized neuropsychological tests for NCI diagnosis [1] and they are frequently abnormal in clinically asymptomatic NCI individuals (reviewed in [14,57,58]). Declines in working and explicit memory also serve as indicators of HIV-NCI progression, particularly with aging [3,9,10]. The beneficial effects of intranasal insulin on these functions in EcoHIV-infected mice, including in older animals (Fig. 2c), indicate that this treatment could mitigate some of the major cognitive deficits in patients with HIV-NCI and potentially lessen the effect of aging on NCI severity.

Third, our results link memory impairment in EcoHIV-infected mice to downregulation of selected neuronal function genes and hippocampal dendritic debarborization without apparent neuronal loss in the region. The joint onset of these changes, their joint reversal by intranasal insulin, and their joint reappearance with insulin withdrawal indicate a strong causal relationship between the hippocampal injury and memory defects in this model. Synaptic but not dendritic injury and impaired memory were observed in transgenic mice expressing Tat in astrocytes [59] suggesting that Tat produced by EcoHIV-infected cells [36] contributes to murine NCI observed here. Synaptodendritic injury, rather than neurodegeneration, is considered the primary defect responsible for NCI in HIV infection [12,60], but it was first documented in brain tissues from AIDS patients with active HIV replication [61,62]. Neuropathological studies in successfully HIV-suppressed individuals with NCI are rare [63]. However, a consensus is building that these individuals have limited HIV-specific brain abnormalities including minimal HIV-related neuroinflammation [6,13,63] and that their NCI correlates with synaptodendritic injury, best represented by reduced combined scores of MAP2 and synaptic marker synaptophysin [64]. Considering our findings here and elsewhere on immunocompetence and unremarkable brain histopathology in chronically EcoHIV-infected mice [24,36,65], we propose that this model mimics neuronal injury aspects of NCI in HIV suppressed people, particularly patients with visuospatial and verbal memory deficits and nonnecrotic functional changes in the hippocampus [66,67].

The mechanisms responsible for the neuronal injury observed here are unknown. The restoration of dendritic arbors and memory after insulin treatment indicates that relevant neurons survive and can return to full functionality, similar to reversal of memory and synaptic deficits in amyloid beta precursor protein (APP) transgenic mice treated with insulin-like growth factor-2 (IGF-2) [68] and transient loss of MAP2 dendritic staining after moderate traumatic brain injury [69]. Common to the three systems are instability of hippocampal dendritic arbors and synaptic termini in response to pathogenic stimuli and dendritic regrowth by surviving neurons either after cessation of the stimulus [69] or upon empirical restorative treatment (68 and this work). The speed of dendritic changes is illustrated in the brain trauma study, where hippocampal MAP2 staining declined 4h after brain impact and recovered partially by 24 h [69]. The proposed mechanism of dendritic structural stability includes reversible MAP2-microtubule association under the control of mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK-ERK) and CaMKII pathways [70]. Notably, CaMKII and its regulator NRGN [71] are transcriptionally downregulated in EcoHIV infection here, as is the neurotrophin BDNF that enhances dendrite formation [72], and they are restored to normal expression with insulin treatment. Although further similarities between EcoHIV model and HIV-NCI in patients on ART must be established, mechanistic studies on the reversible destabilization of hippocampal dendritic arbors by HIV in this model may lead to long-acting treatment of human HIV-NCI.

Finally, our results open avenues for better understanding of beneficial effects of insulin on HIV-NCI in clinically asymptomatic people. The brain is an insulin-sensitive
organ [51] and multiple studies in animals and humans suggest a role for insulin and IGF in maintaining neuronal plasticity and cognitive function (reviewed in [18,51,73]). We have recently shown that murine NCI caused by EcoHIV (but not virus replication) can be prevented by treatment with the glutaminase antagonist 6-diazoo-5-oxo-γ-norleucine [25]. This finding, and downmodulation of the PPAT and TGG5 metabolism-related genes here, suggest that EcoHIV-infected mice demonstrate some of the metabolic dysregulation characterizing human HAND [6,74], possibly explaining some benefits of insulin treatment. The question of potential development of insulin resistance [74] upon long-term intranasal insulin treatment can also be experimentally addressed. Finally, the recent FIV study [23] and the present work indicate that insulin can affect FIV and HIV infection in the brain of their respective hosts. Reduction in HIV brain burdens or expression would mitigate the pathogenic stimulus for cognitive impairment. Exploration of these insulin effects may lead to new antiviral strategies that will also mitigate NCI.

Acknowledgements

We thank Dr O. Arancio, Columbia University, for help in introducing mouse behavioral tests to our laboratory and Ms I. Totillo for help in article preparation. We also recognize the role of Heather Thomas, MBA, as Program Manager of the Johns Hopkins NIMH Center, and support from the JHU Center for AIDS Research.

Author contributions: B.-H.K. performed investigations and formal analysis, and drafted the original article; J.C.M. contributed animal behavior methodology and performed investigations; A.B. performed investigations and formal analysis; E.H., H.H., M.T.N., and R.R. performed investigations; M.J.P. performed formal analysis and edited the article; N.J.H. edited the article; J.C.M. and B.S.S. conceptualized the project and edited the article; D.J.V. conceptualized and supervised the project, performed formal analysis, and wrote the final article.

The current work was supported by P01MH105280 (J.C.M., N.J.H., B.S.S., D.J.V.); R01DA037611 (D.J.V.); P01MH104145 (D.J.V.); P30MH075673 (J.C.M., N.J.H., B.S.S.); R01NS094146 (M.J.P., D.J.V.); and P30AI094189-06 (JHU Center for AIDS Research).

Conflicts of interest

There are no conflicts of interest.

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