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The Effects of THC on HIV Tat Protein-Mediated Neuroinflammation

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THE EFFECTS OF THC ON HIV TAT PROTEIN-MEDIATED NEUROINFLAMMATION

by
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ABSTRACT

CAMILLE MARTIN: THE EFFECTS OF THC ON HIV TAT PROTEIN-MEDIATED NEUROINFLAMMATION

(Under the direction of Dr. Jason Paris)

Although antiretroviral therapies have allowed people living with human immunodeficiency virus (HIV) to achieve normal life expectancies, they cannot cure HIV nor the neurological symptoms associated with infection, termed neuroHIV. NeuroHIV describes a myriad of neurological disorders including mood disorders (depression and anxiety), cognitive impairment, neuropathic pain, and motor disinhibition. The mechanisms by which HIV promotes neurological impairment are not known, but may involve actions of its neurotoxic proteins. One such protein that has been well-characterized is the HIV trans-activator of transcription (Tat). Tat exerts neurotoxic effects via various means, one of which is to activate microglia, the macrophages of the central nervous system, to induce a pro-inflammatory state in the brain. Marijuana (MJ) is more often smoked by HIV-positive individuals than the general population and is demonstrated in basic science models to reduce neuroinflammation. Whether MJ can attenuate HIV-induced neuroinflammation, thus alleviating some neuroHIV burden is not known and is the subject of this thesis. Previous literature suggests that delta-9-tetrahydrocannabinol (THC), one of most well-characterized components of MJ, has anti-inflammatory properties, making it a prime candidate for study. Herein, we exposed human microglia to Tat and a concurrent concentration-response curve of THC (0, 1, 10, or 100, or 1000 nM). We hypothesized that Tat would cause microglial activation that THC could ameliorate. Due to

variance and a shortened time to conduct current experiments due to SARS-CoV-2-related disruptions, we did not observe a significant effect for Tat to activate microglia. However, we did observe THC (10 nM) to attenuate microglial activation compared to other concentrations. Thus, future work should further elucidate the potential anti-inflammatory constituents of MJ for their efficacy on HIV protein-mediated neuroinflammation.

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LIST OF ABBREVIATIONS

BBB	Blood Brain Barrier
cART	Combination Antiretroviral Therapy
CB_1/CB_2	Cannabinoid Receptor 1/2
CBC	Cannabichromene
CBD	Cannabidiol
CNS	Central Nervous System
CUD	Cannabis Use Disorder
EMEM	Eagle's Minimal Essential Medium
Gp120	Glycoprotein 120
HIV	Human Immunodeficiency Virus
HMC3	Human Microglial Clone 3
LRP	Lipoprotein Receptor-Related Protein
MJ	Marijuana
MoCA	Montreal Cognitive Assessment
MOS-HIV CF4	Medical Outcomes Study HIV 4-Item Cognitive Scale
NF-κB	Nuclear Factor Kappa B
NMDA	N-methyl-D-aspartate
PrEP	Pre-Exposure Prophylaxis
ROS	Reactive Oxygen Species
Tat	Trans-Activator of Transcription
THC	Δ-9-Tetrahydrocannabinol

1. Introduction

Despite novel therapeutics that treat human immunodeficiency virus (HIV), no cure exists, so HIV remains prevalent in the U.S. and worldwide. 1.2 million people in the U.S. and 38 million people worldwide are estimated to live with HIV (CDC, 2018; UNAIDS, 2020). HIV can be transmitted through multiple routes, including unprotected anal or vaginal sex, from mother to child during pregnancy (vertical transmission), birth, or breastfeeding, and shared drug injection equipment. The introduction of antiretroviral drugs in 1995 transitioned HIV from an acute to a chronic disease. Currently, there are multiple antiretroviral therapeutics available that are administered in a combinatorial manner (i.e., cART) to manage peripheral viremia. Combination antiretroviral therapy (cART) has reduced the incidence of mortality in HIV+ individuals (HIV-CAUSAL Collaboration, 2010). Since the mid-2000s, pre-exposure prophylaxis (PrEP; a combination of emtricitabine and tenofovir) has been available to prevent HIV infection and can be used up to 72 hours after exposure (CDC, 2020). Despite the benefits of cART, it has limitations. cART cannot cure HIV, and it does not accumulate well in the central nervous system or in the reservoirs where HIV proliferates. Because of these limitations, the neurological symptoms of HIV persist (Heaton et al., 2010; Harezlak et al., 2011; Tozzi et al., 2001; Cysique et al., 2004).

NeuroHIV is a term used to describe the host of neurological symptoms that often accompany HIV infection. It can include conditions such as increased anxiety and depression, behavioral disinhibition, motor impairment, neuropathic pain, and cognitive impairment (Reviewed in Eggers et al., 2017; Navia et al., 1986; Robinson-Papp et al., 2008). Approximately 50% of HIV patients in both the pre- and post-cART era suffer from neuroHIV, further highlighting the need for treatments that alleviate neurological symptoms in HIV patients (Saylor

et al., 2016). In particular, mood disorders such as depression and anxiety are two neuroHIV-associated conditions highly prevalent among people living with HIV (Savetsky et al., 2001; Atkinson et al., 1988). Mood disorders are especially a problem for women, and even more so for women with HIV. In one sample of 357 HIV-positive women, 28.9% met criteria for generalized anxiety, and 32.5% met criteria for a depressive disorder (Yousuf et al., 2020). In comparison, the proportion of American adult women in the general population with generalized anxiety disorder or depression is 7.1% and 10.5%, respectively (SAMHSA, 2020; National Comorbidity Survey, 2007).

Perhaps in part due to these neurological effects, HIV patients use marijuana (MJ) at a higher frequency than the general population. In one study of MJ use in HIV+ patients, 79% reported that they had smoked MJ in their lifetime, and 23% reported smoking within the last month (Prentiss et al., 2004). In contrast, a study that examined MJ use in the general population found that only 8.3% of Americans over the age of 12 had smoked MJ in the past month (Center for Behavioral Health Statistics and Quality, 2016). MJ is the most commonly used illicit drug among HIV+ persons (Sohler et al., 2018).

Reasons that HIV patients reported smoking MJ included pain relief, decreased anxiety and depression, and improved appetite (Prentiss et al., 2004). In another study, 45% of HIV+ participants reported that MJ was highly effective for overall symptom relief, such as reduced stress, pain, and anxiety (Costiniuk et al., 2019). There is some evidence to support these claims; however, findings are mixed. A double-blind, placebo-controlled study that examined the effects of MJ on pain relief in HIV+ patients found that the percentage of subjects with at least 30% pain relief was much higher in the MJ group compared to the placebo group (Ellis et al., 2009). An additional study found that MJ use was associated with a lower likelihood that HIV patients

would use a prescribed opioid analgesic, further exhibiting its pain-relieving properties (Sohler et al., 2018). One study suggests that smoking MJ has beneficial psychological effects in HIV+ men, including increased introspection, better medical management, and better future orientation (Bruce et al., 2020). In addition, cannabinoids, a class of chemicals found in MJ, are analogous to an endogenous class of chemicals produced by the human body called endocannabinoids. Humans have an entire complement of endocannabinoids that comprise this system. One study has even found that endocannabinoids downregulate inflammation *ex vivo* (Jäger et al., 2020). Thus, MJ smokers may not only experience euphoric feelings associated with 'being high'; they also may find additional benefits that cannabinoids provide through the intrinsic endocannabinoid system.

Several studies have revealed insights into which brain regions are involved in HIV/MJ interactions. In one study, comorbid HIV and MJ use were associated with complex neural alterations in multiple brain regions during cognitive interference as measured by the Stroop test. In particular, activation in the left fronto-insular cortex was correlated with cumulative years of MJ use. Activation in this region was highest in the HIV+/MJ+ sample, which suggests this group may have increased impulsivity (Meade et al., 2019). Another study found that chronic MJ use in HIV+ participants had an interactive effect on mean diffusivity in the right global pallidus, suggesting an increase in neuroinflammation in that region (Wang et al., 2020).

There are contradictory findings in the literature regarding whether MJ has a positive or negative impact on the neurological symptoms of HIV, regardless of the area of focus. In terms of its effects on cognition, studies have found both benefits and detriments. Two studies that have shown positive effects describe a weak association between lifetime MJ use in HIV+ persons with improved verbal fluency, and improved ratings of sleep with MJ use (Byrd et al.,

2011; Haney et al., 2007). Other studies show no positive effects or negative effects. Both Wang et al. (2020) and Haney et al. (2007) found that MJ use did not lead to additional neurocognitive impairments (NCI) in their HIV+ participants.

Whether the MJ use is acute or chronic may influence its effects on HIV patients. Recent cannabis use has been shown to have both positive and negative neurocognitive effects. Two studies have shown beneficial anti-inflammatory effects with recent MJ use, shown in one of the studies through lower levels of neuroinflammatory biomarkers (Ellis et al., 2020; Kallianpur et al., 2020). Two other studies found negative effects of current MJ use in HIV, including slower cognitive processing speed and lower scores on the MOS-HIV CF4 test, which is based on self-reports of cognitive functioning (Lorkiewicz et al., 2018; Okafor et al., 2019). However, it is worth noting that although HIV+ current MJ users scored worse on the MOS-HIV CF4 assessment, they did not score differently on the MoCA assessment, a cognition test focused on memory and attention, than non-MJ users (Lorkiewicz et al., 2018). An additional study found that chronic MJ use with HIV may have detrimental effects on brain volume (Kallianpur et al., 2020).

HIV's promotion of neurological dysfunction appears to occur in large part by actions of virotoxic proteins that are produced by infected neural cells. In particular, the trans-activator of transcription (Tat) is a protein produced by HIV that is an important driver of neurotoxicity. Tat damages the nervous system through numerous pathways, both direct and indirect. One direct way is by over-excitation of neurons via increased intracellular Ca^{2+} through activation of L-type Ca^{2+} channels, NMDA receptors, and LRP receptors, which leads to excitotoxicity and cell damage (Napier et al., 2014; Krogh et al., 2014; Liu et al., 2000). In terms of indirect ways, as mitochondria scavenge some excess Ca^{2+}, Tat promotes dysregulation of the mitochondrial

electron transport chain, thereby driving the production of reactive oxygen species (ROS) which leads to cell damage and death (Godai et al., 2019; Teodorof-Deidrich and Spector, 2018). In addition, Tat activates the nervous system's proinflammatory glial cells, astrocytes and microglia, to produce cytokines which can lead to cell damage and death when produced in excess (Kaul et al., 2005; Nath et al., 1999).

It is not known what constituents in HIV interact with MJ to influence neurological outcomes. However, Tat is one neurotoxic protein that is secreted from HIV-infected cells that is known to interact with drugs of abuse (reviewed in Maubert et al., 2016). The damaging neurological effects of HIV infection worsen when HIV interacts with drugs of abuse, such as opioids and psychostimulants (Aksenov et al., 2006; Mahajan et al., 2008). Opioid use can downregulate the user's immune system, making HIV exposure more likely to lead to infection (Nath et al., 2002). Although other drugs of abuse tend to worsen the neurological symptoms of HIV, there have been several proposed mechanisms for cannabinoid protection of HIV infection.

One constituent that may influence the neurological symptoms of HIV is Δ-9-tetrahydrocannabinol (THC). THC, the most well-characterized compound within MJ, is a partial CB_1 and CB_2 agonist. While CB_2 receptors are expressed in immune cells throughout the body, CB_1 receptors are mostly concentrated in the central nervous system (CNS) (Reviewed in Howlett et al.., 2002; Reviewed in Pertwee et al., 2008). CB_1 agonists can suppress L-type and T-type Ca^{2+} channel flux, while CB_2 agonists suppress T-type channel flux. Because Tat is an L-type Ca2+ channel activator, a CB_1 agonist such as THC could theoretically offset Tat's effect (Qian et al., 2017). The CB_2 receptor agonist AM1241 has been shown to protect against the neurodegenerative effects of gp120, another virotoxic HIV protein that has been well-studied (Avraham et al., 2014). Another study found that chronic MJ use may normalize the decreased

glutamate that is associated with HIV (Chang et al., 2006). There is evidence that chronic exposure to inflammatory cytokines, such as the ones produced as a result of Tat activation, promotes psychiatric disorders such as depression (Reviewed in Felger and Lotrich, 2013). In theory, reducing Tat-induced neuroinflammation could alleviate neuroHIV symptoms such as depression and anxiety. The anti-inflammatory properties of MJ make it a promising candidate for study to potentially offset the neuroinflammatory effects of HIV (Lima et al., 2021). In order to investigate the effects of THC on HIV Tat-mediated neuroinflammation, an experiment was conducted exposing human microglia to THC and/or Tat. We hypothesized that Tat would promote microglial activation and combined THC would offset these effects.

2. Materials and Methods

2.1 - Description of Cells and Cell Culture

HMC3 human brain microglial cells

HMC3 cells were purchased from ATCC (Manassas, VA, USA; #CRL-3304). Cells were grown in 75 cm^2 flasks in 13 mL of media (89.9% EMEM, 10% heat-inactivated fetal bovine serum, 0.5% antibiotic/antimycotic mixture), and maintained in a 37^oC incubator (5% CO_2). Media was replaced every 2-3 days and cells did not exceed 90% confluency (maximum passage = 8). For experiments, cells were seeded onto 24-well plates at a density of either 5×10^3 or 1×10^4 cells/ well.

2.2 – Chemicals Involved in Experiment

Chemicals

THC was obtained from the Marijuana Research Laboratory at the University of Mississippi (University, MS, USA). Recombinant Tat_{1-86} was purchased from ImmunoDx (Woburn, MA, USA; #1002).

2.3 – Experimental Procedures

Treatment

Twenty-four hours after seeding, cells were treated with THC (0, 1, 10, 100, or 1000 nM) and/or Tat (50 ng/mL). Cells were incubated for 24 h (37^oC, 5% CO_2) and fixed using paraformaldehyde (4%).

Immunocytochemistry

Fixed cells were permeabilized with Triton X-100 (0.1%) and blocked with bovine serum albumin (1%) for 30 min, respectively. Cells were incubated overnight with an antibody to ionized calcium binding adaptor molecule 1 (Iba-1, rabbit, 1:250; Fujifilm Wako Chemicals, Richmond, VA, USA) followed by a 1 h incubation with a secondary anti-rabbit antibody (AlexaFluor 594, 1:1000; Invitrogen, Carlsbad, CA, USA). Cell nuclei were stained with Hoechst 33342 (ThermoFisher; 1:20,000).

Morphological Assessment

The total number of cells was calculated by counting the nuclear stain. The proportion of cells that were resting (extended, thin processes), active/reactive (ramified, retracted processes), or ameboid/phagocytic (rounded body, no processes) were counted and expressed as a percentage of the total number of cells. A microglial activation scale (1-3) was also used to score the full morphological phenotype. Scoring was conducted as such: 1= Microglia have long processes and are considered resting. 2 = Microglia have partially shortened processes, indicating they are neither fully resting nor phagocytic. 3= Microglia have no visible processes, appear circular, and are considered phagocytic. This scale was adapted from prior work (Paris et al., 2015; Davis et al., 1994; Yoichi, 1999).

Statistical Analyses

Cell activation was assessed using a two-way ANOVA with THC concentration (1, 100, or 1000 nM) and HIV Tat exposure (control or Tat 50 ng/mL) as factors. Group differences were

determined via Fisher's Protected Least Significant Difference *post hoc* tests. No interactions were observed. All analyses were considered significant when $p \leq 0.05$.

3. Results

Exposure to THC (1, 10, 100, or 1000 nM) significantly altered the proportion of cells that were activated [$F(4,22) = 3.64$, $p < 0.05$] (Fig. 1B) or ameboid [$F(4,22) = 3.55$, $p < 0.05$] (Fig. 1C) in morphology. THC (10 nM) significantly increased the proportion of cells that were activated ($p = 0.001$ - 0.049; Fig. 1B), but decreased the proportion that became ameboid/phagocytic ($p = 0.001 – 0.049$; Fig, 1C) compared to all other groups. When assessed as an activation scale, 10 nM THC significantly reduced overall HMC3 activation [$F(4,22) = 2.73$, $p = 0.05$] compared to all groups with the exception of the 1 nM concentration ($p = 0.004$ - 0.03; Fig. 2). No differences were observed in the proportion of resting cells. Due to variance, no significant effect of Tat was observed.

4. Discussion

The initial hypothesis that Tat would cause microglial activation was not upheld, while the hypothesis that THC would reduce activation was partially upheld. The finding that Tat did not cause microglia to become activated is inconsistent with existing literature (Thangaraj et al., 2018; Periyasamy et al., 2019) and our lab's prior findings. However, this is likely due to a high degree of variance in the data regarding the effect of Tat. Additional observations are likely needed, but could not be completed in the current thesis due to restrictions imposed by the SARS-CoV-2 pandemic. We expect additional replications of this experiment to resolve these concerns. In regards to THC, we found it to reduce microglial activation in a concentration-dependent manner such that 10 nM THC significantly reduced activation compared to all other concentrations except 1 nM THC. As such, the anti-inflammatory effects of THC were observed at a 10 nM concentration.

Although the effects of Tat in the current report suffered from high variance, Tat is known to promote neuroinflammation through the activation of astrocytes and microglia (Kaul et al., 2005; Nath et al., 1999). This inflammatory effect is not limited to HIV-infected glial cells, given that surrounding, non-infected neighboring cells can also become activated through a so-called 'bystander effect' (reviewed in Ajasin and Eugenin, 2020). Tat is endocytosed by bystander cells through at least two pathways; one endocytic route is clathrin-dependent, and another is mediated by caveolae (Vendeville et al., 2004; Ferrari et al., 2003). Tat activates cells to produce inflammatory cytokines by hijacking the normal function of a family of transcription factors in the NF-κB signal transduction pathway. The normal function of NF-κB is to regulate the expression of genes encoding various molecules of the immune system including cytokines, chemokines, and cell adhesion molecules. Tat causes greatly increased NF-κB activity, thereby

facilitating the production and release of cytokines, including those that are pro-inflammatory (McElhinny et al., 1995; DeLuca et al., 1996).

There are various pathways by which THC, a CB_1/CB_2 receptor partial agonist, could reduce HIV-induced inflammation, and thus potentially improve neuroHIV symptoms. There is evidence that THC can directly inhibit monocyte activity by decreasing the production of key inflammatory cytokine IL-1β. The same study showed that CB_2 agonist JWH-015 showed similar levels of monocyte IL-1β inhibition, suggesting that THC exerts its effects on monocytes through CB_2 receptors (Rizzo et al., 2019). Additional evidence suggests that THC, as well as CB_2 agonist CP55,940, can prevent Tat from helping excess monocytes adhere to the extracellular matrix of the blood brain barrier (BBB), thus preventing them from causing additional inflammation in the brain (Raborn et al., 2014). Another selective CB_2 agonist, Gp1a, has been shown to decrease levels of proinflammatory markers TNF-α and CCR5, and increase levels of cytokine Fas-ligand in HIV-infected mice (Gorantla et al., 2010). Being able to attenuate the inflammatory effects of Tat could have beneficial impacts on the symptoms of neuroHIV, especially those related to mood disorders.

Mood disturbances such as generalized anxiety disorder and major depression are more common among people with HIV than non-affected individuals (Atkinson et al., 1988). Depression has been linked to an overabundance of inflammatory cytokines acting on the brain (Reviewed in Arseniou et al., 2013). Specifically, Tat expression in mice has been shown to cause anxiety- and depression-like behaviors that recapitulate the clinical condition (Paris et al., 2014; McLaughlin et al., 2017). One study showed that mice whose brains were injected with Tat not only had increased levels of three pro-inflammatory cytokines, but also displayed behaviors associated with depression (Lawson et al., 2011). Currently, however, there is little

evidence that MJ acts as an antidepressant (Reviewed in Feingold and Weinstein, 2021). In contrast, THC has been identified as a potential treatment for anxiety. A low dose of THC has been found to decrease anxiety in post-traumatic stress disorder patients through activation of CB_1 receptors (Raymundi et al., 2020). It is worth noting that not all studies support the idea that THC reduces anxiety, as some note the presence of increased anxiety at higher THC doses (Sharpe et al., 2020). In addition to this drawback, there are more reasons why THC may not be the perfect panacea to neuroHIV.

Because THC produces psychoactive effects, its use as medication is controversial. As of 2022, 37 states in the U.S. allow some form of cannabis use for medicinal purposes, with 11 of those states only allowing "low THC/high CBD" products (State Medical Cannabis Laws, 2022). Another issue with MJ use is the potential for addiction. A 2011 study analyzing a sample of over 7000 lifetime cannabis users found that 8.9% became addicted (Lopez-Quintero et al., 2011). Cannabis use disorder (CUD) is characterized by features such as trying and failing to quit cannabis use, withdrawal, tolerance, and continuing use despite social problems and relationship strain (DSM-5, 2013). One area of concern for people with HIV is that CUD is associated with lower adherence to antiretroviral medications and more HIV symptoms (Bonn-Miller et al., 2014). As such, the use of cannabis must be considered with caution.

There are also sex differences that should be considered for medicinal MJ use. Men and women use MJ at different rates, with men being the more frequent users (Cuttler et al., 2016). In addition, men and women are affected by MJ in different ways, including differing levels of visuospatial memory impairment, withdrawal, and cardiovascular effects (reviewed in Fattore and Fratta, 2010). CUD is also more prevalent in men than women (Hasin et al., 2016). However, while CUD is less prevalent among women, they tend to proceed more rapidly from

first use to cannabis dependence (Cooper and Haney, 2014). Several mechanisms have been proposed to explain sex differences in MJ use and effects, including differences in the cannabinoid receptor system and cannabis metabolism (Calakos et al., 2017). These findings suggest that the effects of MJ on HIV-induced neuroinflammation may differ by sex, as MJ impacts both sexes differently.

Other constituents of MJ have been shown to have benefits and therefore might be useful to study in the future for their impacts on HIV-induced neuroinflammation and neuroHIV. For example, cannabidiol (CBD), an increasingly popular and non-psychoactive MJ constituent, has been shown to reduce anxiety caused by public speaking (Bergamaschi et al., 2011). Another study has shown that CBD reduces the number of extracellular vesicles released from HIV-infected cells, thus reducing their inflammatory effect on neighboring cells (DeMarino et al., 2022). Cannabichromene (CBC) is another MJ constituent that is a CB_2 agonist (Udoh et al., 2019). One study conducted in mice showed that CBC has antidepressant-like effects, as measured by the forced-swim and tail-suspension tests (El-Alfy et al., 2010). There is also evidence that CBC has anti-inflammatory properties (Wirth et al., 1980).

There are a few ways that this experiment could be improved in the future. The SARS-CoV-2 pandemic reduced the amount of time that I was able to spend conducting this experiment to less than two full semesters, thereby decreasing the amount of data generated. Having more observations could resolve the variance in the effects of Tat exposure on microglial activation, potentially adding consistency to the extant literature. Additionally, only one concentration of Tat was used across all treatments; in the future, it may be useful to conduct a concentration-response to determine whether THC-mediated efficacy holds true even under low-to-high conditions of Tat exposure, such as what may occur across clinical samples. Using primary

microglia instead of HMC3 cell lines may be useful as well, since HMC3 cells are resilient and therefore may be less sensitive than human microglia *in vivo*, which would improve the capacity to translate this assay for clinical applications. In addition, it is not known what amount of THC actually accumulates in the brain of smokers. As such, the chosen concentrations of THC used in this experiment may not be optimal. This issue is further complicated by the fact that street MJ has increased in potency over the past two decades (ElSohly et al., 2016). Future studies may expand the concentration-response curve as informed by clinical studies in order to improve translation.

In conclusion, this experiment demonstrated that THC can promote an anti-phagocytic phenotype in HMC3 microglia at a 10 nM concentration. Future studies may better assess the potential anti-inflammatory effects that THC or other Cannabis constituents can exert over HIV or HIV proteins.

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Figure 1

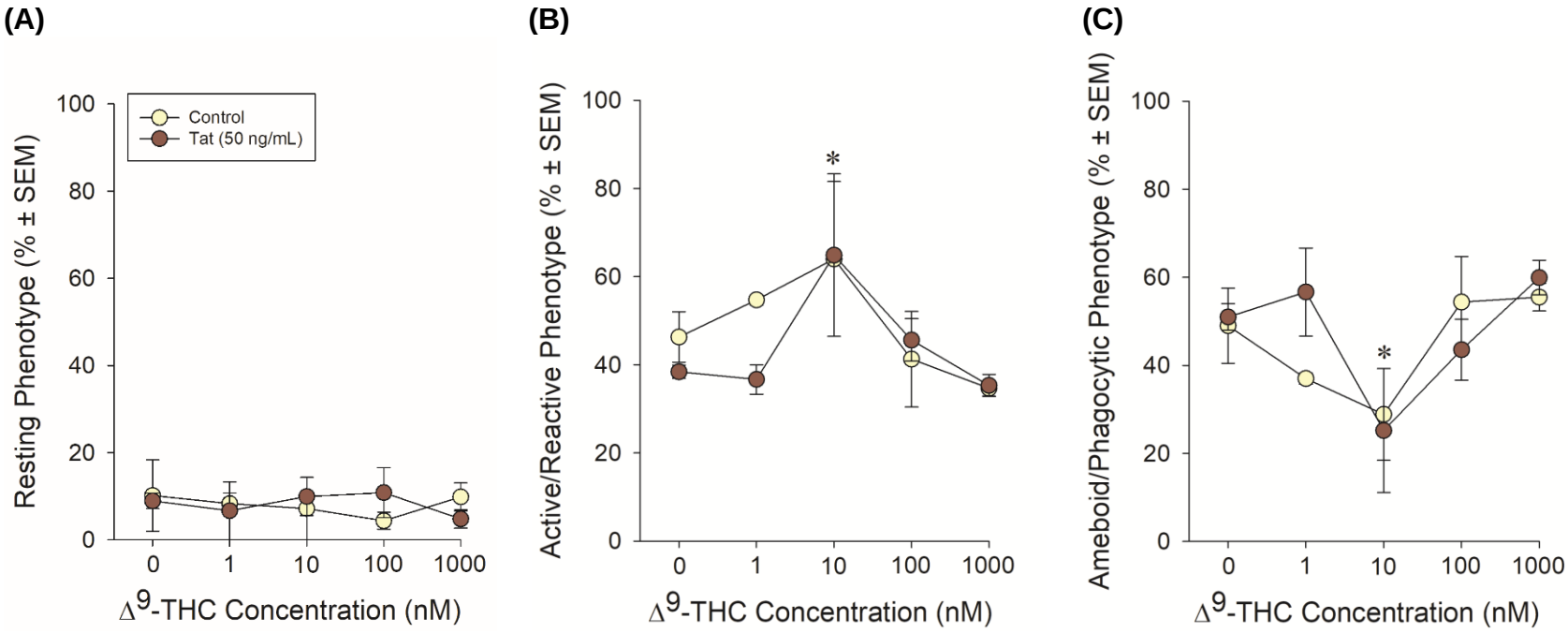
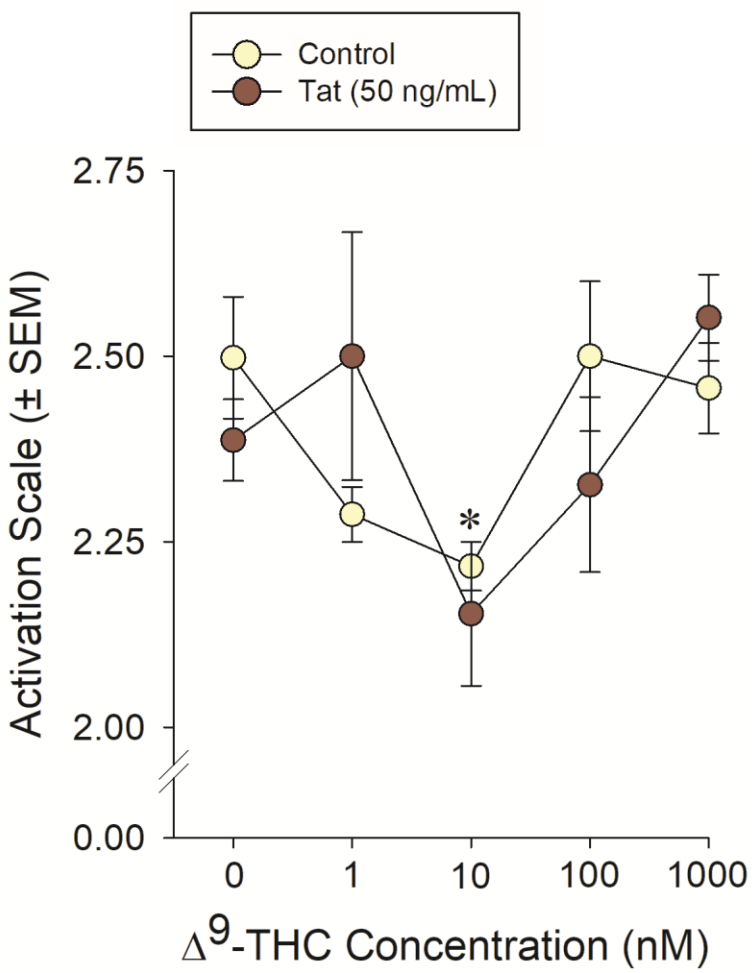

(A)
(B)
(C)
Control
Tat (50 ng/mL)
Resting Phenotype (% ± SEM)
Active/Reactive Phenotype (% ± SEM)
Ameboid/Phagocytic Phenotype (% ± SEM)
0
20
40
60
80
100
0
1
10
100
1000
Δ9-THC Concentration (nM)
*

Figure 2


Control
Tat (50 ng/mL)
Activation Scale (± SEM)
2.75
2.50
2.25
2.00
0.00
*
0
1
10
100
1000
Δ9-THC Concentration (nM)