Review

The Role of Endocannabinoid Signaling in Cortical Inhibitory Neuron Dysfunction in Schizophrenia

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ABSTRACT

Cannabis use has been reported to increase the risk of developing schizophrenia and to worsen symptoms of the illness. Both of these outcomes might be attributable to the disruption by cannabis of the endogenous cannabinoid system's spatiotemporal regulation of the inhibitory circuitry in the prefrontal cortex that is essential for core cognitive processes, such as working memory, which are impaired in schizophrenia. In the healthy brain, the endocannabinoid 2-arachidonylglycerol 1) is synthesized by diacylglycerol lipase in pyramidal neurons; 2) travels retrogradely to nearby inhibitory axon terminals that express the primary type 1 cannabinoid receptor (CB_1R); 3) binds to CB_1R, which inhibits gamma-aminobutyric acid release from the cholecystokinin-containing population of interneurons; and 4) is metabolized by either monoglyceride lipase, which is located in the inhibitory axon terminal, or by α-β-hydrolase domain 6, which is co-localized presynaptically with diacylglycerol lipase. Investigations of the endogenous cannabinoid system in the prefrontal cortex of subjects with schizophrenia have found evidence of higher metabolism of 2-arachidonylglycerol, as well as both greater CB_1R receptor binding and lower levels of CB_1R messenger RNA and protein. Current views on the potential pathogenesis of these alterations, including disturbances in the development of the endogenous cannabinoid system, are discussed. In addition, how interactions between these alterations in the endocannabinoid system and those in other inhibitory neurons in the prefrontal cortex in subjects with schizophrenia might increase the liability to adverse outcomes with cannabis use is considered.

Keywords: Cannabinoid, Cannabis, CB_1R, Cognitive, Inhibitory, Prefrontal cortex

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In recent years, cannabis use has increased in prevalence in parallel with an earlier age of onset of cannabis use during adolescence and a greater content of Δ9-tetrahydrocannabinol, the major psychoactive component of cannabis (1). These trends raise the question of whether the higher rates and earlier onset of use of more potent cannabis may lead to potentially deleterious societal health consequences. One area of potential concern arises from evidence of a relationship between cannabis use and schizophrenia.

Schizophrenia is a serious psychiatric illness associated with high levels of disability and morbidity, elevated rates of suicide, and early mortality (2). The disorder is typically characterized by the triad of 1) psychotic symptoms, such as hallucinations and delusions; 2) negative symptoms, such as alogia and avolition; and 3) impairments in cognitive functions such as working memory and cognitive control. Multiple lines of evidence suggest that cannabis use worsens the course of schizophrenia and may be a risk factor for development of the illness. First, intravenous administration of Δ9-tetrahydrocannabinol has been reported to transiently worsen or induce psychotic symptoms and cognitive deficits in individuals with or without schizophrenia, respectively (3,4). Second, a large number of studies have identified cannabis use as a risk factor for developing schizophrenia (1,5–12). This

elevated risk has been reported to remain significant, though may be attenuated, after correcting for confounding variables such as psychotic symptoms preceding cannabis use, familial risk, parental factors, other psychiatric illness, social background, affiliation with deviant peers, socioeconomic status, trauma, baseline IQ, substance use, and level of education (1,5–12). In addition, this risk is inversely related to the age of onset of cannabis use and directly related to the amount of cannabis use. For example, onset of cannabis use at age 15 relative to age 18 was associated with a higher risk of a psychotic disorder at age 26 (6). Third, cannabis use is associated with an earlier age of onset of schizophrenia (13–15), particularly with higher levels of daily cannabis consumption and when the onset of cannabis use begins before age 15 (16,17). Fourth, individuals with schizophrenia who use cannabis have longer durations of hospital stays and more frequent readmissions (18). However, these lines of evidence do not demonstrate that cannabis use is either necessary or sufficient to develop schizophrenia.

Cannabis use in healthy subjects has been reported to result in deficits in the same cognitive domains that are disturbed in schizophrenia and that have the strongest association with poor functioning and long-term outcomes (19–21). For example, otherwise healthy individuals with prolonged

exposure to high levels of cannabis have performance deficits on neuropsychological tests that persist into periods of abstinence and are more severe in individuals who began using cannabis in adolescence (22). A prospective cohort study found a decline in cognitive function that only occurred in adolescent-onset cannabis users and that did not recover with cessation of cannabis use in adulthood (23). Paradoxically, schizophrenia subjects with a prior history of cannabis use have been reported to have higher performance on multiple cognitive tasks relative to schizophrenia subjects without a cannabis use history (24,25). However, this finding may be explained by the observation that individuals with schizophrenia who have a history of cannabis use have a higher premorbid IQ than those without a history of cannabis use (26). Indeed, higher neurocognitive functioning is also seen in individuals with schizophrenia who abuse other substances (27–29), suggesting that individuals with a more severe course of schizophrenia may lack the cognitive and social capacities to obtain and use illicit substances (27), while a subgroup of schizophrenia subjects with less cognitive impairments may be capable of abusing substances. Alternatively, given the evidence of cannabis use as a risk factor for developing schizophrenia, a subset of individuals at risk for schizophrenia who have relatively normal cognitive functioning may have developed schizophrenia after exposure to cannabis and consequently may have otherwise higher cognitive performance relative to individuals who develop schizophrenia regardless of exposure to cannabis use. However, while epidemiologic studies do not present a fully clear picture of the relationship between cannabis use and cognitive functioning in schizophrenia, direct administration of Δ9-tetrahydrocannabinol to individuals with schizophrenia has been reported to transiently worsen cognitive deficits (4).

Experimental studies in animals have provided more direct evidence of a causal relationship between cannabis use and cognitive impairments. For example, administration of Δ9-tetrahydrocannabinol or other cannabinoid-1 receptor (CB_1R) agonists produced cognitive deficits in adolescent, but not adult, rats (30–32). Similarly, regular administration of Δ9-tetrahydrocannabinol to adolescent monkeys altered the trajectory of developmental improvements in spatial working memory (33). These findings suggest that the long-lasting deleterious effects of Δ9-tetrahydrocannabinol on cognitive function may be more prominent when the exposure occurs during periods of neural circuitry development, such as adolescence, which is also the time period when the symptoms of schizophrenia become clinically apparent.

CORTICAL CIRCUITRY AND COGNITIVE PROCESSES IN SCHIZOPHRENIA

Understanding the mechanisms by which cannabis may disrupt the cognitive functions that are also impaired in schizophrenia requires knowledge of aspects of the cortical circuitry that supports cognitive processes such as working memory. For example, spatial working memory, which is altered in adolescent monkeys by persistent exposure to Δ9-tetrahydrocannabinol (33), depends on a distributed neural circuit that includes the dorsolateral prefrontal cortex (DLPFC) (34). In the DLPFC, layer 3 pyramidal neurons provide reciprocal excitatory connections to other layer 3 pyramidal neurons that sustain circuitry activity during the delay period of working memory tasks (35). In addition, the subpopulation of cortical inhibitory (gamma-aminobutyric acid [GABA]) basket neurons that express the calcium-binding protein parvalbumin provide inhibitory input that tunes pyramidal neuron firing (36). The interaction between layer 3 pyramidal neurons and parvalbumin basket neurons generates gamma frequency (30–80 Hz) oscillations whose power scales with the demands of working memory tasks (37,38). In schizophrenia, DLPFC layer 3 pyramidal neurons and parvalbumin basket neurons are altered. For example, the density of dendritic spines, the postsynaptic targets of excitatory inputs from other pyramidal neurons, are lower on layer 3 pyramidal neurons in schizophrenia (39,40). Furthermore, deficits in messenger RNA (mRNA) levels for parvalbumin (41–45) and in parvalbumin protein levels in basket cell axon terminals (46) are present in the DLPFC in schizophrenia. In addition, deficits in mRNA levels for the GABA synthesizing enzyme glutamic acid decarboxylase 67 (GAD67) (47–52) are particularly prominent in parvalbumin neurons (41), and GAD67 protein levels are lower in parvalbumin neuron axon terminals (52). Thus, the connectivity among pyramidal neurons and parvalbumin basket neurons appears to be impaired in the DLPFC in schizophrenia and could contribute to the alterations in gamma oscillations that underlie working memory deficits (53,54).

Active cannabis use may affect cognitive functioning in schizophrenia by further interfering with the regulation of the already dysfunctional interaction between DLPFC pyramidal neurons and parvalbumin basket neurons. For example, in the primate neocortex, the principal receptor for cannabinoids, CB_1R, is strongly expressed in the DLPFC (55). In the neocortex, CB_1Rs are preferentially localized to the axon terminals of GABA-containing basket neurons that contain the neuropeptide cholecystokinin (CCK) and, to a lesser extent, of some pyramidal neurons (56–58). In fact, the density of CB_1Rs is much higher in the axon terminals of CCK basket cells than of pyramidal cells (59–61). The inhibitory axon terminals from CCK basket neurons target both pyramidal neurons and parvalbumin neurons (56,57). Activation of CB_1Rs suppresses GABA release from CCK neuron axon terminals onto pyramidal neurons (62), and CCK basket neurons also regulate pyramidal neuron activity (56,63,64). Thus, exogenous cannabinoids such as cannabis broadly suppress CCK neuron-mediated inhibition of pyramidal neurons and parvalbumin neurons without the tightly regulated, spatiotemporal selectivity of endocannabinoids.

THE ENDOCANNABINOID SYSTEM

Further exploration of the relationship between cannabis use, cortical circuitry disturbances, and cognitive impairments in schizophrenia also requires knowledge of the endocannabinoid system. The primary endocannabinoid ligands in the brain include anandamide and 2-arachidonylglycerol (2-AG) (65). Anandamide is a partial CB_1R agonist and is found at relatively low concentrations in the brain (65,66). In contrast, 2-AG is a full CB_1R agonist and is found at much higher concentrations than anandamide in the brain (67).

Endocannabinoid System
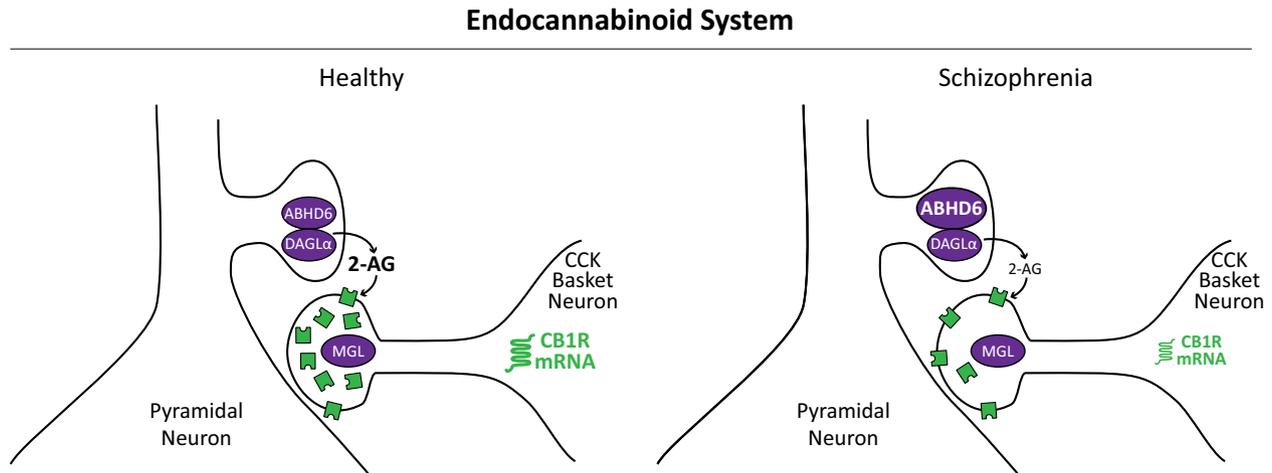
Healthy | Schizophrenia

Figure 1. Schematic illustration of the endogenous cannabinoid system in the dorsolateral prefrontal cortex (DLPFC) in the healthy state and in schizophrenia. Left: The synthesizing enzyme for the endogenous cannabinoid 2-arachidonylglycerol (2-AG), diacylglycerol lipase-α (DAGLα), is localized to the dendritic spines of pyramidal neurons. In response to repeated depolarization, 2-AG is synthesized, travels retrogradely, and activates the endogenous cannabinoid-1 receptor (CB$_1$R) (green) in nearby inhibitory axon terminals from cholecystokinin (CCK)-containing basket neurons. 2-AG is then degraded by either monoglyceride lipase (MGL) localized to CCK neuron axon terminals or by the serine hydrolase α-β-hydrolase domain 6 (ABHD6), which is co-localized with DAGLα in the dendritic spines of pyramidal neurons. Right: In schizophrenia, higher messenger RNA (mRNA) levels for ABHD6 (larger font size) have been reported in the DLPFC. Because ABHD6 is co-localized with diacylglycerol lipase in the dendritic spines of DLPFC pyramidal neurons, higher ABHD6 mRNA levels may lead to higher metabolism of 2-AG directly at the source of 2-AG production, which may, in turn, lower 2-AG activity at CB$_1$Rs. In addition, studies have found lower levels of CB$_1$R mRNA (smaller font size) and protein immunoreactivity and higher CB$_1$R binding in the DLPFC of the same schizophrenia subjects. One potential explanation for this apparent discrepancy may be the presence of higher levels of membrane-bound CB$_1$R due to altered trafficking that are accessible to ligand binding, while the total amount of intracellular CB$_1$R is lower.

Physiological and anatomical lines of evidence suggest that 2-AG serves as a retrograde signal from pyramidal neurons to nearby CCK basket neuron axon terminals that contain high concentrations of the CB$_1$R (Figure 1, left panel). In a process known as depolarization-induced suppression of inhibition (DSI), the rapid depolarization of a pyramidal neuron induces short-term depression of GABA release from local CCK neuron axon terminals. Both CB$_1$R and 2-AG are required for DSI. For example, bath application of 2-AG reduces the amplitude of inhibitory postsynaptic currents (68). Furthermore, 2-AG is synthesized by diacylglycerol lipase (DAGL) (67), which has two isoforms. DAGLα is expressed at much higher levels than DAGLß in adult cortex and is primarily responsible for 2-AG synthesis (69). DAGLα is predominantly localized to pyramidal neurons (58,70–72), and pharmacologic inhibition of DAGL blocks CB$_1$R-mediated DSI (68,73–75).

Once 2-AG has been synthesized and released, it is degraded by monoglyceride lipase (MGL) (76,77) or by α-β-hydrolase domain 6 (ABHD6) (Figure 1, left panel) (78). MGL is localized almost exclusively in presynaptic axon terminals (71,72,79). Consistent with the role of 2-AG in suppressing GABA release, inhibitors of MGL prolong the suppression of inhibitory postsynaptic currents (68,73,80), an effect that is reversible by a CB$_1$R antagonist (68,80). In contrast, inhibitors of fatty acid amide hydrolase, which hydrolyzes anandamide but not 2-AG in vivo (76), have no effect on 2-AG-mediated DSI (68,73,81). Taken together, these data suggest that MGL is anatomically positioned to inactivate 2-AG at its site of action at CB$_1$R in presynaptic inhibitory axon terminals. In contrast, ABHD6 is

co-localized with DAGL in the pyramidal neuron dendritic spines (70,71,78), which allow ABHD6 to regulate 2-AG at its site of production.

In concert, these findings raise the question of whether alterations in the endocannabinoid system in schizophrenia may contribute to, or compensate for, the disturbances in DLPFC circuitry described above. Multiple studies have addressed this question by investigating the status of endocannabinoids and CB$_1$R in the DLPFC in schizophrenia, and we review these studies next. However, it is important to note that the endocannabinoid system is also involved in the regulation of fear and anxiety (82–85) and regulates other neurotransmitter systems in other brain regions, such as glutamatergic signaling in the amygdala (86). Consequently, disturbances in endocannabinoid signaling may also be present in other psychiatric disorders and may have consequences on neurotransmitter function in other brain regions; however, the primary focus of this review article is on endocannabinoid regulation of cortical GABA neuron dysfunction in schizophrenia.

ALTERATIONS IN THE ENDOCANNABINOID SYSTEM IN SCHIZOPHRENIA

The status of endocannabinoid signaling in the DLPFC can first be informed by investigating whether the primary endocannabinoids themselves (i.e., anandamide and 2-AG) are altered in schizophrenia. Initial studies focused on levels of these ligands in the cerebrospinal fluid (CSF) in the illness (Table 1). For example, CSF levels of anandamide were

Table 1. Summary of Key Findings From Studies of Endogenous Cannabinoid Markers in Schizophrenia

Source (Reference)	Schizophrenia Cohort [n]	Technique	Finding in Schizophrenia Subjects
Cerebrospinal Fluid			
(87)	First-episode, antipsychotic-naive [47]	HPLC/MS	Elevated CSF anandamide levels
(88)	Prodromal psychosis [27]	HPLC/MS	Elevated CSF anandamide levels
(90)	[10]	HPLC/MS	Elevated CSF anandamide levels
Blood			
(89)	Acute symptoms, off antipsychotics [12]	LC-APCI-MS	Elevated blood anandamide levels
Imaging			
(99)	[9]	PET [11C]-OMAR	Elevated CB_1R binding in all brain regions, significant in pons
Postmortem Brain			
Endocannabinoid enzymes			
(92)	[42]	qPCR	No change in mRNA levels for endocannabinoid synthesizing (DAGL) and metabolizing (MGL, FAAH) enzymes in DLPFC area 9
(93)	[42]	qPCR	Elevated mRNA levels for 2-AG metabolizing enzyme ABHD6 in DLPFC area 9 of younger subjects; no changes in antipsychotic- or THC-exposed monkeys
CB_1R mRNA and protein			
(101)	[23]	In situ hybridization, immunocytochemistry	Lower CB_1R mRNA and protein levels in DLPFC area 9; no change in antipsychotic-exposed monkeys
(112)	[26]	Immunocytochemistry	Lower CB_1R immunoreactivity in DLPFC area 46, no change in depression
(97)	[37]	qPCR	No change in CB_1R mRNA levels in DLPFC area 46
(104)	[31]	qPCR	No change in CB_1R mRNA levels in DLPFC area 9
CB_1R autoradiography			
(94)	[14]	CB_1R agonist [3H]-CP55940	Elevated CB_1R binding in DLPFC area 9, but not hippocampus, caudate, or putamen
(97)	[37]	[3H]-CP55940	Elevated CB_1R binding in DLPFC area 46 in paranoid subjects
(98)	[47]	CB_1R inverse agonist [3H]-MePPEP	Elevated CB_1R binding in DLPFC areas 9 and 46
(95)	[10]	CB_1R antagonist [3H]-SR141716	Elevated CB_1R binding in anterior cingulate cortex
(96)	[8]	[3H]-CP55940	Elevated CB_1R binding in superficial layers of posterior cingulate cortex
(105)	[21]	[3H]-OMAR	Elevated CB_1R binding in DLPFC area 9 in same schizophrenia subjects with lower CB_1R mRNA and protein levels (101)

2-AG, 2-arachidonoylglyerol; ABHD6, α-β-hydrolase domain 6; CB_1R, cannabinoid-1 receptor; CSF, cerebrospinal fluid; DAGL, diacylglycerol lipase; DLPFC, dorsolateral prefrontal cortex; FAAH, fatty acid amide hydrolase; HPLC/MS, high performance liquid chromatography/mass spectrometry; LC-APCI-MS, liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry; MGL, monoglyceride lipase; mRNA, messenger RNA; PET, positron emission tomography; qPCR, quantitative polymerase chain reaction; THC, tetrahydrocannabinol.

reported to be eightfold higher in a cohort of 47 first-episode, antipsychotic-naive schizophrenia subjects (87) and a cohort of 27 subjects with prodromal psychotic symptoms (88) relative to healthy subjects. Furthermore, a threefold elevation in blood levels of anandamide were reported in a cohort of 12 subjects in an acute phase of schizophrenia and off antipsychotic medications (89). However, CSF studies have not been able to detect quantifiable levels of 2-AG (90), which is the endocannabinoid present in the highest concentration in the brain (67). Thus, the relationship between levels of endocannabinoids measured in the CSF and peripheral blood and endocannabinoid function in brain parenchyma is not clear. In addition, attempts to quantify 2-AG directly in brain parenchyma in human subjects have also not been successful due to the prominent effect of postmortem delay on 2-AG (91).

The status of endocannabinoid signaling in the DLPFC has been further investigated, though indirectly, by quantifying the synthesizing and metabolizing enzymes for 2-AG (Table 1). In the DLPFC of 42 schizophrenia subjects, mRNA levels for DAGLα, DAGLβ, MGL, or fatty acid amide hydrolase did not differ between schizophrenia and healthy subjects (92) (Figure 1, right panel). However, ABHD6 mRNA levels were elevated in the DLPFC of schizophrenia subjects who were younger (<40 years old) and had a shorter illness duration (<15 years of illness) (93). Because of the co-localization of ABHD6 with DAGL in the dendritic spines of DLPFC pyramidal neurons (70,71,78), higher ABHD6 mRNA levels may lead to higher metabolism of 2-AG directly at the source of 2-AG production in dendritic spines, which may, in turn, lead to lower 2-AG levels and binding at CB_1R in the earlier stages of the illness (Figure 1, right panel).

Understanding endocannabinoid signaling in the DLPFC in schizophrenia also requires knowledge of the status of the principal receptor, CB_1R. Postmortem studies employing receptor autoradiography using radiolabeled CB_1R ligands have consistently reported higher CB_1R binding in the frontal cortex in schizophrenia (Table 1) (94–98). For example, a study utilizing the CB_1R agonist [3H]-CP55940 found higher CB_1R binding in DLPFC area 9 in a cohort of 14 schizophrenia subjects relative to healthy subjects (94). Similarly, another study using [3H]-CP55940 found higher levels of CB_1R binding in DLPFC area 46 from a cohort of 37 schizophrenia subjects relative to healthy subjects; however, higher CB_1R binding was only observed in subjects with paranoid schizophrenia (97). CB_1R binding measured using the inverse agonist [3H]-MePPEP was 20% higher in DLPFC areas 9 and 46 in a cohort of 47 schizophrenia subjects (98). In the anterior cingulate cortex, binding of the CB_1R antagonist [3H]-SR141716 was 64% higher in a cohort of 10 schizophrenia subjects (95). In posterior cingulate cortex, levels of CB_1R binding measured using [3H]-CP55940 were 25% higher in the superficial, but not deeper, cortical layers in a cohort of eight schizophrenia subjects (96). Furthermore, a pilot in vivo positron emission tomography study utilizing the novel CB_1R selective ligand $[^{11}C]$-OMAR found higher brain CB_1R binding in a cohort of nine schizophrenia subjects (99) and also an inverse correlation between CB_1R binding and negative symptoms in schizophrenia subjects (100).

While these radiolabeled CB_1R ligand binding studies have consistently reported higher levels of CB_1R binding in schizophrenia, particularly in the DLPFC, studies of CB_1R mRNA and protein levels have provided contrasting results (Table 1). For example, a radioimmunocytochemistry study found lower CB_1R protein levels in DLPFC area 9 of the right hemisphere in a cohort of 23 schizophrenia subjects relative to healthy subjects (101). This study also reported lower CB_1R protein levels using diaminobenzidine-based immunocytochemistry in DLPFC area 9 of the left hemisphere in a subset ($n = 12$) of the same schizophrenia subjects, suggesting that lower CB_1R protein levels are present in both hemispheres of the DLPFC in schizophrenia. This finding appears unlikely to be attributable to a loss of CB_1R-expressing neurons, as the total number of neurons has been reported to be unaltered in the frontal cortex in schizophrenia (102). Furthermore, the density of CB_1R-immunopositive neurons was also reported to not differ, at least in the anterior cingulate cortex, of 15 schizophrenia subjects relative to subjects with major depressive disorder or bipolar disorder or control subjects (103), although this study did not attempt to quantify CB_1R levels. In contrast, two studies of CB_1R mRNA levels by quantitative polymerase chain reaction in DLPFC areas 9 or 46 found no differences from comparison subjects in cohorts of 31 and 37 schizophrenia subjects, respectively (97,104). However, a more detailed in situ hybridization study reported an 11% decrease in CB_1R mRNA levels that was most prominent in layers 2, 3, 5, and 6 in the same 23 schizophrenia subjects that had lower CB_1R protein levels quantified by radioimmunocytochemistry (101). Furthermore, long-term exposure to the typical antipsychotic haloperidol or the atypical antipsychotic olanzapine in monkeys did not affect CB_1R mRNA levels quantified by in situ hybridization in the DLPFC (101).

Because almost all studies of CB_1R mRNA, protein, and binding levels were not conducted in the same subjects, the reason for the apparent discrepancy in CB_1R measures in schizophrenia was not immediately clear. To first address the issue of whether the differences across studies could reflect differences in the cohorts studied, we conducted a receptor binding study using [3H]-OMAR in the same schizophrenia subjects in whom lower CB_1R mRNA and protein immunoreactivity levels had been previously reported (Table 1) (105). Schizophrenia subjects with lower CB_1R mRNA and protein immunoreactivity levels were found to also have higher levels of [3H]-OMAR binding to CB_1R, and [3H]-OMAR receptor binding was negatively correlated with CB_1R mRNA levels (105). The surprising combination of lower CB_1R mRNA and protein immunoreactivity with higher CB_1R binding in the DLPFC of the same schizophrenia subjects suggests at least four different interpretations that can guide further studies that may provide insight into the nature of endocannabinoid system dysfunction in schizophrenia.

First, higher levels of CB_1R binding might reflect higher levels of membrane-bound CB_1R due to altered trafficking of the receptor (Figure 1, right panel). Indeed, in contrast to most G-protein coupled receptors that are found in the plasma membrane, CB_1Rs are largely localized intracellularly (106). Thus, the number of CB_1Rs that are accessible to ligand binding at the membrane could be increased in schizophrenia, while the total amount of intracellular CB_1R is reduced, as suggested by the findings of lower CB_1R mRNA and protein immunoreactivity levels. The recent development of super-resolution microscopy may make it possible to test this hypothesis.

Second, higher levels of CB_1R binding might reflect greater receptor affinity. Unfortunately, CB_1R affinity has generally not been quantified in schizophrenia. One study reported no differences in CB_1R affinity in the DLPFC in schizophrenia but attributed this lack of change in part due to the high levels of assay variance observed in postmortem tissue combined with an insufficient sample size (98). Additional studies that assess CB_1R affinity and binding levels in cohorts of schizophrenia subjects that are large enough to detect changes in affinity in the face of high variance are needed to test this interpretation. In addition, although speculative, higher CB_1R affinity might be related to higher ABHD6 levels in schizophrenia (93). For example, ABHD6 mRNA and CB_1R binding levels were positively correlated in the DLPFC in schizophrenia (93). Because of the co-localization of ABHD6 with DAGL in the dendritic spines of cortical pyramidal neurons (70,71,78), greater expression of ABHD6 mRNA could lead to higher metabolism of 2-AG directly at the source of 2-AG production in dendritic spines, which would, in turn, lower 2-AG activity at CB_1Rs (Figure 1, right panel). Lower 2-AG signaling could then lead to a compensatory upregulation in membrane localization through altered trafficking or higher affinity of the affected CB_1R in that location. Direct testing of this hypothesis would require animal models that overexpress ABHD6.

Third, the GABA neuron axon terminals that normally express CB_1R might not fully develop in schizophrenia, leading to lower overall levels of CB_1R mRNA and protein. For example, the normal increase in CB_1R immunoreactivity

across adolescence in the middle layers of primate DLPFC (107) might not occur in schizophrenia, consistent with findings that other markers of DLPFC GABA neurons fit a model of arrested developmental trajectories in schizophrenia (108–110). Thus, it is possible that CB$_1$R affinity in the remaining CB$_1$R-containing axon terminals increases in schizophrenia as a compensatory response to maintain homeostasis of endocannabinoid signaling. Although longitudinal developmental in vivo positron emission tomography imaging studies of CB$_1$R binding in individuals at high risk for schizophrenia are not currently possible, proof-of-concept tests of this hypothesis might be possible in appropriate animal models.

Fourth, higher levels of CB$_1$R binding may reflect higher CB$_1$R levels in excitatory axon terminals (58,111), while CB$_1$R levels are lower in inhibitory axon terminals. The previous radioimmunocytochemistry study that reported lowered CB$_1$R protein levels in schizophrenia (101) utilized a CB$_1$R antibody against an epitope present on inhibitory, but not excitatory, axon terminals (112), while the [3H]-OMAR ligand binds to all available CB$_1$Rs. Thus, studies that quantify CB$_1$R levels specifically in pyramidal neurons in schizophrenia are required to further test this interpretation.

INTERACTIONS BETWEEN ALTERATIONS IN ENDOCANNABINOID AND GABA SIGNALING IN SCHIZOPHRENIA

These alterations in endocannabinoid signaling may have important effects on GABA neuron disturbances in the DLPFC in schizophrenia. For example, as described earlier, the combination of higher levels of ABHD6, and consequently higher 2-AG metabolism and lower 2-AG levels, may lead to a compensatory upregulation of CB$_1$R affinity and/or membrane localization (Figure 1, right panel). This interpretation is supported by the significant correlation between CB$_1$R binding and ABHD6 mRNA in schizophrenia subjects (93), and the net effect of these changes may be to maintain homeostasis of endocannabinoid regulation of GABA signaling. However, the disturbances in GABA neurons could also be upstream to the changes in the endocannabinoid system in schizophrenia. For example, deficits in mRNA levels for the GABA synthesizing enzyme GAD67 are one of the most consistently reported findings in the DLPFC in schizophrenia (47–52). The resulting deficit in GABA synthesis could evoke compensatory changes, such as downregulation of CB$_1$R expression [levels of GAD67 and CB$_1$R mRNAs are positively correlated in the DLPFC in schizophrenia (101)], to preserve GABA signaling strength. The idea that lower GAD67 expression is upstream of lower CB$_1$R expression in schizophrenia is supported by experimental studies. For example, mice with a GAD67 heterozygous null mutation exhibit lower CB$_1$R mRNA levels, without a change in DAGL mRNA levels, in the medial prefrontal cortex (PFC) (113). This pattern of findings is similar to that seen in schizophrenia. In contrast, mice with a CB$_1$R heterozygous or homozygous null mutation had no change in GAD67 mRNA levels in medial PFC (113). Although the alterations in the endocannabinoid and GABA systems in the DLPFC in schizophrenia are correlated, it is possible that both change in response to another disturbance in the illness.

CANNABIS AND THE ENDOCANNABINOID SYSTEM IN SCHIZOPHRENIA

As noted above, individuals with schizophrenia appear to be particularly susceptible to the cognitive-impairing effects of cannabis. For example, under normal physiological conditions, activation of CB$_1$Rs suppresses GABA release only from the CB$_1$R-containing terminals that synapse onto the pyramidal cells whose activity stimulates endocannabinoid signaling. In contrast, cannabis use suppresses GABA release onto pyramidal cells without spatiotemporal selectivity because exogenous cannabinoids affect all CB$_1$R terminals. The adverse consequences of this indiscriminate activation of CB$_1$Rs may be exacerbated in schizophrenia due to the presence of higher membrane-bound levels of CB$_1$R or higher levels of CB$_1$R affinity (94–98,105).

Furthermore, deficits in GABA synthesis due to lower GAD67 mRNA levels in schizophrenia might be partially compensated for by a reduction in endocannabinoid-mediated suppression of GABA release from CB$_1$R-containing axon terminals due to lower 2-AG signaling. Such a compensation could be driven by greater expression of ABHD6 in the early stages of schizophrenia (93). If this hypothesis is true, then the negative effects of cannabis in schizophrenia may occur because exogenous activation of CB$_1$R by cannabis counteracts the compensatory effect of lower 2-AG signaling, at least in the earlier stages of the illness. Consistent with this interpretation, the deleterious effects of cannabis use appear to be most prominent in younger individuals (10,13,14). Interestingly, administration of a CB$_1$R agonist to adolescent but not adult rats impairs the maturation of inhibitory processes in the PFC (114). Taken together, these data suggest the need for preventative strategies directed toward reducing cannabis use in adolescents at risk for schizophrenia, which is especially important given the increasing prevalence of cannabis use, earlier age of onset of cannabis use during adolescence, rising Δ9-tetrahydrocannabinol content of cannabis in recent years, and recent legalization of recreational cannabis use in multiple states (1). Furthermore, the recently announced National Institutes of Health initiative termed Adolescent Brain Cognitive Development will hopefully provide critical insights into the long-term effects of cannabis use on adolescent brain development by prospectively studying the relationship between substance use and cognitive development in thousands of youth.

ARTICLE INFORMATION

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REFERENCES

1. Radhakrishnan R, Wilkinson ST, D'Souza DC (2014): Gone to pot - a review of the association between cannabis and psychosis. Front Psychiatry 5:54.
2. Insel TR, Scolnick EM (2006): Cure therapeutics and strategic prevention: Raising the bar for mental health research. Mol Psychiatry 11:11–17.
3. D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT, et al. (2004): The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: Implications for psychosis. Neuropsychopharmacology 29:1558–1572.
4. D'Souza DC, Abi-Saab WM, Madonick S, Forselius-Bielen K, Doersch A, Braley G, et al. (2005): Delta-9-tetrahydrocannabinol effects in schizophrenia: Implications for cognition, psychosis, and addiction. Biol Psychiatry 57:594–608.
5. Andreasson S, Allebeck P, Engstrom A, Rydberg U (1987): Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet 2:1483–1486.
6. Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE (2002): Cannabis use in adolescence and risk for adult psychosis: Longitudinal prospective study. BMJ 325:1212–1213.
7. Zammit S, Allebeck P, Andreasson S, Lundberg I, Lewis G (2002): Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: Historical cohort study. BMJ 325:1199–1204.
8. Henquet C, Murray R, Linszen D, van Os J (2005): The environment and schizophrenia: The role of cannabis use. Schizophr Bull 31:608–612.
9. Fergusson DM, Poulton R, Smith PF, Boden JM (2006): Cannabis and psychosis. BMJ 332:172–175.
10. Casadio P, Fernandes C, Murray RM, Di Forti M (2011): Cannabis use in young people: The risk for schizophrenia. Neurosci Biobehav Rev 35:1779–1787.
11. Manrique-Garcia E, Zammit S, Dalman C, Hemmingsson T, Andreasson S, Allebeck P (2012): Cannabis, schizophrenia and other non-affective psychoses: 35 years of follow-up of a population-based cohort. Psychol Med 42:1321–1328.
12. Giordano GN, Ohlsson H, Sundquist K, Sundquist J, Kendler KS (2014): The association between cannabis abuse and subsequent schizophrenia: A Swedish national co-relative control study. Psychol Med 3:1–8.
13. Compton MT, Kelley ME, Ramsay CE, Pringle M, Goulding SM, Esterberg ML, et al. (2009): Association of pre-onset cannabis, alcohol, and tobacco use with age at onset of prodrome and age at onset of psychosis in first-episode patients. Am J Psychiatry 166:1251–1257.
14. Galvez-Buccollini JA, Proal AC, Tomaselli V, Trachtenberg M, Coconcea C, Chun J, et al. (2012): Association between age at onset of psychosis and age at onset of cannabis use in non-affective psychosis. Schizophr Res 139:157–160.
15. Veen ND, Selten JP, van der Tweel I, Feller WG, Hoek HW, Kahn RS (2004): Cannabis use and age at onset of schizophrenia. Am J Psychiatry 161:501–506.
16. Large M, Sharma S, Compton MT, Slade T, Nielssen O (2011): Cannabis use and earlier onset of psychosis: A systematic meta-analysis. Arch Gen Psychiatry 68:555–561.
17. Di Forti M, Sallis H, Allegri F, Trotta A, Ferraro L, Stilo SA, et al. (2014): Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. Schizophr Bull 40:1509–1517.
18. Manrique-Garcia E, Zammit S, Dalman C, Hemmingsson T, Andreasson S, Allebeck P (2014): Prognosis of schizophrenia in persons with and without a history of cannabis use. Psychol Med 44:2513–2521.
19. Green MF (2006): Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. J Clin Psychiatry 67(suppl 9):3–8.
20. Lesh TA, Niendam TA, Minzenberg MJ, Carter CS (2011): Cognitive control deficits in schizophrenia: Mechanisms and meaning. Neuropsychopharmacology 36:316–338.
21. Kahn RS, Keefe RS (2013): Schizophrenia is a cognitive illness: Time for a change in focus. JAMA Psychiatry 70:1107–1112.
22. Lisdahl KM, Gilbart ER, Wright NE, Shollenbarger S (2013): Dare to delay? The impacts of adolescent alcohol and marijuana use onset on cognition, brain structure, and function. Front Psychiatry 4:53.
23. Meier MH, Caspi A, Ambler A, Harrington H, Houts R, Keefe RS, et al. (2012): Persistent cannabis users show neuropsychological decline from childhood to midlife. Proc Natl Acad Sci U S A 109:E2657–E2664.
24. Rabin RA, Zakzanis KK, George TP (2011): The effects of cannabis use on neurocognition in schizophrenia: A meta-analysis. Schizophr Res 128:111–116.
25. Yucel M, Bora E, Lubman DI, Solowij N, Brewer WJ, Cotton SM, et al. (2012): The impact of cannabis use on cognitive functioning in patients with schizophrenia: A meta-analysis of existing findings and new data in a first-episode sample. Schizophr Bull 38:316–330.
26. Ferraro L, Russo M, O'Connor J, Wiffen BD, Falcone MA, Sideli L, et al. (2013): Cannabis users have higher premorbid IQ than other patients with first onset psychosis. Schizophr Res 150:129–135.
27. Joyal CC, Halle P, Lapierre D, Hodgins S (2003): Drug abuse and/or dependence and better neuropsychological performance in patients with schizophrenia. Schizophr Res 63:297–299.
28. McCleery A, Addington J, Addington D (2006): Substance misuse and cognitive functioning in early psychosis: A 2 year follow-up. Schizophr Res 88:187–191.
29. Potvin S, Joyal CC, Pelletier J, Stip E (2008): Contradictory cognitive capacities among substance-abusing patients with schizophrenia: A meta-analysis. Schizophr Res 100:242–251.
30. Schneider M, Koch M (2003): 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 28:1760–1769.
31. O'Shea M, Singh ME, McGregor IS, Mallet PE (2004): Chronic cannabinoid exposure produces lasting memory impairment and increased anxiety in adolescent but not adult rats. J Psychopharmacol 18:502–508.
32. Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N, et al. (2008): 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 33:1113–1126.
33. Verrico CD, Gu H, Peterson ML, Sampson AR, Lewis DA (2014): Repeated delta9-tetrahydrocannabinol exposure in adolescent monkeys: Persistent effects selective for spatial working memory. Am J Psychiatry 171:416–425.
34. Miller EK, Cohen JD (2001): An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167–202.
35. Goldman-Rakic PS (1995): Cellular basis of working memory. Neuron 14:477–485.
36. Gonzalez-Burgos G, Lewis DA (2012): NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr Bull 38:950–957.
37. Gonzalez-Burgos G, Lewis DA (2008): GABA neurons and the mechanisms of network oscillations: Implications for understanding cortical dysfunction in schizophrenia. Schizophr Bull 34:944–961.
38. Howard MW, Rizzuto DS, Caplan JB, Madsen JR, Lisman J, Aschenbrenner-Scheibe R, et al. (2003): Gamma oscillations correlate with working memory load in humans. Cereb Cortex 13:1369–1374.
39. Glantz LA, Lewis DA (2000): Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Arch Gen Psychiatry 57:65–73.
40. Kolluri N, Sun Z, Sampson AR, Lewis DA (2005): Lamina-specific reductions in dendritic spine density in the prefrontal cortex of subjects with schizophrenia. Am J Psychiatry 162:1200–1202.

41. Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, et al. (2003): Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci 23: 6315–6326.

42. Mellios N, Huang HS, Baker SP, Galdzicka M, Ginns E, Akbarian S (2009): Molecular determinants of dysregulated GABAergic gene expression in the prefrontal cortex of subjects with schizophrenia. Biol Psychiatry 65:1006–1014.

43. Fung SJ, Webster MJ, Sivagnanasundaram S, Duncan C, Elashoff M, Weickert CS (2010): Expression of interneuron markers in the dorsolateral prefrontal cortex of the developing human and in schizophrenia. Am J Psychiatry 167:1479–1488.

44. Volk DW, Matsubara T, Li S, Sengupta EJ, Georgiev D, Minabe Y, et al. (2012): Deficits in transcriptional regulators of cortical parvalbumin neurons in schizophrenia. Am J Psychiatry 169:1082–1091.

45. Volk DW, Edelson JR, Lewis DA (2014): Cortical inhibitory neuron disturbances in schizophrenia: Role of the ontogenetic transcription factor Lhx6. Schizophr Bull 40:1053–1061.

46. Glausier JR, Fish KN, Lewis DA (2014): Altered parvalbumin basket cell inputs in the dorsolateral prefrontal cortex of schizophrenia subjects. Mol Psychiatry 19:30–36.

47. Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr, Jones EG (1995): Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatry 52:258–266.

48. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA (2000): Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry 57:237–245.

49. Guidotti A, Auta J, Davis JM, Gerevini VD, Dwivedi Y, Grayson DR, et al. (2000): Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder. Arch Gen Psychiatry 57:1061–1069.

50. Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, et al. (2007): Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. Mol Psychiatry 12:854–869.

51. Duncan CE, Webster MJ, Rothmond DA, Bahn S, Elashoff M, Shannon WC (2010): Prefrontal GABA(A) receptor alpha-subunit expression in normal postnatal human development and schizophrenia. J Psychiatr Res 44:673–681.

52. Curley AA, Arion D, Volk DW, Asafu-Adjei JK, Sampson AR, Fish KN, Lewis DA (2011): Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: Clinical, protein, and cell type-specific features. Am J Psychiatry 168:921–929.

53. Cho RY, Konecky RO, Carter CS (2006): Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. Proc Natl Acad Sci U S A 103:19878–19883.

54. Minzenberg MJ, Firl AJ, Yoon JH, Gomes GC, Reinking C, Carter CS (2010): Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. Neuropsychopharmacology 35:2590–2599.

55. Eggan SM, Lewis DA (2007): Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: A regional and laminar analysis. Cereb Cortex 17:175–191.

56. Karson MA, Tang AH, Milner TA, Alger BE (2009): Synaptic cross talk between perisomatic-targeting interneuron classes expressing cholecystokinin and parvalbumin in hippocampus. J Neurosci 29: 4140–4154.

57. Eggan SM, Melchitzky DS, Sesack SR, Fish KN, Lewis DA (2010): Relationship of cannabinoid CB1 receptor and cholecystokinin immunoreactivity in monkey dorsolateral prefrontal cortex. Neuroscience 169:1651–1661.

58. Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, et al. (2006): Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci 26:5628–5637.

59. Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF (1999): Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19:4544–4558.

60. Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N, Freund TF (2001): Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci 21:9506–9518.

61. Hajos N, Katona I, Naiem SS, Mackie K, Ledent C, Mody I, Freund TF (2000): Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. Eur J Neurosci 12:3239–3249.

62. Klausberger T, Marton LF, O'Neill J, Huck JH, Dalezios Y, Fuentealba P, et al. (2005): Complementary roles of cholecystokinin- and parvalbumin-expressing GABAergic neurons in hippocampal network oscillations. J Neurosci 25:9782–9793.

63. Foldy C, Lee SY, Szabadics J, Neu A, Soltesz I (2007): Cell type-specific gating of perisomatic inhibition by cholecystokinin. Nat Neurosci 10:1128–1130.

64. Karson MA, Whittington KC, Alger BE (2008): Cholecystokinin inhibits endocannabinoid-sensitive hippocampal IPSPs and stimulates others. Neuropharmacology 54:117–128.

65. Katona I, Freund TF (2012): Multiple functions of endocannabinoid signaling in the brain. Annu Rev Neurosci 35:529–558.

66. Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, et al. (2010): International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB(1) and CB(2). Pharmacol Rev 62:588–631.

67. Stella N, Schweitzer P, Piomelli D (1997): A second endogenous cannabinoid that modulates long-term potentiation. Nature 388: 773–778.

68. Hashimotodani Y, Ohno-Shosaku T, Kano M (2007): Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus. J Neurosci 27:1211–1219.

69. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, et al. (2003): Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163:463–468.

70. Yoshida T, Fukaya M, Uchigashima M, Miura E, Kamiya H, Kano M, Watanabe M (2006): Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. J Neurosci 26:4740–4751.

71. Ludanyi A, Hu SS, Yamazaki M, Tanimura A, Piomelli D, Watanabe M, et al. (2011): Complementary synaptic distribution of enzymes responsible for synthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in the human hippocampus. Neuroscience 174: 50–63.

72. Yoshida T, Uchigashima M, Yamasaki M, Katona I, Yamazaki M, Sakimura K, et al. (2011): Unique inhibitory synapse with particularly rich endocannabinoid signaling machinery on pyramidal neurons in basal amygdaloid nucleus. Proc Natl Acad Sci U S A 108: 3059–3064.

73. Szabo B, Urbanski MJ, Bisogno T, Di MV, Mendiguren A, Baer WU, Freiman I (2006): Depolarization-induced retrograde synaptic inhibition in the mouse cerebellar cortex is mediated by 2-arachidonoylglycerol. J Physiol 577:263–280.

74. Hashimotodani Y, Ohno-Shosaku T, Maejima T, Fukami K, Kano M (2008): Pharmacological evidence for the involvement of diacylglycerol lipase in depolarization-induced endocanabinoid release. Neuropharmacology 54:58–67.

75. Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M (2007): Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 27:3663–3676.

76. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. (2002): Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A 99:10819–10824.

77. Blankman JL, Simon GM, Cravatt BF (2007): A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 14:1347–1356.

78. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, et al. (2010): The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci 13:951–957.

79. Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, Freund TF (2004): Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci 20:441–458.

80. Makara JK, Mor M, Fegley D, Szabo SI, Kathuria S, Astarita G, et al. (2005): Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. Nat Neurosci 8:1139–1141.

81. Kim J, Alger BE (2004): Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. Nat Neurosci 7:697–698.

82. Hermanson DJ, Hartley ND, Gamble-George J, Brown N, Shonesy BC, Kingsley PJ, et al. (2013): Substrate-selective COX-2 inhibition decreases anxiety via endocannabinoid activation. Nat Neurosci 16:1291–1298.

83. Bluett RJ, Gamble-George JC, Hermanson DJ, Hartley ND, Marnett LJ, Patel S (2014): Central anandamide deficiency predicts stress-induced anxiety: Behavioral reversal through endocannabinoid augmentation. Transl Psychiatry 4:e408.

84. Brown JA, Horvath S, Garbett KA, Schmidt MJ, Everheart M, Gellert L, et al. (2014): The role of cannabinoid 1 receptor expressing interneurons in behavior. Neurobiol Dis 63:210–221.

85. Shonesy BC, Bluett RJ, Ramikie TS, Baldi R, Hermanson DJ, Kingsley PJ, et al. (2014): Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. Cell Rep 9:1644–1653.

86. Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, Mackie K, et al. (2014): Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. Neuron 81:1111–1125.

87. Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, Faulhaber J, et al. (2004): Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology 29:2108–2114.

88. Koethe D, Giuffrida A, Schreiber D, Hellmich M, Schultze-Lutter F, Ruhrmann S, et al. (2009): Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. Br J Psychiatry 194:371–372.

89. De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, Di Marzo V (2003): Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis 2:5.

90. Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D (1999): Elevated endogenous cannabinoids in schizophrenia. Neuroreport 10:1665–1669.

91. Palkovits M, Harvey-White J, Liu J, Kovacs ZS, Bobest M, Lovas G, et al. (2008): Regional distribution and effects of postmortal delay on endocannabinoid content of the human brain. Neuroscience 152:1032–1039.

92. Volk DW, Eggan SM, Lewis DA (2010): Alterations in metabotropic glutamate receptor 1alpha and regulator of G protein signaling 4 in the prefrontal cortex in schizophrenia. Am J Psychiatry 167:1489–1498.

93. Volk DW, Siegel BI, Verrico CD, Lewis DA (2013): Endocannabinoid metabolism in the prefrontal cortex in schizophrenia. Schizophr Res 147:53–57.

94. Dean B, Sundram S, Bradbury R, Scarr E, Copolov D (2001): Studies on [3H]CP-55940 binding in the human central nervous system: Regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience 103:9–15.

95. Zavitsanou K, Garrick T, Huang XF (2004): Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 28:355–360.

96. Newell KA, Deng C, Huang XF (2006): Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Exp Brain Res 172:556–560.

97. Dalton VS, Long LE, Weickert CS, Zavitsanou K (2011): Paranoid schizophrenia is characterized by increased CB1 receptor binding in the dorsolateral prefrontal cortex. Neuropsychopharmacology 36:1620–1630.

98. Jenko KJ, Hirvonen J, Henter ID, Anderson KB, Zoghbi SS, Hyde TM, et al. (2012): Binding of a tritiated inverse agonist to cannabinoid CB1 receptors is increased in patients with schizophrenia. Schizophr Res 141:185–188.

99. Wong DF, Kuwabara H, Horti AG, Raymont V, Brasic J, Guevara M, et al. (2010): Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. Neuroimage 52:1505–1513.

100. Wong DF, Kuwabara H, Horti AG, Brasic JR, Raymont V, Nandi A, et al. (2012): Cannabinoid receptor subtype 1 (CB1) distribution correlates with neuropsychiatric ratings. Biol Psychiatry Abstract. 71:8S.

101. Eggan SM, Hashimoto T, Lewis DA (2008): Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. Arch Gen Psychiatry 65:772–784.

102. Thune JJ, Uylings HBM, Pakkenberg B (2001): No deficit in total number of neurons in the prefrontal cortex in schizophrenics. J Psychiatr Res 35:15–21.

103. Koethe D, Llenos IC, Dulay JR, Hoyer C, Torrey EF, Leweke FM, Weis S (2007): Expression of CB1 cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. J Neural Transm 114:1055–1063.

104. Uriguen L, Garcia-Fuster MJ, Callado LF, Morentin B, La Harpe R, Casado V, et al. (2009): Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: Effect of antipsychotic treatment. Psychopharmacology (Berl) 206:313–324.

105. Volk DW, Eggan SM, Horti AG, Wong DF, Lewis DA (2014): Reciprocal alterations in cortical cannabinoid receptor 1 binding relative to protein immunoreactivity and transcript levels in schizophrenia. Schizophr Res 159:124–129.

106. Rozenfeld R (2011): Type I cannabinoid receptor trafficking: All roads lead to lysosome. Traffic 12:12–18.

107. Eggan SM, Mizoguchi Y, Stoyak SR, Lewis DA (2010): Development of cannabinoid 1 receptor protein and messenger RNA in monkey dorsolateral prefrontal cortex. Cereb Cortex 20:1164–1174.

108. Hoftman GD, Lewis DA (2011): Postnatal developmental trajectories of neural circuits in the primate prefrontal cortex: Identifying sensitive periods for vulnerability to schizophrenia. Schizophr Bull 37:493–503.

109. Hoftman GD, Volk DW, Bazmi HH, Li S, Sampson AR, Lewis DA (2013): Altered cortical expression of GABA-related genes in schizophrenia: Illness progression vs developmental disturbance. Schizophr Bull 41:180–191.

110. Hyde TM, Lipska BK, Ali T, Mathew SV, Law AJ, Metitiri OE, et al. (2011): Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. J Neurosci 31:11088–11095.

111. Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, et al. (2006): The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J Neurosci 26:2991–3001.

112. Eggan SM, Stoyak SR, Verrico CD, Lewis DA (2010): Cannabinoid CB1 receptor immunoreactivity in the prefrontal cortex: Comparison of schizophrenia and major depressive disorder. Neuropsychopharmacology 35:2060–2071.

113. Eggan SM, Lazarus MS, Stoyak SR, Volk DW, Glausier JR, Huang ZJ, Lewis DA (2012): Cortical glutamic acid decarboxylase 67 deficiency results in lower cannabinoid 1 receptor messenger RNA expression: Implications for schizophrenia. Biol Psychiatry 71:114–119.

114. Cass DK, Flores-Barrera E, Thomases DR, Vital WF, Caballero A, Tseng KY (2014): CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. Mol Psychiatry 19:536–543.