J Neuroimmune Pharm (2006) 1: 410–420
DOI 10.1007/s11481-006-9030-6

ORIGINAL ARTICLE

Abnormal Striatal Dopaminergic Synapses in National NeuroAIDS Tissue Consortium Subjects with HIV Encephalitis

Benjamin B. Gelman · Jeffrey A. Spencer · Charles E. Holzer, III · Vicki M. Soukup

Received: 20 April 2006 / Accepted: 7 June 2006 / Published online: 8 August 2006

Abstract People with human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) have neurological problems that overlap with diseases associated with abnormal dopaminergic (DAergic) synaptic transmission, including subcortical dementia, motor slowing, psychosis, and drug addiction. Previous study has suggested that DAergic tone may be decreased in HIV/AIDS, but biochemical confirmation of that tenet is still lacking. To that end, this study addresses the neurochemical interaction between HIV infection and DAergic synaptic transmission in human brain specimens. Protein markers of DAergic synapses were characterized in homogenates of the corpus striatum from individuals with HIV encephalitis (HIVE) and seronegative controls from the autopsy cohort of the National NeuroAIDS Tissue Consortium. Striatal DAergic markers were abnormal in HIVE. Abnormal presynaptic markers included decreased tyrosine hydroxylase (TH) protein and decreased phosphorylated TH. The presynaptic dopamine reuptake transporter (DAT) was increased reciprocally. Postsynaptic abnormalities included decreased dopamine receptor type 2 (D_2R) and increased D_3R. There was preferential loss of the alternatively spliced long isoform of D_2R relative to the short isoform. Abnormal DAergic synapse proteins were significantly correlated with the HIV Gag mRNA transcripts amplified in striatal extracts. These synaptic changes resemble shifts that occur when DAergic tone is increased experimentally. Increased DAergic tone leads to heightened salience for drugs of abuse, increases behaviors that increase the risk of HIV transmission, and might decrease compliance with antiretroviral medication regimens.

Key words addiction · autopsy · dementia · dopamine · dopaminergic · dopamine receptor · dopamine transporter · dopamine reuptake · HIV encephalitis · national neuroAIDS · tissue consortium · synaptic transmission · tyrosine hydroxylase

This paper was presented at the 12th Annual Society for Neuroimmune Pharmacology Conference on April 6, 2006 in Santa Fe, NM, USA.

B. B. Gelman (✉) · J. A. Spencer
Department of Pathology, Route 0785
University of Texas Medical Branch,
Galveston, Texas 77555-0785, USA
e-mail: bgelman@utmb.edu

B. B. Gelman
Neuroscience and Cell Biology,
University of Texas Medical Branch,
Galveston, TX 77555-0785, USA

C. E. Holzer III
Department of Psychiatry,
University of Texas Medical Branch,
Galveston, TX 77555-0189, USA

V. M. Soukup
Department of Neurology,
University of Texas Medical Branch,
Galveston, TX 77555-0539, USA

Introduction

At least four diseases have clear-cut linkage to abnormal dopaminergic (DAergic) synaptic transmission. In Parkinson's disease (PD), which is a prototypal degeneration of DAergic neurons, the loss of DAergic tone produces movement dysfunction and a subcortical type of dementia that includes impairment of executive function and working memory (Le Moal and Simon 1991; Robbins 2003). Schizophrenia and attention deficit hyperactivity disorder (ADHD) are conditions associated with executive dys-

function and loss of working memory that respond favorably to drugs that modulate DAergic tone in the prefrontal cortex (Kandel et al. 1991; Volkow et al. 2005). Drug addiction, especially the type involving the psychostimulant cocaine, is strongly linked to having excess DAergic tone in mesolimbic "reward circuits" that project through ventromedial striatum (nucleus accumbens) (Koob and Le Moal 1997; Volkow et al. 2004). People infected with human immunodeficiency virus (HIV-1) and/or those with acquired immunodeficiency syndrome (HIV/AIDS) often have HIV-associated subcortical dementia (HAD), motor slowing, neuropsychiatric disturbances including psychosis, and drug addiction (Heaton et al. 1995). For that reason, the interplay between HIV infection and DAergic dysfunction is a topic of some clinical relevancy (Nath et al. 2002; Koutsilieri et al. 2002; Grassi et al. 1997). Brain HIV infection might influence the basic neurobiology and clinical management of DAergic neurological dysfunction. For example, it has been suggested that HIV/AIDS increases the vulnerability to extrapyramidal side effects of antipsychotic medicines; these drugs act in part by blocking dopamine receptors, so altered DAergic tone is a possible mechanism (Berger and Nath 1997). Conversely, addictive behavior and neuropsychiatric problems driven by DAergic circuits can promote behaviors that can increase the risk of HIV transmission to other people (such as needle sharing or risky sexual practices) (Chiasson et al. 1991). Drug addiction and psychosis in HIV/AIDS can also decrease compliance to antiretroviral medication regimens and lead to increased morbidity and mortality rates. Drugs of abuse such as cocaine, morphine, and alcohol can alter brain immunity and/or HIV-1 replication rates in model systems, and could increase HIV neurovirulence or exacerbate HAD (Baldwin et al. 1998; Roth et al. 2002; Koutsilieri et al. 2002). These scenarios suggest that the interaction between HIV infection and DAergic systems has potential impact on disease transmission, progression of virus replication, neurological dysfunction, response to treatment, and comorbidity (Volkow et al. 2004, 2005). To determine more precisely the effect of HIV on DAergic function, we undertook a biochemical characterization of striatal DAergic synapses in people with HIV encephalitis (HIVE), which is the neuropathological substrate of HIV-associated dementia (HAD) in many cases.

Materials and methods

Patients

Twelve human brain samples were characterized biochemically (Table 1). The subjects were recruited into the National NeuroAIDS Tissue Consortium (NNTC) cohort. Subjects with HIV infection had various degrees of neurocognitive dysfunction documented prior to death by using the NNTC testing protocol (Morgello et al. 2001; Woods et al. 2004). When the subjects died, an autopsy was performed and brain specimens were frozen at −80°C and banked at the University of Texas Medical Branch in Galveston, TX (Gelman et al. 2004, 2005). The six people with HIV-1 infection died from end-stage AIDS and were selected for study based on the presence of HIV encephalitis (HIVE) as determined by neuropathological examination (Budka 1991). Specimens were excluded from consideration if they had a

Table 1 Summary of decedents

Ss	HIV (+/−)	HIVE	Age	Sex	Ethnicity	Postmortem interval (h)	Neurocognitive diagnosis	Plasma HIV RNA	Plasma CD4+ lymphocytes
A	Negative	No	40	M	Hispanic	14	N/A	N/A	N/A
B	Negative	No	47	F	African American	18	N/A	N/A	N/A
C	Negative	No	51	F	African American	10	N/A	N/A	N/A
D	Negative	No	34	M	White	24	N/A	N/A	N/A
E	Negative	No	38	M	White	28	N/A	N/A	N/A
F	Negative	No	58	M	White	12	N/A	N/A	N/A
G	Positive	Yes	38	M	White	6	HAD	1843	43
H	Positive	Yes	41	M	White	6	NPIO	>500,000	37
I	Positive	Yes	43	M	White	9	N/A	N/A	N/A
J	Positive	Yes	32	M	White	14	HAD	489,796	77
K	Positive	Yes	43	M	White	Unknown	HAD	>750,000	44
L	Positive	Yes	32	M	White	14	HAD	1,069,444	49

HIV = Human Immunodeficiency Virus type 1; HIVE = HIV encephalitis; HAD = HIV-associated dementia; NPI-O = neurocognitive impairment was present, HAD was highly likely, but a comorbid condition was present; N/A = not available; HIV RNA units are in copies per milliliter; CD4+ lymphocyte counts expressed as cells per cubic millimeter; Plasma samples were collected within 6 months of the autopsy; The order of patients listed in the table is the same order depicted in Figs. 1, 2, and 4.

confounding neuropathological lesion. Six comparison brain specimens were selected from the Galveston NNTC brain repository that did not have HIV-1 infection and did not have encephalitis or neuropathological abnormality, and did not have a recorded history of neurocognitive dysfunction. Raw neurochemical data, clinical data, brain and other organ specimens, blood plasma, cerebrospinal fluid and other resources from this set of subjects, and other subjects are available to the public at no cost by contacting the primary author or the National Coordinating Office of the NNTC (http:\\www.hivbrainbanks.org).

Brain specimen processing

Frozen human postmortem brain hemispheres were dissected and stored at −80°C by using the protocol established by the NNTC (Gelman et al. 2004). A sample of the head of the caudate nucleus, weighing approximately 0.4 g, was excised from the frozen brain slice with a porcelain cutting tool (Dremel Corp., Racine, WI, USA). Samples were placed in 3 volumes of standard radio-immuno-precipitation (RIPA) homogenization buffer (0.05 M Tris–HCl pH 7.4, 0.15 M NaCl, 0.001 M EDTA, 1% IGEPAL, 0.1% SDS) containing protease inhibitor cocktail (Roche, Indianapolis, IN, USA) and phosphatase inhibitor cocktail set II (Calbiochem, San Diego, CA, USA) on ice. RIPA buffer was used to obtain optimal solubilization of membrane protein. Zirconia/silica 0.5-mm microbeads were added and samples were placed in a Minibeadbeater (BioSpec Products, Bartlesville, OK, USA) and pulsed for 20-s intervals and returned to ice for cooling. Pulsing was repeated three times, the samples were centrifuged at 13,000 × *g* at 4°C, and supernatant was used to extract protein. Protein concentration was determined by BCA Assay (Pierce, Rockford, IL, USA).

Western immunoblots and densitometry

Fifty micrograms of protein was mixed with an equal volume of 2× Laemmli Sample Buffer (Bio-Rad Laboratories, Hercules, CA, USA), boiled for 5 min, and then loaded onto 4 – 15% gradient Tris–HCl polyacrylamide gels. Samples were electrophoresed at 150 V for 3 h and tank-transferred to PVDF membranes at 20 V overnight at 4°C in Tris-glycine buffer (0.025 M Tris base, 0.192 M glycine, pH. 8.4). The membranes were blocked in TBST (0.05 M Tris–HCL, 0.15 M NaCl, and 0.1% Tween 20) containing 5% nonfat dry milk for 1 h. Membranes were incubated with primary antibody diluted in fresh block solution overnight at 4°C and were then washed three times at ambient temperature for 5 min each with TBST. Membranes were incubated with appropriate secondary antibodies diluted in TBST for 1 h, washed three times, and then developed with ECL Detection Reagent (Amersham Biosciences, Piscataway, NJ, USA). Membranes were exposed to X-ray film (Kodak, Cedex, France). Primary antibodies used were as follows: β-tubulin (Sigma-Aldrich T7816, St. Louis, MO), dopamine transport protein (DAT; Chemicon ab5802, Temecula, CA, USA), tyrosine hydroxylase (TH) (Sigma T1299), TH phosphorylated at serine 40 (pTH) (Sigma T9577), dopamine type 1 receptor (D_1R) (RDI-D1RABX, Research Diagnostics, Concord, MA, USA), dopamine type 2 receptor (D_2R) (Calbiochem, #324393), dopamine type 3 receptor (D_3R) (RDI-DOP-D3ABR, Research Diagnostics). β-tubulin, TH, and pTH were used at a dilution of 1:5,000; all other primary antibodies were used at a dilution of 1:1,000. Densitometry was performed by using the program 1D Scan (ScanAlytics, Rockville, MD, USA) to quantify band intensity and relative protein abundance.

Isolation of RNA, reverse transcription and PCR

RNA was isolated from caudate by using Tri-Reagent (Sigma) as recommended by the manufacturer. Total RNA was treated with 5 units of DNAse (Invitrogen, Carlsbad, CA, USA) and 2 units of RNAguard (Amersham) per microgram of RNA for 30 min at room temperature, phenol extracted, chloroform extracted, and ethanol precipitated at −80°C. cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad). PCR was performed by using *Taq* DNA Polymerase (Fisher Scientific, Fairlawn, NJ) under the following conditions: 1 cycle of 95°C denaturation for 5 min followed by 95°C denaturation for 30 s, 55°C annealing for 30 s, and 72°C elongation for 30 s. Cycle numbers for GAPDH and Gag were 23 and 45, respectively. cDNA was normalized to the levels of GAPDH in each sample. Primer sequences for GAPDH and Gag were as follows:

GAPDH1 5′-TGATGACATCAAGAAGGTGGTGAA-3′
GAPDH2 5′-TCCTTGGAGGCCATGTGGGCCAT-3′
GAG1 5′-GTAATACCCATGTTTTCAGCAT-3′
GAG2 5′-TCTGGCCTGGTGCAATAGG-3′

Immunohistochemistry of dopamine transport protein

Brain specimens were fixed in 20% formalin and washed. Samples of anterior basal ganglia were excised and embedded in paraffin wax. Tissue blocks were sectioned at a thickness of 6 μm and placed on Superfrost Plus Gold glass slides (Fischer). Slides were baked overnight at 55°C, deparaffinized in three successive 100% xylene baths, and rehydrated with decreasing concentrations of ethanol. Endogenous peroxidase activity was quenched by using 3% H_2O_2 in methanol. Antigen retrieval was performed using microwave-heating in 0.01 M sodium citrate (pH. 6.0)

containing 0.2% Triton X-100 for 20 min at 10% power in a 2-L water bath. Washed slides were blocked in 0.1% nonfat milk and 1% goat serum and were incubated overnight at 4°C with rabbit polyclonal antibody (Chemicon) against DAT diluted 1:200. Biotinylated antirabbit secondary antibody was applied for 1 h, followed by Vectastain ABC avidin–peroxidase complex (Vector, Burlingame, CA). Color was developed using diaminobenzidine (DAB kit Vector). Slides were counterstained in Mayer's hematoxylin and covered after dehydration using Permount (Fisher) containing 25% xylene.

Results

β-tubulin concentration was used as a general neuronal marker. It was not significantly different in the people with HIVE, and suggests that the two groups contained equivalent amounts of neuronal protein (Fig. 1a, b). Three different presynaptic DAergic markers were abnormal in people with HIVE. The dopamine reuptake transport protein (DAT) is a specific presynaptic DAergic marker; it controls the concentration of dopamine in the synaptic cleft by transporting it across the presynaptic membrane (Rudnick and

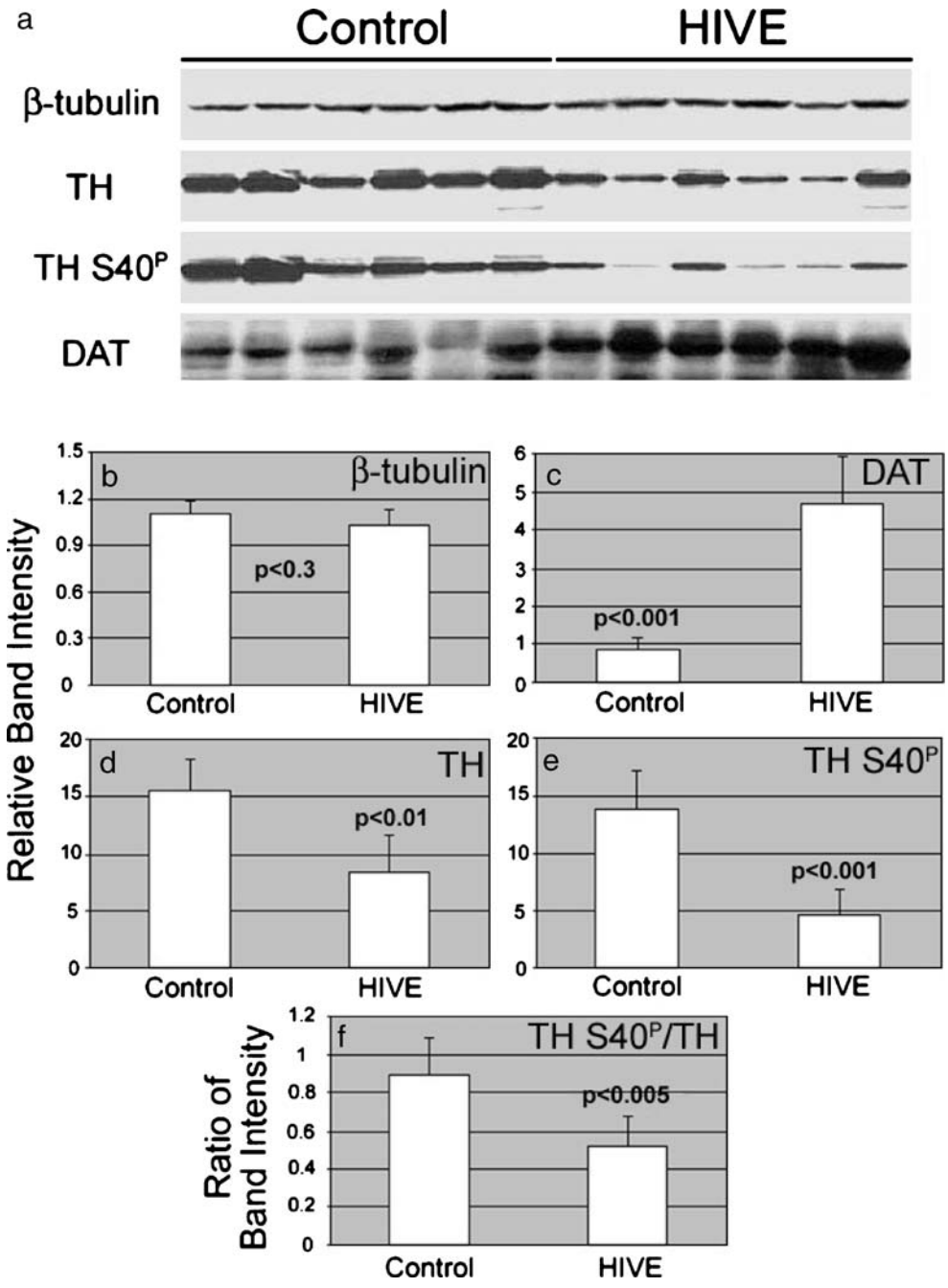

Fig. 1 Presynaptic markers of dopaminergic synapses in the striatum of people with HIVE compared to HIV seronegatives. (a) Western blots of DAT, TH phosphorylated on serine 40, TH, and β-tubulin. (b–f) Band intensities in panel (a) were quantified by using densitometry. β-tubulin concentration (b) was unchanged between controls and HIVE. DAT concentration was increased and TH was decreased significantly in HIVE (c, d). TH phosphorylated on serine 40 was significantly decreased in HIVE (e). The ratio of phospho-TH to total TH was decreased significantly (f), which reflects a decrease in posttranscriptional TH phosphorylation. The sequencing of subjects in panel (a), left to right, matches the order in Figs. 2 and 4, and Table 1.

Clark 1993). DAT band intensity was increased significantly in HIVE (by over 4-fold; $p < 0.001$) (Fig. 1a, c). TH is a presynaptic protein that is the rate-limiting enzyme of the dopamine synthetic pathway. The TH band intensity was decreased by 50% ($p < 0.01$) in HIVE (Fig. 1a, d). TH can be posttranslationally phosphorylated at serine 40 (pTH) to a catalytically more active form (Wolf and Roth 1990; Lewis et al. 1987; Lindgren et al. 2000, 2001; Kansy et al. 2004; Haavik et al. 1989). When phosphospecific antibody was used against pTH, these band intensities showed a sharp decrease (69%; $p < 0.001$) (Fig. 1a, e). The ratio of pTH to TH, which reflects the major posttranslationally regulated component of enzyme catalysis, was decreased significantly ($p < 0.005$) (Fig. 1f).

Altered presynaptic DAergic drive can produce changes in gene expression in striatal postsynaptic neurons (Burt et al. 1977; Creese and Snyder 1979; Seeman 1980). To determine if DAergic synapses were perturbed postsynaptically, the concentration was measured of three genomically distinct dopamine receptors, D_1R, D_2R, and D_3R. D_2R protein was significantly decreased in HIVE; D_3R protein was increased significantly; D_1R protein was not changed (Fig. 2). Two alternatively spliced D_2R molecules were detected in the immunoblots. The long splice variant (D_2L) band migrates at 59 kDa and contains 444 amino acids; the short form (D_2S) migrates at 47 kDa and contains 415 amino acids (Dal Toso et al. 1989). D_2L was strongly decreased ($p < 0002$); D_2S was decreased to a lesser extent ($p < 0.029$). There is evidence that D_2S is preferentially synthesized by presynaptic neurons and is the "dopamine autoreceptor" (D_2Ra), whereas D_2L is the prevalent isoform synthesized in the postsynaptic medium spiny neuron (Khan et al. 1998; Centonze et al. 2002).

To determine the anatomical distribution of abnormal striatal DAergic synapses, we performed immunohistochemistry to localize DAT, which was sharply increased in HIVE and is representative of these DAergic anomalies. Figure 3 illustrates punctate DAT deposits that are typical of its synaptic localization in striatum (Ciliax et al. 1995). Abnormal DAergic synapses in HIVE had increased DAT immunostaining that was broadly distributed in striatum. Increased DAT staining in HIVE was not restricted to foci that contained HIVE changes, such as microglial nodules or multinucleated cells (not illustrated).

Finally, we asked whether having abnormal DAergic synapses was related to having increased HIV replication in striatum. Figure 4 shows that all of the subjects with HIVE had amplified HIV Gag transcripts and none of the

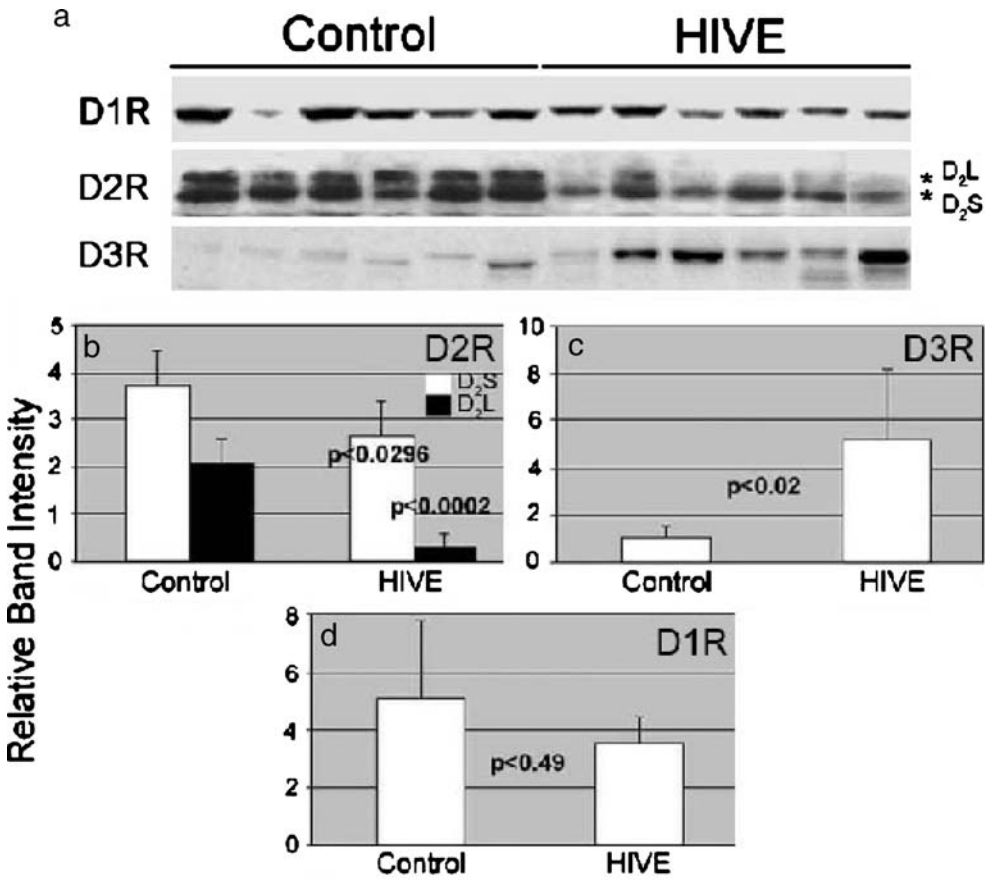

Fig. 2 Postsynaptic markers of dopaminergic synapses in the striatum of people with HIVE compared to HIV seronegatives. (a) Western blots of three dopamine receptors, D_1R, D_2R, and D_3R. (b–d) Band intensities shown in panel (a) were quantified by using densitometry. The concentration of the D_2R long isoform (D_2L) was sharply and significantly decreased in HIVE (b). The D_2R short isoform (D_2S) was significantly decreased (b). The concentration of D_3R was increased significantly (c). D_1R was not changed (d). The sequencing of subjects in panel (a), left to right, matches the order in Figs. 1 and 4, and Table 1.

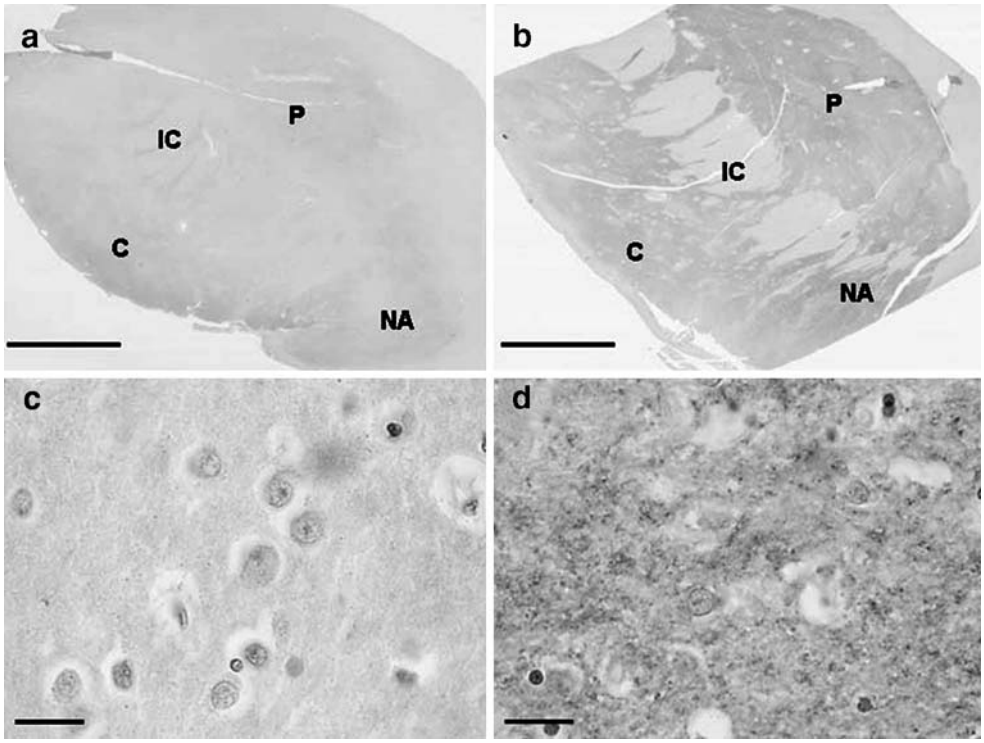

Fig. 3 Example of increased immunostaining of DAT in rostral neostriatum of a subject with HIVE (b, d) and a seronegative control (a, c). Whole mounts show a very diffuse pattern of increased DAT staining intensity in the subject with HIVE (b). High-power magnification shows punctuate deposits of DAT in a typical synaptic staining pattern (c, d). Slides were counterstained with hematoxylin. C = caudate nucleus, P = putamen, IC = internal capsule, NA = nucleus accumbens. Scale bar is 5 mm in (a) and (b), and 2 μm in (c) and (d).

uninfected controls did. The amount of HIV Gag transcript amplified in HIVE was correlated positively and significantly with DAT and D_3R (Table 2). TH, pTH, and D_2L were negatively, but not significantly, correlated with HIV Gag.

Discussion

These data provide broad biochemical evidence that striatal DAergic synapses are abnormal in NNTC subjects with HIVE. Four separate gene products enriched in DAergic synapses were abnormal. The panel of anomalies included examples of both pre- and postsynaptic neuronal markers. The fact that there were strong increases in some DAergic markers, with sharp decreases in others, implies that the changes are not likely to be the result of postmortem protein degradation, generalized neurotoxicity, or dropout of synapses. Abnormal presynaptic DAergic markers included a sharp increase in DAT concentration, and a reciprocal decrease in TH concentration. Down-regulation of TH synthesis leads to a long-term decrease in the rate of dopamine synthesis because TH activity is the rate-controlling step (Tank et al. 1986a, 1986b; Fossom et al. 1992; Meller et al. 1987). Moreover, there was a posttranslational decrease in catalytically active pTH(ser40), which rapidly suppresses the rate of dopamine synthesis further (Wolf and Roth 1990; Lewis et al. 1987; Lindgren et al. 2000, 2001; Kansy et al. 2004; Haavik et al. 1989). These results suggest that the rate of dopamine synthesis in presynaptic neurons is sharply decreased in HIVE. Another potential presynaptic anomaly in HIVE was the decrease in D_2S; this

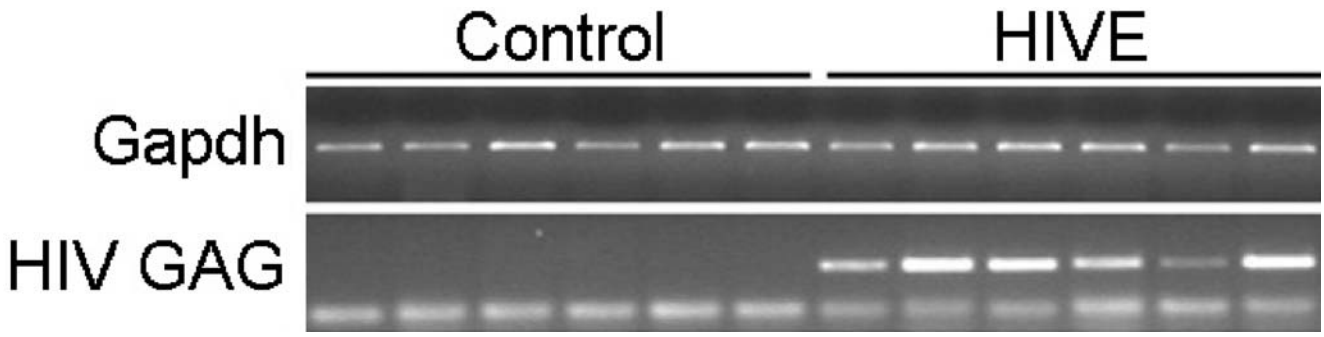

Fig. 4 HIV Gag transcripts in human striatum were amplified by using RT-PCR. Seronegative subjects (HIV-negative, control) did not contain HIV Gag transcripts. All six HIVE patients had amplified HIV Gag transcripts at various concentrations. cDNA was normalized to the levels of Gapdh. The sequencing of subjects, left to right, matches the order in Figs. 1 and 2, and Table 1.

Table 2 Amplified HIV Gag mRNA and the composition of striatal DAergic synapses

Dopaminergic synaptic protein	*n*	*r*	*p*
Abnormal presynaptic markers			
Tyrosine hydroxylase (TH)	6	−0.40	0.432
Tyrosine hydroxylase phosphorylated	6	−0.57	0.218
Dopamine reuptake protein (DAT)	6	0.83	0.040*
Abnormal postsynaptic markers			
Dopamine receptor 2S (D_2S)	6	−0.50	0.313
Dopamine receptor 2L (D_2L)	6	−0.57	0.234
Dopamine receptor 3 (D_3R)	6	0.91	0.012*

n = Number of subjects; *r* = correlation coefficient; *p* = probability value; * = significant correlation.

splice variant of the D_2R gene may be the presynaptic D_2R "autoreceptor" (D_2Sa) (Khan et al. 1998; Centonze et al. 2002). A decrease in D_2Sa expression in HIVE is consonant with changes in presynaptic TH and DAT, because D_2Sa modulates gene transcription and protein function of those markers (Meller et al. 1987; Dickinson et al. 1999; Lindgren et al. 2001; Kimmel et al. 2001; Zahniser and Doolen 2001; Mayfield and Zahniser 2001). The concentration of two different postsynaptic DAergic markers was perturbed in HIVE. D_2L protein was significantly decreased and D_3R was significantly increased. D_1R was unchanged. Both postsynaptic changes are highly selective phenotypic shifts because D_1R, D_2L, and D_3R arise from separate genes and undergo unique transcriptional and posttranslational regulation. Because all of the markers were differently regulated in HIVE (increased, decreased, or unchanged), a simple dropout of presynaptic DAergic neurons or postsynaptic medium spiny neurons is not a likely scenario. Instead, the panel suggests that functional adaptation occurred in viable pre- and postsynaptic neurons (as opposed to neuronal dropout or degeneration; Gelman et al. 2004). All told, the straightforward biochemical measurement of DAergic synaptic markers has produced convergent, self-reinforcing lines of evidence that striatal DAergic synapses are not normal in people with HIVE.

To determine the functional significance of abnormal DAergic synapses in HIVE, we compared changes in HIVE to those that occur when dopamine availability is manipulated experimentally (Fig. 5). The biochemical changes in HIVE resemble perturbations that occur when DAergic tone (synaptic dopamine availability) is increased chronically. For example, decreased TH and increased DAT expression in presynaptic neurons both occur in cocaine abuse when DAergic tone and dopamine receptor occupancy are high. This adaptation probably serves to decrease the synthesis of dopamine and increase reuptake from the synaptic cleft, leading to a compensatory dampening of DAergic tone (Little et al. 1998, 1999; Staley et al. 1994; Chen et al. 1999). Conversely, these shifts are opposite to what is observed in PD striatum and animal models in which DAergic tone and receptor occupancy are low. This adaptation probably serves to increase dopamine synthesis and decrease reuptake, to compensate for decreased DAergic tone (Joyce et al. 1997; Blanchard et al. 1994; Harrington et al. 1996; Uhl et al. 1994). A similar interpretation follows from results of the postsynaptic markers, because synaptic dopamine concentration also modulates dopamine receptor expression (Burt et al. 1977; Creese and Snyder 1979; Seeman 1980). Thus, in cocaine addicts with high DAergic drive and high receptor occupancy, striatal D_2R expression is driven downward, while D_3R is generally increased (Staley and Mash 1996; Mash and Staley 1999; Segal et al. 1997; Volkow et al. 2004; Nader and Czoty 2005). Conversely, striatal D_2R is increased and D_3R is decreased in people and animals with low receptor

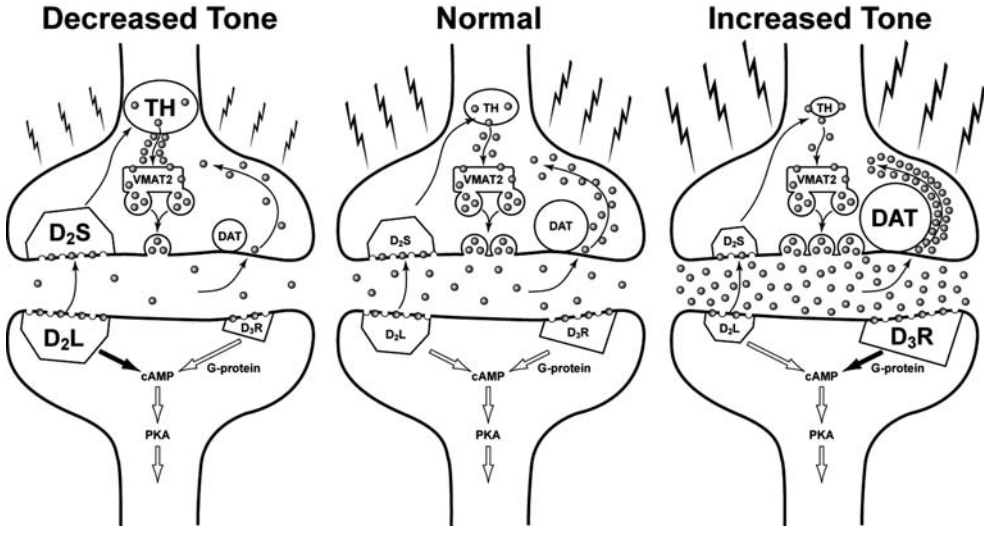

Fig. 5 Three synapses are illustrated that depict adaptations to experimental manipulation of DAergic tone. The size of the symbol reflects the concentration of each protein in striatal synapses. The synapse at right depicts changes produced when the concentration of dopamine in the synaptic cleft is increased experimentally. TH decreases and DAT increases in the presynaptic bouton; D_2R decreases and D_3R increases in the postsynaptic bouton. The synapse at left depicts changes in the opposite direction after DAergic tone is decreased, such as in Parkinson's disease. The changes observed in HIVE striatum suggested increased DAergic tone.

occupancy and tone (Gerfen et al. 1990; Herrero et al. 1996; Quik et al. 2000; Bezard et al. 2001; Wade et al. 2001; Thobois et al. 2004). All told, the panel of changes in HIVE indicates that striatal dopaminergic synapses underwent coordinated adaptations that resemble what happens when DAergic tone is increased, and are in contrast to what happens when tone is decreased. That surprising conclusion disagrees with some of the conclusions suggested by clinical observation alone. For example, patients with HIV/AIDS can exhibit Parkinsonian-like extrapyramidal signs, which can suggest that DAergic tone is decreased in dorsolateral striatal circuits involved with motor control (Hriso et al. 1991; Lopez et al. 1999; Mirsattari et al. 1998; Berger and Nath 1997; Berger and Arendt 2000). However, correlative neuroanatomical evidence in clinically examined subjects to support that suggestion is not yet available. Retrospective postmortem measurements suggested that DAergic tone may be blunted in AIDS because of the drop-out of DAergic neurons in pars compacta of the substantial nigra, but these decedents did not have a Parkinsonian syndrome (Reyes et al. 1991; Marcario et al. 2004). Other evidence seems to agree with the suggestion that DAergic tone is increased in HIV infection. For example, increasing dopamine availability accelerated infection in the simian immunodeficiency virus (SIV) macaque model of HIV infection (Koutsilieri et al. 2002), and exacerbated the neuropathology of SIVE (Czub et al. 2001).

An increase in striatal DAergic tone could have various clinical consequences. One highly important group of DAergic circuits to consider is the "reward circuitry." Brainstem DAergic neurons in the ventral tegmental area (VTA) project to the ventromedial part of striatum, including the nucleus accumbens (NA). The NA has rich connections with the limbic lobe and coordinates reward-seeking and motivational behavior. There is evidence that when these circuits are "overloaded," the increase in synaptic dopamine reinforces reward-seeking behavior, akin to addiction behavior or compulsive gambling in humans (Koob and Le Moal 1997; Esch and Stefano 2004). Addictive drugs such as cocaine produce permanent neural sensitization in brain mesolimbic systems that lead to a compulsive "wanting to take drugs" that can last for years (White and Kalivas 1998). Cocaine increases dopamine availability by directly blocking DAT and reinforces drug-seeking behavior in these reward circuits (Volkow et al. 2004). Hyperdopaminergic mutant mice lacking DAT gene function also exhibit a "high salience for reward" (Esch and Stefano 2004). In turn, chronically increased synaptic dopamine concentration is associated with both pre- and postsynaptic changes in gene expression. The premier example is striatal D_2R transcription, which is driven downward when occupancy is high, and increases when receptor occupancy is low (Gerfen et al. 1990; Staley and Mash 1996; Herrero et al. 1996; Segal et al. 1997; Mash and Staley 1999; Quik et al. 2000; Bezard et al. 2001; Wade et al. 2001; Thobois et al. 2004; Nader and Czoty 2005). Decreased expression of striatal D_2R is consistently observed in people who are addicted to drugs, particularly cocaine. Volkow et al. (2004) suggest that drug abuse increases D_2R occupation, which then drives D_2R expression downward. Having less available D_2R "desensitizes" the reward circuit and causes the addicted person to seek more stimulation. The decreased striatal D_2R expression that we observed in HIVE is therefore consistent with a postsynaptic response to chronic overstimulation, as occurs in cocaine abuse and therapies that stimulate dopaminergic transmission (Thobois et al. 2004; Nader and Czoty 2005). Low striatal D_2R expression, for any reason, can increase vulnerability to the reinforcing effects of cocaine (e.g., self-administration in monkeys) (Nader and Czoty 2005). Therefore, decreased D_2R in HIVE and the other convergent biochemical changes we have described [D_3R, TH, pTH(ser40), DAT] are observed when DAergic tone is increased. These results imply that HIVE could increase vulnerability to situations in which striatal DAergic tone is increased pathologically, such as drug addiction. This suggestion raises several clinically relevant hypotheses for future investigation:

(1) HIVE may exacerbate the rewarding properties of cocaine and other addictive drugs by stimulating DAergic circuits and driving down striatal D_2R expression.
(2) HIV infection could increase need for DAergic reinforcement, which, in turn, can lead to behavior that increases the risk of HIV transmission.
(3) Aggressively treating drug addiction in people with HIV/AIDS, by reducing reward-seeking behavior, could decrease the rate of HIV transmission to others.
(4) Suppressing HIV replication in the brain, by reducing DAergic tone, could improve the probability of successfully treating drug addiction.
(5) Suppressing synaptic dopamine concentration pharmacologically might be useful to prevent HIV-induced overloading of DAergic synapses in some circuits.

These new neurochemical findings are comparable in some aspects with previously reported results using PET scanning. Striatal D_2R binding availability was decreased in 10 people with HAD (Wang et al. 2004), but to a lesser extent than the decreased D_2L concentration in HIVE. A potential reason for the difference of intensity is that [^{11}C] raclopride binding, as measured in PET, is not specific to D_2L. Raclopride binds to D_2L, D_2S, and D_3R. Based on our biochemical quantification of all three of these binding partners, raclopride binding in HIVE would reflect a mixture of *increased* D_3R binding, sharply decreased D_2L binding, and modestly decreased D_2S binding. The com-

peting effect of increased D_3R is one scenario that explains why decreased PET tracer binding is not as pronounced as the decrease in D_2L concentration. PET scanning has also suggested that striatal presynaptic neurons are perturbed in HAD, i.e., binding availability of the presynaptic DAergic marker DAT was decreased (Wang et al. 2004). In seeming contrast, DAT protein was sharply increased in HIVE in our panel of changes. Experimentally, it is known that DAT binding availability is strongly influenced by factors other than the concentration of DAT protein or mRNA (Wilson et al. 1996). For example, DAT binding is sensitive to changes in the concentration of dopamine in the synaptic cleft, which competes with exogenous [^{11}C]cocaine PET tracer molecules and limits available DAT binding sites (Gatley et al. 1995, 1997). Thus, an increase in the concentration of synaptic dopamine in HIVE (i.e., increased DAergic tone) could effectively compete with PET tracer molecules and sharply decrease the number of available binding sites. Another potential scenario is that the increased DAT reflects redistribution due to increased membrane internalization and trafficking of the protein, which decreases PET tracer binding due to a lack of access to the extracellular compartment (Melikian 2004; Zahniser and Doolen 2001). And finally, ligand affinity of DAT protein is sensitive to posttranslational modifications that include oligomerization and phosphorylation (Wilson et al. 1996). In sum, the interrelationship between DAT protein, its distribution within in the synapse, its conformational variants, and its binding affinities is very complicated. When comparing PET results to these postmortem protein measurements, it also is important to recognize that the two patient populations were not equivalent: PET was performed on people who were clinically classified to have HAD with unknown neuropathology (Wang et al. 2004), whereas DAT was measured in decedents with a confirmed neuropathological diagnosis of HIVE. HIVE is the neuropathological substrate of HAD in the majority of cases (Wiley and Achim 1994).

We conclude that biochemical measurements in autopsy brain specimens show that striatal DAergic synapses are abnormal in NNTC subjects with HIV encephalitis. Based on correlative changes that are described in the experimental literature, the overall pattern of abnormality in HIVE probably reflects adaptation to increased DAergic tone. More extensive multidisciplinary and translational studies are needed to determine whether abnormal synapses reflect altered physiological function of specific striatal DAergic circuits in the manner that we have suggested. The influence of HIV/AIDS on DAergic systems is ripe for a critical reappraisal.

Acknowledgements We thank Joshua G. Lisinicchia and Alyson R. Clayborn for technical assistance, and Jean Richey, RN, and the excellent nursing program staff of the Texas NeuroAIDS Research Center. Mr. Steve Schuenke assisted with illustration. This research was supported by the National Institutes of Health, R24 NS45491 and R01 MH69200.

References

Baldwin GC, Roth MD, Tashkin DP (1998) Acute and chronic effects of cocaine on the immune system and the possible link to AIDS. J Neuroimmunol 83:133–138

Berger JR, Nath A (1997) HIV dementia and the basal ganglia. Intervirology 40:122–131

Berger JR, Arendt G (2000) HIV dementia: the role of the basal ganglia and dopaminergic systems. J Psychopharmacol 14:214–221

Bezard E, Devero S, Prunier C, Ravenscroft P, Chalon S, Guilloteau D, Crossman AR, Bioulac B, Brotchie JM, Gross CE (2001) Relationship between the appearance of symptoms and the level of nigrostriatial degeneration in a progressive MPTP-lesioned macaque model of Parkinson's disease. J Neurosci 21:6853–6861

Blanchard V, Raisman-Vozari R, Vyas S, Michel PP, Javoy-Agid F, Uhl G, Agid Y (1994) Differential expression of tyrosine hydroxylase and membrane dopamine transporter genes in subpopulations of dopaminergic neurons of the rat mesencephalon. Brain Res Mol Brain Res 22:29–38

Budka H (1991) Neuropathology of human immunodeficiency virus infection. Brain Pathol 1:163–175

Burt DR, Creese I, Snyder SH (1977) Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196:326–328

Centonze D, Usiello A, Gubellini P, Pisani A, Borrelli E, Bernardi G, Calabresi P (2002) Dopamine D_2 receptor-mediated inhibition of dopaminergic neurons in mice lacking D_2L receptors. Neuropsychopharmacology 27:723–726

Chen L, Segal DM, Moraes CT, Mash DC (1999) Dopamine transporter mRNA in autopsy studies of chronic cocaine users. Brain Res Mol Brain Res 73:181–185

Chiasson MA, Stoneburner RL, Hildebrandt DS, Ewing WE, Telzak EE, Jaffe HW (1991) Heterosexual transmission of HIV-1 associated with the use of smokable freebase cocaine (crack). AIDS 5:1121–1126

Ciliax BJ, Heilman C, Demchyshyn LL, Pristupa ZB, Ince E, Hersch SM, Niznik HB, Levey AI (1995) The dopamine transporter: immunochemical characterization and localization in brain. J Neurosci 15:1714–1723

Creese I, Snyder SH (1979) Nigrostriatal lesions enhance striatal [^{3}H]apomorphine and [^{3}H]spiroperidol binding. Eur J Pharmacol 56:277–281

Czub S, Koutsilieri E, Soppers S, Czub M, Stahl-Hennig C, Müller JG, Pedersen V, Gsell W, Heeney JL, Gerlach M, Gosztonyi G, Riederer P, Ter Meulen V (2001) Enhancement of central nervous system pathology in early simian immunodeficiency virus infection by dopaminergic drugs. Acta Neuropathol 101:85–91

Dal Toso R, Sommer B, Ewert M, Herb A, Prichett D, Bach A, Shivers B, Seeburg PH (1989) The dopamine D_2 receptor: two molecular forms generated by alternative splicing. EMBO J 8:4025–4034

Dickinson SD, Sabeti J, Larson GA, Giardina K, Rubinstein M, Kelly MA, Grandy DK, Low MJ, Gerhardt GA, Zahniser NR (1999) Dopamine D_2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. J Neurochem 72:148–156

Esch T, Stefano GB (2004) The neurobiology of pleasure, reward

processes, addiction and their health implications. Neuroendocrinology Lett 25:235 – 251

Fossom LH, Sterling CR, Tank AW (1992) Regulation of tyrosine hydroxylase gene transcription rate and tyrosine hydroxylase mRNA stability by cyclic AMP and glucocorticoid. Mol Pharmacol 42:908 – 989

Gatley SJ, Volkow ND, Fowler JS, Dewey SL, Logan J (1995) Sensitivity of striatal [^{11}C]cocaine binding to decreases in synaptic dopamine. Synapse 20:137–144

Gatley SJ, Volkow ND, Gifford AN, Ding YS, Logan J, Wang GJ (1997) Model for estimating dopamine transporter occupancy and subsequent increases in synaptic dopamine using positron emission tomography and carbon-11-labeled cocaine. Biochem Pharmacol 53:43 – 52

Gelman BB, Soukup VM, Keherly MJ, Holzer CE, Richey FJ, Lahart CJ (2004) Acquired neuronal channelopathies in HIV-associated dementia. J Neuroimmunol 157:111–119

Gelman BB, Soukup VM, Holzer CE III, Fabian RH, Schuenke KW, Keherly MJ, Richey FJ, Lahart CJ (2005) Lysosome expansion in white matter: potential correlate of altered membrane turnover in HIV-associated dementia. J AIDS 39:422 – 425

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 253:1429 – 1432

Grassi MP, Perin C, Clerici F, Zocchetti C, Borella M, Cargnel A, Mangoni A (1997) Effects of HIV seropositivity and drug abuse on cognitive function. Eur Neurol 37:48 – 52

Haavik J, Schellilng D, Campbell DG, Andersson KK, Flatmark T, Cohen P (1989) Identification of protein phosphatase 2A as the major tyrosine hydroxylase phophatase in adrenal medulla and corpus striatum: evidence from the effects of okadaic acid. FEBS Lett 251:36 – 42

Harrington KA, Augwood SJ, Kingsbury AE, Foster OJ, Emson PC (1996) Dopamine transporter (DAT) and synaptic vesicle amine transporter (VMAT2) gene expression in the substantia nigra of control and Parkinson's disease. Brain Res Mol Brain Res 36:157–162

Heaton RK, Grant I, Butters N, White DA, Kirson D, Atkinson JH, McCutchan JA, Taylor M, Kelly MD, Ellis RJ, Wolfson T, Velin R, Marcotte TD, Hesselink JR, Jernigan TL, Chandler J, Wallace M, Abramson I, The HNRC Group (1995) The HNRC 500—neuropsychology of HIV infection at different disease stages. HIV Neurobehavioral Research Center. J Int Neuropsychol Soc 1:231–251

Herrero MT, Augood SJ, Asensi H, Agid Y, Obeso JA, Emson PC (1996) Effects of L-DOPA-therapy on dopamine D2 receptor mRNA expression in the striatum of MPTP-intoxicated parkinsonian monkeys. Brain Res Mol Brain Res 42:149 –155

Hriso E, Kuhn T, Masdeu JC, Grundman M (1991) Extrapyramidal symptoms due to dopamine-blocking agents in patients with AIDS encephalopathy. Am J Psychiatry 148:1558–1561

Joyce JN, Smutzer G, Whitty CJ, Myers A, Bannon M (1997) Differential modification of dopamine transporter and tyrosine hydroxylase mRNAs in midbrain of subjects with Parkinson's, Alzheimer's with parkinsonism, and Alzheimer's Disease. Mov Disord 12:885 – 897

Kandel ER, Schwartz JH, Jessell TM (1991) Disorders of mood, depression, mania, and anxiety disorders. In Principles of Neuroscience, 3rd edition. Appleton and Lange, Norwalk, CT, pp 869 – 886

Kansy JW, Daubner SC, Nishi A, Sotogaku N, Lloyd MD, Nguyen C, Lu L, Haycock JW, Hope BT, Fitzpatrick PF, Bibb JA (2004) Identification of tyrosine hydroxylase as a physiological substrate for Cdk5. J Neurochem 91:374 – 384

Khan ZU, Mrzljak L, Gutierrez A, DeLa Calle A, Goldman-Rakic PS (1998) Prominence of the dopamine D2 short isoform in dopaminergic pathways. Proc Natl Acad Sci U S A 95:7731–7736

Kimmel HL, Joyce AR, Carroll FI, Kuhar MJ (2001) Dopamine D1 and D2 receptors influence dopamine transporter synthesis and degradation in the rat. J Pharmacol Exp Ther 298:129 –140

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52–58

Koutsilieri E, Sopper S, Scheller C, ter Meulen V, Riederer P (2002) Involvement of dopamine in the progression of AIDS Dementia Complex. J Neural Transm 109:399 – 410

Le Moal M, Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 71: 155 – 234

Lewis EJ, Harrington CA, Chikaraishi DM (1987) Transcriptional regulation of the tyrosine hydroxylase gene by glucocorticoid and cyclic AMP. Proc Natl Acad Sci U S A 84:3550 – 3554

Lindgren N, Xu ZQ, Lindskog M, Herrera-Marschitz M, Goiny M, Haycock J, Goldstein M, Hokfelt T, Fisone G (2000) Regulation of tyrosine hydroxylase activity and phosphorylation at Ser(19) and Ser(40) via activation of glutamate NMDA receptors in rat striatum. J Neurochem 74:2470 – 2477

Lindgren N, Xu ZQ, Herrera-Marschitz M, Haycock J, Hokfelt T, Fisone G (2001) Dopamine D(2) receptors regulate tyrosine hydroxylase activity and phosphorylation at Ser40 in rat striatum. Eur J Neurosci 13:773 – 780

Little KY, McLaughlin DP, Zhang L, McFinton PR, Dalack GW, Cook EH Jr, Cassin BJ, Watson SJ (1998) Brain dopamine transporter mRNA and binding sites in cocaine users: a post mortem study. Arch Gen Psychiatry 55:793 – 799

Little KY, Zhang L, Desmond T, Frey KA, Dalack GW, Cassin BJ (1999) Striatal dopaminergic abnormalities in human cocaine users. Am J Psychiatry 156:238 – 245

Lopez OL, Smith G, Meltzer CC, Becker JT (1999) Dopamine systems in human immunodeficiency virus-associated dementia. Neuropsych Neuropsychol Behav Neurol 12:184 –192

Marcario JF, Manaye KF, Santa Cruz KS, Mouton PR, Berman NE, Cheney PD (2004) Severe subcortical degeneration in macaques infected with neurovirulent simian immunodeficiency virus. J Neurovirol 10:387–399

Mash DC, Staley JK (1999) D3 dopamine and kappa opioid receptor alterations in human brain of cocaine-overdose victims. Ann N Y Acad Sci 877:507–522

Mayfield RD, Zahniser NR (2001) Dopamine 2 receptor regulation of the dopamine transporter expressed in *Xenopus laevis* oocytes is voltage-dependent. Mol Pharmacol 59:113 –121

Melikian HE (2004) Neurotransmitter transporter trafficking: endocytosis, recycling and regulation. Pharmacol Ther 104:17−27

Meller E, Bohmaker K, Namba Y, Friedhoff AJ, Goldsteint M (1987) Relationship between receptor occupancy and response at striatal dopamine autoreceptors. Mol Pharmacol 31:592 – 598

Mirsattari SM, Power C, Nath A (1998) Parkinsonism with HIV dementia. Mov Disord 13:684 – 689

Morgello S, Gelman BB, Kozlowski PB, Vinters HV, Masliah E, Cornford M, Cavert W, Marra C, Grant I, Singer E (2001) The national neuroAIDS tissue consortium. Neuropathol Appl Neurobiol 27:326 –335

Nader MA, Czoty PW (2005) PET Imaging of dopamine D_2 receptors in monkey models of cocaine abuse: genetic predisposition versus environmental modulation. Am J Psychiatry 162:1473 – 1482

Nath A, Hauser KF, Wojna V, Booze RM, Maragos W, Prendergast M, Cass W, Turchan JT (2002) Molecular basis for interactions of HIV and drugs of abuse. J AIDS 31(Suppl 2):S62 – S69

Quik M, Police S, He L, DiMonte DA, Langston JW (2000) Expression of D(3) receptor messenger RNA and binding sites in monkey striatum and substantia nigra after nigrostriatal

degeneration: effect of levodopa treatment. Neuroscience 98: 263 – 273
Reyes MG, Faraldi F, Senseng CS, Flowers C, Fariello R (1991) Nigral degeneration in acquired immune deficiency syndrome (AIDS). Acta Neuropathol (Berl) 82:39 – 44
Robbins TW (2003) Dopamine and cognition. Curr Opin Neurol 16 (suppl 2):S1– S2
Roth MD, Tashkin DP, Choi R, Jamieson BD, Zack JA, Baldwin GC (2002) Cocaine enhances human immunodeficiency virus replication in a model of severe combined immunodeficient mice implanted with human peripheral blood leukocytes. J Infect Dis 185:701–705
Rudnick G, Clark J (1993) From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. Biochim Biophys Acta 1144:249 – 263
Seeman P (1980) Brain dopamine receptors. Pharmacol Rev 32:229 –313
Segal DM, Moraes CT, Mash DC (1997) Up-regulation of D3 dopamine receptor mRNA in the nucleus accumbens of human cocaine fatalities. Brain Res Mol Brain Res 45:335 – 339
Staley JK, Hearn L, Ruttenber AJ, Wetli CV, Mash DC (1994) High affinity cocaine recognition sites on the dopamine transporter are elevated in fatal cocaine overdose victims. J Pharmacol Exp Ther 271:1678 – 1685
Staley JK, Mash DC (1996) Adaptive increase in D_3 dopamine receptors in the brain reward circuits of human cocaine fatalities. J Neurosci 16:6100 – 6106
Tank AW, Curella P, Ham L (1986a) Induction of mRNA for tyrosine hydroxylase by cyclic AMP and glucocorticoids in a rat pheochromocytoma cell line: evidence for the regulation of tyrosine hydroxylase synthesis by multiple mechanisms in cells exposed to elevated levels of both inducing agents. Mol Pharmacol 30: 497 – 503
Tank AW, Ham L, Curella P (1986b) Induction of tyrosine hydroxylase by cyclic AMP and glucocorticoids in a rat pheochromocytoma cell line: effect of the inducing agents alone or in combination on the enzyme levels and rate of synthesis of tyrosine hydroxylase. Mol Pharmacol 30:486 – 496
Thobois S, Vingerhoets F, Fraix V, Xie-Brustolin J, Mollion H, Costes N, Mertens P, Benabid AL, Pollak P, Broussolle E (2004) Role of dopaminergic treatment in dopamine receptor down-regulation in advanced Parkinson disease. A positron emission tomographic study. Arch Neurol 61:1705 –1709
Uhl GR, Walther D, Mash D, Faucheux B, Javoy-Agid F (1994) Dopamine transporter messenger RNA in Parkinson's disease and control substantia nigra. Ann Neurol 35:494 – 498
Volkow ND, Fowler JS, Wang GJ, Swanson JM (2004) Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Mol Psychiatry 9:557– 569
Volkow ND, Wang GJ, Fowler JS, Ding Y-S (2005) Imaging the effects of methylphenidate on brain dopamine: new model on its therapeutic actions for attention-deficit/hyperactivity disorder. Biol Psychiatry 57:1410 –1415
Wade TV, Rothblat DS, Schneider JS (2001) Changes in striatal dopamine D3 receptor regulation during expression of and recovery from MPTP-induced parkinsonism. Brain Res 905:111–119
Wang GJ, Chang L, Volkow ND, Telang F, Logan J, Ernst T, Fowler JS (2004) Decreased brain dopaminergic transporters in HIV-associated dementia patients. Brain 127:2452–2458
White FJ, Kalivas PW (1998) Neuroadaptations involved in amphetamine and cocaine addiction. Drug Alcohol Depend 51:141–153
Wiley CA, Achim CL (1994) HIV encephalitis is the pathologic correlate of dementia in AIDS. Ann Neurol 36:673 – 676
Wilson JM, Levey AI, Bergeron C, Kalasinsky K, Ang L, Peretti F, Adams VI, Smialek J, Anderson WR, Shannak K, Deck J, Niznik HB, Kish SJ (1996) Striatal dopamine, dopamine transporter, and vesicular monoamine transporter in chronic cocaine users. Ann Neurol 40:428 – 439
Wolf ME, Roth RH (1990) Autoreceptor regulation of dopamine synthesis. Ann N Y Acad Sci 604:323 – 343
Woods SP, Rippeth JD, Frol AB, Levy JK, Ryan E, Soukup VM, Hinkin CH, Lazzaretto D, Cherner M, Marcotte TD, Gelman BB, Morgello S, Singer EJ, Grant, I, Heaton RK (2004) Interrater reliability of clinical ratings and neurocognitive diagnoses in HIV. J Clin Exp Neuropsychol 26:759 – 778
Zahniser NR, Doolen S (2001) Chronic and acute regulation of Na+/Cl-dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. Pharmacol Ther 92:21– 55