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Pathogenesis of HIV-associated sensory neuropathy: evidence from *in vivo* and *in vitro* experimental models

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Abstract

HIV-associated sensory neuropathy (HIV-SN) is a frequent neurological complication of HIV infection and its treatment with some antiretroviral drugs. We review the pathogenesis of the viral-induced and drug-induced causes of the neuropathy, and its primary symptom, pain, based on evidence from *in vivo* and *in vitro* models of HIV-SN. Viral coat proteins mediate nerve fibre damage and hypernociception through direct and indirect mechanisms. Direct interactions between viral proteins and nerve fibres dominate axonal pathology, while somal pathology is dominated by indirect mechanisms that occur secondary to virus-mediated activation of glia and macrophage infiltration into the dorsal root ganglia. The treatment-induced neuropathy and resulting hypernociception arise primarily from drug-induced mitochondrial dysfunction, but the sequence of events initiated by the mitochondrial dysfunction that leads to the nerve fibre damage and dysfunction still are unclear. Overall, the models that have been developed to study the pathogenesis of HIV-SN and hypernociception associated with the neuropathy are reasonable models of HIV-SN, and have provided useful insights in to the pathogenesis HIV-SN. As the models are developed they may ultimately lead to identification of therapeutics targets for the prevention or treatment of this common neurological complication of HIV infection.

Keywords

HIV-associated sensory neuropathy (HIV-SN), antiretroviral toxic neuropathy (ATN), gp120, neuropathic pain, axonal degeneration, intra-epidermal nerve fibre, highly-active antiretroviral therapy (HAART), nucleoside reverse transcriptase inhibitor (NRTI).

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Introduction

Between 30 and 60% of individuals infected with the human immunodeficiency virus (HIV) develop HIV-associated sensory neuropathy (HIV-SN), a peripheral sensory neuropathy that is frequently painful, and is characterised by length-dependent degeneration of myelinated and unmyelinated nerve fibres (*Keswani, et al., 2002; Ferrari, et al., 2006*). The virus, the immune response to the virus, and antiretroviral drugs, in particular the nucleoside reverse transcriptase inhibitors (NRTIs), are all potentially neurotoxic, and these three factors, acting alone or in combination, are probably the most important mediators of HIV-SN (*Keswani, et al., 2002; Ferrari, et al., 2006*). However, our knowledge of how these factors damage sensory neurones is limited, which has impeded the development of effective prophylactic or therapeutic interventions for HIV-SN. Consequently, HIV-SN continues to be a major cause of disability and an area of therapeutic need despite the introduction of less neurotoxic treatment regimens, and improved virologic control (*Smyth, et al., 2007; Ellis, et al., 2010; Maritz, et al., 2010; Phillips, et al., 2010*).

In the last decade, significant progress has been made in developing and characterising *in vivo* and *in vitro* experimental models of HIV-SN. Here we review studies that have investigated primary lesions to the peripheral nervous system by HIV or antiretroviral drugs. We discuss whether the *in vivo* models adequately reflect the pathological processes that occur in HIV-SN, and how data from *in vivo* and *in vitro* studies have advanced our understanding of the pathophysiology of HIV-SN and its primary symptom, pain. We do not provide a detailed description of the animal models of HIV-SN that have been developed since they have recently been reviewed (*Bhangoo, et al., 2010*). The review is divided into two parts: part one describes experimental models of HIV-mediated neuropathy, part two describes experimental models of antiretroviral drug-induced neuropathy and the neurotoxic interaction that occurs between the virus and antiretroviral drugs.

Virus-mediated neurotoxicity in the peripheral nervous system

HIV-associated sensory neuropathy is characterised by distal axonal degeneration and reduced nerve fibre density along the peripheral nerve trunk (*de la Monte, et al., 1988; Chaunu, et al., 1989; Rizzuto, et al., 1995*), neuronal loss in the dorsal root ganglia (DRG) (*Rance, et al., 1988*), and dying-back of peripheral and central terminals of peripheral nerve fibres (*Rance, et al., 1988; Holland, et al., 1997; Polydefkis, et al., 2002*). Even in the absence of clinical features of HIV-SN, post-mortem neuro-anatomical studies have demonstrated immune cell infiltration and inflammatory mediator release in peripheral nerve trunks and DRG of individuals who had advanced disease (*de la Monte, et al., 1988; Mah, et al., 1988; Yoshioka, et al., 1994; Rizzuto, et al., 1995; Nagano, et al., 1996; Bradley, et al., 1998; Jones, et al., 2005*). Neuronal damage in HIV-infected individuals is unlikely to result from active infection of neurones, especially since neurones do not express CD4 receptors,

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which are required for viral entry into a cell (*Dubois-Dalcq, et al., 1995; Jones, et al., 2005; Acharjee, et al., 2010*). Rather, the infiltration of activated immune cells into peripheral nerves indicates that peripheral nerve damage may occur indirectly through inappropriate activation of immuno-competent cells by the virus or viral proteins (*Tyor, et al., 1995; Keswani, et al., 2002*). Alternatively, the damage may be mediated by direct viral-mediated neurotoxicity, similar to that observed in the central nervous system, but such evidence is scarce in the peripheral nervous system (*Ahmed, et al., 2009; Acharjee, et al., 2010*). Because active viral infection of nerves does not appear to be important in the neurotoxity of HIV, research on the neuropathology of HIV-SN has focused on experimental models employing soluble viral gene products rather than active virus. However, there have been several studies which have employed recombinant HIV-1 clones generated from virus derived from peripheral nerves or brain tissue of HIV-infected individuals (*Zhang, et al., 2003; Jones, et al., 2005*), or models employing lentivirus infections that result in immunodeficiency in felids (*Zhu, et al., 2007*) and non-human primates (*Laast et al., 2007; Laast et al., 2011; Lehmann, et al., 2011*) .

The primary viral gene product used *in vivo* and *in vitro* to investigate the pathogenesis of HIV-mediated neurotoxicity is HIV-1 gp120, the coat protein that mediates the binding to and transmission of HIV into permissive cells through interactions with the CD4 protein and the chemokine co-receptors, CCR5 and CXCR4. Even in its soluble form, as occurs when gp120 is shed from viral particles or released from infected cells, the molecule is able to activate sensitive cells (*Conti, et al., 2004*). Additionally, the HIV-1 viral protein R (Vpr), an accessory protein that regulates HIV-1 infectivity through a variety of mechanisms and that has been identified in macrophage-like cells in the DRGs of HIV-infected individuals, has been used experimentally to assess the neurotoxicity of viral gene products *in vitro* (*Romani and Engelbrecht, 2009; Acharjee, et al., 2010*).

Pathogenesis of HIV-SN (Figure 1)

Acute perineural application of gp120 to rat sciatic nerves caused acute axonal swelling and increased tumour necrosis factor (TNF)-a expression in the nerve trunk at the site of application (*Herzberg and Sagen, 2001*), in the DRG of the exposed nerve, and in glial cells in the dorsal horn of the spinal cord at the level the exposed nerve enters the spinal cord (*Zheng, et al., 2011b*). In addition, macrophage infiltration into the nerve at the site of gp120 application has been demonstrated within one week of gp120 application (*Wallace, et al., 2007a*). When the macrophage accumulation in the nerve trunk was at its greatest, about two weeks after gp120 application, there was associated dying-back of distal nerve fibre endings in the epidermis, and macrophage infiltration, activation of satellite cells, and inflammatory mediator production occurred in the DRG (*Wallace, et al., 2007a; Wallace, et al., 2007b*). The aforementioned changes were preceded by expression of ATF3, a marker of cellular stress and neuronal injury, in DRG neurones (*Wallace, et al., 2007a*). Thus, perineural

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application of gp120 produced a localised inflammatory reaction similar to that seen in peripheral nerve trunks of individuals infected with HIV (*de la Monte, et al., 1988; Mah, et al., 1988; Nagano, et al., 1996*), and these localised changes were associated with pathological changes characteristic of the distal nerve fibre ending and DRG changes that occur in HIV-SN (*Yoshioka, et al., 1994; Nagano, et al., 1996; Bradley, et al., 1998; Skopelitis, et al., 2007; Zhou, et al., 2007*). However, unlike individuals infected with HIV, acute perineural application of gp120 in rats did not cause significant demyelination and axonal degeneration at the site of gp120 application, or apoptosis in DRG cells (*de la Monte, et al., 1988; Chaunu, et al., 1989; Rizzuto, et al., 1995; Wallace, et al., 2007a*). These differences from the clinical state may reflect the acute and localised exposure to gp120 in the model compared to the chronic and generalised exposure to gp120 that occurs in HIV-infected individuals. Indeed, in a primate model of HIV-SN in which macaque monkeys were infected with simian immunodeficiency virus (SIV), the infection resulted in neuronal loss in the trigeminal nerve ganglia of the monkeys (*Laast et al., 2007*) and reduced conduction velocity in C-fibres (*Laast et al., 2011*) that correlated with accumulation of infected macrophages in the DRG. Similarly, in a feline model of HIV-SN in which cats were infected with feline immunodeficiency virus (FIV), infection was associated with reduced axonal density and dying-back of distal nerve fibre endings in the sural nerve (*Zhu et al., 2007*).

Studies using cultured primary rat and human DRG neurones have shown that the neurotoxic effects of gp120 may be directly mediated by activation of chemokine receptors on the surface of neurones, or indirectly through the activation of Schwann cells and macrophages. Exposing co-cultures of rat DRG neurones and Schwann cells to gp120 caused dose-dependent neurite degeneration and neuronal apoptosis via caspase-3 and mitochondrial-dependent neuronal apoptosis involving inositol triphosphate-dependent calcium release from endoplasmic reticulum (*Keswani, et al., 2003b; Hoke, et al., 2009*). Activation of pro-apoptotic c-Jun N-terminal kinase-dependent pathways also may have contributed to the neurite retraction and apoptosis induced by gp120 (*Bodner, et al., 2004*). The toxicity observed by Keswani et al (*2003b*) required the presence of Schwann cells, and based on the available evidence, they proposed that binding of gp120 to CXCR4 receptors on Schwann cells stimulated the release of the chemokine CCL5, which activated CCR5 receptors on DRG neuronal cells. This neuronal activation induced TNF-α release from the neuronal cells which, through an autocrine and/or paracrine mechanism, activated neuronally-expressed TNFR1 receptors, leading to cell death (*Keswani, et al., 2003b; Melli, et al., 2006*). This proposed mechanism recently gained support from *in vivo* experiments, which showed increased expression of TNF-α in the DRG of exposed nerves following perineural application of gp120 in rats (*Zheng, et al., 2011b*). And, consistent with the findings of Keswani and colleagues, exposing mixed primary neuronal/glial/macrophage cultures from transgenic rats expressing human CD4/CCR5 receptors to recombinant HIV-1 clones containing the C2V3 region of the HIV *env* gene derived from virus isolated from peripheral

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nerves of HIV-infected individuals activated Schwann cells and induced neurite retraction (*Jones, et al., 2005*).

Activation of the non-specific immune response probably also contributes to the indirect neurotoxicity of the virus. *In vivo*, trigeminal nerve ganglionitis in SIV-infected macaques was associated with infiltrating mononuclear cells, evidence of neuronophagia, and neuronal loss and replacement by satellite cells and immune cells (*Laast et al., 2007*), while *in vitro*, gp120 induced neuronal cell lysis in DRG cultures through activation of complement pathways (*Apostolski et al., 1994*), and several studies have reported that inflammatory mediators released by HIV-infected macrophages may contribute to the indirect neurotoxicity of the virus (*Zhang, et al., 2003; Jones, et al., 2005; Hahn, et al., 2008*). Hahn and colleagues (*2008*) showed that supernatant from cultured human monocytic cells infected with HIV inhibited neurite growth in cultured human foetal DRGs even when neuronally-expressed CCR5 and CXCR4 receptors were neutralised. Inhibition of neurite growth was associated with depolarisation of the mitochondrial membrane and generation of reactive oxygen species. Unfortunately, the substances in the supernatant that mediated the neurotoxicity were not determined, but presumably inflammatory substances such as interleukin(IL)-1β and TNF-α, which are involved in neuronal death in the central nervous system of HIV-infected individuals (*Kaul and Lipton, 2006*), and which have been found in the DRG and peripheral nerve trunks of people infected with HIV (*Yoshioka, et al., 1994; Nagano, et al., 1996; Jones, et al., 2005*), may have been responsible. Indeed, some recombinant HIV-1 clones derived from virus extracted from peripheral nerves of HIV-infected individuals increased the expression of IL-1β and TNF-α mRNA in cultured rat DRG, with the most likely source being macrophages (*Jones, et al., 2005*), and perineural application of gp120 in rats increased expression of TNF-α in the DRG (*Zheng, et al., 2011b*). The ability of recombinant HIV-1 virions to induce production of inflammatory mediators by macrophages has been shown to be dependent on gp120 activating CXCR4 and CCR5 receptors on these cells (*Zhang, et al., 2003*). Mitochondrial dysfunction similar to that reported by Hahn and colleagues (*2008*) *in vitro* was recently reported by Lehmann and colleagues (*2011*) in axonal mitochondria isolated from macaque monkeys infected with SIV. Although they did not identify the mechanism by which SIV infection induced mitochondrial dysfunction, the data from Hahn and colleagues (*2008*) and Lehmann and colleagues (*2011*) indicates that mitochondrial dysfunction may contribute to the virus-induced neuropathy.

In addition to indirect neurotoxicity, there is experimental evidence for direct neurotoxicty of the virus on peripheral nerve fibres, especially along axons, which may also contribute to the neuronal degeneration and dying-back of epidermal nerve fibres observed in affected patients. Using compartmentalised culture systems, it has been demonstrated that selective application of gp120 to the neurites of cultured rat DRG neurones, in the absence of Schwann cells, resulted in direct gp120-mediated neurite degeneration (*Melli, et al., 2006*).

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However, when Schwann cells were present in the axonal side chambers the magnitude of gp120-mediated axonal degeneration was reduced, probably by shielding the axon from gp120. The direct axonal toxicity of gp120 was mediated through an axonal caspase-3 dependent mechanism, which was initiated following activation of neuronal CXCR4 and CCR5 receptors. Similar caspase-3 dependent axonopathy, which occurs independently of somal apoptotic pathways, has been reported following traumatic nerve injury in rats (*Buki, et al., 2000*). Recombinant HIV-1 virions also caused cell death in human neuroblastoma cells from the LAN-2 cell line through a gp120-dependent effect mediated through activation of CXCR4 and CCR5 receptors (*Zhang, et al., 2003*).

Thus, similar to what has been observed in models of HIV-induced neurotoxicity in the central nervous system (*Kaul and Lipton, 2006*), gp120 mediated activation of glia and macrophages in the peripheral nervous system can produce indirect neurotoxicity through the release of inflammatory mediators. These *in vitro* effects fit with the clinical findings of macrophage infiltration and inflammatory cytokine release in peripheral nerve trunks and DRG of HIV-infected individuals (*Yoshioka, et al., 1994; Nagano, et al., 1996; Bradley, et al., 1998; Skopelitis, et al., 2007; Zhou, et al., 2007*). In addition, the virus, and in particular the gp120 protein, may cause direct axonopathy. This direct effect may contribute to the dying back of epidermal nerve fibres that is observed in HIV-infected individuals.

Neuropathic pain-like behaviours in animal models of HIV-SN (Figure 2)

Pain is the primary symptom of HIV-SN (*Smyth, et al., 2007; Wadley, et al., 2011*), and consistent with this feature of the disease, perineural exposure of rat sciatic nerve to gp120 resulted in thermal (*Herzberg and Sagen, 2001*) and mechanical hypernociception; where hypernociception is defined here as enhanced nociceptive sensitivity (*Herzberg and Sagen, 2001; Oh, et al., 2001; Wallace, et al., 2007a; Wallace, et al., 2007b; Zheng, et al., 2011b*). Oh and colleagues (*2001*) showed that injection of gp120 or chemokines that activate CXCR4 and CCR5 receptors into the foot pad of rats produced significant mechanical hypernociception. At the cellular level, these same ligands caused Substance P release, and induced direct depolarisation of cultured rat DRG, which were putative nociceptive DRG cells (*Oh, et al., 2001*). Thus, binding of gp120 to its chemokine receptors causes activation of nociceptive neurones, and this activity may have contributed to the hypernociception observed in animals exposed to gp120 and the pain reported by HIV-infected individuals. Similar increases in neuronal excitability also occurred when the viral accessory protein, Vpr, was applied to cultured rat and human DRG cells, indicating that viral gene products other than gp120 may also contribute to the development of hypernociception, but it is unknown what cell types were being assessed (*Acharjee, et al., 2010*).

Exposure of nerve fibres to gp120 through intraplantar injection or direct application to the sciatic nerve trunk probably only results in acute exposure to gp120 before it is

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denatures or cleared by the immune system. Yet, pain-like behaviours described by several investigators lasted several weeks, indicating that more long-term changes in the processing of nociceptive signals had occurred (*Herzberg and Sagen, 2001; Wallace, et al., 2007a; Wallace, et al., 2007b; Zheng, et al., 2011b*). The sustained thermal (*Herzberg and Sagen, 2001*) and mechanical (*Herzberg and Sagen, 2001; Wallace, et al., 2007a; Wallace, et al., 2007b; Zheng, et al., 2011b*) hypernociception observed in rats, correlated loosely with increased TNF-α expression at the site of gp120 application (*Herzberg and Sagen, 2001*), in the DRG of the exposed nerve and in the dorsal horn of the spinal cord at the level of entry of the exposed nerve (*Zheng, et al., 2011b*), and macrophage accumulation at the site of gp120 application (*Wallace, et al., 2007a; Wallace, et al., 2007b*). All these changes are important factors in the development of chronic pain-like behaviours in animal models of neuropathic pain (*Thacker, et al., 2007*). Furthermore, hypernociception to cold and mechanical stimuli also developed in immunodeficient transgenic mice expressing Vpr in monocytoid cells (*Acharjee, et al., 2010*). This hypernociception was associated with increased expression of interferon-a, but not IL-6, in DRG and sciatic nerves of the mice. Thus inflammatory changes caused by at least two viral gene products of HIV have been shown to induce inflammatory changes in peripheral nerves associated with hypernociception.

In addition to changes in the peripheral nervous system, exposing the sciatic nerve of rats to gp120 resulted in activation of microglia and astrocytes in the dorsal horn of the spinal cord (*Herzberg and Sagen, 2001; Wallace, et al., 2007a; Wallace, et al., 2007b; Zheng, et al., 2011b*). The time course of this gliosis coincided with the development of peak hypernociception (*Wallace, et al., 2007a*), and its maintenance for up to 30 days post gp120 exposure (*Herzberg and Sagen, 2001*). Zheng and colleagues recently demonstrated this gliosis is associated with expression of TNF-α by astrocytes and microglia, and that neutralising TNF-α attenuated gp120-mediated mechanical hypernociception in rats, as did neutralising TNF-α in the DRG (*Zheng, et al., 2011b*). Thus, increased expression of TNF-α in the central and peripheral nervous system appears to have a pivotal role in the maintenance of the hypernociceptive state in animals exposed to gp120. Another possible contributor to hypernociception is the pro-nociceptive chemokine CCL2, which is increased in the DRG of the exposed nerve root (*Wallace, et al., 2007b; White, et al., 2007*). The DRG and CNS changes described in animals exposed to gp120 are consistent with those described in other rodent models of painful peripheral neuropathy (*Latremoliere and Woolf, 2009*). Thus, the pain experienced by individuals with HIV-SN may arise from direct stimulation of nociceptive neurones by gp120 or Vpr, and be augmented and sustained through inflammatory reactions in peripheral nerves, DRG and spinal cord dorsal horn, resulting in peripheral and central sensitisation of nociceptive pathways.

In summary, *in vivo* and *in vitro* evidence indicates that HIV may cause nerve pathology via direct and indirect mechanisms, with direct mechanisms dominating the axonal

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pathology and indirect mechanism dominating the somal pathology. This dual effect of the virus on peripheral nerve fibres, in terms of direct and indirect mechanisms of action, and somal versus axonal sites of action, may be additive or synergistic, enhancing the overall nerve pathology caused by HIV infection, leading to the high incidence of neuropathy in the infected population. However, data from *in vivo* and *in vitro* studies needs to be considered in the context of the pharmacological doses of gp120 used in these studies (*Klasse and Moore, 2004*), the acute onset of the toxicity in the models compared to the slower progression to clinically apparent neuropathy in HIV-infected individuals, and the potentially confounding effects of species differences in receptor affinity for viral gene products and differences in receptor tropism depending on the source of the gp120 used. To overcome these problems, further use of more sophisticated experimental models, such as those models that employ recombinant HIV-1 clones derived from nervous system tissues of infected patients, and transgenic animal expressing viral gene products or myelogenous cells expressing human CD4, CXCR4 or CCR5 receptors, needs to occur.

Antiretroviral drug mediated neurotoxicity in the peripheral nervous system

The use of nucleoside reverse transcriptase inhibitors (NRTIs) zalcitabine, didanosine and stavudine to treat HIV infection is strongly associated with the development of HIV-SN (*Dalakas, 2001; Dalakas, et al., 2001; Cherry, et al., 2009; Ellis, et al., 2010*). Of the three drugs, stavudine is the only one currently in widespread use (*WHO, et al., 2010*). Experimental evidence for the pathological processes by which NRTIs may cause neuropathy is growing and indicates a pivotal role for mitochondrial dysfunction. However, elucidation of the specific neuropathological processes that ultimately cause nerve fibre dysfuncion are confounded in HIV-infected individuals by concomitant viral-induced nerve damage. Therefore, delineation of the mechanisms that underlie the drug-induced component of the neuropathy has required the development of *in vitro* and *in vivo* models of pure NRTI-induced neurotoxicity.

Toxicity of NRTIs in vitro

Zalcitabine, didanosine and stavudine induce dose-dependent inhibition of neurite outgrowth and mitochondrial DNA synthesis in neuronal cell line PC-12 cells (*Cui, et al., 1997*), neurite degeneration in disassociated embryonic and neonatal rat DRG neurones (*Keswani, et al., 2003a; Bodner, et al., 2004; Keswani, et al., 2004*), and reduced soma size and neurite length in feline DRG neurones (*Zhu, et al., 2007*). The reduced soma size and neurite length in cultured feline DRG neurones was associated with reduced expression of mRNA for brain-derived neurotrophic factor (BDNF) and its receptor, TrkB. Accordingly, these effects could be reversed by administration of BDNF, indicating that the impaired neuronal growth caused by NRTI's may be secondary to reduced expression and responsiveness to neurotrophic factors (*Zhu, et al., 2007*). In addition to impairing neuronal growth, didanosine induced c-Jun N-terminal kinase-dependent apoptosis of the neonatal

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DRG cells (*Bodner, et al., 2004*), while zalcitabine reduced PC-12 cell proliferation (*Cui, et al., 1997*) and resulted in a loss of mitochondrial membrane integrity and calcium-dependent necrosis in embryonic rat DRG neurones (*Keswani, et al., 2003a; Keswani, et al., 2004*). Thus, NRTI-induced cell death may be mediated by apoptotic and necrotic mechanisms. The reduced neuronal growth and increased cell death induced by some NRTIs probably involves disrupted mitochondrial function due to drug-dependent inhibition of mitochondrial DNA polymerase and depletion of mitochondrial DNA (*Keilbaugh, et al., 1997; Kakuda, 2000*). As such, the *in vitro* data supports the findings of Dalakas and colleagues (*2001*) who reported that the majority of HIV-positive individuals who developed a neuropathy after starting zalcitabine therapy had enlarged, vacuolated mitochondria, and depleted mitochondrial DNA in peripheral nerves and Schwann cells. However, direct disruption of mitochondrial proteins by the drugs may have also contributed to the mitochondrial dysfunction observed in neuronal tissues *in vitro* and *in vivo* (*Skuta, et al., 1999*). In the following section, we describe the evidence for, and mechanism of, NRTI-induced neurotoxicity *in vivo*.

Neuropathological features of NRTI-induced neuropathy in vivo

In the first animal model of NRTI-induced neuropathology, Anderson and colleagues (*1992*) showed that chronic oral administration of zalcitabine to rabbits resulted in reduced sural nerve conduction velocities and amplitudes. These changes were associated with dose and duration-dependent neuropathology, including myelin splitting, intramyelinic oedema, demyelination/ remyelination, and axonal loss. The ultrastructural features of the nerve pathology induced by zalcitabine in rabbits, led the authors to conclude that the primary event involved damage to Schwann cells, with secondary axonopathy (*Feldman, et al., 1992*). Similarly, decreases in conduction velocity (*Chen and Levine, 2007*), myelin hypertrophy and disruption of Remak bundles (*Bhangoo, et al., 2007*), and morphological changes in neuronal cell mitochondria (*van Steenwinckel, et al., 2008*) have been reported to occur in rats within a week of a single parenteral dose of zalcitabine. Thus, even acute exposure to zalcitabine increases risk of neuropathy, which is consistent with the epidemiological data showing that any previous exposure to zalcitabine, didanosine or stavudine increases risk of developing HIV-SN (*Smyth, et al., 2007; Cherry, et al., 2009*). However, this acute exposure was not sufficient to cause significant demyelination, remyelination , axonal degeneration (*Bhangoo, et al., 2007; van Steenwinckel, et al., 2008*), or decreases in intraepidermal nerve fibre (IENF) density in hind paw glabrous skin of rats (*Siau, et al., 2006*), indicating that these pathological features may require sustained exposure to the drug. Indeed, intraperitoneal injections of zalcitabine three times a week for three weeks did decrease IENF density in the plantar skin of the hind paw of rats (*Wallace, et al., 2007b*), which is consistent with the time-course of HIV-SN developing in individuals starting antiretroviral therapy (*Husstedt, et al., 2001*).

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Development of *in vivo* models using didanosine and stavudine has proven difficult. Whereas chronic, twice daily oral administration of didanosine to rats induced myelin splitting and intramyelin oedema in peripheral nerve fibres (*Schmued, et al., 1996; Patterson, et al., 2000*), Zhu and colleagues (*2007*) failed to induce nerve fibre loss, electrophysiological changes or dying back of intraepidermal nerve fibres in cats administered oral didanosine daily for 6 weeks. Moreover, Warner and colleagues (*1995*) failed to observe morphological or electrophysiological evidence of peripheral nerve toxicity in rabbits given once daily oral didanosine for 24 weeks, even though the drug was given at near-lethal doses and plasma levels confirmed systemic drug exposure. This study also failed to demonstrate any neurotoxic effects of chronic once daily oral administration of high-dose stavudine. This lack of overt neuropathology in animals administered stavudine has also been reported in rats administered the drug daily for six weeks at doses previously shown to induce the development of hypernociception to a noxious mechanical stimulus (*Weber, et al., 2007; Makweya, et al., 2008*). Moreover, no signs of neuropathy were reported in monkeys administered the drug on a daily basis for one year (*Kaul, et al., 1999*), although the details of how neuropathy was assessed in the monkeys was not provided. The variability in response to didanosine and the lack of neurotoxic effect of stavudine on peripheral nerves in previously reported animal models has meant that zalcitabine-based animal models have formed the basis of investigations into the mechanisms of NRTI-induced neuropathology and neuropathic pain-like behaviours, despite the drug no longer being used clinically.

Pathogenesis of NRTI-induced neuropathy (Figure 3)

The mechanisms underlying neuropathy in animals given zalcitabine have been studied primarily in the context of the neuropathic pain-like behaviours in rodents. In a series of experiments, Levine and colleagues (*Joseph, et al., 2004; Joseph and Levine, 2004; Joseph and Levine, 2006; Chen and Levine, 2007*) demonstrated that a single intravenous injection, or chronic daily oral dosing, of zalcitabine in rats resulted in dose-dependent mechanical and thermal hypersensitivity of the hind paws, which in the case of intravenous injection, developed rapidly and was sustained for at least twenty days. The hypernociception was associated with a reduction in C-fibre conduction velocity and a change in the distribution of the interspike interval, but not with a decrease in firing threshold or an increase in response to sustained stimulation with threshold or supra-threshold stimuli (*Joseph and Levine, 2004; Chen and Levine, 2007*). The behavioural and electrophysiological changes appear to have been related to increased calcium signalling in the nerve fibres (*Joseph, et al., 2004*) and activation of pro-apoptotic caspase pathways (*Joseph and Levine, 2004*); data which supports a hypothesis of drug-induced mitochondrial damage leading to altered calcium homeostatis and activation of apoptotic pathways. Yet, the same group of investigators also showed that disrupting the mitochondrial electron transport chain and preventing mitochondrial phosphorylation attenuated zalcitabine-induced hypernociception (*Joseph and Levine, 2006*). These findings are counterintuitive given the authors' earlier

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findings, and the standard explanation of disrupted mitochondrial function causing the neuropathy in rodents and humans. Indeed, Lehmann and colleagues (*2011*) recently showed increased mitochondrial DNA mutation deletions and decreased mitochondrial protein levels, including the catalytic subunit of cytochrome *c* oxidase I, in sural nerves biopsies of HIV-infected individuals who had been exposed to neurotoxic antiretroviral regimens and who had developed HIV-SN. Thus data from affected HIV-infected individuals complements a model of mitochondrial dysfunction.

Recently, Wallace and colleagues (*2007b*) demonstrated that zalcitabine, injected intraperitoneally three times a week for three weeks, induced mechanical, but not thermal, hypersensitivity and anxiety-like behaviour in rats. Overt pathological changes in peripheral nerves, including dying back of intraepidermal nerve fibres and macrophage infiltration into the DRG of exposed nerves were also seen. The infiltration of macrophages was accompanied by increased expression of the pro-nociceptive chemokine CCL2, which could have contributed to the animals' hypernociceptive state. Another possible contributor to hypernociception that develops after exposure to zalcitabine is TNF-a. Zheng and colleagues (*2011c*) showed that a single intraperitoneal injection of zalcitabine in rats that induced sustained mechanical hypernociception was associated with increased expression of the pro-nociceptive cytokine TNF-a by DRG neurones. Unfortunately, neither group of investigators determined whether neutralising TNF-a or CCL2 in the DRG attenuated the hypernociception, so the contribution of these molecules to the hypernociception remains speculative. Indeed, with regards to CCL2, Bhangoo and colleagues (*2007*) reported that a single intraperitoneal injection of zalcitabine in rats, which induced mechanical hypersensitivity, failed to induce upregulation of CCR2, the receptor for CCL2, in dissociated DRG neurones, or to increase the responsiveness of these cells to application of the chemokine. Rather, Bhangoo and colleagues reported enhanced mRNA expression of the chemokine CXCR4 in DRG neurones and glia, and its ligand, CXCL12, in glia. Blocking CXCR4 blocked the development of the zalcitabine-induced mechanical hypersensitivity, demonstrating the involvement of CXCR4 activation in the development of the hypernociception. Thus, chemokines may be involved in the development of NRTI-induced neuropathy, but the nature and extent of that involvement, and whether there are differences across animal models, is unclear.

The behavioural and neuropathological changes induced by sustained exposure to zalcitabine were also associated with modest increases in dorsal horn astrocyte and microglial activation (*Wallace, et al., 2007b*). However, blocking microglial activation did not attenuate zalcitabine-induced mechanical hypersensitivity, indicating that the microgliosis was not significantly contributing to the hypersensitivity in this model of neuropathic pain. A more recent study also shows that spinal microgliosis does not appear to be responsible for the development of zalcitabine-induced SN (*Zheng, et al., 2011a*). Yet, a single intraperitoneal

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injection of zalcitabine did result in release of TNF-a by activated astrocytes, and neutralisation of this TNF-a attenuated zalcitabine-induced hypernociception (*Zheng, et al., 2011c*). These conflicting data on the role of spinal glia in mediating the hypernociception induced by zalcitabine may reflect differences in experimental design (e.g., chronic versus single administration of zalcitabine), or they may reflect the selective importance of activated astrocytes rather than microglia in mediating the central sensitization of nociceptive pathways. Whatever the case may be, there is accumulating evidence for changes in the dorsal horn contributing to NRTI-induced hypernociception. In a recent study, Renn and colleagues (2011) reported increases in BDNF concentrations in the dorsal horn of mice after a single intravenous injection of stavudine, and that this rise in dorsal horn BDNF was correlated with mechanical hypersensitivity and enhanced activity in dorsal horn wide dynamic range neurones. And, this hypersensitivity was reduced in BDNF heterozygous mice ($BDNF^{+/-}$) compared to homozygous ($BDNF^{+/+}$) mice, and in animals depleted of BDNF by intrathecal administration of trkB chimeric protein.

Models of combined NRTI and virus-induced neuropathy

Wallace and colleagues (*2007b*) demonstrated that chronic intraperitoneal administration of zalcitabine induced sustained mechanical hypernociception and some evidence of nerve fibre pathology in rats, but in contrast to their gp120-model of HIV-SN (*Wallace, et al., 2007a*), this treatment did not induce macrophage infiltration of the nerve trunk or the expression of markers of neuronal stress, such as ATF3 and c-Jun, in DRG neurones. Thus, the overt neurotoxicity of the drug was limited compared to that induced by gp120 alone. However, in a model that more closely mimics the clinical situation, where patients exposed to antiretroviral drugs typically also have an underlying HIV infection, the authors reported that the algesic effects of acute perineural exposure to gp120 in rats was enhanced by administration of zalcitabine, and that this enhanced mechanical hypersensitivity was associated with increased expression of CCL2 in the DRG and increased microgliosis in the dorsal horn of the spinal cord. Other studies have also shown positive neurotoxic interactions between NRTIs and viral components. In transgenic mice expressing gp120 under a GFAP promoter, constitutive expression of gp120 within the nervous system did not induce any significant neuropathology, but administration of didanosine in the drinking water of the mice, at a dose that in itself was not neurotoxic, resulted in the development of hypersensitivity to noxious heat. This neurotoxicity was associated with loss of unmyelinated axons in peripheral nerves and dying back of intraepidermal nerve fibres; all features of the pathology seen in humans with HIV-SN (*Keswani, et al., 2006*). In a feline model of HIV-SN using FIV, administering didanosine to cats infected with FIV enhanced the virus induced loss of intraepidermal nerve fibres. Moreover, addition of didanosine to *ex vivo* preparations of DRG cells from the cats, enhanced the reductions in neurite length and soma size, and the reductions in mitochondrial cytochrome C oxidase subunit I and BDNF mRNA induced by FIV (*Zhu, et al., 2007*). The enhanced toxicity of NRTIs, even those like didanosine that do

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not produce reliable animal models of pure NTRI-induced neuropathy, when given in combination with virus or viral antigens, may explain the continued problem of HIV-SN in individuals on antiretroviral therapy since the use of zalcitabine was stopped. It remains to be determined whether this positive virus-drug interaction will maintain the rate of HIV-SN even with the substitution of stavudine for reverse transcriptase inhibitors like tenofovir, which exhibit very low levels of mitochondrial toxicity *in vitro* (*Lee, et al., 2003*).

In summary, animal models of pure NRTI-induced neuropathy have yielded mixed results, with only zalcitabine producing reliable models. Even then, the different zalcitabine models have identified slightly different mechanisms for the nerve pathology, especially with regards to the involvement of the chemokine CCL2. But even within models, the nature of the mitochondrial dysfunction induced by zalcitabine, and its contribution to the development of the neuropathy, is equivocal. For example, zalcitabine induced hypernociception was associated with mitochondrial dysfunction sufficient to initiate mitochondrial-dependent caspase signalling, but maintenance of the hypernociception was dependent on sustained functional mitochondria, implying adequate mitochondrial function. Similar findings were reported in another model of drug-induced neuropathy, vincristine-induced neuropathy, suggesting possible overlap in the underlying mechanisms of these two drug-induced neuropathies (*Joseph and Levine, 2004; Joseph et al., 2006*). Thus, further investigation into either model may yield answers pertinent to both models. Models of combined NRTI and virus-induced neuropathy, however, have provided useful insight into the positive neurotoxic interactions between the two, and may help explain the continuing problem of HIV-SN in the era of combination antiretroviral therapy.

Conclusion

Models of virus and drug-induced HIV-SN have provided significant insight into the pathological processes that underlie the morphological, and to a lesser extent, the electrophysiological changes seen in patients with the neuropathy. The models of virus-induced neuropathy strongly support direct and indirect mechanisms of viral toxicity, with indirect damage involving inappropriate activation of immuno-competent cells and the subsequent release of inflammatory mediators in peripheral nerves. The *in vivo* models of the drug-induced neuropathy provide evidence supporting mitochondrial dysfunction as a key process in the development of neuropathological changes, but the sequelae of events initiated by the mitochondrial dysfunction that leads to the neuropathy still is unclear. In general, the in vivo models have construct validity, and the models of pure drug-induced neuropathy and combined drug and virus-induced neuropathy developed by Wallace and colleagues (2007b) appear to have predictive validity also. For example, congruent with efficacy in clinical trials for painful HIV-SN, amitriptyline did not reduce mechanical hypersensitivity in these rat models, but the mixed CB1/CB2 cannabinoid receptor agonist WIN 55,212-2 did (*Phillips, et al., 2010*). Also, the models have started to address the interaction between the viral or drug

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induced neuropathy and co-morbid conditions that may affect the development and progression of the painful symptoms of the neuropathy. For example, depression has been linked to the presence of pain in individuals with HIV-SN (Lucey, et al., 2011) and Wallace and colleagues demonstrated interaction between the presence of pain and anxiety-like behaviour in rat models of HIV-SN (*Wallace, et al., 2008*). Yet, there remains significant scope for the models to also address co-morbid medical conditions such as diabetes mellitus, isoniazid therapy for tuberculosis infection and nutritional deficiencies (e.g., vitamin B_{12}), that are common in the HIV-infected population and which may increase the risk of developing HIV-SN. Indeed, as we move forward in the development of animal models of HIV-SN we anticipate advances in models that address co-morbid conditions, but probably the most significant advances will come with development of models that better reflect viral exposure of the peripheral nervous system in infected individuals, such as the FIV and SIV models employed by Zhu and colleagues (2007) and Laast and colleagues (2007, 2011), respectively, and the modern drugs and drug combinations that are used clinically to treat HIV. Ultimately, the goal of the models is to provide insight into potential therapeutic targets that may either prevent or attenuate the development of the neuropathy, or treat the symptoms of the neuropathy, of which pain is the primary symptom. To date the models have not identified potential targets for either of these two goals, and neither have the models identified a clear link between the nature of the nerve damage and whether pain develops of not. However the continued development, study and refinement of animal models of HIV-SN, such as recent investigations into changes in gene expression in the dorsal horn and DRG cells of animals exposed to systemic NRTIs and gp120 (*Dorsey et al., 2009; Maratou, et al., 2009; Renn et al., 2011*), hold promise for the identification of new and efficacious therapeutic targets for this debilitating neuropathy that is common in the HIV-positive population, and whose symptoms are resistant to conventional treatments.

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References

Acharjee S, Noorbakhsh F, Stemkowski PL, Olechowski C, Cohen EA, Ballanyi K, Kerr B, Pardo C, Smith PA, Power C (2010). HIV-1 viral protein R causes peripheral nervous system injury associated with in vivo neuropathic pain. Faseb J 24: 4343-4353.

Ahmed F, MacArthur L, De Bernardi MA, Mocchetti I (2009). Retrograde and anterograde transport of HIV protein gp120 in the nervous system. Brain Behav Immun 23: 355-364.

Anderson TD, Davidovich A, Arceo R, Brosnan C, Arezzo J, Schaumburg H (1992). Peripheral neuropathy induced by 2',3'-dideoxycytidine. A rabbit model of 2',3'-dideoxycytidine neurotoxicity. Lab Invest 66: 63-74.

Apostolski S, McAlarney T, Hays AP, Latov N (1994). Complement dependent cytotoxicity of sensory ganglion neurons mediated by the gp120 glycoprotein of HIV-1. Immunol Invest 23: 47-52.

Bhangoo SK, Petty L, White FA (2010). Animal models of HIV-associated painful sensory neuropathy. In: Animal Models of Pain - Neuromethods 49. Ma C, Zhang J-M (Eds). Humana Press, New York, pp 171-179.

Bhangoo SK, Ren D, Miller RJ, Chan DM, Ripsch MS, Weiss C, McGinnis C, White FA (2007). CXCR4 chemokine receptor signaling mediates pain hypersensitivity in association with antiretroviral toxic neuropathy. Brain Behav Immun 21: 581-591.

Bodner A, Toth PT, Miller RJ (2004). Activation of c-Jun N-terminal kinase mediates gp120IIIB- and nucleoside analogue-induced sensory neuron toxicity. Exp Neurol 188: 246-253.

Bradley WG, Shapshak P, Delgado S, Nagano I, Stewart R, Rocha B (1998). Morphometric analysis of the peripheral neuropathy of AIDS. Muscle Nerve 21: 1188-1195.

Buki A, Okonkwo DO, Wang KKW, Povlishock JT (2000). Cytochrome c Release and Caspase Activation in Traumatic Axonal Injury. J Neurosci 20: 2825-2834.

Chaunu MP, Ratinahirana H, Raphael M, Henin D, Leport C, Brun-Vezinet F, Leger JM, Brunet P, Hauw JJ (1989). The spectrum of changes on 20 nerve biopsies in patients with HIV infection. Muscle Nerve 12: 452-459.

Chen X, Levine JD (2007). Mechanically-evoked C-fiber activity in painful alcohol and AIDS therapy neuropathy in the rat. Mol Pain 3: 5.

Published in final form: Kamerman PR, Moss PJ, Weber J, Wallace VCJ, Rice ASC, Huang W. Pathogenesis of HIV-associated sensory neuropathy: evidence from in vivo and in vitro experimental models. *Journal of the Peripheral Nervous System* 17: 19-31, 2012.
DOI: 10.1111/j.1529-8027.2012.00373.x, PMID: 22462664

Cherry CL, Affandi JS, Imran D, Yunihastuti E, Smyth K, Vanar S, Kamarulzaman A, Price P (2009). Age and height predict neuropathy risk in patients with HIV prescribed stavudine. Neurology 73: 315-320.

Conti L, Fantuzzi L, Del Corno M, Belardelli F, Gessani S (2004). Immunomodulatory effects of the HIV-1 gp120 protein on antigen presenting cells: implications for AIDS pathogenesis. Immunobiology 209: 99-115.

Cui L, Locatelli L, Xie MY, Sommadossi JP (1997). Effect of nucleoside analogs on neurite regeneration and mitochondrial DNA synthesis in PC-12 cells. J Pharmacol Exp Ther 280: 1228-1234.

Dalakas MC (2001). Peripheral neuropathy and antiretroviral drugs. J Peripher Nerv Syst 6: 14-20.

Dalakas MC, Semino-Mora C, Leon-Monzon M (2001). Mitochondrial alterations with mitochondrial DNA depletion in the nerves of AIDS patients with peripheral neuropathy induced by 2'3'-dideoxycytidine (ddC). Lab Invest 81: 1537-1544.

de la Monte SM, Gabuzda DH, Ho DD, Brown RH, Jr., Hedley-Whyte ET, Schooley RT, Hirsch MS, Bhan AK (1988). Peripheral neuropathy in the acquired immunodeficiency syndrome. Ann Neurol 23: 485-492.

Dorsey SG, Leitch CC, Renn CL, Lessans S, Smith BA, Wang XM, Dionne RA (2009). Genome-wide screen identifies drug-induced regulation of the gene giant axonal neuropathy (Gan) in a mouse model of antiretroviral-induced painful peripheral neuropathy. Biol Res Nurs 11: 7-16.

Dubois-Dalcq M, Altmeyer R, Chiron M, Wilt S (1995). HIV interactions with cells of the nervous system. Curr Opin Neurobiol 5: 647-655.

Ellis RJ, Rosario D, Clifford DB, McArthur JC, Simpson D, Alexander T, Gelman BB, Vaida F, Collier A, Marra CM, Ances B, Atkinson JH, Dworkin RH, Morgello S, Grant I (2010). Continued high prevalence and adverse clinical impact of human immunodeficiency virus-associated sensory neuropathy in the era of combination antiretroviral therapy: the CHARTER Study. Arch Neurol 67: 552-558.

Feldman D, Brosnan C, Anderson TD (1992). Ultrastructure of peripheral neuropathy induced in rabbits by 2',3'-dideoxycytidine. Lab Invest 66: 75-85.

Ferrari S, Vento S, Monaco S, Cavallaro T, Cainelli F, Rizzuto N, Temesgen Z (2006). Human immunodeficiency virus-associated peripheral neuropathies. Mayo Clin Proc 81: 213-219.

Published in final form: Kamerman PR, Moss PJ, Weber J, Wallace VCJ, Rice ASC, Huang W. Pathogenesis of HIV-associated sensory neuropathy: evidence from in vivo and in vitro experimental models. *Journal of the Peripheral Nervous System* 17: 19-31, 2012.
DOI: 10.1111/j.1529-8027.2012.00373.x, PMID: 22462664

Hahn K, Robinson B, Anderson C, Li W, Pardo CA, Morgello S, Simpson D, Nath A (2008). Differential effects of HIV infected macrophages on dorsal root ganglia neurons and axons. Exp Neurol 210: 30-40.

Herzberg U, Sagen J (2001). Peripheral nerve exposure to HIV viral envelope protein gp120 induces neuropathic pain and spinal gliosis. J Neuroimmunol 116: 29-39.

Hoke A, Morris M, Haughey NJ (2009). GPI-1046 protects dorsal root ganglia from gp120-induced axonal injury by modulating store-operated calcium entry. J Peripher Nerv Syst 14: 27-35.

Holland NR, Stocks A, Hauer P, Cornblath DR, Griffin JW, McArthur JC (1997). Intraepidermal nerve fiber density in patients with painful sensory neuropathy. Neurology 48: 708-711.

Husstedt IW, Bockenholt S, Kammer-Suhr B, Evers S (2001). Pain therapy in HIV-associated polyneuropathy. Schmerz 15: 138-146.

Jones G, Zhu Y, Silva C, Tsutsui S, Pardo CA, Keppler OT, McArthur JC, Power C (2005). Peripheral nerve-derived HIV-1 is predominantly CCR5-dependent and causes neuronal degeneration and neuroinflammation. Virology 334: 178-193.

Joseph EK, Chen X, Khasar SG, Levine JD (2004). Novel mechanism of enhanced nociception in a model of AIDS therapy-induced painful peripheral neuropathy in the rat. Pain 107: 147-158.

Joseph EK, Levine JD (2004). Caspase signalling in neuropathic and inflammatory pain in the rat. Eur J Neurosci 20: 2896-2902.

Joseph EK, Levine JD (2006). Mitochondrial electron transport in models of neuropathic and inflammatory pain. Pain 121: 105-114.

Kakuda TN (2000). Pharmacology of nucleoside and nucleotide reverse transcriptase inhibitor-induced mitochondrial toxicity. Clin Ther 22: 685-708.

Kaul M, Lipton SA (2006). Mechanisms of neuroimmunity and neurodegeneration associated with HIV-1 infection and AIDS. J Neuroimmune Pharmacol 1: 138-151.

Kaul S, Dandekar KA, Schilling BE, Barbhaiya RH (1999). Toxicokinetics of 2',3'-didehydro-3'-deoxythymidine, stavudine (D4T). Drug Metab Dispos 27: 1-12.

Keilbaugh SA, Hobbs GA, Simpson MV (1997). Effect of 2',3'-dideoxycytidine on oxidative phosphorylation in the PC12 cell, a neuronal model. Biochem Pharmacol 53: 1485-1492.

Published in final form: Kamerman PR, Moss PJ, Weber J, Wallace VCJ, Rice ASC, Huang W. Pathogenesis of HIV-associated sensory neuropathy: evidence from in vivo and in vitro experimental models. *Journal of the Peripheral Nervous System* 17: 19-31, 2012.
DOI: 10.1111/j.1529-8027.2012.00373.x, PMID: 22462664

Keswani SC, Chander B, Hasan C, Griffin JW, McArthur JC, Hoke A (2003a). FK506 is neuroprotective in a model of antiretroviral toxic neuropathy. Ann Neurol 53: 57-64.

Keswani SC, Jack C, Zhou C, Hoke A (2006). Establishment of a rodent model of HIV-associated sensory neuropathy. J Neurosci 26: 10299-10304.

Keswani SC, Leitz GJ, Hoke A (2004). Erythropoietin is neuroprotective in models of HIV sensory neuropathy. Neurosci Lett 371: 102-105.

Keswani SC, Pardo CA, Cherry CL, Hoke A, McArthur JC (2002). HIV-associated sensory neuropathies. AIDS 16: 2105-2117.

Keswani SC, Polley M, Pardo CA, Griffin JW, McArthur JC, Hoke A (2003b). Schwann cell chemokine receptors mediate HIV-1 gp120 toxicity to sensory neurons. Ann Neurol 54: 287-296.

Klasse PJ, Moore JP (2004). Is there enough gp120 in the body fluids of HIV-1-infected individuals to have biologically significant effects? Virology 323: 1-8.

Laast VA, Pardo CA, Tarwater PM, Queen SE, Reinhart TA, Ghosh M, Adams RJ, Zink MC, Mankowski JL (2007). Pathogenesis of simian immunodeficiency virus-induced alterations in macaque trigeminal ganglia. J Neuropathol Exp Neurol 66: 26-34.

Laast VA, Shim B, Johanek LM, Dorsey JL, Hauer PE, Tarwater PM, Adams RJ, Pardo CA, McArthur JC, Ringkamp M, Mankowski JL (2011). Macrophage-mediated dorsal root ganglion damage precedes altered nerve conduction in SIV-infected macaques. Am J Pathol 179: 2337-2345.

Latremoliere A, Woolf CJ (2009). Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 10: 895-926.

Lee H, Hanes J, Johnson KA (2003). Toxicity of nucleoside analogues used to treat AIDS and the selectivity of the mitochondrial DNA polymerase. Biochemistry 42: 14711-14719.

Lehmann HC, Chen W, Borzan J, Mankowski JL, Hoke A (2011). Mitochondrial dysfunction in distal axons contributes to human immunodeficiency virus sensory neuropathy. Ann Neurol 69: 100-110.

Lucey BP, Clifford DB, Creighton J, Edwards RR, McArthur JC, Haythornthwaite J (2011). Relationship of depression and catastrophizing to pain, disability, and medication adherence in patients with HIV-associated sensory neuropathy. AIDS Care 23: 921-928.

Published in final form: Kamerman PR, Moss PJ, Weber J, Wallace VCJ, Rice ASC, Huang W. Pathogenesis of HIV-associated sensory neuropathy: evidence from in vivo and in vitro experimental models. *Journal of the Peripheral Nervous System* 17: 19-31, 2012.
DOI: 10.1111/j.1529-8027.2012.00373.x, PMID: 22462664

Mah V, Vartavarian LM, Akers MA, Vinters HV (1988). Abnormalities of peripheral nerve in patients with human immunodeficiency virus infection. Ann Neurol 24: 713-717.

Makweya S, Mphahlele N, Kamerman P (2008). The effect of stavudine on peripheral nerves. In: 12th World Congress on Pain, Glasgow, Scotland.

Maratou K, Wallace VC, Hasnie FS, Okuse K, Hosseini R, Jina N, Blackbeard J, Pheby T, Orengo C, Dickenson AH, McMahon SB, Rice AS (2009). Comparison of dorsal root ganglion gene expression in rat models of traumatic and HIV-associated neuropathic pain. Eur J Pain 13: 387-398.

Maritz J, Benatar M, Dave JA, Harrison TB, Badri M, Levitt NS, Heckmann JM (2010). HIV neuropathy in South Africans: frequency, characteristics, and risk factors. Muscle Nerve 41: 599-606.

Melli G, Keswani SC, Fischer A, Chen W, Hoke A (2006). Spatially distinct and functionally independent mechanisms of axonal degeneration in a model of HIV-associated sensory neuropathy. Brain 129: 1330-1338.

Nagano I, Shapshak P, Yoshioka M, Xin K, Nakamura S, Bradley WG (1996). Increased NADPH-diaphorase reactivity and cytokine expression in dorsal root ganglia in acquired immunodeficiency syndrome. J Neurol Sci 136: 117-128.

Oh SB, Tran PB, Gillard SE, Hurley RW, Hammond DL, Miller RJ (2001). Chemokines and glycoprotein120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. J Neurosci 21: 5027-5035.

Patterson TA, Schmued LC, Sandberg JA, Slikker W (2000). Temporal development of 2',3'-dideoxyinosine (ddI)-induced peripheral myelinopathy. Neurotoxicol Teratol 22: 429-434.

Phillips TJ, Cherry CL, Cox S, Marshall SJ, Rice AS (2010). Pharmacological treatment of painful HIV-associated sensory neuropathy: a systematic review and meta-analysis of randomised controlled trials. PLoS One 5: e14433.

Polydefkis M, Yiannoutsos CT, Cohen BA, Hollander H, Schifitto G, Clifford DB, Simpson DM, Katzenstein D, Shriver S, Hauer P, Brown A, Haidich AB, Moo L, McArthur JC (2002). Reduced intraepidermal nerve fiber density in HIV-associated sensory neuropathy. Neurology 58: 115-119.

Rance NE, McArthur JC, Cornblath DR, Landstrom DL, Griffin JW, Price DL (1988). Gracile tract degeneration in patients with sensory neuropathy and AIDS. Neurology 38: 265-271.

Published in final form: Kamerman PR, Moss PJ, Weber J, Wallace VCJ, Rice ASC, Huang W. Pathogenesis of HIV-associated sensory neuropathy: evidence from in vivo and in vitro experimental models. *Journal of the Peripheral Nervous System* 17: 19-31, 2012.
DOI: 10.1111/j.1529-8027.2012.00373.x, PMID: 22462664

Renn CL, Leitch CC, Lessans S, Rhee P, McGuire WC, Smith BA, Traub RJ, Dorsey SG (2011). Brain-derived neurotrophic factor modulates antiretroviral-induced mechanical allodynia in the mouse. J Neurosci Res 89: 1551-1565.

Rizzuto N, Cavallaro T, Monaco S, Morbin M, Bonetti B, Ferrari S, Galiazzo-Rizzuto S, Zanette G, Bertolasi L (1995). Role of HIV in the pathogenesis of distal symmetrical peripheral neuropathy. Acta Neuropathol 90: 244-250.

Romani B, Engelbrecht S (2009). Human immunodeficiency virus type 1 Vpr: functions and molecular interactions. The Journal of general virology 90: 1795-1805.

Schmued LC, Albertson CM, Andrews A, Sandberg JA, Nickols J, Slikker W, Jr. (1996). Evaluation of brain and nerve pathology in rats chronically dosed with ddI or isoniazid. Neurotoxicol Teratol 18: 555-563.

Siau C, Xiao W, Bennett GJ (2006). Paclitaxel- and vincristine-evoked painful peripheral neuropathies: loss of epidermal innervation and activation of Langerhans cells. Exp Neurol 201: 507-514.

Skopelitis E, Aroni K, Kontos AN, Konstantinou K, Kokotis P, Karandreas N, Kordossis T (2007). Early detection of subclinical HIV sensory polyneuropathy using intraepidermal nerve fibre density quantification: association with HIV stage and surrogate markers. Int J STD AIDS 18: 856-860.

Skuta G, Fischer GM, Janaky T, Kele Z, Szabo P, Tozser J, Sumegi B (1999). Molecular mechanism of the short-term cardiotoxicity caused by 2',3'-dideoxycytidine (ddC): modulation of reactive oxygen species levels and ADP-ribosylation reactions. Biochem Pharmacol 58: 1915-1925.

Smyth K, Affandi JS, McArthur JC, Bowtell-Harris C, Mijch AM, Watson K, Costello K, Woolley IJ, Price P, Wesselingh SL, Cherry CL (2007). Prevalence of and risk factors for HIV-associated neuropathy in Melbourne, Australia 1993-2006. HIV Med 8: 367-373.

Thacker MA, Clark AK, Marchand F, McMahon SB (2007). Pathophysiology of Peripheral Neuropathic Pain: Immune Cells and Molecules. Anesth Analg 105: 838-847.

Tyor WR, Wesselingh SL, Griffin JW, McArthur JC, Griffin DE (1995). Unifying hypothesis for the pathogenesis of HIV-associated dementia complex, vacuolar myelopathy, and sensory neuropathy. J Acquir Immune Defic Syndr Hum Retrovirol 9: 379-388.

van Steenwinckel J, Brisorgueil MJ, Fischer J, Verge D, Gingrich JA, Bourgoin S, Hamon M, Bernard R, Conrath M (2008). Role of spinal serotonin 5-HT2A receptor in 2',3'-dideoxycytidine-induced neuropathic pain in the rat and the mouse. Pain 137: 66-80.

Wadley AL, Cherry CL, Price P, Kamerman PR (2011). HIV Neuropathy Risk Factors and Symptom Characterization in Stavudine-Exposed South Africans. J Pain Symptom Manage 41: 700-706.

Wallace VC, Blackbeard J, Pheby T, Segerdahl AR, Davies M, Hasnie F, Hall S, McMahon SB, Rice AS (2007a). Pharmacological, behavioural and mechanistic analysis of HIV-1 gp120 induced painful neuropathy. Pain 133: 47-63.

Wallace VC, Blackbeard J, Segerdahl AR, Hasnie F, Pheby T, McMahon SB, Rice AS (2007b). Characterization of rodent models of HIV-gp120 and antiretroviral-associated neuropathic pain. Brain 130: 2688-2702.

Wallace VC, Segerdahl AR, Blackbeard J, Pheby T, Rice AS (2008). Anxiety-like behaviour is attenuated by gabapentin, morphine and diazepam in a rodent model of HIV anti-retroviral-associated neuropathic pain. Neurosci Lett 448: 153-156.

Warner WA, Bregman CL, Comereski CR, Arezzo JC, Davidson TJ, Knupp CA, Kaul S, Durham SK, Wasserman AJ, Frantz JD (1995). Didanosine (ddl) and stavudine (d4T): absence of peripheral neurotoxicity in rabbits. Food Chem Toxicol 33: 1047-1050.

Weber J, Mitchell D, Kamerman PR (2007). Oral administration of stavudine induces hyperalgesia without affecting activity in rats. Physiol Behav 92: 807-813.

White FA, Jung H, Miller RJ (2007). Chemokines and the pathophysiology of neuropathic pain. Proc Natl Acad Sci U S A 104: 20151-20158.

WHO, UNAIDS, Unicef (2010). Towards universal access: scaling up priority HIV/AIDS interventions in the health sector: progress report 2010. WHO, Geneva, p 145.

Yoshioka M, Shapshak P, Srivastava AK, Stewart RV, Nelson SJ, Bradley WG, Berger JR, Rhodes RH, Sun NC, Nakamura S (1994). Expression of HIV-1 and interleukin-6 in lumbosacral dorsal root ganglia of patients with AIDS. Neurology 44: 1120-1130.

Zhang K, Rana F, Silva C, Ethier J, Wehrly K, Chesebro B, Power C (2003). Human Immunodeficiency Virus Type 1 Envelope-Mediated Neuronal Death: Uncoupling of Viral Replication and Neurotoxicity. J Virol 77: 6899-6912.

Zheng FY, Xiao WH, Bennett GJ (2011a). The response of spinal microglia to chemotherapy-evoked painful peripheral neuropathies is distinct from that evoked by traumatic nerve injuries. Neuroscience 176: 447-454.

Published in final form: Kamerman PR, Moss PJ, Weber J, Wallace VCJ, Rice ASC, Huang W. Pathogenesis of HIV-associated sensory neuropathy: evidence from in vivo and in vitro experimental models. *Journal of the Peripheral Nervous System* 17: 19-31, 2012.
DOI: 10.1111/j.1529-8027.2012.00373.x, PMID: 22462664

Zheng W, Ouyang H, Zheng X, Liu S, Mata M, Fink DJ, Hao S (2011b). Glial TNFalpha in the spinal cord regulates neuropathic pain induced by HIV gp120 application in rats. Mol Pain 7: 40.

Zheng X, Ouyang H, Liu S, Mata M, Fink DJ, Hao S (2011c). TNFalpha is involved in neuropathic pain induced by nucleoside reverse transcriptase inhibitor in rats. Brain Behav Immun 25: 1668-1676.

Zhou L, Kitch DW, Evans SR, Hauer P, Raman S, Ebenezer GJ, Gerschenson M, Marra CM, Valcour V, Diaz-Arrastia R, Goodkin K, Millar L, Shriver S, Asmuth DM, Clifford DB, Simpson DM, McArthur JC (2007). Correlates of epidermal nerve fiber densities in HIV-associated distal sensory polyneuropathy. Neurology 68: 2113-2119.

Zhu Y, Antony JM, Martinez JA, Glerum DM, Brussee V, Hoke A, Zochodne D, Power C (2007). Didanosine causes sensory neuropathy in an HIV/AIDS animal model: impaired mitochondrial and neurotrophic factor gene expression. Brain 130: 2011-2023.

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Figure legends

Figure 1. Pathogenesis of HIV-induced peripheral nerve fibre damage. In dorsal root ganglia activation of Schwann cells and recruitment of macrophages following gp120-mediated activation of chemokine receptors CXCR4 and/or CCR5 causes the release of inflammatory mediators by these cells, ultimately causes apoptosis. In the nerve trunk, activation of apoptotic pathways by gp120 contributes to axonal degeneration and intraepidermal nerve fibre die-back. Axonal Schwann cells provide a barrier to gp120-mediated axonal damage. The details of pathways shown with dashed lines are uncertain.

Figure 2. HIV-induced hypernociception. Activation of chemokine receptors CXCR4 and/or CCR5 by gp120 causes membrane depolarization, sensitization of TRPV1 and bradykinin(BK) receptors, and release of Substance P, all of which contribute to the development of acute hypernociception. gp120 also causes macrophage infiltration along the nerve trunk and the release of pro-hypernociceptive cytokine TNF-α. Sustained exposure to gp120 leads to release of pro-hypernociceptive molecules CCL2 AND TNF-α in the dorsal root ganglia (dashed line). Ultimately, hyperactivity in the affected primary afferents results in gliosis in the dorsal horn of the spinal cord, which contributes to the maintenance of the hypernociceptive state. Viral protein R (Vpr) also may contribute to the development of hypernociception through membrane depolarization and release of interferon-γ in the dorsal root ganglion (dashed lines). However the mechanisms behind these processes are largely unknown. The details of pathways shown with dashed lines are uncertain.

Figure 3. Drug-induced hypernociception. The nucleotide reverse transcriptase inhibitor zalcitabine induces mitochondrial dysfunction associated with altered intracellular calcium homoeostasis and cytochrome C release, which initiates apoptotic pathways. These apoptotic pathways can lead to hypernociception through an imprecisely known mechanism. Activation of apoptotic pathways in the axon may contribute to drug-induced die-back of intra-epidermal nerve fibres. In addition, Zalcitabine causes activation of Schwann cells and macrophage infiltration into the dorsal root ganglia. Activated Schwann cells express the chemokine CXCL12, which probably mediates hypernociception through activation of CXCR4 receptors expressed on neurone. The dorsal root ganglion neurones also express the pro-nociceptive molecules CCR2 and TNF-α. An astrocytosis develops in the dorsal horn of the spinal cord following exposure to zalcitabine, which contributes to the development of hypernociceptive state through expression of TNF-α. The details of pathways shown with dashed lines are uncertain.

Figure 1

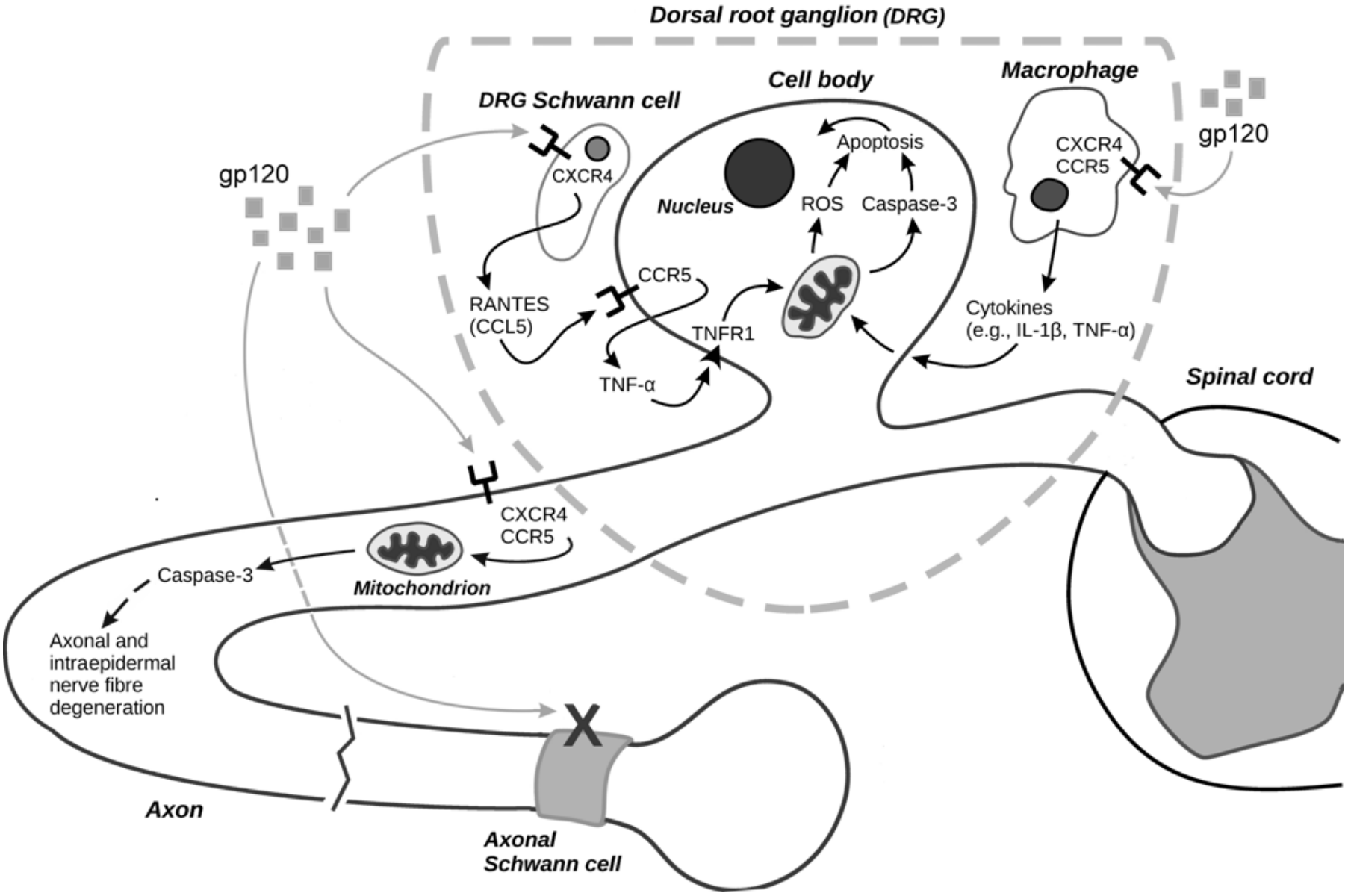

Dorsal root ganglion (DRG)
DRG Schwann cell
Cell body
Macrophage
gp120
CXCR4
Nucleus
Apoptosis
ROS
Caspase-3
CXCR4
CCR5
gp120
RANTES
(CCL5)
CCR5
TNFR1
TNF-α
Cytokines
(e.g., IL-1β, TNF-α)
Spinal cord
CXCR4
CCR5
Caspase-3
Mitochondrion
Axonal and
intraepidermal
nerve fibre
degeneration
Axon
Axonal
Schwann cell

Figure 2

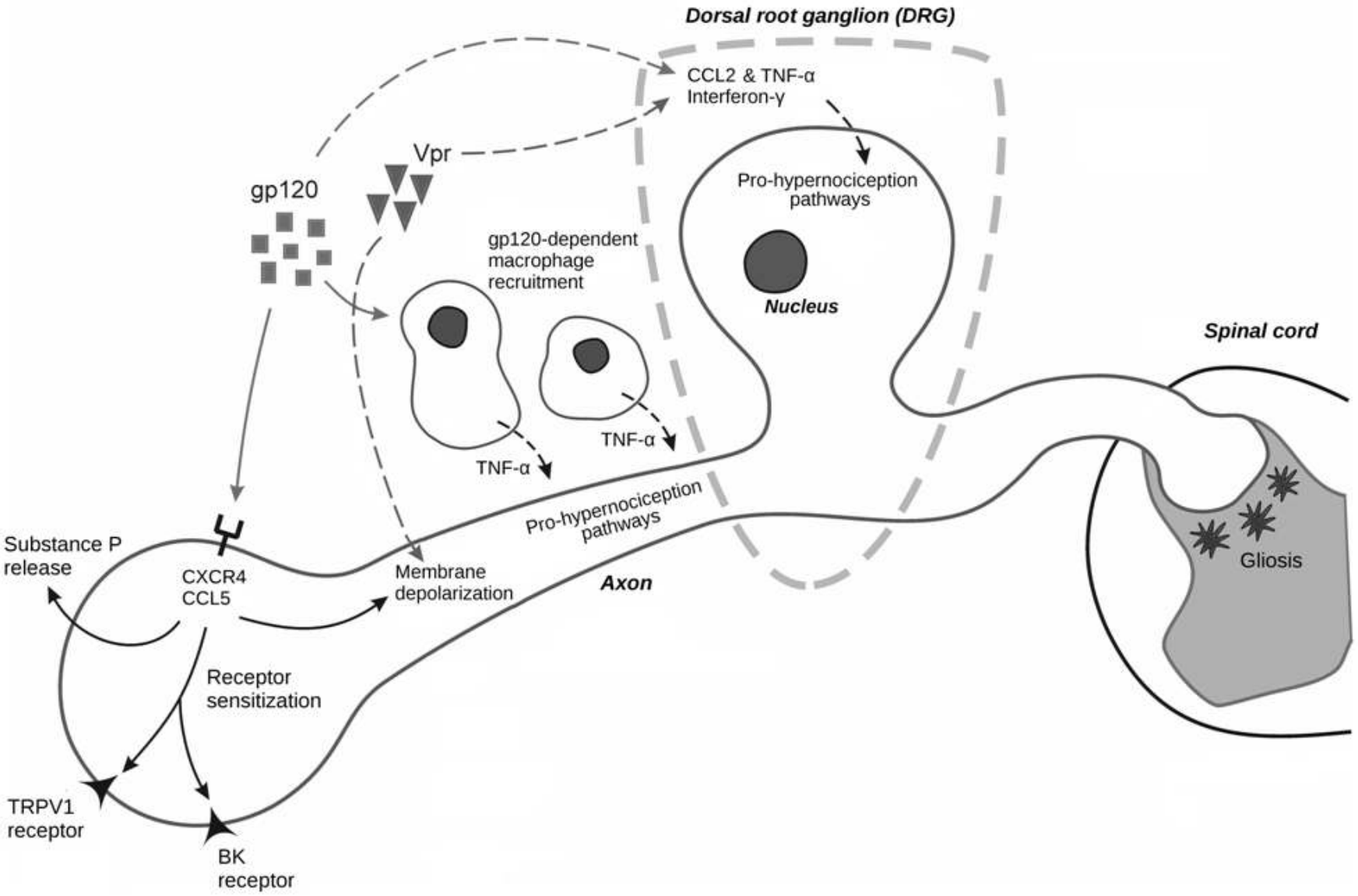
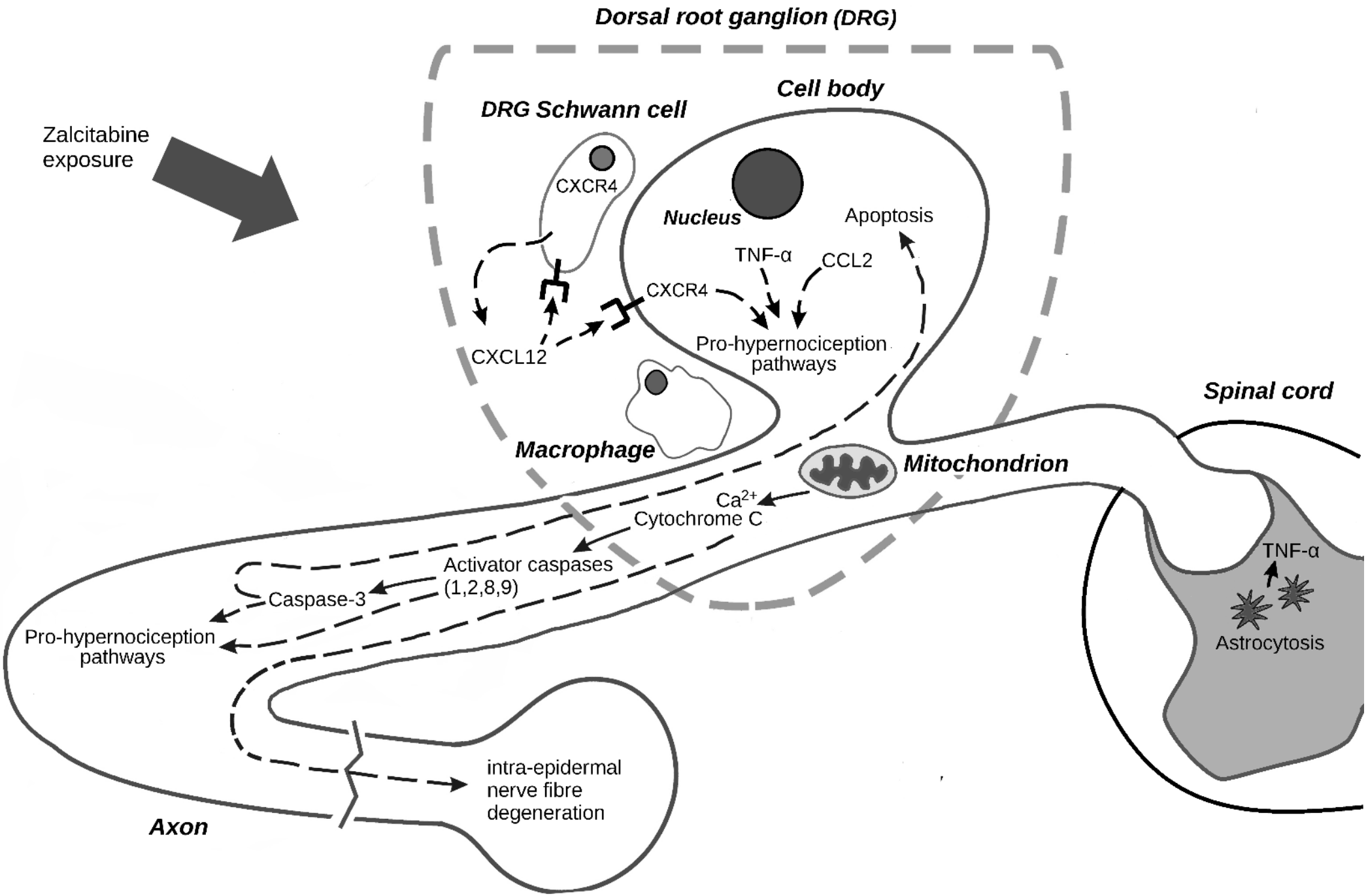

Dorsal root ganglion (DRG)
CCL2 & TNF-α
Interferon-γ
Vpr
gp120
Pro-hypernociception pathways
gp120-dependent macrophage recruitment
Nucleus
Spinal cord
TNF-α
TNF-α
Pro-hypernociception pathways
Substance P release
CXCR4
CCL5
Membrane depolarization
Axon
Gliosis
Receptor sensitization
TRPV1 receptor
BK receptor

Figure 3


Dorsal root ganglion (DRG)
Cell body
DRG Schwann cell
Zalcitabine exposure
CXCR4
Nucleus
Apoptosis
TNF-α
CCL2
CXCR4
CXCL12
Pro-hypernociception pathways
Spinal cord
Macrophage
Mitochondrion
Ca2+
Cytochrome C
Activator caspases (1,2,8,9)
Caspase-3
Pro-hypernociception pathways
TNF-α
Astrocytosis
intra-epidermal nerve fibre degeneration
Axon