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Susceptibility of the adolescent brain to cannabinoids: long-term hippocampal effects and relevance to schizophrenia

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Clinical studies report associations between cannabis use during adolescence and later onset of schizophrenia. We examined the causal relationship between developmental cannabinoid administration and long-term behavioral and molecular alterations in mice. Mice were administered either WIN 55,212-2 (WIN), a cannabinoid receptor 1 (CB1) agonist or vehicle (Veh) during adolescence (postnatal day 30–35) or early adulthood (postnatal day 63–70). Behavioral testing was conducted after postnatal day 120 followed by biochemical assays. Adolescent cannabinoid treatment (ACU) leads to deficits in prepulse inhibition and fear conditioning in adulthood. Metabotropic glutamate receptors type 5 (mGluR5), a receptor critically involved in fear conditioning and endocannabinoid (eCB) signaling, is significantly reduced in the ACU mouse hippocampus. Next, we examined expression profiles of genes involved in eCB synthesis (diacylglycerol lipase (DGL)) and uptake (monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH)) in the experimental mice. We find evidence of increased MGL and FAAH in ACU mice, reflecting increases in eCB uptake and degradation. These data suggest that administration of cannabinoids during adolescence leads to a behavioral phenotype associated with a rodent model of schizophrenia, as indexed by alterations in sensorimotor gating and hippocampal-dependent learning and memory deficits. Further, these deficits are associated with a reduction in hippocampal mGluR5 and a sustained change in eCB turnover, suggesting reduced eCB signaling in the ACU hippocampus. These data suggest that significant cannabis use during adolescence may be a contributory causal factor in the development of certain features of schizophrenia and may offer mGluR5 as a potential therapeutic target.

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Introduction

Cannabis is the most commonly abused illicit drug in the United States, with about 60% of the 2.4 million marijuana initiates in 2010 being < 18 years of age.[1] Daily marijuana use is now at a 30-year peak level among high-school seniors.[2] This is of particular health concern given the large body of literature that shows an association between adolescent cannabis use and adult onset of psychosis.[3–8] A recent systematic review of longitudinal studies of cannabis use and subsequent psychotic outcomes reported a 40% increased risk of psychotic outcome in individuals who had ever used cannabis (pooled adjusted odds ratio = 1.41, 95% confidence interval 1.20 ± 1.65).[9] The risk rose in a dose-dependent fashion with greater cannabis exposure (odds ratio = 2.09, 1.54 ± 2.84). Schizophrenia did not develop days or weeks after cannabis use but years later, suggesting that cannabis use during a critical period of brain maturation may lead to long-term effects. These human studies demonstrate associations but do not demonstrate causality.

$\Delta 9$-Tetrahydrocannanol ($\Delta 9$-THC), the main psychoactive constituent of *Cannabis sativa*, binds to cannabinoid receptors in the brain. To date, two G-protein-coupled cannabinoid receptors, cannabinoid receptor 1 (CB1) and CB2, have been discovered. CB1 receptors are highly expressed in the brain, particularly in the cortex, hippocampus and striatum. The principal endogenous ligands for the CB receptors include the endocannabinoids (eCBs) 2-AG (2–arachidonoylglycerol) and anandamide (N-arachidonoylethanolamide, AEA), with 2-AG being predominant in the hippocampus.[10–12] The key synthetic enzyme for 2-AG is diacylglycerol lipase (DGL), whereas several routes for AEA synthesis have been described. Inactivation of these eCBs occur predominantly through monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH) for 2-AG and AEA, respectively.[13] In the brain, eCBs are synthesized on demand in post-synaptic neurons, released into the synapse where they activate presynaptic CB1 receptors in a retrograde manner to inhibit neurotransmitter release. In the rodent and human hippocampus, CB1 receptors are predominantly expressed on gamma-aminobutyric acid (GABA) terminals,[14–16] implicating a role in GABA neurotransmission. eCBs are key activity-dependent molecules in the regulation of synaptic transmission in the brain and are involved in numerous neural processes, including memory and cognition.

Previous studies show that Δ^9-tetrahydrocannabinol (Δ^9-THC) or CB1 receptor agonists administered prenatally or peri-pubertally leads to behavioral deficits in rodents,[17–22] suggesting that adolescence may be a developmental period

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during which the brain is susceptible to the effects of exogenous cannabinoids. In addition, human post-mortem studies have identified alterations in the eCB system in schizophrenia.[23–26] We conducted a series of experiments in mice to (i) examine the cause–effect relationship between adolescent cannabinoid treatment (ACU) and development of schizophrenia-like behaviors and (ii) to determine whether exposure to exogenous cannabinoids during adolescence has a lasting impact on metabotropic glutamate receptors type 5 (mGluR5) and CB1 receptor expression, genes critically involved in eCB signaling.

Methods

Behavioral experiments. C57BL6 mice were obtained from Jackson Laboratories, (Bar Harbor, ME), housed on a 12:12-h light–dark cycle in a temperature- and humidity-controlled environment with *ad libitum* access to food and water. Mice were administered either a CB1 agonist (WIN 55,212-2, $2 \, mg \, kg^{-1}$) or vehicle ($n = 10$ per group) for 3–5 or 10 consecutive days by intraperitoneal injection. Injections were administered to mice starting on either postnatal day 30 or 63 to reflect adolescence and adulthood, respectively. After drug administration, the animals were left undisturbed until postnatal day 120 at which time they underwent behavioral testing. Behavioral tests included locomotor activity, prepulse inhibition (PPI), social interaction and fear conditioning in that order (Figure 1). Two weeks after behavioral tests were completed, the mice were killed, brains immediately removed, hippocampus dissected, snap frozen and processed for immunoblotting studies. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the UT Southwestern Medical Center and are in accordance with the National Institutes of Health policy on the care and use of laboratory animals.

Fear conditioning. Fear conditioning was measured in boxes equipped with a metal grid floor connected to a scrambled shock generator (Med Associates, St Albans, VT). For training, mice were individually placed in the chamber. After 2 min, the mice received two tone-shock pairings (30 s white noise, 95 dB tone co-terminated with a 2 s, 0.5 mA foot shock, 1 min inter-trial interval). The following day, memory of the context was measured by placing the mice into the same chambers and freezing was measured every 5 s for 5 min. Forty-eight hours after training, memory for the white noise cue was measured by placing the mice in a box with altered floors and walls, different lighting and a vanilla smell. Freezing was measured every 4–5 s for 3 min, then the noise cue was turned on for an additional 3 min and freezing was measured every 5 s.

Baseline startle and PPI. Startle was measured using a San Diego Instruments SR-Lab Startle Response System (San Diego, CA). Mice were placed into the Plexiglas holders and allowed to acclimate to the chamber and background white noise (70 dB) for 5 min. After the acclimation period, startle stimuli (120 dB, 40 ms, white noise) were presented with an average interstimulus interval of 20 s (range 13–27 s). The maximum startle amplitude was measured. The Plexiglas

holders were wiped and allowed to dry between mice. Startle was measured using a San Diego Instruments SR-Lab Startle Response System (San Diego, CA). Mice were placed into the Plexiglas holders and allowed to acclimate to the chamber and background white noise (70 dB) for 5 min. After the acclimation period, six startle stimuli (120 dB, 40 ms, white noise) were presented with an average interstimulus interval of 15 s (range 7–23 s, these data were not used to calculate PPI), followed by 40-startle stimuli preceded by a prepulse stimulus (20 ms prepulse preceding the 120-dB stimulus by 100 ms). The prepulse intensities were 0, 4, 8 or 16 dB above the background noise and were presented in a pseudorandom order. The Plexiglas holders were wiped and allowed to dry between mice.

Locomotor activity. Mice were placed individually into a clean, plastic mouse cage ($18 \, cm \times 28 \, cm$) with minimal bedding. Each cage was placed into a dark Plexiglas box. Movement was monitored by five photobeams in one dimension (Photobeam Activity System, San Diego Instruments, San Diego, CA) for 2 h, with the number of beam breaks recorded every 5 min. Movement was characterized in three ways: repetitive beam breaks of a single beam is classified as stereotypy, consecutive beam breaks of $\geqslant 2$ beams is classified as ambulatory movements and total beam breaks during each 5-min interval.

Social interaction. A mouse was placed in the center of a novel open field environment ($44 \, cm \times 44 \, cm$, walls 30 cm high) in a dimly lit room and allowed to explore for 5 min. A small plastic chamber (the 'interaction box', $8.5 \, cm \times 4.5 \, cm$) was placed along one wall of the arena. After 5 min, the test mouse was removed and a novel, unfamiliar mouse was placed into the interaction box. Small holes in the interaction box allow the mice to see, hear and smell each other. The test mouse was returned to the center of the open field environment and allowed to explore for another 5 min. The test mouse was monitored from above by a video camera connected to a computer running video-tracking software (Ethovision 3.0, Noldus, Leesburg, VA). The time the test mouse spent in the area immediately adjacent (within 8 cm) to the interaction chamber was recorded as the interaction time. Total activity within the arena was also measured.

Immunoblotting experiments. Bilateral hippocampi from each mouse were pulverized on dry ice and homogenized in buffer ($1 \times$ phosphate-buffered saline containing 1% sodium dodecyl sulfate, 1 mM phenylmethanesulfonylfluoride, $20 \, mg \, ml^{-1}$ leupeptin, $10 \, mg \, ml^{-1}$ pepstatinA, $2 \, mg \, ml^{-1}$ aprotinin). In all, 20 mg protein per sample was loaded in duplicate on a 10% polyacrylamide gel, transferred to nitrocellulose membrane, blocked for 30 min at room temperature (5% non-fat dry milk, 0.1% Tween20, 50 mM Tris-buffered saline; TBS, pH7.5) and then incubated overnight at $4 \, ^\circ C$ with mGluR5 (1:1000), CB1 (1:1000), MGL (1:1000), FAAH (1: 1000), DGL (1:500) or norbin (1:350). After washing, blots were incubated with respective secondary antibody for 30 min. β-Tubulin or vcp (valosin-containing protein) was used as a loading control. Immunoreactive proteins were detected using enhanced

chemiluminescence (Amersham, NJ) using Fuji film (Light Labs Company, Dusseldorf, Germany). Film-based images of immunoblots were scanned and bands of interest quantified using ImageQuant software (Amersham,UK) blind to the treatment group. Antibodies were obtained commercially: CB1 and mGluR5 (Abcam, Cambridge, MA), MGL (Thermoscientific, Rockford, IL), FAAH, DGL and norbin (Santa Cruz Biotechnology, Santa Cruz, CA).

Statistical analysis. For behavioral data, two-way analyses of variance were used to examine significant main effects of age (adolescent or adult), drug (WIN 55,212-2 or vehicle) and age × drug interactions. Significant findings were further analyzed using *post hoc t* tests. Immunoblotting data were quantified and analyzed by unpaired *t*-tests for each gene target. Pearson's Product Moment correlations were conducted to determine relationships between measured target molecules and behavior. Values outside two s.d.s away from the mean were considered outliers and not included in the statistical analyses. All statistical analyses were conducted using Statistica software (StatSoft Inc., Tulsa, OK, USA). Significance was taken as $P < 0.05$ for all experiments.

Results

ACU leads to long-lasting behavioral deficits. Mice treated with CB1 agonist during adolescence show significant long-lasting deficits in sensorimotor gating and hippocampal-dependent contextual learning in adulthood. These deficits were not observed in mice treated with the CB1 agonist during adulthood.

PPI: Mice treated with WIN 55,212-2 during adolescence display significant deficits in PPI. There is a significant interaction of age ($F = 12.37$, df (1,36), $P = 0.0012$), no effect
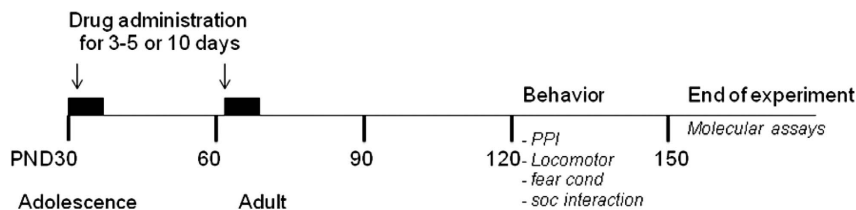

Figure 1 Overview of experimental timeline. Mice were administered cannabinoid receptor 1 agonist (WIN 55,212-2) or vehicle for either 3–5 or 10 days starting at postnatal day (PND) 30 or PND 63, tested behaviorally after PND 120 and killed 2 weeks later. Cond, conditioning; PPI, prepulse inhibition; soc, social.
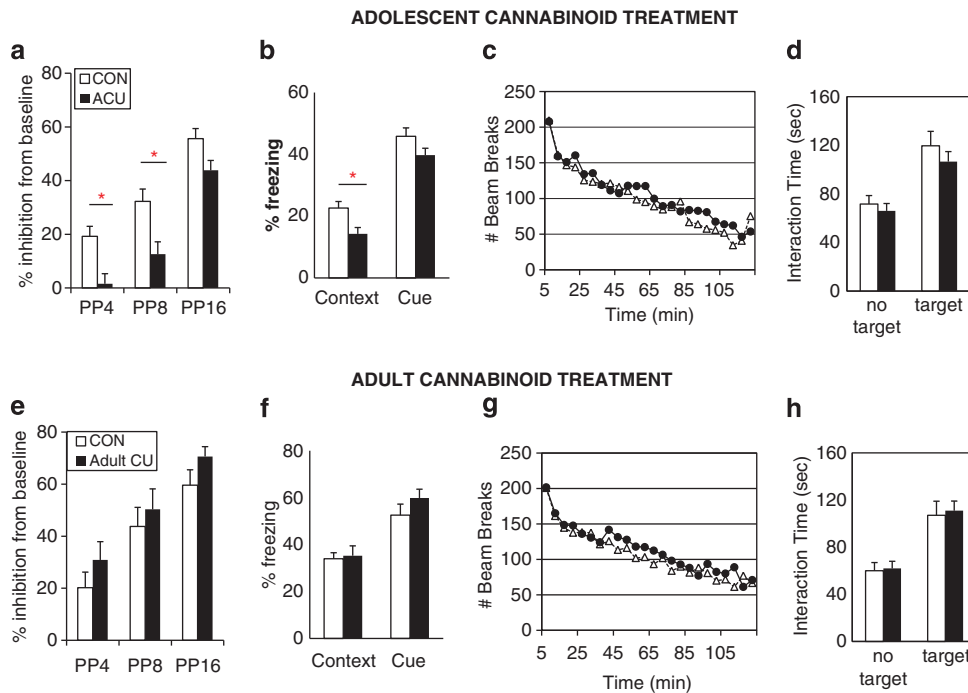

Figure 2 Adolescent cannabinoid treatment (ACU) leads to long-term behavioral deficits. Mice were administered WIN 55,212-2 (white bars) or vehicle (black bars) for 10 days during adolescence (**a–d**) or adulthood (**e–h**) and tested behaviorally as 4-month-old adults (*n* = 10 mice per group). (**a, e**) Prepulse inhibition: the startle response was measured with prepulse intensities 0, 4, 8, or 16 dB above the background noise presented in a pseudorandom order. (**b, f**) Contextual and cued fear conditioning show significant deficits in contextual but not cued freezing in ACU, but not adult CU, mice compared with control (CON) mice. (**c, g**) Locomotor activity: movement was monitored by five photobeams in one dimension for 2 h. Data are expressed as the average number of beam breaks at each 5-min bin per 2-hour test. (**d, h**) Social interaction: time spent by test mouse in the area immediately adjacent to the interaction chamber either with or without a novel, unfamiliar mouse. All data are presented as means + s.e.m. Asterisk represents significant differences at $P \leqslant 0.05$.

of drug (F = 0.44, df (1,36), $P = 0.51$) and a significant age × drug interaction (F = 6.09, df (2,27), $P = 0.018$). *Post hoc* analyses show that ACU mice show reduced PPI at prepulse intensities 4 dB ($t = 2.1$, df 18, $P = 0.049$) and 8 dB ($t = 2.2$, df 18, $P = 0.041$) above background. Adult-CU mice, however, do not show any significant change in PPI at any of the prepulse intensities (all t between 0.6 and 1.6, all $P > 0.13$) (Figure 2a and 2e).

Fear conditioning: Mice treated with WIN 55,212-2 during adolescence display significant deficits in contextual learning. There is a significant interaction of age (F = 72, df (1,36), $P < 0.0001$), drug (F = 8.1, df (1,36), $P = 0.007$) and a significant age × drug interaction (F = 8.1, df, p = 0.007). *Post hoc* analyses show that ACU mice show reduced freezing compared with controls ($t = 2.85$, df 18, $P = 0.011$). Adult-CU mice, however, do not show any significant change in the contextual fear conditioning ($t = 0.27$, df 18, p = 0.78), Similarly, two-way analysis of variance conducted for cued fear conditioning shows a significant interaction of age (F = 57.3, df (1,36), $P < 0.001$), drug (F = 5.4, df (1,36), $P = 0.03$) and a significant age × drug interaction (F = 5.6, df (1,36), $P = 0.02$). *Post hoc* analyses show that ACU mice show reduced freezing compared with controls ($t = 2.34$, df 18, $P = 0.031$). Adult-CU mice, however, do not show any significant change in cued fear conditioning ($t = 1.22$, df 18, $P = 0.24$,). Mice treated with WIN 55,212-2 during adolescence for a shorter duration between 3–5 days did not display any fear-conditioning deficits in the contextual ($t = 0.62$, df 38, $P = 0.54$) or cued paradigms ($t = 0.83$, df 38, $P = 0.41$) (Figure 2b and 2f).

Social interaction: There were no significant differences in interaction time between groups. There were no interactions of age (F = 0.78, df (1,36), $P = 0.38$), drug (F = 0.22, $P = 0.64$) or age × drug interaction (F = 0.80, df (1,36), $P = 0.38$) (Figure 2d and 2h).

Locomotor activity: There were no significant differences in total locomotor activity. There were no interactions of age (F = 1.54, df (1,36), $P = 0.22$), drug (F = 1.14, df (1,36), $P = 0.29$) or age × drug interaction (F = 0.002, df (1,36), $P = 0.96$) (Figure 2c and 2g).

Molecular assays. mGluR5 is critically involved in fear conditioning with mGluR5 knockout mice showing impairments in acquisition of fear, deficits in the ability to extinguish contextual fear and poor performance in novelty detection,[27,28] all tasks that involve the hippocampus. We measured mGluR5 protein expression in the hippocampus of the ACU mice and found significant reductions in mGluR5 levels ($t = 3.02$, df 18, $P = 0.007$) (Figure 3). In addition, mGluR5 receptor activation is one mechanism for activating eCB synthesis and release, suggesting that eCB signaling might be altered in ACU mice. We measured CB1, DGL (synthetic enzyme for 2-AG), MGL (metabolic enzyme for 2-AG) and FAAH (metabolic enzyme for AEA) protein levels in the hippocampus of the experimental mice. We found significant increases in both MGL ($t = 4.2$, df 13, $P = 0.001$)
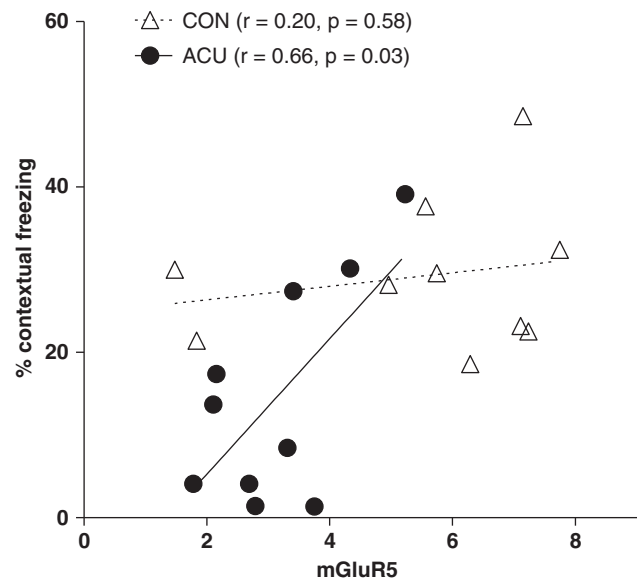
Figure 4 Correlations between contextual freezing behavior and hippocampal mGluR5 protein levels in adolescent cannabinoid treatment (ACU) and control (CON) mice ($n = 10$ mice per group). Siginificant correlations are seen between contextual freezing in ACU mice but not CON mice.
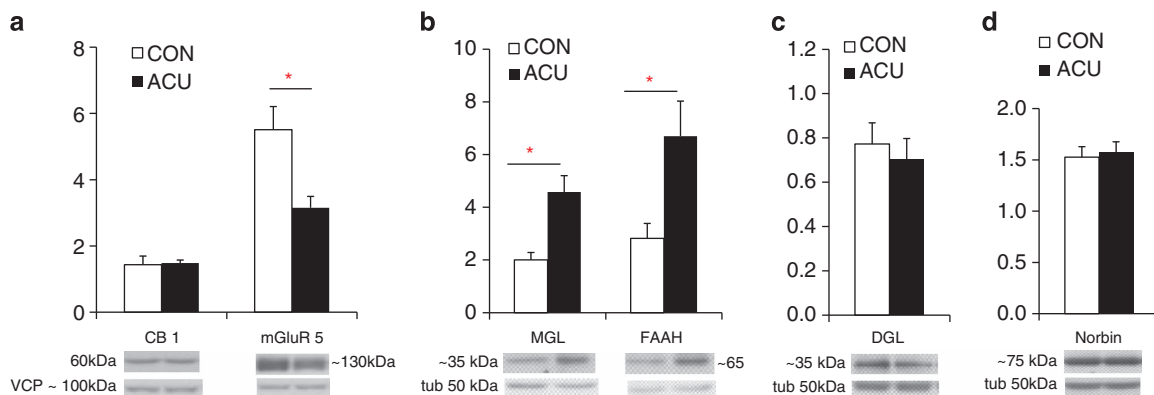
Figure 3 Adolescent cannabinoid treatment (ACU) leads to altered expression of genes involved in endocannabinoid signaling in the hippocampus of ACU and control (CON) mice in adulthood ($n = 10$ mice per group). (**a**) Levels of hippocampal cannabinoid receptor 1 (CB1), mGluR5, (**b**) monoacylglycerol lipase (MGL), fatty acid amide hydrolase (FAAH), (**c**) diacylglycerol lipase (DGL) and (**d**) norbin protein relative to loading controls in ACU (white bars) and CON (black bars). All data are presented as means + s.e.m. Asterisk represents significant differences at $P \leqslant 0.05$. vcp, valosin-containing protein.
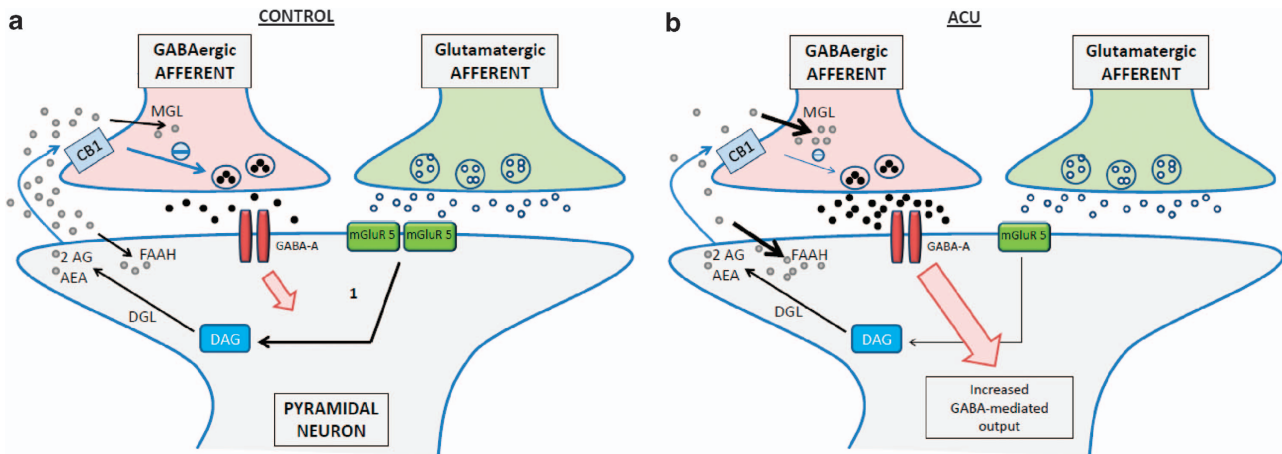
Figure 5 Model of hippocampal deficits induced by adolescent cannabinoid administration. Panel (**a**) shows a control endocannabinoid (eCB) synapse and panel (**b**) shows adolescent cannabinoid treatment (ACU)-induced changes in the adult. Adult mice treated with cannabinoids during adolescence express significantly lower levels of mGluR5, known to stimulate eCB synthesis. This is associated with an upregulation of monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH), enzymes associated with eCB degradation. The net effect is lower eCB production and increased degradation, which would lower eCB levels and reduce cannabinoid receptor 1 (CB1) activation. As CB1 activation inhibits gamma-aminobutyric acid (GABA) release, lower CB1 activation would be expected to lead to greater GABA release in the ACU hippocampus. AEA, N-arachidonoylethanolamide, 2-AG, 2–arachidonoylglycerol; DAG, diacyl glycerol; DGL, diacylglycerol lipase.

and FAAH ($t=2.8$, df_{15}, $P=0.014$) but no changes in CB1 ($t=0.17$, df_{18}, $P=0.86$) or DGL ($t=0.56$, df_{14}, $P=0.59$) between groups. Lastly, we quantified norbin, an endogenous mGluR5 ligand,[29] which was not altered in ACU mice ($t=0.35$, df 16, $P=0.73$).

Receptor-behavior correlations. Given the critical role for mGluR5 in contextual fear conditioning, we examined correlations between contextual freezing and mGluR5 protein levels. In ACU mice, we find strong significant correlations ($r=0.66$, $P=0.039$) that are not seen in vehicle-treated mice ($r=0.20$, $P=0.58$). There were no correlations between freezing behavior and hippocampal CB1 levels in ACU mice ($r=0.31$, $P=0.39$) or controls ($r=0.35$, $P=0.32$) (Figure 4).

Discussion

We find that cannabinoid treatment leads to certain schizophrenia-like behaviors in an age of exposure-dependent manner, providing evidence supportive of the notion that cannabis use during adolescence may have a contributory causative role in schizophrenia. Mice treated with cannabinoids during adolescence exhibit sensorimotor-gating deficits and impaired learning and memory in adulthood, physiological functions known to be affected in schizophrenia. We also find reduced expression of hippocampal mGluR5 receptors, levels of which correlate with the learning and memory deficits in ACU mice.

ACU-induced behavior. Cannabinoid treatment during adolescence, but not adulthood, leads to disruptions of PPI and contextual fear conditioning. PPI deficits are well characterized in schizophrenia and reflect an inability to 'gate' sensory stimuli.[30] Our findings are consistent with previous studies showing a persistent reductions in PPI following Δ9-THC or synthetic cannabinoid treatments during

adolescence.[19,21] Contextual fear conditioning is known to be dependent on the hippocampus. There is high density of CB1 receptors in the hippocampus[31] and direct injection of cannabinoids into the hippocampus acutely impairs memory,[32] possibly by desynchronizing hippocampal neuronal assemblies.[33] Microinjections of THC into the hippocampus, but not other brain regions relevant to maze learning, impair learning in radial arm maze.[34] In addition, hippocampal morphological changes are reported following chronic administration of cannabinoids.[35,36] Thus, cannabinoids affect hippocampal structure and function, a brain region repeatedly implicated in schizophrenia pathophysiology. Declarative memory, critically dependent on the hippocampal,[37–39] is one of the most consistent impairments in memory in schizophrenia.[40–42] These deficits include impairments in the flexible use of learned information,[43–45] deficits in recall[46] and contextual memory.[47] Abnormal hippocampal activation is observed while schizophrenia volunteers perform declarative memory tasks.[48–50] These deficits may be similar to the hippocampal-dependent contextual fear-conditioning deficits induced by ACU in our mouse model. Previous studies have reported deficits in object recognition and spatial learning in cannabinoid-treated rats,[19,32,51–54] supporting the idea that ACU leads to hippocampal deficits.[55,56]

ACU-induced molecular changes. The ACU-induced reductions in mGluR5 are of particular interest. mGluR5 knockout mice show reduced hippocampal long-term potentiation associated with deficits in PPI that can be reversed by chronic clozapine treatment.[57] Administration of mGluR5 antagonists (MPEP (2-Methyl-6-(phenylethynyl)-pyridine), MTEP (3-((2-methyl-4-thiazolyl)ethynyl)pyridine)) augments psychotomimetic effects of NMDA (N-methyl-D-aspartate) receptor antagonists[58] while mGluR5 agonists and positive allosteric modulators attenuate these effects.[59,60] Modulators of mGluR5 are being developed as novel treatments for schizophrenia.[61] Further, mGluR5 is critically involved in fear

conditioning,[27] with mGluR5 knockout mice showing impairments in acquisition of fear and deficits in the ability to extinguish contextual fear and performing poorly on detecting novelty.[28] This is consistent with our data showing reduced mGLuR5 in the ACU hippocampus.

Next, we examined expression of eCB genes. The rationale for this was twofold. Firstly, modulation of the eCB system by exogenous cannabinoids could impact the trajectory of the developing eCB system. Secondly, we found reduced mGluR5, the activation of which is known to mobilize eCBs. We find that the enzymes involved in eCB inactivation, MGL and FAAH are significantly increased in the ACU hippocampus. This observation, together with reduced mGluR5, suggests that there is reduced eCB synthesis and greater eCB degradation. Both 2-AG and AEA are present in high concentrations during adolescence[62] in the rodent brain, and treatment with THC during adolescence acutely increases 2-AG concentrations.[62] It is possible that chronic administration of exogenous cannabinoids alters the dynamics of the eCB system in an attempt to maintain normal eCB signaling. This could be in the form of reducing synthesis of eCB, increasing degradation or reduction in CB1 receptor expression. We find evidence of reduced synthesis and increased breakdown of eCB but did not find any long-term change in CB1 receptor expression. This is, however, consistent with a previous rodent studies that quantified CB1 expression following adolescent THC treatment[22] but differs from other studies.[63] Another study reports acute but transient reductions in CB1 expression following chronic administration of the synthetic cannabinoid agonist, CP-55940, during adolescence.[64] A similar pattern of CB1 transient downregulation is seen in human PET (positron emission tomography) imaging studies of the CB1 receptor in chronic cannabis, but, with abstinence, CB1 receptor density returned to normal levels.[65] It has also been proposed that adolescent CB1 receptors contribute to learning impairments in ACU mice by virtue of their functional properties.[66] Adolescent CB1 receptors are less functionally active during adolescence, desensitize and develop tolerance to THC more slowly than the adult rodent which may be one reason that adolescent rodents find Δ^9-THC less aversive compared with adults.[53] This delay in CB1 homeostatic adaptation, not CB1 density, has been postulated to contribute to the long-term cognitive deficits in ACU mice.[66] It is also possible that long-term ACU-induced gene expression changes are mediated via epigenetic mechanisms. Drugs of abuse can alter specific gene programs by altering chromatin structure on specific gene promoters,[67] and histone-associated heterochromatin structural changes are known to occur in hippocampal long-term potentiation and memory formation.[68] Our data suggests that ACU leads to persistent changes in eCB system in the hippocampus that may impact long-term plasticity[69,70] and subsequent hippocampal-dependent learning and memory as seen in schizophrenia.

Model of ACU-induced hippocampal deficits: relevance to schizophrenia. CB1Rs are almost exclusively expressed on GABA-containing interneurons,[16,71] but may also exist on glutamate terminals.[72–74] Cholecystokinin-containing axon terminals contain high levels of CB1 receptors in rodents[14,71] and humans[23] and are more sensitive to the effects of CB1

receptor agonists than pyramidal cell axon terminals.[73] The data we present suggests that ACU-induced schizophrenia-like behaviors are associated with a reduction in eCB signaling, which would be expected to increase GABA release (Figure 5). There are several reports of reductions in GAD67, the synthetic enzyme for GABA, in the prefrontal cortex[75] and hippocampus[76] in schizophrenia, interpreted as reductions in GABA neurotransmission in schizophrenia. On the other hand, a recent human magnetic resonance spectroscopy (MRS) study finds increases in GABA in the medial prefrontal cortex of unmedicated schizophrenia volunteers.[77] This MRS study is consistent with our model (Figure 5) showing that ACU leads to a long-term reduction in eCB signaling that is predicted to enhance GABA release. One possible explanation for differences in GABA levels in schizophrenia could be explained by the fact that schizophrenia is a heterogeneous illness, and individuals with schizophrenia who have significant premorbid adolescent cannabis use may have a discrete pathophysiology. In fact, individuals with schizophrenia and a history of adolescent cannabis use exhibit a clinically distinct profile with earlier onset of illness,[78–82] more severe positive symptoms[80,83,84] and discrete cognitive deficits[85–94] compared with affected individuals without a cannabis use history. These data may suggest that individuals with schizophrenia and significant history of adolescent cannabis use may have a distinct underlying neurobiology associated with differential clinical profile.

Limitations of the study. There are several considerations to take into account. One is that we treated mice the commonly used synthetic CB1 agonist, WIN 55,212-2. This compound is a full agonist at the CB1 receptor, while Δ9-THC is a partial agonist at CB1, raising the possibility that the two compounds might not have identical effects. Secondly, molecular assays were conducted on mice that had behavioral testing 2 weeks before being killed. It is possible that the behavioral testing could influence expression levels of the proteins examined. Thirdly, there are some discrepancies between studies on behaviors induced by adolescent cannabinoids/Δ9-THC treatment. This might reflect experimental design differences, such as age at treatment, duration of treatment or drug doses used, which might be of particularly importance in terms of the magnitude and duration of receptor desensitization.

Summary. In summary, we report that the adolescent use of cannabinoids has long-term impact on the brain and induces schizophrenia-like behaviors, including hippocampal learning and memory deficits. Adult administration of cannabinoids did not have lasting effects. We also demonstrate that ACU induces a long-term reorganization of the eCB system and a reduction in mGluR5 that correlates with ACU-induced hippocampal deficits. These data may add to the literature suggesting candidacy of mGluR5 as a therapeutic target for schizophrenia, perhaps specifically for those individuals with a history of significant adolescent cannabis use.

Conflict of interest

The authors declare no conflict of interest.

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