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Differential involvement of p38 and JNK MAP kinases in HIV-1 Tat and gp120-induced apoptosis and neurite degeneration in striatal neurons

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Abstract

The role of p38 and c-jun-N-terminal kinases 1/2 (JNK), members of the mitogen-activated protein kinase (MAPK) family, in mediating the toxic effects of HIV-1 Tat and gp120 were explored in primary mouse striatal neurons in vitro. Both Tat and gp120 caused significant increases in p38 and JNK MAPK phosphorylation, caspase-3 activity, neurite losses and cell death in striatal neurons. Tat-induced increases in caspase-3 activity were significantly attenuated by an inhibitor of JNK (SP600125), but not by an inhibitor of p38 (SB203580), MAPK. However, despite preventing increases in caspase-3 activity, JNK inhibition failed to avert Tat-induced neuronal losses suggesting that the reductions in caspase-3 activity were insufficient to prevent cell death caused by Tat. Alternatively, gp120-induced increases in caspase-3 activity, neurite losses and neuronal death were prevented by p38, but not JNK, MAPK inhibition. Our findings suggest that gp120 induces neuronal dysfunction and death through actions at p38 MAPK, while Tat kills neurons through actions that are independent of p38 or JNK MAPK, or through the concurrent activation of multiple proapoptotic pathways.

Keywords

Caspase-3; neurotoxicity; AIDS; neurodegeneration; basal ganglia

INTRODUCTION

Mitogen-activated protein kinase (MAPK) signaling transduces a wide spectrum of cellular processes including cell growth, differentiation, and apoptosis. MAPKs include extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, which have

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been linked with a diverse range of biological activities including regulation of programmed cell death (Ono and Han, 2000). Activation of ERKs typically contribute to cell differentiation, proliferation and survival (Ono and Han, 2000); whereas JNK and p38 MAPK, in contrast, are activated by pro-inflammatory cytokines and environmental stress, and can promote apoptosis (Davis, 2000). All three MAPK pathways can be activated independently, and their involvement in apoptosis differs among cell types, context, and the particular experimental paradigm used.

Our understanding of the specific cell surface receptors and effectors involved in Tat and gp120-mediated cytotoxicity is incomplete. Both viral protein toxins can activate JNK and/or p38 MAPKs in non-neural cells (Kumar et al., 1998; Mischiati et al., 1999; Gu et al., 2001; Bodner et al., 2002; Kapasi et al., 2002; Barber et al., 2004), as well as in macrophages/microglia (Kaul and Lipton, 1999; D'Aversa et al., 2004), although in some cell types gp120 toxicity occurs independently of p38 and JNK MAPK activation (Biard-Piechaczyk et al., 2000). HIV or simian immunodeficiency virus (SIV) exposed microglia and astroglia can express JNK or p38 MAPK (Barber et al., 2004). Inhibition of p38 or JNK MAPK in microglia prevents the activation of inducible nitric oxide synthase and limits the production of toxic byproducts, including nitric oxide and tumor necrosis factor- α , among others (Bhat et al., 1998; Si et al., 2004; Yi et al., 2004). For example, attenuation of p38 MAPK activity prevented gp120-induced hippocampal neuron death; however, this only occurred if activated macrophages/microglia were present suggesting that p38 MAPK expression by macrophages/microglia indirectly regulates neurotoxicity (Kaul and Lipton, 1999).

Tat and gp120 are intrinsically toxic to neurons (Nath, 1999; Petito et al., 1999; Kaul et al., 2001; Song et al., 2003). Recent studies suggest that the neurotoxicity caused by both viral proteins may be regulated by MAPKs. Gp120-induced increases in JNK phosphorylation, as well as intracellular Ca^{2+} levels and ERK phosphorylation, in rat hippocampal neurons (Khan et al., 2004); however, the role of JNK in survival was not assessed. In another study, alterations in JNK through manipulation of its upstream kinase mixed-lineage kinase-3 (MLK3) prevented gp120-CXCR4 triggered death in cultured rat hippocampal neurons (Bodner et al., 2002). Importantly, gp120-CXCR4 interactions that lead to apoptosis may differ among cell types (Bodner et al., 2003). Inhibition of p38 MAPK reduces gp120 toxicity in human neurons (Hu et al., 2005). Extracellular Tat likely causes death in striatal neurons through multiple apoptotic pathways (Singh et al., 2004), which may differ among different cell and neuronal types.

The neurotoxic effects of HIV-1 proteins were explored in striatal neurons because the striatum is a major site of infection (Achim et al., 1994; Berger and Nath, 1997) and sustain highest viral loads in the CNS (Wiley et al., 1998; Avison et al., 2003). HIV dementia has been described as a subcortical dementia (Berger and Nath, 1997), aspects of which can include features of parkinsonism (Mirsattari et al., 1998) and dopamine dysfunction (Nath et al., 2000b). Moreover, striatal neurons are unique in their response to neurotoxic insults (Miller et al., 1999; Mitchell et al., 1999; Calabresi et al., 2000; Whetsell, Jr., 2002; Goody et al., 2003). Marginal neuronal losses are reported in the putamen of non-demented individuals with AIDS at autopsy (Everall et al., 1995), while more dramatic neuronal losses

occur in the globus pallidus of nonhuman primates infected with SIV (Marcario et al., 2004). Neuroimaging shows structural and/or neurochemical defects within the basal ganglia of patients with HIV (Avison et al., 2004; Yiannoutsos et al., 2004; Sporer et al., 2005), aspects of which may be reversible (e.g., see Filippi et al., 1998; Avison et al., 2004), suggesting the therapeutic importance of understanding basic signaling events underlying neurodegeneration in the basal ganglia.

The high susceptibility to the striatum to HIV and an incomplete understanding of the role of p38 and JNK MAPK in mediating HIV-1 neurotoxicity, combined with the unique response profile of striatal neurons to excitotoxic insults, prompted us to explore Tat and gp120 toxicity in highly-enriched, striatal neuron populations *in vitro*. The results reported herein suggest that Tat and gp120 are intrinsically lethal to striatal neurons through partially overlapping but fundamentally different apoptotic signaling events. Importantly, p38 MAPK activation alone mediated gp120-induced neurite dystrophy and cell death in striatal neurons, while neither p38 nor JNK inhibition was sufficient to prevent Tat-induced neurite losses or neuronal death.

EXPERIMENTAL PROCEDURES

Isolation and treatment of striatal neuron cultures

Highly-enriched, striatal neuronal cultures from embryonic day 14 (E14) ICR mice were prepared in accordance with NIH Guidelines and following approval by the local IACUC as previously described (Goody et al., 2003; Singh et al., 2003; Singh et al., 2004), which includes efforts to use the minimum number of mice possible and any potential discomfort. Fetal striata were dissected and striatal cells isolated and grown for 6 to 8 days *in vitro* (DIV) prior to assay in serum-free DMEM/F-12 medium supplemented with 2% B-27, 1 μ g/ml linoleic acid, 25- μ g/ml insulin, and 1% antibiotic/antimycotic. Striatal neurons were seeded in identical densities (4×10^5 cells/ well) and grown on poly-D-lysine (0.1 mg/ml) coated Cellocate coverslips (Eppendorf Inc., Westbury, NY) inserted into 24-well plates at 35 °C in 5% CO₂ / 95% air. Recombinant Tat (Tat₁₋₇₂ or a non-toxic deletion mutant Tat₃₁₋₆₁) isolates from HIV-1_{BRU} were prepared as previously described (Ma and Nath, 1997). Recombinant gp120_{IIIB} (Protein Sciences Corporation, Meriden, CT) or denatured control gp120_{IIIB} (boiled for 1 h) were assessed. After 6–8 days *in vitro*, neuron-enriched cultures of mouse striata were continuously exposed for 0–72 h with HIV-1 Tat (100 nM) or gp120 (500 pM) applied extracellularly. Striatal cultures consist of ~97% neurons, and most of these are spiny projection neurons (medium spiny neurons) (Gerfen and Wilson, 1996), as determined using previously described morphological and immunocytochemical criteria (Goody et al., 2003; Singh et al., 2004). To inhibit p38 and JNK1/2 MAPKs, respectively, cells were pretreated for 60 min with SB203580 (10 μ M; Tocris Cookson, Inc.; Ellisville, MO) or SP600125 (10 μ M; Biosource International, Camarillo, CA).

Neuron viability

Neuron viability was assessed by repeatedly photographing the same neurons at 24 h intervals before/after experimental treatments using an inverted microscope with phase contrast optics and 40 $\times$ objective as previously described (Singh et al., 2004). Neuron death

was defined by rigorous criteria, including dissolution of Nissl substance, cytoplasmic swelling and vacuolization, and eventual fragmentation of the cell body (Singh et al., 2004).

Neurite length

Neurite length was estimated as part of the neuron viability studies using a modification of a procedure described by Sholl (1953). A calibrated template consisting of concentric circles was overlaid and centered over neuronal cell bodies. The number of intersections between circles and neurites was recorded as an estimate of neurite length using phase-contrast microscopy and a 40× objective as previously described (Hauser et al., 2000). Cultured neurons were systematically, but arbitrarily sampled as previously described (Hauser et al., 2000). Neurons with overlapping neurites or neurites that could not be unambiguously associated with a single cell body of origin were excluded from the sample.

Caspase-3 activity and immunoblotting

Caspase-3 activity was measured and Western immunoblotting performed as previously described (Singh et al., 2003; Singh et al., 2004). Because, either p38 or JNK MAPKs may be tonically active in unstimulated neurons in the present study, relative changes in phosphorylation levels in viral protein- versus vehicle-treated controls were reported.

Statistical analysis

Statistical comparisons were made using one-way and repeated measures ANOVA and *post hoc* comparisons performed using Duncan's test (Statistica, StatSoft, Tulsa, OK). In the case of the measurements of neurite length in the Tat-treated neurons and some of the viability experiments, the variances were non-homogeneous and Kruskal-Wallis nonparametric ANOVA was performed at 24, 48, and 72 h. Experiments consisted of independent samples of cells pooled from separate mice. Data are presented as the mean $\pm$ the SEM.

RESULTS

HIV-1 Tat and gp120 exposure cause striatal neuronal losses in vitro

Both Tat and gp120 were significantly toxic to striatal neurons at 24 h (Fig. 1A) or 48 h (data not shown) following exposure as previously reported (Gurwell et al., 2001; Singh et al., 2004). The toxic actions of both virotoxins were highly selective and toxicity was not observed when nontoxic, mutant Tat (Tat₃₁₋₆₁) or denatured gp120 (20 min boiling) were used (Fig. 1A).

HIV-1 Tat and gp120 increase p38 and JNK MAPK phosphorylation in striatal neurons

Both HIV-1 Tat (100 nM) and gp120 (500 pM) variably increased the phosphorylation of JNK and/or p38 MAPKs at 4 h (Figs. 1B–E; 2A–D). The concentrations of Tat and gp120 used consistently induce apoptosis in striatal neurons (Singh et al., 2004). Gp120 caused a more rapid phosphorylation of p38 MAPK within 5 min compared to Tat, which did not show significant increases until 15 min following exposure (Fig. 2A–B). Moreover, unlike Tat exposure, which caused sustained elevations in p38 MAPK, gp120 induced biphasic increases in p38 phosphorylation characterized by a sustained initial increase (5 min to 1 h)

followed by a decline to near pretreatment levels at 2 h and subsequent increase at 4 h (Fig. 2B).

Marked elevations in JNK phosphorylation were noted within 15 min of exposure to either Tat or gp120 (Fig. 2C–D). However, gp120 elicited robust increases in JNK1/2 phosphorylation (Fig. 2D), while Tat caused slight (~1.4-fold) albeit significant increases in JNK1 phosphorylation and marginal increases in JNK2 phosphorylation at 15 min and 4 h (Fig. 2C). In general, Tat-induced increases in JNK were modest and quite variable. Although some temporal differences in the pattern of phosphorylation were recorded, it is important to note that either Tat or gp120 alone appeared to increase the activity of both MAPKs to varying degrees.

HIV-1 Tat and gp120-induced caspase-3 activation and neuronal death are differentially regulated by p38 and JNK MAPK inhibitors

To determine whether activation of JNK or p38 MAPKs mediate Tat or gp120-induced neurodegeneration and explore possible involvement of caspase-3, striatal neuron cultures were pretreated with SB203580 or SP600125 for 60 min prior to Tat (100 nM) or gp120 (500 pM) exposure. Neuron viability and neurite length were assessed using time-lapse photomicroscopy (Fig. 3). Caspase-3 was measured enzymatically (Fig. 4A,B). Interestingly, inhibition of p38 MAPK (SB203580, 10 μ M) completely prevented gp120-induced caspase-3 activation (Fig. 4A), but had no effect on caspase-3 activation caused by Tat (Fig. 4B). By contrast, inhibiting JNK (SP600125, 10 μ M) significantly attenuated Tat, but not gp120, induced increases in caspase-3 activity ($P < 0.05$) (Fig. 4B). This indicates that Tat and gp120 increase caspase-3 activity via independent upstream signals involving JNK and p38 MAPKs, respectively.

To assess the role of each MAPK in Tat or gp120-induced neuronal death, striatal neurons were pretreated with each inhibitor as described above. Interestingly, neither JNK (SP600125, 10 μ M) nor p38 (SB203580, 10 μ M) MAPK inhibition was able to prevent Tat-induced neuronal losses (Fig. 4C). Alternatively, as might be predicted based on caspase-3 activity, p38 MAPK inhibition completely prevented gp120-induced neuronal death (Fig. 4D). Therefore, despite the fact that JNK inhibition significantly reduced caspase-3 levels, Tat was nevertheless able to kill neurons via JNK- and caspase-3 independent mechanisms.

Tat and gp120-induced neurite losses

Tat and gp120 caused significant neurite losses compared to vehicle-treated controls at most times that were assessed (Kruskal-Wallis ANOVA) (Fig. 5A). Although the inhibition of p38 MAPK (SB203580) enhanced the variability among individual neurons in response to Tat, neurite length did not differ from control or Tat exposed neurons (Fig. 5A). On the other hand, gp120 significantly reduced neurite length and this could be prevented by p38 MAPK inhibition (Fig. 5B). JNK inhibition (SP600125) did not prevent Tat or gp120 alterations in neurites (Fig. 5A–B). The findings support the notion that gp120 acts through p38 MAPK to cause neuritic pruning, while Tat acts via alternate (non-p38 MAPK) or multiple pathways.

DISCUSSION

The present study shows that Tat and gp120 increase p38 and JNK MAPK phosphorylation in primary striatal neurons and this likely contributes to HIV-1-induced neurotoxicity. Although there are some reports that Tat can activate p38 MAPK in non-neuronal cells, few studies have examined the direct effects of either viral protein on p38 or JNK MAPK signaling in neurons. Recently, p38 MAPK was found to mediate gp120-induced apoptosis in primary human neurons (Hu et al., 2005). We previously found that exposure to Tat or gp120 increased caspase-3 activity and apoptosis in striatal neurons (Singh et al., 2004). However, caspase-3 inhibition only blocked gp120-, but not Tat-, induced neuronal losses, and Tat alone additionally activated endonuclease-G (endo-G) suggesting that Tat acted via both caspase-dependent and caspase-independent pathways (Singh et al., 2004). These earlier findings prompted us to explore potential differences in proapoptotic signaling between Tat and gp120. In particular, this included an examination of unique Tat-driven signaling events that could potentially trigger apoptosis independent of caspase-3. Moreover, viral protein-induced activation of p38 and JNK MAPK, and effector caspases, occurs within minutes to hours, and likely precedes neurite losses and cell death by hours or days (Singh et al., 2004; Mattson et al., 2005). For this reason, we studied the sequential phosphorylation of p38 and JNK MAPK at 0–4 h, caspase-3 activity at 4 h, and cellular changes at 24 h intervals. The present results show that there are indeed distinct differences in the events preceding striatal neuron death, as well as those mediating death itself.

Our findings agree with previous studies in non-neuronal cells indicating that Tat and gp120 can contribute to different aspects of HIV disease progression through the activation/inactivation of several MAPKs, including JNK and p38 (Kumar et al., 1998; Mischiati et al., 1999; Kan et al., 2000; Kutsch et al., 2000; Liu et al., 2002). However, unlike earlier studies that have assessed individual viral proteins and MAPKs separately in neurons (Lannuzel et al., 1997; Bodner et al., 2002; Kolson, 2002), we find that JNK and p38 MAPKs are concurrently activated and may act in a coordinated manner to mediate HIV-induced neurotoxicity. In addition, we find that the inhibition of JNK MAPK does not prevent gp120-induced striatal neuron death. This is contrary to a report that JNK mediates gp120-induced death of rat hippocampal neurons (Bodner et al., 2002), which regulated JNK activity by manipulating mixed-lineage kinase 3 (MLK3) (Bodner et al., 2002). Although the reason for the discrepancy is uncertain, especially since both studies used gp120_{IIIIB} derived from the same CXCR4-tropic viral strain, the divergence might result from inherent differences between striatal neurons and other neuron types as noted earlier. Another explanation is that MLK3 has also been recently reported to modulate p38 MAPK activity (in addition to JNK) (Kim et al., 2004), suggesting that p38 MAPK may have also been involved in gp120-induced hippocampal neuron losses. Lastly, although our striatal cultures are highly enriched in neurons (Singh et al., 2004), with negligible microglia (~0.14%) or astroglia (~1%), we cannot exclude the possibility that contaminating glia might mediate some aspects of Tat or gp120 neurotoxicity (Vesce et al., 1997; El-Hage et al., 2005). Studies in progress are specifically addressing this issue.

The unique temporal patterns of p38 and JNK MAPK phosphorylation seen with each viral protein may convey differing information to downstream apoptotic effectors. For example,

sustained activation of JNK can uncouple the neuroprotective effects of ERK from its upstream activator MEK, which differs from acute activation (Shen et al., 2003). Importantly, p38 MAPK inhibition prevented gp120-induced caspase-3 activation and cell death, while JNK inhibition had no effect on gp120 neurotoxicity, indicating that p38 MAPK activation is necessary for gp120 mediated neuronal death. By contrast, despite the ability to induce caspase-3 via JNK, JNK inhibition failed to prevent Tat-induced increases in neuronal death, suggesting that Tat can act independently of p38 or JNK MAPKs and caspase-3 to induce neuronal death. Tat has been suggested to induce apoptosis via a caspase-dependent, as well as a caspase-independent pathway involving endo-G, while gp120 may act exclusively via a caspase-dependent cascade (Singh et al., 2004). Assuming Tat activates both caspase-dependent and independent pathways concurrently, then the blockade of JNK-induced increases in caspase-3 would likely fail to rescue dying neurons as was observed in the present study.

Signals originating at synapses can trigger or prevent apoptotic death of the neuronal cell body, and synaptic losses may occur independent of death of the entire neuron (Mattson and Duan, 1999; Mattson, 2000). Thus, we predicted that p38 and JNK MAPK might contribute to neurite losses without causing overt cell death. Gp120, in particular, induced neurite losses that were prevented by p38 MAPK inhibition; however, the neurite losses coincided temporally with destruction of the neuronal cell body. The same findings were observed following Tat exposure. Therefore, our findings offered few clues as to whether neurite reductions contributed to cell death or vice versa, but did show that p38 MAPK is involved in both gp120-induced neurite losses and cell death. Lastly, despite modest increases in neurite length in some neurons in controls at 24 h suggesting some growth (e.g., Fig. 5A), mean neurite lengths declined markedly from pretreatment values with Tat or gp120 exposure. This suggested that reductions were due to pruning of existing neurites rather than growth inhibition. This is an important distinction, since both JNK and p38 MAPK are reported to influence the outgrowth of new neurites (Campbell and Holt, 2003; Eom et al., 2004), in addition to promoting both degenerative or regenerative processes (Herdegen and Waetzig, 2001; Hashimoto and Masliah, 2003).

Although JNK and p38 MAPKs can act independently through activator protein-1 (AP-1) and ATF-2, respectively, these MAPKs may also interact via direct protein-protein interactions and/or by co-activating separate but related transcription factors. Recent evidence suggests that p38 and JNK MAPKs can act in a coordinated manner when mediating excitotoxic effects in cerebellar granule neurons (Chen et al., 2003). Interestingly, in combination, Tat and gp120 can be synergistically neurotoxic. The present results suggest that the coordinated and/or sustained activation of both p38 and JNK MAPKs may underlie the synergistic neurotoxicity seen with concurrent Tat and gp120 exposure (Bansal et al., 2000; Nath et al., 2000a). Alternatively, p38 and/or JNK MAPKs may interact with other mixed-lineage kinase-driven pathways (Gallo and Johnson, 2002), such as ERK, to further regulate caspase-dependent and independent neuronal losses underlying neuro-AIDS. These findings may have important therapeutic implications since drugs such as minocycline that mediate neuroprotective effects through p38 MAPK (Lin et al., 2001) have been proposed as a potential treatment for HIV dementia (Si et al., 2004).

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ABBREVIATIONS

Ac-DEVD-AMC	Ac-Asp-Glu-Val-Asp- 7-amino-4-methylcoumarin
Ac-Asp-Glu-Val-Asp-aldehyde	Ac-DEVD-CHO
AIDS	acquired immunodeficiency syndrome
SP600125	anthra[1,9-cd]pyrazol-6(2H)-one
CXCR4	alpha chemokine receptor 4
JNK	caspase-3; c-Jun N-terminal kinase 1,2
endo-G	endonuclease-G
ERK	extracellular signal-regulated kinase
HIV	human immunodeficiency virus
SB 203580	[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1 H-imidazole]
MAPK	mitogen-activated protein kinase
MLK3	mixed-lineage kinase-3
SIV	simian immunodeficiency virus
Tat	transactivator of transcription

REFERENCES

- Achim CL, Wang R, Miners DK, Wiley CA. Brain viral burden in HIV infection. *J. Neuropathol. Exp. Neurol.* 1994; 53:284–294. [PubMed: 8176412]
- Avison, M.; Berger, JR.; McArthur, JC.; Nath, A. HIV meningitis and dementia. In: Nath, A.; Berger, JR., editors. *Clinical Neurovirology*. New York: Marcel Dekker, Inc.; 2003. p. 251-276.
- Avison MJ, Nath A, Greene-Avison R, Schmitt FA, Greenberg RN, Berger JR. Neuroimaging correlates of HIV-associated BBB compromise. *J. Neuroimmunol.* 2004; 157:140–146. [PubMed: 15579291]
- Bansal AK, Mactutus CF, Nath A, Maragos W, Hauser KF, Booze RM. Neurotoxicity of HIV-1 proteins gp120 and tat in the rat striatum. *Brain Res.* 2000; 879:42–49. [PubMed: 11011004]
- Barber SA, Uhrlaub JL, DeWitt JB, Tarwater PM, Zink MC. Dysregulation of mitogen-activated protein kinase signaling pathways in simian immunodeficiency virus encephalitis. *Am. J. Pathol.* 2004; 164:355–362. [PubMed: 14742241]
- Berger JR, Nath A. HIV dementia and the basal ganglia. *Intervirology.* 1997; 40:122–131. [PubMed: 9450229]
- Bhat NR, Zhang P, Lee JC, Hogan EL. Extracellular signal-regulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-alpha gene expression in endotoxin-stimulated primary glial cultures. *J. Neurosci.* 1998; 18:1633–1641. [PubMed: 9464988]
- Biard-Piechaczyk M, Robert-Hebmann V, Richard V, Roland J, Hipskind RA, Devaux C. Caspase-dependent apoptosis of cells expressing the chemokine receptor CXCR4 is induced by cell

- membrane-associated human immunodeficiency virus type 1 envelope glycoprotein (gp120). *Virology*. 2000; 268:329–344. [PubMed: 10704341]
- Bodner A, Maroney AC, Finn JP, Ghadge G, Roos R, Miller RJ. Mixed lineage kinase 3 mediates gp120IIIB-induced neurotoxicity. *J. Neurochem*. 2002; 82:1424–1434. [PubMed: 12354290]
- Bodner A, Toth PT, Oh SB, Lu M, Tran PB, Chin RK, Ren D, Miller RJ. CD4 dependence of gp120IIIB-CXCR4 interaction is cell-type specific. *J. Neuroimmunol*. 2003; 140:1–12. [PubMed: 12864967]
- Calabresi P, Centonze D, Gubellini P, Marfia GA, Pisani A, Sancesario G, Bernardi G. Synaptic transmission in the striatum: from plasticity to neurodegeneration. *Prog. Neurobiol*. 2000; 61:231–265. [PubMed: 10727775]
- Campbell DS, Holt CE. Apoptotic pathway and MAPKs differentially regulate chemotropic responses of retinal growth cones. *Neuron*. 2003; 37:939–952. [PubMed: 12670423]
- Chen RW, Qin ZH, Ren M, Kanai H, Chalecka-Franaszek E, Leeds P, Chuang DM. Regulation of c-Jun N-terminal kinase, p38 kinase and AP-1 DNA binding in cultured brain neurons: roles in glutamate excitotoxicity and lithium neuroprotection. *J. Neurochem*. 2003; 84:566–575. [PubMed: 12558976]
- D'Aversa TG, Yu KO, Berman JW. Expression of chemokines by human fetal microglia after treatment with the human immunodeficiency virus type 1 protein Tat. *J. Neurovirol*. 2004; 10:86–97. [PubMed: 15204927]
- Davis RJ. Signal transduction by the JNK group of MAP kinases. *Cell*. 2000; 103:239–252. [PubMed: 11057897]
- El-Hage N, Gurwell JA, Singh IN, Knapp PE, Nath A, Hauser KF. Synergistic increases in intracellular Ca^{2+} , and the release of MCP-1, RANTES, and IL-6 by astrocytes treated with opiates and HIV-1 Tat. *Glia*. 2005; 50:91–106. [PubMed: 15630704]
- Eom DS, Choi WS, Oh YJ. Bcl-2 enhances neurite extension via activation of c-Jun N-terminal kinase. *Biochem. Biophys. Res. Commun*. 2004; 314:377–381. [PubMed: 14733915]
- Everall I, Barnes H, Spargo E, Lantos P. Assessment of neuronal density in the putamen in human immunodeficiency virus (HIV) infection. Application of stereology and spatial analysis of quadrats. *J Neurovirol*. 1995; 1:126–129. [PubMed: 9222349]
- Filippi CG, Sze G, Farber SJ, Shahmanesh M, Selwyn PA. Regression of HIV encephalopathy and basal ganglia signal intensity abnormality at MR imaging in patients with AIDS after the initiation of protease inhibitor therapy. *Radiology*. 1998; 206:491–498. [PubMed: 9457204]
- Gallo KA, Johnson GL. Mixed-lineage kinase control of JNK and p38 MAPK pathways. *Nat. Rev. Mol. Cell Biol*. 2002; 3:663–672. [PubMed: 12209126]
- Gerfen, CR.; Wilson, CJ. The basal ganglia. In: Swanson, LW.; Bjorklund, A.; Hokfelt, T., editors. *Integrated Systems of the CNS, Part III. Cerebellum, Basal Ganglia, Olfactory System*. Vol. 12. New York: Elsevier; 1996. p. 371–468. *Handbook of Chemical Neuroanatomy*. Bjorklund, A. and Hokfelt, T. Ref Type: Serial (Book, Monograph)
- Goody RJ, Martin KM, Goebel SM, Hauser KF. Dynorphin A toxicity in striatal neurons via an alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/Kainate receptor mechanism. *Neurosci*. 2003; 116:807–816.
- Gu Y, Wu RF, Xu YC, Flores SC, Terada LS. HIV Tat activates c-Jun amino-terminal kinase through an oxidant-dependent mechanism. *Virology*. 2001; 286:62–71. [PubMed: 11448159]
- Gurwell JA, Nath A, Sun Q, Zhang J, Martin KM, Chen Y, Hauser KF. Synergistic neurotoxicity of opioids and human immunodeficiency virus-1 Tat protein in striatal neurons *in vitro*. *Neuroscience*. 2001; 102:555–563. [PubMed: 11226693]
- Hashimoto M, Masliah E. Cycles of aberrant synaptic sprouting and neurodegeneration in Alzheimer's and dementia with Lewy bodies. *Neurochem. Res*. 2003; 28:1743–1756. [PubMed: 14584828]
- Hauser KF, Houdi AA, Turbek CS, Elde RP, Maxson W III. Opioids intrinsically inhibit the genesis of mouse cerebellar granule cell precursors *in vitro*: Differential impact of μ and δ receptor activation on proliferation and neurite elongation. *Eur. J. Neurosci*. 2000; 12:1281–1293. [PubMed: 10762357]
- Herdegen T, Waetzig V. The JNK and p38 signal transduction following axotomy. *Restor. Neurol. Neurosci*. 2001; 19:29–39. [PubMed: 12082227]

- Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine potentiates HIV-1 gp120-induced neuronal apoptosis. *J. Infect. Dis.* 2005; 191:886–889. [PubMed: 15717263]
- Kan H, Xie Z, Finkel MS. HIV gp120 enhances NO production by cardiac myocytes through p38 MAP kinase-mediated NF-kappaB activation. *Am. J. Physiol Heart Circ. Physiol.* 2000; 279:H3138–H3143. [PubMed: 11087273]
- Kapasi AA, Patel G, Franki N, Singhal PC. HIV-1 gp120-induced tubular epithelial cell apoptosis is mediated through p38-MAPK phosphorylation. *Mol. Med.* 2002; 8:676–685. [PubMed: 12520084]
- Kaul M, Garden GA, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature.* 2001; 410:988–994. [PubMed: 11309629]
- Kaul M, Lipton SA. Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* 1999; 96:8212–8216. [PubMed: 10393974]
- Khan MZ, Brandimarti R, Patel JP, Huynh N, Wang J, Huang Z, Fatatis A, Meucci O. Apoptotic and antiapoptotic effects of CXCR4: is it a matter of intrinsic efficacy? Implications for HIV neuropathogenesis. *AIDS Res. Hum. Retroviruses.* 2004; 20:1063–1071. [PubMed: 15585097]
- Kim KY, Kim BC, Xu Z, Kim SJ. Mixed lineage kinase 3 (MLK3)-activated p38 MAP kinase mediates transforming growth factor-beta-induced apoptosis in hepatoma cells. *J. Biol. Chem.* 2004; 279:29478–29484. [PubMed: 15069087]
- Kolson DL. Neuropathogenesis of central nervous system HIV-1 infection. *Clin. Lab Med.* 2002; 22:703–717. [PubMed: 12244593]
- Kumar A, Manna SK, Dhawan S, Aggarwal BB. HIV-Tat protein activates c-Jun N-terminal kinase and activator protein-1. *J. Immunol.* 1998; 161:776–781. [PubMed: 9670954]
- Kutsch O, Oh J, Nath A, Benveniste EN. Induction of the chemokines interleukin-8 and IP-10 by human immunodeficiency virus type 1 tat in astrocytes. *J. Virol.* 2000; 74:9214–9221. [PubMed: 10982368]
- Lannuzel A, Barnier JV, Hery C, Huynh VT, Guibert B, Gray F, Vincent JD, Tardieu M. Human immunodeficiency virus type 1 and its coat protein gp120 induce apoptosis and activate JNK and ERK mitogen-activated protein kinases in human neurons. *Ann. Neurol.* 1997; 42:847–856. [PubMed: 9403476]
- Lin S, Zhang Y, Dodel R, Farlow MR, Paul SM, Du Y. Minocycline blocks nitric oxide-induced neurotoxicity by inhibition p38 MAP kinase in rat cerebellar granule neurons. *Neurosci. Lett.* 2001; 315:61–64. [PubMed: 11711215]
- Liu X, Jana M, Dasgupta S, Koka S, He J, Wood C, Pahan K. Human immunodeficiency virus type 1 (HIV-1) tat induces nitric-oxide synthase in human astroglia. *J. Biol. Chem.* 2002; 277:39312–39319. [PubMed: 12167619]
- Ma M, Nath A. Molecular determinants for cellular uptake of Tat protein of human immunodeficiency virus type 1 in brain cells. *J. Virol.* 1997; 71:2495–2499. [PubMed: 9032389]
- Marcario JK, Manaye KF, SantaCruz KS, Mouton PR, Berman NE, Cheney PD. Severe subcortical degeneration in macaques infected with neurovirulent simian immunodeficiency virus. *J. Neurovirol.* 2004; 10:387–399. [PubMed: 15765810]
- Mattson MP. Apoptotic and anti-apoptotic synaptic signaling mechanisms. *Brain Pathol.* 2000; 10:300–312. [PubMed: 10764050]
- Mattson MP, Duan W. "Apoptotic" biochemical cascades in synaptic compartments: roles in adaptive plasticity and neurodegenerative disorders. *J. Neurosci. Res.* 1999; 58:152–166. [PubMed: 10491580]
- Mattson MP, Haughey NJ, Nath A. Cell death in HIV dementia. *Cell Death. Differ.* 2005
- Miller GW, Gainetdinov RR, Levey AI, Caron MG. Dopamine transporters and neuronal injury. *Trends Pharmacol. Sci.* 1999; 20:424–429. [PubMed: 10498956]
- Mirsattari SM, Power C, Nath A. Parkinsonism with HIV infection. *Mov Disord.* 1998; 13:684–689. [PubMed: 9686775]
- Mischianti C, Pironi F, Milani D, Giacca M, Mirandola P, Capitani S, Zauli G. Extracellular HIV-1 Tat protein differentially activates the JNK and ERK/MAPK pathways in CD4 T cells. *AIDS.* 1999; 13:1637–1645. [PubMed: 10509564]
- Mitchell IJ, Cooper AJ, Griffiths MR. The selective vulnerability of striatopallidal neurons. *Prog. Neurobiol.* 1999; 59:691–719. [PubMed: 10845758]

- Nath A. Pathobiology of human immunodeficiency virus dementia. *Semin. Neurol.* 1999; 19:113–127. [PubMed: 10718533]
- Nath A, Haughey N, Jones M, Anderson C, Bell J, Geiger JD. Synergistic neurotoxicity by human immunodeficiency virus proteins Tat and gp120: protection by memantine. *Ann Neurol.* 2000a; 47:186–194. [PubMed: 10665489]
- Nath A, Jones M, Maragos W, Booze R, Mactutus C, Bell J, Hauser KF, Mattson M. Neurotoxicity and dysfunction of dopamine systems associated with AIDS dementia. *Psychopharmacol.* 2000b; 14:222–227.
- Ono K, Han J. The p38 signal transduction pathway: activation and function. *Cell Signal.* 2000; 12:1–13. [PubMed: 10676842]
- Petito CK, Kerza-Kwiatecki AP, Gendelman HE, McCarthy M, Nath A, Podack ER, Shapshak P, Wiley CA. Review: neuronal injury in HIV infection. *J. Neurovirol.* 1999; 5:327–341. [PubMed: 10463855]
- Shen YH, Godlewski J, Zhu J, Sathyanarayana P, Leaner V, Birrer MJ, Rana A, Tzivion G. Cross-talk between JNK/SAPK and ERK/MAPK pathways: sustained activation of JNK blocks ERK activation by mitogenic factors. *J. Biol. Chem.* 2003; 278:26715–26721. [PubMed: 12738796]
- Sholl DA. Dendritic organization in the neurons of the visual and motor cortices of the cat. *J. Anat. (London).* 1953; 87:387–407. [PubMed: 13117757]
- Si Q, Cosenza M, Kim MO, Zhao ML, Brownlee M, Goldstein H, Lee S. A novel action of minocycline: inhibition of human immunodeficiency virus type 1 infection in microglia. *J. Neurovirol.* 2004; 10:284–292. [PubMed: 15385251]
- Singh IN, Goody RJ, Dean C, Ahmad NM, Lutz SE, Knapp PE, Nath A, Hauser KF. Apoptotic death of striatal neurons induced by HIV-1 Tat and gp120: differential involvement of caspase-3 and endonuclease G. *J. Neurovirol.* 2004; 10:141–151. [PubMed: 15204919]
- Singh IN, Goody RJ, Goebel SM, Martin KM, Marinova Z, Hirschberg D, Yakovleva T, Bergman T, Bakalkin G, Hauser KF. Dynorphin A (1–17) induces apoptosis in striatal neurons through alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor-mediated cytochrome c release and caspase-3 activation. *Neurosci.* 2003; 122:1013–1023.
- Song L, Nath A, Geiger JD, Moore A, Hochman S. Human immunodeficiency virus type 1 Tat protein directly activates neuronal N-methyl-D-aspartate receptors at an allosteric zinc-sensitive site. *J. Neurovirol.* 2003; 9:399–403. [PubMed: 12775422]
- Sporer B, Linke R, Seelos K, Paul R, Klopstock T, Pfister HW. HIV-induced chorea Evidence for basal ganglia dysregulation by SPECT. *J. Neurol.* 2005; 252:356–358. [PubMed: 15726276]
- Vesce S, Bezzi P, Rossi D, Meldolesi J, Volterra A. HIV-1 gp120 glycoprotein affects the astrocyte control of extracellular glutamate by both inhibiting the uptake and stimulating the release of the amino acid. *FEBS Lett.* 1997; 411:107–109. [PubMed: 9247152]
- Whetsell WO Jr. The mammalian striatum and neurotoxic injury. *Brain Pathol.* 2002; 12:482–487. [PubMed: 12408235]
- Wiley CA, Soontornniyomkij V, Radhakrishnan L, Masliah E, Mellors J, Hermann SA, Dailey P, Achim CL. Distribution of brain HIV load in AIDS. *Brain Pathol.* 1998; 8:277–284. [PubMed: 9546286]
- Yi Y, Lee C, Liu QH, Freedman BD, Collman RG. Chemokine receptor utilization and macrophage signaling by human immunodeficiency virus type 1 gp120: Implications for neuropathogenesis. *J. Neurovirol.* 2004; 10(Suppl 1):91–96. 91-6. [PubMed: 14982745]
- Yiannoutsos CT, Ernst T, Chang L, Lee PL, Richards T, Marra CM, Meyerhoff DJ, Jarvik JG, Kolson D, Schifitto G, Ellis RJ, Swindells S, Simpson DM, Miller EN, Gonzalez RG, Navia BA. Regional patterns of brain metabolites in AIDS dementia complex. *Neuroimage.* 2004; 23:928–935. [PubMed: 15528093]

Fig. 1.

Tat and gp120 increased striatal neuron death at 24 h *in vitro* (A). Tat or gp120 induced significant neuronal losses ($*P < 0.05$ versus vehicle-treated control cultures; Kruskal-Wallis ANOVA & post hoc test), while inactive mutant Tat (Tat₃₁₋₆₁) or denatured gp120 failed to cause marked neurotoxicity; mean $\pm$ SEM from $n = 6$ experiments (A). Neuronal losses were assessed by time-lapse photography at 0, 24, 48 and 72 h (24 h data shown) (A). Phosphorylated p38 (p-p38) and JNK (p-JNK) MAPK were increased by Tat or gp120 at 4 h (B,C). Both viral proteins significantly increased p38 phosphorylation in striatal neurons at 4

h ($*P < 0.05$ versus vehicle controls) (D). Tat increased phosphorylated JNK1 and JNK2 levels (p-JNK) at 4 h ($*P < 0.05$ versus vehicle control cultures) (E), while gp120 variably increased JNK2 at this time ($*P < 0.05$ versus vehicle control cultures) (E). Tat and gp120 concentrations in these experiments were 100 nM and 500 pM, respectively. Mean $\pm$ SEM from $n = 4$ experiments.

Fig. 2.

Time-dependent increases in Tat and gp120-induced p38 and JNK MAPK phosphorylation in cultured striatal neurons. Gp120 increased phosphorylation p38 MAPK (p-p38) at 5 min following exposure, while Tat (100 nM) did not cause significant increases until 15 min (A–B) (* $P < 0.05$ vs. pretreatment levels) (A–B). Although Tat and gp120 exposure caused overall increases in JNK activation, subtle differences in the timing and extent of JNK1 vs. JNK2 phosphorylation were noted (* $P < 0.05$ vs. pretreatment levels) (C–D); Tat-induced increases in JNK were more modest and variable. Tat and gp120 concentrations in these experiments were 100 nM and 500 pM, respectively. Mean $\pm$ SEM from $n = 3$ experiments.

Fig. 3.

Time-lapse photomicrographs showing the effects of gp120 and MAPK inhibitors on striatal neurodegeneration prior to (0 h) and at 24 and 72 h following gp120 exposure (A–L). Gp120 exposure (D–F) caused destruction of the cell body (arrowheads) and neurites compared to vehicle-treated controls (A–C). The gp120-induced neurodegenerative changes were prevented by p38 MAPK inhibition (SB203580) (G–I), but not JNK inhibition (SP600125) (J–L). Tat caused significant neuronal death that was unaffected by JNK or p38 MAPK inhibition. Scale bar in C = 25 μ m (A–C), in F = 25 μ m (D–F), in L = 25 μ m (G–L).

Fig. 4.

At 4 h following viral protein exposure, p38 MAPK inhibition (SB203580) and JNK inhibition (SP600125) selectively attenuated Tat (100 nM) and/or gp120 (500 pM) induced increases in caspase-3 activity in cultured striatal neurons (A–B). SP600125 exposure markedly reduced Tat-induced increases caspase-3 activity, while SB203580 selectively blocked gp120-induced increases in caspase-3 activity (* $P < 0.05$ vs. vehicle-treated controls; # $P < 0.05$ vs. treatment with Tat or gp120 alone); mean $\pm$ SEM from $n = 4$ experiments (A–B). At 48 h following viral protein exposure, p38 MAPK, but not JNK

inhibition prevented gp120-induced neuronal death as assessed using time-lapse photomicroscopy; while neither p38 nor JNK MAPK inhibition rescued striatal neurons from the neurotoxic effects of Tat (C–D) (* $P < 0.05$ versus vehicle-treated controls; # $P < 0.05$ vs. treatment with gp120 alone) (C–D); mean $\pm$ SEM from $n = 3$ experiments.

Fig. 5.

Effects of Tat, gp120, and MAPK inhibitors on neurite length represented as the change from pretreatment values. Tat caused significant neurite losses compared to vehicle-treated controls or Tat treatment with the JNK inhibitor SP600125 at 48 and 72 h ($*P < 0.05$ vs. vehicle-exposed controls; Kruskal-Wallis ANOVA & *post hoc* test) (A). Inhibition of p38 MAPK had no consistent effects on Tat-induced neurite losses (SB203580-treated neurons neither differed from control nor Tat exposed neurons). By contrast, significant gp120-induced reductions in neurite length at 24, 48, and 72 h ($*P < 0.02$ vs. vehicle-treated controls) were prevented by p38 MAPK inhibition ($\#P < 0.01$ vs. gp120-treated cultures) (B). JNK inhibition (SP600125) failed to prevent gp120-induced neurite losses ($*P < 0.05$ vs. vehicle-treated controls; ANOVA & Duncan's test) (B); data in A and B are the mean $\pm$ SEM from $n = 3$ experiments.