Modulation of the Endogenous Cannabinoid System as a Therapeutic Target in the Treatment of Mental Health Disorders

by

Harrison Stratton

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Graduate Supervisory Committee:

Michael Shafer, Co-Chair
Michael Foster Olive, Co-Chair
Jie Wu, Member

ARIZONA STATE UNIVERSITY

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ABSTRACT

Development of effective therapeutic interventions for the treatment of mental health disorders has been a significant driving force in the search to understand the human brain. Current treatments for mental health disorders rely on modulating neurotransmitter systems such as norepinephrine (NE), serotonin (5-HT), dopamine (DA) and γ-aminobutyric acid (GABA) to achieve clinically relevant relief of symptoms. While many medications are available to the clinician that individually target these neural systems, treatment often results in patients reporting unwanted side effects or experiencing incomplete relief. To counter this lack of treatment efficacy, further investigation of other avenues for achieving similar or better outcomes and potentially reach patients refractory to common therapies must be undertaken. One of these potential new target systems is the endogenous cannabinoid system (ECS), which is currently composed of cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). These metabotropic seven transmembrane (7-TM) loop G-protein coupled receptors (GPCR) are responsible for mediating the effects of acute *Cannabis* ingestion as well as modulating several core functions of the nervous system including emotion, memory, and learning behavior. Due ubiquitous expression of ECS proteins, there is broad overlap between brain regions that show high levels of receptor expression and those thought to be involved in the etiology of a range of mental health disorders including depression, anxiety and schizophrenia. Consequently, modulation of cannabinoid receptor function is a novel and potentially clinically relevant mechanism for influencing the levels of other neuromodulators and neurotransmitters, such as dopamine, that are known to play crucial

roles in the progression of mental illness. In addition, characterization of endogenous cannabinoids and cannabinoid receptors with respect to their normal physiological function and possible roles in pathophysiology may provide insight for the development of future ECS-based therapies.

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CHAPTER 1

INTRODUCTION

Increasing awareness regarding the prevalence of mental health disorders has, over the course of the last three decades, occurred alongside dramatic advances in technology that have allowed investigation into the fundamental neurobiological mechanisms underlying these disorders[1]. This work has resulted in a vast body of scientific knowledge seeking to address the physiological substrates that are responsible for the development and progression of mental health disorders, but it has not been easy to directly translate this knowledge into definitively effective therapies. This problem of treatment efficacy is, at least in part, related to the highly stigmatized nature of our population's perceptions concerning the nature of mental illness[1]. This is exemplified by the fact that despite significant advances in the field of mental health research, less than 50 percent of those suffering from depression receive adequate treatment[1–3]. Many overlapping factors produce this lack of treatment efficacy, including social constructs and the physiological side effects of the pharmacological tools used in the course of treatment[1–5]. Consequently, the search to find new molecules to alleviate the symptoms of mental health disorders while reducing the side effect profile of the drug(s) used is of the utmost importance. Emerging lines of evidence point toward new treatment possibilities that are centered on modulation of the endogenous cannabinoid system (ECS) to exert their therapeutic effect.

Cannabis sativa is the most commonly used illicit drug worldwide[6]. With a deep history of *Cannabis* use among humans dating back millennia, it is only within the last

century that possession of any form of the *Cannabis* plant has become illegal[7]. The primary psychotropic compound found within *Cannabis* is delta-9-terahydrocannabinol (Δ^9-THC) as shown in Figure 1 and table 1. This molecule produces euphoric sensations typically cited as part of the negative consequences of *Cannabis* ingestion[6,8]. While this one substance is responsible for the illegality of *Cannabis*, there are hundreds of related, yet diverse compounds, known as phytocannabinoids, found within the resin of *Cannabis sativa* that have unknown mechanisms of action[9–11]. These various cannabinoid molecules must be individually studied for potential therapeutic effects that could be produced without the psychotropic side effects of THC. Likely, there are components of the plant that have previously gone unnoticed, which could provide new avenues for modulation of the human central nervous system and the treatment of disease[7]. Over the past decade, there has been significant academic interest in exploring the full breadth of the neuromodulatory effects produced by the constituent components of *Cannabis sativa*. This resurgence has paralleled the emergence of a new population of individuals that ingest *Cannabis* regularly to treat a variety of medical conditions, often with the recommendation of a medical professional[12–14]. However, without firm scientific evidence regarding the efficacy of many of the purported medical benefits claimed by individuals within this community, these patients cannot receive the same level of care received by patients undergoing conventional therapy. To address this disparity in knowledge regarding the delivery of efficacious treatment, evidence of the medical properties of *Cannabis* must be collected through rigorous scientific inquiry to adequately treat this new population of patients. Consequently, there exists great potential for development of new pharmacological therapies from the plethora of

phytocannabinoid compounds that could act on targets in the central nervous system (CNS)[8–10]. Many of these compounds have been isolated but remain largely uncharacterized in terms of their physiological and pharmacological effects in humans. Rigorous investigation of these phytocannabinoids could reveal new modulatory mechanisms that may prove efficacious in the treatment of mental health disorders. In addition, characterization of endogenous cannabinoids and cannabinoid receptors with respect to their normal physiological function and possible roles in pathophysiology may provide insight for the development of novel ECS modulation-based therapies.

	CB1	CB2
Agonist	CP 55,940, Arachidonoyl-Glycerol (2-AG),	JWH-015, JWH-051, JWH-133, CP 55,940
Partial Agonist	Anandamide (AEA), Δ9-Tetrahydrocannabinol (THC, Dronabinol), WIN 55,212-2,	WIN 55,212-2, Anandamide (AEA), Δ9-Tetrahydrocannabinol, 2-Arachydonyl-Glycerol (2-AG)
Antagonist/Inverse Agonist	SR 141617A, LY 320315, AM 251	SR144528, AM630

Table 1 – Cannabinoid Receptor Ligands
Various synthetic, endogenous, and plant derived ligands of cannabinoid receptors 1 and 2 grouped based on their activity profile at each receptor target. As shown above, cannabinoids derived from plant sources are primarily partial agonists at the cannabinoid receptors and have additional activity at other receptors that are not considered members of the cannabinoid family.

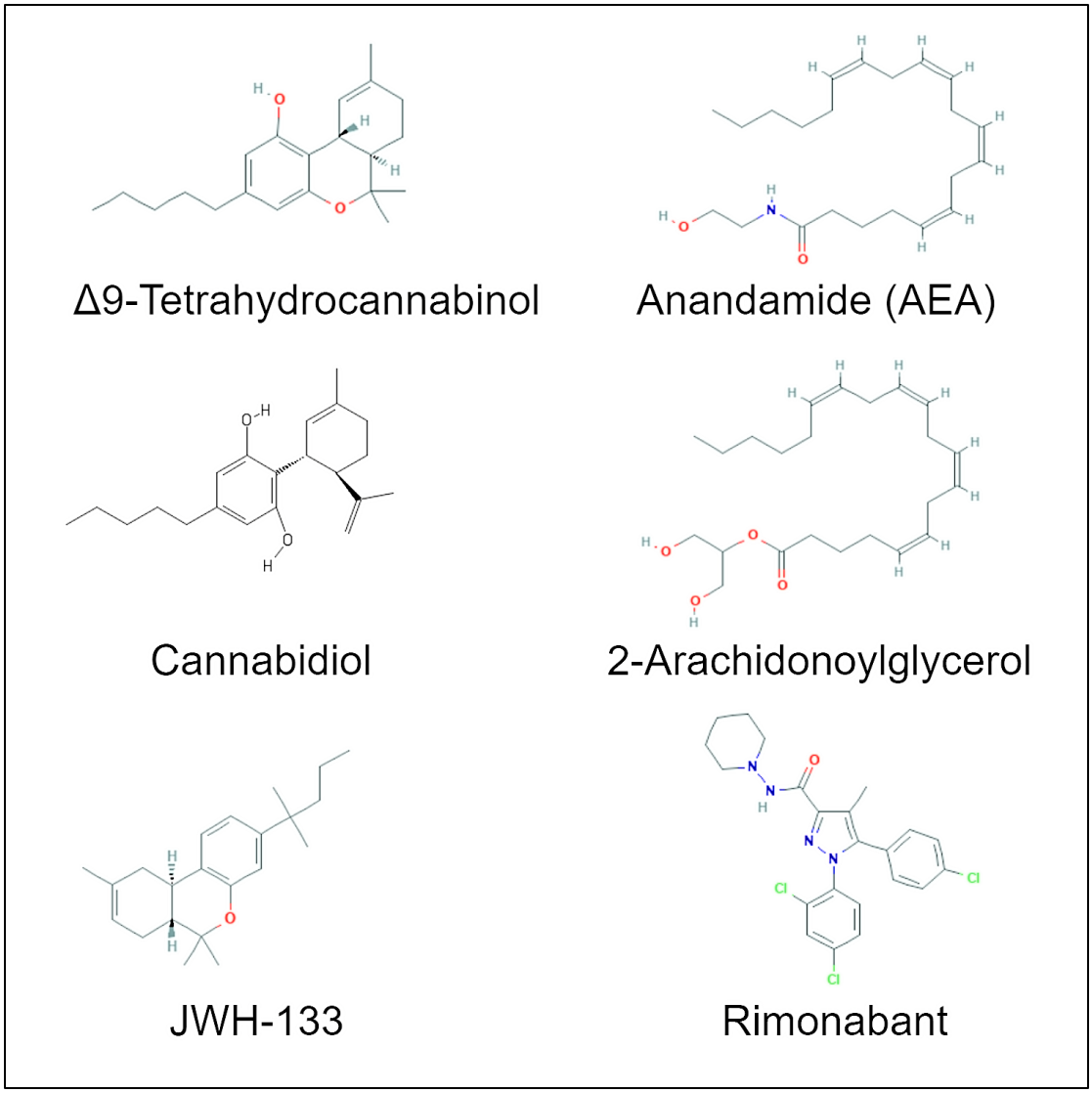

Figure 1 – Molecular Structure of Endogenous and Synthetic Cannabinoids
Delta-9-Tetrahydracannabinol (d9-THC) is thought to be the primary psychoactive constituent of *Cannabis sativa* and is known to act at CB1 receptors to produce a range of effects on cognition, emotion as well as physiological functions such as cardiac output. Anandamide and 2-Arachidonoylglycerol are the primary endogenous cannabinoids which are derived from lipid precursors found in the membranes of cells throughout the body. Rimonabant is a CB1 receptor inverse agonist that was marketed for a short time but withdrawn after negative effects including suicidal ideation were observed in patients receiving it to control weight gain and JWH-133 is a potent and highly selective CB2 receptor agonist used to research the endogenous cannabinoid system.

Studies investigating the role of the ECS in mediating mental health disorders as well as healthy physiological processes is still quite young relative to investigation of other neuronal signaling systems. Most of the evidence from human studies has focused on the effects of THC with recent animal studies of cannabinoid (CB) receptor psychopharmacology making use of newly synthesized and highly specific targeted ligands to better understand this receptor system. To facilitate understanding of this system, a short overview of ECS anatomy and physiology is presented below with additional consideration given to potential new cannabinoid receptors as well as other targets of endogenous cannabinoid ligands. Each subsequent section addresses a major mental health disorder including affective disorders, psychotic disorders, and anxiety disorders along with a discussion of how the ECS could potentially be involved in each and how they could be impacted by the development of new therapies targeting the ECS. Specifically, the pathological features of the underlying neural physiology in each of these disorders will be discussed. It is from this perspective of dysfunctional neural physiology that recent discoveries regarding the endogenous cannabinoid system can be applied toward the development of novel therapeutics strategies targeting patients suffering from mental health disorders.

The Endogenous Cannabinoid System

The symptoms associated with mental health disorders are widely believed to be the result of deleterious changes in the underlying functionality of molecular pathways mediating neuronal communication within the central nervous system[1]. Aberrant patterns of neuronal activity are present in the brain, it can lead to alterations in the behavioral

profile that characterizes each of us as individuals. A variety of neurotransmitter receptor proteins are expressed in neural tissue, forming functionally nested control systems that perform sensory integration and guide motor output. These systems interact in a variety of ways to regulate cellular communication through the release of neurotransmitter signaling molecules such as acetylcholine, glutamate, and gamma amino butyric acid (GABA), which represent some of the most well characterized signaling systems[15–18]. Cannabinoid receptors are another subclass of this protein signaling system that is defined by these proteins' ability to bind molecules derived from the *Cannabis sativa* plant, as well as other synthetic cannabinoid molecules[15]. Unfortunately, research into the endogenous cannabinoid system has lagged far behind that of other receptor families due to the precarious nature of the legal situation surrounding sources of cannabinoid ligands. With the advent of synthetic cannabinoid molecules, significant strides have been made in understanding the anatomy and physiology of this receptor class. Much of this work has demonstrated that the endogenous cannabinoid system like other neuromodulatory systems is widely expressed and thus represents a novel and powerful means of targeting the underlying pathological properties of neural networks in patients suffering from mental health disorders[19–21].

The endogenous cannabinoid system is currently composed of cannabinoid receptor 1 (CB1) and cannabinoid receptor (CB2) with recent evidence heavily suggesting a possible third member currently designated G-protein receptor 55 (GPR55)[15,18,22]. These CB receptors are widely distributed throughout mammalian tissue and early immunohistochemistry studies have revealed CB1 receptors are the most abundant receptors in neural tissue, with evidence of neural CB2 only emerging late this

past decade[15,23]. Previously, CB2 had been considered the peripheral CB receptor since it is expressed at high concentrations within the spleen and is found on B cells, NK cells, and lymphocytes[23]. Neuroanatomical localization of the CB1 receptor has shown abundant protein expression in the neo-cortex, hippocampus, amygdala, striatum, and midbrain nuclei[15–20,24]. The CB1 receptor has been found primarily on presynaptic axonal terminals (Figure 2) with some expression observed on post synaptic cell bodies. All the CB receptor proteins are members of the super family of seven transmembrane loop G-protein coupled receptors (GPCR's) bound to an inhibitory internal enzymatic complex (Gi/o) that is released upon receptor activation[15,25]. Activation of CB1 initiates a signaling cascade inhibiting adenylyl cyclase and presynaptic N p/q type calcium channels, in addition to activating MAP kinase proteins and presynaptic inwardly rectifying potassium channels[15,25,26]. This signaling cascade results in cannabinoid mediated retrograde synaptic plasticity. This occurs through synthesis of cannabinoids in the post synaptic density and is dependent upon the arrival of presynaptic signals. These cannabinoids, produced after signal arrival, diffuse away from the synapse and act on the terminal itself, ultimately influencing its long-term vesicular output[15,25]. The effects of CB2 activation are very similar to that of CB1 but have not been entirely functionally characterized within brain tissue[23,27,28]. CB receptors have also been identified on astrocytes, with the demonstration that these receptors are functionally relevant on both neurons and glia[27]. This evidence regarding CB mediated neuron to glia communication speaks further to their relevance as a robust signaling system within the CNS.

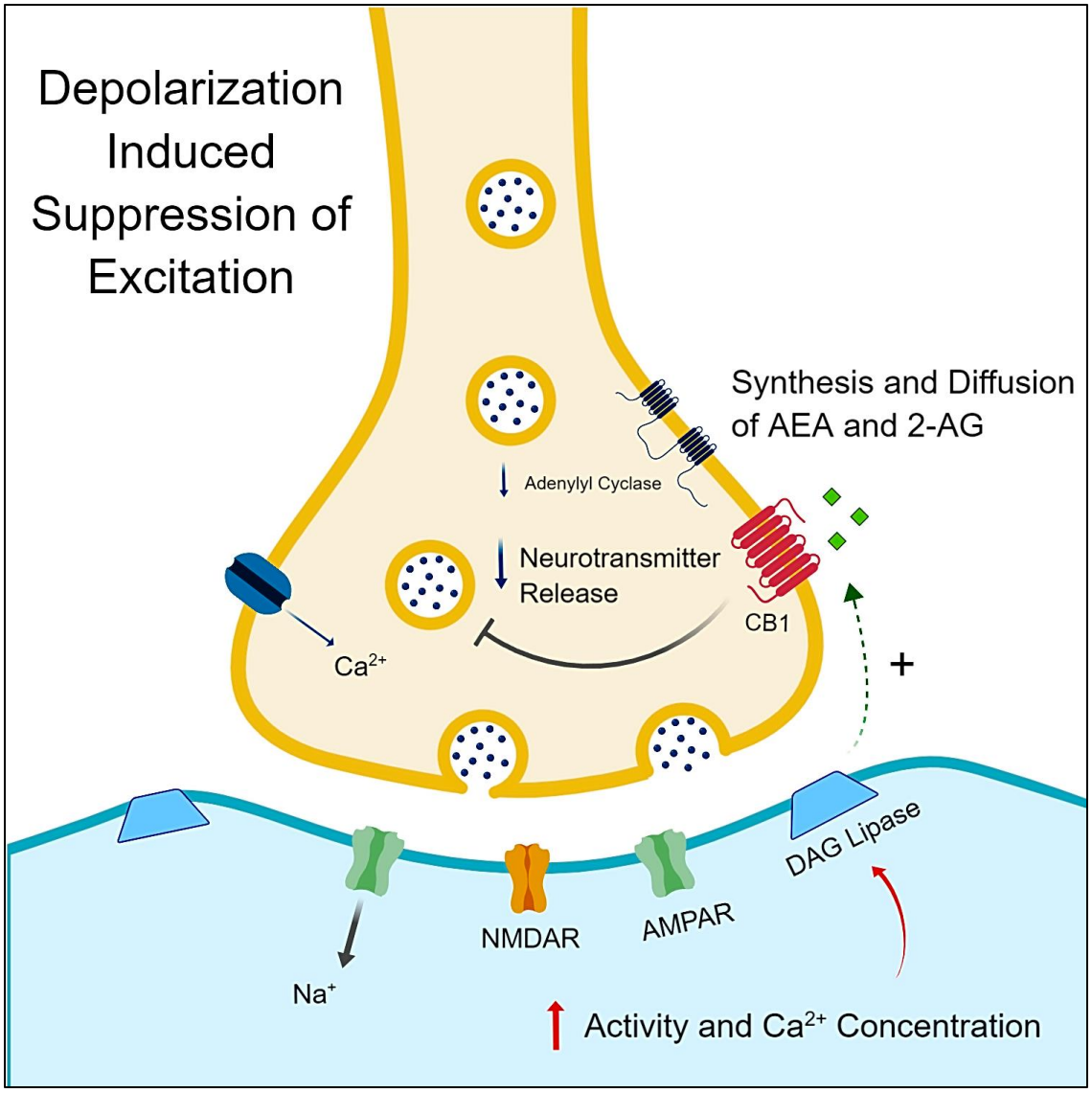

Figure 2 – ECS Modulation of Excitatory Synaptic Activity
Release of excitatory neurotransmitter glutamate from presynaptic terminal binds to post synaptic receptors causing depolarization of the membrane and induces the synthesis of both 2-AG and AEA, depending on the specific cellular population. On-demand synthesis of 2-AG and AEA leads to retrograde control of excitation through activating CB1R causing inhibition of adenylate cyclase via G_i and subsequent decreased neurotransmitter release from the presynaptic terminal, which is thought to aid in the prevention of excitoxicity during periods of sustained input activity. Similar effects can be observed at synaptic terminals containing other transmitters, such as GABA, which means cannabinoids serve as a means of controlling short term synaptic plasticity.

There are several endogenous molecules that serve as ligands at cannabinoid receptor binding sites, all of which possess a molecular motif making them lipid soluble. The solubility of these compounds allows them to serve as long-term messengers that can perpetuate retrograde signals, which persist in the membrane far after the passage of an action potential. This longevity is part of the therapeutic potential of these compounds, as their effects tend to have significant duration due to the lipophilic structure of these ligands[15,18]. This elongation of action creates a broader window of therapeutic action and reduces the chance of lethal overdose. Anandamide (AEA, Figure 3) is the most well documented endogenous cannabinoid and is locally synthesized from ethanolamine and arachidonic acid precursors in an on-demand fashion based on recent levels of cellular activity[8,15,18]. This molecule and its relative compounds are produced through hydrolysis of N-arachidonoyl phosphatidyl ethanolamine (NAPE) by phospholipase D. Synthesis is based on intracellular levels of calcium and cyclic AMP (cAMP). While basal anandamide concentrations are kept low, the endocannabinoid 2-arachidonoyl glycerol (2-AG) is found in much higher concentrations throughout the brain. The primary pathway for synthesis of 2-AG as shown in Figure 4, employs the hydrolysis of phosphatidylinositol by phospholipase C (PLC) and diacylglycerol lipase (DAG). Binding of these natural ligands is differential, with anandamide serving as a low efficacy agonist at both receptors whereas 2-AG has high levels of efficacy at both receptor sites. Along with their unique synthesis pathways, anandamide and 2-AG have separate degradation processes with anandamide being broken down by fatty acid amine hydrolases (FAAH) and 2-AG by monoacylglycerol lipase (MAG)[8,15,18,29–32].

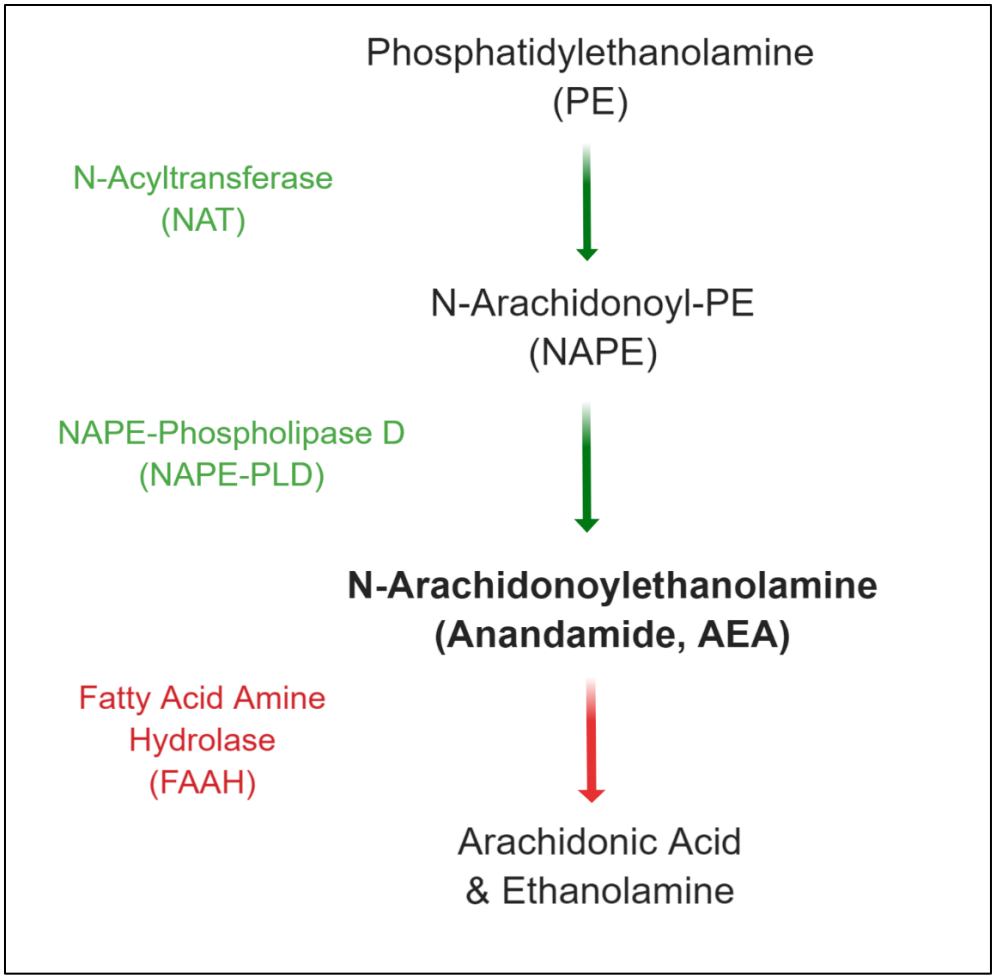

Figure 3 – Biosynthetic pathway of Anandamide
Enzymatic biosynthesis of the endogenous cannabinoid N-Arachidonoylethanolamine (Anandamide, AEA) from phosphatidylethanolamine precursor and subsequent hydrolysis by Fatty Acid Amine Hydrolase.

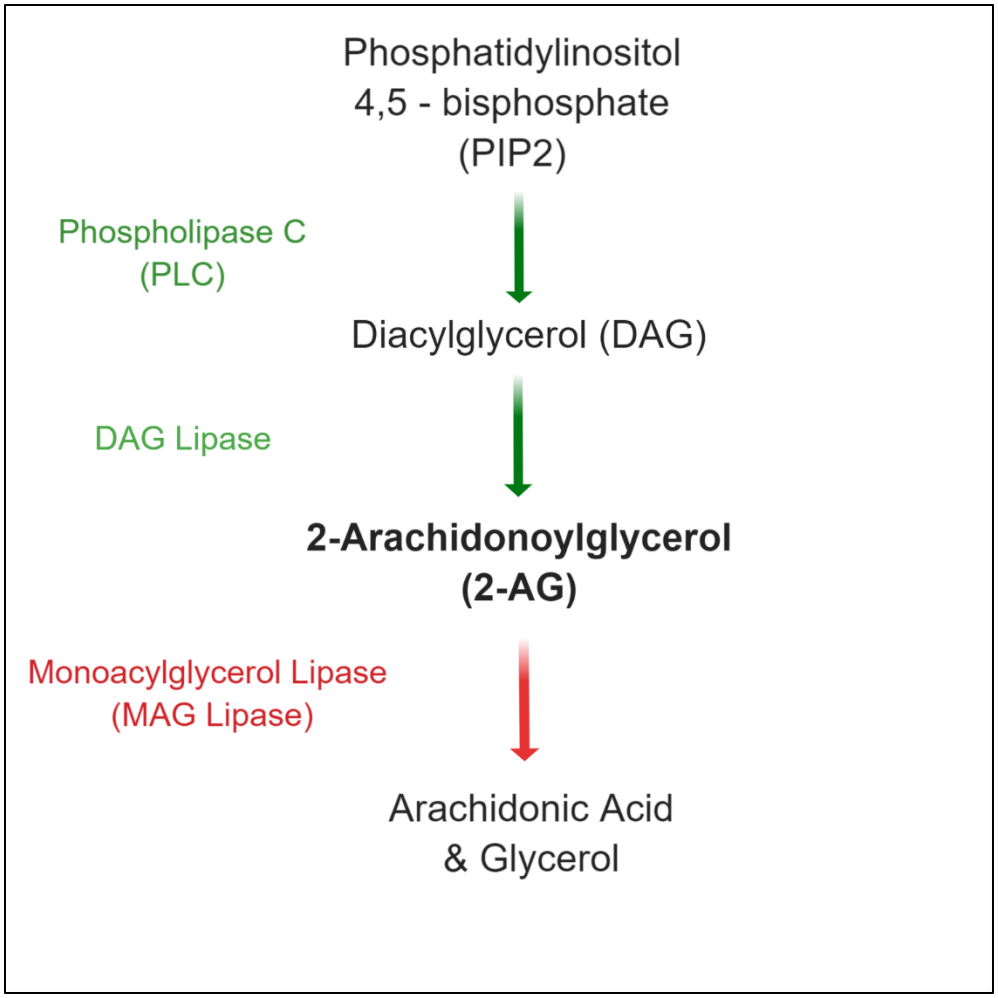

Figure 4 – Biosynthesis of 2-Arachidonoylglycerol
One of the pathways regulating the enzymatic biosynthesis of 2-arachidonoylglycerol (2-AG) from one of its phospholipid precursors, which is a process that is heavily dependent upon levels of intracellular calcium.

Manipulation of the ECS can be performed using direct agonists or antagonists of CB receptors and concentrations of endogenous ligands can be altered by pharmacologically interfering with enzymatic degradation pathways[12–15,33]. Phytocannabinoids that act at CB receptors include Δ^9-THC, Δ^8-THC, cannabinol, and cannabidiol with the former facilitating the primary psychoactive effects of acute

Cannabis ingestion and the latter maintaining little psychoactive capacity. These natural ligands are not known for their high selectivity and only with the advent of synthetic compounds has localization been clearly performed[6,8–11]. An example of this abundant cross selectivity is the transient receptor potential vanilloid 1 receptors (TRPV1), which is activated by many of the same molecules that trigger CB receptor activation[34,35]. This indicates the presence of a highly complex lipid signaling system that is dependent upon the local composition of lipids in the cellular membrane, along with dependence on the local receptor expression profile. Together, these two variables interact to produce an averaged CB signal that can have dramatic effects on synaptic responses to incoming depolarization. As a result, high endocannabinoid densities can be found at synapses after periods of sustained cellular firing[36]. These lipids in the cellular membrane act on CB1 receptors and produce a physical constant that regulates potassium and calcium currents through the synapse[15,25,32,37–39]. This inhibitory regulation of synaptic current explains the effects of strong CB agonists where application can result in reduced motor coordination, mood elevation, memory storage deficits, and increased appetite[15,39–42]. With this effect on appetite in mind, blockade of the CB1 receptor has been investigated as a method for treating obesity. Models in mice have shown lean body phenotype is associated with high levels of endogenous cannabinoids. Also, weight loss among obese rats with administration of CB1 antagonists has been observed[43]. The CB1 antagonist SR141716A (Rimonabant) was developed for this purpose in humans and was briefly released into some market's where patients in clinical trials and subsequently following release of the drug experienced increases in anxiety, depression, and suicidal ideation, likely due to the distribution of CB1 receptors in the basal ganglia (Table 2). After

considering the effects of this antagonist, it was removed from the market even though the drug was effective at achieving reductions in weight[44].

The ECS provides an optimal target for therapeutic intervention regarding mental health disorders. The ubiquitous expression of CB proteins includes many of the key systems targeted by existing therapies, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs)[6,13,15–18,45–55]. This is illustrated by the fact that Δ^9-THC, when applied alone and in moderate doses, produces an antidepressant effect on human subjects [41,56]. Synthetic encapsulated Δ^9-THC is available as a schedule 3 drug in the United States known as Dronabinol for the treatment of nausea, vomiting and other symptoms associated with chemotherapy treatment[57]. The recent discovery of neural CB2 receptors suggests this family of receptors is still largely unexplored in the brain and further inquiry should be conducted to identify possible new therapeutic targets[27,28,58]. Neurophysiological characterization of other receptor systems has demonstrated how differences in receptor density and localization provide key insight into how that specific neurotransmitter system participates in the general process of cognition. Further exploratory studies investigating the interactions between CB1 and CB2 are required to elucidate how this system functions at a molecular and cellular level.

CHAPTER 2

MENTAL HEALTH DISORDERS AND THE ENDOGENOUS CANNABINOID SYSTEM

Affective Disorders

One of the most prolific classifications of mental health disorders are those that disrupt processing of emotional stimuli, known as affective or mood disorders[1,2]. Within this classification, we find smaller groups with distinct symptoms including bi-polar disorder (BP), major depressive disorder (MDD), dysthymic disorder, and cyclothymic disorder. Approximately 9.5% of American adults aged 18-44 have experienced an affective disorder from one of these categories in the past 12 months; BP and MDD comprise most cases and will be the focus of this discussion[1]. The neuroanatomical substrates underlying the expression of affective disorders have been the subject of great debate within the mental health community. There are large bodies of evidence pointing toward improper of storage and excessive focus on negatively biased memories as a key pathological feature observed in depression[1,2,59–63]. Patients suffering from affective disorders often have decreased neuronal activity in the dorsal frontal cortex as well as dysfunctional activity with cell loss observed in the hippocampus and amygdala[1,60,64]. To counter pathological activity in these brain regions, modern antidepressant drugs have been designed to target specific proteins involved in the uptake of serotonin and norepinephrine[1–5]. This eventually leads to an increase in the basal levels of these neurotransmitters found in the synapses of neurons across the brain. Patients experience elevated dopamine release due to these alterations in basal serotonin and norepinephrine

levels and generally experience elevated mood as a result. Although these pharmacological interventions are effective, especially when coupled with behavioral treatments, there remains a large population that does not respond to conventional treatment alone[1,2,4,5]. This treatment-resistant group could benefit from altering the basal norepinephrine and serotonin levels using a different intermediary than conventional drugs. Manipulation of the ECS could serve as a means for providing this therapeutic modulation of the neural substrates underlying the disease pathology.

Major Depressive Disorder (MDD)

Diagnosis of MDD generally occurs after the administration of a variety of tests from the DSM-V to determine whether there is a disruption in the processing of emotional cues. Tests are routinely performed by a psychologist or psychiatrist, with additional observation of symptoms such as fatigue, lowered mood, suicidal ideation, lack of appetite, and disruption of normal sleeping patterns. Broad fluctuations in symptomology with this disorder can lead to difficulties in diagnosis, as patients can sometimes be hesitant to initially consult a specialized physician concerning their condition[1]. This may be observed by the increasing number of patients diagnosed with MDD by their family or general practitioner instead of a mental health professional. Conventional treatments for MDD modulate serotonin and norepinephrine, increasing dopaminergic tone and generally resulting in relief of depressive symptoms[1,2].

Bipolar Disorder 1 and 2

Patients suffering from bipolar disorder, formerly referred to as manic-depression, experience symptoms characterized by cyclic fluctuations between periods of manic happiness and deep depression[61,62]. About 2.6% of American adults have been diagnosed or were bipolar within the past twelve months[1]. The large changes in mood are often disruptive to relationships and can interfere with a patient's ability to maintain steady work. The distinction between the two forms of the disorder is primarily determined by examining the extent of the manic episodes, as bipolar 2 patients generally do not manifest with as potent manic symptoms. Those suffering from bipolar 2 disorder can often be confused with a person suffering intermittent depression and diagnosis must be performed with respect to long term symptomology. Lithium is one of the most common mood stabilizers and has gained popularity in treating bipolar disorder even though it has a very small therapeutic window[1,2,61,62].

The ECS and Affective Disorders

Investigating the ECS could enhance our ability to treat mental health disorders in the future by providing alternative molecular routes for modulating serotonin and norepinephrine. For example, CB1 receptors have been identified in the raphe nucleus of the midbrain, which is a major nucleus containing noradrenergic cell groups[53,54]. These noradrenergic cells project axons throughout the brain to provide modulation of glutamate and GABA at specific sites including cells of the amygdala, striatum, and forebrain. In models of depression, there are deficits in noradrenergic signaling that

ultimately result in decreased dopaminergic tone and can result in negatively biased mood[60]. In addition, there are abundant CB1 receptors expressed in the amygdala, a major neural substrate of emotional processing, as well as the hippocampus, which participates in memory storage and retrieval[20,21,41,55,64–69]. This indicates that CB receptors are likely involved in the emotional coding of memories and could potentially present a method for modulating aberrant ideation on negative emotions. To this effect, mice overexpressing CB2 receptors have a phenotype that leaves them less vulnerable to negative stressors [47]. In contrast, human studies have shown that individuals with certain variations of the CB1 gene have a higher potential for being resistant to pharmacological treatment for depression and therefore could be more at risk if depression occurs[69]. Taken together, these lines of evidence suggest that the ECS is involved in the processing of emotional memories and could potentially provide a novel means of countering the deleterious behavior associated with mental illness[70].

In addition to its action with pharmacological plasticity, the CB1 receptor has recently been implicated in the neurogenesis of inhibitory neurons within the hippocampus[40,46,53]. This finding is significant because perturbations in memory formation are observed in affective disorders along with deficits in hippocampal cell growth. Conventional therapies that targets serotonergic systems have also been shown to induce neurogenesis, which has led to the development of the neurogenesis hypothesis regarding depression[71–73]. This extends the role of cannabinoids in memory beyond molecular plasticity to a new level that includes developmental plasticity in response to environmental interactions. This evidence suggests that the ECS could be involved in regulating emotional memory and this may explain why we see antidepressant effects with THC administration.

Schizophrenia and Psychotic Disorders

Approximately 1.1% of the US adult population currently suffers from a psychotic disorder of some kind, which is relatively low when compared with other mental health disorders[1,74]. The chronic, severe nature of the symptoms associated with this disorder makes it particularly challenging to treat and can have drastic effects on a patient's long-term health. The manifestation of psychosis varies based on the individual, but generally produces behavioral, emotional, and cognitive symptoms often characterized by somatosensory hallucinations, inabilities to execute planning, and erratic control of emotion[56,57,75,76]. Three major classes of symptoms referred to as positive, negative and cognitive have been developed to describe the range of behaviors observed in patients diagnosed with schizophrenia. The positive symptoms reported by patients include hallucinations and delusions that can affect all motor and sensory systems with varying levels of intensity. Negative symptoms incorporate those that have to do with disruption of the patient's emotional state, like that observed within affective disorders which can sometimes complicate the diagnosis of schizophrenia as it is confused with other less severe disorder initially[58,67,77–79]. Lastly, the cognitive symptoms of schizophrenia can be subtle, with slight perturbations to higher order executive functions that can often only be detected with the administration of highly specialized tests by a mental health professional. Among these categories, there is a large degree of variation in expression of the disorder that is likely due to subtle variations in underlying pathological features[1,80,81]. In the face of this variation, some progress has been made in recognizing common elements between individuals with evidence pointing toward

deficits in limbic dopamine signaling and frontal cortical hypo-activity contributing to the disorder[7,31,39,82].

The ECS and Schizophrenia

The involvement of the ECS in the emergence of schizophrenia has been highly debated and there is little controlled research involving human subjects to adequately infer therapeutic relationships. There is evidence to suggest that *Cannabis* use can improve cognitive function in certain patients with schizophrenia[83–85]. This concept is contrary to much of the evidence in the schizophrenia research community, where many report *Cannabis* as a potential factor that can precipitate the emergence of psychosis[58,68,74,78]. While conflicting results exist, some studies have shown that *Cannabis* abuse can exacerbate psychotic symptoms in patients already predisposed to schizophrenia. Many of these studies rely upon correlations drawn from interviews of clinical populations suffering from schizophrenia that have either recently used *Cannabis* or have a history of past recreational use[7,83,86–89]. The inherently broad array of symptoms experienced by schizophrenic patients makes designing studies to investigate this population more difficult than studies involving other mental health disorders. These studies are often subject to confounding factors in the form of selection bias and recall bias, which means they need to be carefully evaluated before conclusions can extrapolated to the wider human population. Several studies using post-mortem human brain tissue demonstrate alterations in CB receptor localization and density, though with conflicting outcomes with some demonstrating increased density of CB1 and others

demonstrating no significant difference or reduced CB1 expression compared to control samples. Since there are few adequate mouse models that can fully replicate the symptoms of schizophrenia, our progression towards understanding the underlying neural physiology of schizophrenia has been slower than for other mental health disorders[90]. The few models that do exist in rodents have shown that agonism of the ECS can reduce the severity of the negative symptoms of schizophrenia but has little effect on the positive symptoms of the disorder[91,92] . Black et. al. suggests that antagonism of CB1 with co-administration of anti-psychotics improves cognitive function and reduces the side-effect profiles of anti-psychotics. An example of this is observed in schizophrenia patients who use *Cannabis* and also display higher levels of success in measures of psychomotor speed when compared with those who do not use *Cannabis*[64,83,93]. As discussed, prior evidence suggests the pathophysiology of schizophrenia may be linked to deficits in limbic dopamine signaling. Thus far, CB receptors 1 and 2 have been reported in high density in limbic and para-limbic areas and are involved in dopamine signaling, suggesting a possible link between dysfunction of the ECS and schizophrenia and highlighting the need for further investigation into this system for possible therapeutic manipulation.

Anxiety/Fear Disorder

Anxiety is a psychophysiological state characterized by somatic, emotional, cognitive, and behavioral components, which affects roughly 40 million Americans above 18 years of age[1,2,70,93]. In a clinical setting, humans are diagnosed with anxiety disorders through the professional administration of tests from the

Diagnostic and Statistical Manual of Mental Disorders (DSM-V)[70]. Based upon certain criteria, clinicians can separate the observed behaviors into a variety of subtypes of anxiety including Generalized Anxiety Disorder (GAD), Panic Disorder, Post Traumatic Stress Disorder (PTSD), Obsessive Compulsive Disorder (OCD), or a Phobia of some kind. While anxiety, is to some extent relevant for survival of an organism in the face of natural stressors, its persistence beyond situational arousal is generally considered pathological[94].

The etiology of anxiety disorders is unclear. It is known changes occur to the primary modulators of synaptic transmission including shifts in serotonin, nor-epinephrine, and dopamine release[72,80,95,96]. These same neuromodulators are associated with depression and many hypothesize these disorders are intertwined, with treatments for both disorders overlapping heavily[67,80]. For example, studies of mood in humans and rodents have shown severe or long-lasting stress can change the anatomical distribution of neuromodulators in the brain and therefore affect behavior. Other investigations have shown individuals with certain anxiety disorders possess variations in neuroanatomical structures identified to play a crucial role in controlling memory and mood[76,82,97]. Moreover, environmental factors, such as trauma or a major life-altering event, could trigger the emergence of anxious behavior in those who have an inherited susceptibility[98]. Treatment for anxiety usually consists of combined behavioral therapy with pharmacological manipulation using classical drugs including benzodiazepines, buspirone, selective serotonin or nor-epinephrine reuptake inhibitors, and older tricyclic antidepressants[70,80]. These options have had limited success as their efficacy is highly patient dependent and the side effects are often intolerable, leading to discontinuation of

therapy. This suggests pharmacotherapies with better tolerability and broader efficacy must be developed, which is where the ECS is poised to play a crucial role[7,84,85,99].

The ECS and Anxiety

Cannabinoid receptors have been identified in many regions that are associated with anxiety, such as the amygdala, hippocampus, ventromedial forebrain, and periaqueductal grey[86–88]. However, interpretation of this localization and its effects on anxiety in rodents has been difficult. Many studies of the effects of cannabinoids on anxiety in mammals show varied outcomes, which can often be attributed to differences in dose, drug, or experimental design. There have been major attempts to parallel classifications of behavior in humans to models of anxiety in rodents with limited success based upon the paradigm and behavior of interest. Measures of time spent in open versus enclosed environments, such as the elevated plus maze, have been used to great effect in the study of anxiety in rodents[84]. This method is based on the logic that rodents have evolved in an environment where enclosed spaces are relatively safe and anxiety free, and the open spaces represent the opposite. Therefore, when an animal is placed in such a maze, the time spent out in the open and time spent in enclosed spaces can be reported as a ratio representing the anxiety level of the animal. Even though great attempts to standardize these methods have been made, interpretation of rodent behavior is still somewhat subjective. Clearly this situation requires remediation and further study should

be dedicated to focusing the lens of experimentation on standardized models of anxiety in rodents.

There are several means of altering tone within the ECS, including direct agonism, direct antagonism, and blocking enzymatic pathways of cannabinoid degradation to increase endogenous levels. Studies using direct agonists of the ECS including Delta -9- THC, WIN55-212,2, anandamide, and others have shown that CB1 is responsible for the primary psychoactive effects associated with *Cannabis* ingestion. The nature of the behavioral effects of these drugs on anxiety is biphasic, with low doses producing anxiolytic effects and anxiogenic effects emerging when high doses are administered[89,89,90,100]. In a mouse model of anxiety, the behavioral traits associated with anxiety can be ameliorated by injection of CB1 agonist into the dorsal hippocampus. Additionally, blockade of CB1 results in an anxiogenic response when CB1 antagonist AM251 is directly injected into the core of the amygdala or when it is administered systemically. The synthetic compound URB597 is an inhibitor of FAAH and when administered with anandamide can produce anxiety, but if either is given alone, they produce anxiolytic effects[89]. Since CB1 is psychoactive, its use to treat anxiety is clouded due to its side effects, such as euphoria during the initial stages of blood plasma saturation. To this end, CB2 has been investigated as a potential anxiolytic target since it does not possess the psychoactive properties associated with CB1 activation. Studies of CB2 and behavior have shown rodents with higher CB2 expression are resistant to anxiety and have differential responses to benzodiazepines, likely due to changes in GABAergic tone. Studies of this receptor and its relationship to anxious behavior are still limited and must be further explored before any strong conclusions can be drawn.

Since there is evidence the ECS interacts with many different modulatory systems, including current anxiety treatments, exploration of this receptor family could open a new taxonomy of lipid-like drugs[92].

CHAPTER 3

FUTURE INSIGHTS FOR THERAPEUTIC DEVELOPMENT

The ubiquitous expression of CB receptors on cells in the brain presents a wide array of possibilities for altering neuronal activity and potentially treating pathological activity[8,24,54,73]. Given the complexity of the ECS, further studies must be conducted to establish a baseline for comparison to models of disease. Genetic knock-out mice have been one of the most supportive tools in recent efforts to understand the cannabinoid system as they lack CB receptor expression. Behavioral assessment of the mice under application of various cannabinoids will allow the assessment of specific pharmacological activity for various ligands. These results can then be translated into human trials used to formally test the potential of the compound. Currently, no clinical trials can be performed using herbal derivatives of *Cannabis* even though the material likely contains potent modulators of psychological state. Future research aimed at isolating specific herbal ligands for behavioral and pharmacological evaluation in animal models is the next step. In addition, combinations of cannabinoids should be studied together due to the moderate selectivity of many ligands. It is likely that what is observed with *Cannabis* is due to the mixture of cannabinoids found within the plant itself and not due to specific ligands such a THC alone[101]. The phytocannabinoids within *Cannabis* have already undergone evolutionary development through coexistence with mammals and are likely an important reservoir of information for the understanding and modulation of our endogenous neurological systems.

The lipophilic nature of cannabinoid ligands confers them an inherent advantage over other molecules because they can traverse the blood brain barrier (BBB) and are incorporated into the cell membrane. Embedded within and free to move along the plane of the plasma membrane, cannabinoid ligands probabilistically interact with CB receptors. The probability of CB receptor activation scales with the density of ligands in the membrane is a complex value due to the partial activity of many molecules at these receptors[42]. This suggests that drugs designed to target cannabinoid receptors will have a longer half-life and have a high therapeutic index as the hydrophobic nature of the molecule reduces the chances of overdose. Properties such as these represent key advantages of cannabinoids that could be beneficial in scenarios where current treatments carry risks of abuse and non-compliance, such as in bipolar disorder or schizophrenia.

The emergence of incredibly powerful genetic and molecular tools, including fluorescent probes, has provided the tools to study the very foundations of mental health in our own cells. To truly understand the physiological mechanisms of the ECS, we must look beyond cultural stigmas to envision new medical frontiers wherever they may present. From the modest research conducted thus far, it is apparent the ECS plays a crucial role in normal neurophysiology and in many functions that we consider core elements of our human existence, such as memory and emotion. It is also probable that the ECS is involved in mediating symptoms of mental health disorders, and dysfunction of the ECS may even be involved in the pathogenesis of mental health disorders. With current research so focused on determining the underlying mechanisms and potential therapeutic opportunities of mental health disorders, it is enormously short-sighted to ignore such an integral and robust signaling system. Investigating the ECS, especially in

conjunction with other neural signaling systems will likely provide a more comprehensive understanding of normal neurophysiology and pathophysiology, as well as present novel techniques for modulating the symptoms of mental health disorders.

REFERENCES

1. Kessler R. Prevalence, Severity, and Comorbidity of 12-Month DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*. 2005;62(6):617.

2. Belmaker RH, Agam G. Major depressive disorder. *The New England journal of medicine*. 2008;358(1):55-68. doi:10.1056/NEJMra073096

3. Bschor T, Adli M. Treatment of depressive disorders. *Deutsches Ärzteblatt international*. 2008;105(45):782-792. doi:10.3238/arztebl.2008.0782

4. Inoue T, Sasaki K, Boku S, et al. Sertraline treatment of patients with major depressive disorder who failed initial treatment with paroxetine or fluvoxamine. *Progress in neuro-psychopharmacology & biological psychiatry*. 2012;38(2):223. doi:10.1016/j.pnpbp.2012.04.001

5. Leucht C, Huhn M, Leucht S. Amitriptyline versus placebo for major depressive disorder. *Cochrane database of systematic reviews (Online)*. 2012;12:CD009138.

6. Soyka M. Cannabinoids and mental health. *Schmerz (Berlin, Germany)*. 2003;17(4):268-273. doi:10.1007/s00482-003-0226-x

7. Busquets-Garcia A, Puighermanal E, Pastor A, Torre R de la, Maldonado R, Ozaita A. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. *Biological psychiatry*. 2011;70(5):479-486. doi:10.1016/j.biopsych.2011.04.022

8. Straiker A, Hu SS-J, Long JZ, et al. Monoacylglycerol Lipase Limits the Duration of Endocannabinoid-Mediated Depolarization-Induced Suppression of Excitation in Autaptic Hippocampal Neurons. *Molecular pharmacology*. 2009;76(6):1220. doi:10.1124/mol.109.059030

9. Atwood BK, Lee D, Straiker A, Widlanski TS, Mackie K. CP47,497-C8 and JWH073, commonly found in “Spice” herbal blends, are potent and efficacious CB(1) cannabinoid receptor agonists. *European journal of pharmacology*. 2011;659(2-3):139.

10. Ross RA. Anandamide and vanilloid TRPV1 receptors. *British journal of pharmacology*. 2003;140(5):790-801. doi:10.1038/sj.bjp.0705467

11. Adamczyk P, Miszkiel J, McCreary AC, Filip M, Papp M, Przegaliński E. The effects of cannabinoid CB1, CB2 and vanilloid TRPV1 receptor antagonists on cocaine addictive behavior in rats. *Brain research*. 2012;1444(0):45-54. doi:10.1016/j.brainres.2012.01.030

12. Morgan NH, Stanford IM, Woodhall GL. Functional CB2 type cannabinoid receptors at CNS synapses. *Neuropharmacology*. 2009;57(4):356-368. doi:10.1016/j.neuropharm.2009.07.017

13. Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Progress in lipid research*. 2011;50(2):193-211. doi:10.1016/j.plipres.2011.01.001

14. Straiker A, Mackie K. Cannabinoids, electrophysiology, and retrograde messengers: challenges for the next 5 years. *The AAPS journal*. 2006;8(2):E272-E276. doi:10.1007/BF02854897

15. Hampson RE, Miller F, Palchik G, Deadwyler SA. Cannabinoid receptor activation modifies NMDA receptor mediated release of intracellular calcium: Implications for endocannabinoid control of hippocampal neural plasticity. *Neuropharmacology*. 2011;60(6):944-952. doi:10.1016/j.neuropharm.2011.01.039

16. Walsh S, Mnich K, Mackie K, Gorman AM, Finn DP, Dowd E. Loss of cannabinoid CB.sub.1 receptor expression in the 6-hydroxydopamine-induced nigrostriatal terminal lesion model of Parkinson's disease in the rat. *Brain research bulletin*. 2010;81(6):543. doi:10.1016/j.brainresbull.2010.01.009

17. El-Alfy AT, Ivey K, Robinson K, et al. Antidepressant-like effect of Δ 9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. *Pharmacology, Biochemistry and Behavior*. 2010;95(4):434-442. doi:10.1016/j.pbb.2010.03.004

18. Mackie K. Understanding cannabinoid psychoactivity with mouse genetic models. *PLoS biology*. 2007;5(10):e280. doi:10.1371/journal.pbio.0050280

19. Trillou CR, Delgorge C, Menet C, Arnone M, Soubrié P. CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *International journal of obesity*. 2004;28(4):640-648. doi:10.1038/sj.ijo.0802583

20. Kelly DL, McMahon RP, Huestis MA, et al. Effects of the cannabinoid-1 receptor antagonist rimonabant on psychiatric symptoms in overweight people with schizophrenia: a randomized, double-blind, pilot study. *Journal of clinical psychopharmacology*. 2011;31(1):86-91. doi:10.1097/JCP.0b013e318204825b

21. Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Molecular pharmacology*. 2005;67(5):1697-1704. doi:10.1124/mol.104.006882

22. Lau T, Schloss P. The cannabinoid CB 1 receptor is expressed on serotonergic and dopaminergic neurons. *European journal of pharmacology*. 2008;578(2):137-141. doi:10.1016/j.ejphar.2007.09.022

23. Kleijn J, Wiskerke J, Cremers TIFH, Schoffelmeer ANM, Westerink BHC, Pattij T. Effects of amphetamine on dopamine release in the rat nucleus accumbens shell region depend on cannabinoid CB1 receptor activation. *Neurochemistry international*. 2012;60(8):791. doi:10.1016/j.neuint.2012.03.002

24. Häring M, Marsicano G, Lutz B, Monory K. Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. *Neuroscience*. 2007;146(3):1212-1219. doi:10.1016/j.neuroscience.2007.02.021

25. Schulze DR, Carroll FI, McMahon LR. Interactions between dopamine transporter and cannabinoid receptor ligands in rhesus monkeys. *Psychopharmacology*. 2012;222(3):425-438. doi:10.1007/s00213-012-2661-9

26. Lane DA, Chan J, Fitzgerald ML, Kearn CS, Mackie K, Pickel VM. Quinpirole elicits differential in vivo changes in the pre- and postsynaptic distributions of dopamine D^sub 2^ receptors in mouse striatum: relation to cannabinoid-1 (CB^sub 1^) receptor targeting. *Psychopharmacology*. 2012;221(1):101. doi:10.1007/s00213-011-2553-4

27. Lupica CR, Riegel AC. Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. *Neuropharmacology*. 2005;48(8):1105-1116. doi:10.1016/j.neuropharm.2005.03.016

28. Ferreira SG, Teixeira FM, Garção P, et al. Presynaptic CB(1) cannabinoid receptors control frontocortical serotonin and glutamate release–species differences. *Neurochemistry international*. 2012;61(2):219.

29. Oropeza VC, Mackie K, Bockstaele EJV. Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. *Brain research*. 2007;1127(1):36-44. doi:10.1016/j.brainres.2006.09.110

30. Carvalho AF, Bockstaele EJV. Cannabinoid modulation of noradrenergic circuits: Implications for psychiatric disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2012;38(1):59-67. doi:10.1016/j.pnpbp.2012.01.008

31. Laviolette SR, Grace AA. The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. *Cellular and Molecular Life Sciences*. 2006;63(14):1597-1613. doi:10.1007/s00018-006-6027-5

32. Shearman LP, Rosko KM, Fleischer R, et al. Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. *Behavioural pharmacology*. 2003;14(8):573-582. doi:10.1097/01.fbp.0000104880.69384.38

33. Radbruch L, Nauck F. Review of cannabinoids in the treatment of nausea and vomiting. *Schmerz (Berlin, Germany)*. 2004;18(4):306.

34. Atwood BK, Straiker A, Mackie K. CB_2: therapeutic target-in-waiting. *Progress in neuro-psychopharmacology & biological psychiatry*. 2012;38(1):16.

35. Hamilton JP, Gotlib IH. Neural Substrates of Increased Memory Sensitivity for Negative Stimuli in Major Depression. *Biological psychiatry*. 2008;63(12):1155-1162. doi:10.1016/j.biopsych.2007.12.015

36. Yu T, Guo M, Garza J, et al. Cognitive and neural correlates of depression-like behaviour in socially defeated mice: an animal model of depression with cognitive dysfunction. *The International Journal of Neuropsychopharmacology*. 2011;14(03):303. doi:10.1017/S1461145710000945

37. Mantere O, Suominen K, Leppämäki S, Valtonen H, Arvilommi P, Isometsä E. The clinical characteristics of DSM-IV bipolar I and II disorders: baseline findings from the Jorvi Bipolar Study (JoBS). *Bipolar disorders*. 2004;6(5):395-405. doi:10.1111/j.1399-5618.2004.00140.x

38. Miller K. Bipolar disorder: etiology, diagnosis, and management. *Journal of the American Academy of Nurse Practitioners*. 2006;18(8):368-373. doi:10.1111/j.1745-7599.2006.00148.x

39. Cousins DA, Butts K, Young AH. The role of dopamine in bipolar disorder. *Bipolar disorders*. 2009;11(8):787-806. doi:10.1111/j.1399-5618.2009.00760.x

40. Samuels BA, Hen R. Neurogenesis and affective disorders. *European Journal of Neuroscience*. 2011;33(6):1152-1159. doi:10.1111/j.1460-9568.2011.07614.x

41. Marco EM, Laviola G. The endocannabinoid system in the regulation of emotions throughout lifespan: a discussion on therapeutic perspectives. *J Psychopharmacol (Oxford)*. 2012;26(1):150-163. doi:10.1177/0269881111408459

42. Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology*. 2002;159(4):379-387. doi:10.1007/s00213-001-0946-5

43. McLaughlin RJ, Gobbi G. Cannabinoids and emotionality: a neuroanatomical perspective. *Neuroscience*. 2012;204:134-144. doi:10.1016/j.neuroscience.2011.07.052

44. Katona I, Rancz EA, Acsady L, et al. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2001;21(23):9506-9518.

45. Domschke K, Arolt V, Deckert J, et al. Cannabinoid receptor 1 (CNR1) gene: impact on antidepressant treatment response and emotion processing in major depression. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*. 2008;18(10):751-759. doi:10.1016/j.euroneuro.2008.05.003

46. Jiang W, Zhang Y, Xiao L, et al. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *The Journal of clinical investigation*. 2005;115(11):3104-3116. doi:10.1172/JCI25509

47. Garcia-Gutierrez MS, Perez-Ortiz JM, Gutierrez-Adan A, Manzanares J. Depression-resistant endophenotype in mice overexpressing cannabinoid CB2 receptors. *British journal of pharmacology*. 2010;160(7):1773-1773. doi:10.1111/j.1476-5381.2010.00819.x

48. Serra G, Fratta W. A possible role for the endocannabinoid system in the neurobiology of depression. *Clinical practice and epidemiology in mental health : CP & EMH*. 2007;3(1):25-25. doi:10.1186/1745-0179-3-25

49. Katona I, Sperlágh B, Maglóczky Z, et al. GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience*. 2000;100(4):797-804. doi:10.1016/S0306-4522(00)00286-4

50. Katona I, Urbán GM, Wallace M, et al. Molecular composition of the endocannabinoid system at glutamatergic synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006;26(21):5628-5637. doi:10.1523/JNEUROSCI.0309-06.2006

51. Katona I, Sperlagh B, Sik A, et al. Presynaptically Located CB1 Cannabinoid Receptors Regulate GABA Release from Axon Terminals of Specific Hippocampal Interneurons. *Journal of Neuroscience*. 1999;19(11):4544.

52. Mackie K, Katona I. Get stoned in GABAergic synapses. *Nature neuroscience*. 2009;12(9):1081-1083. doi:10.1038/nn0909-1081

53. Wolf SA, Ullrich O, Kempermann G, et al. Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell communication and signaling : CCS*. 2010;8(1):12-12. doi:10.1186/1478-811X-8-12

54. Clinical: Clinical Review - Schizophrenia. *GP (London, England : 1994)*. 2010:49.

55. Cuesta MJ, Ugarte MD, Goicoa T, Eraso S, Peralta V. A taxometric analysis of schizophrenia symptoms. *Psychiatry research*. 2007;150(3):245-253. doi:10.1016/j.psychres.2006.01.019

56. Freedman R. Neuronal Dysfunction and Schizophrenia Symptoms. *The American Journal of Psychiatry*. 2007;164(3):385-390.

57. Kendall T. Treating negative symptoms of schizophrenia. *BMJ (Clinical research ed)*. 2012;344:e664. doi:10.1136/bmj.e664

58. Lodge DJ, Grace AA. Developmental pathology, dopamine, stress and schizophrenia. *International Journal of Developmental Neuroscience*. 2011;29(3):207-213. doi:10.1016/j.ijdevneu.2010.08.002

59. Schwarcz G, Karajgi B, McCarthy R. Synthetic delta-9-tetrahydrocannabinol (dronabinol) can improve the symptoms of schizophrenia. *Journal of clinical psychopharmacology*. 2009;29(3):255.

60. Zuardi AW, Crippa JAS, Hallak JEC, Moreira FA, Guimarães FS. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas médicas e biológicas / Sociedade Brasileira de Biofísica .[et al]*. 2006;39(4):421-429.

61. Metrik J, Kahler CW, McGeary JE, Monti PM, Rohsenow DJ. Acute Effects of Marijuana Smoking on Negative and Positive Affect. *Journal of Cognitive Psychotherapy*. 2011;25(1):31-46. doi:10.1891/0889-8391.25.1.31

62. Wilson N, Cadet JL. Comorbid mood, psychosis, and marijuana abuse disorders: a theoretical review. *Journal of addictive diseases*. 2009;28(4):309-319. doi:10.1080/10550880903182960

63. Bersani G, Orlandi V, Kotzalidis GD, Pancheri P. Cannabis and schizophrenia: impact on onset, course, psychopathology and outcomes. *European Archives of Psychiatry and Clinical Neurosciences*. 2002;252(2):86-92. doi:10.1007/s00406-002-0366-5

64. Coulston CM, Perdices M, Tennant CC. The neuropsychological correlates of cannabis use in schizophrenia: lifetime abuse/dependence, frequency of use, and recency of use. *Schizophrenia research*. 2007;96(1-3):169-184. doi:10.1016/j.schres.2007.08.006

65. Pierre JM. Psychosis Associated With Medical Marijuana: Risk vs. Benefits of Medicinal Cannabis Use. *American Journal of Psychiatry*. 2010;167(5):598-599. doi:10.1176/appi.ajp.201 0.09121762

66. Hambrecht M, Hafner H. Cannabis, vulnerability, and the onset of schizophrenia: an epidemiological perspective. *Australian and New Zealand Journal of Psychiatry*. 2000;34(3):468-468. doi:10.1046/j.1440-1614.2000.00736.x

67. Meyer U, Feldon J. Epidemiology-driven neurodevelopmental animal models of schizophrenia. *Progress in neurobiology*. 2010;90(3):285-326. doi:10.1016/j.pneurobio.2009.10.018

68. Spano MS, Fadda P, Frau R, Fattore L, Fratta W. Cannabinoid self-administration attenuates PCP-induced schizophrenia-like symptoms in adult rats. *European Neuropsychopharmacology*. 2010;20(1):25-36. doi:10.1016/j.euroneuro.2009.09.004

69. Black MD, Pichat P, Arad M, et al. AVE1625, a cannabinoid CB1 receptor antagonist, as a co-treatment with antipsychotics for schizophrenia: improvement in cognitive function and reduction of antipsychotic-side effects in rodents. *Psychopharmacology*. 2011;215(1):149-163. doi:10.1007/s00213-010-2124-0

70. Bandelow B, Sher L, Bunevicius R, et al. Guidelines for the pharmacological treatment of anxiety disorders, obsessive–compulsive disorder and posttraumatic stress disorder in primary care. *International Journal of Psychiatry in Clinical Practice*. 2012;16(2):77-84. doi:10.3109/13651501.2012.667114

71. Cannabinoid-induced enhanced interaction and protein levels of serotonin 5-HT2A and dopamine D2 receptors in rat prefrontal cortex. *Journal of Psychopharmacology*. 2012;26(10):1333-1347. doi:10.1177/0269881112450786

72. Christianson JP, Ragole T, Amat J, et al. 5-hydroxytryptamine 2C receptors in the basolateral amygdala are involved in the expression of anxiety after uncontrollable traumatic stress. *Biological psychiatry*. 2010;67(4):339-345. doi:10.1016/j.biopsych.2009.09.011

73. Coulston CM, Perdices M, Tennant CC. The neuropsychology of cannabis and other substance use in schizophrenia: review of the literature and critical evaluation of methodological issues. *The Australian and New Zealand Journal of Psychiatry*. 2007;41(11):869-884. doi:10.1080/00048670701634952

74. Miyamoto S, Miyake N, Jarskog LF, Fleischhacker WW, Lieberman JA. Pharmacological treatment of schizophrenia: a critical review of the pharmacology and clinical effects of current and future therapeutic agents. *Molecular Psychiatry*. 2012;17:1206.

75. Jockers-Scherübl MC, Rentzsch J, Danker-Hopfe H, et al. Adequate antipsychotic treatment normalizes serum nerve growth factor concentrations in schizophrenia with and without cannabis or additional substance abuse. *Neuroscience letters*. 2006;400(3):262-266. doi:10.1016/j.neulet.2006.02.056

76. Ashton CH, Moore PB. Endocannabinoid system dysfunction in mood and related disorders. *Acta Psychiatrica Scandinavica*. 2011;124(4):250-261. doi:10.1111/j.1600-0447.2011.01687.x

77. A G, F.M L, C.W G, et al. Cerebrospinal anandamie levels are elevatd in acute schizophrenia and are inversley correlated with psychotic symptoms. *Neuropsychopharmacology*. 2004;29:2108.

78. D'Souza DC, Sewell RA, Ranganathan M. Cannabis and psychosis/schizophrenia: human studies. *European archives of psychiatry and clinical neuroscience*. 2009;259(7):413-431. doi:10.1007/s00406-009-0024-2

79. Hamera E, Schneider JK, Deviney S. Alcohol, cannabis, nicotine, and caffeine use and symptom distress in schizophrenia. *The Journal of nervous and mental disease*. 1995;183(9):559.

80. Farach FJ, Pruitt LD, Jun JJ, Jerud AB, Zoellner LA, Roy-Byrne PP. Pharmacological treatment of anxiety disorders: Current treatments and future directions. *Journal of anxiety disorders*. 2012;26(8):833. doi:10.1016/j.janxdis.2012.07.009

81. Ashton CH. Adverse effects of cannabis and cannabinoids. *British journal of anaesthesia*. 1999;83(4):637.

82. Haller J, Varga B, Ledent C, Freund TF. CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. *Behavioural Pharmacology*. 2004;15(4):299. doi:10.1097/01.fbp.0000135704.56422.40

83. Witkin JM, Tzavara ET, Nomikos GG. A role for cannabinoid CB1 receptors in mood and anxiety disorders. *Behavioural pharmacology*. 2005;16(5-6):315-331. doi:10.1097/00008877-200509000-00005

84. Rutkowska M, Jamontt J, Gliniak H. Effects of cannabinoids on the anxiety-like response in mice. *Pharmacological reports : PR*. 2006;58(2):200.

85. Anxiolytic-like effect induced by the cannabinoid CB1 receptor agonist, arachydonilcyclopropylamide (ACPA), in the rat amygdala is mediated through the D1 and D2 dopaminergic systems. *Journal of Psychopharmacology*. 2011;25(1):131-140. doi:10.1177/0269881110376688

86. Onaivi ES. Neuropsychobiological Evidence for the Functional Presence and Expression of Cannabinoid CB2 Receptors in the Brain. *NPS*. 2006;54(4):231-246. doi:10.1159/000100778

87. Campos AC, Guimarães FS. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology*. 2008;199(2):223. doi:10.1007/s00213-008-1168-x

88. Rodgers RJ, Evans PM, Murphy A. Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-naïve and plus-maze-experienced mice. *Behavioural Pharmacology*. 2005;16(5-6):405.

89. Haller J, Barna I, Barsvari B, et al. Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. *Psychopharmacology*. 2009;204(4):607-616. doi:10.1007/s00213-009-1494-7

90. Zarrindast M-R, Sarahroodi S, Arzi A, Khodayar MJ, Taheri-Shalmani S, Rezayof A. Cannabinoid CB1 receptors of the rat central amygdala mediate anxiety-like behavior: interaction with the opioid system. *Behavioural pharmacology*. 2008;19(7):716-723. doi:10.1097/FBP.0b013e3283123c83

91. Moreira FA, Aguiar DC, Terzian ALB, Guimarães FS, Wotjak CT. Cannabinoid type 1 receptors and transient receptor potential vanilloid type 1 channels in fear and anxiety-two sides of one coin? *Neuroscience*. 2012;204:186-192. doi:10.1016/j.neuroscience.2011.08.046

92. Moreira FA. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract Res Clin Endocrinol Metab*. 2009;23(1):133.

93. Byers AL, Yaffe K, Covinsky KE, Friedman MB, Bruce ML. High Occurrence of Mood and Anxiety Disorders Among Older Adults: The National Comorbidity Survey Replication. *Arch Gen Psychiatry*. 2010;67(5):489-496. doi:10.1001/archgenpsychiatry.2010.35

94. Chorpita BF, Barlow DH. The Development of Anxiety: The Role of Control in the Early Environment. *Psychological bulletin*. 1998;124(1):3-21. doi:10.1037/0033-2909.124.1.3

95. Fadok JP, Argilli E, Garelick MG, et al. *Activation of Dopamine Neurons Is Critical for Aversive Conditioning and Prevention of Generalized Anxiety*. Vol 14.; 2011. doi:10.1038/nn.2808

96. Opponency Revisited: Competition and Cooperation Between Dopamine and Serotonin | Neuropsychopharmacology. https://www.nature.com/articles/npp2010151. Accessed March 21, 2019.

97. Crippa JA, Zuardi AW, Martín-Santos R, et al. Cannabis and anxiety: a critical review of the evidence. *Human Psychopharmacology: Clinical and Experimental*. 2009;24(7):515-523. doi:10.1002/hup.1048

98. Fong M. Assessment and diagnosis of DSM-IV anxiety disorders. *Journal of counseling and development*. 1999;77(2):209.

99. Witkin JM, Tzavara ET, Davis RJ, Li X, Nomikos GG. A therapeutic role for cannabinoid CB 1 receptor antagonists in major depressive disorders. *Trends in pharmacological sciences*. 2005;26(12):609-617. doi:10.1016/j.tips.2005.10.006

100. García-Gutiérrez MS, Manzanares J. The cannabinoid CB1 receptor is involved in the anxiolytic, sedative and amnesic actions of benzodiazepines. *Journal of psychopharmacology (Oxford, England)*. 2010;24(5):757-765. doi:10.1177/0269881109106910

101. Central nervous system effects of haloperidol on THC in healthy male volunteers. *Journal of Psychopharmacology*. 2010;24(11):1697-1708. doi:10.1177/0269881109358200

102. Cristino L, de Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience*. 2006;139(4):1405-1415. doi:10.1016/j.neuroscience.2006.02.074

103. Hebert-Chatelain E, Reguero L, Puente N, et al. Cannabinoid control of brain bioenergetics: Exploring the subcellular localization of the CB1 receptor. *Molecular Metabolism*. 2014;3(4):495-504. doi:10.1016/j.molmet.2014.03.007

104. Moldrich G, Wenger T. Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study☆. *Peptides*. 2000;21(11):1735-1742. doi:10.1016/S0196-9781(00)00324-7

105. Eggan SM, Lewis DA. Immunocytochemical Distribution of the Cannabinoid CB1 Receptor in the Primate Neocortex: A Regional and Laminar Analysis. *Cereb Cortex*. 2007;17(1):175-191. doi:10.1093/cercor/bhj136

106. Zarate J, Churruca I, Echevarría E, et al. Immunohistochemical localization of CB1 cannabinoid receptors in frontal cortex and related limbic areas in obese Zucker rats: Effects of chronic fluoxetine treatment. *Brain Research*. 2008;1236:57-72. doi:10.1016/j.brainres.2008.07.100

107. Ibarra-Lecue I, Pilar-Cuéllar F, Muguruza C, et al. The endocannabinoid system in mental disorders: Evidence from human brain studies. *Biochemical Pharmacology*. 2018;157:97-107. doi:10.1016/j.bcp.2018.07.009

108. Herkenham M, Lynn AB, Little MD, et al. Cannabinoid receptor localization in brain. *PNAS*. 1990;87(5):1932-1936.

109. Mátyás F, Urbán GM, Watanabe M, et al. Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. *Neuropharmacology*. 2008;54(1):95-107. doi:10.1016/j.neuropharm.2007.05.028

110. Gong J-P, Onaivi ES, Ishiguro H, et al. Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. *Brain research*. 2006;1071(1):10-23. doi:10.1016/j.brainres.2005.11.035

111. Lanciego JL, Barroso-Chinea P, Rico AJ, et al. Expression of the mRNA coding the cannabinoid receptor 2 in the pallidal complex of *Macaca fascicularis*. *Journal of Psychopharmacology*. 2011;25(1):97-104. doi:10.1177/0269881110367732

112. Brusco A, Tagliaferro P, Saez T, Onaivi ES. Postsynaptic localization of CB2 cannabinoid receptors in the rat hippocampus. *Synapse*. 2008;62(12):944-949. doi:10.1002/syn.20569

113. Suárez J, Llorente R, Romero-Zerbo SY, et al. Early maternal deprivation induces gender-dependent changes on the expression of hippocampal CB1 and CB2 cannabinoid receptors of neonatal rats. *Hippocampus*. 2009;19(7):623-632. doi:10.1002/hipo.20537

114. Suárez J, Romero-Zerbo SY, Rivera P, et al. Endocannabinoid system in the adult rat circumventricular areas: An immunohistochemical study. *Journal of Comparative Neurology*. 2010;518(15):3065-3085. doi:10.1002/cne.22382

115. Zhang H-Y, Gao M, Liu Q-R, et al. Cannabinoid CB_2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. *Proceedings of the National Academy of Sciences*. 2014;111(46):E5007-E5015. doi:10.1073/pnas.1413210111

116. Ashton JC, Friberg D, Darlington CL, Smith PF. Expression of the cannabinoid CB2 receptor in the rat cerebellum: An immunohistochemical study. *Neuroscience Letters*. 2006;396(2):113-116. doi:10.1016/j.neulet.2005.11.038

117. Baek J-H, Yiwen heng, Darlington CL, Smith PF. Cannabinoid CB2 receptor expression in the rat brainstem cochlear and vestibular nuclei. *Acta Oto-Laryngologica*. 2008;128(9):961-967. doi:10.1080/00016480701796944

118. Koch M, Habazettl I, Dehghani F, Korf H-W. The rat pineal gland comprises an endocannabinoid system. *Journal of Pineal Research*. 2008;45(4):351-360. doi:10.1111/j.1600-079X.2008.00597.x

119. Atwood BK, Lopez J, Wager-Miller J, Mackie K, Straiker A. Expression of G protein-coupled receptors and related proteins in HEK293, AtT20, BV2, and N18 cell lines as revealed by microarray analysis. *BMC genomics*. 2011;12(1):14-14. doi:10.1186/1471-2164-12-14

APPENDIX A

TABLES OF CB1 AND CB2 RECEPTOR EXPRESSION

Location	Cell Type	Density	Reference
Olfactory Bulb & Tubercle	Neurons	Moderate	Cristino et. al 2006[102], Hebert-Chatelain et. al 2014[103], Moldrich & Wenger 2000[104]
Cerebral Cortex	Neurons in layers II-III, in upper layer V, and in layer VI (soma only, no axonal localization) Layers I & IV	High Low (layer IV overall lowest density in frontal lobe)	Eggan & Lewis 2007[105], Hebert-Chatelain et. al 2014, Moldrich & Wenger 2000, Zarate et. al 2008[106]
Pre-frontal Cortex	Neurons	High	Eggan & Lewis 2007, Hebert-Chatelain et. al 2014
Cingulate Cortex	Glial cells, neurons	Moderate	Ibarra-Lecue et. al 2018[107], Eggan & Lewis 2007, Hebert-Chatelain et. al 2014, Moldrich & Wenger 2000
Thalamic Nuclei	Neuron & glial cells	Low	Cristino et. al 2006, Moldrich & Wenger 2000
Hypothalamus	Neurons	Low	Moldrich & Wenger 2000
Hippocampus: CA1, CA2 & CA3	Pyramidal neurons & interneurons	Moderate-High (highest in CA1)	Cristino et. al 2006, Eggan & Lewis 2007, Herkenham et. al 1990, Moldrich & Wenger 2000
Hippocampus CA1	Interneuron-like cells	High	Eggan & Lewis 2007, Moldrich & Wenger 2000
Dentate Gyrus	Neurons	High	Cristino et. al 2006, Eggan & Lewis 2007, Moldrich & Wenger 2000
Subiculum	Neurons	High	Eggan & Lewis 2007, Moldrich & Wenger 2000
Entorhinal Cortex	Neurons	Moderate-High	Moldrich & Wenger 2000
Amygdala	Neuron & glial cells	Moderate-High	Cristino et. al 2006, Eggan & Lewis 2007, Herkenham et. al 1990[108], Moldrich & Wenger 2000
Caudate-Putamen/Striatum	Neurons, DA Neurons	High	Herkenham et. al 1990, Moldrich & Wenger 2000
Globus Pallidus (GPe & GPi)	Neurons	High	Herkenham et. al 1990, Moldrich & Wenger 2000
Substantia Nigra PR	Neurons	High	Cristino et. al 2006, Eggan & Lewis 2007, Moldrich & Wenger 2000
Nucleus Accumbens	--	Moderate	Eggan & Lewis 2007, Moldrich & Wenger 2000

Ventral Tegmental Area	DA Neurons (D_1, D_2)	High	Martin et. al 2008[109],
Periaqueductal gray, Pons, Area Postrema	--	Low	Moldrich & Wenger 2000
Cerebellum: all layers	Inhibitory Interneurons	High	Moldrich & Wenger 2000
Cerebellum: Purkinje cells	Neurons	High	Herkenham et. al 1990, Moldrich & Wenger 2000
Cerebellum: Molecular layer	Dendrites	Moderate	Herkenham et. al 1990, Moldrich & Wenger 2000
Cerebellum: Granular Layer	Mossy fibers & neuropil	Moderate	Herkenham et. al 1990, Moldrich & Wenger 2000
Olfactory Bulb, Piriform Cortex, Cingulate Cortex, Medial Forebrain Bundle and Substantia Nigra	Perivascular glial fibers	Moderate-High	Moldrich & Wenger 2000

Table 2 – Localization and Expression of Cannabinoid Receptor 1 in the CNS
Distribution and expression levels of cannabinoid receptor 1 protein observed in various regions throughout the central nervous system. CB1 is expressed widely and in high densities making it rather easy to identify when compared to CB2.

Location	Cell Type	Reference
Olfactory Bulb & Tubercle	Neurons	Gong et al. 2006[110]
Cerebral Cortex	Pyramidal-like neurons layers III & V	Gong et al. 2006, Lanciego et. al 2011[111]
Thalamic Nuclei	Neuron & glial cells	Gong et al. 2006
Hippocampus: CA2 & CA3	Pyramidal neurons & interneurons	Brusco et al. 2008[112], Gong et al. 2006
Hippocampus CA1 & CA3	Interneuron-like cells	Lanciego et al. 2011
Amygdala	Neuron & glial cells	Garcia-Gutierrez et al. 2010[47], Gong et al. 2006
Strata Oriens & Radiatum	Neuropil	Suarez et. al 2008, 2009[113,114]
Corpus Callosum	Glial cells	Gong et al. 2006
Globus Pallidus	Neurons	Lanciego et al. 2011
Periaqueductal gray, Substantia Nigra	Neurons	Brusco et al. 2008, Gong et al. 2006
Ventral Tegmental Area	DA Neurons	Zhang et al. 2014[115]

Cerebellum: Molecular Layer	Parallel varicose fibers and neuropil	Ashton et al. 2006[116], Baek et al. 2008, Gong et al. 2006
Cerebellum: Purkinje cells	Neurons	Baek et al. 2008[117], Suarez et al. 2008, 2009
Cerebellum: Granular Layer	Mossy fibers & neuropil	Baek et al. 2008, Suarez et al. 2008, 2009
Pineal Gland	Pinealocytes & intrapineal nerve fibers	Koch et al. 2008[118]
Retina	Inner photoreceptor segments, inner nuclear layer, ganglion cell layer	Lopez et al. 2010[119]
Nucleus Accumbens	--	Garcia-Gutierrez et al. 2010
Cingulate Cortex	--	Garcia-Gutierrez et al. 2010

Table 3 - Localization of Cannabinoid Receptor 2 in the CNS
Distribution of cannabinoid receptor 2 throughout regions of the central nervous system as determined using immunocytochemical analysis as well as *in situ* hybridization of mRNA transcripts. This latter method provides much better resolution of cellular transcription because of the lack of specificity in commercially available CB2 antibodies and the low expression levels of CB2 protein in the CNS.