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Dose-dependent neurocognitive deficits following postnatal day 10 HIV-1 viral protein exposure: Relationship to hippocampal anatomy parameters

Sylvia Fitting[a,b], Kristen A. McLaurin[a], Rosemarie M. Booze[a], Charles F. Mactutus[a,*]

[a] University of South Carolina, Behavioral Neuroscience Program, Department of Psychology, Columbia, SC 29208, USA
[b] University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

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ABSTRACT

Despite the availability of antiretroviral prophylactic treatment, pediatric human immunodeficiency virus type 1 (HIV-1) continues to be a significant risk factor in the post-cART era. The time of infection (i.e., during pregnancy, delivery or breastfeeding) may play a role in the development of neurocognitive deficits in pediatric HIV-1. HIV-1 viral protein exposure on postnatal day (P)1, preceding the postnatal brain growth spurt in rats, had deleterious effects on neurocognitive development and anatomical parameters of the hippocampus (Fitting et al., 2008a,b). In the present study, rats were stereotaxically injected with HIV-1 viral proteins, including Tat_{1-86} and gp120, on P10 to further examine the role of timing on neurocognitive development and anatomical parameters of the hippocampus (Fitting et al., 2010). The dose-dependent virotoxin effects observed across development following P10 Tat_{1-86} exposure were specific to spatial learning and absent from prepulse inhibition and locomotor activity. A relationship between alterations in spatial learning and/or memory and hippocampal anatomical parameters was noted. Specifically, the estimated number of neurons and astrocytes in the hilus of the dentate gyrus explained 70% of the variance of search behavior in Morris water maze acquisition training for adolescents and 65% of the variance for adults; a brain-behavior relationship consistent with observations following P1 viral protein exposure. Collectively, late viral protein exposure (P10) results in selective alterations in neurocognitive development without modifying measures of somatic growth, preattentive processing, or locomotor activity, as characterized by early viral protein exposure (P1). Thus, timing may be a critical factor in disease progression, with children infected with HIV earlier in life being more vulnerable to CNS disease.

1. Introduction

Pediatric human immunodeficiency virus type 1 (HIV-1) predominantly results from "vertical" mother-to-child transmission (MTCT), occurring during active labor and delivery, pregnancy and/or postnatal breastfeeding (Kourtis et al., 2001; AIDSinfo, 2017). Marked decreases in the prevalence of pediatric HIV-1 were observed following the implementation of interventions aimed at preventing MTCT, including treatment with combination antiretroviral therapy (cART; Luzuriaga and Mofenson, 2016). cART is commonly prescribed to HIV-1-infected mothers during pregnancy (Suksomboon et al., 2007; Volmink et al., 2007; Townsend et al., 2008; Briand et al., 2013; Reliquet et al., 2014; Townsend et al., 2014; Wang et al., 2016; Peters et al., 2017) and HIV-1 infected newborns and/or children (Riordan and Bugembe, 2009; Chadwick et al., 2011; van der Plas et al., 2013; Bitnun et al., 2014; Mutwa et al., 2014; Shiau et al., 2017). Nevertheless, despite the availability of antiretroviral prophylactic treatment, vertical MTCT continues to be a significant risk factor in the post-cART era, with approximately 160,000 children being newly infected in 2016, most of whom live in resource-limited settings (UNAIDS, 2017).

Additionally, the advent of cART shifted the global epidemic into a new phase as HIV-1 seropositive children survive into adulthood (Sohn and Hazra, 2013; Crowell et al., 2014; Smith and Wilkins, 2015). By 2020, it is estimated that approximately 1.94 million children will be living with HIV-1 (Penazzato et al., 2014). The chronic nature of pediatric HIV-1 in the post-cART era has enormous implications for children's neurocognition and development (Vijayan et al., 2009; Brady et al., 2010; Paramesparan et al., 2010; Sohn and Hazra, 2013; Crowell et al., 2014). Specifically, high rates of subtle to severe neurocognitive deficits have been reported in HIV-1 infected children on cART that survive to adulthood (Burns et al., 2008; Webb et al., 2009; Paramesparan et al., 2010; Crowell et al., 2014). The causes of these neurocognitive deficits, despite effective cART, are multifactorial and likely include continued viral replication in the central nervous system (CNS), ongoing neuroinflammation, irreversible CNS injury prior to cART initiation, neurotoxic effects of cART, and socioeconomic and

* Corresponding author at: Behavioral Neuroscience Program, Department of Psychology, University of South Carolina, Columbia, SC 29208.
E-mail addresses: sfitting@email.unc.edu (S. Fitting), mactutus@mailbox.sc.edu (C.F. Mactutus).

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psychosocial constraints (Crowell et al., 2014).

Pediatric HIV-1 infection presents a very different clinical picture compared to HIV-1-infection in adulthood (Sohn and Hazra, 2013; Crowell et al., 2014). In the post-cART era, HIV-1 infected children commonly exhibit high rates of chronic neurological impairment, including significant delays in cognitive development, motor skills, and language (Lindsey et al., 2007; Van Rie et al., 2008; Walker et al., 2013). The rates of disease progression within the pediatric HIV-1 population, however, have been shown to dramatically vary and are likely related to differences in viral load, strain of HIV, time of HIV infection, and/or genetic vulnerabilities (Belman, 1997; Rigardetto et al., 1999; Chearskul et al., 2002; Crowell et al., 2014). A relationship between the time of perinatal infection and the course of the disease in infants has also been suggested by other studies (Blanche et al., 1990, 1994; Becquet et al., 2012) with a greater sensitivity of the immature brain to the devastating effects of HIV-1 (Meeker et al., 2004; Becquet et al., 2012). To date, however, the relative importance of timing and its effects on neurocognitive development has been relatively understudied in pediatric HIV-1.

In human pediatric HIV/AIDS cases, the time of infection is broadly defined as 'occurring in the period shortly before and after birth', which describes both prenatal (i.e., 20th to 28th week of gestation) and postnatal (i.e., 7–28 days after birth) infection (Wiley et al., 1994; Donovan and Palumbo, 2010; CDC, 2014). Technological limitations of HIV-1 testing prevent us from determining either the exact timing or degree of early postnatal transmission of HIV-1. DNA or RNA polymerase chain reactions (PCR), viral culture, and p24 antigen tests, although able to detect the virus itself, are not able to define the route of infection before the age of 2–3 months (Ogundele and Coulter, 2003; CDC, 2014). Due to experimental limitations and ethical constraints in human pediatric AIDS research, an animal model that is (A) translational to the important health issues surrounding pediatric AIDS, and (B) able to determine the timing of MTCT and its effects on the CNS in a precise and controlled manner, is of great clinical relevance in defining the different rates of progression in pediatric HIV/AIDS. In the present study, stereotaxic injections of HIV-1 viral proteins at postnatal day 10 (P10), including the transactivator of transcription ($Tat_{1–86}$) and envelope glycoprotein 120 (gp120), provides an opportunity to elucidate the effect of neurotoxic proteins on neurodevelopment (Carryl et al., 2015).

Neurotoxic viral proteins, including Tat and gp120, are active in the CNS, even when peripheral immune system function is restored under cART, and may be partially responsible for the neuroanatomical alterations commonly observed in HIV-1 seropositive individuals (e.g., synaptodendritic alterations: Ellis et al., 2007; Gelman and Nguyen, 2010; Desplats et al., 2013; neuronal loss: Del Valle et al., 2000; Jones et al., 2000; Nath et al., 2000). Tat, the transactivating protein for retroviral replication (Cann et al., 1985; Li et al., 2010), is produced very early after infection, and is necessary for viral expression, cell-to-cell virus transmission and disease progression (Sodroski et al., 1985; Ensoli et al., 1993; Chang et al., 1997; Karn, 1999; Lin et al., 2003; Ensoli et al., 2006; Richter and Palu, 2006). Gp120, a structural viral gene product, is vital for viral entry into the CNS (Hao and Lyman, 1999) and may cause neurotoxicity by binding to cell surface receptors, ultimately leading to neuronal death (Lipton, 1991; Corasaniti et al., 2001a; Haughey and Mattson, 2002). Preclinical assessments, including both *in vitro* and *in vivo* studies, have extensively examined both Tat and gp120 (Brenneman et al., 1988; Lipton et al., 1995; Nath et al., 1996; Cheng et al., 1998; Aksenov et al., 2001; Corasaniti et al., 2001b; Bruce-Keller et al., 2003; Aksenov et al., 2006; Aksenova et al., 2006; Fitting et al., 2006a; Aksenov et al., 2008; Adams et al., 2010; Zhu et al., 2011; Bertrand et al., 2013; Fitting et al., 2013; Hahn et al., 2013; Bertrand et al., 2014, 2015; Marks et al., 2016), providing strong evidence for the role of HIV-1 viral proteins in neurocognitive and neuroanatomical alterations in adulthood.

Although preclinical and clinical studies have characterized HIV-1 associated neurocognitive disorders (HAND) in adults (e.g., review, Woods et al., 2009; Heaton et al., 2010), neurocognitive deficits in HIV-infected children are poorly understood (Crowell et al., 2014). A limited number of preclinical studies have focused on the effects of early exposure to HIV-1 viral toxic proteins on the development of neurocognitive disorders, independent of the virus itself (Bussiere et al., 1999; Fitting et al., 2008b; Webb et al., 2009; Moran et al., 2014a; Fitting et al., 2015; McLaurin et al., 2017a). Specifically, stereotaxic injections of the viral proteins Tat and/or gp120 on P1 revealed deleterious effects on multiple reflexive, preattentive (e.g., prepulse inhibition (PPI)), and neurocognitive assessments (e.g., Morris water maze), as well as alterations in anatomical parameters of the hippocampus (Fitting et al., 2008a,b; Moran et al., 2014a). A histological dose-response study that injected HIV-1 proteins at P10 indicated that timing may have a differential effect on the anatomical parameters of the hippocampus in adult rats (~5 months) compared to the P1 study (Fitting et al., 2008a; Fitting et al., 2010). Stereotaxic injections of HIV-1 viral proteins on P1 in rats were chosen to mimic early transmission (i.e., *in utero*) of the virus, whereas HIV-1 protein delivery on P10 more closely resembles HIV-1 protein entry into the CNS at labor/delivery in humans.

Thus, the present study investigated the effects of perinatal P10 HIV-1 protein neurotoxicity using a series of neurocognitive assessments throughout development and adulthood to address two interrelated aims. First, dose-response functions were used to examine the short and long-term effects of $Tat_{1–86}$ and gp120 on developmental milestones and neurocognition. Measures of somatic growth, including body weight and eye opening, and motor development, including locomotor activity, were used to examine developmental milestones. Neurocognitive assessments included PPI, to examine sensorimotor gating and temporal processing, and the Morris water maze, to examine spatial learning and memory. Assessments were chosen as indexes of neuropsychological assessments in humans that have been shown to be impaired in pediatric HIV-1 infection (Boivin et al., 1995; Newell et al., 2003; Jeremy et al., 2005; Lodha et al., 2005; Martin et al., 2006; Willen, 2006; Lindsey et al., 2007; Baillieu and Potterton, 2008; Van Rie et al., 2009; Webb et al., 2009; Paramesparan et al., 2010; Le Doare et al., 2012; Walker et al., 2013). Second, the relationship between neurocognitive deficits and neuroanatomical alterations (Fitting et al., 2010) was investigated. Thus, the present study provides an opportunity to determine whether the developing CNS is more vulnerable to later HIV-1 viral protein exposure period (i.e., P10) relative to the earlier published findings at P1 (Fitting et al., 2008a,b; Moran et al., 2014a), aiding in the understanding of the role of timing in chronic neurological impairment in pediatric HIV-1.

2. Results

2.1. Somatic growth

2.1.1. Body weight

Fig. 1 illustrates the mean (± *S.E.M.*) body weight data across different test days for each treatment group (Tat: A, Gp120: B). Body weight increased significantly with age across all groups. Separate ANOVAs were conducted for each testing set with Tat dose treatment (4 levels) or gp120 dose treatment (4 levels) as a between-subjects factor, and when appropriate, a within-subjects test day factor. For Tat or gp120, ANOVAs demonstrated no treatment effect or treatment by day interaction for any of the test sets. Planned contrast analyses for Tat or gp120 revealed no overall treatment effect, no dose-dependent treatment effect, and hence no threshold effect. Results indicated that the HIV-1 viral protein doses produced no general growth deficits when injected on P10 consistent with the findings of the previous P1 study (Fitting et al., 2008b).

2.1.2. Eye opening

Fig. 2 illustrates the median (± *Interquartile Range*) for eye opening