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Effects of Opiates and HIV Proteins on Neurons: The Role of Ferritin Heavy Chain and a Potential for Synergism

Lindsay Festa¹ and Olimpia Meucci^{1,2,*}

¹Department of Pharmacology and Physiology, Drexel University College of Medicine, 245 N. 15th Street, Philadelphia PA, USA

²Department of Microbiology and Immunology, Drexel University College of Medicine, 245 N. 15th Street, Philadelphia PA, USA

Abstract

Human immunodeficiency virus 1 (HIV-1) and its associated proteins can have a profound impact on the central nervous system. Co-morbid abuse of opiates, such as morphine and heroin, is often associated with rapid disease progression and greater neurological dysfunction. The mechanisms by which HIV proteins and opiates cause neuronal damage on their own and together are unclear. The emergence of ferritin heavy chain (FHC) as a negative regulator of the chemokine receptor CXCR4, a co-receptor for HIV, may prove to be important in elucidating the interaction between HIV proteins and opiates. This review summarizes our current knowledge of central nervous system damage inflicted by HIV and opiates, as well as the regulation of CXCR4 by opiate-induced changes in FHC protein levels. We propose that HIV proteins and opiates exhibit an additive or synergistic effect on FHC/CXCR4, thereby decreasing neuronal signaling and function.

Keywords

CXCR4; ferritin; gp120; HIV; neuron; opiates; tat

INTRODUCTION

Human immunodeficiency virus (HIV) continues to have a significant impact both globally and throughout the United States. Even though estimated incidences of HIV in the United States have remained relatively stable over the past few years, there are still approximately 50,000 new cases reported annually [1]. A major risk factor for HIV exposure and eventual progression to acquired immune deficiency syndrome (AIDS) is intravenous drug use (IVDU), accounting for approximately one-third of all HIV cases [2]. Abuse of opiates has also been linked to accelerated disease progression and increased neurotoxicity [3, 4, 5].

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*Address correspondence to this author at the Drexel University College of Medicine, 245 North 15th Street, NCB rm#8221, Philadelphia, PA 19102, USA; Tel: (215) 762 2597; Fax: (215) 762 2299; omeucci@drexelmed.edu.

CONFLICT OF INTEREST

Declared none.

The introduction of highly active antiretroviral therapy (HAART), also known as combination antiretroviral therapy (cART), has increased quality of life and life expectancy in individuals with HIV; however, it should be noted that patients with presumed transmission *via* IVDU have significantly shorter life expectancies even with HAART [6, 7]. Despite these advances, the prevalence of HIV-associated neurocognitive disorders (HAND) has increased [8, 9]. It is important to determine the mechanisms by which HIV proteins cause neural dysfunction and how opiates potentially interact *via* an additive or synergistic effect. This review will discuss the role of HIV proteins and opiates on neuronal signaling and functioning, and the potential mechanisms underlying interaction between the two, namely through regulation of the iron-sequestering protein ferritin heavy chain (FHC).

NEUROPATHOGENESIS OF HIV-1 INFECTION

Infiltration of HIV into the CNS occurs early after initial infection; however, it is unclear whether the virus remains in the CNS or if it is cleared, only to re-enter at a later time point [8]. Either way, the infection typically remains asymptomatic for years before appearance of neurological complications (neuroAIDS), including HAND [10]. The “Trojan Horse hypothesis” is the most commonly accepted model of CNS penetration. According to this model, HIV-1 enters the brain as a passenger in cells trafficking through the blood-brain barrier (BBB), namely infected monocytes and T cells [11]. From there, cells of the brain can be directly infected, specifically perivascular macrophages and microglia since they possess CD4 and chemokine receptors necessary for HIV-1 entry [12]. Other primary cells of the CNS (neurons, astrocytes, oligodendrocytes) are generally not infected by HIV-1 and if they are, such as in the case of astrocytes, they do not substantially contribute to viral replication [10].

Symptomatically, CNS HIV-1 infection presents as short-term memory loss, reduced concentration, depression, apathy and personality changes [13]. In addition, both sensory and motor problems are observed in these patients [14]. Depending on the severity and nature of cognitive impairments, affected individuals can be categorized into three groups: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), or HIV-1-associated dementia (HAD) [15]. HAART has been successful in precluding many end-stage complications of AIDS, including reducing the prevalence of HAD; however, the longer life expectancy has been followed by an increase in mild neurocognitive impairment with 25% of treated HIV-infected individuals developing at least one neurological symptom, based on a recent study [16].

HIV-1 infection of the CNS is characterized by several different neuropathological changes broadly termed HIV encephalitis (HIVE) [17]. These changes include formation of multinucleated giant cells [18], presence of microglial nodules, infiltration of lymphocytes [19], activated CNS macrophages [20], dendritic pruning [21], and synaptic/neuronal loss [22]. Dendritic injury is correlated with clinical symptoms [23]. Thus, even though only macrophages and microglia are actively infected in the CNS, other cells of the brain, namely neurons, can undergo damage and contribute to the development of a “toxic” environment. The mechanisms behind neuronal damage in the context of HIV-1 infection, specifically through the viral proteins tat and gp120, will be discussed in detail in the following section.

As mentioned earlier, drug abusers who are HIV-positive show more rapid disease progression, as well as greater neurological symptoms. When compared to non-drug abusing HIV patients, several differences emerge including greater blood-brain barrier disruption [24], enhanced microglia activation and turnover [25, 26], and greater frequency of HIVE [27]. This research, in tandem with findings suggesting that drugs alone can cause CNS damage, points to an additive, or potentially synergistic, effect between HIV-1 and drugs of abuse, including opiates. Research has been conducted to investigate the interaction between HIV and opiates, but lack of suitable *in vivo* models has made it hard to delineate the impact of co-morbid HIV infection and opiate abuse on neuronal structure and function.

VIRAL PROTEINS: MULTIPLE MECHANISMS OF NEURONAL INJURY

While evidence for neuronal injury has been found both in imaging studies [28] and post-mortem tissue [22], the mechanisms behind HIV-induced neuron damage are still not fully characterized. HIV proteins, such as envelope glycoprotein gp120 and trans-activator of transcription (tat), seem to injury neurons through two different, but not mutually exclusive, mechanisms. Other HIV proteins, such as vpr, nef, and gp41, are known to cause neuronal damage; however, they have not been studied as extensively and thus will not be discussed in detail in this review. The “direct injury” hypothesis states that neurons can be directly harmed, though not infected, by HIV proteins, while the “bystander effect” theory suggests that neuronal injury is caused by secretion of neurotoxins shed by infected macrophages and microglia. Additionally, despite not being productively infected, astrocytes can also contribute to neuronal damage during HIV infection through the release of various neurotoxins [17]. There is plenty of evidence supporting an indirect mechanism of neuronal injury and death by HIV-infected microglia and macrophages. However, a direct injury model cannot be overlooked since neurons express receptors that enable both gp120 and tat to directly bind to them. Direct binding of viral proteins to neurons may play a key role in how they potentially interact with opiates in the CNS. A comprehensive review of this topic has recently been published [29].

Briefly, HIV-1 infects microglia and perivascular macrophages using CD4 and CCR5 and/or CXCR4 chemokine receptors. Infected cells can then release various neurotoxins, which induce neuronal cell injury and death [30, 31]. These neurotoxins primarily modulate damage through two prominent modes of Ca^{2+} entry: L-type calcium channels and NMDA channels [32]. Changes in intracellular Ca^{2+} are also involved in gp120-induced neurotoxicity [33]. Rise in neuronal Ca^{2+} levels is believed to be a final common pathway in neuronal injury, leading to Ca^{2+} overload, p38 MAPK activation, release of mitochondrial cytochrome *c*, caspase activation and free-radical formation ($\text{NO}^{\bullet}$) [17]. NMDA glutamate-receptor antagonists, NMDA-mediated channel blockers [31], L-type voltage-dependent calcium channel antagonists [32] and $\text{NO}^{\bullet}$ synthase inhibitors [34] were all shown to attenuate gp120-induced neurotoxicity *in vitro*. The activity of p38 MAPK is also a key step in apoptosis caused by Ca^{2+} overload through NMDA channels. Activation of p38 MAPK was found in gp120-treated mixed cerebrocortical cultures, which was dependent on microglia, and subsequent inhibition of p38 attenuated gp120-induced neuronal injury [35]. It should be noted that while both gp120 and tat increase phosphorylation of p38, inhibition of p38 did not prevent tat-induced neuronal damage and loss, suggesting that tat injures

neurons through p38-independent mechanisms [36]. Caspase activation also plays an essential role in apoptosis. In mixed cerebrocortical cultures, neurons showed a significant increase in caspase-3, caspase-8, and caspase-9 when treated with gp120 [37]. Release of cytochrome *c* preceded caspase-3 activity and inhibition of caspase-9 prevented gp120-induced neuronal apoptosis, suggesting that caspase cascades are important in HIV-associated neurodegeneration. *In vivo* [38] and post mortem tissue [37] studies have further confirmed the role of caspase-3 activation in HIV-associated neurotoxicity. In addition to caspase inhibitors, brain-derived neurotrophic factor (BDNF) exhibits a neuroprotective effect on caspase-3 activation caused by gp120 by decreasing the surface levels of CXCR4, but not CCR5, on neurons [39]. *In vivo* experiments using BDNF heterogeneous mice suggest that gp120 reduces the level of BDNF in the brain and increases caspase-3 activity, indicating that a reciprocal relationship between the two may exist [40]. While these experiments were done in the presence of glia and astrocytes (albeit minimal), these findings hint at a potential direct mechanism of neuronal injury *via* the interaction of gp120 and the chemokine receptors CXCR4 and CCR5. This direct mechanism of neuronal injury will be discussed in detail later.

Infected microglia and macrophages have also been shown to secrete inflammatory cytokines, such as tumor necrosis factor (TNF- α) and interleukin-1 beta (IL-1 β) [30]. TNF- α itself can directly induce neuronal apoptosis *via* TNF- α receptors [41]; however, both TNF- α and IL-1 β can stimulate macrophages to release L-cysteine, which can activate NMDA receptors and lead to apoptosis [30]. Addition of TNF- α neutralizing antibodies to mixed cerebrocortical cultures treated with gp120 prevented neuronal apoptosis [30]. Some cytokines, however, have exhibited neuroprotective effects, such as transforming growth factor beta 1 (TGF- β 1). TGF- β 1 prevented gp120-induced neuronal death, as well as neuronal Ca²⁺ overloading, even in the presence of NMDA [42]. TGF- β 1 has also been shown to inhibit the secretion and activity of TNF- α [43], suggesting another mechanism by which TGF- β 1 exhibits neuroprotective functions.

While neurons cannot be productively infected with HIV, they can bind several different viral proteins, including tat and gp120. Elevated levels of tat mRNA have been found in patients with HIV-1 dementia [44] and HIVE [45], suggesting that tat plays a role in HIV-associated neurodegeneration. Furthermore, tat has been shown to induce neuronal apoptosis in the absence of glia, supporting a direct injury model for this viral protein [46]. Tat has been found to bind to and activate non-NMDA [47, 48] and NMDA receptors [49, 50]. Activation of these receptors disrupts Ca²⁺ homeostasis, eventually leading to neurotoxicity [51]. In addition, tat can directly phosphorylate the NR2A and NR2B subunits of the NMDA receptor [49] and blockers of NR2B/NR2B and NR2A/NR2B homo/heterodimers can attenuate tat neurotoxicity [50]. As mentioned earlier, increases in Ca²⁺ levels lead to a cascade of events that cause apoptosis, typically through the activation of caspases. While broad permanent caspase inhibitors can inhibit tat-induced neuronal death [46], tat is also able to activate an alternative neurotoxic pathway involving endonuclease G, a mitochondria released apoptotic DNase [52, 53]. This may explain why inhibition of p38 MAPK activity did not prevent tat-induced neuron damage and death [36].

In addition to activating receptors on the cell membrane, tat can be directly taken into neurons *via* low-density lipoprotein receptor-related proteins (LRP) [54]. This internalization of tat disrupts the metabolic balance of LRP ligands such as amyloid precursor protein, amyloid β -protein, and apolipoprotein E4, suggesting a distinct neurotoxic pathway from surface-bound tat. The LRP receptor, in the presence of tat, can also form a macromolecular complex with postsynaptic density protein-95 (PSD-95), NMDAR, and neuronal nitric oxide synthase (nNOS), causing apoptosis through the production of NO^{*} in primary human astrocytes and neurons [55]. Interestingly, the formation of this complex led to apoptosis in cells negative, as well as positive, for NMDA receptors.

The envelope glycoprotein gp120, which is cleaved from the precursor protein gp160, interacts with the chemokine receptor CCR5 (R5 viruses), CXCR4 (X4 viruses), or both (R5X4 viruses) [56]. Despite the fact that neurons lack the CD4 receptor necessary for productive infection, the association between chemokine receptors and gp120 are CD4-independent, suggesting potential direct neurotoxic effects [57]. R5 viruses primarily cause initial infection; however, increased incidence of CXCR4-using strains in AIDS patients has been found at later stages of the disease, correlating with the onset of neurocognitive symptoms and neuronal damage [58]. Also, neuronal CXCR4 is overexpressed, while CCR5 is downregulated, in patients with HIVE [59]. Thus, it appears that CXCR4 potentially plays a crucial role in mediating direct neuronal injury by gp120.

Before the neurotoxic effects of gp120 on neurons are discussed, it is important to understand the normal homeostatic roles of CXCR4, a G-protein coupled receptor (GPCR), and its endogenous ligand CXCL12/stromal derived factor-1 alpha (SDF-1 α). CXCR4 and CXCL12 are constitutively expressed in the developing and mature brain and have been implicated in neuronal proliferation, maturation and survival [60, 61]. In the adult brain, CXCL12 mainly acts *via* mechanisms dictating neuronal survival and neurotransmission. CXCL12 activates Akt and several of its antiapoptotic targets, including NF- κ B and MDM2 [62], and prevents phosphorylation of the transcriptional repressor protein Rb, which in turn reduces the binding of E2F-1 and its accumulation in the cytosol [63]. Regulation of Rb and E2F-1 and downstream apoptotic genes enhances neuronal survival. Further, Rb protein is necessary for the neuroprotective effect of CXCL12 [64]. CXCL12 has also been found to inhibit caspase activation, which, as discussed earlier, is implicated in apoptosis [65]. More importantly, CXCL12 can also modulate NMDA subunit composition both *in vitro* and *in vivo*, altering excitotoxic responses. Selective down regulation of the NR2B subunit by CXCL12 controls calcium influx mediated by stimulation of extrasynaptic receptors and the ensuing cell death, while maintaining pro-survival pathways dependent on synaptic receptors [66]. Furthermore, unpublished findings from our group show that CXCL12/CXCR4 positively regulate dendritic spine density in cortical neurons, highlighting a novel physiologic mechanism that might be altered in neuroAIDS. This is an important concept considering that with the advent of HAART, dendritic pruning and synaptic injury are more frequently reported than neuronal loss. Overall, CXCL12 and its receptor CXCR4 have important neuronal survival properties and disruption of this signaling pair can have severe effects on the brain. The importance of the CXCL12/CXCR4 axis in brain homeostasis is further emphasized by the consequences of CXCL12 cleavage by matrix metalloproteases

(MMPs), which generates the neurotoxic product aa5-67 CXCL12. This cleaved form of CXCL12 is unable to properly interact with CXCR4 and rather stimulates an alternate receptor, CXCR3 – coupled to neurotoxicity [67]. Thus, cleavage of CXCL12 not only impairs the normal neuroprotective function of CXCR4, but also actively leads to neuronal injury *via* CXCR3. Importantly, upregulation of MMPs and cleavage of CXCL12 have been reported in HIV brains and suggested to contribute to neuropathology in humans [68].

Binding of gp120 to CXCR4 exhibits some similar properties to CXCL12. Both of these ligands can activate MAP kinases, such as ERK1/2, when bound to neurons [69], and can induce CXCR4 internalization and dimerization, albeit differently [70]. However, gp120 activates cell signaling pathways that are distinct from CXCL12. Unlike CXCL12, gp120 is unable to stimulate the Akt pathway associated with neuronal survival [62]. Instead, gp120 induces pathways associated with apoptosis, such as p38 and caspase-3, even in the absence of glia and CXCR4 internalization [36, 71]. E2F-1 translational activity, as well as two downstream targets (Cdc2 and Puma), were upregulated in neurons treated with gp120. In addition, E2F-1 levels were elevated in the nucleus of neurons in the brains of HIV-positive patients with dementia compared to HIV-negative individuals and neurologically normal HIV-positive patients [72]. CXCL12 [73], CXCR4 inhibitors and G_i-signaling inhibitors [74] all reduced or blocked gp120-induced neurotoxicity, demonstrating that gp120 is directly interacting with neurons.

The above-mentioned findings show that both HIV-1 proteins tat and gp120 interact directly with neurons and activate neurodegenerative pathways. It should be clear that the bystander hypothesis and direct effect model are in no way exclusive from each other and occur in tandem in the CNS to induce damage. HIV-1 has devastating effects on the CNS and efforts must be continued in order to elucidate its mechanisms and develop potential therapies.

OPIOID RECEPTORS: POTENT ANALGESICS AND MEDIATORS OF NEUROTOXICITY

Opioid receptors are GPCRs, expressed in the CNS and immune systems, which mediate powerful analgesic effects in acute and chronic pain situations [75]. Unfortunately, morphine and other opioid peptides, such as heroin and codeine, have several side effects such as respiratory depression, confusion, and blurred vision. Additionally, they are highly addictive, with tolerance and dependence occurring after prolonged use [76]. While the mechanisms of opioid tolerance and addiction have been intensely studied for years, not until recently have potential mechanisms of opioid-induced neurotoxicity been investigated [77]. Recent evidence suggests that activation of opioid receptors in the CNS can induce apoptosis and neuronal damage.

Opioid receptors are divided into three distinct classes: mu (μ , MOR), delta (δ , DOR), and kappa (κ , KOR) [78]. Morphine is a full agonist for the MOR and a partial agonist for the DOR [79]; however, knockout of MORs completely blocked analgesia by morphine, suggesting the effect of morphine is primarily mediated through MORs [80]. In addition, MORs are believed to play a critical role in mediating both the beneficial and harmful effects of clinically important drugs [81]. Until recently, the intracellular signaling pathways

activated by μ -receptors have not been explored in detail [82–84]. Long-term morphine treatment, both *in vitro* and *in vivo*, significantly decreased pERK1/2 levels, a kinase associated with cell survival and gene regulation [85]. In addition to disrupting cell survival pathways, morphine has been found to induce apoptosis in different brain regions, such as the frontal cortex and hippocampal CA1, CA2, and CA3 regions [86]. Chronic morphine-induced apoptosis, through caspase-3, was somewhat mediated by NMDARs and was associated with morphine tolerance *in vitro* and *in vivo* [87]. It should be noted that astrocytes, compared to both neurons and microglia, are resistant to morphine-induced apoptosis [88]. Additionally, morphine can activate both pathways of apoptosis: the intrinsic pathway mediated by the upregulation of JNK3 [89] and Bad [90], and the extrinsic pathway activated *via* FasL, Fas and FADD/p53 [90, 91]. While morphine is able to activate some pro-survival signaling pathways, it appears that long-term morphine use and its associated tolerance leads to a reduction in survival signaling and an increase in proapoptotic pathways.

Along with apoptosis, morphine induces a number of other insults in the brain. For instance, dendritic spine function and morphology is greatly altered by morphine treatment. Most excitatory synapses are located on dendritic spines and thus they play a major role in experience-dependent learning and memory [92]. Furthermore, spines can protect neurons from excitotoxicity by acting as cellular compartments that sequester calcium, preventing large calcium increases in the neuronal cell body [93]. Several studies have found that morphine decreases the complexity of dendritic spines, the total dendrite length, and density of spines [94, 95], while also shrinking preexisting spines [96]. The mechanisms behind these morphological changes are not completely clear; however, it appears that morphine suppresses the activity of neurogenic differentiation 1 transcription factor (NeuroD), which functions to stabilize dendritic spines [97]. It seems that MOR internalization is a key factor dictating the effects of opioids on dendritic spines [98]. Since dendritic injury is a key component of HAND, as well as the one pathological change that correlates with cognitive symptoms, it is important to elucidate the mechanisms behind opioid-induced dendritic changes and how they might interplay with HIV proteins.

INTERACTIONS BETWEEN CXCR4 AND μ -OPIOID RECEPTORS: HETEROLOGOUS DESENSITIZATION AND THE ROLE OF FERRITIN HEAVY CHAIN

Chemokine and opioid receptors are both expressed in the immune system, as well as the CNS. Based on this mutual expression pattern, the interaction between these two receptors has been studied extensively. Through the process of bi-directional heterologous desensitization, opioids can alter the chemotactic properties of chemokines [99–101] and chemokines can affect the analgesic properties of opioids [102]. While the mechanism behind heterologous desensitization is unclear, multiple processes seem to factor in, such as receptor phosphorylation, G-protein coupled kinases (GRK), and tyrosine kinases [103, 104]. It should be noted that the relationship is not always bi-directional, as opioid receptors can desensitize CXCR1 and CXCR2, but these chemokine receptors have no effect on opioid receptor properties [103].

Until recently, the focus on heterologous desensitization between chemokine receptors and opioid receptors has been predominantly in the immune system; however, new evidence suggests novel mechanisms by which this regulation occurs in the CNS. Stimulation of neurons co-expressing CXCR4 and MORs with morphine or MOR agonist [D-Ala², N-Me-Phe⁴, Gly-ol⁵]enkephalin (DAMGO) inhibited intracellular pathways (ERK1/2 and Akt) activated by CXCL12, preventing CXCL12-mediated neuroprotection [105, 106]. Additionally, morphine reduced CXCL12-induced phosphorylation of CXCR4, suggesting a different mechanism of heterologous desensitization than has been reported in immune cells [106]. Studies in primary neurons indicated that morphine (or DAMGO) do not reduce the surface level expression levels of CXCR4, as it has been reported to do with CCR5 in astrocytes [107]; however, these opiates upregulate protein levels of ferritin heavy chain (FHC), a negative regulator of CXCR4 [105, 108]. *In vitro* and *in vivo* data show that MOR stimulation of FHC requires *de novo* protein synthesis – an effect that leads to impairment of CXCR4 signaling [106]. These data suggest a potential mechanism by which opiates can alter signaling *via* CXCR4, a major HIV-1 co-receptor, without changing the co-receptor levels, thus possibly playing a dual role in HIV-induced neuropathology.

Ferritin is a highly conserved binding protein that is widely expressed in the body. Its main functions consist of iron sequestration and storage, playing an essential role in iron homeostasis [109]. The ferritin protein is made up of two components, ferritin heavy chain and ferritin light chain (FLC), which combine to form the 24-subunit protein. FHC mediates the oxidation of Fe²⁺ to Fe³⁺, while FLC is important for the nucleation of the iron core [110]. Each cell type has varying H:L ratios, which are affected by several different factors such as iron, cytokines, and secondary messengers [111]. In the CNS, FHC protein is predominantly seen in microglia, oligodendrocytes and neurons; however, FLC is present, albeit in smaller quantities [112]. According to recent data, uptake of circulating FHC, but not FLC, into endosomes and lysosomes is mediated by the human transferrin receptor [113]; however, this remains somewhat controversial and additional research needs to confirm this finding.

As mentioned earlier, FHC was found to be a CXCR4-binding protein, a novel mechanism possibly outside of its iron sequestration and storage functions. Furthermore, treatment of cell lines with CXCL12 caused a time-dependent increase in FHC association with CXCR4, as well as phosphorylation and nuclear translocation of FHC [108]. Phosphorylation was important both for inhibiting CXCL12-mediated ERK1/2 signaling and inducing nuclear translocation of FHC, suggesting a potential mechanism for the CXCR4-signaling disruption. It is unclear how nuclear FHC modulates CXCR4 function; however, FHC is able to bind to DNA, where it may potentially inhibit CXCR4 signaling [114]. Nuclear ferritin and its potential role in the interaction between opiates and HIV proteins will be discussed in the next section.

Abnormal expression of ferritin has been implicated in a variety of neurodegenerative diseases such as restless leg syndrome [115], Alzheimer's disease [116], Parkinson's disease [117], and most importantly in the context of this review, neuroAIDS [118]. Preliminary *in vivo* data from our lab have found an increase of FHC expression in the cortex of opiate drug abusers, as well as HIV-positive individuals with marked neurological deficits, which was

inversely correlated with the levels of CXCR4 phosphorylation [119]. This implicates FHC as a potentially mediator in the neurological complications of both opiate drug abuse and HIV. Ongoing studies in our lab are currently investigating this issue in more detail. The goal is to elucidate the mechanisms involved in morphine-induced increases in FHC, as well as the potential effect of HIV proteins on the expression and localization of FHC. The interplay between opiates and HIV and the possible convergence of pathways on FHC may prove to be an important factor in HIV-induced neuropathogenesis.

INTERACTION BETWEEN HIV PROTEINS AND OPIATES: A POTENTIAL ROLE FOR FERRITIN HEAVY CHAIN

It should be evident based on the above-mentioned research that HIV proteins and opiates can induce neurological dysfunction and damage on their own through a variety of pathways. What remains to be explained is how viral proteins and opiates interact to induce damage to the CNS. Some studies suggested that morphine could possibly protect neurons in HIV neuropathogenesis as morphine can reduce toxicity by the R5-tropic gp120_{BaL}, but not gp120_{IIIB} (X4-tropic), *via* the release of CCL5 from astrocytes [120]. The impact of this finding, however, should be debated. First, while R5 viruses are typically found during the beginning of infection, as the disease progresses and eventually develops into AIDS, X4 and dual-tropic viruses emerge. Thus, the neuroprotective effect might be useful in terms of initial infection but neurological symptoms correlate with the emergence of X4 or dual-tropic strains and morphine does not reduce their toxicity. Also, the immune suppression action of morphine needs to be taken into account.

On the other hand, a number of studies have investigated the potential additive or synergistic effects of morphine with the HIV proteins tat and gp120. *In vitro* and *in vivo* data have found that co-administration of morphine and either tat or gp120 cause enhanced microglia activation [121], increased release of inflammatory cytokines (IL-6, IL-1 β , TNF- α) [121, 122], synergism on neuronal apoptosis [3, 123] and alteration of dendritic spines and dendrites [122]. While these studies point out that opiates and viral proteins can interact with one another to cause significant neurotoxicity, they do not address the potential mechanisms underlying these observations. Ferritin heavy chain and its ability to disrupt homeostatic CXCR4 signaling may be an effector through which opiates and viral proteins additively or synergistically damage the CNS.

As mentioned before, gp120 can bind to CXCR4, altering neuronal function and survival. While this viral protein can activate ERK1/2 and other CXCR4-dependent intracellular pathways, it cannot trigger phosphorylation of Akt in neurons [62], which is also significantly reduced by the upregulation of FHC induced by morphine or DAMGO. Therefore, downregulation of Akt pathways by both gp120 and morphine may be additive, if not synergistic. It remains to be established whether binding of gp120 to CXCR4 may alter FHC levels as observed with morphine.

Nuclear translocation of FHC is another mechanism that may prove to be important in mediating the disruptive effects on CXCR4. Ferritin has typically been thought of as a cytoplasmic iron-storage protein; however, several reports have found the presence of

ferritin in cell nuclei [112, 124, 125]. Ferritin that is present in the nucleus is comprised of the same ferritin found in the cytoplasm (i.e. FHC/FLC) and both come from the same genes, suggesting a specific nuclear translocation mechanism [126]. CXCL12 stimulation caused phosphorylation and nuclear translocation of FHC [108]. FHC phosphorylation seems to be necessary to disrupt CXCR4 signaling as a phosphorylation deficient mutant did not exhibit inhibitory effects [108]. These findings also suggest that phosphorylation of FHC is important for subcellular localization; however, the role of nuclear FHC in CXCR4 signaling is still unclear. The up-regulation of FHC by morphine inhibited CXCR4 signaling, but it is unknown if the increase in total FHC levels are associated with increased nuclear translocation of this protein; our lab is currently investigating this issue. We are also interested in studying the effect of gp120 on nuclear translocation of FHC. Since gp120 has been shown to inhibit CXCR4 function, changes in FHC cellular localization may be one way it disrupts CXCR4.

The relationship between ferritin and the tumor suppressor p53 is one that could prove to be essential in HIV neuropathology. Though the direction of the association is unclear, evidence suggests that ferritin expression is regulated by p53 in cell lines [127, 128]. Furthermore, FHC was found to bind to p53, activate its transcription, and stabilize p53 protein levels under oxidative stress [129]. The activation of p53 can lead to a variety of cellular responses, including apoptosis. The viral protein gp120, through binding to CXCR4, induces p53 activity and its proapoptotic target Apaf-1 in neurons; the CXCR4 inhibitor AMD3100 attenuated gp120-stimulated p53 and Apaf-1 effects [130]. It remains to be seen whether morphine-induced changes in FHC levels cause a direct association with p53 and if this activates downstream pro-apoptotic targets of p53. If it does, this could be a mechanism by which opiates and gp120 cause greater neuronal injury [3] and neurological symptoms in drug-abusing HIV patients.

Secretion of TNF- α and IL-1 β by HIV-1 infected microglia and macrophages has been well characterized. These inflammatory cytokines, in the context of HIV infection alone, can cause neuronal damage through TNF- α receptors and activation of NMDA receptors [30]. Co-treatment of morphine and the viral protein, tat, was previously found to enhance microglia activation and thus, the secretion of these inflammatory cytokines. Interestingly, TNF- α and IL-1 β both alter the expression of FHC in certain cell types. IL-1 β was found to decrease the level of surface transferrin receptor in a monocyte cell line [131], while increasing the secretion of ferritin from hepatoma cells [132]. TNF- α also exhibited these properties and upregulated levels of FHC mRNA and protein [131]. TNF- α mediated upregulation of FHC is iron-independent [133] and increased during apoptosis [134].

The effects of TNF- α and IL-1 β on the expression of FHC have not been investigated in the brain. It seems feasible that these cytokines could alter FHC levels in both neurons and microglia in HIV-infected patients. Release of TNF- α and IL-1 β from infected macrophages in the CNS could increase FHC levels in neurons, which in turn would disrupt the neuronal survival signaling properties of CXCR4, similar to the effect of morphine. Additionally, the cytokines could stimulate the secretion of ferritin from macrophages, which neurons could take up *via* the transferrin receptor or another receptor. It would be important to investigate potential differences between neurons and glia cells in their response to these cytokines. It is

possible that the different cell types in the CNS could respond distinctively and exhibit varying abilities in regulating FHC levels. A potential up-regulation of FHC by TNF- α and IL-1 β , either through increased transcription/translation or uptake of serum ferritin, could explain the higher levels of FHC (and decreased levels of phosphorylated CXCR4) we found in neurons of opiate-abusing HIV patients [119, Pitcher *et al.* unpublished data]. Interestingly, nuclear ferritin is upregulated by TNF- α and IL-1 β [125], and thus, it would be important to see if this occurs in neurons, and, if it does, could it cause inhibition of CXCR4 signaling?

Viral proteins and morphine in the brain could alter levels of FHC through different mechanisms, which could then converge on disruption of CXCR4. Inability of CXCL12 to signal through CXCR4 significantly decreases proper neuronal signaling and survival, potentially altering important repair mechanisms that can limit the neurocognitive deficits seen in patients with HAND. Fig. (1) presents potential pathways that viral proteins and inflammatory cytokines may use to regulate FHC levels, as well as our current understanding of opiate-induced elevated FHC expression. These cellular pathways regulating FHC may contribute to neuronal dysfunction in HIV/DU patients.

CONCLUSIONS

The mechanisms mediating HIV- and morphine-induced neuropathology are intricate, involving many different pathways and causing significant damage on their own. Opiate-abusers who are also HIV-positive are at a greater risk for neurological dysfunction, as it appears that there is at least an additive effect of morphine and viral proteins in the CNS, though the pathways implicated in this have been unclear thus far. The emergence of FHC as a mediator of neuronal function and survival, however, may prove to be important in understanding HIV-1 neuropathogenesis in the context of drug abuse. Future studies should examine the role of HIV-1 infection in FHC regulation, as it is possible that viral proteins and inflammatory cytokines may regulate its transcription and translation. Additionally, further understanding of the pathological mechanisms specific to neuronal FHC, as well as the role of elevated FHC in opiate-induced neuronal dysfunction, will be important.

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References

1. Prejean J, Song R, Hernandez A, et al. Estimated HIV Incidence in the United States, 2006–2009. *PLoS One*. 2011; 6:e17502. [PubMed: 21826193]
2. Center for Disease Control. Drug-associated HIV Transmission Continues in the United States. Atlanta: US Department of Health and Human Services; 2007.
3. Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine potentiates HIV-1 gp120-induced neuronal apoptosis. *J Infect Dis*. 2005; 191:886–9. [PubMed: 15717263]
4. Hauser KF, El-Hage N, Buch S, et al. Impact of opiate-HIV-1 interactions on neurotoxic signaling. *J Neuroimmune Pharmacol*. 2006; 1:98–105. [PubMed: 18040795]
5. Bokhari SM, Hedge R, Callen S, et al. Morphine Potentiates Neuropathogenesis of SIV Infection in Rhesus Macaques. *J Neuroimmune Pharmacol*. 2011; 6:626–39. [PubMed: 21431470]

6. Cooper DA. Life and death in the cART era. *Lancet*. 2008; 372:266–7. [PubMed: 18657689]
7. Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet*. 2008; 372:293–8. [PubMed: 18657708]
8. Gonzalez-Scarano F, Martin-Garcia J. The neuropathogenesis of AIDS. *Nat Rev Immunol*. 2005; 5:69–81. [PubMed: 15630430]
9. Vivithanaporn P, Gill MJ, Power C. Impact of current antiretroviral therapies on neuroAIDS. *Expert Rev Anti Infect Ther*. 2011; 9:371–4. [PubMed: 21504390]
10. Lawrence DM, Major EO. HIV-1 and the brain: connections between HIV-1 associated dementia, neuropathology and neuroimmunology. *Microbes Infect*. 2002; 4:301–8. [PubMed: 11909740]
11. Haase AT. Pathogenesis of lentivirus infections. *Nature*. 1986; 322:130–6. [PubMed: 2425264]
12. Ghafouri M, Amini S, Khalili K, Sawaya BE. HIV-1 associated dementia: symptoms and causes. *Retrovirology*. 2006; 3:28. [PubMed: 16712719]
13. Reger M, Welsh R, Razani J, Martin DJ, Boone KB. A meta-analysis of the neuropsychological sequelae of HIV infection. *J Int Neuropsychol Soc*. 2002; 8:410–24. [PubMed: 11939699]
14. Ances BM, Ellis RJ. Dementia and neurocognitive disorders due to HIV-1 infection. *Semin Neurol*. 2007; 27:86–92. [PubMed: 17226745]
15. Antinori A, Arendt G, Becker JT, et al. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology*. 2007; 69:1798–9.
16. Vivithanaporn P, Heo G, Gamble J, et al. Neurologic disease burden in treated HIV/AIDS predicts survival: a population-based study. *Neurology*. 2010; 75:1150–8. [PubMed: 20739646]
17. Kaul M, Garden GA, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature*. 2001; 410:988–94. [PubMed: 11309629]
18. McArthur JC, Steiner J, Sacktor N, Nath A. Human immunodeficiency virus-associated neurocognitive disorders: Mind the gap. *Ann Neurol*. 2010; 67:699–714. [PubMed: 20517932]
19. McArthur JC, Sacktor N, Selnes O. Human immunodeficiency virus-associated dementia. *Semin Neurol*. 1999; 19:129–50. [PubMed: 10718534]
20. Glass JD, Johnson RT. Human immunodeficiency and the brain. *Annu Rev Neurosci*. 1996; 19:1–26. [PubMed: 8833434]
21. Masliah E, Ge N, Achim CL, Hansen LA, Wiley CA. Selective neuronal vulnerability in HIV encephalitis. *J Neuropathol Exp Neurol*. 1992; 51:585–93. [PubMed: 1484289]
22. Thompson KA, McArthur JC, Wesselingh SL. Correlation between neurological progression and astrocyte apoptosis in HIV-associated dementia. *Ann Neurol*. 2001; 49:745–52. [PubMed: 11409426]
23. Masliah E, Heaton RK, Marcotte TD, et al. Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC Group. The HIV Neurobehavioral Research Center. *Ann Neurol*. 1997; 42:963–72. [PubMed: 9403489]
24. Zhang L, Looney D, Taub D, et al. Cocaine opens the blood-brain barrier to HIV-1 invasion. *J Neurovirol*. 1998; 4:619–26. [PubMed: 10065903]
25. Tomlinson GS, Simmonds P, Busuttill A, Chiswick A, Bell JE. Upregulation of microglia in drug users with and without pre-symptomatic HIV infection. *Neuropathol Appl Neurobiol*. 1999; 25:369–79. [PubMed: 10564526]
26. Anthony IC, Ramage SN, Carnie FW, Simmonds P, Bell JE. Does drug abuse alter microglial phenotype and cell turnover in the context of advancing HIV infection? *Neuropathol Appl Neurobiol*. 2005; 31:325–38. [PubMed: 15885069]
27. Bell JE, Arango JC, Anthony IC. Neurobiology of multiple insults: HIV-1-associated brain disorders in those who use illicit drugs. *J Neuroimmune Pharmacol*. 2006; 1:182–91. [PubMed: 18040783]
28. Brew BJ, Bhalla RB, Fleisher M, et al. Cerebrospinal fluid beta 2 microglobulin in patients infected with human immunodeficiency virus. *Neurology*. 1989; 39:830–4. [PubMed: 2657485]
29. Lindl KA, Marks DR, Kolson DL, Jordan-Scuitto KL. HIV-associated neurocognitive disorder: pathogenesis and therapeutic opportunities. *J Neuroimmune Pharmacol*. 2010; 5:294–309. [PubMed: 20396973]

30. Genis P, Jett M, Bernton EW, et al. Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease. *J Exp Med.* 1992; 176:1703–18. [PubMed: 1460427]
31. Giulian D, Wendt E, Vaca K, Noonan CA. The envelope glycoprotein of human immunodeficiency virus type 1 stimulate release of neurotoxins from monocytes. *Proc Natl Acad Sci USA.* 1993; 90:2769–73. [PubMed: 8464887]
32. Lipton SA. AIDS-related dementia and calcium homeostasis. *Ann NY Acad Sci.* 1994; 747:205–24. [PubMed: 7847672]
33. Dreyer EB, Kaiser PK, Offermann JT, Lipton SA. HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science.* 1990; 248:364–7. [PubMed: 2326646]
34. Dawson VL, Dawson TM, Uhl GR, Snyder SH. Human immunodeficiency virus type 1 coat protein neurotoxicity mediated by nitric oxide in primary cortical cultures. *Proc Natl Acad Sci USA.* 1993; 90:3256–9. [PubMed: 8097316]
35. Medders KE, Sejbuk NE, Maung R, Desai MK, Kaul M. Activation of p38 MAPK is required in monocytic and neuronal cells for HIV glycoprotein 120-induced neurotoxicity. *J Immunol.* 2010; 185:4883–95. [PubMed: 20855878]
36. Singh IN, El-Hage N, Campbell ME, et al. Differential involvement of p38 and JNK MAP kinases in HIV-1 Tat and gp120-induced apoptosis and neurite degeneration in striatal neurons. *Neuroscience.* 2005; 135:781–90. [PubMed: 16111829]
37. Garden GA, Budd SL, Tsai E, et al. Caspase cascades in human immunodeficiency virus-associated neurodegeneration. *J Neurosci.* 2002; 22:4015–24. [PubMed: 12019321]
38. Acquas E, Bachis A, Nosheny RL, Cernak I, Mocchetti I. Human immunodeficiency virus type 1 protein gp120 causes neuronal cell death in the rat brain by activating caspases. *Neurotox Res.* 2004; 5:605–15. [PubMed: 15111237]
39. Bachis A, Major EO, Mocchetti I. Brain-derived neurotrophic factor inhibits human immunodeficiency virus-1/gp120-mediated cerebellar granule cell death by preventing gp120 internalization. *J Neurosci.* 2003; 23:5715–22. [PubMed: 12843275]
40. Nosheny RL, Bachis A, Acquas E, Mocchetti I. Human immunodeficiency virus type 1 glycoprotein gp120 reduces the level of brain-derived neurotrophic factor *in vivo*: potential implication for neuronal cell death. *Eur J Neurosci.* 2004; 20:2857–64. [PubMed: 15579139]
41. Pulliam L, Zhou M, Stubblebine M, Bitler CM. Differential modulation of cell death proteins in human brain cells by tumor necrosis factor alpha and platelet activating factor. *J Neurosci Res.* 1998; 54:530–8. [PubMed: 9822163]
42. Meucci O, Miller RJ. gp120-induced neurotoxicity in hippocampal pyramidal neuron cultures: protective action of TGF-beta 1. *J Neurosci.* 1996; 16:4080–8. [PubMed: 8753870]
43. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Immunol.* 1998; 16:137–61. [PubMed: 9597127]
44. Wesselingh SL, Power C, Glass JD, et al. Intracerebral cytokine messenger RNA expression in acquired immunodeficiency syndrome dementia. *Ann Neurol.* 1993; 33:576–82. [PubMed: 8498837]
45. Wiley CA, Baldwin M, Achim CL. Expression of HIV regulatory and structural mRNA in the central nervous system. *AIDS.* 1996; 10:843–7. [PubMed: 8828741]
46. Bonavia R, Bajetto A, Barbero S, Albin A, Noonan DM, Schettini G. HIV-1 Tat causes apoptotic death and calcium homeostasis alterations in rat neurons. *Biochem Biophys Res Commun.* 2001; 288:301–8. [PubMed: 11606043]
47. Magnuson DS, Knudsen BE, Geiger JD, Brownstone RM, Nath A. Human immunodeficiency virus type 1 tat activates non-N-methyl-D-aspartate excitatory amino acid receptors and causes neurotoxicity. *Ann Neurol.* 1995; 37:373–80. [PubMed: 7695237]
48. Cheng J, Nath A, Knudsen B, et al. Neuronal excitatory properties of human immunodeficiency virus type Tat protein. *Neuroscience.* 1998; 82:97–106. [PubMed: 9483506]
49. Haughey NJ, Nath A, Mattson MP, Slevin JT, Geiger JD. HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. *J Neurochem.* 2001; 78:457–67. [PubMed: 11483648]

50. O'Donnell LA, Agrawal A, Jordan-Sciutto KL, et al. Human immunodeficiency virus (HIV)-induced neurotoxicity: roles for the NMDA receptor subtypes. *J Neurosci.* 2006; 26:981–90. [PubMed: 16421318]
51. Haughey NJ, Holden CP, Nath A, Geiger JD. Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. *J Neurochem.* 1999; 73:1363–74. [PubMed: 10501179]
52. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature.* 2001; 412:95–9. [PubMed: 11452314]
53. Singh IN, Goody RJ, Dean C, et al. Apoptotic death of striatal neurons induced by human immunodeficiency virus-1 Tat and gp120: Differential involvement of caspase-2 and endonuclease G. *J Neurovirol.* 2004; 10:141–51. [PubMed: 15204919]
54. Liu Y, Jones M, Hingtgen CM, et al. Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. *Nat Med.* 2000; 6:1380–7. [PubMed: 11100124]
55. Eugenin EA, King JE, Nath A, et al. HIV-tat induces formation of an LRP-PSD-95-NMDAR-nNOS complex that promotes apoptosis in neurons and astrocytes. *Proc Natl Acad Sci USA.* 2007; 104:3438–43. [PubMed: 17360663]
56. Berger EA, Doms RW, Fenyo EM, et al. A new classification for HIV-1. *Nature.* 1998; 391:240. [PubMed: 9440686]
57. Hesselgesser J, Halks-Miller M, DelVecchio V, et al. CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons. *Curr Biol.* 1997; 7:112–21. [PubMed: 9024623]
58. Clapham PR, McKnight A. HIV-1 receptors and cell tropism. *Br Med Bull.* 2001; 58:43–59. [PubMed: 11714623]
59. Petito CK, Roberts B, Cantando JD, Rabinstein A, Duncan R. Hippocampal injury and alterations in neuronal chemokine co-receptor expression in patients with AIDS. *J Neuropathol Exp Neurol.* 2001; 60:377–85. [PubMed: 11305873]
60. Lazarini F, Tham TN, Casanova P, Arenzana-Seisdedos F, Dubois-Dalcq M. Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. *Glia.* 2003; 42:138–48.
61. Li M, Ransohoff RM. Multiple roles of chemokine CXCL12 in the central nervous system: a migration from immunology to neurobiology. *Prog Neurobiol.* 2008; 84:116–31. [PubMed: 18177992]
62. Khan MZ, Brandimarti R, Patel JP, et al. Apoptotic and antiapoptotic effects of CXCR4: is it a matter of intrinsic efficacy? Implications for HIV neuropathogenesis. *AIDS Res Hum Retroviruses.* 2004; 20:1063–71. [PubMed: 15585097]
63. Khan MZ, Brandimarti R, Musser BJ, Resue DM, Fatatis A, Meucci O. The chemokine receptor CXCR4 regulates cell-cycle proteins in neurons. *J Neurovirol.* 2003; 9:300–14. [PubMed: 12775414]
64. Khan MZ, Brandimarti R, Shimizu S, Nicolai J, Crowe E, Meucci O. The chemokine CXCL12 promotes survival of postmitotic neurons by regulating Rb protein. *Cell Death Differ.* 2008; 15:1663–72. [PubMed: 18583990]
65. Cardone MH, Roy N, Stennicke HR, et al. Regulation of cell death protease caspase-9 by phosphorylation. *Science.* 1998; 282:1318–21. [PubMed: 9812896]
66. Nicolai J, Burbassi S, Rubin J, Meucci O. CXCL12 inhibits expression of NMDA receptor's NR2B subunit through a histone deacetylase-dependent pathway contributing to neuronal survival. *Cell Death Dis.* 2010; 1:e33. [PubMed: 21364640]
67. Zhang K, McQuibban GA, Silva C, et al. HIV-induced metalloproteinase processing of the chemokine stromal cell derived factor-1 causes neurodegeneration. *Nat Neurosci.* 2003; 6:1064–71. [PubMed: 14502291]
68. Vergote D, Butler GS, Ooms M, et al. Proteolytic processing of SDF-1alpha reveals a change in receptor specificity mediating HIV-associated neurodegeneration. *Proc Natl Acad Sci USA.* 2006; 103:19182–7. [PubMed: 17148615]

69. Lazarini F, Casanova P, Tham TN, et al. Differential signalling of the chemokine receptor CXCR4 by stromal cell-derived 1 and the HIV glycoprotein in rat neurons and astrocytes. *Eur J Neurosci.* 2000; 12:117–25. [PubMed: 10651866]
70. Toth PT, Ren D, Miller RJ. Regulation of CXCR4 receptor dimerization by chemokine SDF-1 α and the HIV-1 coat protein gp120: a fluorescence resonance energy transfer (FRET) study. *J Pharmacol Exp Ther.* 2004; 310:8–17. [PubMed: 15014135]
71. Bardi G, Sengupta R, Khan MZ, Patel JP, Meucci O. Human immunodeficiency virus gp120-induced apoptosis of human neuroblastoma cells in the absence of CXCR4 internalization. *J Neurovirol.* 2006; 12:211–18. [PubMed: 16877302]
72. Shimizu S, Khan MZ, Hippensteel RL, Parker A, Raghupathi R, Meucci O. Role of transcription factor E2F1 in CXCR4-mediated neurotoxicity and HIV neuropathology. *Neurobiol Dis.* 2007; 25:17–26. [PubMed: 17011204]
73. Meucci O, Fatatis A, Simen AA, Bushell TJ, Gray PW, Miller RJ. Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proc Natl Acad Sci USA.* 1998; 95:14500–5. [PubMed: 9826729]
74. Pandey V, Bolsover SR. Immediate and neurotoxic effects of HIV protein gp120 act through CXCR4 receptor. *Biochem Biophys Res Commun.* 2000; 274:212–5. [PubMed: 10903920]
75. Peterson PK, Molitor TW, Chao CC. The opioid-cytokine connection. *J Neuroimmunol.* 1998; 83:63–9. [PubMed: 9610674]
76. Raehal KM, Bohn LM. Mu opioid receptor regulation and opiate responsiveness. *AAPS J.* 2005; 7:E587–91. [PubMed: 16353937]
77. Zhang Y, Chen Q, Yu LC. Morphine: a protective or destructive role in neurons? *Neuroscientist.* 2008; 14:561–70. [PubMed: 18349442]
78. Pasternak GW. Multiple opiate receptors: déjà v all over again. *Neuropharmacology.* 2004; 47 (Suppl 1):312–23. [PubMed: 15464147]
79. Raynor K, Kong H, Chen Y, et al. Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Mol Pharmacol.* 1994; 45:330–4. [PubMed: 8114680]
80. Matthes HW, Maldonado R, Simonin F, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature.* 1996; 383:819–23. [PubMed: 8893006]
81. Kieffer BL. Opioids: first lessons from knockout mice. *Trends Pharmacol Sci.* 1999; 20:19–26. [PubMed: 10101958]
82. Polakiewicz RD, Schiefer SM, Gingras AC, Sonenberg N, Comb MJ. mu-Opioid receptor activates signaling pathways implicated in cell survival and translational control. *J Biol Chem.* 1998; 273:23534–41. [PubMed: 9722592]
83. Kramer HK, Simon EJ. mu and delta-opioid receptor agonists induce mitogen-activated protein kinase (MAPK) activation in the absence of receptor internalization. *Neuropharmacology.* 2000; 39:1707–19. [PubMed: 10884553]
84. Meriney SD, Gray DB, Pilar G. Morphine-induced delay of normal cell death in the avian ciliary ganglion. *Science.* 1985; 228:1451–3. [PubMed: 2990029]
85. Ferrer-Alcón M, Garcia-Fuster MJ, La Harpe R, Garcia-Sevilla JA. Long-term regulation of signaling components of adenylyl cyclase and mitogen-activated protein kinase in the pre-frontal cortex of human opiate addicts. *J Neurochem.* 2004; 90:220–30. [PubMed: 15198681]
86. Atici S, Cinel L, Cinel I, et al. Opioid neurotoxicity: comparison of morphine and tramadol in an experimental rat model. *Int J Neurosci.* 2004; 114:1001–11. [PubMed: 15527204]
87. Mao J, Sung B, Ji RR, Lim G. Neuronal apoptosis associated with morphine tolerance: evidence for an opioid-induced neurotoxic mechanism. *J Neurosci.* 2002; 22:7650–61. [PubMed: 12196588]
88. Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology.* 2002; 42:829–36. [PubMed: 12015209]
89. Fan XL, Zhang JS, Zhang XQ, Ma L. Chronic morphine treatment and withdrawal induce up-regulation of c-Jun N-terminal kinase 3 gene expression in rat brain. *Neuroscience.* 2003; 122:997–1002. [PubMed: 14643766]
90. Emeterio EP, Tramullas M, Hurle MA. Modulation of apoptosis in the mouse brain after morphine treatments and morphine withdrawal. *J Neurosci Res.* 2006; 83:1352–61. [PubMed: 16496378]

91. Yin D, Woodruff M, Zhang Y, et al. Morphine promotes Jurkat cell apoptosis through pro-apoptotic FADD/P53 and anti-apoptotic PI3K/Akt/NF-kappaB pathways. *J Neuroimmunol.* 2006; 174:101–7. [PubMed: 16529824]
92. Lamprecht R, LeDoux J. Structural plasticity and memory. *Nat Rev Neurosci.* 2004; 4:45–54. [PubMed: 14708003]
93. Segal M. Dendritic spines, synaptic plasticity and neuronal survival: activity shapes dendritic spines to enhance neuronal viability. *Eur J Neurosci.* 2010; 31:2178–84. [PubMed: 20550565]
94. Robinson TE, Kolb B. Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. *Synapse.* 1999; 33:160–6. [PubMed: 10400894]
95. Li Y, Wang H, Niu L, Zhou Y. Chronic morphine exposure alters the dendritic morphology of pyramidal neurons in visual cortex of rats. *Neurosci Lett.* 2007; 418:227–31. [PubMed: 17466454]
96. Liao D, Lin H, Law PY, Loh HH. Mu-opioid receptors modulate stability of dendritic spines. *Proc Natl Acad Sci USA.* 2005; 102:1725–30. [PubMed: 15659552]
97. Zheng H, Zeng Y, Chu J, Kam AY, Loh HH, Law PY. Modulations of NeuroD activity contributes to the differential effects of morphine and fentanyl on dendritic spine stability. *J Neurosci.* 2010; 30:8102–10. [PubMed: 20554861]
98. Liao D, Grigoriants OO, Wang W, Wiens K, Loh HH, Law PY. Distinct effects of individual opioids on the morphology of spines depend on upon the internalization of mu opioid receptors. *Mol Cell Neurosci.* 2007; 35:456–69. [PubMed: 17513124]
99. Liu Y, Blackburn DJ, Chuang LF, Killam KF Jr, Chuang RY. Effects of *in vivo* and *in vitro* administration of morphine sulfate upon rhesus macaque polymorphonuclear cell phagocytosis and chemotaxis. *J Pharmacol Exp Ther.* 1992; 263:533–9. [PubMed: 1432688]
100. Rogers TJ, Steele AD, Howard OM, Oppenheim JJ. Bidirectional heterologous desensitization of opioid and chemokine receptors. *Ann NY Acad Sci.* 2000; 917:19–28. [PubMed: 11268344]
101. Chen C, Li J, Bot G, Szabo I, Rogers TJ, Liu-Chen LY. Heterodimerization and cross-desensitization between the mu-opioid receptor and the chemokine CCR5 receptor. *Eur J Pharmacol.* 2004; 483:175–86. [PubMed: 14729105]
102. Szabo I, Chen XH, Xin L, et al. Heterologous desensitization of opioid receptors by chemokines inhibits chemotaxis and enhances the perception of pain. *Proc Nat Acad Sci USA.* 2002; 99:10276–81. [PubMed: 12130663]
103. Grimm MC, Ben-Baruch A, Taub DD, et al. Opiates transdeactivate chemokine receptors: delta and mu opiate receptor-mediated heterologous desensitization. *J Exp Med.* 1998; 188:317–25. [PubMed: 9670044]
104. Steele AD, Szabo I, Bednar F, Rogers TJ. Interactions between opioid and chemokine receptors: heterologous desensitization. *Cytokine Growth Factor Rev.* 2002; 13:209–22. [PubMed: 12486875]
105. Patel JP, Sengupta R, Bardi G, Khan MZ, Mullen-Przeworski A, Meucci O. Modulation of neuronal CXCR4 by the mu-opioid agonist DAMGO. *J Neurovirol.* 2006; 12:492–500. [PubMed: 17162664]
106. Sengupta R, Burbassi S, Shimizu S, et al. Morphine increases brain levels of ferritin heavy chain leading to inhibition of CXCR4-mediated survival signaling in neurons. *J Neurosci.* 2009; 29:2534–44. [PubMed: 19244528]
107. Mahajan SD, Schwartz SA, Aalinkeel R, Chawda RP, Sykes DE, Nair MP. Morphine modulates chemokine gene regulation in normal human astrocytes. *Clin Immunol.* 2005; 115:323–32. [PubMed: 15893700]
108. Li R, Luo C, Mines M, Zhang J, Fan GH. Chemokine CXCL12 induces binding of ferritin heavy chain to the chemokine receptor CXCR4, alters CXCR4 signaling, and induces phosphorylation and nuclear translocation of ferritin heavy chain. *J Biol Chem.* 2006; 281:37616–27. [PubMed: 17056593]
109. Hintze KJ, Theil EC. Cellular regulation and molecular interactions of the ferritins. *Cell Mol Life Sci.* 2006; 63:591–600. [PubMed: 16465450]
110. Torti FM, Torti SV. Regulation of ferritin genes and proteins. *Blood.* 2002; 99:3505–16. [PubMed: 11986201]

111. White K, Munro HN. Induction of ferritin subunit synthesis by iron is regulated at both the transcriptional and translational levels. *J Biol Chem.* 1988; 263:8938–42. [PubMed: 3379054]
112. Friedman A, Arosio P, Finazzi D, Koziorowski D, Galazka-Friedman J. Ferritin as an important player in neurodegeneration. *Parkinsonism Relat Disord.* 2011; 17:423–30. [PubMed: 21550835]
113. Li L, Fang CJ, Ryan JC, et al. Binding and uptake of H-ferritin are mediated by human transferrin receptor-1. *Proc Natl Acad Sci USA.* 2010; 107:3505–10. [PubMed: 20133674]
114. Surguladze N, Thompson KM, Beard JL, Connor JR, Fried MG. Interactions and reactions of ferritin with DNA. *J Biol Chem.* 2004; 279:14694–702. [PubMed: 14734543]
115. Connor JR, Boyer PJ, Menzies SL, et al. Neuropathological examination suggests impaired brain iron acquisition in restless leg syndrome. *Neurology.* 2003; 61:304–9. [PubMed: 12913188]
116. Quintana C, Guitierrez L. Could a dysfunction of ferritin be a determinant factor in the aetiology of some neurodegenerative diseases? *Biochim Biophys Acta.* 2010; 1800:770–82. [PubMed: 20447447]
117. Gotz ME, Double K, Gerlach M, Youdim MB, Riederer P. The relevance of iron in the pathogenesis of Parkinson's disease. *Ann NY Acad Sci.* 2004; 1012:193–208. [PubMed: 15105267]
118. Deisenhammer F, Miller RF, Brink NS, Harrison MJ, Thompson EJ. Cerebrospinal fluid ferritin in HIV infected patients with acute neurological episodes. *Genitourin Med.* 1997; 73:181–3. [PubMed: 9306897]
119. Pitcher J, Shimizu S, Burbassi S, Meucci O. Disruption of neuronal CXCR4 function by opioids: preliminary evidence of ferritin heavy chain as a potential etiological agent in neuroAIDS. *J Neuroimmunol.* 2010; 224:66–71. [PubMed: 20627326]
120. Avdoshina V, Biggio F, Palchik G, Campbell LA, Mocchetti I. Morphine induces the release of CCL5 from astrocytes: potential neuroprotective mechanism against the HIV protein gp120. *Glia.* 2010; 58:1630–9. [PubMed: 20578038]
121. Bokhari SM, Yao H, Bethel-Brown C, et al. Morphine enhances Tat-induced activation in murine microglia. *J Neurovirol.* 2009; 15:219–28. [PubMed: 19462331]
122. Fitting S, Xu R, Bull C, et al. Interactive comorbidity between opioid drug abuse and HIV-1 Tat: chronic exposure augments spine loss and sublethal dendritic pathology in striatal neurons. *Am J Pathol.* 2010; 177:1397–410. [PubMed: 20651230]
123. Gurwell JA, Nath A, Sun Q, et al. Synergistic neurotoxicity of opioids and human immunodeficiency virus-1 Tat protein in striatal neurons *in vitro*. *Neuroscience.* 2001; 102:555–63. [PubMed: 11226693]
124. Pountney D, Trugnan G, Bourgeois M, Beaumont C. The identification of ferritin in the nucleus of K562 cells, and investigation of a possible role in the transcriptional regulation of adult beta-globin gene expression. *J Cell Sci.* 1999; 112:825–31. [PubMed: 10036232]
125. Thompson KJ, Fried MG, Ye Z, Boyer P, Connor JR. Regulation, mechanisms and proposed function of ferritin translocation to cell nuclei. *J Cell Sci.* 2002; 2165–77. [PubMed: 11973357]
126. Surguladze N, Patton S, Cozzi A, Fried MG, Connor JR. Characterization of nuclear ferritin and mechanisms of translocation. *Biochem J.* 2005; 388:731–40. [PubMed: 15675895]
127. Faniello MC, Di Sanzo M, Quaresima B, et al. p53-mediated downregulation of H ferritin promoter transcriptional efficiency *via* NF-Y. *Int J Biochem Cell Biol.* 2008; 40:2110–9. [PubMed: 18372207]
128. Zhang F, Wang W, Tsuji Y, Torti SV, Torti FM. Post-transcriptional modulation of iron homeostasis during p53-dependent growth arrest. *J Biol Chem.* 2008; 283:33911–8. [PubMed: 18819919]
129. Lee JH, Jang H, Cho EJ, Youn HD. Ferritin binds and activates p53 under oxidative stress. *Biochem Biophys Res Commun.* 2009; 389:399–4. [PubMed: 19716362]
130. Khan MZ, Shimizu S, Patel JP, et al. Regulation of neuronal P53 activity by CXCR4. *Mol Cell Neurosci.* 2005; 30:58–66. [PubMed: 16005638]
131. Fahmay M, Young SP. Modulation of iron metabolism in monocyte cell line U937 by inflammatory cytokines: changes in transferrin uptake, iron handling and ferritin mRNA. *Biochem J.* 1993; 296:175–81. [PubMed: 8250840]

132. Tran TN, Eubanks SK, Schaffer KJ, Zhou CY, Linder MC. Secretion of ferritin by rat hepatoma cells and its regulation by inflammatory cytokines and iron. *Blood*. 1997; 90:4979–86. [PubMed: 9389717]
133. Miller LL, Miller SC, Torti SV, Tsuji Y, Torti FM. Iron-independent induction of ferritin H chain by tumor necrosis factor. *Proc Natl Acad Sci USA*. 1991; 88:4946–50. [PubMed: 2052577]
134. Cozzi A, Levi S, Corsi B, et al. Role of iron and ferritin in TNFalpha-induced apoptosis in HeLa cells. *FEBS Lett*. 2003; 537:187–92. [PubMed: 12606055]

Fig. 1.

Simplified schematic of cellular pathways regulating FHC that may contribute to neuronal dysfunction in HIV/DU patients: In addition to the classical iron-dependent pathways and uptake of extracellular protein, intracellular levels of FHC can be regulated by various external factors, such as opiates and HIV-1 infection. While morphine-induced FHC changes appear to be primarily mediated by post-transcriptional mechanisms, pro-inflammatory cytokines that are typically elevated by HIV infection ($\text{TNF-}\alpha$ and $\text{IL-1}\beta$) can stimulate both transcription and translation of FHC, as reported in monocytes and other cells. FHC inhibits homeostatic protective signaling mediated by the CXCL12/CXCR4 axis

in neurons, without inducing significant alterations of CXCR4 expression – thus, leaving the co-receptor available for interaction with the viral protein gp120. It is still unclear whether viral proteins such as gp120 can also influence FHC expression. Furthermore, FHC is also found in the nucleus where it is involved in protection of DNA from oxidative stress and, possibly, transcriptional regulation. However, it is currently unknown what controls FHC nuclear import/export and whether FHC interaction with CXCR4 alters this process. Overall, opiates and viral proteins can synergistically alter FHC, acting at multiple steps. This may ultimately lead to neuronal dysfunction or injury caused by loss of neuroprotective mechanisms in a highly toxic inflammatory environment, such as the HIV-infected brain (MOR: m-opioid receptor; DU: drug users).