Spring 4-28-2022

Ameliorative Effects of Minor Cannabinoids over HIV-1 Tat-Mediated Visceral Pain

Charlie Worth

AMELIORATIVE EFFECTS OF MINOR CANNABINOIDS OVER HIV-1 TAT-MEDIATED VISCERAL PAIN

by

Charlie Worth

A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford

May 2022

Approved By:

_____

Advisor: Professor Jason Paris

_____

Reader: Professor Nicole Ashpole

_____

Reader: Professor Gregg Roman

ACKNOWLEDGEMENTS

I would like to thank the Sally McDonnell Barksdale Honors College for giving me countless resources, including funding, to help me pursue academic success inside and outside the field of research. I would like to thank the National Institute on Drug Abuse (NIDA) for also providing funds that supported this project (R00 DA039791; R01 DA05251). I would also like to thank everyone who has helped me along my research journey, including: Dr. Paris, my thesis advisor, for never failing to offer help, words of encouragement, and unending knowledge on subject areas that truly interest me, as well as for opening up his lab and all of its wonderful and fascinating resources (which I brag to my other friends about); Mrs. Fakhri Mahdi, for her lab expertise and guidance as I learned my responsibilities in the lab; Emaya Moss, for being my direct student mentor from the day I joined the lab, and for never putting me down when I struggled, but rather always ensuring I persevere and get the job done; Salahuddin Mohammed, for his wisdom, mentorship, and friendly conversations that always made me feel like I was an asset in the lab; Alaa Qrareya, for continuously offering aid, as well as playing a major role in making the third floor of Faser Hall feel like a home on campus. I have made so many fond memories in the lab; the summer I spent working there was perhaps my most enjoyable season of life. Words cannot express my gratitude for the members of the Paris Lab.

ABSTRACT

CHARLIE WORTH: AMELIORATIVE EFFECTS OF MINOR CANNABINOIDS OVER HIV-1 TAT-MEDIATED VISCERAL PAIN

(Under the direction of Dr. Jason Paris)

As the total number of people living with HIV continues to rise across the world, an effective HIV treatment is still sought after. While modern-day advanced therapies exist for mitigating much of the negative effects of HIV, the virus remains evasive and problematic in the central nervous system. Thus, even with treatment, many people living with HIV continue to suffer from a plethora of symptoms. However, a large proportion of HIV-positive patients claim to feel a reduction in those persevering symptoms after cannabis usage. This anecdotal evidence has sparked interest in the efficacy of cannabis constituents for HIV therapy. This investigation first studies the *in vitro* effects of a known anti-inflammatory, corticosterone, on human microglial cells that were exposed to one of the most well-characterized HIV virotoxins, the transactivator of transcription (Tat). Tat is known to cause significant impairments in central nervous system processes through several proinflammatory mechanisms, and corticosterone was shown to curtail these inflammatory effects. In addition, Tat expression was also shown to be correlated with worsened cognitive functioning *in vivo*, as seen by poorer performances of mice expressing Tat in a 5-choice serial reaction time test. Finally, several minor cannabinoids were shown to reduce peripheral pain in Tat-expressing mice in an acetic acid writhing assay.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

cART	Combined Antiretroviral Therapeutics
CB1	Cannabinoid Receptor 1
CB2	Cannabinoid Receptor 2
CBDA	Cannabidiolic Acid
CBDV	Cannabidivarin
CBG	Cannabigerol
CBGA	Cannabigerolic Acid
CBN	Cannabinol
CNS	Central Nervous System
CORT	Corticosterone
DMSO	Dimethyl Sulfoxide
EMEM	Eagle's Minimum Essential Medium
FBS	Fetal Bovine Serum
HIV	Human Immunodeficiency Virus
HMC3	Human Microglia Cell Line-3
IFNα	Interferon-alpha
IL-	Interleukin
NF-κB	Nuclear Factor-Kappa B
NMDAR	N-Methyl-D-Aspartate Receptor
PLWH	People Living With HIV
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
Tat	Transactivator of Transcription
TNFα	Tumor Necrosis Factor Alpha
5-CSRTT	5-Choice Serial Reaction Time Test
Δ9-THC	Delta-9-tetrahydrocannabinol

1. INTRODUCTION

There is an increasing interest in the suspected ability of cannabinoid usage as a treatment for medical ailments. One specific area of its potential medical application is its efficacy in the management of pain associated with a variety of diseases. Of patients suffering from such diseases, those infected with human immunodeficiency virus (HIV) are especially noteworthy. As of 2019, there were approximately 38 million people living with HIV (PLWH) world-wide, with an increase of 1.9 million cases in 2019 alone (CDC, 2021). The first-line treatment for HIV is combined antiretroviral therapeutics (cART). While cART are efficacious peripherally, they do not accumulate well in the central nervous system (CNS) and they cannot eradicate HIV from latent reservoirs within the body, particularly those in the CNS. This leads to many HIV patients experiencing neuropathic pain, regardless of current treatment methods.

One particular viral protein that may contribute to the manifestation of several symptoms present in HIV patients is the HIV-1 transactivator of transcription (Tat), which has previously been studied with respect to neurocognitive disorders related to HIV infection. In cell cultures, Tat exerts direct and indirect actions to damage or kill neurons. Directly, Tat can promote neurotoxicity via actions at the lipoprotein receptor-related protein, which promotes downstream excitotoxicity via activation of *N*-methyl-d-aspartate receptors (NMDARs) (Haughey et al., 2001; Prendergast et al., 2002). Tat can also activate NMDARs directly along with other cation channels (including voltage-gated calcium channels and sodium channels) (Haughey et al, 1999; Fitting et al., 2014). Tat disrupts intracellular processes that buffer these ions, including mitochondrial-mediated processes that are associated with the generation of reactive oxygen and nitrogen species (ROS, RNS) (El Amine et al., 2018; Fan & He, 2016). In addition to its direct neurotoxic

effects, Tat also exerts indirect neurotoxic actions that are mediated by glial cells. For instance, astrocytes are important for proper neuronal function given their ability to buffer ions and neurotransmitters, such as glutamate. Tat impairs the ability for astrocytes to uptake glutamate, thereby promoting neuronal excitotoxicity (Zhou et al., 2004). Furthermore, Tat also acts on the immune glial cells of the brain, microglia, by promoting inflammatory cytokine release that can further damage neurons (El-Hage et al., 2005; Li et al., 2005; Sheng et al., 2000; Sorrel & Hauser, 2014; Yang et al., 2010). These effects are also observed *in vivo* in transgenic mouse models that conditionally-express the HIV-1 Tat protein (Bruce-Keller et al., 2008; Holman et al., 2016; Kim et al, 2003; Marks et al., 2016).

It has been suggested that between 40-74% of HIV patients consume cannabis (Costiniuk et al., 2019). According to many of cannabis-consuming PLWH, this cannabinoid use is associated with a reduction in pain, which may identify cannabinoid usage as a novel potential pain management treatment for patients suffering with neuropathic pain, particularly those infected with HIV (Dosenovic et al., 2017). Cannabinoids may even be more desirable than other modern-day pain management strategies; gabapentinoid or opioid interventions are often prescribed to HIV patients without successful retraction of pain (Oh et al., 2021). There may also be benefits from combining cannabis use with established treatment methods (Rasche et al., 2018). Several studies have indicated smoked cannabis in particular as having analgesic effects on HIV-related neuropathy; even experimentally induced pain has been shown to be reduced via smoked cannabis consumption (Lynch & Campbell, 2011). These data often coincide

with results gathered from surveys and questionnaires given to HIV patients currently using cannabis (Woolridge et al., 2005; Costiniuk et al., 2019).

The most well-studied constituent of cannabis is delta-9-tetrahydrocannabinol (Δ9-THC), which acts as a partial agonist at cannabinoid receptors 1 and 2 (CB1 and CB2, respectively). Notably, activation of these receptors reduces inflammation, while unmitigated inflammation promotes and sustains pain, particularly neuropathic pain (Morales et al., 2016). Outside of Δ9-THC, there are over 120 minor cannabinoids in cannabis that may exert similar effects and, because Δ9-THC is psychoactive, its therapeutic potential is limited. In large part, psychoactivity is mediated by CB1. However, CB2 is expressed in immune cells and promotes anti-inflammation, thus minor cannabinoids that target CB2 would be preferential (Turcotte et al., 2016). Other minor cannabinoids may act at non-CB sites such as ion channels (i.e. transient receptor superfamily channels) to reduce pain (Starkus et al., 2019).

Along with neuropathic pain, another common symptom that remains difficult to treat in PLWH is cachexia, characterized by weight loss and loss of appetite. Cannabinoids have also been shown to have efficacy in regards to the treatment of this symptom. Cannabinoids have been observed as being superior to placebo for the outcome of weight change and appetite in patients suffering from HIV-related cachexia (Mücke et al., 2016). In particular, dronabinol (a Δ9-THC analogue) has been associated with a "modest benefit" for HIV-associated weight loss and is currently in use successfully to increase the appetites of HIV patients suffering from cachexia (Murnion 2015; Croxford 2003). Two hormones responsible for the feelings of hunger and satiety, ghrelin and leptin, respectively, have been shown to be present in the blood at increased levels upon

cannabis administration, without causing a significant effect on insulin levels (Riggs et al., 2012). As with neuropathic pain, cannabinoids have also shown efficacy for managing HIV-related cachexia when used in conjunction with current advanced HIV therapies (Rasche et al., 2018). This further suggests cannabinoids may offer relief on more than one front for HIV patients.

Along with these benefits, cannabinoids have been shown to have a relatively good safety profile. One common worry about the usage of cannabinoids for medicinal purposes is abuse liability; however, data suggest that non-psychoactive minor cannabinoids, such as CBD, exert low potential for abuse (Chayasirisobhon, 2019). Many different cannabinoids have been studied through various routes of administration, which has produced an increasingly sizeable body of evidence that supports the claim that the risk of adverse effects from cannabinoids is generally low and that they are generally well-tolerated. Data from one survey suggests that the overwhelming majority of cannabinoid users experience no side effects and claim their cannabis usage leads to improved symptoms with respect to their illness (Schnelle et al., 1999).

While much of the data regarding the efficacy of cannabinoids seem promising, there also seems to be some limitations to its usefulness. A meta-analysis suggests that mental health symptoms may be increased after cannabinoid usage in HIV-patients (Mücke et al., 2018). Furthermore, there is existing data that suggests high dosages of cannabinoids may bring about adverse effects or exaggerate pain intensity (Beaulieu & Ware, 2007). Additionally, a cross-sectional study of HIV-patients that use cannabis found that nearly half of these users report memory deterioration associated with their cannabis use (Woolridge et al., 2005).

In this investigation, we hypothesized that HIV-Tat expression in mice would impair cognitive function and Tat-mediated inflammation in human microglial cells would be attenuated with a known anti-inflammatory, corticosterone (CORT). Additionally, we hypothesized that the algesic effects of Tat would be reversed in Tat-expressing mice with the administration of several different minor cannabinoids.

2. MATERIALS AND METHODS

The use of mice in these studies was pre-approved by the Institutional Animal Care and Use Committees at the University of Mississippi. All experiments were conducted in accordance with ethical guidelines defined by the National Institutes of Health (NIH Publication No. 85-23).

2.1 - Subjects and Housing

Behavioral studies used adult male and female mice that expressed (or did not) a GFAP-driven, doxycycline-inducible, HIV-1_{IIIB} *tat*$_{1-86}$ transgene (N=8+231; Bruce-Keller et al., 2008). Female mice were tested only when in the proestrus phase of the estrous cycle. Tat(-) mice that did not express the *tat* transgene and Tat(+) mice that did express the *tat* transgene were generated in the vivarium at the University of Mississippi. Mice were young adults (between two and five months) at the time of testing, were housed 3-5/cage, and were maintained in a temperature- and humidity-controlled room on a 12:12 h light/dark cycle (lights off at 09:00 h) with *ad libitum* access to food and water.

2.2 - Chemicals

Chemicals used *in vitro*: Human microglia cell line-3 (HMC3) cells were treated with vehicle or low-to-high corticosterone (32, 100, 320 nM dissolved in DMSO and diluted 1:10,000 in media; #27840, Sigma-Aldrich), and vehicle or HIV-1 Tat$_{1-86}$ (50 ng/mL diluted to concentration in dd$H_2$0; #1002-2, ImmunoDx, Woburn, MA). The chosen Tat concentration was derived from one known to induce functional deficits in glia and neurons similar to those observed in HIV (El-Hage et al., 2005).

Chemicals used *in vivo*: Mice were treated with vehicle [10% EtOH, 10% cremophor, 90% saline (0.9%)] or minor cannabinoids: CBGA, CBDA, CBG, CBN,

CBDV (10 mg/kg, i.p.; obtained from the Marijuana Research Laboratory, University of Mississippi, University, MS). To induce HIV-1 *tat*$_{1-86}$ transgene expression (or not), Tat(+) and Tat(-) control mice were administered doxycycline (30 mg/kg, s.c.; #14422, Cayman Chemical, Ann Arbor, MI) for 5 days with an additional two days of doxycycline washout prior to behavioral testing (to minimize any potential non-specific behavioral effects of doxycycline).

2.3 - Cell Culture of HMC3 Human Microglia

HMC3 cells were obtained from ATCC (#CRL2266; Manassas, VA) and maintained in growth medium: 89.5% EMEM/F12 (Life Technologies, Carlsbad, CA), 10% heat-inactivated fetal bovine serum (FBS; Thermo Scientific Hyclone, Logan, UT), and 0.5% antibiotic/antimycotic mixture (Life Technologies). For experiments, cells were seeded onto 24-well plates at a density of 5,000 cells/well. All cells were passaged between 6 and 12 times. For all experiments, cells were seeded on day 1, underwent experimental manipulations on day 2 (i.e. treatment with Tat and/or corticosterone), and were fixed in 4% paraformaldehyde 24 h later.

2.4 - Immunocytochemistry and Assessment of Microglial Activation

Fixed cells were incubated with antibodies against Iba-1 (Wako Pure Chemical Industries, 019-19741; 1:200) and were visualized following further staining with an anti-rabbit secondary (AlexaFluor 594; 1:500) and a Hoechst 33342 (1:10,000) nuclear stain. The total number of cells was counted in each field with at least 25 cells counted per field. Each condition was represented by two technical replicates per plate and each plate was considered one observation. All observations were independent experiments.

To quantify the morphology of HMC3 cells, each cell was scored by a blinded observer based on the following activation scale: (1) “resting” identified by an elongated morphology, (2) “activated/reactive” identified by a ramified morphology, or (3) “phagocytic” identified by an amoeboid morphology (Davis et al., 1994; Yoichi, 1999; Ladeby, 2005) (Fig 1).

2.5 - 5-Choice Serial Reaction Time Test

Cognitive ability to acquire nose-poke operant responding was assessed in Tat(-) and Tat(+) mice. Using a 5-choice serial reaction time test (5-CSRTT; Zantiks Ltd., Cambridge, UK) mice were trained to nose-poke for a reward of vanilla-flavored Ensure® (35 µL). Mice were not food-deprived. In brief, mice underwent up to 100 trials per day for 21 d. Each session began with a free reinforcer. Each trial consisted of the activation of a house light for 5s, followed by the lighting of 3 of 5 apertures for 20s. Mice had 20s to nose-poke one of the 3 lit apertures followed by an inter-trial interval of 25 s. The number of correct nose-pokes was calculated as an index of cognitive capacity to acquire operant behavior.

2.6 - Acetic Acid Writhing Test

Visceral algesia was determined via the acetic acid writhing test (Ross et al., 2011). In brief, mice were treated with vehicle or minor cannabinoids 30 min prior to testing. At the time of testing, mice were administered an i.p. injection of acetic acid (0.7%) and were immediately placed under a clear cylinder. Behavior was recorded for 30 min and scored later by an observer that was blinded to the treatment condition. The number of writhes per 5 min and the total number of writhes over 30 min were scored.

2.7 - Statistical Analyses

Microglial activation was assessed via separate two-way ANOVAs (Fig. 2) with corticosterone condition (0, 32, 100, or 320 nM) and Tat condition (control or Tat 50 ng/mL) as factors. Behavior in the 5-CSRTT was analyzed by day or week via repeated measures ANOVA with Tat condition [Tat(-) or Tat(+)] as the between-subjects factor and time (days or weeks) as the within-subjects factor (Fig. 3). Significant differences following main effects were determined using Fisher's Protected Least Significant Difference *post-hoc* tests. Interactions were delineated via simple main effects and main effect contrasts with alpha controlled for multiple comparisons. Behavior in the acetic acid writhing test was assessed via Student's independent, one-tailed *t*-tests for each treatment condition (Fig. 4). All analyses were considered significant when $p < 0.05$.

3. RESULTS

3.1 – HIV-1 Tat Activated Human Microglia *In Vitro*

Exposing human microglial cells (HMC3) to Tat (50 ng/mL) significantly reduced the proportion of cells that were resting [$F(1,181) = 15.29$, $p < 0.05$] (Fig. 2A) and significantly increased the proportion of cells that were activated in a phagocytic state [$F(1,178) = 37.70$, $p < 0.05$] (Fig. 2B). Tat significantly interacted with a known anti-inflammatory, corticosterone, to alter the activation state of microglia [$F(3,178) = 3.43$, $p < 0.05$] (Fig. 2C).

3.2 – HIV-1 Tat Impaired Cognitive Function *In Vivo*

In a 5-CSRTT, exposure to Tat significantly interacted with performance across the 21 days of testing [$F(20,120) = 2.00$, $p < 0.05$] (Fig. 3A). Compared to control, mice expressing HIV-1 Tat averaged significantly fewer correct nose-pokes per session during week 1 ($p = 0.01$), week 2 ($p = 0.006$), and week 3 ($p = 0.03$; Fig. 3B). Irrespective of genotype, the proportion of correct nose-pokes significantly reduced in week 3 compared to responding in week 1 ($p = 0.0003$; Fig. 3B).

3.3 - HIV-1 Tat Promoted Visceral Pain in Mice That was Alleviated by Minor Cannabinoids

In males or females, HIV-1 Tat expression resulted in an increased cumulative number of writhes in response to acetic acid [$t_{males}(14) = 1.85$, $p < 0.05$; $t_{females}(18) = 2.28$, $p < 0.05$] (Fig. 4). In males, pretreatment with the minor cannabinoids CBGA or CBN attenuated Tat-mediated writhing; whereas, pretreatment with CBG [$t(18) = 3.44$, $p < 0.05$], CBDA

[$t(18) = 2.88$, $p < 0.05$], or CBDV [$t(18) = 2.44$, $p < 0.05$] did not (Fig. 4). In females, pretreatment with CBGA, CBG, or CBN attenuated Tat-mediated writhing; but, CBDA [$t(19) = 3.99$, $p < 0.05$] and CBDV [$t(17) = 2.64$, $p < 0.05$] did not (Fig. 4).

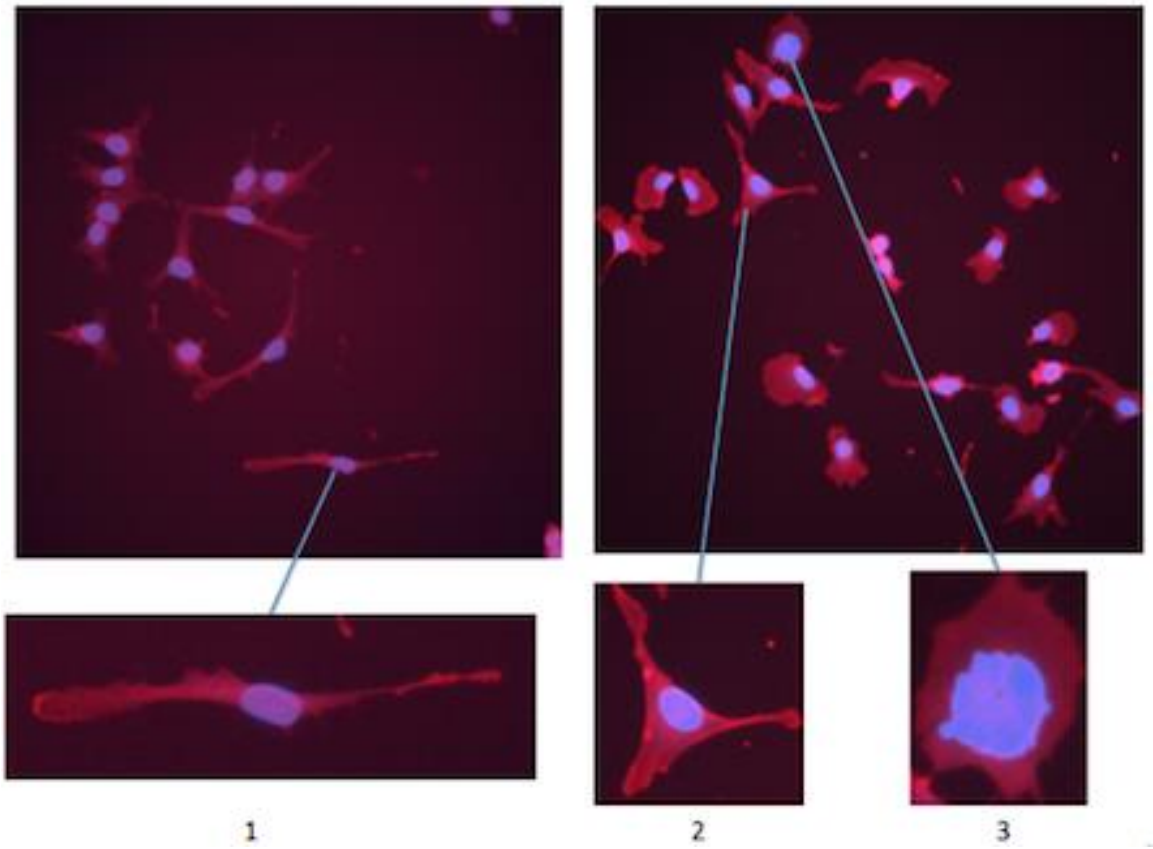
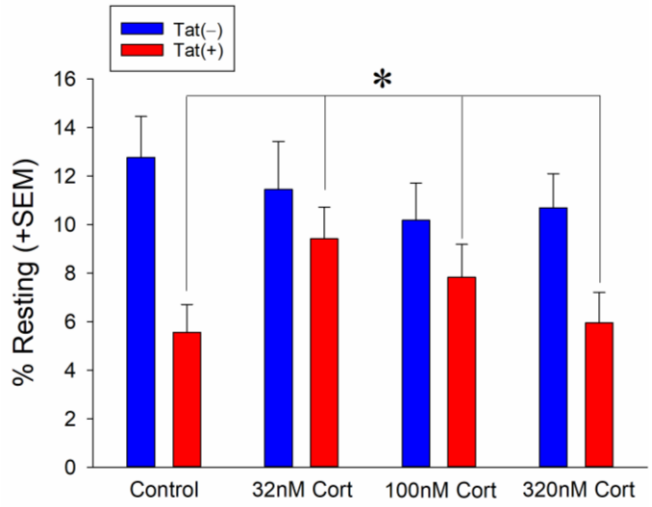
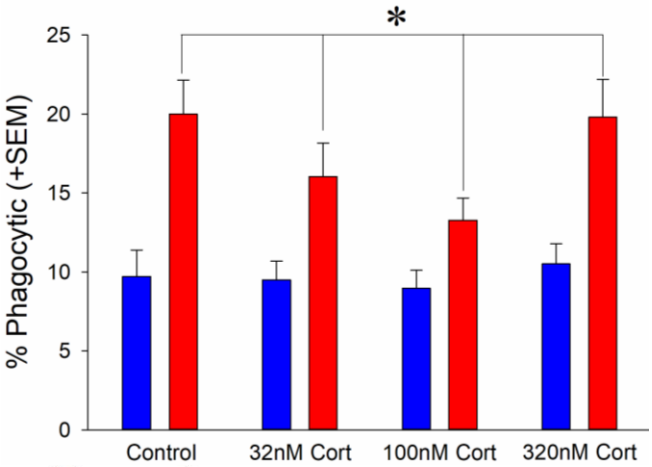


Figure 1: Examples of the values assigned to each of the three possible morphologies of HMC3 cells included in the activation scale.

2A



2B



2C

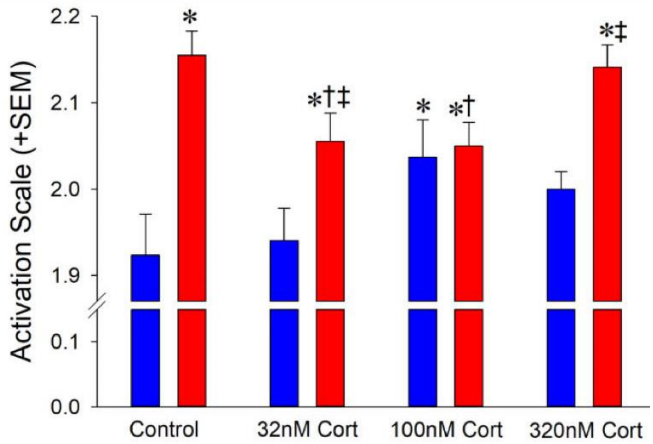


Figure 2: Differences in the percent of resting (1A), phagocytic (1B), and mean activation scale (1C) of HMC3 cells with or without exposure to corticosterone (0, 32, 100, 320 nM) and/or Tat (50 ng/mL). * indicates significant difference from media control; † indicates significant difference from Tat-exposed control; ‡ indicates significant difference from respective media-control of the same corticosterone concentration; $p < 0.05$.

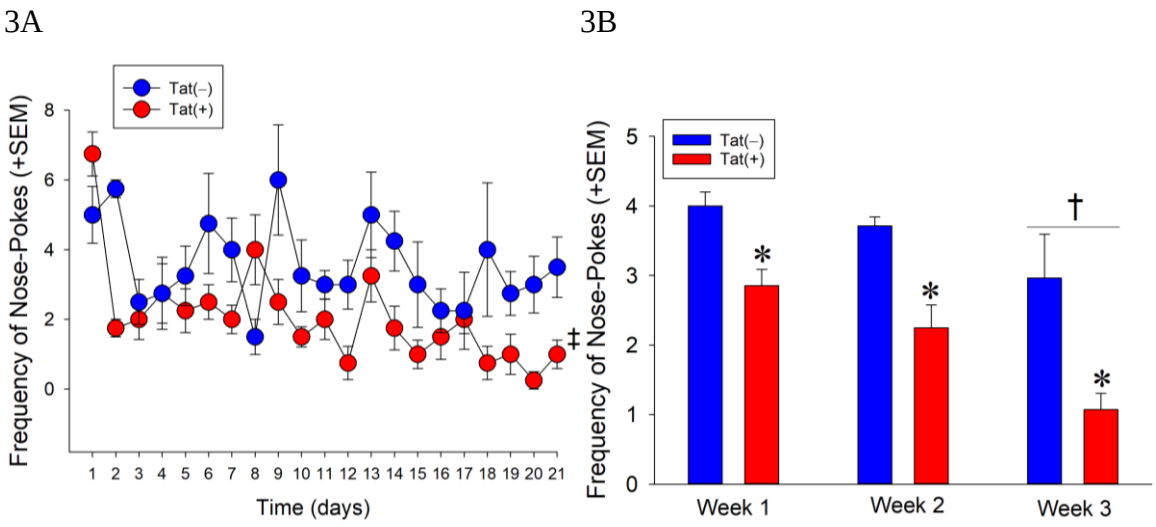


Figure 3: Frequency of correct nose-pokes by mice in the 5-CSRTT shown across all 21 days (Fig 3A) and as grouped into three weeks (Fig 3B). ‡ indicates interaction between Tat expression and time; * indicates significant main effect for Tat(+) to differ from Tat(-) mice; † indicates significant main effect for week 3 to differ from week 1; $p < 0.05$.

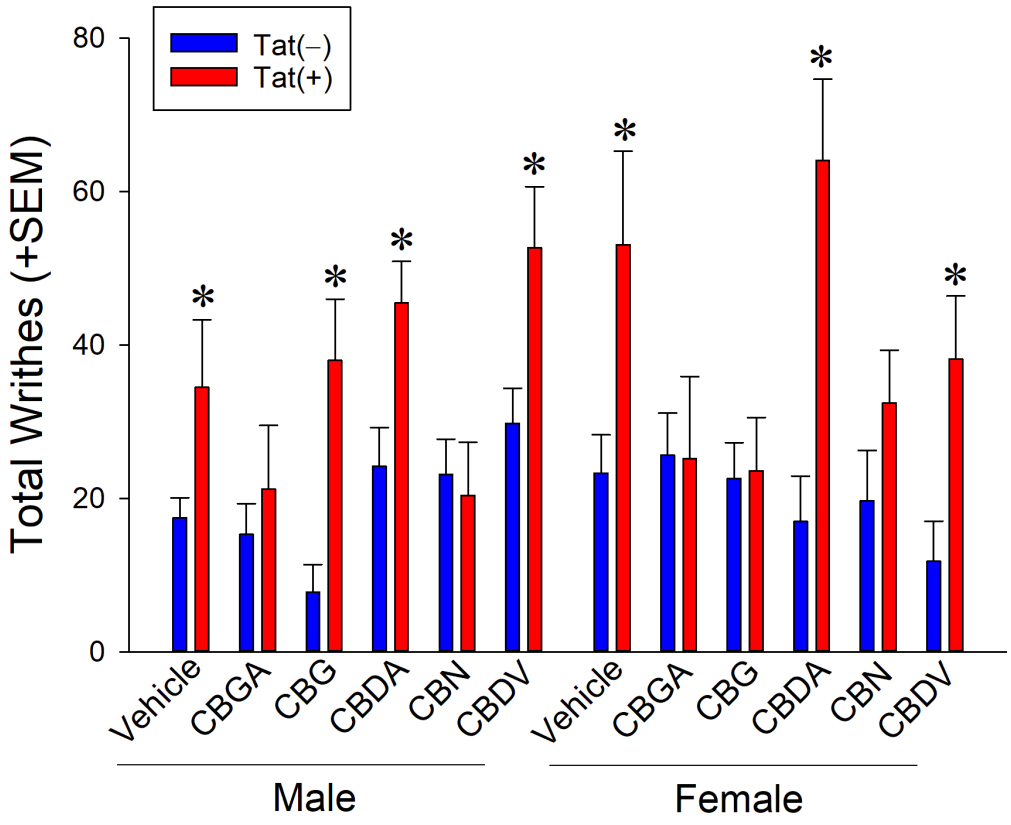


Figure 4: Total writhes of Tat(-) or Tat(+) mice in the acetic acid writhing assay after administration of vehicle or each of the five minor cannabinoids tested separated by sex. * indicates Tat(+) group significantly differs from respective Tat(-) control administered the same compound.

4. DISCUSSION

The hypotheses that HIV-Tat exposure would activate microglia, produce a cognitive detriment, and augment visceral pain were upheld. The hypothesis that the anti-inflammatory, corticosterone, would attenuate Tat-mediated inflammation in human microglial cells was also upheld and was observed to be concentration-dependent. Lastly, the hypothesis that potentially anti-inflammatory minor cannabinoids would curtail the algesic effects of Tat was upheld as well. Together, these data suggest that Tat protein is sufficient to promote a proinflammatory response that can increase visceral pain and natural product anti-inflammatories may ameliorate these effects. These data support prior findings and extend them.

As expected with respect to the current literature, the presence of Tat in HMC3 culture promoted an inflammatory response from the cells, as seen in a significant increase in the proportion of cells that were characterized by a phagocytic morphology, as well as a significant decrease in the proportion of cells that assumed a resting morphology. It should be noted that these two significant observations are not inextricably linked to one another despite their similarities; in other words, a significant difference in the proportion of morphologically phagocytic cells does not always guarantee a significant and opposite difference in the proportions of those that are resting, and *vice versa*. The inclusion of the middle ground, "active/reactive" morphology allows for a transition state to be assessed. The activation scale accounts for all activation states observed in a culture and we believe provides a more statistically robust outcome.

It is well known that the HIV-1 Tat protein promotes a neuroinflammatory profile. As shown in the data presented in this study, Tat expression induces microglia to take on a phagocytic, ameboid morphology, which is characteristically associated with an

onset of inflammatory reactions. Specifically, Tat has been shown to interact with the miRNA-34a-NLRC5-NF-κB signaling axis, ultimately resulting in an up-regulation of NF-κB, in turn promoting the expression of several proinflammatory cytokines (i.e. IL-1β and IL-6); this interaction has been studied *in vivo* on rhesus macaques models (Periyasamy et al., 2019). This is consistent with prior data that detected a marked elevation of both cytokines (TNFα, IFNα, IL-7, and IL-10) and chemokines (IP-10, MCP-1, and MIP-1α) in the cerebrospinal fluid of HIV-positive humans compared to HIV-negative humans (de Almeida et al., 2016). Additionally, this activated state has also been referred to as a "senescence-like phenotype," as an up-regulation in senescence markers such as p16 and p21 have been previously observed *in vitro* (Thangaraj et al., 2021).

Neuroinflammation may contribute to many pathologies among PLWH, including cognitive dysfunction. A particularly relevant cross-sectional study showed significantly more cognitive impairment in PLWH when compared to HIV-negative subjects, regardless of whether or not the PLWH were undergoing treatment with cART (Milanini et al., 2020). In addition to this, PLWH with fully suppressed HIV RNA (via cART) have also been shown to have decreased gray matter volume and white matter structural irregularities (Underwood et al., 2017). White matter irregularities have been proposed to be an important factor associated with cognitive dysfunction in HIV-infected humans receiving treatment (Underwood et al., 2017).

There was a marked discrepancy between Tat(+) and Tat(-) mice in their ability to learn an association between a stimulus (the onset of light) and a reward (Fig. 3A, 3B), supporting the notion that Tat may impair associative learning. Notably, both groups of

mice declined in performance by week 3 compared to their performance in week 1. This is suspected to be due to an enhanced number of correct nose-pokes due to random chance in the first week. As mice habituate to the task in later weeks, fewer responses were made, better parsing the groups. In support, the first day of the experiment recorded the single-highest average correct nose-poke count for the Tat(+) mice.

Natural products that can ameliorate neuroinflammation may improve HIV-related neurological outcomes. As previously mentioned, the rates of cannabis usage amongst PLWH may be up to 74%. Along with anecdotal evidence of HIV-related pain reduction from cannabis ingestion, data from the existing literature also support this claim. One randomized placebo-controlled study involving PLWH saw a median reduction of 34% in chronic pain, compared to 17% in the placebo group (Abrams et al., 2007). The exact constituents of cannabis that might confer anti-inflammatory effects are not known. However, herein we have found three lead constituents in the current experiment.

The data from the acetic writhing assay suggests that certain minor cannabinoids offer a reduction in visceral pain caused by Tat expression. As expected, Tat expression was associated with an increase in writhes for the vehicle condition in both sexes. In males, CBGA and CBN administration significantly resulted in less writhes in Tat(+) mice when compared to Tat(+) mice administered vehicle. Both CBGA and CBN had the same effect in females, as well as CBG. Although not significant, it is also worth noting that across both sexes, CBDA appeared to potentially increase writhes. Only five of the 120+ minor cannabinoids were studied, supporting the need for further research into other minor cannabinoids that may be of interest. Furthermore, the acetic acid writhing

assay is often used as a way to screen for a compound's ability to reduce visceral pain; the effective minor cannabinoids in this study may be potential compounds for study in more specific modes of pain (e.g. thermal or mechanical pain).

Notably, there were sex differences in the efficacy of some minor cannabinoids assessed. Though not significant, female mice tended to have more exaggerated cumulative writhes when compared to males. Also worth noting is that a significant attenuation of writhes was seen across both sexes after administration of CBGA and CBN, but CBG only attenuated writhes in females. These findings suggest a potential sex-determined difference in response to certain minor cannabinoids. Sex differences are not novel in the realm of Tat's effects on the brain; male Tat(+) mice have been shown to down-regulate inhibitory GABAergic synaptic activity, where female Tat(+) mice saw an up-regulation (Xu et al., 2022). Sex differences have also previously been reported regarding action of the endocannabinoid system (Cooper & Craft, 2018; Rubino & Parolaro, 2011). As such, sex-differences should be considered when identifying cannabinoids that may be efficacious in the treatment of HIV-related pain.

Future studies should seek to extend the data presented in the current work. The use of primary human microglia may offer a more applicable *in vitro* model for the study of Tat on these cells. Many more minor cannabinoids and other cannabis constituents exist but were not assayed, thus a wide array of compounds may have desirable effects. More specific pain assays may be used to narrow the exact mechanisms by which these constituents confer their analgesic properties (i.e. tail-flick test, Hargreaves test, etc).

In conclusion, these data show that Tat expression both *in vitro* and *in vivo* can negatively impact healthy CNS function. Corticosterone, a known anti-inflammatory,

significantly reversed the HMC3 morphological transition from a resting state to an activated, pro-inflammatory state. Several minor cannabinoids exert a similarly desirable effect in mice expressing Tat, as seen in a reduction in visceral pain.

REFERENCES

Abrams, D. I.; Jay, C. A.; Shade, S. B.; Vizoso, H.; Reda, H.; Press, S.; Kelly, M. E.; Rowbotham, M. C.; Petersen, K. L. (2007). Cannabis in painful HIV-associated sensory neuropathy: a randomized placebo-controlled trial. *Neurology*, *68*(7), 515--521.

Beaulieu P, Ware M, 2007. Reassessment of the role of cannabinoids in the management of pain. Curr Opin Anaesthesiol. 2007 Oct;20(5):473-7. doi: 10.1097/ACO.0b013e3282efd175. PMID: 17873600.

Bruce-Keller AJ, Turchan-Cholewo J, Smart EJ, Geurin T, Chauhan A, Reid R, Xu R, Nath A, Knapp PE, Hauser KF, 2008. Morphine causes rapid increases in glial activation and neuronal injury in the striatum of inducible HIV-1 Tat transgenic mice. *Glia*; 56: 1414–27.

Centers for Disease Control and Prevention. *HIV Surveillance Report, 2019*; vol.32. http://www.cdc.gov/hiv/library/reports/hiv-surveillance.html. Published May 2021. Accessed 18 April 12, 2022.

Chayasirisobhon S, 2019. Cannabis and Neuropsychiatric Disorders: An Updated Review. Acta Neurol Taiwan. 2019 Jun 15;28(2):27-39. PMID: 31867704.

Cooper ZD, Craft RM, 2018. Sex-Dependent Effects of Cannabis and Cannabinoids: A Translational Perspective, *Neuropsychopharmacology* 43 (2018) 34–51.

Costiniuk CT, Saneei Z, Salahuddin S, Cox J, Routy J-P, Rueda S, et al., 2019. Cannabis Consumption in People Living with HIV: Reasons for Use, Secondary Effects, and Opportunities for Health Education. *Cannabis and Cannabinoid Research*; **4**(3): 204–213.

Croxford JL. Therapeutic potential of cannabinoids in CNS disease. CNS Drugs. 2003;17(3):179-202. doi: 10.2165/00023210-200317030-00004. PMID: 12617697.

Davis EJ, Foster TD, Thomas WE. Cellular forms and functions of brain microglia. *Brain Res Bull.* 1994;34:73–78.

de Almeida SM, Rotta I, Jiang Y, et al., 2016. Biomarkers of chemotaxis and inflammation in cerebrospinal fluid and serum in individuals with HIV-1 subtype C versus B. *J Neurovirol*.;22(6):715-724. doi:10.1007/s13365-016-0437-4

Dosenovic S, Jelicic Kadic A, Miljanovic M, Biocic M, Boric K, Cavar M, Markovina N, Vucic K, Puljak L, 2017. Interventions for Neuropathic Pain: An Overview of Systematic Reviews. *Anesth Analg*. 125:643–652, 2017

El-Amine R, Germini D, Zakharova VV, et al., 2018. HIV-1 Tat protein induces DNA damage in human peripheral blood B-lymphocytes via mitochondrial ROS production. *Redox Biol*. 2018;15:97-108. doi:10.1016/j.redox.2017.11.024

El-Hage N, Gurwell JA, Singh IN, Knapp PE, Nath A, Hauser KF, 2005. Synergistic increases in intracellular Ca^{2+}, and the release of MCP-1, RANTES, and IL-6 by astrocytes treated with opiates and HIV-1 Tat. *Glia*; 50: 91–106.

Fan Y, He JJ, 2016. HIV-1 Tat Promotes Lysosomal Exocytosis in Astrocytes and Contributes to Astrocyte-mediated Tat Neurotoxicity. *J Biol Chem*.; 291(43): 22830-22840. doi:10.1074/jbc.M116.731836

Fitting S, Knapp PE, Zou S, et al., 2014. Interactive HIV-1 Tat and morphine-induced synaptodendritic injury is triggered through focal disruptions in Na^+ influx, mitochondrial instability, and Ca^{2+} overload. *J Neurosci*. 2014;34(38):12850-12864. doi:10.1523/JNEUROSCI.5351-13.2014

Haughey NJ, Holden CP, Nath A, Geiger JD, 1999. Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein tat. *J Neurochem*; 73: 1363–1374. doi: 10.1046/j.1471-4159.1999.0731363.x.

Haughey, N.J., Nath, A., Mattson, M.P., Slevin, J.T. and Geiger, J.D, 2001. HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. Journal of Neurochemistry, 78: 457-467.

Holman C, Piper SK, Grittner U, Diamantaras AA, Siegerink B, Kimmelman J, Dirnagl U, 2016. Where have all the rodents gone? The effects of attrition on preclinical research on stroke and cancer. *PLoS Biol*; 14: e1002331.

Kim BO, Liu Y, Ruan Y, Xu ZC, Schantz L, He JJ, 2003. Neuropathologies in transgenic mice expressing human immunodeficiency virus type 1 Tat protein under the regulation of the astrocyte-specific glial fibrillary acidic protein promoter and doxycycline. *Am J Pathol*; 162: 1693–707.

Ladeby R, Wirenfeldta M, Garcia-Ovejerob D, Fengera C, Dissing-Olesena L, Dalmaua I, Finsena B. Microglial cell population dynamics in the injured adult central nervous system. *Brain Res Rev.* 2005;48:196–206.

Li JC, Lee DC, Cheung BK, Lau AS, 2005. Mechanisms for HIV Tat upregulation of IL-10 and other cytokine expression: kinase signaling and PKR-mediated immune response. *FEBS Lett*; 579: 3055–62.

Lynch ME, Campbell F, 2011. Cannabinoids for treatment of chronic non-cancer pain; a systematic review of randomized trials. *Br J Clin Pharmacol* 2011;72:735-44.

Marks WD, Paris JJ, Schier CJ, Denton MD, Fitting S, McQuiston AR, Knapp PE, Hauser KF, 2016. HIV-1 Tat causes cognitive deficits and selective loss of parvalbumin, somatostatin, and neuronal nitric oxide synthase expressing hippocampal CA1 interneuron subpopulations. *J Neurovirol*; 22: 747–62.

Milanini B, Allen I, Paul R, et al., 2020. Frequency and Predictors of HIV-Related Cognitive Impairment in East Africa: The Africa Cohort Study (AFRICOS). *J Acquir Immune Defic Syndr*.: 83(2): 157-164. doi:10.1097/QAI.0000000000002242

Morales P., Hernandez-folgado L., Goya P., Jagerovic N, 2016. Cannabinoid receptor 2 (CB 2) agonists and antagonists: A patent update. *Expert Opin. Ther. Pat.* 2016;26:843–856. doi: 10.1080/13543776.2016.1193157

Mücke M, Carter C, Cuhls H, Prüß M, Radbruch L, Häuser W, 2016. Cannabinoide in der palliativen Versorgung : Systematische Übersicht und Metaanalyse der Wirksamkeit, Verträglichkeit und Sicherheit [Cannabinoids in palliative care: Systematic review and meta-analysis of efficacy, tolerability and safety]. Schmerz. 2016 Feb;30(1):25-36. German. doi: 10.1007/s00482-015-0085-2.

Mücke M, Weier M, Carter C, Copeland J, Degenhardt L, Cuhls H, Radbruch L, Häuser W, Conrad R, 2018. Systematic review and meta-analysis of cannabinoids in palliative medicine. J Cachexia Sarcopenia Muscle. 2018 Apr;9(2):220-234. doi: 10.1002/jcsm.12273. Epub 2018 Feb 5. PMID: 29400010; PMCID: PMC5879974.

Murnion B. Medicinal cannabis. Aust Prescr. 2015 Dec;38(6):212-5. doi: 10.18773/austprescr.2015.072. Epub 2015 Dec 1. PMID: 26843715; PMCID: PMC4674028

Oh G, Brouwer ES, Abner EL, et al., 2021. Predictors of chronic opioid therapy in Medicaid beneficiaries with HIV who initiated antiretroviral therapy. *Sci Rep*. 2021;11(1):15503. Published 2021 Jul 29. doi:10.1038/s41598-021-94690-8

Periyasamy P, Thangaraj A, Bendi VS, Buch S. HIV-1 Tat-mediated microglial inflammation involves a novel miRNA-34a-NLRC5-NFκB signaling axis. *Brain Behav Immun*. 2019;80:227-237. doi:10.1016/j.bbi.2019.03.011

Prendergast MA, Rogers DT, Mulholland PJ, Littleton JM, Wilkins LH, Jr, Self RL, Nath A., 2002. Neurotoxic effects of the human immunodeficiency virus type-1 transcription factor Tat require function of a polyamine sensitive-site on the N-methyl-D-aspartate receptor. *Brain Res* 2002; 954: 300–7.

Rasche, T., Emmert, D., Stieber, C. et al., 2018. Cannabinoidtherapie in der Praxis. *Urologe* 57**,** 558–562 (2018). https://doi.org/10.1007/s00120-018-0636-0

Riggs PK, Vaida F, Rossi SS, Sorkin LS, Gouaux B, Grant I, Ellis RJ, 2012. A pilot study of the effects of cannabis on appetite hormones in HIV-infected adult men. Brain Res. 2012 Jan 11;1431:46-52. doi: 10.1016/j.brainres.2011.11.001. Epub 2011 Nov 7.

Ross GR, Gade AR, Dewey WL, Akbarali HI. Opioid-induced hypernociception is associated with hyperexcitability and altered tetrodotoxin-resistant Na+ channel function of dorsal root ganglia. Am J Physiol Cell Physiol. 2012 Apr 15;302(8):C1152-61. doi: 10.1152/ajpcell.00171.2011. Epub 2011 Dec 21. PMID: 22189556; PMCID: PMC3330728.

Rubino T, Parolaro D, 2011. Sexually dimorphic effects of cannabinoid compounds on emotion and cognition, *Front Behav Neurosci* 5 (2011) 64.

Schnelle M, Grotenhermen F, Reif M, Gorter RW, 1999. Ergebnisse einer standardisierten Umfrage zur medizinischen Verwendung von Cannabisprodukten im deutschen Sprachraum [Results of a standardized survey on the medical use of cannabis products in the German-speaking area]. Forsch Komplementarmed. 1999 Oct;6 Suppl 3:28-36. German. doi: 10.1159/000057154. PMID: 10575286

Sheng WS, Hu S, Hegg CC, Thayer SA, Peterson PK, 2000. Activation of human microglial cells by HIV-1 gp41 and Tat proteins. *Clin Immunol*; 96: 243–51.

Sorrell ME, Hauser KF, 2014. Ligand-gated purinergic receptors regulate HIV-1 Tat and morphine related neurotoxicity in primary mouse striatal neuron-glia co-cultures. *J Neuroimmune Pharmacol*; 9: 233–44.

Starkus J, Jansen C, Shimoda LMN, Stokes AJ, Small-Howard AL, Turner H. Diverse TRPV1 responses to cannabinoids. *Channels (Austin)*. 2019;13(1):172-191. doi:10.1080/19336950.2019.1619436

Thangaraj A, Chivero ET, Tripathi A, et al. HIV TAT-mediated microglial senescence: Role of SIRT3-dependent mitochondrial oxidative stress [published correction appears in Redox Biol. 2021 Mar 3;:101909]. *Redox Biol*. 2021;40:101843. doi:10.1016/j.redox.2020.101843

Turcotte C, Blanchet MR, Laviolette M, Flamand N, 2016. The CB_2 receptor and its role as a regulator of inflammation. *Cell Mol Life Sci*. 2016;73(23):4449-4470. doi:10.1007/s00018-016-2300-4

Underwood J, Cole JH, Caan M, et al., 2017. Gray and White Matter Abnormalities in Treated Human Immunodeficiency Virus Disease and Their Relationship to Cognitive Function. *Clin Infect Dis*.; 65(3): 422-432. doi:10.1093/cid/cix301

Woolridge E, Barton S, Samuel J, Osorio J, Dougherty A, Holdcroft A, 2005. Cannabis use in HIV for pain and other medical symptoms. J Pain Symptom Manage. 2005 Apr;29(4):358-67

Xu C, Yadav-Samudrala BJ, Xu C, et al., 2022. Inhibitory Neurotransmission Is Sex-Dependently Affected by Tat Expression in Transgenic Mice and Suppressed by the Fatty Acid Amide Hydrolase Enzyme Inhibitor PF3845 via Cannabinoid Type-1 Receptor Mechanisms. *Cells*. 2022;11(5):857. Published 2022 Mar 2. doi:10.3390/cells11050857

Yang Y, Wu J, Lu Y, 2010. Mechanism of HIV-1-TAT induction of interleukin-1beta from human monocytes: involvement of the phospholipase C/protein kinase C signaling cascade. *J Med Virol*; 82: 735–46.

Yoichi K. Activated and phagocytic microglia. In: Walz W, editor. *Cerebral ischemia: molecular and cellular pathophysiology.* New Jersey: Humana Press; 1999. pp. 251–271.

Zhou BY, Liu Y, Kim B, Xiao Y, He JJ, 2004. Astrocyte activation and dysfunction and neuron death by HIV-1 Tat expression in astrocytes. *Mol Cell Neurosci*; 27:296–305