

## EMERGING INSIGHTS ON THE MOLECULAR MECHANISMS OF HIV NEUROPATHOGENESIS

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### **ABSTRACT**

HIV is an infectious retrovirus that had devastating impacts when it first became prevalent in the United States and world wide. HIV primarily infects the lymphatic system, however, HIV can travel and infect other systems such as the nervous system. This review focuses on the players of HIV neuropathogenesis at the molecular level. HIV neuropathogenesis can lead to neurocognitive conditions such as HIV-associated neurocognitive disorders (HAND) and HIV-associated dementia (HAD). These disorders can have devastating impacts on one's neurological health, therefore, investigations of molecular mechanisms are important in attempts to discover new drug targets. Current HIV drugs do not target HIV protein production, and HIV proteins are a huge factor in HIV neuropathogenesis. Molecular mechanisms that have been recently investigated are the roles of HIV proteins in neuronal dysfunction, ways the structure of the neurons become impaired, and the mitochondrial damage caused by HIV infection. Understanding the molecular basis of HIV neuropathogenesis is critical to fighting this battle of HIV infection impacts within the brain.

### **INTRODUCTION**

Human immunodeficiency virus (HIV) is a retrovirus that received great attention when discovered in the early 1980s and has been infecting humans ever since. Approximately thirty-nine million individuals are currently infected with HIV globally (<https://www.who.int/news-room/fact-sheets/detail/hiv-aids>). HIV commonly infects macrophages, CD4+ and CD8+ T-cells, monocytes, lymphocytes, microglia, and astrocytes (Jadhav and Nema 2021). Macrophages, CD4+ and CD8+ T-cells, monocytes, and lymphocytes are commonly found within the lymphatic system but can traffic into the central nervous system (CNS) (Killingsworth and Spudich 2022). Microglia and astrocytes are specific to the CNS, however, macrophages are also usually present within the CNS (Betts et al. 2022). Macrophages are cells that engulf infectious agents. For example, macrophages can engulf an antigen bound to an antibody complex to help clear out infections. CD4+ T-cells are cells that activate other immune cells to do their job (Betts et al. 2022). A CD4+ T-cell can signal another cell to start producing antibodies (Betts et al. 2022). CD8+ T-cells are cells that can attack the pathogens. CD8+ T-cells will destroy infected cells by destroying the cell membrane (Betts et al. 2022). Monocytes are the stage before a cell becomes a macrophage. Lymphocytes are the stage before a cell becomes T-cell or B-cell. A T-lymphocyte is an immature T-cell, and a B-lymphocyte is an immature B-cell. Microglia are the immune cells of the CNS and they act very similarly to macrophages (Betts et al. 2022). Microglia are involved in tissue repair for the brain, growth, and development of new neural tissues, and innate immunity (Thangaraj et al. 2018). Astrocytes are CNS cells that are essential for many chemical regulations within the CNS and important for the formation of the blood-brain barrier (Betts et al. 2022). HIV commonly enters cells through the CCR5 and CXCR4 chemokine receptors on cells, which all of the cells described above have (Friedman-Levi et al. 2021).

Antiretroviral therapy (ART) has greatly increased HIV life expectancies (Wandeler 2016). However, this has resulted in a higher incidence of HIV-associated neurocognitive disorders (HAND) (Sharma et al. 2021). The most severe form of HAND is HIV-associated dementia (HAD) (Killingsworth and Spudich 2022). Common clinical symptoms of HAND can consist of memory decline, changes in behavior, decline in mental clarity and conciseness, concentration issues, and motor impairment (Killingsworth and Spudich 2022). Those affected by HAND or HAD can severely be impaired in their

daily lives. Therefore, research relating to how to treat HAND and HAD will be an essential factor in finding a cure for HIV neuropathogenesis.

A significant challenge for HIV neuropathogenesis treatment is HIV's abilities to remain latent, compartmentalize, cerebrospinal fluid escape (CSF escape), and its ability to form a reservoir within the brain (Handoko et al. 2021; Killingsworth and Spudich 2022; Oliveria et al. 2023; Sharma et al. 2021). HIV latency occurs when a cell becomes infected with HIV but then the cell stops producing new virions (Bednar et al. 2015). The HIV virus integrates into the host cell genome but then does not replicate new virions, resulting in a cell containing the HIV genome that does not express any HIV (Bednar et al. 2015). This phenomenon can make HIV a difficult virus to treat because it has latent cells that will not be active and therefore not targeted by ART (Chen et al. 2022). HIV compartmentalization is when the HIV within your lymphatic system or blood is slightly different from HIV within your CNS/brain (Killingsworth and Spudich 2022). CSF escape is when the HIV virus is undetectable within the blood but is still detectable within the CSF despite being on ART (Handoko et al. 2021). HIV reservoir formation is defined as a high amount of latent cells concentrated within a certain organ system (Chen et al. 2022). HIV brain reservoir formation is a high concentration of latent cells within the brain that form this reservoir of HIV that is not actively replicating (Chen et al. 2022). Common sites when HIV DNA has been observed within the brain are the frontal cortex, basal ganglia, and occipital cortex (Oliveira et al 2023).

While HIV cannot infect neurons directly, HIV proteins can exit infected cells and enter into the neurons causing neuronal disruption and dysregulation (Kannan et al. 2022). Common HIV proteins that cause disruption to neurons are Tat, gp120, gp41, Vpr, Rev, Vpu, and Nef (Jadhav and Nema 2021). HIV proteins can enter the neuron via fusion with extracellular vesicles or endocytosis of proteins floating around levels of the body (Hu et al. 2020; Kannan et al 2022). Furthermore, HIV ART does not impact HIV protein production since the virus integrates into the host cell permanently (Santerre et al. 2019). Therefore, these individuals can live with a lot of neurocognitive issues even when on HIV medication.

HIV proteins can cause toxic effects on neurons and their cellular organelles. Common structures of the neurons that are impacted by HIV proteins are the dendritic spines and the synapses (Hu et al. 2020; Kannan et al 2022; Liu et al. 2018). These structures on the neuron are very important for cellular communication to perform bodily functions (Betts et al. 2022). Therefore, if any cellular component has a mechanism to damage these important structures, communication is impaired and can lead to neurocognitive disorders that we see in HAND and HAD. A common organelle affected by HIV proteins is the mitochondrion. Mitochondria are essential for creating adenosine triphosphate (ATP) for cells to carry out cellular functions (Thangaraj et al. 2018). The HIV protein Tat can cause the mitochondria to produce more reactive oxygen species (ROS) that cause damage to the mitochondria (Darbinian et al. 2020). As a result, this process leads to neuronal damage and an increased risk for HAND and HAD (Darbinian et al. 2020). Furthermore, HIV proteins can impair mitochondrial degradation pathways that lead to a build up of damaged mitochondria within the cell that can cause neuronal dysfunction (Darbinian et al. 2020).

Molecular mechanisms of HIV neuropathogenesis demands further investigation to identify new drug targets to give people living with HIV a better quality of life. In this review, I will focus on the molecular mechanisms of HIV neuropathogenesis. I will focus on HIV proteins, extracellular vesicles, and mitochondrial dynamics in an attempt to bring together an overarching view of the numerous ways HIV affects neurons.

### **TAT'S EFFECT ON CREB AND BDNF**

HIV proteins can enter neurons and cause cellular dysfunction. HIV Tat (trans-activator of transcription) is a viral protein produced from the RNA genome of HIV (Ajasin and Eugenin 2020). Tat's role is to promote and enhance the transcription of HIV components (Ajasin and Eugenin 2020). Tat is one of the first proteins to be produced from an infected cell and it has been known to cause neurodegeneration (Santerre et al. 2019). In addition, Tat can decrease the length, thickness, and number of dendrites (Liu et al. 2018). Dendrites are the end branches of neurons that receive signals from other neurons. Tat and its production are not affected by HIV ART after the HIV DNA has already been integrated into the host cell's genome (Santerre et al. 2019). Overall, Tat's presence within neurons can cause neuronal

dysregulation for individuals suffering from HIV and this viral protein entry can lead to formation of HAND.

The Tat protein has been analyzed for its effects on neuronal regulators. HIV Tat can disrupt the cAMP response element-binding protein (CREB protein) and its downstream product, brain-derived neurotrophic factor (BDNF) (Liu et al. 2018; Santerre et al. 2019; Shrestha et al. 2022). CREB is a transcription factor that is key for memory, learning, and synaptic plasticity (Liu et al. 2018; Shrestha et al. 2022). BDNF is a protein that is important for synaptic transmission, synaptic plasticity control, and synaptic growth promotion (Liu et al. 2018). Since BDNF is a downstream target of CREB, if CREB becomes affected so will BDNF (Liu et al. 2018; Santerre et al. 2019; Shrestha et al. 2022). Cell cultures that are treated with the Tat protein show a decrease in CREB and BDNF (Santerre et al. 2019). Therefore, Tat reduces the production of these proteins by very complex mechanisms.

Tat increases a transcription factor p73, and that increases the production of a downstream transcription factor p53 (Santerre et al. 2019). Tat induces p53 expression and allows for p53 to bind to miR-34a promoter to create increased copies of miR-34a (Fig. 1) (Santerre et al. 2019). miR-34a is a microRNA which are short noncoding RNAs that can promote the degradation of mRNA or repress translation of an mRNA (Rashid et al. 2023). miR-34a in particular promotes HIV pathogenesis (Rashid et al. 2023). In human neuron cells, Tat up-regulated the expression of miR-34a and decreased in CREB and BDNF expression (Santerre et al. 2019). Therefore, the CREB promoter was analyzed and a transcription factor, E2F3 was found to contain miR-34a binding sites (Santerre et al. 2019). Tat causes a decrease in the expression of E2F3 by miR-34a binding to E2F3 mRNA to inhibit its production and therefore E2F3 is not produced to bind to the CREB promoter (Santerre et al. 2018). However, if overexpressed E2F3 was induced in cells with Tat, that was enough to rescue the CREB promoter (Santerre et al. 2019). Therefore, E2F3 plays an essential role in proper neuronal function (Santerre et al. 2019).

Tat was found to be the main cause of upregulation of different factors that lead to a decrease of CREB and BDNF. Tat increased the production of p73 which therefore increased p53 to activate the production of miR-34a (Santerre et al. 2019). This increased production of miR-34a due to Tat allowed this miR-34a to bind to the E2F3 transcription factor mRNA and inhibit its production (Santerre et al. 2019). The E2F3 transcription factor is essential for binding to the CREB promoter to allow CREB proteins to be produced so it can produce the downstream target BDNF which are both essential for neuronal function (Santerre et al. 2019). Without E2F3, CREB and BDNF cannot be produced. (Santerre et al. 2019). Without the production of these essential proteins, the neurons can miscommunicate and this can eventually lead to neurocognitive disorders (Santerre et al. 2019).

Phosphorylation of the CREB protein/transcription factor is essential to bind to the calcium/cyclic AMP response element (CRE) in the BDNF promoter (Fig. 1) (Liu et al. 2018). BDNF is a downstream product of CREB, so the CREB protein helps induce BDNF transcription (Liu et al. 2018). Phosphorylation of the CREB protein at Serine133 (Ser133) is essential for the CREB protein to work (Liu et al. 2018). One of the phosphatases known to dephosphorylate CREB at Ser133 is serine/threonine phosphatase 1 (PP1) (Liu et al. 2018). PP1 phosphorylation can be suppressed/positively regulated by inhibitor protein 2 (I-2) at threonine320 (Liu et al. 2018). I-2 can bind to PP1 at Threonine320 (Thr320) to positively regulate it to dephosphorylate target proteins (Liu et al. 2018). However, PP1 has a catalytic subunit that allows for autodephosphorylation (Liu et al. 2018). Autodephosphorylation is when a molecule can remove a phosphate group from itself. Primary mouse cortical neuron cells treated with Tat caused I-2 mRNA levels to increase and that PP1 expression was increased for the first six hours and then went back to baseline measures (Liu et al. 2018). This resulted in a 50% decrease of CREB phosphorylation at Ser133 when neurons were exposed to the Tat protein (Liu et al. 2018). BDNF mRNA levels were also decreased by 50% when exposed to Tat (Liu et al. 2018). Overall, the logic here was that Tat increases I-2 mRNA levels for increased I-2 proteins that go and bind to PP1 at Thr320 to cause PP1 to dephosphorylate target proteins like CREB, resulting in neuronal dysfunction (Liu et al. 2018). The dephosphorylation of CREB affects its downstream product BDNF, causing damaging effects on the neurons (Liu et al. 2018). Tat expression led to impairments of dendritic spines in neurons (Liu et al. 2018). Therefore, future research on how to inhibit the Tat protein would be beneficial in treating HIV neuropathogenesis.

**gp120'S EFFECT ON CREB AND BDNF**

Tat is not the only protein that has been shown to have effects on the CREB/BDNF pathway. HIV gp120 is the outside envelope protein that allows for cell adherence to the CD4+ T-cell receptor (Shrestha et al. 2022). gp120 can be taken up by the neurons through the CXCR4 receptor via lipid-raft mediated pinocytosis or endocytosis (Shrestha et al. 2022). CREB phosphorylation at Ser133 by protein kinase A (PKA) impacts spatial memory (Shrestha et al. 2022). CREB mRNA, protein levels, and phosphorylation levels at Ser133 were decreased in the presence of gp120 (Shrestha et al. 2022). This suggests that there is a potential link of CREB dephosphorylation via gp120. CREB phosphorylation is important for the transcription of downstream products like BDNF (Liu et al. 2018). The Sirt1 protein was analyzed and gp120 was found to decrease Sirt1 mRNA levels but increase miR-134 and miR-34a levels (Shrestha et al. 2022). Sirt1 protein is found within neuronal nuclei, microglia, and astrocytes (Xu et al. 2018). Sirt1 protein is essential for regulating homeostasis, circadian rhythm, and has neuroprotective roles in neuronal diseases (Xu et al. 2018). miR-34a leads to a suppression of E2F3 which is essential for CREB (Shrestha et al. 2022). Overall gp120 can inhibit the Sirt1 protein, from there that leads to an increase in miR-34a that decreases E2F3 and inhibits CREB production (Fig. 2) (Shrestha et al. 2022). The lack of CREB alters the hippocampus, can increase the risk of different neurodegenerative diseases, and can increase the risk of memory dysfunction (Shrestha et al. 2022).

Tat and gp120 can both be taken up by neurons and can cause damaging effects. Piecing all of this information together, it seems that Tat induces p73 production that allows for an increase of p53 to make more miR-34a levels (Fig. 1) (Santeree et al. 2019). From there, miR-34a can suppress E2F3 mRNA to reduce E2F3 production. Therefore, there is no E2F3 transcription to bind to the CREB promoter to produce CREB protein which is essential for downstream production of BDNF (Santeree et al. 2019). During this process, Tat also allows for an increase in I-2 levels (Liu et al. 2018). I-2 binds to Thr320 on PP1 to allow for PP1 to dephosphorylate CREB to inactivate it (Liu et al. 2018). An inactivated CREB protein cannot bind to the CRE element in the BDNF promoter, resulting in a decreased production of BDNF (Liu et al. 2018). Together these studies illustrate that these two mechanisms of CREB suppression and CREB dephosphorylation lead to impairment of the CREB protein production. Tat and gp120 can both induce miR-34a levels but through different mechanisms. Tat induces miR-34a from an increased production of p53 that promotes binding to the miR-34a promoter (Santeree et al. 2019). Whereas gp120 induces miR-34a by decreasing Sirt1 protein levels (Shrestha et al. 2022).

Tat and gp120 can cause impairment to CREB/BDNF through various mechanisms. When both of these proteins enter a neuron, the two can hit the neuron hard and cause extreme neuro-dysregulation (Liu et al. 2018; Santeree et al. 2019; Shrestha et al. 2022). This allows for multiple ways of miR-34a production to be increased to decrease E2F3 production to the point where miniscule amounts would be left and not enough CREB would be produced for a healthy neuron (Liu et al. 2018; Santeree et al. 2019; Shrestha et al. 2022). The mechanism for how gp120 and Tat impair CREB/BDNF is imperative to understand for discovering new drug targets to allow for the production of CREB/BDNF since these are key neuronal regulators.

**IMPACT OF EXTRACELLULAR VESICLES IMPACT ON HIV NEUROPATHOGENESIS**

Extracellular vesicles (EVs) are an essential form of communication for cells within the CNS (Hu et al. 2020). EVs occur when a cell releases a membrane with different cellular components, including proteins, into the CNS that can be taken up by other cells (Hu et al. 2020; Kannan et al. 2022). Cells that are infected with HIV can release EVs with HIV proteins that can be taken up by other cells to cause impairment in the recipient cell (Hu et al. 2020). Two cells in the nervous system I will be focusing on that release EVs are the astrocytes and microglia. Astrocyte EVs are known as astrocyte-derived extracellular vesicles (ADEVs) (Hu et al. 2020). Microglia EVs are known as microglia-derived EVs (MDEVs) (Kannan et al. 2022). EVs from HIV primary infected cells and biofluids continue to carry HIV viral proteins despite patient treatment with ART (Hu et al. 2020) Therefore, this leads to a buildup of HIV proteins within the CNS that can enter neurons and can cause HAND (Hu et al. 2020).

ADEVs cause dysregulation to neuronal synapses. Synapses are structures at the ends of the neurons where signals meet and can be sent from one neuron to another neuron (Betts et al. 2022). MicroRNA, miR-7 is known to have an important part in neuronal homeostasis but it also targets neuroligin 2 (NLGN2) (Hu et al. 2020). Neuroligins (NGLNs) are cell adhesion proteins that regulate synaptic function, structure, and modification (Hu et al. 2020). miR-7 was upregulated in the brains of HIV-infected individuals and SIV-infected rhesus macaques (Fig. 3) (Hu et al. 2020). Furthermore, the Tat protein was found to be responsible for the upregulation of miR-7 in human primary astrocytes (Hu et al. 2020). Tat infected astrocytes produced ADEVs with increased levels of miR-7 (Hu et al. 2020). A luciferase reporter assay showed miR-7 binds to the 3'UTR of NLGN2 inhibiting its function (Hu et al. 2020). Primary rat hippocampal neurons exposed to Tat ADEVs had a decrease in postsynaptic density protein 95 (PSD95), a synaptic scaffolding protein essential for the regulation of localization and trafficking of glutamate receptors (Hu et al. 2020; Yoo et al. 2019). PSD95 is also an excitatory postsynaptic marker (Hu et al. 2020). There was also a decrease in gephyrin (Hu et al. 2020). Gephyrin is a scaffold protein that is essential for maintaining the inhibitory synaptic transmission of neurotransmitters (Choi and Ko 2015). Additionally, neurons exposed to Tat ADEVs had synaptic injury with loss of inhibitory and excitatory synapses (Hu et al. 2020). A signal sent across an inhibitory synapse decreases neuronal activity while a signal sent across an excitatory synapse increases neuronal activity (Huang et al. 2018). This research demonstrated the loss of synapses and synaptic injury is damaging to neurons. Synapses are important for the establishment and maintenance of neuronal communication; they allow for information to be transferred from one neuron to another (Hu et al. 2020). Therefore, if a mechanism interrupts this synapse function, it can lead to neuronal impairment. This study adds to the picture of how HIV affects the CNS leading to HAND and HAD.

MDEVs and the NLRP3 inflammasome complex can affect the neurons (Kannan et al. 2022). The NLRP3 inflammasome is a component of the innate immune system that regulates the secretion of proinflammatory cytokines and is a contributor to HAND (Kannan et al. 2022; Kelley et al. 2019). In human primary rat cortical and hippocampal neurons (HPM) Tat MDEVs caused NLRP3 and glutamic acid decarboxylase 65 (GAD65) expression to be increased while PSD95 expression was decreased (Kannan et al. 2022). GAD65 is an enzyme that synthesizes gamma-aminobutyric acid which is a CNS inhibitory neurotransmitter (Budhram et al. 2021). Neurons exposed to MDEVs had fewer dendritic spines (Kannan et al. 2022). In immortalized murine microglia cells (BV2 cells) and HPM, Tat caused an increase in the amount of exosomes released (Kannan et al. 2022). Furthermore, NLRP3 and its downstream product, IL-1 $\beta$  levels, were increased in EVs (Kannan et al. 2022). IL-1 $\beta$  is a pro-inflammatory cytokine that promotes inflammation (Lopez-Castejon and Brough 2011). In Tat exposed BV2 cells, there was a decrease in vesicular glutamate transporter 1 (vGlut1) expression but an increase in Gephyrin (Kannan et al. 2022). Tat enters into microglia cells activates the NLRP3 inflammasome complex that leads to the production of IL-1 $\beta$  (Fig. 3) (Kannan et al. 2022). From there, NLRP3 and IL-1 $\beta$  are packaged into MDEVs and released to infect other neurons (Kannan et al. 2022). Once neurons take up these MDEVs the neuron goes through cellular changes; there is an increase in GAD65 and gephyrin but a decrease in PSD95 and vGlut1 expression (Kannan et al. 2022). The effect of MDEVs result in dendritic spine and synaptodendritic injury (Kannan et al. 2022). Dendrites are essential for regular neuronal function. Dendrites are the main piece of the neuron that receives most of the signals from other neurons (Betts et al. 2022).

The main idea of EVs is that these mechanisms can result in HAND. microRNAs are a factor in ADEVs infecting neurons resulting in defects of synapses (Hu et al. 2020). Inflammatory molecules affect neuronal functions that result in dendritic spine loss (Kannan et al. 2022). The key differentiating factor between these MDEVs and ADEVs is the gephyrin expression levels. In the ADEVs, there was a decrease in levels that resulted in synapse impairment, but in the MDEVs, there was an increase in levels that can lead to dendritic spine loss (Hu et al. 2022; Kannan et al. 2022). Gephyrin is associated with inhibitory synapse markers, so in the ADEVs, the decrease could represent the fact that Tat impairs synapses in general so inhibitory and excitatory synapses would both be decreased. However, the increase within the MDEVs could demonstrate that Tat impairs neuronal synapse signaling because it is expressing an

increase of an inhibitory protein. There needs to be a balance within the neuronal synapses of excitatory and inhibitory, too much of one can result in impaired transmission of signals (Huang et al. 2018).

Regarding the MDEVs, more proteins are observed. PSD95, vGlut1, gephyrin and GAD65 were analyzed. PSD95 and vGlut1 are both associated with excitatory synapses while gephyrin and GAD65 are both associated with inhibitory synapses (Kannan et al. 2022). Both gephyrin and GAD65 were upregulated while PSD95 and vGlut1 were both downregulated (Kannan et al. 2022). This could explain the increase of inhibitory synapses that lead to an imbalance of too many inhibitory synapses that lead to improper signaling transport within the neurons. Ultimately this leads to neuronal dysfunction that we see in HAND. There has also been discussion that damage to the synapses and dendrites can spread to the whole neuron resulting in apoptosis (Kannan et al. 2022). Therefore, if a cell is hit with both mechanisms that cause synapse impairment and dendritic spine loss, the cell may just die. However, this could also lead to neuronal dysfunction if you have many neurons impaired already by HIV Tat EVs and then others are dying. Overall, Tat causes impairments in EVs that are exported from infected cells to neurons causing neuronal damage.

### **gp120 EFFECTS ON SYNAPSES AND DENDRITIC SPINES**

As mentioned, HIV protein gp120 can be released from infected cells and be taken up by neurons to cause dysregulation. Infected HIV cells like macrophages and microglia can release inflammatory cytokines that can bind to neuronal receptors and cause synaptic injury by decreasing the number of excitatory synapses (Zhang et al. 2019). The loss of excitatory synapses correlates with HAND (Zhang et al. 2019).

gp120 causes a loss of excitatory synapses resulting in neuronal dysfunction (Zhang et al. 2019). gp120 binds to the CXCR4 coreceptor on microglia and stimulates the microglia to produce and release inflammatory cytokines like IL-1 $\beta$  (Fig. 4) (Zhang et al. 2019). IL-1 $\beta$  causes Src family kinases (SFKs) to phosphorylate the N-methyl-D-aspartate receptor (NMDARs) (Zhang et al. 2019). The NMDAR is a neuron ion channel that is important for synaptic plasticity and memory (Friedman-Levi et al. 2021). This phosphorylation stimulates the synthesis of the GABA neurotransmitters to block the synapses producing an inhibitory synapse (Zhang et al. 2019). Under normal conditions, p38 MAPK regulates the amount of inhibitory to excitatory synapses (Zhang et al. 2019). However, when a cell is exposed to IL-1 $\beta$  by gp120 the SFKs override p38 MAPK and overproduce the amount of inhibitory synapses leading to neurocognitive dysfunction (Zhang et al. 2019).

This is not the first time IL-1 $\beta$  expression has led to inhibitory synapses. As mentioned, in microglia cells affected by Tat produce IL-1 $\beta$  and release this into MDEVs to be taken up by the neurons causing synaptic injury (Fig. 3) (Kannan et al. 2022). IL-1 $\beta$  clearly plays a significant role in synaptic injury through more than one mechanism. Therefore, the role of IL-1 $\beta$  in neuronal injury and HIV neuropathogenesis should be further investigated.

gp120 turns glutamate (Glu) neurotoxic and then they both go on to activate the NMDAR to have a spontaneous excitatory postsynaptic current (sEPSC) (Fig. 4) (Liu et al. 2023). Glu is an excitatory neurotransmitter that is essential for cognitive behavior and memory (Liu et al. 2023). When gp120 interacts with Glu it changes it from having a good function to a neuropathogenic function (Liu et al. 2023). From there both of these proteins interact with NMDAR to have it be in a sEPSC frequency which is bad for the cell (Liu et al. 2023). sEPSC affects dendritic spine structure and lowers dendritic spine numbers (Liu et al. 2023). The effects on the spines result in decreased neurotransmission of signals (Liu et al. 2023). The significance of the low levels tested within the rat cerebrocortical neurons is to show the neuropathogenic effects on the brain since modern ART has lowered the levels of HIV infection within the body. Even at low levels of gp120, it is still causing neuropathic effects resulting in the prevalence of HAND. In conclusion, gp120 has detrimental effects on the NMDAR. NMDAR seems to be a similar factor that has a crucial role in mediating neuronal function and structures due to gp120. gp120's presence disrupts the neurons that cause cognitive impairments that are related to the symptoms we see in HAND.

gp120 causes increased production of proBDNF which leads to synaptic loss within the striatum. (Speidell et al. 2020). The striatum is part of the basal ganglia and it is important for motor function (Baez-Mendoza and Schultz 2013). proBDNF is a precursor protein of BDNF that is produced in greater

amounts in the presence of gp120 (Speidell et al. 2020). Mature BDNF binds to tyrosine kinase receptor (TrkB) and pan p75 neurotrophin receptor (p75NTR) receptors to regulate synaptic plasticity (Speidell et al. 2020). Therefore, it is essential that mature BDNF binds to both receptors to regulate synaptic plasticity (Speidell et al. 2020).

However, proBDNF tends to favor the binding of the p75NTR receptor promoting an imbalance of the TrkB/p75NTR ratio leading to dendritic spine disfiguration and reduced synaptic function (Speidell et al. 2020). TrkB is essential in dendritic spine formation and increasing synaptic function (Speidell et al. 2020). p75NTR harms dendritic morphology and synaptic function (Speidell et al. 2020). TrkB has an essential role in dendritic spine formation and synaptic regulation, and it is properly activated by mature BDNF, however when proBDNF is present it favors binding to p75NTR causing an imbalance that results in improper inactivation of TrkB that results in neuronal damage (Speidell et al. 2020). gp120 causes a promotion of proBDNF production that leads to binding of the p75NTR receptor resulting in an increased amount of unstable and immature dendritic spine subtypes and motor learning impairments. (Speidell et al. 2020). Motor learning is dependent on synapses, so therefore since there was a result of motor learning impairments, this means that the synapses were also impaired by gp120 and proBDNF (Speidell et al. 2020). Overall, gp120 leads to an increased production of proBDNF that results in neuronal dysfunctions that can be associated with HAND (Speidell et al. 2020).

proBDNF accumulates in neurons when gp120 is present. ProBDNF is usually cleaved to mature BDNF via the furin protease (Allen et al. 2022). gp120 affects the last step in glycolysis that allows for a decrease of phosphoenolpyruvate (PEP) to pyruvate by pyruvate kinase muscle isoform 2 (PKM2) dimerization (Fig. 5) (Allen et al. 2022). As a result, there is a reduction in ATP production due to PKM2 dimerization and there is increased glycolysis to try and compensate for this ATP reduction (Allen et al. 2022). The increase of glycolysis allows for an increase of glycolytic metabolites that can contribute to the formation of advanced glycation end products (AGEs) (Allen et al. 2022). These AGEs build up in the cell and bind to proBDNF to prevent proper cleavage to mature BDNF (Allen et al. 2022). Therefore, gp120 allows for an increase in proBDNF by blocking proper cleavage that allows for the proBDNF isoform to build up in the cell and cause neuronal damage (Allen et al. 2022).

Synapses are an essential part of the neurons that are crucial for function. When gp120 acts on different cellular mechanisms that contribute to synaptic and dendritic spine loss we see how HAND is occurring. Together, it seems that gp120 can allow for the NMDAR to become phosphorylated to produce GABA neurotransmitters to induce inhibitory synapses and it can also bind directly to the NMDAR and allow no uptake a Glu that is an essential neurotransmitter (Liu et al. 2023; Zhang et al. 2019). NMDAR appears to play multiple roles in contributing to HIV neuropathogenesis that warrants further research. gp120 also prevents BDNF from being cleaved properly by the accumulation of AGEs that keep it in the precursor form. proBDNF creates an imbalance of the TrkB/p75NTR ratio that results in synaptic and dendritic spine losses (Allen et al. 2022; Speidell et al. 2020). Finding a drug target that helps induce proper cleavage of BDNF could have a possible positive impact on reducing synaptic and dendritic spine impairment.

### **MITOCHONDRIAL DAMAGE FROM HIV**

HIV proteins can damage the mitochondria of neurons and alter neuronal function. Tat has been shown to interfere with mitochondrial dynamics (Thangaraj et al. 2018). Tat reduces mitochondrial membrane potential, reduces ATP production, reduces oxygen consumption rate, and interferes with mitophagy (Teodorof-Diedrich and Spector 2018; Thangaraj et al. 2018). This is significant because loss of mitochondrial membrane potential is associated with mitochondrial injury and damage (Teodorof-Diedrich and Spector 2018).

Mitophagy is when damaged or depolarized mitochondria are sent to the lysosome for degradation (Teodorof-Diedrich and Spector 2018; Thangaraj et al. 2018). Mitophagy is an essential cellular process to maintain cellular metabolism and reduce cell stress (Teodorof-Diedrich and Spector 2018). In a normal healthy cell, damaged mitochondria segments are separated from healthy mitochondria segments via dynamin 1-like protein (Drp1) (Fig. 6) (Thangaraj et al. 2018). Then PTEN-induced putative kinase 1 (PINK1) is expressed on the outer membrane of damaged mitochondria

(Thangaraj et al. 2018). From there, PINK1 phosphorylates itself to recruit parkin RBR E3 ubiquitin-protein ligase (PRKN) to the outer mitochondrial membrane for ubiquitination (Thangaraj et al. 2018). The ubiquitinated mitochondrial membrane recruits sequestosome 1 (SQSTM1), an autophagic receptor protein, to bind to the ubiquitinated membrane (Thangaraj et al. 2018). Then, microtubule-associated protein 1 light chain 3 beta (MAP1LC3B) engulfs the damaged mitochondria to form the mitophagosome (Thangaraj et al. 2018). This process forms the mitophagosomes to be sent to the lysosome for degradation (Thangaraj et al. 2018).

In HIV-infected microglia, Tat caused an accumulation of SQSTM1 and MAP1LC3B-II (Thangaraj et al. 2018). Since these markers are on mitochondria that should be degraded by the lysosome, accumulation of these means there is incomplete mitophagy (Thangaraj et al. 2018). Tat also decreased lysosomal-associated membrane protein 2 (LAMP2) which is a marker protein that is expressed when mitophagosomes are fused with lysosomes (Thangaraj et al. 2018). An increased amount of impaired mitochondria in the neuron results in microglia activation that causes neuroinflammation (Thangaraj et al. 2018). Chronically activated microglia can lead to HAND (Thangaraj et al. 2018).

Multiple studies have replicated similar findings in mitophagy marker expression levels. HIV proteins activate mitophagy markers to localize to the damaged mitochondria in neurons but then export to the lysosome is impaired (Teodorof-Diedrich and Spector 2018). Tat and gp120 increased DRP1 expression promoting mitochondrial fragmentation (Teodorof-Diedrich and Spector 2018). DRP1 is needed for mitochondrial separation of healthy and damaged mitochondria (Teodorof-Diedrich and Spector 2018; Thangaraj et al. 2018). Therefore, an increase of this protein with exposure to HIV proteins shows that these proteins are promoting damage to the mitochondria. Mitophagy markers, PINK1, PRKN, and Drp1 expression levels were increased when exposed to Tat (Teodorof-Diedrich and Spector 2018; Thangaraj et al. 2018). Autophagosome markers, beclin 1 (BECN1), MAP1LC3B-II, and SQSTM1 expression levels were increased due to Tat exposure (Thangaraj et al. 2018). Upregulation and accumulation of these markers provide evidence that HIV proteins cause damage to the mitochondria and that there is incomplete degradation of these damaged mitochondria. This results in inflammation that can lead to HAND.

Tat can also cause damage to mitochondrial DNA (mtDNA) (Darbinian et al. 2020). mtDNA damage can lead to improper functioning of the mitochondria. Tat caused increased reactive oxygen species (ROS) in mitochondria (Darbinian et al. 2020). An increase in ROS results in damage to the mtDNA and is commonly seen in neurodegenerative disorders (Darbinian et al. 2020). Tat caused decreased ATP5A1 gene expression which resulted in decreased ATP production and caused a decrease in 8-oxoguanine DNA glycosylase-1 (OGG1) (Darbinian et al. 2020). OGG1 is an enzyme that can help repair mtDNA damage, however, in the presence of Tat this protein is downregulated (Darbinian et al. 2020). Therefore, Tat severely impairs mitochondrial function because Tat causes damage but also inhibits mitochondrial repair mechanism (Darbinian et al. 2020). Mitochondrial damage and incomplete degradation of these damaged mitochondria cause a buildup of inflammation within the neurons which can lead to HAND.

Piecing everything together, Tat damages the mitochondria DNA and impairs mitochondrial function (Fig. 6). The mitochondria are now damaged and need to be degraded. However, Tat impairs the maturation of mitophagosome and blocks fusion with the lysosome and proper degradation. (Darbinian et al. 2020; Teodorof-Diedrich and Spector 2018; Thangaraj et al. 2018). Tat's presence acts as a blockade for proper degradation and causes a buildup of proteins and damaged mitochondria. This accumulation can probably damage the last steps of mitochondrial degradation because there is too much disruption within the cell to be able to carry out proper cellular functions. Research on drug targets that delve into mitophagy proteins and damaged mitochondria clearance should be further investigated.

Another important factor in mitochondrial function is mitochondria-associated endoplasmic reticulum membranes (MAMs) (Arjona et al. 2023). Association between the mitochondria and endoplasmic reticulum (ER) is essential for calcium and lipid transport and for postsynaptic dynamics within neurons (Arjona et al. 2023). HIV proteins like Tat can cause stress on the ER that results in improperly folded proteins within the neurons and astrocytes (Arjona et al. 2023). Tat can also cause calcium imbalances that are an important transport within this system (Arjona et al. 2023).

Tat interferes with interactions of MAM tethering proteins that are essential for proper fusion of MAMs between the mitochondria and ER (Arjona et al. 2023). The protein duo interaction of PTPIP51 and MAM tethering protein, VAPB, is essential for memory formation and synaptic activity regulation (Arjona et al. 2023). Tat promotes phosphorylation of PTPIP51 by Lyn and Src kinases to change PTPIP51's localization to not go to the mitochondria to prevent PTPIP51 from associating with VAPB (Fig. 7) (Arjona et al. 2023). Therefore, these VAPB and PTPIP51 cannot interact to have proper MAM transport leading to mitochondrial dysfunction and stress (Arjona et al. 2023). This failed interaction results in disruption of calcium signaling between the two organelles resulting in decreased synaptic activity which is essential for proper neuronal function (Arjona et al. 2023). Therefore, this can result in HAND.

The interesting factor is that this is not the first time we see Src kinases interacting with synaptic function. As mentioned, a Src kinase causes phosphorylation of NDMAR to produce GABA neurotransmitters to create inhibitory synapses (Fig. 4) (Zhang et al. 2019). Src kinases phosphorylate PTPIP51 to change proper localization to the mitochondria to mess with synaptic activity (Arjona et al. 2023). The common denominator of these two functions that impairs synaptic function which leads to neurological issues is the Src kinase. Therefore, the role of Src kinase in HIV neuropathogenesis should be investigated and potentially inhibited.

HIV proteins can cause mtDNA damage and functional damage (Darbinian et al. 2020). The improper mitochondria clearance and disassociation of mitochondrial proteins and ER proteins can have downstream effects that can impair the synapses or lead to a buildup of mitochondria that can cause inflammation (Arjona et al. 2023; Thangaraj et al. 2018). The bigger picture is that when HIV proteins enter the neurons they disrupt the mitochondria in many ways that increase neuronal dysfunction, supporting HAND formation in HIV-infected individuals.

## **CONCLUSION**

This review highlighted some of the molecular neuropathic mechanisms of HIV. The HIV Tat and gp120 proteins can be released from infected cells and taken up by neurons. From there, these proteins can harm and damage the cell in many ways. These proteins can result in harm to neuronal structures like the synapses and dendritic spines.

Future studies should include a deeper analysis of the role of the Src kinases to discover a more conclusive role in how Src kinases support HIV neuropathogenesis. A common cause of dysregulation within the neurons is HIV proteins such as Tat and gp120. Damage to the mitochondria, synapses, dendritic spines, and the overall neuron seem to be influenced by HIV proteins' presence within the neuron. A promising area of investigation for reduction of HAND and HAD is aiming to impair and inhibit Tat and gp120. The creation of synthetic proteins could also potentially help treat neuroinflammation by creating proteins that are harmed by HIV. For example, creating a synthetic E2F3 protein that can bind to the CREB promoter to produce the CREB protein in a neuron where HIV Tat is present would allow proper CREB and BDNF production since Tat inhibits this process. Creation of synthetic neuronal regulator proteins that are usually depleted by HIV could help restore protein levels to normal and allow for normal neuronal functions. HIV neuropathogenesis can lead to neurological problems that impair the quality of life for individuals. The goal of this review is to elaborate on the molecular mechanisms of HIV neuropathogenesis to help inform the search for new drug targets.

Literature Cited:

- Ajasin D and Eugenin EA. 2020. HIV-1 Tat: role in bystander toxicity. *Frontiers in Cellular and Infection Microbiology*. 10:1-15. 10.3389/fcimb.2020.00061
- Allen CNS, Arjona SP, Santerre M, De Lucia C, Koch WJ, and Sawaya BE. 2022. Metabolic reprogramming in HIV-associated neurocognitive disorders. *Frontiers in Cellular Neuroscience*. 16:1-21. 10.3389/fncel.2022.812887
- Arjona SP, Allen CNS, Santerre M, Gross S, Soboloff J, Booze R, and Sawaya BE. 2023. Disruption of mitochondrial-associated ER membranes by HIV-1 tat protein contributes to premature brain aging. *CNS Neuroscience Therapeutics*. 29:365-377. 10.1111/cns.14011
- Báez-Mendoza R and Schultz W. 2013. The role of the striatum in social behavior. *Frontiers in Neuroscience*. 7:1-14. 10.3389/fnins.2013.00233
- Bednar MM, Sturdevant CB, Tompkins LA, Arrildt KT, Dukhovlinova E, Kincer LP, and Swanstrom R. 2015. Compartmentalization, viral evolution, and viral latency of HIV in the CNS. *Current HIV/AIDS Reports*. 12:262-271. 10.1007/s11904-015-0265-9
- Betts JG, Young KA, Wise JA, Johnson E, Poe B, Kruse DH, Korol O, Johnson JE, Womble M, and DeSaix P. 2022. *Anatomy and physiology*. 2nd ed. OpenStax. Houston, TX
- Budhram A, Sechi E, Flanagan EP, Dubey D, Zekeridou A, Shah SS, Gadoth A, Naddaf E, McKeon A, Pittock SJ, and Zalewski NL. 2021. Clinical spectrum of high-titre GAD65 antibodies. *Journal of Neurology, Neurosurgery, and Psychiatry*. 92:645-654. 10.1136/jnnp-2020-325275
- Chen J, Zhou T, Zhang Y, Luo S, Chen H, Chen D, Li C, and Li W. 2022. The reservoir of latent HIV. *Frontiers in Cellular and Infection Microbiology*. 12:1-15. 10.3389/fcimb.2022.945956
- Choi G and Ko J. 2015. Gephyrin: a central GABAergic synapse organizer. *Experimental and Molecular Medicine*. 47:e158. 10.1038/emm.2015.5
- Darbinian N, Darbinyan A, Merabova N, Selzer ME, and Amini S. 2020. HIV-1 and HIV-1-tat induce mitochondrial DNA damage in human neurons. *Journal of HIV AIDS*. 6:1-29. 10.16966/2380-5536.176
- Friedman-Levi Y, Liraz-Zaltsman S, Shemesh C, Rosenblatt K, Kesner EL, Ginberg G, Carmichael ST, Silva AJ, and Shohami E. 2021. Pharmacological blockers of CCR5 and CXCR4 improve recovery after traumatic brain injury. *Experimental Neurology*. 338:113604. 10.1016/j.expneurol.2021.113604
- Handoko R, Chan P, Jagodzinski L, Pinyakorn S, Ubolyam S, Phanuphak N, Sacdalan C, Kroon E, Dumrongpisutikul N, Paul R, Valcour V, Ananworanich J, Vasana S, Spudich S, and SEARCH010/RV254 Study Team. 2021. Minimal detection of cerebrospinal fluid escape after initiation of antiretroviral therapy in acute HIV-1 infection. *AIDS*. 35:777-782. <https://doi.org/10.1097/qad.0000000000002786>.
- Hu G, Niu F, Liao K, Periyasamy P, Sil S, Liu J, Dravid SM, and Buch S. 2020. HIV-1 tat-induced astrocytic extracellular vesicle miR-7 impairs synaptic architecture. *Journal of Neuroimmune Pharmacology*. 15:538-553. 10.1007/s11481-019-09869-8
- Huang Z, Khaled HG, Kirschmann M, Gobes SM, and Hahnloser RH. 2018. Excitatory and inhibitory synapse reorganization immediately after critical sensory experience in a vocal learner. *Elife*. 7:e37571. 10.7554/eLife.37571
- Jadhav S and Nema V. 2021. HIV-associated neurotoxicity: the interplay of host and viral proteins. *Mediators of Inflammation*. 2021:1-11. 10.1155/2021/1267041
- Kannan M, Singh S, Chemparathy DT, Oladapo AA, Gawande DY, Dravid SM, Buch S, and Sil S. 2022. HIV-1 tat induced microglial EVs leads to neuronal synaptodendritic injury: microglia-neuron

- cross-talk in neuroHIV. *Extracellular Vesicles and Circulating Nucleic Acids*. 3:133-149. 10.20517/evcna.2022.14
- Kelley N, Jeltema D, Duan Y, and He Y. 2019. The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *International Journal of Molecular Sciences*. 20:1-24. 10.3390/ijms20133328
- Killingsworth L and Spudich S. Neuropathogenesis of HIV-1: insights from across the spectrum of acute through long-term treated infection. *Seminars in Immunopathology*. 44:709-724. <https://doi.org/10.1007/s00281-022-00953-5>
- Liu J, Xie J, Dutta D, and Xiong H. 2023. HIV-1 envelope protein gp120 modulation of glutamate effects on cortical neuronal synapses: implications for HIV-1-associated neuropathogenesis. *International Journal of Physiology, Pathophysiology and Pharmacology*. 15:75-87. PMC10349318
- Liu Y, Zhou D, Feng J, Liu Z, Hu Y, Liu C, and Kong X. 2018. HIV-1 protein Tat1-72 impairs neuronal dendrites via activation of PP1 and regulation of the CREB/BDNF pathway. *Virologica Sinica*. 33:261-269. <https://doi.org/10.1007/s12250-018-0031-4>
- Lopez-Castejon G and Brough D. 2011. Understanding the mechanism of IL-1 $\beta$  secretion. *Cytokine Growth Factor Review*. 22:189-195. 10.1016/j.cytogfr.2011.10.001
- Oliveira MF, Pankow A, Vollbrecht T, Kumar NM, Cabalero G, Ignacio C, Zhao M, Vitomirov A, Gouaux B, Nakawawa M, Murrell B, Ellis RJ, and Gianella S. 2023. Evaluation of archival HIV DNA in brain and lymphoid tissues. *Journal of Virology*. 97: e00543-23. <https://doi-org.ezproxy.tcnj.edu/10.1128/jvi.00543-23>
- Rashid F, Zaongo SD, Song F, and Chen Y. 2023. The diverse roles of miRNAs in HIV pathogenesis: current understanding and future perspectives. *Frontiers in Immunology*. 13:1-13. 10.3389/fimmu.2022.1091543
- Santerre M, Bagashev A, Gorecki L, Lysek KZ, Wang Y, Shrestha J, Del Carpio-Cano F, Mukerjee R, and Sawaya BE. 2019. HIV-1 Tat protein promotes neuronal dysregulation by inhibiting E2F transcription factor 3 (E2F3). *Journal of Biological Chemistry*. 294:3618-3633. 10.1074/jbc.RA118.003744.
- Sharma V, Creegan M, Tokarev A, Hsu D, Slike BM, Sacdalan C, Chan P, Spudich S, Ananworanich J, Eller MA, Krebs SJ, Vasani S, Bolton DL, RV254/SEARCH010, and RV304/SEARCH013 Study Teams. 2021. Cerebrospinal fluid CD4+ T cell infection in humans and macaques during acute HIV-1 and SHIV infection. *PLOS Pathogens*. 17:e1010105. <https://doi.org/10.1371/journal.ppat.1010105>.
- Shrestha J, Santerre M, Allen CNS, Arjona SP, Merali C, Mukerjee R, Chitralla KN, Park J, Bagashev A, Bui V, Eugenin EA, Merali S, Kaul M, Chin J, and Sawaya BE. 2022. HIV-1 gp120 impairs spatial memory through cyclic AMP response element-binding protein. *Frontiers in Aging Neuroscience*. 14:1-19. 10.3389/fnagi.2022.811481
- Speidell A, Asuni GP, Wakulski R, and Mochetti I. 2020. Up-regulation of the p75 neurotrophin receptor is an essential mechanism for HIV-gp120 mediated synaptic loss in the striatum. *Brain, Behavior, and Immunity*. 89:371-379. 10.1016/j.bbi.2020.07.023
- Teodorof-Diedrich C and Spector SA. 2018. Human immunodeficiency virus type 1 gp120 and tat induce mitochondrial fragmentation and incomplete mitophagy in human neurons. *Journal of Virology*. 92:1-16. 10.1128/JVI.00993-18.
- Thangaraj A, Periyasamy P, Liao K, Bendi VS, Callen S, Pendyala G, and Buch S. 2018. HIV-1 tAT-mediated microglial activation: role of mitochondrial dysfunction and defective mitophagy. *Autophagy*. 14:1596-1619. 10.1080/15548627.2018.1476810
- Wandeler G, Johnson LF, and Egger M. 2016. Trends in life expectancy of HIV-positive adults on antiretroviral therapy across the globe: comparisons with general population. *Current Opinion in HIV and AIDS*. 11:492-500. 10.1097/COH.0000000000000298
- Xu J, Jackson CW, Khoury N, Escobar I, and Perez-Pinzon MA. 2018. Brain SIRT1 mediates metabolic homeostasis and neuroprotection. *Frontiers in Endocrinology*. 9:1-16. 10.3389/fendo.2018.00702

Yoo KS, Lee K, Oh JY, Lee H, Park H, Seok Park Y, and Kyu Kim H. 2019. Postsynaptic density protein 95 (PSD-95) is transported by KIF5 to dendritic regions. *Molecular Brain*. 12:1-12. <https://doi.org/10.1186/s13041-019-0520-x>

Zhang X, Green MV, and Thayer SA. 2019. HIV gp120-induced neuroinflammation potentiates NMDA receptors to overcome basal suppression of inhibitory synapses by p38 MAPK. *Journal of Neurochemistry*. 148:499-515. 10.1111/jnc.14640.

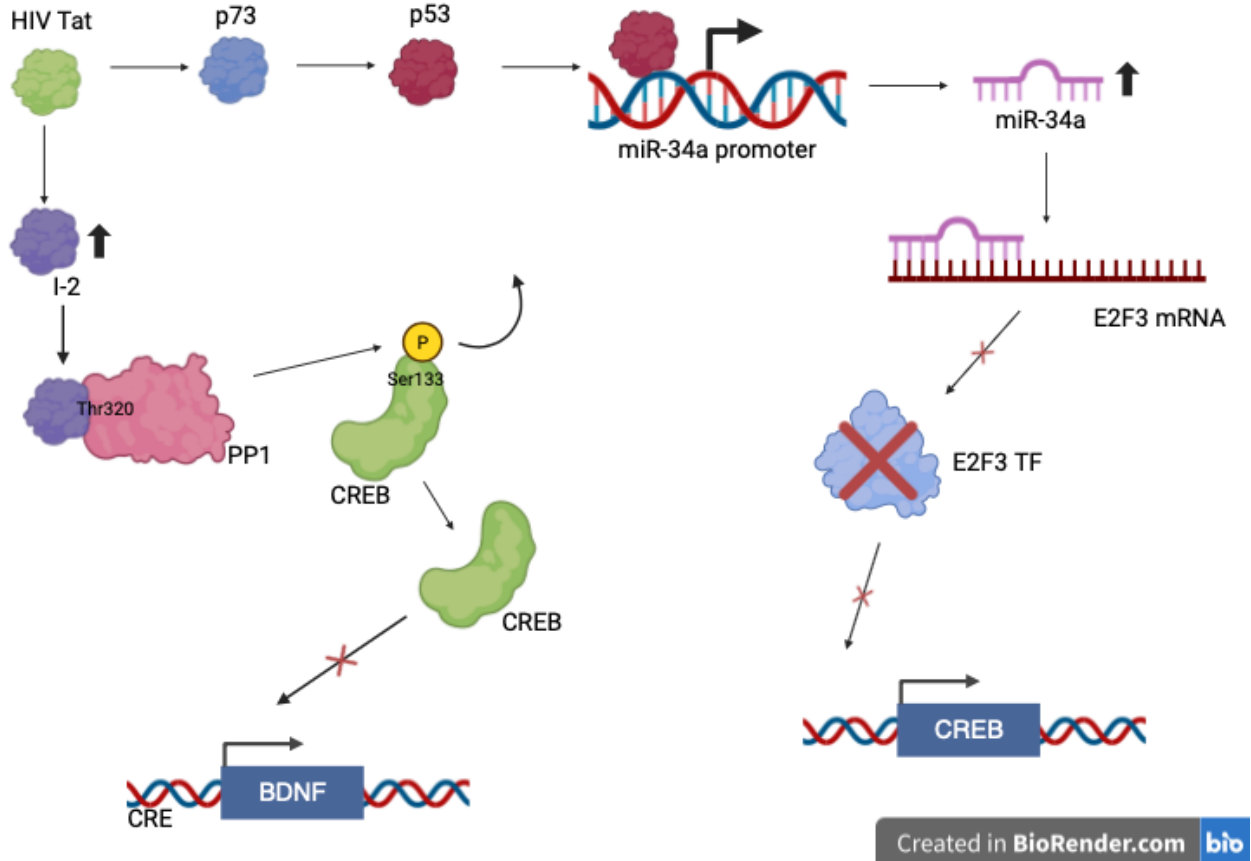


Figure 1: HIV Tat promotes p73 production and p73 promotes p53 production. p53 binds to the miR-34a promoter to increase miR-34a production. miR-34a binds to the E2F3 transcription factor mRNA to inhibit production of it. Therefore, there is no E2F3 present to bind to the CREB promoter to produce CREB protein. HIV Tat can also increase I-2 levels and I-2 binds to PP1 at Thr320. That causes PP1 to act as a dephosphorylase and it removes the phosphate from Ser11 on the CREB protein, leading to improper function of the CREB protein. This results in CREB not being able to bind to the CRE element in the BDNF promoter, resulting in BDNF production. (Liu et al. 2018; Santerre et al. 2019)

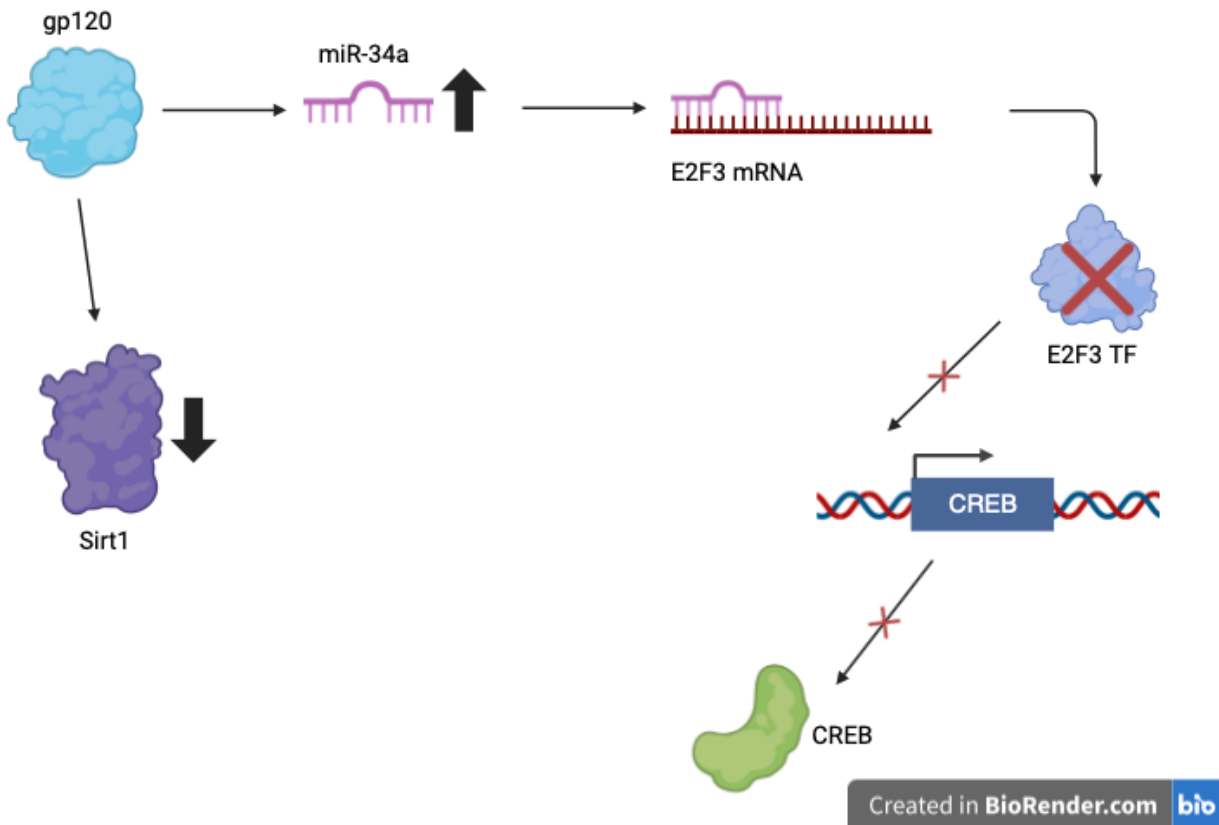


Figure 2: HIV gp120 affects the production of the CREB protein. gp120 increases the levels of miR-34a to bind to the E2F3 transcription factor mRNA to inhibit E2F3 production. Therefore, we are left without a transcription factor to bind to the CREB promoter to produce CREB protein. gp120 also downregulates the production of Sirt1, an important protein found to have neuroprotective roles. (Shrestha et al. 2022).

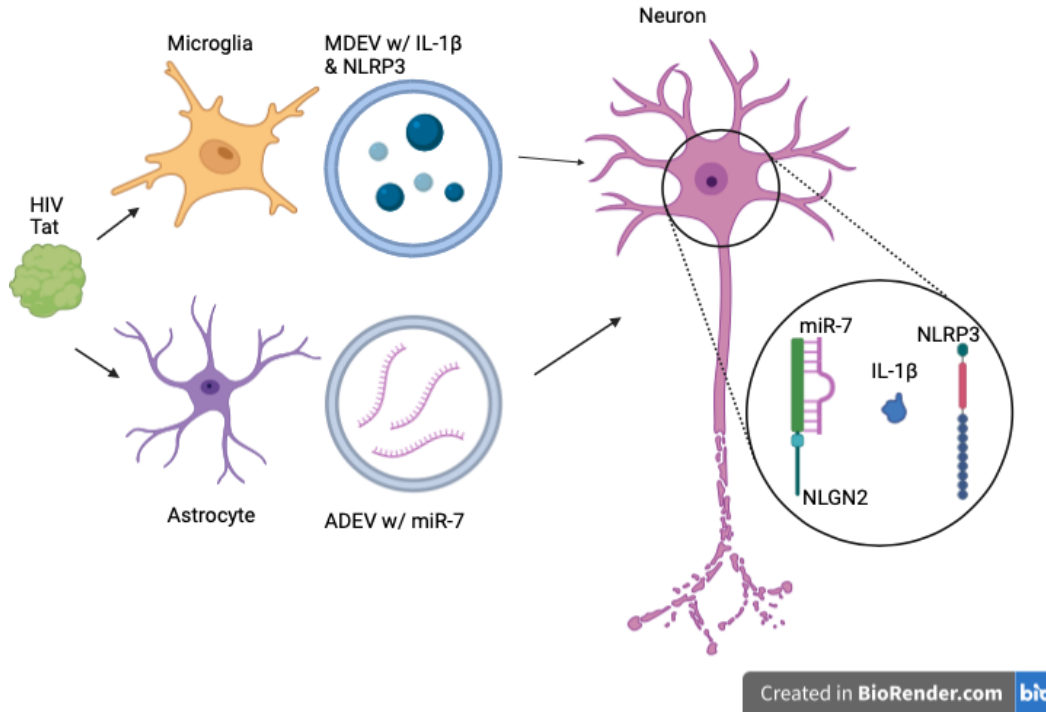


Figure 3: HIV Tat protein infects the microglia and astrocyte. The activated microglia upregulates the production of IL-1 $\beta$  and NLRP3 due to Tat and the astrocyte upregulates miR-7 production. Once these vesicles infuse within the neuron miR-7 binds to the 3'UTR region of the NLGN2 to cause synaptic dysfunction. IL-1 $\beta$  and NLRP3 enter the neuron and alter the levels of synaptic proteins to cause dendritic spine injury. (Hu et al. 2020; Kannan et al 2022)

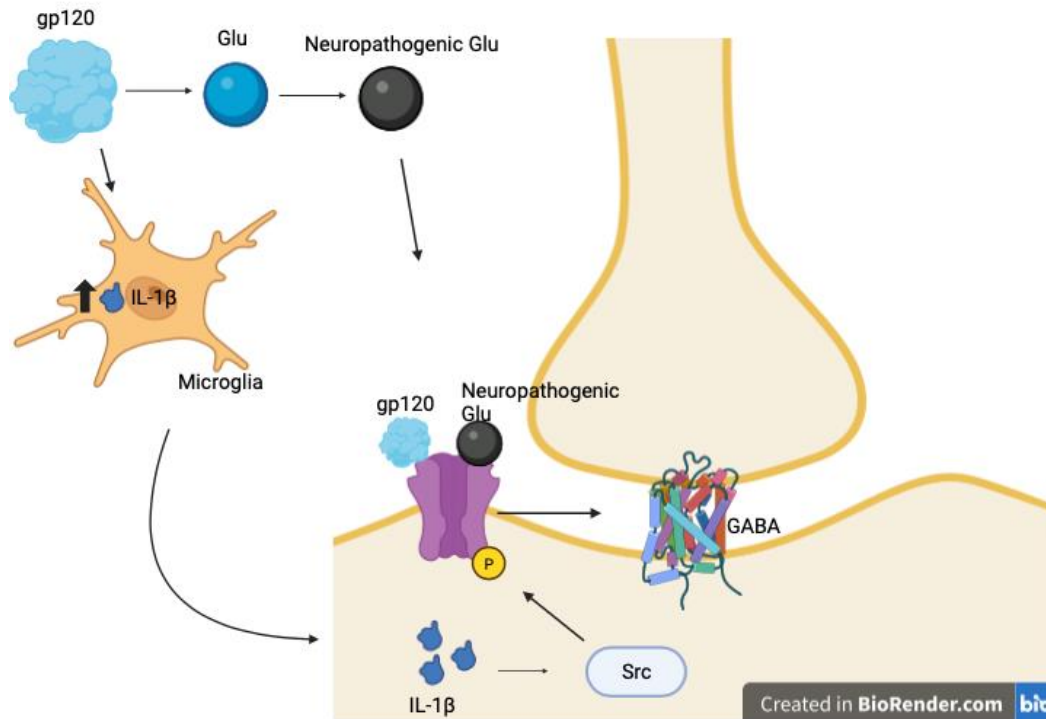


Figure 4: gp120 can enter the microglia and cause increased production in IL-1 $\beta$ . IL-1 $\beta$  enters the neuron to activate the Src Kinase. The Src Kinase phosphorylates the NMDAR to increase the production of GABA to have increased inhibitory synapses. Gp120 can also alter Glu into a neuropathic form and then these two proteins interact with the NMDAR to cause sEPSC. (Liu et al. 2023; Zhang et al. 2019)

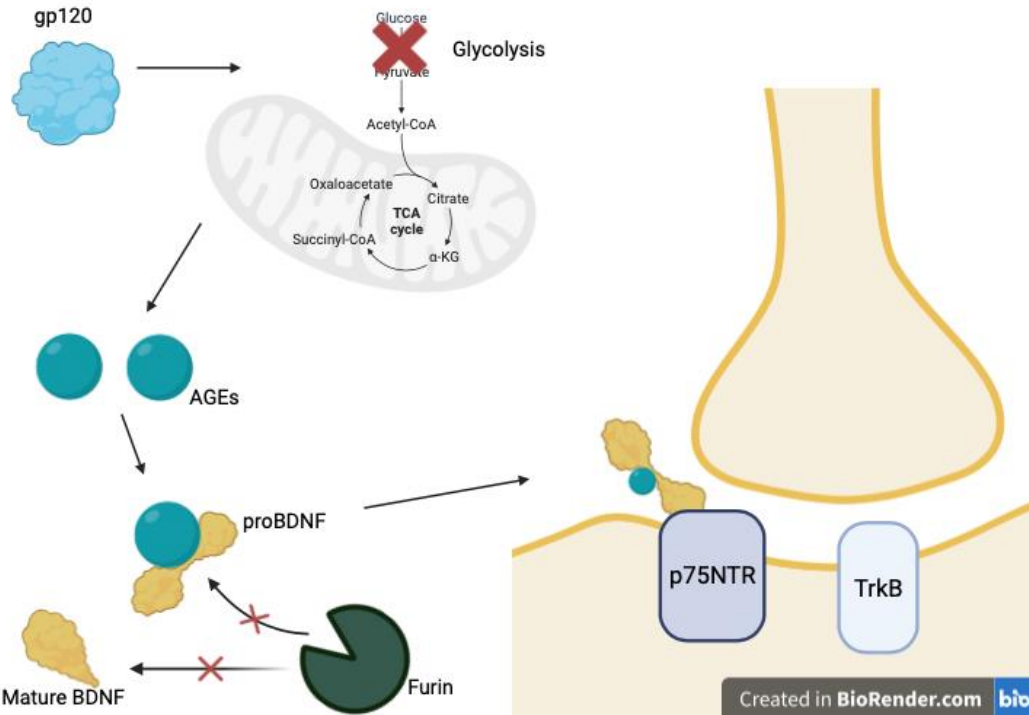
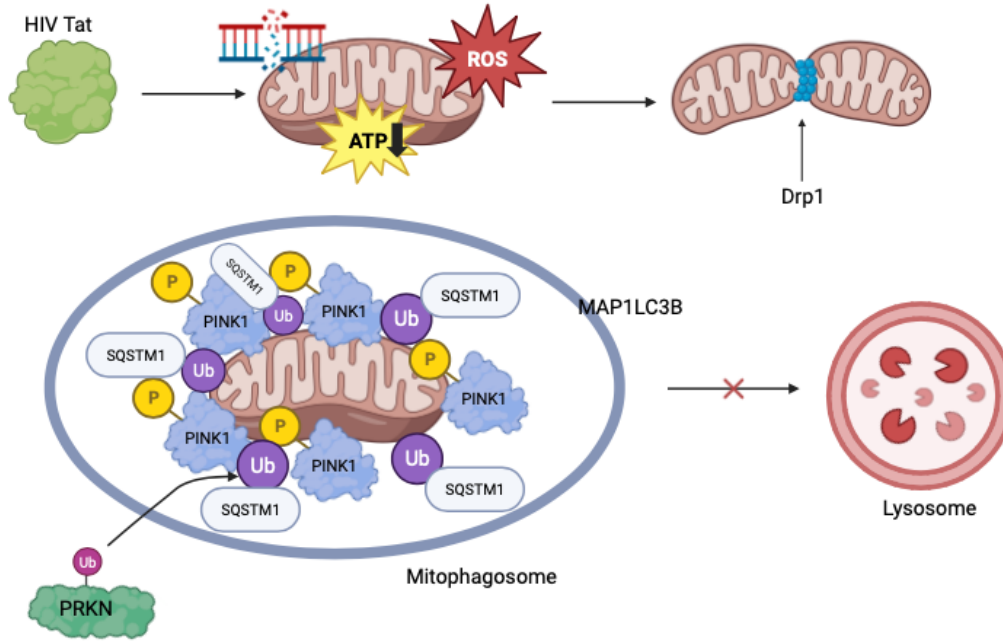


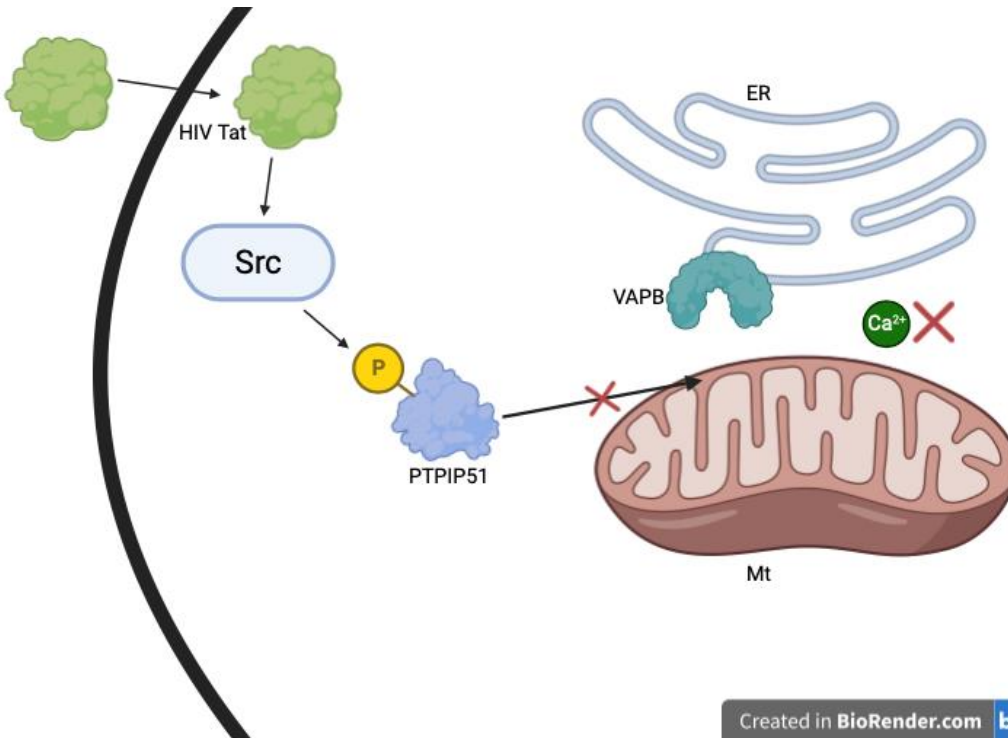
Figure 5: gp120 effects the last step in glycolysis to lead to the production of AGEs that bind to proBDNF to prevent cleavage into matureBDNF. This immature form, proBDNF favors binding to the p75NTR receptor and causes an imbalance between the p75NTR receptor and the TrkB receptor to cause dendritic spine and synapse impairment. (Allen et al. 2022; Speidell et al. 2020)



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Figure 6: HIV Tat causes mitochondrial damage such as DNA damage, decreased membrane potential, increased ROS species, and decreased ATP production. From there, increased concentrations of DRP1, PINK1, SQSTM1, and MAP1LC3B increased. DRP1 separates damaged mitochondrial segments from the healthy mitochondrial segment. PINK1 concentrates out to the outer mitochondrial membrane and autophosphorylates. The phosphorylation of PINK recruits PRKN to ubiquitinate the mitochondrial membrane. SQSTM1 binds to the ub and then MAP1LC3B is recruited to engulf the mitochondria with the proteins attached to make up the mitophagosome. However, Tat does not allow full maturation of this mitophagosome and it doesn't properly fuse with the lysosome for proper degradation of damaged mitochondria.

(Darbinian et al. 2020; Teodorof-Diedrich and Spector 2018; Thangaraj et al. 2018)



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Figure 7: HIV Tat infects the neuron and then activates the Src Kinase to phosphorylate PTPIP51. The phosphorylation of PTPIP51 causes it to not localize to the mitochondria resulting in no interaction between its partner protein in the ER, VAPB. Therefore, the two can not interact to allow for proper vesicle transfer which affects calcium levels. The imbalance of calcium levels affects neuronal health. (Adapted from Arjona et al. 2022)