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HIV-1 Proteins, Tat and gp120, Target the Developing Dopamine System

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Abstract

In 2014, 3.2 million children (< 15 years of age) were estimated to be living with HIV and AIDS worldwide, with the 240,000 newly infected children in the past year, i.e., another child infected approximately every two minutes [1]. The primary mode of HIV infection is through mother-to-child transmission (MTCT), occurring either *in utero*, intrapartum, or during breastfeeding. The effects of HIV-1 on the central nervous system (CNS) are putatively accepted to be mediated, in part, *via* viral proteins, such as Tat and gp120. The current review focuses on the targets of HIV-1 proteins during the development of the dopamine (DA) system, which appears to be specifically susceptible in HIV-1-infected children. Collectively, the data suggest that the DA system is a clinically relevant target in chronic HIV-1 infection, is one of the major targets in pediatric HIV-1 CNS infection, and may be specifically susceptible during development. The present review discusses the development of the DA system, follows the possible targets of the HIV-1 proteins during the development of the DA system, and suggests potential therapeutic approaches. By coupling our growing understanding of the development of the CNS with the pronounced age-related differences in disease progression, new light may be shed on the neurological and neurocognitive deficits that follow HIV-1 infection.

Keywords

Pediatric HIV/AIDS; development; Tat; gp120; dopamine; neurocognitive impairment

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

1. PEDIATRIC HIV/AIDS INFECTION

1.1. BACKGROUND

The first cases of acquired immune deficiency syndrome (AIDS) were reported around 1980 in young homosexual men [2, 3]. The possibility of vertical spread of the AIDS disease from mother to child was confirmed with the first reports of children diagnosed with AIDS [4–6]. These reports were fundamental in establishing that neither sexual contact or injection drug use was required for transmission of AIDS; forcing a change of public perception from AIDS as a ‘gay epidemic’ [1]. The subsequent increase in the total number of AIDS-diagnosed children increasing to nearly 300 in 1986 could not portend the looming pandemic [7]. Now, over 30 years since the first cases were reported, the magnitude of the global epidemic of HIV/AIDS has surpassed all expectations; the latest data on the world HIV/AIDS pandemic are presented in Table 1 [1]. Of the total estimate of 35 million people living with HIV, 3.2 million are children (<15 years of age, 2.9–3.5 million) and 4 million are young people (15–24 years of age, 3.6–4.6 million), 29% of whom are adolescents (15–19 years of age). Providing antiretroviral therapies for pregnant women living with HIV has significantly lowered the number of children newly infected with HIV [8–10]. Nevertheless, 18 years after starting the use of combined antiretroviral therapy (cART), 240,000 new pediatric HIV infections still occurred worldwide in 2013; i.e., thus another infant is infected approximately every two minutes. The vast majority of infected children (87.5%) live in Sub-Saharan Africa [1].

The major source of HIV-1 infection in children occurs through vertical mother-to-child transmission (MTCT) with the ability of the virus being transmitted dependent upon timing: *in utero*, at parturition, and postnatally. Timing of exposure also confers a difference in route of exposure: prenatally, with the beginning of separation of the placenta from the uterine wall (anytime after the 20th week, but most often in the 3rd trimester (> 28th week)), at parturition, *via* exposure of the infant to maternal blood as well as genital tract secretions, and postnatally, *via* the mother’s milk by breastfeeding [11].

Prior to the availability of pharmacologic interventions, the incidence of MTCT of HIV-1, even in high resource countries such as the United States and Europe, was approximately 25%. A precipitous drop in MTCT was to be subsequently possible [12] following the groundbreaking demonstration provided by the Pediatric AIDS Clinical Trial Group 076 trial that antepartum, intrapartum, and neonatal zidovudine (AZT) treatment reduced MTCT by 67% [13]. Across the following two decades, the administration of antiretroviral (ARV) therapy during pregnancy and at delivery, and to the infant, has successfully produced a marked and sustained reduction in the transmission of HIV to infants to less than 2% in high resource countries [14]. Nevertheless, in more health resource-limited countries, MTCT rates of 23%–35% continue to be reported [15–17]. A caveat to the success in resource rich countries is also notable. The rate of MTCT may be as high as 6–7% despite use of cART, an outcome that appears attributable to the presence of higher viral loads or an increased rate of preterm delivery [18, 19]. Reductions in rate of MTCT are further challenged by the fact that drugs of abuse may increase MTCT of HIV, even in the presence of cART, *via* their effects on induction of preterm birth and increasing viral plasma load [20, 21]. Moreover,

despite the continuing decrease in number of new pediatric HIV infections with current treatment protocols [22], there are approximately 16 million women aged 15 years and older living with HIV. A primary concern remains the increased vulnerability to, and risk of, HIV infection among adolescent girls and young women in Sub-Saharan Africa [1]. In this light, the reduction and prevention of MTCT have precipitated a significant and growing population of children uninfected with HIV, but with significant *in utero* and neonatal exposure to antiretroviral drugs. The long-term safety of such early exposures is not within the purview of this review, but its significance is discussed elsewhere, and is an urgent need [23–25]. Clearly, the above facts and reasons provide hope and optimism, yet on the other hand, challenges remain and urgently need to be addressed [1].

Marked differences in rates of clinical disease progression occur between children and adults. Indeed, age-specific differences in disease expression have been noted throughout the HIV/AIDS epidemic [4, 26] highlighting the critical importance of investigating the effects of HIV/AIDS on developmental processes. A bimodal distribution was suggested in early reports with rapid progression of ~ 25% of HIV-1-infected children to AIDS or death within their first year, whereas the other 75% of children displayed adult-like rates of disease progression [27, 28]. A more comprehensive study of perinatally infected children (~N=4000) demonstrated significant age-dependent differences in the progression of the disease of children younger than 5 years of age [29]. Independent of CD4+ T-cell count or the level of plasma viremia, younger children had an increased risk of progression to AIDS, or death, compared with their older counterparts. As follow-up was censored at the end of 1995, this estimate of disease progression is prior to the introduction of cART.

In an effort to provide an improved estimate of infant mortality as a function of timing of HIV infection (perinatally vs postnatally), a collective analysis was performed on all available intervention cohorts and randomized trials on prevention of HIV MTCT in Africa (N = 12,112 children of HIV-infected women representing 12 individual studies) [30]. Children infected with HIV showed an approximate 10-fold increase in mortality rate per 1,000 children compared to those not infected with HIV (38.2% vs 3.9%). Out of the HIV-infected children that died after 1 year of acquiring HIV, 26% of the children were infected postnatally and 52% were infected perinatally, relative to 4% of the children not infected with HIV [30]. These striking differences implicate both timing and route of infection as critical factors for HIV infection as well as disease progression.

Since the mid-1990s, with the initiation of highly active antiretroviral therapy (HAART) in the United States, the mortality rate of perinatally HIV-infected children has been reduced by over 90%, i.e., from 7.2 in 1994 to 0.6 in 2006 (per 100 child-years). Further, the mean age at death doubled from approximately 9 to 18 years of age across this pre-to-post HAART interval [31]. Nevertheless, HIV-infected children show 10–30-fold higher mortality rates compared to age-matched uninfected children [32]. The causes of death have also changed with the majority caused by end-stage AIDS, sepsis, and renal failure and fewer attributable to opportunistic infections [31].

The dramatic decline in mortality rate for perinatally HIV-infected children, however, has not come without morbidity. In the next subsection, an overview is provided concerning the

most common neurological and neuropsychological outcomes of pediatric HIV/AIDS, both prior and subsequent to the introduction of cART. The era preceding (in addition to the era following) the introduction of cART is reviewed, as access to medication is still limited in the more resource-limited countries of the world. Findings presented in this review are drawn from both clinical and preclinical studies, the latter with both *in vitro* and *in vivo* approaches.

1.2. Neurological and Neuropsychological Findings

While cells of the immune system are the principal targets of HIV-1 infection, the effects of the virus on the nervous system are very serious and devastating. It appears that the CNS is the most adversely affected organ in the body, after the immune system. HIV-1 entry into the brain and infection of the CNS are critical elements in the development of neurological disorders, such as motor and cognitive dysfunction. HIV-1-associated progressive encephalopathy (PE), specifically seen in the pre-cART era, refers to HIV-1-infected children with progressive CNS dysfunction and is putatively held as comparable to HIV-1-associated dementia (HAD) in adults. PE is characterized by impaired brain growth, retardation of developmental milestones, progressive motor dysfunction, as well as attentional and cognitive deficits [33–37]. PE in infants may have different pathophysiologic mechanisms than those underlying PE in older children [38]. The strong reduction of encephalopathy that has been reported with increasing age [39] suggests differential progression of HIV-1 infection and may reflect greater sensitivity of the immature brain to the devastating effects of HIV-1 [40]. Infants infected early, prior to the marked postnatal increase in rate of brain growth, may be most vulnerable and progress more rapidly compared to infants infected later, when the rate of brain development has already peaked. Additionally, different rates of disease progression may be anticipated depending on the factors of viral load, strain of HIV, and/or genetic vulnerabilities [41, 42].

Now during the post-cART era, PE has significantly decreased and survival and quality of life in HIV-1-infected children has dramatically improved. However, as HIV-1 is now considered to be a chronic disease [43], high rates of neurocognitive impairment are being observed in HIV-1-infected children as they enter adolescence and young adulthood [44, 45]. Table 2 lists important reviews on pre- and post-cART pediatric HIV/AIDS infection, with particular focus on those which highlight (1) differences between adults and children [46–48], (2) the predictors for vertical transmission from mother-to-child [27], (3) underlying mechanisms of HIV-1 encephalopathy in children [49], (4) neurocognitive development [45, 50], (5) the DA system [51], and (6) antiretroviral therapy [26, 52–54].

A distinct feature in pediatric NeuroAIDS is an unusual calcification of brain tissue in the basal ganglia with widespread astrogliosis; an outcome also found in the cerebral cortex pre-cART [7, 37, 55, 56]. Several studies of pediatric HIV-1 infection of the nervous system give evidence for basal ganglia involvement [41, 56–59] with reviews mentioning basal ganglia calcification as a hallmark of pediatric CNS HIV-1 infection [55]. In addition to basal ganglia involvement, the prefrontal cortex (PFC) has been demonstrated to be specifically susceptible in HIV-1-infected children, with executive function showing the greatest decline as a function of HIV disease progression, especially in the post-cART era

[60]. The close anatomical relationship between the basal ganglia and the cerebral cortex suggests that basal ganglia dysfunction may regulate cortical information flow [61]. A list of frequent neuropathological and neurological features seen in pediatric HIV/AIDS infection in the pre- and post-cART era is given in Table 3.

Although the pathogenesis of HIV-1 encephalopathy is complex, many of its clinical features can be attributed to abnormalities in striatal pathways, and particularly in the DA system. Symptoms manifest extrapyramidal dysfunction that is characterized by rigidity/stiffness, ambulation difficulties/shuffling gait, dysarthria/drooling/swallowing dysfunction, hypomimetic/inexpressive faces, and bradykinesia [50, 62]. In advanced Parkinson's disease (PD) similar observations are noted, which suggest a profound abnormality of the striatal DA system [51, 63]. The disruption of the DA system during maturation is also suggested to influence subsequent cortical specification, particularly the prefrontal cortical areas [61]. Poor attention, deficits in working memory, and an increased slowing of information processing can be associated with frontal cortex dysfunction and are prominent cognitive neuropsychological complications of pediatric HIV-1 infection [50, 59, 64]. In the next section some more detail will be provided regarding the involvement of the DA system in HIV-1 infection.

1.3. Involvement of the Dopamine (DA) System

It was recognized some time ago that HIV-1-associated cognitive and motor symptoms may result from damage to the DA system [reviews: 63, 65–69]. DA dysfunctions in HIV-1-infected patients were initially suggested by clinical manifestations of slowing in psychomotor tasks, tremor, apathy, and altered posture and gait [70, 71]; i.e., symptoms resembling those associated with DA deficiency in PD [72, 73]. Further evidence of DA deficits was confirmed when the PD-like symptoms in HIV-infected patients were exacerbated following treatment with DA-blocking drugs (e.g., prochlorperazine, perphenazine, trifluoperazine, or even low-dose haloperidol) [74–76]. Thus, these observations implicate damage to the integrity of the brain DA system consequent to HIV-1 infection.

Alterations in DA activity in HIV-1-infected patients have been associated with neurological impairments at different stages of disease progression, as indexed by significantly decreased cerebrospinal fluid (CSF) concentrations of DA and homovanillic acid (HVA) [68, 73, 77–79]. Levels of immunoreactive tyrosine hydroxylase (TH) (the rate-limiting enzyme of dopamine synthesis) were significantly reduced in the substantia nigra (SN) in HIV-infected brains, relative to controls [80]. Decreased DA concentrations in the CNS of HIV-1-infected individuals are not necessarily restricted to a few brain regions, but rather may be significantly decreased across multiple cortical and subcortical regions, e.g., frontocortical areas, the basal ganglia (BG), caudate (CA), putamen (Put), globus pallidus (GP), and SN [81].

Notably, the decreases in DA concentration in various brain regions are directly related to specific neuropsychological disorders found in HIV-infected individuals [82]. A significant relationship between cART medication adherence and neurocognitive impairments in HIV patients, with or without cocaine dependence, was revealed by mean T-scores under 40 on

most measures [83]. By reference, a T-score of 40 is one standard deviation below a normally distributed mean T-score of 50.

Structural magnetic resonance imaging (MRI) techniques subsequently revealed a volume reduction in the BG, posterior cortex, and deep gray and white matter [84, 85]. Functional MRI techniques also unveiled alterations in HIV-infected patients with neurocognitive impairments: significant cerebral hemodynamic changes with increased cerebral blood volume in the deep and cortical gray matter, decreased N-acetyl aspartate/creatine ratio, and elevated levels of choline in white matter [86, 87]. Indeed, a number of recent human imaging studies [83, 88, 89] and ultrasound scans [79] confirm the DA system as highly compromised by HIV infection.

It is well-appreciated that the DA transporter (DAT) plays a pivotal role in DA homeostasis by maintaining stable synaptic DA concentrations and regulation of disease and brain processes, notably cognitive function [61, 90]. The DAT is reduced in HIV patients, especially in those with cognitive/motor deficits [88]; DAT deficits correlate with cognitive/motor impairments [89]. As reported in our own and others work, DAT is directly targeted by HIV-1 proteins resulting in transporter impairment [91–97].

The involvement of the DA system in HIV-1 infection is further supported by studies reporting enhanced effects of HIV-1 proteins by various drugs of abuse [65, 92, 93, 98–109]. The findings indicate that HIV encephalitis occurs more frequently in HIV-1-infected adult drug users than in HIV-infected patients without a history of drug use and also show an earlier death rate than the non-drug using individuals [110]. The development and maintenance of addictive behavior is specifically associated with the nucleus accumbens (NAc) (ventral striatum); a region innervated by DA neurons originating in the ventral tegmental area (VTA) with projections to different regions of the frontal cortex [111]. It has been repeatedly suggested that alterations in the DA system may exaggerate the neurological manifestations of HIV-1 infection *via* a selective sensitivity of DA neurons to HIV-1 and/or HIV-1 related viral proteins as well as possible modulation by drugs that act on the DA system [65, 92, 98, 106, 107, 112]. It is further suggested that the BG are involved in cognitive processes closely linked to cortical areas, such as the frontal cortex [61].

The significant role of the DA system in the development of neurological dysfunction in brains of pediatric HIV-1 patients is highlighted by additional findings on motor control. Motor function, which relies on BG function, is compromised in HIV-1-infected individuals [113–115]. The use of DA agonists in pediatric patients with HIV-1 infection and PD-like features has demonstrated consistent improvement in motor function [62]. A sustained improvement in all symptoms with levodopa (L-DOPA) has been reported in pediatric patients [62] and stands in contrast to the variable results observed in adults [116]. An early developmental alteration of the DA system may underlie the differences seen between adults and children infected with HIV-1.

Collectively, the extant data suggest that the DA system is a clinically relevant target in chronic HIV-1 infection, is one of the major targets in pediatric HIV-1 CNS infection, and may be specifically susceptible during development [117]. The following sections of this

review provide a focus on the development of the DA system, identifies some possible targets of the HIV-1 proteins during the development of the DA system, and discusses potential therapeutic approaches. By coupling our growing understanding of the development of the CNS with the pronounced age-related differences in disease progression, new light may be shed on the neurological and neurocognitive deficits that follow HIV-1 infection.

2. NEURAL DEVELOPMENT OF THE DA SYSTEM

2.1. Projection Pathways of the Mesencephalic (Midbrain) DA System

The regional distribution of DA within the CNS has been demonstrated to be organizationally complex. There are several major DA-containing nuclei, which exhibit heterogeneity in terms of their projection areas. Furthermore, these nuclei exhibit afferent projections of different lengths [111]. These long-length afferents (that will be focused on in this review) comprise DA projections to the telencephalon that originate from the ventral mesencephalon (midbrain). The cell bodies of the midbrain DA system comprise the retrorubral nucleus (RRF) (A8), the SN (area A9) and the VTA (area A10) [111]. These most prominent DA cell groups contain approximately 75% of the total number of brain DA cells [118]. The projections of these midbrain DA-containing cell groups form three principle axonal pathways: (1) nigrostriatal; (2) mesolimbic; and (3) mesocortical DA projections [111, 119, 120].

The DA neurons of the SN, that are located in the lateral part of the ventral midbrain, give rise to the nigrostriatal afferent system that innervates the dorsolateral striatum (corresponding to the caudate-putamen in humans). The majority of neurons in the nigrostriatal projection system are DAergic with less than 5% being non-DA-containing [111]. The nigrostriatal pathway regulates control of voluntary movement; PD is associated with its degeneration [121, 122]. Recently, its involvement has also been demonstrated in HIV-1 dementia with HIV-1-infected patients displaying motor dysfunctions similar to those seen in Parkinson's patients [123].

The DA neurons of the VTA, that are located in the ventromedial part of the midbrain, form the mesolimbic afferent system, that projects to subcortical limbic regions including the NAc, septum, amygdala, and hippocampus. The mesolimbic pathway is involved in reward behavior and is implicated in neurobiological theories of addiction, particularly because of its connection to the NAc [61, 124]. Disruption of DA function in the NAc by drugs of abuse, such as cocaine, is exaggerated with HIV-1 proteins [93, 125] and may underlie the increased prevalence of neurological complications in HIV-1-infected drug users relative to HIV-infected patients without a history of drug use [110]. DA neurons are also lost in the mesolimbic pathway in PD, although that loss is delayed relative to the nigrostriatal pathway that is characteristically described in this disease. Because deficits do not become apparent until there is a reduction of 80–90% in the number of neurons, their loss here is more asymptomatic in nature.

The DA neurons projecting to the cerebral cortex are distinguished by their different origins in the ventral mesencephalon [126]. The neural pathway originating from the DA cell bodies

in the VTA primarily projects to the deeper cortical layers (V–VI) of the frontal cortex. The second class of DA afferents originates in the SN, are highly collateralized, and project to the superficial cortical layers (I–III) in the anterior cingulate cortex [126]. The mesocortical pathway is essential to the functional integrity of the dorsolateral prefrontal cortex and is involved in emotion, motivation, and memory [61, 124]. Disruptions of this system are associated with schizophrenia (negative symptoms), addictive behavioral disorders, as well as attention-deficit hyperactivity disorder (ADHD). Selective lesions of the DA neurons of the mesocortical DA system in rats or primates have given evidence that DA has a major role in regulating cortical neurons of the prefrontal cortex and cognitive functions, such as memory and attentional processes [61, 127]. HIV-1-infected patients with severe HAD demonstrate a 40% decrease of dendritic area in the frontal cortex and a 40–60% decrease of dendritic spine density, compared to controls without dementia [128, 129].

The question arises how, and specifically when, the DA system develops. The early development of DA neurons is characterized by their birth, specification, and migration to their final positions [130]. Neurotransmitter chemistry, process growth, and synapse formation further characterize this development [131]. Subsequently, contact and interactions between the midbrain nuclei and other neural nuclei, such as the striatal target and cortical areas, are established by axonal extension, terminal differentiation, and synapse formation [130]. Thus, compared to HIV-1 in adults, the unique problem in pediatric HIV-1 infection may be an alteration of the DA system during development. Effects on the maturation of a healthy DA system may include early alterations in DA transmission and function, ultimately producing disturbances on later subcortical and cortical specification. Analysis of pediatric HIV-1 infection and its effects on the developing DA system provides evidence for a role of DA on motor and cognitive dysfunction in HIV-1-infected children. Because of the profound abnormalities in the striatal DA system, the present review specifically has a focus on the development of the striatal DA system including the projections from the SN to the striatum and its effects on the associated cortical areas, such as the frontal cortex.

The next section will look more closely at the development of the striatal DA system, by focusing on data that are collected from rat models, with some additional data derived from human studies. The maturational profile of the CNS in rats is linked to the nervous system development in humans by using the brain growth spurt as a frame of reference [132]. According to the brain growth spurt construct, brain development in the first 10 embryonic days (E) of a rat are comparable to the first 12 gestation weeks in a human (1st trimester), with the following 10 days in a rat (E10–E21) being comparable to the human 2nd trimester (13–28th gestation week), and the last 12 weeks of gestation in a human (3rd trimester or 29–40th gestation week) being comparable to postnatal days (P)1–10 in a rat. The timeline of the development of the DA system is summarized in Fig. (1).

2.2. Timeline of the Development of the DA System

2.2.1. Tyrosine Hydroxylase (TH)—Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthetic pathway of catecholamines (i.e., DA, noradrenaline and adrenaline) [133]. In the midbrain, TH is expressed early during ontogeny and can be used

as a specific DAergic neuronal marker. In rats, TH⁺ cells can be detected in the midbrain as early as E11-12; TH is also detected in axons and axonal growth cones at this stage [134]. At E13-14, the first terminal fields of TH⁺ labeled cells are detected in the striatum [124, 134]. The localization of TH⁺ neurons in the human fetus displays similar essential features to those of the rat fetus [135]. Neurons showing TH⁺ immunoreactivity in the human fetal nervous system are present at 12 weeks of gestation (end of the 1st trimester) with numerous TH⁺ labeled axons in the 12-to-15-week human fetal brain. As with rats, TH⁺ cells can be traced rostrally with terminal fields located in the ventrolateral caudate nucleus, an area receiving the greatest number of TH⁺ labeled axons of any region in the telencephalon [135]. Due to the differentiation of the DA terminals postnatally, an increase in striatal TH activity is noted between birth and postnatal day P30 [130]. The maturation seen from E18 into adulthood is characterized by an increase in cell number and continued aggregation and migration of the labeled neurons rather than the development of new axonal pathways [136]. It is suggested that decreases in TH may be a crucial factor in the neurological manifestations of HIV-1 infection. Specifically, during the maturation period when TH⁺ neurons and axonal projections stabilize, an alteration in TH levels may be especially disturbing to the normal development of the DA system.

2.2.2. The First Detection of DA Neurons (mRNA, Autoreceptors)—Once ventral midbrain neurons acquire a DAergic identity, and thus an achievement of final commitment, a set of genes involved in the maturation of DA is activated. At E12.5 DA neurons express the *c-ret* proto-oncogene and GFR α 1 (mRNA) and endogenous DA levels are detectable in the developing rat midbrain (A9, A10). The concentration of DA neurons increases sharply at E16, attaining a plateau before birth [124]. Besides DA gene expression, D₂-like autoreceptors are detected in the midbrain of rats at E13–E14, two days after TH⁺ immunoreactivity, with their number increasing thereafter, and their expression prior to the functional onset of DA neurotransmission. Because DA pathways and functional neurotransmission are not yet established, DA is accumulated in normal development of ventral midbrain neurons until E16 (21st week of gestation in humans) [124].

Marked neuronal degeneration in the SN of HIV-infected patients has been repeatedly documented [137, 138]. Specifically, a significant decrease in neuron density and total neuron size is observed in the SN of HIV-infected brains compared to normal control brains, as well as relative to non-pigmented neurons of the SN [138]. The SN of HIV-1-infected adult human brain autopsy material also demonstrated a significant reduction of TH⁺ immunoreactive levels relative to seronegative controls, suggesting the regulation of TH expression by HIV-1 and/or HIV-1-related viral proteins [80]; however, no changes were found in the levels of neuron specific enolase (NSE) in the SN of HIV-1 brains [80]. Perhaps this decrease in TH expression levels is not due to DA cell death, but rather that HIV-1 and/or its related viral proteins regulate and act on TH expression with the consequence of decreased DA production [80].

2.2.3. The Formation of DAergic Connections—The first DA fibers in the striatum, as well as the initial extended axons into the striatum, can be seen at E14-16 (18–21st week of gestation in humans), with the majority of DAergic projections to the striatum taking

place between E18 and P4 [130, 139, 140]. Glial cell line-derived neurotrophic factor immunoreactivity coincides with increased DAergic innervation, suggesting a regulation of DAergic innervation of the striatum [139]. It should be noted that the importance of glial cells is also seen along the ventral surface of the mesencephalon where nascent DA cells (E13) align along the radial glia (at E15-18) that provide the pathway for migration of the DA cells [141]. In the human fetus, at approximately 28 weeks of age, a considerable number of DA axons and synapses are present [139]. In the postnatal rat, the proportion of DA varicosities forming synapses and the mean size of DA varicosities slowly attain their peak (P5-7 in the striatum and P12-14 in the NAc); an age approximating the time of birth in humans, and extending to several years after birth [132, 142]. The first DA-containing fibers that reach the cortex originate from the VTA to innervate the deep cortical layers (V–VI) by the 17th day of gestation (~the 23rd week of gestation in humans) and are most developed in the PFC [126, 136, 143]. Even though a large number of DA-containing fibers can be observed in some subareas of the PFC at P2, the bulk of DAergic innervation starts before birth in humans (~the 32nd week of gestation) and at P4 in rats, with changes in the morphology and density of the DA-containing fibers continuing to increase until P60 in rats [143]. In contrast, DAergic projections to layer I–III (originating from the SN) reach their targets during the 1st and 2nd postnatal weeks in rats (~birth in humans), becoming gradually thicker and more densely varicose (~4th postnatal week) [126].

During the time window of DA innervation and synaptogenesis, neurons are specifically sensitive to disturbances in their synaptic environment (e.g., changes in DA levels, altered neurotransmitter activity). A match between proper targets and proper presynaptic elements is necessary for the maturation of the DA neurons and the DA system [124]. An interaction between either the target or the presynaptic elements with a neurotoxin may induce specific abnormal changes in the synaptic environment leading to an alteration in development of the DA system [142]. Reduction of striatal target size induced by excitotoxic lesion in immature rats causes apoptosis of SN TH⁺ neurons, supporting the view that striatum plays a role in the survival and maintenance of nigral DA neurons [124]. Further, early postnatal lesions of the SN in rats resulted in disintegration of striatal compartmentation with a shrunken striatum reflecting a massive cell loss in the matrix compartment (whereas the striosomal cells - predominantly innervated by the VTA - become evenly distributed) [144]. Regarding the development of the PFC, small lesions to DA neurons in the VTA at P1 disrupted development of the PFC with a reduction of the cortical thickness by 6% [143]. It should be noted that specific differences exist between rodents and humans in the DAergic innervations of the cerebral cortex, with more expansive cortical DAergic projections in humans (i.e., including motor, premotor and supplementary motor areas) with much larger terminal fields [126]. The more pronounced development of the cerebral cortex and cortical DA in humans has been implicated in a wide range of mental processes and needs to be considered when extrapolating findings in rodents.

2.2.4. The Establishment of DA Neurotransmission—DA neurotransmission is established in rodents around E15-16, much later than the activation of the actual maturation of DA properties [124]. During embryonic development, DA synthesis (TH), storage, and neurotransmission appear to develop asynchronously. In the ventral midbrain, measurable

DA is detected around E12.5, whereas specific high-affinity DA uptake in rat midbrain cells is only found beginning at E16, but with a sharp increase between E16 and E18 (~22–24th weeks of gestation in humans), followed by a plateau before birth [124]. Whereas the endogenous DA levels appear early in the midbrain, DA in the striatum is first detected at E14–E16. The onset of DA uptake and its subsequent increase coincides with the arrival and the establishment of the first contacts of presynaptic DA fibers with their target striatal neurons [124]. The detection of the DAT (mRNA) at E15–16 (~20–22nd weeks of gestation in humans) is consistent with the appearance of high-affinity DA uptake, which is detected at E15 [124]. DAT is critical in terminating DA neurotransmission and maintaining DA homeostasis in the CNS through recycling synaptic DA into the neuronal terminals [145]. Direct cell interactions condition DAT gene expression in developing midbrain DA neurons, with the DAT gene remaining repressed until DA axons begin to reach their target innervation fields [124]. Only subsequently is DAT gene expression and functional uptake detectable. DA uptake is a critical step required only when synaptic transmission is established, which occurs when developing DA neurons reach their target fields and when accompanied by increased expression of other DA pre- and post-synaptic markers (TH, DA receptors). The late appearance of DA uptake and its subsequent increase could explain the elevation in extracellular DA concentration in early postnatal midbrain organotypic cultures, which might be due to spontaneous release and is markedly increased between P0 to P30 relative to the mature striatum [124]. The order in which the developmental events occur for midbrain DAergic neurons is similar in rats and humans but the length of the developmental time period is significantly extended in humans [124]. The finding that alterations or injury to DAT may contribute to HIV-1-associated neurological complications is supported *in vivo* by a human MRI study demonstrating decreased DAT availability in HAD patients [88]. Reduced levels of DAT ligand binding were found in HIV-1 patients with cognitive and motor deficits, specifically in the putamen and ventral striatum (NAc) [88]. Functional maturation is essentially associated with the evolving anatomical organization of the human and rat brain, such as the formation of connections and axonal pathways. Synaptically released DA is reutilized through presynaptic DAT reuptake, which is a fundamental process for regulating extracellular levels of DA [146].

2.2.5. DA Receptors—DA receptors are located pre- and post-synaptically at DAergic synapses. Postsynaptically they play a role in cell-to-cell communication whereas presynaptically they modulate the release and synthesis of DA (autoreceptors). DA function is putatively held to be mediated by five distinct membrane bound receptors. D₁ and D₅-members belong to the D₁ receptor subtype, which stimulate adenylate cyclase, whereas D₂, D₃, D₄-members belong to the D₂ receptor subtype, which inhibit such activity. Although within each subtype the receptors share similar molecular structures and comparable pharmacological profiles, their localization and relative distribution provide distinguishing characteristics [147]. The most ubiquitous receptors are the D₁ and D₂ receptors, with their highest concentration occurring in the striatum (50 pmole/g and 20 pmole/g, respectively) [120, 147, 148]. D₃ receptors are primarily located in limbic areas, albeit in low concentrations in the shell of the NAc (1 pmole/g); D₄ receptors are specifically localized in limbic and cortical regions (2 pmole/g) [148–150]. In the PFC, the presence of the D₄ receptors are recognized as dominant, but there is substantial overlap of receptor subtype

expression in the main brain regions innervated by DA neurons [61]. Whereas D₁- and D₂-receptor mRNAs in the striatum appear to be expressed early in prenatal development (E14), the majority of D₁- and D₂-receptor binding activity in the striatum develops in the postnatal period. The increase in DAergic innervation correlates well with binding activity, suggesting that the developmental regulation of D₁- and D₂-receptor synthesis is independent of D₁- and D₂-receptor gene transcription [140]. The D₁ receptors are predominantly expressed by striatal output neurons projecting to the SN; D₂ receptors reside mainly in output neurons projecting to the GP [120, 148]. Developmentally, D₁ receptor mRNA is present as early as E14 in the striatum, displays peak levels on P5, and subsequently declines to its lower adult levels (20% of peak values) [140]. In contrast, although D₂ receptor mRNA levels are also detectable by E14, they decline from P0–P5, and then increase to adulthood [140]. Presynaptic regulation of nerve terminal activity is one principle role of D₁- and D₂-receptor subtypes, in the SN and the striatum, respectively [61]. Functionally, striatal D₁- and D₂-receptor binding sites increase throughout development, but maximally between P7 and adulthood [140]. Pharmacological studies using D₁- or D₂-receptor antagonists have clearly demonstrated involvement of the DA system in coordination of voluntary movements in rodents [120]. The generation of animals with genetic mutations of the DA-receptors (D₁–D₅) and their behavioral analysis has revealed that each of these mutants presents a locomotor phenotype. However, a more complex interaction among the DA receptors exists for the regulation of voluntary movements, with the D₁- and D₂-receptor interaction most prominent [120].

From a developmental perspective, it should be emphasized that the roles that receptors play in the CNS may differ during maturation, as well as in certain disease states; a fact that is attributable to selective subunit switching and subtype expression [111, 147]. Not surprisingly, neonatal lesions of the DA system (D₁- and D₂-receptor) precipitated different behavioral and neurochemical effects than lesions made in adulthood [147, 151].

2.2.6. DA Metabolism—Released DA is converted to dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) after reuptake by the nerve terminal [119]. Whereas DOPAC is the major metabolite in rats, the major brain metabolite in humans and primates is HVA [119]. In research studies, the accumulation of HVA in the brain or CSF is often used as an index of the functional activity of DA neurons in the brain [119]. In the rat brain, it has been demonstrated that short-term accumulation of DOPAC in the striatum is an accepted index of activity in DA neurons of the nigrostriatal pathway [119]. Early developmental alterations of DA metabolism and its contribution to long-lasting abnormalities in the DA system are supported by a study assessing midbrain cell cultures that reported differential changes in mature and immature rat cell cultures [152]. During a hypoxic challenge, mature and immature DA cells differ in the regulation of the extra- and intracellular DA levels, as indexed by alterations of DA metabolism. In immature cells, the low synthetic capacity of TH, along with low capacities of transport and storage processes, decreased extracellular DA levels relative to that observed in mature cells [152]. If the same is true for pediatric HIV-1 infection, this could be a critical factor in the long-term modulation of TH expression and subsequent long-term behavioral and/or neurological abnormalities induced by HIV-1 and its related proteins [152]. Assessment of DA

metabolism in HIV-1-infected patients, revealed a lower level of HVA in the CSF, compared to healthy control subjects, suggesting depressed DAergic activity [77]. The decreased CSF concentrations of HVA also correlated with impaired performance on neuropsychological tests, specifically those for motor speed, attention, concentration and executive control [77]. The concentrations of DA and HVA in the caudate nucleus were both significantly reduced in post-mortem brain tissue from AIDS patients, relative to control subjects [68]. These data suggest that the motor dysfunctions and neuroleptic sensitivity that have been observed in AIDS patients is attributable to deficits of striatal DA innervation, as found in PD [68].

In the next section of this review the specific targets of the viral proteins, Tat and gp120, in the developing DA system are discussed as being critically involved in the neurological and neurocognitive complications associated with HIV-1 infection. As the virus does not infect neurons per se, HIV-1 proteins are putatively accepted as the causative agents of the observed neuronal loss in HIV-1-infected patients. The expression levels of the viral toxins in the brain of HIV patients pre-mortem is not well-established. However, the quantity of Tat detected in sera of HIV-1-infected individuals may be similar to the concentrations of Tat found in the supernatant of HIV-1-infected H9 cells [153]. The quantity of Tat detected in the sera of HIV-1-infected patients varies appreciably (out of $n = 33$, Tat was detected in sera of 12 HIV-1-infected patients and no Tat was detected in 21 HIV-1-infected patients). It is well-known that non-response to cART (9–45%), also referred to as immune-virological discordance, is associated with a low CD4+ cell count (CD4+ cell counts of < 200 cells/ μ L) and high viral load before initiation of cART [154, 155]. As the Tat protein controls transcription of the lentivirus HIV genome, it is commonly acknowledged that HIV-1-infected patients with low CD4+ cell counts and high viral load have detectable Tat expression.

3. SPECIFIC TARGETS OF THE VIRAL PROTEINS, TAT AND GP120, IN THE DEVELOPING DA SYSTEM

Pediatric HIV-1 research has been demonstrated to involve profound abnormalities of the striatal DA system, including the BG [63]. The BG and its DAergic influence have also been associated with cognitive processes, and are closely linked to cortical areas such as the frontal cortex [61]. Poor attentional abilities and an increased slowing of information processing in pediatric HIV-1 infection are among the most prominent cognitive neuropsychological complications in HIV-1-infected children [45, 51, 59, 64, 156].

The developmental effects of HIV-1, and its impact on the DA system, have been addressed by our group with the use of the HIV-1 transgenic (Tg) rat [108, 109, 157–159]. With the exception of a Gag-Pol deletion, which renders them noninfectious, the HIV genome is intact in the HIV-1 Tg rat [160]. Further, as the HIV-1 transgene is present from conception it provides a compelling approach to model pediatric AIDS.

Young adult HIV-1 Tg rats are generally healthy, as we have reported over a series of studies. Adult female HIV-1 Tg rats display similar growth rates as controls [109, 161], show normal estrous cyclicity [159], and reveal no body weight loss through 11 months of

age [109]. Notably, the health status of our HIV-1 Tg animals would not have been anticipated from the original description of the HIV-1 Tg rat [160]. The HIV-1 Tg rat, as originally described, had several severe pathological phenotypes associated with transgene expression (then located on chromosome 2 and 9), including prominent neurological disorders (hind limb paralysis), three grades of cataracts, and wasting at the relatively early adult age of 5–9 months. Quite in contrast, the HIV-1 Tg animals employed in our research studies are a healthy derivation of these originally described phenotypes, provided on an inbred F344 background, with the transgene now limited to chromosome 9. HIV-1 protein expression in the HIV-1 Tg rat is under the control of the natural HIV-1 promoter, LTR, with protein expression in mononuclear phagocytes/astrocytes, but not in neurons [277] - a pattern resembling that noted in HIV-1+ human brains. Protein expression in the HIV-1 Tg rat is regulated by the viral LTR and uses rat cyclin-T as a cofactor to regulate Tat production [160]. HIV-1 proviral DNA thereby produces viral proteins without active viremia. As suggested by Peng *et al.*, (2010), the moderate phenotype more closely resembles HIV-associated neurocognitive disorders (HAND), (expression of viral proteins without productive viral synthesis), is suitable for developmental studies, and should be considered distinct from the original descriptions of the most severe phenotypes of the initially derived HIV-1 Tg rat [162].

Although HIV-1 Tg animals have light to moderate cataracts, our studies using light levels of 22 lux (dim) and 100 lux (bright) found similar visual abilities of HIV-1 Tg rats and controls to detect light cues [158, 159]. Thus, the ability of the HIV-1 Tg rats to respond to brief dim visual cues in small behavioral testing chambers is intact. Although visual acuity may be impaired by the presence of cataracts, these HIV-1 Tg animals are well-suited for behavioral testing using visual stimuli (i.e., light cues) in standard operant chambers. Accordingly, executive function and attention, as the most affected cognitive processes in pediatric AIDS, were tested in adult HIV-1 Tg rats with lifelong exposure to HIV-1 proteins [161]. We found chronic exposure to HIV-1 proteins primarily impairs sustained attention as well as inhibition and flexibility as core components of executive function. In future studies, the cognitive deficits that define pediatric AIDS can be modeled and demonstrated in the HIV-1 Tg rat, providing opportunities to develop therapeutics for HIV-1-infected children.

A recent time-limited (6-month) longitudinal study in adult animals (5–11 months old) provided compelling evidence for functional alterations in the brain DA system, as indexed by alterations in gating, novelty, episodic memory, and cocaine-induced sensitization [157]. The developmental period for this study was well before the occurrence of severe neurological symptoms or clinical signs of wasting [162]. Alterations in sensorimotor gating were suggested by the leftward shift in the prepulse inhibition (PPI) curve; the HIV-1 Tg rats were also hyperreactive to auditory startle stimuli. It is of particular note that alterations in brainstem auditory evoked potentials (BAEP), a measure of temporal processing, are one of the earliest neurological abnormalities of HIV-1-positive individuals [259–261]. A weaker novelty response was also apparent in the HIV-1 Tg rats over repeated monthly evaluations, albeit they displayed a robust initial activity response comparable to control rats. Evidence for impaired long-term episodic memory was revealed by decreased within-session habituation of the HIV-1 Tg rats across 3-day periods, a pattern that emerged over

the repeated monthly evaluations. Finally, differential cocaine-induced sensitization was noted in the HIV-1 Tg rats, as revealed in initiation of sensitization (i.e., a 10-day cocaine protocol) as well as in expression of sensitization with a cocaine challenge injection following a 7-day period of abstinence. Thus, the HIV-1 Tg rat displays neurocognitive and behavioral alterations that implicate sustained, if not permanent, perturbations of the brain DA system. Further, these data are of particular note given the resemblance of the noninfectious status of the HIV-1 Tg rat to the brain proinflammatory immune responses and suppression of infection in HIV-1+ patients on cART.

Alterations in DAergic system markers were expected in the HIV-1-Tg animals, given the neurocognitive and behavioral findings cited above. Western blotting revealed differences between the HIV-1 Tg and control groups regarding DAergic markers [108]. Protein levels of monoamine oxidase A (MAO-A) were significantly lower in the midbrain of the HIV-1 Tg rats, relative to controls. No significant changes were observed in DAT, dopamine D₁ receptor, and TH protein expression in the absence of methamphetamine treatment. However, HIV-1 Tg animals that received methamphetamine displayed a different pattern of alterations; in HIV-1 Tg rats, MAO-A protein expression was significantly higher, and TH protein expression was significantly lower, than that of the control rats. Thus, the chronic expression of HIV-1 proteins induced significant alterations in DA-related behavioral and neurochemical measures, and furthermore, these alterations were especially prominent with challenges to the DA system (i.e., methamphetamine).

In adolescent rats, cognitive alterations were examined in PPI [108] and alterations in the DA system were noted in TH protein levels or expression and/or in DAT mRNA [108, 157]. Thus, low-level, life-long, exposure to HIV-1 proteins (including Tat and gp120) significantly impact the developing DA system with both behavioral and neurochemical impairments. These DAergic impairments occur under conditions in the HIV-1 Tg rat which resemble the brain proinflammatory immune responses and suppression of infection in HIV-1+ children under cART. Interestingly, a recent study using [¹⁸F]fallypride positron emission tomography (PET) reported D_{2/3} receptor deficits in young HIV-1 Tg rats. The HIV-1 Tg rats had smaller striatal volumes, lower expression levels of TH, and significantly lower binding potential in the dorsal striatum relative to control rats [163].

Other findings support the notion of selective sensitivity of DA neurons to HIV-1 proteins [65, 92, 93, 98, 105, 112]. With the knowledge of the DAergic targets of the HIV-viral proteins (especially Tat and gp120), the development of selective therapeutic treatments may be possible and may reduce the neuropsychological complications that are associated with pediatric HIV-1 infection.

3.1. Transactivator of Transcription (Tat)

The alteration of the DA system by Tat is supported by a number of studies [65, 92, 93, 98, 105–107, 157, 164, 165]. One question that arises is if the impairment of DA neurotransmission in the nigrostriatal system by Tat is a result of diminished activity of TH, the rate-limiting DA synthesizing enzyme, or whether Tat acts directly on DA cells, targeting the DAT and DA receptors.

The effects of Tat on TH have been shown *in vitro* and *in vivo*. *In vitro* treatment of DA rat PC12 cells with synthetic HIV-1 Tat₁₋₈₆ protein or Tat cDNA inhibited the expression of TH in the culture medium [166]. The *in vivo* data indicated a loss of TH immunoreactivity in fibers on the injected side one week after injection of Tat into the rat striatum, which was accompanied by a loss in the nigral DA neuron cell bodies [166]. A reduced staining of neurons expressing TH was noted in the SN that was followed by a loss of TH⁺ neurons at later time points [166]. Continuous, stable Tat₁₋₈₆ production in the rat striatum and hippocampus was obtained using stereotaxic injection of genetically engineered rat C6 glioma cells [164]. Tat was retrogradely transported from the striatum to the SN through TH⁺ fibers; impaired motor function (rotarod performance), neurotoxicity, and reactive gliosis were also observed [164]. Tat production in the striatum significantly decreased TH immunoreactivity in the SN, with no effects on TH immunoreactivity in the VTA. Immunoreactivity for both OX42 and GFAP was also dramatically increased in the SN, suggesting Tat-induced astrocyte and microglia activation. Additionally, another study that investigated Tat₁₋₇₂ effects on DA terminals in the rat striatum, demonstrated silver staining was observed after two and seven days in the ipsilateral cortex, indicating that Tat may be transported to the cortex [102]. These results suggest that Tat can induce a selective block of TH gene expression with the ability of Tat being transported by TH⁺ immunoreactive fibers to different areas of the brain, such as the SN and cortex [102, 164, 166]. It can be assumed that the entry of HIV-1 into the system of the fetus is unlikely to occur before the 20th week of gestation, with the highest risk for vertical transmission occurring at birth. At that time TH-labeled neuronal groups and fibers have already been formed and therefore, have the potential to transport Tat to striatal and cortical DA target fields, as seen in adult animals [102, 164, 166]. However, the early interference of HIV-1 Tat with the ability to inhibit the expression of TH may cause an alteration in the continued aggregation and migration of the TH-labeled neurons leading to reduced TH levels and consequently alterations in DA system development.

The suggestion of Tat not only targeting TH, but also acting on DA levels has been supported by microdialysis studies, demonstrating a decrease in striatal DA overflow and/or content 48 h after intrastriatal infusion of Tat (25 µg) [93, 105, 106, 167]. DA overflow, evoked by super-physiological concentrations (100 mM) of KCl was assessed 24, 48 and 72 h after administration of Tat into the striatum. Tat-induced a linear decline of DA overflow with evoked DA being reduced to a greater extent after 48 h in the Tat group relative to the vehicle control group [106]. The findings of a Tat-induced reduction in evoked DA transmission suggests a loss of DAergic synaptic reuptake function, most likely mediated by impairment of the DAT.

Similarly, primary cell cultures from E18 rat midbrain indicated a significant decrease in [³H]WIN 35428 binding 48 h after 50 nM Tat₁₋₇₂ exposure [112]. WIN 35428 is a phenyltropane-based DA reuptake inhibitor, structurally derived from cocaine; tritiated WIN 35428 is frequently used to map binding of novel ligands to the DAT. Thus, a decrease in [³H]WIN 35428 binding after Tat₁₋₇₂ exposure suggests a decreased ability of Tat to bind DAT ligands [112]. No significant change in immunoreactive DAT protein levels was noted that could account for the decreased specific ligand binding. Collectively, these observations

suggest functional alterations of DAT protein underlie the compromised DAT ligand binding [112].

The Tat-induced inhibition on the uptake of DA into neurons occurs by Tat protein directly interacting with the DAT protein [92, 165]. Assessment of DAT function using rat striatal synaptosomes demonstrated that HIV-1 Tat₁₋₈₆ protein decreases DA uptake in a time- and dose-dependent manner, and moreover, that this Tat:DAT binding is reversible [92]. Interestingly, Tat inhibits DAT function through a protein kinase C (PKC) and trafficking-dependent mechanism which impacts DAergic tone *via* regulation of DAT and VMAT2 proteins [95]. More recent studies using integrated computational modeling, simulation, protein mutagenesis and molecular pharmacology techniques have discovered a key intermolecular interaction between Tat and DAT. In particular, residue Tyr470 in DAT is critical for Tat inhibition of DAT. Thus, the underlying allosteric mechanisms on the DAT for the actions of Tat have begun to be determined [96]. Understanding this Tat:DAT protein-protein interaction may allow the development of compounds that specifically block the Tat binding to DAT, yet maintain normal DA synaptic function. Such compounds would be ideal for stabilizing DAergic tone in HIV-1+ brain tissue.

Collectively, these results suggest that Tat directly induces functional impairment of the DAT *via* an allosteric mechanism, and thus DA is not able to be taken up into the presynaptic terminal and remains in the synaptic cleft. From a developmental point of view, it has been shown that DAT and DA uptake is detected much later than DA mRNA, around the 20th week of gestation, remaining repressed until DA axons begin to reach their target [124]. If DAT is targeted early in development, the maintenance of DA homeostasis in the CNS is disrupted and altered already very early in development when the establishment of axonal pathways are undergoing stabilization [124]. As mentioned in recent studies, a match between appropriate targets and presynaptic elements is requisite for the maturation of embryonic DA neurons [124]; match failure leads to an altered and disrupted DA system early in development.

The importance of D₁ receptor-controlled pathways in Tat neurotoxicity has been indicated in both rat fetal midbrain cell cultures [80] as well as in rat hippocampal cell cultures [98]. The blockade of D₁ receptors in cultured midbrain neurons with the specific D₁ antagonist, SCH 23390, attenuated cell death caused by a 50 nM dose of HIV-1 Tat₁₋₇₂ [80], suggesting that Tat neurotoxicity is the result of activation of D₁-mediated pathways. The authors note that overactivation of D₁ receptors is an anticipated consequence of Tat-mediated inhibition of DAT activity, as has been suggested by other recent studies [98, 168, 169]. Another finding is that Tat exposure led to decreased binding of [³H]SCH 23390, but did not alter the level of D₁ immunoreactive protein, suggesting that Tat treatment alters the function of D₁ receptors [80]. Specific binding of [³H]SCH 23390 to D₁ receptors might be dependent on the oxidative status of the protein. It is known that specific ligand binding to the D₁ receptor requires participation of both SS and SH groups at the recognition site and that receptor function is sensitive to oxidative modification [170, 171]. Because Tat neurotoxicity is associated with increased protein oxidation, D₁ receptors may be a target for Tat-induced oxidative damage [80]. In contrast, it should be noted that the specific D₁ receptor antagonist SCH 23390 (10 μM) did not affect 50 nM Tat-induced neurotoxicity in rat

hippocampal cultures, although a suppression of cocaine-mediated potentiation of Tat-induced neurotoxicity was noted, suggesting that Tat-induced neurotoxicity is not restricted to a D₁ receptor mechanism [112]. However, the nonsignificant effect of the D₁ receptor antagonist SCH 23390 might be due to the fact that D₁ and D₂ receptors occur with their highest concentration in the striatum, whereas the D₃ receptors are mainly located in limbic areas [120, 147–149]. D₁ receptors appear to be predominantly expressed by striatal output neurons projecting to the SN [148]. The involvement of DA receptors is supported by the often seen hypersensitivity to DA receptor blockade in HIV-1-infected children that may contribute to a selective abnormality of the striatal DA system [63, 172].

Collectively, Tat induces an overactivation of D₁ receptors, which may be a consequence of increased extracellular DA due to a Tat-mediated allosteric inhibition of DAT activity. Due to the fact that vertical transmission of HIV-1 occurs during a time window of synaptogenesis, axonal growth and DA innervation, the disruption and alteration of DA transmission may have devastating effects on the maturation of the DA system. Further, Tat targeting the expression of TH is an additional factor that might cause an alteration of DA levels reducing the number of available DA neurons early in development. Between P0 and P30 in rats, corresponding to the 28th week of gestation through 24 weeks after birth in humans, TH activity increases from 10% to 75%. If this process is targeted by Tat, the differentiation of DA terminals may be disturbed leading to an irreversible disruption of the DA system early in development. Another complicating factor is the transport of Tat from one area to another, such as from the striatum to the SN and cortical regions, increasing the devastating effects of Tat in regions remote from the original insult.

There is also growing evidence for effects of Tat on non-DA targets related to neurocognitive impairments. In a series of studies [161, 173–175] we have investigated the long-term effects of Tat and gp120 on the mature hippocampus following intrahippocampal injection during the neonatal period [174]. Specifically, neonatal Tat exposure produced a selective loss of neurons in the cornu ammonis fields (CA)2/3 and hilus of the dentate gyrus (DGH) [174]. A similar outcome has been reported in humans suggesting a selective vulnerability of CA3 hippocampal neurons to AIDS-related injury [176]. Further, the Tat-induced decrease of neuron cell number in the DGH were predictive of the spatial memory impairments subsequently detected in adulthood [175], strongly supporting a critical role of Tat in pediatric HIV-1 neuropathogenesis and neurocognitive impairment of HIV-1-infected children.

A follow-up study examined the neurotoxic profile of Tat₁₋₈₆ following direct P1 injections into the hippocampus [161]. Tat₁₋₈₆ appeared to have more profound effects on the developing nervous system than the Tat₁₋₇₂ used in the prior study [175]. Neither Tat₁₋₈₆ nor gp120 injections into the neonatal hippocampus, at doses sufficient to induce neurotoxicity, had any statistically detectable effect on expression of the inflammatory cytokine IL-1 β or the inflammatory factors NF- κ B and I- κ B. The sequences of both Tat₁₋₇₂ and Tat₁₋₈₆ differ mainly in the presence of the second exon encoded region of the protein. The second exon contains an RGD sequence that partially provides the recognition sequence for cell surface integrin binding [177]. Integrins have an important role in early hippocampal development and the formation of correct connectivity. Thus, it might be expected for Tat₁₋₈₆ to have

more profound developmental effects, relative to Tat₁₋₇₂. Additional investigations into the actions of the second tat exon during hippocampal development are needed to clarify this issue.

Interestingly, we found no evidence for a long-term dose-response effect on the estimates of the total neuron number after Tat was injected into the hippocampus of the neonatal rat at P10 [173]. However, Tat injection at this later point in development dose-dependently increased the number of astrocytes and oligodendrocytes. Given the differential consequences of P1 vs P10 Tat injections, it appears the CNS is most vulnerable during early development. The neuronal loss caused by Tat occurs prior to the marked increase in rate of brain growth, and moreover, the variants of the Tat protein (Tat₁₋₇₂ vs Tat₁₋₈₆) may have important differential effects on neural development.

3.2. Glycoprotein 120 (gp120)

CNS injection, or expression, of gp120 may cause widespread neuronal injury, degeneration, and death *in vivo* as well as *in vitro* [178]. Neuronal cell cultures that display such sensitivity to gp120 include those from the hippocampus [179], cortex [178, 180, 181], cerebellar granule cells [182], and the dorsal root ganglia [183], but also DA neurons [184, 185].

When injected into the rat striatum, gp120 evoked caspase-3-mediated neuronal degeneration proximal to injection site but also at the distal SN pars compacta [185]. The striatum receives DAergic projections from the SN. As gp120 can be internalized by neurons [182] which exhibit activated caspase-3, it may be that gp120 causes DA cell loss when it is taken up by nigrostriatal fibers and retrogradely transported to the DA cell bodies [185]. Consistent with this suggestion, the observed cell loss reflects a selective degeneration of TH⁺ neurons in the A9 region of the SN, which projects into the dorsal striatum. DA levels in the dorsal striatum were significantly lower in gp120-treated rats relative to controls. Moreover, an accumulation of gp120 immunoreactivity has been observed inside TH⁺ neurons of the SN when gp120 was delivered into the striatum [186]. Collectively, these results are consistent with a model of HIV-1 neurotoxicity in which neuronal degeneration occurs following the retrograde transport of HIV-1 proteins to the neuronal cell bodies.

The processes of DA neurons have also been found to be adversely affected by gp120 in primary mesencephalic cultures [184]. DA cells of the rat midbrain displayed a reduction in their ability to transport DA as well as a concentration-dependent reduction in the length of their dendrites following a 3-day exposure to gp120 (10^{-12} to 10^{-8} M). The alteration of DA uptake and process length suggests, respectively, a reduction in the number of transport sites ($V_{\max}$) and an overall simplification of the neuropil; the downregulation of DA transport by gp120 is also supported [187]. Effects of gp120 (10^{-12} to 10^{-8} M) exposure on survival of TH⁺ and NSE⁺ cells failed to detect any significant changes in cell number compared to non-treated cultures [184]. Although neither DA cell number or the total number of neuronal cells were decreased, collectively, these results are consistent with decreased DAergic function and a reduction in the neuronal cytoarchitecture [184]. Immunohistochemical studies have shown that the majority of DA neurons in SN and the VTA are immunoreactive for NMDA-R₁ receptors [188]. Since glutamate release is also associated with gp120-

induced neurotoxicity, NMDA receptor activation could contribute to the blockage of DA uptake and downregulation of DA transport by increased intracellular Ca^{2+} . DA transport is critical for differentiation and maturation of neuronal cells [189]; if there is a prolonged decrease in levels, especially during a critical period of development, long-term consequences would be expected for the morphological and functional development of the brain [152].

The neurotoxic effect of gp120 on neuronal dendrites is also supported by the assessment of post-mortem human forebrain samples at 16–18 weeks of gestation [190]. Following 4 weeks of gp120 exposure, human primary CNS cultures displayed gliosis and dendritic injury similar to that described in HIV-1-infected patients with HAD [129]. Gp120-induced neurotoxicity evident in neuronal and dendritic alterations was supported very early in this line of research [191]. A significant reduction of dendritic processes was noted and accompanied by an increase in pathology as displayed by greater fragmentation, varicosities, and beading [190]. The reduction of dendritic complexity was accompanied by decreased MAP2 staining as well as an increase in total GFAP staining, an astrocyte marker [190]. It may very well be that the neurocognitive impairments associated with HIV-1 infection reflect an accumulation of synaptodendritic injuries leading to reduced dendritic complexity, cell death, and cognitive decline [190].

Neonatal animals treated on P1 with gp120 displayed alterations in the DA system in adulthood as indicated by alterations in PPI and their attenuation by apomorphine (APO) [192]. A number of preclinical studies support the involvement of the DA system in sensorimotor gating and PPI is well-accepted model for studying deficits in sensory information processing, such as those characteristic of schizophrenia [193]. Event-related brain potentials (ERP) have been demonstrated to be the earliest readily quantifiable alterations noted in the progression of HIV-1-infected patients to HAD, indicating a relationship between HIV-1 infection and sensory gating [194, 195]. Interestingly, the effects of neonatal gp-120 were far greater in adulthood than when the animals were tested as weanlings [196]; observations consistent with latent long-term neurotoxic effects of gp120. The observed behavioral alterations of gp120-treated animals to a DA agonist may be related to an interference with the signaling properties of DA neurons. Future research is necessary to determine how APO (a D_1 - and D_2 -receptor agonist) attenuates the gp120-induced alterations in PPI. A previous study suggested NMDA involvement with regulating PPI *via* a D_1 -regulated pathway [197]. Another possibility is that APO may be overactivating the remaining D_1 receptors, as a direct acting D_1 stimulant, which would help normalize PPI. Interactions between DA and gp120-induced neurotoxicity suggest damage to D_1 receptors plays an important role in regulation of PPI. The convergence of observations of deficits in sensorimotor gating with the administration of single HIV-1 proteins (Tat or gp120), and in the HIV-1 Tg rat, as measured by PPI, indicates the investigation of the neural substrates of PPI, may yield fundamental information regarding the neural systems involved in HIV-1 pathophysiology.

4. TREATMENT POSSIBILITIES

The long-course and complex regimens of ARV prophylaxis treatment have proven to virtually eliminate early transmission rates in resource-rich countries from 30–40% to less than 2% [52, 53, 198, 199]. Following the adult ARV development, the treatment of pediatric HIV infection has evolved from monotherapy with AZT, to a dual therapy with nucleoside reverse transcriptase inhibitors (NRTIs) and subsequently to multi-drug therapy involving a combination of three or more ARV drugs [53]. Nevertheless, even in the presence of ARV prophylaxis, vertical transmission is still the highest risk of HIV-1 transmission in children, with 1800 new HIV-1 cases being reported for Western and Central Europe and North America by UNAIDS for 2013 [1]. One problem that occurs consequent to the frequent use of ARV drugs, such as AZT, is the mutation of HIV-1 to AZT-resistant variants in HIV-1-infected mothers and children [200]. One suggestion for the resistance of HIV-1 to AZT is the decreased kinase activity demonstrated in T-lymphocytic leukemia cell lines and the resistance of taking up an AZT anabolite into cellular DNA [201].

Regarding treatment of pediatric HIV-1 infection, it is well-known that antiretroviral therapies (AVT), such as HAART have greatly improved the outcome of HIV-1-infected children, with a major increase of survival time [26, 202] as well as promising global improvements in cognitive function [203]. Like AZT, HAART suppresses HIV-1 replication in both plasma and cerebrospinal fluid (CSF), and targets the HIV-1 T cell reservoirs that are persistent and present in HIV-1-infected children [202]. However, in contrast to an increase in survival rate, neurological dysfunctions, such as motor and cognitive impairments, have been reported in a steady number with no decline in incidence and prevalence compared to other complications of immunodeficiency [204, 205]. Incomplete adherence to HAART seems to correlate with cognitive impairment, indicating that drug resistance emergence in the CSF, blood and the CNS may have a crucial impact with compromising the penetration of the blood-brain barrier (BBB) for certain ARV agents [204]. Further, the treatment of pediatric HIV-1 infection is much more difficult relative to treating HIV infection in adults because of the lack of drug approval, inadequate drug formulation, and a lack of pharmacokinetic data for children.

The observed neuronal degeneration in the striatal DA system of pediatric HIV-1 patients supports the use of DA agonists as a therapeutic approach, such as it is done in PD. Whereas the results of DA agonists in adults have been variable, studies assessing pediatric HIV-1-infected children with PD-like features have shown to produce consistent improvement in motor function [62, 116]. DA agonists have been proposed to have therapeutic effects, such as L-DOPA effects in PD [62, 206]. L-DOPA treatment, which is a precursor of DA and restores DA levels, resulted in improvement of ambulation, alertness, activity, and facial expression that were seen within 2 weeks and were sustained for at least 6 months [62]. Another study assessing the interaction of neonatal gp120 with the DA system suggests that D₁/D₂ agonist therapy mitigates outcomes, such as with attentional processes [192]. Further, *in vitro* cell culture studies support the notion that HIV-1 components, such as the HIV-1 protein Tat, act on the D₁ receptors of the DA system [80, 98]. It is suggested that an interaction between DA and Tat- and gp120- induced neurotoxicity may involve D₁ receptor

regulation and this could play an important role in the regulation of cognitive processes. However, when considering a DA agonist as a treatment approach in HIV-1 infection, it is important to consider findings that have shown that increased DA availability, as with selegiline treatment (a selective monoamine oxidase type B inhibitor), can enhance CNS viral replication, CNS vacuolization, and encephalitic lesions [207]. L-DOPA, as well as selegiline, accelerated infection causing vacuolization and increased viral load in the SIV-infected macaque model [207]. Further, it has been reported that DA-stimulated transcription through the NF- κ B element has a critical role in mediating DA responsiveness [208]. Interestingly, a study assessing the effects of DA on HIV-1 infection demonstrated that antioxidant treatment (glutathione, one of the main components of cellular defense mechanisms against oxidative stress, and its indirect precursor, N-acetylcysteine) reduced DA-induced HIV-1 activation, indicating that alterations in cellular redox states may underlie the observed effect [209]. Cellular vulnerability to HIV-1 may thus be closely linked with excessive DA levels that are accompanied by alterations in intracellular oxidant/antioxidant levels [209].

Regarding the role of reactive oxygen species (ROS) in HIV-1 infection, antioxidants have been proposed as a therapeutic approach [210–212]. Oxidative stress has been repeatedly demonstrated to be an early indicator of HIV-1 protein-induced neurotoxicity in synaptosomes [165], midbrain cells [213], and hippocampal cell cultures [98]. HIV-1-induced inhibition of antioxidant enzymes, such as superoxide dismutase (SOD), may alter the pro-oxidant/antioxidant balance of host cells [211]. The antioxidant enzyme, SOD, has been demonstrated to protect against HIV-1 gp120-induced neuronal apoptosis in the striatum of rats [214]. Another possible agent in attenuating gp120- and Tat-induced oxidative stress is 17 β -estradiol (E₂) [215], which additionally prevents the loss of DAT function [165]. The additional action of E₂ on the DA system without excessive exposure of the CNS to DA is promising and may be a particularly efficacious therapeutic treatment for the neurological complications associated with pediatric HIV-1 infection. In this line of research, HIV-1 protein-mediated inhibition of DAT activity has been demonstrated in SK-N-SH cells with Tat and gp120 inhibiting DA uptake into striatal synaptosomes [165]. Further, the Tat and gp120-induced increase in oxidative stress in SK-N-SH cells, as indexed by increased levels of dichlorofluorescein (DCFH) fluorescence, was significantly attenuated by E₂ (100 nM). The protective effect of E₂ is supported by *in vivo* data [216] with a significant reduction of apoptotic cell death following i.c.v. injection of gp120 (100 ng/rat/day) into the neocortex. In contrast to studies indicating that E₂ protection is *via* an antioxidant mechanism independent of receptor binding [217], the neuroprotective effects of E₂ are demonstrated *in vivo* and *in vitro* to involve E₂ receptors. Specifically, the E₂ receptor antagonist ICI 182,780 blocked the neuroprotective effects of E₂. These findings indicate that Tat- and gp120-induced oxidative stress appears to be mediated, at least in part, by E₂ receptors [165, 215, 216, 218].

Synaptodendritic injury has been observed in response to HIV-1 Tat protein exposure. Tat protein-induced synaptodendritic damage is highly specific and dependent upon the presence of the cysteine region of the Tat protein (aa 21–32) [219]. Moreover, Tat protein-induced synaptodendritic damage occurs prior to cell death, at very low Tat concentrations,

and may be reversible [220]. Synaptic alterations in pyramidal cells of the hippocampus [221], as well as decreased spine density in medium spiny neurons [222], have also been reported in mice conditionally expressing the HIV-1 Tat protein in astrocytes. Similarly, we have recently reported that HIV-1 Tg rats have altered dendritic spines in the nucleus accumbens medium spiny neurons [159]. The HIV-1 Tg rats had a reduction of longer spines and an increase in shorter, less projected spines, indicating a population shift to a more immature spine phenotype as a consequence of constitutive, low-level, exposure to HIV-1 proteins. Likewise, gp120 expressing mice demonstrate synaptic dysfunction [223, 224].

Collectively, these single protein (Tat or gp120) transgenic mice and HIV-1 Tg rat studies suggest that exposure to HIV-1 proteins, individually, or in concert, affects synaptodendritic processes. The neurocognitive effects of HIV-1 appear to correlate with synaptic loss and dysfunction, rather than with cell death per se [225]. As a potential restorative-based therapeutic approach, it has been well-established that E₂ rapidly increases dendritic spine density *in vivo* [226, 227], through regulation of the actin cytoskeleton in spines [228]. Phytoestrogens are diphenolic structures found in plants, which possess similar chemical and structural properties to 17 β -estradiol [229, 230]. We reported that phytoestrogens promoted recovery from the synaptodendritic damage caused by HIV-1 Tat [220], an effect most likely mediated *via* the beta form of the estrogen receptor.

Even though synaptodendritic damage correlates with the neurocognitive impairments associated with HIV-1 infection [225, 231], the extent of synaptic restoration that is possible remains unaddressed. Other critical questions that beg to be addressed are whether a critical therapeutic window might exist for promoting the most efficacious neurorestoration, as well as, what the key developmental windows are for maintaining synaptic integrity. Despite the correlation between spine loss and neurocognitive impairments, unfortunately, there are few studies of therapeutic pathways for enhancing HIV-1 dendritic recovery [232, 233]; however, it has been clearly established that estrogen promotes robust spine formation *via* modulation of F-actin [228, 234]. Our studies suggest that phytoestrogens, presumably acting through F-actin, provide a novel venue for promoting neurorestoration [220] and deserve further examination. Estrogen receptors play a key role in modulating dendritic spine dynamics [228, 234–237], suggesting the estrogen beta receptor could be used as a therapeutic target to reduce HIV-1-induced synaptodendritic injury, and/or to promote restoration from synaptodendritic damage.

We discovered the neurorestorative potential of the phytoestrogens by assessing their ability to prevent, as well as to enhance recovery from, the synaptodendritic alterations caused *in vitro* by HIV-1 Tat₁₋₈₆ [220]. Specifically, the effects of phytoestrogens, daidzein and liquiritigenin, on restoration of the dendritic network were determined following withdrawal of HIV-1 Tat, and the role of estrogen receptors in the phytoestrogen-induced recovery process was determined through the use of tamoxifen [220]. In brief, we found that HIV-1 Tat-induced synaptodendritic injury could be recovered by the phytoestrogens, and that this neurorestoration was mediated through an estrogen receptor-mediated mechanism. Even though it is currently unknown if such reversals can promote recovery from HIV-1-associated neurological and neurocognitive disorders, phytoestrogenic compounds may

nevertheless prevent, or at least attenuate, cumulative injury to the dendritic network. It is unknown what the developmental consequences might be from early treatments with phytoestrogenic, estrogen beta receptor targeted compounds, and the specific neurorestorative effects on the DA system.

In summary, treatment possibilities need to be thought of carefully with possible antiretroviral, antioxidant, and neuroprotective/neurorestorative therapeutic approaches. It is proposed that treatment studies should include agents that target CNS pathology, including neuroprotective substances (such as estrogen beta receptor compounds and phytoestrogens), with specific actions either on maintaining or restoring DA synaptic integrity.

CONCLUSION

Pediatric HIV-1 infection presents a different clinical picture compared to HIV-1-infection in adults, indicating the importance of investigating perinatally acquired HIV/AIDS, and thus, the effects of HIV/AIDS on development. The present review paper highlights that HIV-1-infected children experience a wide spectrum of motor, cognitive, and adaptive developmental deficits with underlying neurological problems. As effects occur in a developing nervous system, neurological consequences of HIV-1 appear earlier in the course of infection and progress more rapidly in HIV-1-infected children relative to HIV-1-infected adults. The underlying CNS abnormalities in pediatric HIV-1 infection are complex, but many of its clinical features can be attributed to abnormalities of the DA system with involvement of the striatal/cortical pathway. Fig. (1) summarizes the targets of gp120 and Tat on the developing DA system. With this review we hope that future research will focus more on establishing functional, structural or expression-based changes of DAergic markers in pediatric HIV-1 patients and thus provide promising therapeutic interventions.

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ABBREVIATIONS

6-OHDA	6-hydroxydopamine
AIDS	Acquired immune deficiency syndrome
Area A10	Ventral tegmental area (VTA)
Area A8	Retrorubral nucleus (RRF)
Area A9	Substantia nigra (SN)
ARV	Antiretroviral
AZT	Azidothymidine or Zidovudine
BAEP	Brainstem auditory evoked potentials
BBB	Blood-brain barrier

CA	Caudate
CG	Cingulate gyrus
CNS	Central nervous system
CP	Caudate-putamen
CT	Computer tomography
DA	Dopamine
DAT	Dopamine transporter
E	Embryonic day
E₂	17 β -estradiol
ERK	Extracellular-regulated kinase
ERP	Event-related brain potential
FGF1	Fibroblast growth factor 1
GFAP	Glial fibrillary acidic protein
GP	Globus pallidus
Gp120	Glycoprotein 120
HAD	HIV-1-associated dementia
HIV	Human immunodeficiency virus
HIVE	HIV-1 encephalitis
HVA	Homovanillic acid
JNK	c-Jun N-terminal kinase
LTP	Long-term potentiation
MRI	Magnetic resonance imaging
NAc	Nucleus accumbens
NeoR	Neomycin B hexa-arginine conjugate
NMDA	N-methyl-D-aspartate
NSE	Neuron specific enolase
P	Postnatal day
PD	Parkinson's disease
PE	HIV-1-associated progressive encephalopathy
PFC	Prefrontal cortex
PPI	Prepulse inhibition
Put	Putamen

ROS	Reactive oxygen species
SIV	Simian immunodeficiency virus
SN	Substantia nigra
SOD	Superoxide dismutase
TAT	Transactivator of Transcription
Tg	Transgenic
TH	Tyrosine hydroxylase
VTA	Ventral tegmental area

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Fig. (1).

Potential targets of gp120 and Tat in the developing DA system. Important milestones in the development of the striatal DA system in rats. X-axes indicate the time pattern of the maturation of the CNS in rats, which corresponds to the nervous system development in humans (the brain growth spurt as a frame of reference [132]). The units of time for humans and rats are in months and days, respectively [275, 276]. (1) Immunoreactivity for TH in the midbrain is first observed at E11–12 [134]; DA is first detected in the midbrain at E12–13 [124]. (2) DA autoreceptors are first detected in the midbrain of rats at E13–E14 [124]. (3) The establishment of DA neurotransmission (DAT) occurs in rodents around E15–16 [124]. (4) Synapses are first observed in SN at E18 [130]. (5) TH⁺ fibers are first observed in the striatum at E13–14 [124]. (6) DA receptor mRNA is first expressed in the striatum at E14 [140]. (7) First DA fibers in the striatum, the first extension of axons into the striatal anlage can be seen at E14–16, with the most DA projections to the striatum at E18–P4 [130, 139, 140]. (8) The first DA-containing fibers reach the frontal cortex at E17 [126, 136, 143]; with DA innervation starting at P2 with DAergic fibers continuing to increase until P60 [143]. (9) Largest increase in synapses in SN at P15–30 [130]. (10) Differentiation of DA terminals takes place postnatally, indicated by large increases in TH activity and DA uptake at P0–30 [130]. (11) Formation of synapses increases gradually to reach a peak at P5–7 in CP and P12–14 in NAc [142]. (12) Increase in D-R binding activity in striatal complex is a postnatal development [140]. HIV-1 entry between the 20th gestation week and the termination of breastfeeding as the time window for vertical transmission. Note: E, embryonic day; CA, caudate; CG, cingulate gyrus; CP, caudate-putamen; DA, dopamine; DAT, dopamine transporter; D-R, dopamine receptor; HIV-1, human immunodeficiency virus type 1; NAc, nucleus accumbens; P, postnatal day; Put, putamen; SN, substantia nigra; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

Table 1

World estimates of the HIV & AIDS epidemics at the end of 2013 [1].

		Estimate*	Range*
Number of people living with HIV/AIDS in 2013	Children	3.2	2.9–3.5
	Total	35	33.2–37.2
People newly infected with HIV in 2013	Children	0.24	0.21–0.28
	Total	2.1	1.9–2.4
AIDS deaths in 2013	Children	0.19	N/A
	Total	1.5	1.4–1.7

* Million.

Table 2

Reviews concerning pediatric HIV/AIDS infection.

Reference	Title
[57]	Neurological and neuropathological features in children
[238]	Pathology of HIV-1 infection of the central nervous system
[239]	Clinical comparison of adults and pediatric NeuroAIDS
[58]	Neurologic and neuropsychologic findings
[49]	HIV-1 encephalopathy in children
[41]	Infants, children, and adolescents
[240]	Imaging of pediatric central nervous system HIV infection
[27]	Clinical and biological disease progression
[47]	Neurological findings in pediatric HIV/AIDS. Clinical features
[241]	Neurocognitive functioning and effects of combined therapy
[156]	Neurocognitive outcomes in pediatric HIV
[242]	Global perspective on neurologic/neurodevelopmental sequela
[45]	A review of pediatric HIV effects on neurocognitive development
[51]	Dopaminergic deficits and future treatments
[54]	Pediatric antiretroviral therapy
[31]	Complications related to HIV or its treatment
[52]	Antiretroviral therapy in children: recent advances
[50]	Neurodevelopment in children born to HIV-infected mothers
[53]	Pharmacotherapy of pediatric HIV infection
[48]	Immunology of pediatric HIV infection

Table 3

List of neurological findings in pediatric HIV/AIDS infection with corresponding references.

Findings	Reference
Neuroimaging Findings (CT scan, MRI)	
<ul style="list-style-type: none"> impaired brain growth cerebral atrophy white matter abnormalities ventricular enlargements calcification of basal ganglia calcification of frontal white matter 	[34, 37, 56, 240, 243–252]
Neuropathological Findings	
<ul style="list-style-type: none"> gliosis in deep cerebral white matter and basal ganglia reactive astrocytosis scattered macrophages multinucleated giant cells microglial nodules 	[56, 244, 246, 253, 254]
<i>In situ</i> hybridization Findings	
<ul style="list-style-type: none"> mRNA-expressing cells in frontal cortex (layer IV) HIV-1-infected macrophages HIV-1-infected multinucleated giant cells neuronal degeneration 	[244, 255–257]
Electrophysiological Findings	
<ul style="list-style-type: none"> dysfunction of brainstem auditory pathways alteration in occipital VEPs VEPs over parietal and temporal areas not well formed delay in ERPs, e.g., N1, N2, P3, PA3 component 	[34, 194, 258–261]
Psychometric Findings	
<i>Developmental delays/decline</i> <ul style="list-style-type: none"> loss/deviation from the normal progression of developmental skills failure to acquire (i.e., language; fine and gross motor skills) 	[241–244, 262–267]
<i>Cognitive dysfunction</i> <ul style="list-style-type: none"> intellectual deterioration attentional/learning deficits verbal functioning 	[34, 242–245, 268–270]
<i>Social-emotional dysfunction</i>	[245]

Findings	Reference
<i>Motor dysfunction</i> <ul style="list-style-type: none">pyramidal tract signs (i.e., general muscle weakness, spasticity)extrapyramidal and cerebellar signs (i.e., rigidity, ataxia, tremor)	[34, 113–115, 242, 243, 258, 262, 264, 271–274]