Specific Aims

Marijuana (Cannabis sativa) is the mostly commonly used drug among adolescents, and its perceived risk has dropped in recent years. Recently, recreational and medical laws have increased the availability Cannabis to the public. Availability and low perceived risk usually precedes a usage spike.(1, 2) This is extremely concerning because adolescents are particularly susceptible to long-term decreases in cognition associated with Cannabis usage.(3-7) *Despite common long-term use, little is known about the mechanisms and neuroadaptations in vivo that underlie this adolescent specific issue which occurs with chronic use.*

The phytocannabinoid delta-9-tetrahydrocannabinol (Δ9-THC) is the primary psychoactive component of Cannabis.(9) Δ9-THC primarily exerts its cognitive effects through the changes in synaptic plasticity at the CB1 receptor, a presynaptic 7-transmembrane domain GPCR-coupled receptor(10-13). CB1 receptors are expressed widely throughout the brain on both GABAergic and glutamatergic synapses and work has shown that CB1Rs mediated LTD in glutamatergic synapses is sufficient for Δ9-THC's cognitive effects. (12, 14, 15) However, CB1R mediated synaptic plasticity in GABAergic synapses is necessary for Δ9-THC cognitive deficits. (16) *This points to a circuit level mechanism of Δ9-THC's cognitive deficits*. CB1Rs are primarily present on GABAergic cells(17), and endocannabinoids preferentially target inhibitory perisomatic synapses on cortical projection neurons(18) Taken together, these data suggest enhancing GABAergic CB1R (GABA:CB-1) signaling downregulates excitatory synapses. Given the importance of age in these cognitive deficits, the mechanism of these changes probably lies in the interference of normal brain development. During adolescence grey matter volume decreases while white matter volume increases(19-21) and the PFC is the last location to fully mature(22). The PFC is a critical region for executive function and cognition. Adolescence is then a critical period for maturation of executive function and cognition. Given the role cannabinoid signaling in facilitating glutamatergic LTD, I propose that adolescent cannabis usage *enhances* GABAergic signaling in the PFC, leading to synaptic pruning of glutamatergic cortical projection neurons in the frontal lobe.

To test this hypothesis, I will directly investigate GPCR cascades in GABA:CB-1 neurons. CB1Rs are typically $G_{i/o}$ coupled, and corresponding activation of the $G_{i/o}$ cascades leads to decreased cAMP and therefore suppressed signaling(23). However, CB1Rs may shift Gi/o coupling to G_s under specific conditions, therefore enhancing GABAergic signaling (24). Increases in GABAergic signaling can lead to LTD at excitatory synapses, and eventual synapse loss. Indeed, excitatory synapses are preferentially pruned in adolescence while inhibitory synapses endure in adolescence(25), and loss of excitatory transmission is sufficient for Δ9-THC's cognitive effects(12). This demonstrates a vital knowledge gap in what GPCR cascades modulate Δ9-THC's cognitive effects. *To this end, I will investigate the behavioral and synaptic changes associated with different GPCR signaling cascades in cortical GABA:CB-1 neurons.*

Aim1: GPCR signaling in GABA:CB-1 cortical neurons mimic the cognitive effects of Δ9-THC.

1.1 Establishment of Cre expression in GABA and CB1 cortical neurons

1.2 Effects of $G_{i/o}$ and G_s stimulation of CB1-containing GABAergic cortical neurons on cognitive behavior.

1.3 Effects of chronic G_s and $G_{i/o}$ stimulation of CB1-containing GABAergic cortical neurons on IPSCs.

Aim 2 Alterations in PFC GABA:CB-1 signaling affect synaptic plasticity of cortical primary afferents

2.1 Chronic GABAergic stimulation reduces cortico-striatal projections and spine density.

2.3 Chronic GABAergic stimulation reduces cortico-hippocampal projections and spine density.

2.3 Chronic GABAergic stimulation and apoptosis.

Completion of the proposed experiments will significantly advance our understanding of how Δ9-THC exerts its negative effects on adolescents, a pressing concern given in the increased accessibility of Cannabis. This key insight into the mechanisms of normal brain maturation will also address NIMH's high priority of utilizing physiological models to understand the brain circuits in normal and abnormal mental function.

A. Significance

Marijuana (Cannabis sativa) has been used recreationally and therapeutically throughout history, and for many years studies concerning potential long-term deficits on the brain had mixed and disputed results.(26) It's now thought that chronic marijuana consumption does have some subtle long-term effects on the brain in the realm of cognition, including executive function and memory. But these effects become much more pronounced if data from human studies is parsed by the initial age of Cannabis usage(6, 7). Indeed, animal models show that adolescent, but not adult, chronic exposure to THC results in behavioral deficits, and corresponding changes in physiology, in learning and memory (3-5) This is extremely concerning, as Cannabis is the most frequently used drug in adolescence and because high schoolers' perceived risk of marijuana usage has decreased while usage has increased since 1992(1). It is of further concern that as the number of states with medical or recreational marijuana laws rise, the legal status of marijuana may influence its perceived availability, risk, and use among adolescents(2). This upswing in availability and decline of perceived risk highlights our urgent need to understand the neurobiology underlying these age-specific cognitive deficits.

Background:

Given the long-term cognitive deficits we see in marijuana users are correlated to their onset of usage, the mechanism of these changes probably lies in the interferences of brain development. The adolescent brain is not fully developed; during adolescence grey matter volume decreases while white matter volume increases, and these changes are through to arise from increases in synaptic pruning and increases in myelination. (19-21) The PFC is the last location to fully mature(22) and is a critical region for executive functions, including cognition. Adolescence is then a critical period for maturation of executive function, and exploring the cannabinoids in maturation of the PFC is critical to understanding these deficits.

Cannabinoids are an extremely prevalent system which act through a multiplicity of cascades effected through various receptors interactions on different neuronal subpopulations(27). To ascertain the mechanism behind the deleterious effects of marijuana during adolescence, we must first parse the relevant substances within Cannabis. The phytocannabinoids most enriched in Cannabis sativa are cannabidiol (CBD) and Δ9-THC. Marijuana inhalation and IP administration of THC produce similar memory impairments in rats, and changes in CBD dose did not contribute to, and in some instances may counteract, THC's effects on cognition in both rats and humans. (13, 28, 29) These findings support the theory of Δ9-THC as the primary psychoactive component of Cannabis, and in this proposal we will focus on determining Δ9-THC's effects on cognition.(9)

Δ9-THC primarily exerts its cognitive effects through the changes in synaptic plasticity at CB1R(10-13). Although CB1 Receptors are expressed widely throughout the brain presynaptically at both GABAergic and glutamatergic synapses, work has shown that CB1Rs mediate LTD in glutamatergic synapses and this is sufficient for Δ9-THC's cognitive effects(12, 14, 15). However, reducing GABAergic signaling is also sufficient to relieve Δ9-THC induced cognitive deficits(16), indicating a circuit level mechanism of Δ9-THC. Because of the relative distribution of CB1Rs show that they are primarily expressed on a subset of GABAergic interneurons(17), and fact that Δ9-THC is a full agonist at GABAergic CB1Rs(30), in this proposal I will focus on Δ9-THC's effects on CB1R- expressing GABAergic interneurons. Indeed, endocannabinoids preferentially target inhibitory perisomatic synapses on cortical projection neurons(18), the same subpopulation which is preferentially pruned in adolescence. I propose that adolescent cannabis usage results in enhanced GABAergic signaling in the PFC, leading to synaptic pruning of glutamatergic cortical projection neurons in the frontal lobe.

Intriguingly during adolescence there is another change in a GPCR system in the PFC. Dopaminergic signaling also modulates cognition, and during adolescence D2 receptors begin to strongly inhibit interneurons(31-33). D2 receptors are shown to colocalize presynaptically with CB1 on GABAergic cells in the PFC(34). Both CB1 and D2 are typically $G_{i/o}$ coupled, and corresponding activation of either normally leads to decreased cAMP

and therefore suppressed GABAergic signaling. Recent evidence demonstrates that co-activation of D2 and CB1 receptors can reduce GABAergic signaling in the PFC and VTA(34, 35) However previous work has shown co-activation of D2 and CB1 receptors change $G_{i/o}$ coupling to G_s, therefore enhancing GABAergic signaling(24). Since increases in GABAergic signaling can lead to LTD at excitatory synapses, and eventual synapse loss, the nature of CB1 coupling is of particular interest given the loss of excitatory transmission in the cognitive effects of Δ9-THC in adolescents. Therefore there is vital knowledge gap in how synergistic actions of CB1 and D2 receptors within the PFC change their signaling cascades and contribute to the cognitive effects of Δ9-THC*. To this end, I will manipulate the downstream effects of GPCR signaling cascades in cortical GABAergic neurons to examine their effects on cognition.*

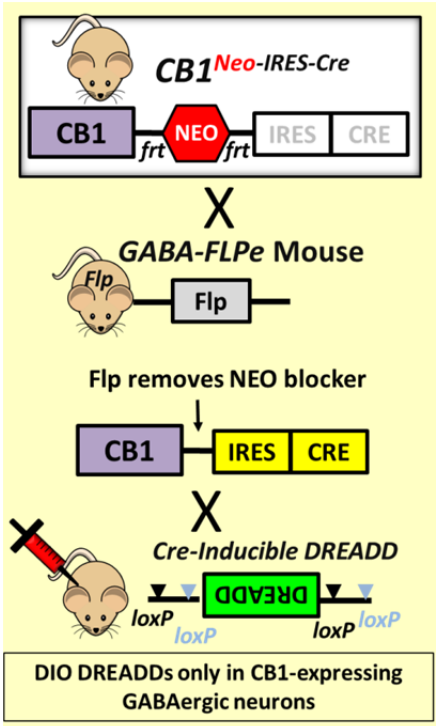

Figure 1: Breeding scheme for cell-specific expression of DREADDs

B. Innovation

These experiment will expand current scope of the field of Cannabis research. As described above, the cannabinoid system is exceedingly complex, containing multiple receptors on several cell types, and acts presynaptically to mediate synaptic plasticity through a multiplicity of signaling cascades, depending on the conditions of receptor activation. Despite these complications, researchers have ascertained that CB-1 signaling is common at GABAergic interneurons and this signaling is necessary for Δ9-THC. However, the mechanism for their contributions to aberrant cognition is unknown, as there is evidence that simultaneous signaling of D2 and CB-1 receptors may recruit either stimulating or repressing GPCRs. Because of changes in the internal environment in adolescence, namely loss of glutamatergic synapses and increases in D2 signaling, it is essential to interrogate and manipulate changes in CB-1 signaling in vivo.

This proposal side-steps the issue of simultaneous activation of CB-1 and D2 receptors, and instead probes the response of GABAergic cells to either G_s or $G_{i/o}$ stimulation, both postulated mechanisms of coupled CB1 and D2 interactions GABAergic interneurons. By probing GABAergic cells in this way we mimic the chronic stimulation of these cells by Δ9-THC without off target effects. Coupled with our plasticity analysis in Aim 2, we will elucidate the role of adolescent GABA:CB-1 signaling on behavior and synaptic plasticity.

The novel mouse line proposed will allow specific manipulation of GABAergic subpopulations of interest in vivo. Prior to this proposal, isolating CB1-expressing glutamatergic or GABAergic cellular populations required both developmental knock-out, and additions of molecular antagonists to electrophysiological baths, both of which fail to stimulate the intact system to achieve these results. These experiment will describe the *first* cell-type specific manipulation of CB-1 signaling in vivo.

C. Approach:

Aim1: GPCR signaling in CB1-containing GABAergic cortical neurons mimic the cognitive effects of Δ9-THC.

Rationale: The mechanism underflying the cognitive deficits stemming from chronic Δ9-THC have proven difficult to ascertain. Different groups have attributed these effects to deficits in glutamatergic signaling, or increases in GABAergic signaling in vitro and in adult animals. However, in adolescents, but not adults, there are persisting deficits in cognition after prolonged abstinence. This indicates interactions between mechanisms

of Δ9-THC action and brain maturation. The primary process underlying adolescent brain maturation is the decrease in excitatory synapses, while inhibitory synapses are spared. In addition, the dopaminergic system (which modulates cognition and executive function) also undergoes changes during adolescence. In adolescence the D2 receptors on interneurons become strongly active, altering the inhibitory GABAergic signaling. Therefore, I will focus on GABAergic cells. However, the intracellular signaling cascades of D2 and CB1 and their potential crosstalk is still disputed. Both D2 and CB1 receptors are inhibitory G-protein coupled receptors, and stimulation of either reduces intracellular cAMP. When stimulated together, some groups have found a transition to excitatory G_s-protein coupling, increasing cAMP and GABAergic signaling(24, 36). In contrast, another research group found co-activation of D2 and CB1 receptors promoted LTD at these inhibitory synapses, decreasing GABAergic signaling through decreases in cAMP.(34, 35) To elucidate the mechanism of reduced cognition in adolescents we must understand the results of stimulation of either G_s or $G_{i/o}$ signaling on GABAergic cells in vivo. *I hypothesize that as CB1 receptors on GABAergic neurons are stimulated in adolescent mice, CB1 preferentially G_s couples, as opposed to its established $G_{i/o}$ coupling.* To this end, I will develop a mouse model of cell type specific Cre expression, and inject flexed $G_{i/o}$ or G_s coupled DREADDs into the forebrain. Then we will stimulate these cells via CNO while delivering Δ9-THC to control animals. We will then assay changes in cognitive behavior resulting from GABA:CB-1 stimulation or inhibition and measure changes electrophysiological properties of these neurons.

Methods:

Animals: For Aim 1.1, I will develop the mouse line used in Aim 1.2, 1.3, and Aim 2. For experiments in Aim 1.2 and 1.3 I will use 54 4-week old healthy mice from a novel transgenic mouse line. These mice will be crosses of Dlx5/6-Flpe mice, which generate myc-tagged FLPe recombinase in GABAergic neurons in the forebrain(37), and a CB1 Cre recombinase driver line. The Cre is preceded by a frt-flanked stop cassette (see **Fig1**). In this way, we will express Cre specifically in CB1 positive GABA neurons. In each subsequent aim for sufficient power and breadth, I will want N=18/treatment group ($G_{i/o}$, G_s and Δ9-THC), with n=9 males and n=9 females to account for any effects of pubertal hormones. N=6/treatment group will be controls per condition.

Validation of mouse line: Aim 1.1 will be devoted to the validation of our mouse line, using 3 generations to confirm GABA:CB-1^{Cre} expression. Validation will involve RT-PCRs to assay for Cre expression in tissue, mating to a GFP reporter line to visualize Cre incorporation into DNA, and then characterizing the anatomical expression of Cre using histology to confirm the limited expression pattern of our GABA:CB-1 mice(38). After characterization of GABA:CB-1^{Cre} mice, the investigation of the GABA:CB-1 subpopulation will continue.

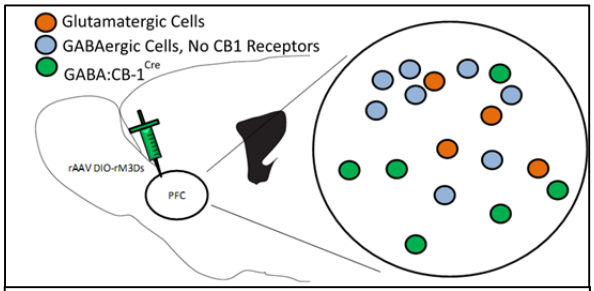

Figure 2: Schematic of DREADD expression patterns. Injection of DIO-DREADDs into the PFC will spatially and cell type specifically express rM3Ds (G_s coupled) or hM4Di ($G_{i/o}$ coupled)

Treatments and Schedule: In Aim 1.2, 36 mice will undergo intracranial surgeries at 4 weeks, into the prefrontal cortex, and 18 mice will have sham-surgeries. The purpose is to administer AAVs containing flexed DREADDS; 18 animals will receive hM4Di $G_{i/o}$-coupled and 18 will receive rM3Ds G_s-coupled DREADDs. This means that our DREADDs will be spatially restricted and cell type specific (See **Figs 1, 2**). Because these mice will be younger than adults, they will require altered stereotaxic coordinates for the PFC. These are estimated using a correction factor calculated by regression equation $F' = F_a - 0.66 (bl - 3.8)$, where F' is the predicted coordinate, F_a is the coordinate given by a mouse brain atlas, and bl is the bregma-lambda distance of the mouse(39). The coordinates for an adult are AP: +1.7, ML:+.75, and DV:-2.5. After waiting 2 weeks for DREADD expression at the synapse, I will chronically administer CNO (0.3 mg per kg) to n=9 $G_{i/o}$ and n=9 G_s and Δ9-THC(2mg per kg) to n=9 sham-surgery animals. The control animals will be given .1% sterile saline to control for effects of DREADD transduction and handling. These daily injections will

continue throughout the duration of these experiments. After a week of stimulation, I will test the animals from Aim 1.2 on several behavioral paradigms of cognition. After this test period, we will use these same animals for the electrophysiology in Aim 1.3.

Behavioral Tasks: In Aim 1.2 we will several tasks to assay cognitive function in mice. First I will utilize the Novel Object Recognition (NOR) task, to assay hippocampal learning and memory. In brief, animals are familiarized with two identical objects, and then one object is substituted for a novel object. Increased time spent with novel object is taken as a measure of increased learning and memory. To assay attention deficits, I will use the multiple-choice serial- reaction test (5-CSRTT) (**Fig 3**). 5-CSRTT involves simultaneous monitoring of five nose-poke apertures for a light-cue associated with food reward. Accuracy and speed nose poke responses are taken as measures of attentional capacity and incorrect responses are taken as a measure of impulsivity.

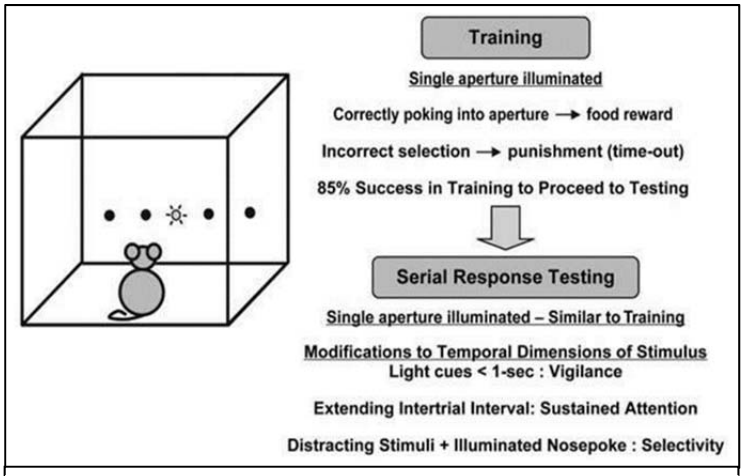

Figure 3: Schematic of the multiple choice serial reaction test, and its related behaviors, from "Assessments of Cognitive Impairments in Mutant Mice (8)

Electrophysiology: In Aim 1.3, we will prepare acute ex-vivo slices from p63-p70 animals, post behavioral testing, to examine cortical GABAergic synaptic properties, specifically long-term depression at these synapses and mIPSCs. To assay mIPSCs, we will perform whole-cell patch clamping in a bath containing CNQX, APV and TTX to remove any effects glutamatergic or Na+ signaling. We will patch ganglion cells in Layer 5 of the cortex and stimulate both distally, in layer 2/3 and locally in layer 5(<50μm away). PPR will be assayed as amplitude of the second IPSC divided by the amplitude of the first stimulated IPSC to determine the level of LTD. To assay in the influence of another dopaminergic signaling on these signaling cascades, I will apply a selective D2/D3 agonist to the bath, quinpirole, then measuring the resulting changes in IPSC 15 minutes after application.

Possible Results: In this experiment we will determine if prolonged $G_{i/o}$ or G_s stimulation of CB1-expressing GABA cells in the PFC reproduce the cognitive deficits and changes in IPSCs induced by Δ9-THC administered peripubertally. If my hypothesis is correct, we would expect chronic G_s stimulation in GABA:CB-1 interneurons to mimic the behavioral deficits of Δ9-THC administered to sham surgery animals. This will manifest as reduced time spent with the novel object, and reduced correct nose pokes in 5-CSRTT. Similarly, in electrophysiology we would expect to see an increase mIPSC frequency, but not amplitude in both of these groups. We would also expected the PPR to decrease in these, consistent with an increase in GABA release. I also expect quinpriole to suppress GABA release in our saline administered groups, but enhance these effects in our CNO and Δ9-THC treated animals. However, if D2 and CB1 signaling preferentially activate $G_{i/o}$ cascades, then we will see the opposite: $G_{i/o}$ and Δ9-THC behavioral and electrophysiological covariance and will be induced with the CNO stimulation and Δ9-THC group. If in fact, my hypothesis is proven false, and alternative explanation may be that relief of inhibition may allow outgrowth and preservation of excitatory synapses, which are then more available to later downregulation. Lastly, I do not expect to see any differences between sexes, which will allow me to combine these results for further analysis.

Potential Problems: As the rest of the proposal depends on the generation of cell specific Cre recombinase expression, the transgenic mouse line is an essential technique. Although transgenic tools and techniques are well know, if needs be we can supplement our experiments with CCK^{Cre} driver lines, as emerging evidence suggest that CCK-positive interneurons also express CB1Rs(40). If the coordinates as predicted must be altered, then we can pilot more effective PFC coordinates through trial and error. Although preparing healthy

ex-vivo slices is difficult, we have preliminary data from both virally transfected and non-transfected cells indicating that viruses do not inherently alter basal cell properties (not shown) and as DREADDs are only activated by the designer drug CNO, so we don't anticipate changes to any baseline properties of our cells. Finally, our DREADDs are derived from two different species, as neither human nor rat derived DREADDs are coupled to both G_s and $G_{i/o}$ cascades. However, the source of the sequences should not matter, as administration of the designer drug CNO stimulates both DREADDs, and by including saline treated groups we control for the expression of these DREADDs.

Aim 2 Alterations in PFC GABAergic signaling affect cortical primary afferents

Rationale: Much of the rodent literature has focused on hippocampal plasticity and Δ9-THC's effects on memory. Deficits arising from the GABAergic neurons as gained prominence and indeed, we examine the role of GABAergic signaling in behavior in Aim 1. However, GABAergic signaling is a common in inhibitory interneurons, which are present in large numbers in the PFC, and GABAergic interneurons often synapse onto glutamatergic neurons. Since there is evidence that glutamatergic LTD is sufficient for cognitive effects of Δ9-THC, perhaps it is due changes in cortical efferent projecting neurons. Indeed, human research has seen functional differences in the corticostriatal and cortico-hippocampal pathways of intoxicated cannabis users(41). *I hypothesize that increasing GABAergic signaling in the forebrain of adolescent mice will lead to decreases in the number of mature stubby spine on the dendrites of projection neurons, a proxy measure of synaptic strength*. Therefore, in this Aim, I will inject several brain regions associated with attention and memory with retrograde tracers, coupled with long term stimulation of G_s DREADDs. We will then analyze stained cell bodies in the PFC for changes in spine density and morphology.

Methods:

Animals: In this Aim, I will use N=100 total 4-wk old GABA:CB1^{Cre} mice, 50 males and 50 females.

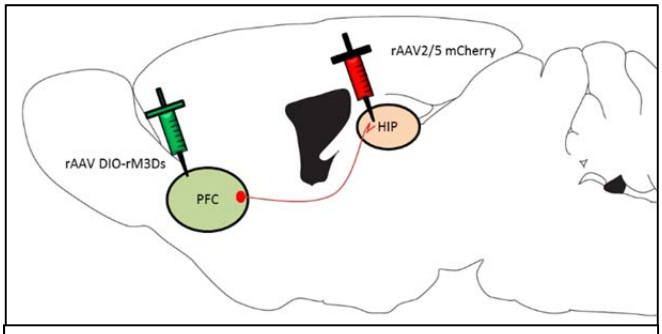

Figure 4: Schematic of example dual injections. In addition to PFC injections of DIO-DREADDs, retrograde mCherry will be injected into the Striatum or Hippocampus (pictured

Treatment: All animals in this AIM will undergo stereotaxic surgeries at 4 weeks of age. I will inject rAAV-DIO-rM3Ds (G_s-coupled DREADDs) into the PFC of all (N=100) animals. While the animals are still under anesthesia, I will also stereotaxically inject retrograde rAAV2/5-mCherry into either the striatum (n=50, adult coordinates AP: +0.7, ML: +1.4, DV: -2.8) or the dentate gyrus of the hippocampus (n=50, adult coordinates AP: -2.2, ML:+2.5 DV -2.1). After a 2-week quiescent period allowing for robust expression of our AAVs, there will be mCherry in the cell bodies and dendrites of projection neurons of the PFC, in addition to G_s DREADDs on GABA:CB-1^{Cre} cells (see **Fig 4**). This will allow visualization of changes in projection neurons from the PFC as a result of increased GABAergic signaling.

Five saline males and five females of each brain region (described in **Table 1**, right) will be sacrificed prior to CNO administration, deemed "pretreatment." The remaining animals will have a 7 day course of i.p. injections of CNO to agonize G_s signaling in CB1 GABAergic cells, or injection of .1% saline as a control. 5 animals/ treatment group will be sacrificed 24 hours after the last CNO injection, to measure the effects pf chronic stimulation on primary cortical afferents. The remaining animals will be sacrificed 14 days after the last CNO injections, mimicking a withdrawal and abstinence period. By sacrificing groups at 3 time points, we can analyze any effects of

Table 1: Treatment groups in Aim 2

	CNO		Saline		Totals
	Males	Female	Males	Female	
Hip	10	10	15	15	60
Str	10	10	15	15	60

stimulation on cortical projection neurons, while and controlling for the aging process in our analysis (**Fig 5,** right).

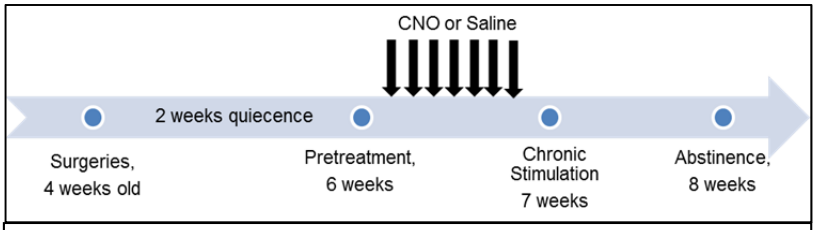

Figure 5: Summary of experimental timeline in Aim 2. Animals will be sacrificed at 3 time points: pretreatment, after chronic stimulation, and after a week of abstinence.

Outputs: Animals will be perfused with 4% Paraformaldehyde, post-fixed, cryoprotected, and sectioned at 250μM for spine analysis or 30μM for Terminal deoxynucleotidyl transferase (TdT) d UTP Nick- End Labeling (TUNEL) staining(42). Confocal microscopy will be used to obtain images of PFC projection neurons at MSU's Center for Advanced Microscopy, and analysis of dendritic spine density and spine type (stubby, mushroom, or thin) will be assessed using NeuronStudio software. The resulting quantitation of spine density and morphology in this study will provide critical information concern CB1 GABAergic signaling on PFC Primary Projection Neurons, and provide insight into aberrant synaptic pruning in adolescent Δ9-THC usage. TUNEL staining marks DNA breaks by endonucleases thereby providing insight whether potential effect of Δ9-THC in adolescence are due to aberrant cell death, as CB1 stimulation can cause apoptosis (43-45).

Potential Results: I do not predict to see any differences dependent upon sex, allowing data analysis to combine males and females in our analysis. I predict stimulation of GABAergic signaling through CNO administration will decrease the spine density and reduce the number of mature spines on PFC PNs just after chronic stimulation. After a week of abstinence, I expect an increase in spine counts, consistent with improved cognitive outcomes with abstinence in adolescents(46, 47) And given the predicted recovery of spines, I predict no change in apoptotic signals within each time point.

Possible Problems: Many of potential issues from Aim 1 arise again in Aim 2. Transgenic line difficulties may be overcome with utilization of CCK^{Cre} driver lines, although it would cloud any interpretation of the proposed studies. Effective surgical targeting in young animals may prove difficult in this model. Similar to Aim1, we can address this with trial and error targeting. If theory underlying my proposed method in Aim2 depends upon the results of Aim 1. If my predictions for Aim 1 are incorrect, I can easily change injections to the inhibitory hM4Di DREADD, instead. Finally, we will examine both males and females in our studies in compliance with NIH's mission to consider sex as a biological variable, and in an effort to address potential sex differences in marijuana usage(48).

Completion of the proposed experiments will significantly advance our understanding of Δ9-THC's effects on cortical GABAergic signaling and the resulting changes in synaptic pruning and plasticity in adolescents. Understanding these mechanisms are of urgent importance given the increased accessibility of Cannabis, and will address key NIH priorities, such as considering sex as a biological variable and utilizing physiological models to understand the brain circuits in normal and abnormal mental function. The proposed experiments will provide ample training benefits in a variety of techniques, including animal behavior, transgenic and viral vector approaches, electrophysiology and spine morphology. This host of skills will masterfully prepare the applicant for a future in functional neuroscience research.

References

1. Johnston LD, O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. . Monitoring the Future: national results on adolescent drug use: Overview of key findings, 2012. Ann Arbor, MI: Institute for Social Research, the University of Michigan2013.
2. Stolzenberg L, D'Alessio SJ, Dariano D. The effect of medical cannabis laws on juvenile cannabis use. The International journal on drug policy. 2016;27:82-8. Epub 2015/07/01. doi: 10.1016/j.drugpo.2015.05.018. PubMed PMID: 26123892.
3. Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N, Thompson MR, Dawson B, Mallet PE, Kashem MA, Matsuda-Matsumoto H, Iwazaki T, McGregor IS. Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2008;33(5):1113-26. Epub 2007/06/22. doi: 10.1038/sj.npp.1301475. PubMed PMID: 17581536.
4. Schneider M, Koch M. Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2003;28(10):1760-9. Epub 2003/07/31. doi: 10.1038/sj.npp.1300225. PubMed PMID: 12888772.
5. Schneider M, Schomig E, Leweke FM. Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. Addiction biology. 2008;13(3-4):345-57. Epub 2008/09/11. doi: 10.1111/j.1369-1600.2008.00117.x. PubMed PMID: 18782382.
6. Jacobus J, Tapert SF. Effects of Cannabis on the Adolescent Brain. Current pharmaceutical design. 2014;20(13):2186-93. PubMed PMID: 23829363; PMCID: PMC3930618.
7. Meier MH, Caspi A, Ambler A, Harrington H, Houts R, Keefe RSE, McDonald K, Ward A, Poulton R, Moffitt TE. Persistent cannabis users show neuropsychological decline from childhood to midlife. Proceedings of the National Academy of Sciences. 2012;109(40):E2657–E64. doi: 10.1073/pnas.1206820109.
8. Rodriguiz RM WW. Assessments of Cognitive Deficits in Mutant Mice. In: Levin ED BJ, editor. Animal Models of Cognitive Impairment. Boca Raton (FL): CRC Press/Taylor & Francis; 2006.
9. Gaoni Y, Mechoulam R. Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. Journal of the American Chemical Society. 1964;86(8):1646-7. doi: 10.1021/ja01062a046.
10. Lichtman AH, Martin BR. Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology (Berl). 1996;126(2):125-31. Epub 1996/07/01. PubMed PMID: 8856831.
11. Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F, Koehl M, Abrous DN, Mendizabal-Zubiaga J, Grandes P, Liu Q, Bai G, Wang W, Xiong L, Ren W, Marsicano G, Zhang X. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Cell. 2012;148(5):1039-50. Epub 2012/03/06. doi: 10.1016/j.cell.2012.01.037. PubMed PMID: 22385967.
12. Fan N, Yang H, Zhang J, Chen C. Reduced expression of glutamate receptors and phosphorylation of CREB are responsible for in vivo Δ9-THC exposure-impaired hippocampal synaptic plasticity. Journal of neurochemistry. 2010;112(3):691-702. doi: 10.1111/j.1471-4159.2009.06489.x. PubMed PMID: PMC2809144.
13. Hoffman AF, Oz M, Yang R, Lichtman AH, Lupica CR. Opposing actions of chronic Δ(9)-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. Learning & memory (Cold Spring Harbor, NY). 2007;14(1-2):63-74. doi: 10.1101/lm.439007. PubMed PMID: PMC1828281.
14. Chen R, Zhang J, Fan N, Teng ZQ, Wu Y, Yang H, Tang YP, Sun H, Song Y, Chen C. Delta9-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. Cell. 2013;155(5):1154-65. Epub 2013/11/26. doi: 10.1016/j.cell.2013.10.042. PubMed PMID: 24267894; PMCID: PMC3918429.
15. Auclair N, Otani S, Soubrie P, Crepel F. Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. Journal of neurophysiology. 2000;83(6):3287-93. Epub 2000/06/10. PubMed PMID: 10848548.
16. Varvel SA, Anum E, Niyuhire F, Wise LE, Lichtman AH. Delta(9)-THC-induced cognitive deficits in mice are reversed by the GABA(A) antagonist bicuculline. Psychopharmacology (Berl). 2005;178(2-3):317-27. Epub 2004/08/24. doi: 10.1007/s00213-004-1988-2. PubMed PMID: 15322725.
17. Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. The European journal of neuroscience. 1999;11(12):4213-25. Epub 1999/12/14. PubMed PMID: 10594647.

18. Trettel J, Fortin DA, Levine ES. Endocannabinoid signalling selectively targets perisomatic inhibitory inputs to pyramidal neurones in juvenile mouse neocortex. The Journal of physiology. 2004;556(Pt 1):95-107. Epub 2004/01/27. doi: 10.1113/jphysiol.2003.058198. PubMed PMID: 14742727; PMCID: PMC1664891.
19. Sowell ER, Thompson PM, Holmes CJ, Batth R, Jernigan TL, Toga AW. Localizing age-related changes in brain structure between childhood and adolescence using statistical parametric mapping. NeuroImage. 1999;9(6 Pt 1):587-97. Epub 1999/05/21. doi: 10.1006/nimg.1999.0436. PubMed PMID: 10334902.
20. Jernigan TL, Trauner DA, Hesselink JR, Tallal PA. Maturation of human cerebrum observed in vivo during adolescence. Brain : a journal of neurology. 1991;114 (Pt 5):2037-49. Epub 1991/10/01. PubMed PMID: 1933232.
21. Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL. Brain development during childhood and adolescence: a longitudinal MRI study. Nature neuroscience. 1999;2(10):861-3. Epub 1999/09/24. doi: 10.1038/13158. PubMed PMID: 10491603.
22. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, Nugent TF, 3rd, Herman DH, Clasen LS, Toga AW, Rapoport JL, Thompson PM. Dynamic mapping of human cortical development during childhood through early adulthood. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(21):8174-9. Epub 2004/05/19. doi: 10.1073/pnas.0402680101. PubMed PMID: 15148381; PMCID: PMC419576.
23. Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid Receptors and Their Ligands: Beyond CB(1) and CB(2). Pharmacological Reviews. 2010;62(4):588-631. doi: 10.1124/pr.110.003004. PubMed PMID: PMC2993256.
24. Glass M, Felder CC. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1997;17(14):5327-33. Epub 1997/07/15. PubMed PMID: 9204917.
25. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. Science (New York, NY). 1986;232(4747):232-5. Epub 1986/04/11. PubMed PMID: 3952506.
26. Crean RD, Crane NA, Mason BJ. An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. Journal of addiction medicine. 2011;5(1):1-8. Epub 2011/02/16. doi: 10.1097/ADM.0b013e31820c23fa. PubMed PMID: 21321675; PMCID: PMC3037578.
27. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-Mediated Control of Synaptic Transmission. Physiological reviews. 2009;89(1):309-80.
28. Niyuhire F, Varvel SA, Martin BR, Lichtman AH. Exposure to marijuana smoke impairs memory retrieval in mice. J Pharmacol Exp Ther. 2007;322(3):1067-75. Epub 2007/06/26. doi: 10.1124/jpet.107.119594. PubMed PMID: 17586723.
29. Ilan AB, Gevins A, Coleman M, ElSohly MA, de Wit H. Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. Behavioural pharmacology. 2005;16(5-6):487-96. Epub 2005/09/09. PubMed PMID: 16148455.
30. Laaris N, Good CH, Lupica CR. Delta9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus. Neuropharmacology. 2010;59(1-2):121-7. Epub 2010/04/27. doi: 10.1016/j.neuropharm.2010.04.013. PubMed PMID: 20417220; PMCID: PMC2882293.
31. Tseng KY, O'Donnell P. Dopamine modulation of prefrontal cortical interneurons changes during adolescence. Cerebral cortex (New York, NY : 1991). 2007;17(5):1235-40. Epub 2006/07/05. doi: 10.1093/cercor/bhl034. PubMed PMID: 16818475; PMCID: PMC2204087.
32. Tseng KY, O'Donnell P. D2 dopamine receptors recruit a GABA component for their attenuation of excitatory synaptic transmission in the adult rat prefrontal cortex. Synapse (New York, NY). 2007;61(10):843-50. Epub 2007/07/03. doi: 10.1002/syn.20432. PubMed PMID: 17603809; PMCID: PMC2190290.
33. Gorelova N, Seamans JK, Yang CR. Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. Journal of neurophysiology. 2002;88(6):3150-66. Epub 2002/12/06. doi: 10.1152/jn.00335.2002. PubMed PMID: 12466437.
34. Chiu CQ, Puente N, Grandes P, Castillo PE. Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(21):7236-48. Epub 2010/05/28. doi: 10.1523/jneurosci.0736-10.2010. PubMed PMID: 20505090; PMCID: PMC2905527.
35. Pan B, Hillard CJ, Liu QS. D2 dopamine receptor activation facilitates endocannabinoid-mediated long-term synaptic depression of GABAergic synaptic transmission in midbrain dopamine neurons via cAMP-protein kinase A

signaling. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008;28(52):14018-30. Epub 2008/12/26. doi: 10.1523/jneurosci.4035-08.2008. PubMed PMID: 19109485; PMCID: PMC2656602.
36. Jarrahian A, Watts VJ, Barker EL. D2 dopamine receptors modulate Galpha-subunit coupling of the CB1 cannabinoid receptor. J Pharmacol Exp Ther. 2004;308(3):880-6. Epub 2003/11/25. doi: 10.1124/jpet.103.057620. PubMed PMID: 14634050.
37. De Marco Garcia NV, Fishell G. Subtype-selective electroporation of cortical interneurons. Journal of visualized experiments : JoVE. 2014(90):e51518. Epub 2014/09/02. doi: 10.3791/51518. PubMed PMID: 25177832; PMCID: PMC4243608.
38. Gofflot F, Wendling O, Chartoire N, Birling MC, Warot X, Auwerx J. Characterization and Validation of Cre-Driver Mouse Lines. Current protocols in mouse biology. 2011;1(1):1-15. Epub 2011/01/01. doi: 10.1002/9780470942390.mo100103. PubMed PMID: 26068985.
39. Qiao X, Noebels JL. Developmental analysis of hippocampal mossy fiber outgrowth in a mutant mouse with inherited spike-wave seizures. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1993;13(11):4622-35. Epub 1993/11/01. PubMed PMID: 8229188.
40. Whissell PD, Cajanding JD, Fogel N, Kim JC. Comparative density of CCK- and PV-GABA cells within the cortex and hippocampus. Front Neuroanat. 2015;9. doi: 10.3389/fnana.2015.00124. PubMed PMID: 26441554; PMCID: PMC4585045.
41. Bhattacharyya S, Falkenberg I, Martin-Santos R, Atakan Z, Crippa JA, Giampietro V, Brammer M, McGuire P. Cannabinoid modulation of functional connectivity within regions processing attentional salience. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2015;40(6):1343-52. Epub 2014/09/25. doi: 10.1038/npp.2014.258. PubMed PMID: 25249057; PMCID: PMC4397391.
42. Kyrylkova K, Kyryachenko S, Leid M, Kioussi C. Detection of apoptosis by TUNEL assay. Methods in molecular biology (Clifton, NJ). 2012;887:41-7. Epub 2012/05/09. doi: 10.1007/978-1-61779-860-3_5. PubMed PMID: 22566045.
43. Downer EJ, Fogarty MP, Campbell VA. Tetrahydrocannabinol-induced neurotoxicity depends on CB(1) receptor-mediated c-Jun N-terminal kinase activation in cultured cortical neurons. British Journal of Pharmacology. 2003;140(3):547-57. doi: 10.1038/sj.bjp.0705464. PubMed PMID: PMC1574055.
44. Campbell VA. Tetrahydrocannabinol-induced apoptosis of cultured cortical neurones is associated with cytochrome c release and caspase-3 activation. Neuropharmacology. 2001;40(5):702-9. Epub 2001/04/20. PubMed PMID: 11311898.
45. Chan GC, Hinds TR, Impey S, Storm DR. Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1998;18(14):5322-32. Epub 1998/07/03. PubMed PMID: 9651215.
46. Ganzer F, Broning S, Kraft S, Sack PM, Thomasius R. Weighing the Evidence: A Systematic Review on Long-Term Neurocognitive Effects of Cannabis Use in Abstinent Adolescents and Adults. Neuropsychology review. 2016. Epub 2016/04/30. doi: 10.1007/s11065-016-9316-2. PubMed PMID: 27125202.
47. Roten A, Baker NL, Gray KM. Cognitive performance in a placebo-controlled pharmacotherapy trial for youth with marijuana dependence. Addictive Behaviors. 2015;45:119-23. doi: http://dx.doi.org/10.1016/j.addbeh.2015.01.013.
48. Marusich JA, Craft RM, Lefever TW, Wiley JL. The impact of gonadal hormones on cannabinoid dependence. Experimental and clinical psychopharmacology. 2015;23(4):206-16. Epub 2015/08/04. doi: 10.1037/pha0000027. PubMed PMID: 26237318; PMCID: PMC4696547.