Brain, Behavior, and Immunity xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Methamphetamine augment HIV-1 Tat mediated memory deficits by altering the expression of synaptic proteins and neurotrophic factors

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ARTICLE INFO

Keywords:
HIV-1 Tat
Methamphetamine
Memory deficits
Synapsin1
PSD-95
BDNF
p-CaMKII

ABSTRACT

Methamphetamine (METH) abuse is common among individuals infected with HIV-1 and has been shown to affect HIV replication and pathogenesis. These HIV-1 infected individuals also exhibit greater neuronal injury and higher cognitive decline. HIV-1 proteins, specifically gp120 and HIV-1 Tat, have been earlier shown to affect neurocognition. HIV-1 Tat, a viral protein released early during HIV-1 replication, contributes to HIV-associated neurotoxicity through various mechanisms including production of pro-inflammatory cytokines, reactive oxygen species and dysregulation of neuroplasticity. However, the combined effect of METH and HIV-1 Tat on neurocognition and its potential effect on neuroplasticity mechanisms remains largely unknown. Therefore, the present study was undertaken to investigate the combined effect of METH and HIV-1 Tat on behavior and on the expression of neuroplasticity markers by utilizing Doxycycline (DOX)-inducible HIV-1 Tat (1-86) transgenic mice. Expression of Tat in various brain regions of these mice was confirmed by RT-PCR. The mice were administered with an escalating dose of METH (0.1 mg/kg to 6 mg/kg, i.p) over a 7-day period, followed by 6 mg/kg, i.p METH twice a day for four weeks. After three weeks of METH administration, Y maze and Morris water maze assays were performed to determine the effect of Tat and METH on working and spatial memory, respectively. Compared with controls, working memory was significantly decreased in Tat mice that were administered METH. Moreover, significant deficits in spatial memory were also observed in Tat-Tg mice that were administered METH. A significant reduction in the protein expressions of synapsin 1, synaptophysin, Arg3.1, PSD-95, and BDNF in different brain regions were also observed. Expression levels of Calmodulin kinase II (CaMKII), a marker of synaptodendritic integrity, were also significantly decreased in HIV-1 Tat mice that were treated with METH. Together, this data suggests that METH enhances HIV-1 Tat-induced memory deficits by reducing the expression of pre- and postsynaptic proteins and neuroplasticity markers, thus providing novel insights into the molecular mechanisms behind neurocognitive impairments in HIV-infected amphetamine users.

1. Introduction

Despite the availability of combination antiretroviral therapy (cART), HIV-1 infected patients remain at risk of developing HIV-associated neurocognitive disorders (HAND). The prevalence of the most severe form of HAND, HIV-associated dementia (HAD), has declined in the post-cART era (Valcour, 2011; Maschke, 2000). However, patients continue to suffer from minor forms of HAND, namely asymptomatic neurocognitive impairment (ANI) and minor neurocognitive disorder (MND) (Nightingale, 2014; Heaton, 2010). Moreover; evidence also indicates a shifting pattern of neurocognitive impairment in HIV patients, from deficits in motor ability, the speed of information processing, and verbal speed in the pre-cART era to deficits in memory and executive function in the post-cART era (Heaton, 2011). HIV-1 infection in the brain is characterized by the presence of activated astrocytes (Ton and Xiong, 2013), decreased number of neurons (Kaul et al., 2001), alterations in dendritic and synaptic densities (Atluri, 2013; Everall, 1999), decreased expression of synaptic plasticity genes (Atluri, 2013), and decreased dopaminergic transporters (Chang, 2008). The development HAND and pathological changes observed in HIV-1 infected individuals can be attributed to direct and indirect toxic effects of HIV viral proteins (gp120, Nef, Tat, and Vpr) released from infected

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https://doi.org/10.1016/j.bbi.2018.04.018
Received 28 May 2017; Received in revised form 29 April 2018; Accepted 30 April 2018

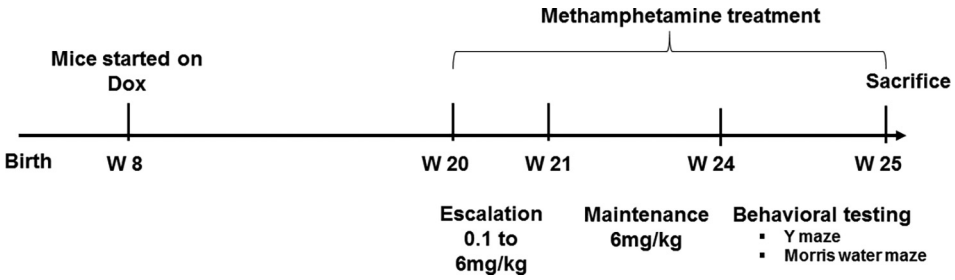

Fig. 1. Schematic representation of the experimental timeline: Six-week-old male and female WT mice and HIV-1 Tat mice were administered doxycycline for 12 weeks before starting administration of METH as described in materials and methods. Animals were subjected to behavioral testing by Y maze and Morris water maze assays on week 24. Animals were euthanized at week 25 and different brain regions were harvested to identify the expression of various synaptic proteins and neurotrophic factors.

brain cells such as microglia, perivascular macrophages and astrocytes (Saylor, 2016; Hong and Banks, 2015).

HIV-1 transactivator of transcription, Tat, is released early during the infection and is important for a variety of viral functions such as HIV-1 mRNA splicing (Jablonski, 2009), capping (Chiu, 2001; Chiu, 2002; Zhou, 2003), translation (Braddock, 1993; Braddock, 1990; Charnay, 2009; SenGupta, 1990), and reverse transcription (Apolloni, 2007; Harrich, 1997; Kameoka, 2001). Tat contributes to neuronal toxicity by various mechanisms including disruption of blood brain barrier (BBB) permeability (Zhong, 2008; Leibrand, 2017; Woollard, 2014), increased oxidative stress (Kim, 2015; Agrawal, 2012; Cota-Gomez, 2011; Wallace, 2006; Toborek, 2003), increased apoptosis (Perry, 2005; Eugenin, 2007; Mattson et al., 2005), increase in the release of pro-inflammatory cytokines (CCL5, IL-1β, IL-6, IL-8, and TNF-α) (Nookala and Kumar, 2014; Nookala, 2013; Haij, 2015; Planès, 2016; Serrero et al., 2017), and excitotoxicity through involvement of NMDA receptors, glutamate and calcium influx into cells (Krogh, 2014; Haughey, 2001). Further, HIV-1 Tat has also been shown to suppress long-term potentiation (LTP) (Behnisch et al., 2004) and impair spatial learning and memory in both mouse (Fitting, 2013; Li, 2004) as well as rat models (Fitting et al., 2008; Harricharan, 2015).

Illicit drugs such as methamphetamine (METH) and others has been shown to be associated with increased risk of HIV -1 infection (Scott et al., 1999; Corsi and Booth, 2008; Shoptaw and Reback, 2007). METH is a psychostimulant that exerts its actions by primarily affecting variety of neurotransmitter systems, including dopaminergic (Ares-Santos, 2014; Parsegian and See, 2014; Ares-Santos et al., 2013), serotonergic (Chiu, 2012; Seminerio, 2011), gamma amino butyric acid (GABA)-ergic (Munoz, 2016; Wearne, 2016), and glutamatergic systems (Jayanthi, 2014; Mark et al., 2004), eventually leading to structural and functional abnormalities in the brain (Belcher et al., 2005; Berman, 2008). Consistent with HIV-1 Tat-mediated cognitive impairments, METH use has also been shown to impair learning and memory in the human population and in animal models (Kesby, 2015; Scott, 2007; Siegel et al., 2010). In view of the existing evidence that Tat and METH affect same brain regions and share similar mechanisms of neurotoxicity, we sought to determine the possible additive/synergistic role of Tat and METH in causing cognitive impairments. There has been several studies addressing the combined effect of methamphetamine and HIV-1 gp120 (Hoefer, 2015; Kesby, 2015; Henry, 2013; Henry, 2014). However, only limited information is available regarding the combined effect of HIV-1 Tat and methamphetamine (Kesby et al., 2016). Further, there is no information available regarding the role of neuroplasticity genes in regulation of cognitive functions in context of methamphetamine/HIV-1 Tat-mediated cognitive impairments. We utilized HIV-1 Tat transgenic mouse model to assess HIV-1 Tat-mediated cognitive impairments and whether methamphetamine augments Tat-mediated deficits in cognitive functions.

2. Materials and methods

2.1. Animals

Doxycycline-inducible HIV-1 Tat transgenic mice (C57/BL6J) were obtained from Dr. Kurt Hauser at Virginia Commonwealth University (VCU). These mice express HIV-1 Tat in the brain using a *tet-on* inducible system and GFAP promoter (Das et al., 2016; Kim, 2003; Bruce-Keller, 2008). The Tat-Tg mice present many clinical findings of HIV-1 infection, including infiltration of blood cells, neuronal apoptosis, astrocytosis, reduced gray matter density, dendritic degeneration and inflammation (Kim, 2003; Carey, 2013). Moreover, deficits in learning and memory have also been observed in Tat-Tg mice (Fitting, 2013; Carey, 2012). Both male (M) and female (F) mice were used for the experiments and were divided into four groups each: Control M (n = 9), Tat M (n = 7), METH M (n = 9), Tat + METH M (N = 7), Control F (n = 9), Tat F (n = 8), METH F (n = 12) and Tat + METH F (n = 8). Mice were administered DOX (6 g/kg) through formulated chow starting at eight weeks of age. DOX was administered for 12 weeks before starting behavioral experiments. Mice were housed in an animal facility in groups of 3–5 per cage and were allowed unlimited access to food and water. Twelve h light/dark cycle was employed by turning on the lights from 6:00 AM to 6:00 PM. Behavioral testing was performed between 8:00 AM and 5:00 PM. All procedures were approved and followed in accordance with UMKC Institutional Animal Care and Use Committee.

2.2. Methamphetamine (METH) treatment

To simulate chronic METH use in human abusers, we employed an escalating dosing regimen in mice. METH (Sigma, St. Louis, MO) was dissolved in phosphate buffered saline (PBS) and was administered intraperitoneally twice a day. We escalated the dose of METH stepwise from 0.1 mg/kg to 6 mg/kg over the 7-day duration. After the escalation period, 6 mg/kg METH was given b.i.d for 4 weeks. Control mice received an equivalent volume of PBS intraperitoneally (Fig. 1).

2.3. Y-Maze

The effect of METH, Tat and Tat + METH on short term memory was evaluated using a Y-maze. The maze has three interconnected closed arms, that are 12 in. in length each and are 120^0 from each other. The animal was placed into the end of one of the arms and was allowed to freely explore the maze for 5 min. The starting arm positions of mice were chosen at random. The movement of the animal inside the Y-maze was recorded by an overhead camera using ANY-maze (version 4.99z) behavioral tracking software. The arms were thoroughly cleaned with disinfectant and 70% ethanol to eliminate any residual odors of previous mice. The number of arm entries were counted and acted as a marker of locomotor activity. The number of spontaneous alternations, as defined by entry into three different arms in sequence (triad), serves as a measure of working memory. Percentage spontaneous alternations was calculated from number of triads and arm entries using the following equation: % Spontaneous alternations = (number of triads/total number of arm entries-2) × 100.

2.4. Morris water maze

The Morris water maze is a standard test employing a circular tank