Priority Communication

Adolescent Δ^9-Tetrahydrocannabinol Exposure and Astrocyte-Specific Genetic Vulnerability Converge on Nuclear Factor-κB–Cyclooxygenase-2 Signaling to Impair Memory in Adulthood

Yan Jouroukhin, Xiaolei Zhu, Alexey V. Shevelkin, Yuto Hasegawa, Bagrat Abazyan, Atsushi Saito, Jonathan Pevsner, Atsushi Kamiya, and Mikhail V. Pletnikov

ABSTRACT

BACKGROUND: Although several studies have linked adolescent cannabis use to long-term cognitive dysfunction, there are negative reports as well. The fact that not all users develop cognitive impairment suggests a genetic vulnerability to adverse effects of cannabis, which are attributed to action of Δ9-tetrahydrocannabinol (Δ^9-THC), a cannabis constituent and partial agonist of brain cannabinoid receptor 1. As both neurons and glial cells express cannabinoid receptor 1, genetic vulnerability could influence Δ^9-THC–induced signaling in a cell type–specific manner.

METHODS: Here we use an animal model of inducible expression of dominant-negative disrupted in schizophrenia 1 (DN-DISC1) selectively in astrocytes to evaluate the molecular mechanisms, whereby an astrocyte genetic vulnerability could interact with adolescent Δ^9-THC exposure to impair recognition memory in adulthood.

RESULTS: Selective expression of DN-DISC1 in astrocytes and adolescent treatment with Δ^9-THC synergistically affected recognition memory in adult mice. Similar deficits in recognition memory were observed following knockdown of endogenous *Disc1* in hippocampal astrocytes in mice treated with Δ^9-THC during adolescence. At the molecular level, DN-DISC1 and Δ^9-THC synergistically activated the nuclear factor-κB–cyclooxygenase-2 pathway in astrocytes and decreased immunoreactivity of parvalbumin-positive presynaptic inhibitory boutons around pyramidal neurons of the hippocampal CA3 area. The cognitive abnormalities were prevented in DN-DISC1 mice exposed to Δ^9-THC by simultaneous adolescent treatment with the cyclooxygenase-2 inhibitor, NS398.

CONCLUSIONS: Our data demonstrate that individual vulnerability to cannabis can be exclusively mediated by astrocytes. Results of this work suggest that genetic predisposition within astrocytes can exaggerate Δ^9-THC–produced cognitive impairments via convergent inflammatory signaling, suggesting possible targets for preventing adverse effects of cannabis within susceptible individuals.

Keywords: Adolescence, Astrocytes, Cannabis, Cognitive dysfunction, Gene-environment interaction, Hippocampus

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Cannabis is the most commonly used illicit drug of abuse in the United States (1). Although several studies have reported no long-term cognitive impairments after cannabis use, chronic cannabis exposure during adolescence has been associated with persistent deficits in some cognitive domains, including attention, memory, and processing speed (2–4). The *Cannabis sativa* plant includes more than 400 different chemical constituents, of which about 70 are cannabinoids (5). Cannabis-induced adverse effects are mediated by Δ9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive constituent of cannabis and a partial agonist of brain cannabinoid receptor 1 (CNR1) (6). However, the mechanisms underlying Δ^9-THC–induced long-lasting behavioral and cognitive abnormalities remain unknown.

Although neurons highly express CNR1, the role of glial CNR1 is being increasingly appreciated (7,8). Two recent studies have shown that detrimental effects of Δ^9-THC on learning and memory in mice are mediated by astrocyte CNR1 (9,10), activation of nuclear factor-κB (NF-κB) signaling, and upregulation of cyclooxygenase-2 (COX-2) that might lead to excessive glutamate release by astrocytes (11).

Notably, not all cannabis users demonstrate cognitive impairment, suggesting a genetic vulnerability to adverse effects of cannabis (12–14). Similarly, preclinical studies have reported that mice carrying mutations in candidate genes for psychiatric disorders exhibit greater responses to adverse effects of Δ^9-THC on memory (15–19). However, the underlying molecular mechanisms of how genetic mutations could moderate cognitive effects of cannabis remain unknown. Further, although astrocytes appear to play a major role in mediating effects of Δ^9-THC on memory (9,10), the molecular

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underpinning of how genetic risk factors could interact with Δ^9-THC in a cell type–specific manner to impair cognitive abilities has never been studied. To address these questions, we used an animal model of selective astrocyte expression of a rare, highly penetrant mutation, a dominant-negative (DN) form of disrupted in schizophrenia 1 (DISC1) (18,20–28).

DISC1 is a gene disrupted by the balanced (1;11) (q42.1; q14.3) translocation, segregating in a Scottish family with several major psychiatric disorders (29,30). Although the *DISC1* locus has not been reported in recent genome-wide association studies (31), rare mutations of large effects contribute to behavioral and cognitive abnormalities (32), and have important roles in mechanistic studies (33,34). It is in this context that we use a C-terminus truncated form of full-length *DISC1* as a DN molecular tool (DN-DISC1). In this study, we sought to determine the molecular basis of gene by environment (G×E) interaction in astrocytes and elucidate how G×E interplay could shape individual vulnerability to adverse cognitive effects of cannabis on cognitive abilities.

METHODS AND MATERIALS

Animals

To evaluate the cell-specific role of astrocytes in G×E interaction, mice expressing DN-DISC1 in astrocytes (aDN-DISC1) were exposed to chronic Δ^9-THC treatment (8 mg/kg, subcutaneous, daily) for 3 weeks from postnatal day 30 (P30) and on. Twenty-one days later, mice were assessed in a series of behavioral tests. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee.

Behavioral Tests

The following tests were used: open field, spontaneous alternation, spatial recognition in Y-maze, novel object recognition test (NORT), novel place recognition test (NPRT), and fear conditioning, as previously described (23–25,35).

Adeno-associated Virus Injections

Adeno-associated virus (AAV)-Gfa-enhanced green fluorescent protein (EGFP)-mir30-Disc1 or (AAV)-Gfa-EGFP-mir30-control were injected in the CA2/CA3 areas of the hippocampus at P15 to P17.

Isolation of RNA and RNA Sequencing Analyses

Total RNA was purified from mouse hippocampus upon completion of Δ^9-THC. RNA sequencing (RNA-seq) analysis was done as described in Supplement 1.

Biochemistry

Expression of phosphorylated p65, phosphorylated nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (phospho-IκBα), and COX-2 was assessed with standard Western blotting in primary astrocytes or hippocampal tissue samples. DISC1-IκBα binding was analyzed with co-immunoprecipitation as previously described (36).

Immunohistochemistry

We evaluated 67-kDa glutamate decarboxylase–positive ($GAD67^+$) presynaptic boutons within parvalbumin-positive (PV^+) branches on the surface of pyramidal neurons of the CA1 and CA3 areas of the hippocampus.

Pharmacological Treatment With the COX-2 Inhibitor

Effects of COX-2 inhibition on cognitive deficits were assessed with the COX-2 inhibitor, NS398 (10 mg/kg, subcutaneous, daily).

Measurement of Glutamate in Hippocampal Tissue and in Culture Medium

Glutamate concentration in the hippocampus or primary astrocyte culture was assayed using Kusakabe's method (37) or glutamate assay, respectively (for detailed information, please see Supplement 1).

RESULTS

aDN-DISC1 and Adolescent Δ^9-THC Impair Memory in Adult Mice

Based on our prior studies (26,28), we hypothesized that expression of aDN-DISC1 would synergistically interact with adolescent Δ^9-THC exposure to affect learning and memory in adult mice. To test this hypothesis, we treated control or aDN-DISC1 male and female mice with single daily injections of Δ^9-THC (8 mg/kg, subcutaneous) starting at P30 for 3 weeks (14) to span mouse adolescence (P30–P51), which corresponds to human adolescence from 12 to 19 years of age (38–41). Upon completion of treatment, the mice were left undisturbed for another 3 weeks before behavioral testing was commenced (Figure 1A).

For male mice, compared with other groups, aDN-DISC1 mice treated with Δ^9-THC exhibited synergistically impaired performance in the spatial recognition test in the Y-maze (significant aDN-DISC1 × Δ^9-THC interaction [$F_{1,33} = 4.46$, $p = .045$]) and the NORT (significant aDN-DISC1 × Δ^9-THC interaction [$F_{1,33} = 14.48$, $p < .001$]), as well as significantly worse performance in the NPRT compared with aDN-DISC1 mice treated with vehicle ($p < .05$) (Figure 1B–D). For female mice, the aDN-DISC1-Δ^9THC combination produced synergistic impairment in the spatial recognition test in the Y-maze only (significant aDN-DISC1 × Δ^9-THC interaction [$F_{1,43} = 12.88$, $p < .001$]) (Figure 1E–G). Memory deficits were not associated with group differences in general exploratory activity during the training phase for the above tests, distance traveled in the Y-maze, or novelty-induced activity. Effects of Δ^9-THC were found in neither the forced swim test, nor the context- or cue-dependent delay fear conditioning (Figures S1–S3 in Supplement 1). Thus, our results suggest that adolescent treatment with Δ^9-THC and expression of aDN-DISC1 synergistically affect recognition memory in adult mice. As the most robust behavioral effects were found in aDN-DISC1 male mice, we focused on male mice in all subsequent tests.

To examine whether the above synergistic effects were dependent on adolescent Δ^9-THC exposure (37), adult control and aDN-DISC1 mice were treated using the same protocol. We found no significant effects of adult Δ^9-THC exposure on recognition memory in aDN-DISC1 mice (Figure S4 in Supplement 1).

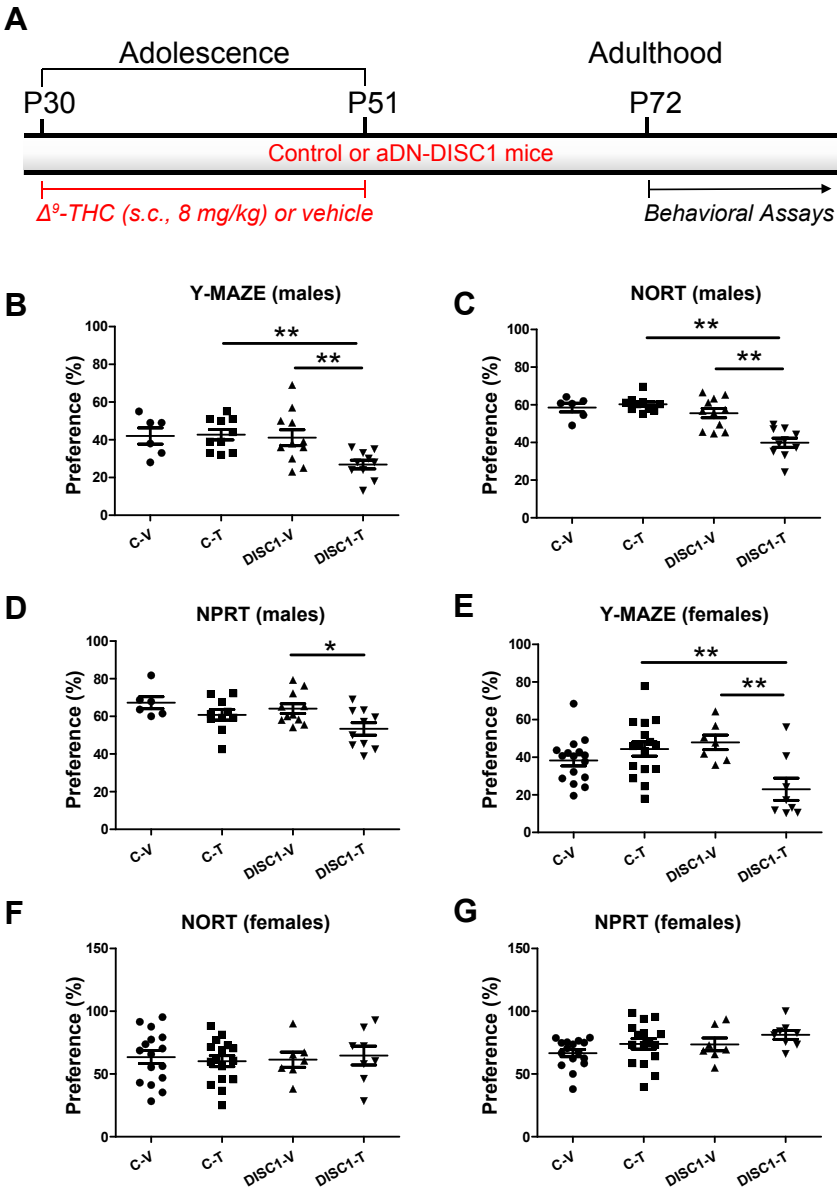

Figure 1. Cognitive impairments in astrocyte dominant-negative disrupted in schizophrenia 1 (aDN-DISC1) mice. **(A)** Schematic diagram of the treatment protocol. **(B–G)** In all graphs, the y-axis depicts the preference (%); the x-axis depicts the experimental groups: control mice treated with vehicle (C-V) (6 male mice and 16 female mice), control mice treated with Δ9-tetrahydrocannabinol (Δ^9-THC) (C-T) (10 male mice and 16 female mice), aDN-DISC1 mice treated with vehicle (DISC1-V) (11 male mice and 7 female mice), and aDN-DISC1 mice treated with Δ^9-THC (DISC1-T) (10 male mice and 8 female mice). **(B)** Spatial recognition memory in the Y-maze. Compared with other groups, the male DISC1-T group (n = 10 mice) exhibited the significantly decreased preference for the previously blocked arm. Two-way analysis of variance (ANOVA) of the preference data revealed a significant effect of DN-DISC1 ($F_{1,33}$ = 5.49, p = .025) and significant aDN-DISC1 x Δ^9-THC interaction ($F_{1,33}$ = 4.46, p = .045); Fisher's least significant difference (LSD) post hoc test showed that the male DISC1-T group was significantly different from both the male C-T group (n = 10 mice) and male DISC1-V group (n = 11 mice). $^{**}p < .01$. **(C)** Novel object recognition test (NORT). Compared with other groups, the male DISC1-T group exhibited a significantly decreased preference for the novel object. Two-way ANOVA of the preference data revealed a significant effect of DN-DISC1 ($F_{1,33}$ = 26.12, $p < .001$), a significant effect of Δ^9-THC ($F_{1,33}$ = 9.26, p = .005), and a significant aDN-DISC1 x Δ^9-THC interaction ($F_{1,33}$ = 14.48, $p <$ .001); Fisher's LSD post hoc test showed that the male DISC1-T group was different from both the male C-T and male DISC1-V (n = 11 mice) groups. $^{**}p < .01$. **(D)** Novel place recognition test (NPRT). Compared with other groups, male DISC1-T exhibited a significantly decreased preference for the novel place of one of two identical objects. Two-way ANOVA of the preference data revealed a significant effect of Δ^9-THC ($F_{1,33}$ = 7.89, p = .008). Planned post hoc tests showed a significantly decreased preference in the male DISC1-T group compared with the male DISC1-V group, but there was no difference in the preference between the male DISC1-T and male C-T groups (p = .074). $^{*}p <$ .05. **(E)** Spatial recognition memory in the Y-maze. Compared with other groups, the female DISC1-T group (n = 8 mice) exhibited the significantly decreased preference for the previously blocked arm. Two-way ANOVA of the preference data revealed a significant effect of Δ^9-THC ($F_{1,43}$ = 4.74, p = .035) and a significant aDN-DISC1 × Δ^9-THC interaction ($F_{1,43}$ = 12.88, $p < .001$); Fisher's LSD post hoc test showed that the female DISC1-T group was different from the female DISC1-V (n = 7 mice) and female C-T (n = 16 mice) groups. $^{**}p < .01$. **(F)** NORT. No group differences were found. **(G)** NPRT. No group differences were found. P, postnatal day; s.c., subcutaneous.

Although expression of aDN-DISC1 in the brain reaches the maximum by late adolescence, there is expression of aDN-DSC1 during late gestation and the early postnatal period (28) that coincides with astrocyte proliferation and maturation (42,43). To evaluate a possible contribution of early expression of aDN-DISC1 to the cognitive phenotypes, we turned off expression of aDN-DISC1 using doxycycline (DOX)-containing food beginning at P21 onward (Figure S5A in Supplement 1). We observed no significant cognitive effects in any group (Figure S5B–D in Supplement 1), suggesting that developmental expression of aDN-DISC1 unlikely contributed to the cognitive abnormalities.

We next wondered whether a different psychoactive compound could also interact with aDN-DISC1 to impair recognition memory (44,45). To evaluate this possibility, control and aDN-DISC1 adolescent male mice were treated with amphetamine (1 mg/kg, intraperitoneal). No significant changes in the same memory tests were found in either group (Figure S6 in Supplement 1).

We also evaluated whether expression of DN-DISC1 in neurons could also lead to the cognitive deficits after

adolescent Δ^9-THC treatment. We generated mice with expression of neuronal DN-DISC1 by crossing tetracycline-responsive element DN-DISC1 mice with *CamkII*-tetracycline-controlled transactivator mice to express DN-DISC1 in forebrain neurons (23). Control and neuronal DN-DISC1 male mice were treated with the same treatment (Figure S7 in Supplement 1), suggesting a cell type–specific G×E interaction to impair recognition memory.

The Hippocampus Is Sufficient for Mediating the Major Effects of Interaction

The hippocampus plays the critical role in spatial recognition memory (46–48). The cognitive effects of exogenous cannabinoids have been linked to adverse effects on hippocampal neuronal circuits (49–52). In addition, we previously reported strong expression of aDN-DISC1 in the hippocampus compared with the frontal cortex (28). Thus, we evaluated the contribution of hippocampal aDN-DISC1 to memory deficits observed in Δ^9-THC–treated mice. To address this question and alter expression of aDISC1 with a different genetic tool, we engineered an AAV vector to knock down endogenous *Disc1* selectively in astrocytes, AAV-Gfa-EGFP-mir30-Disc1, or control (scrambled) vector, AAV-Gfa-EGFP-mir30-control (Figure S8 in Supplement 1). AAV-Gfa-EGFP-mir30-Disc1 decreased expression of *Disc1* in vivo (Figure S8A in Supplement 1) and transduced astrocytes only (Figure S8B in Supplement 1).

Because prior studies have suggested that Δ^9-THC exposure may affect CA1-CA3 circuits (9,53,54), AAV-Gfa-EGFP-mir30-Disc1 and AAV-Gfa-EGFP-mir30-control were injected in the CA2-CA3 areas of the hippocampus at P16 to let the effects of knockdown (KD) take place by P30, when Δ^9-THC treatment was commenced. Three weeks later, behavioral testing was initiated (Figure 2A). Consistent with our earlier findings (Figure 1), compared with the AAV-Gfa-EGFP-mir30-control, the AAV-Gfa-EGFP-mir30-Disc1 synergistically impaired spatial memory in the Y-maze test (significant *Disc1*-KD × Δ^9-THC interaction [$F_{1,22}$ = 13.90, p = .001]) and NORT (significant *Disc1*-KD × Δ^9-THC interaction [$F_{1,22}$ = 4.35, p = .05]), but not the NPRT, in mice treated with Δ^9-THC during adolescence (Figure 2B–D). These effects were unlikely related to nonspecific changes in locomotion or exploratory activity (Figure S9 in Supplement 1). Upon completion of behavioral tests (P90), we confirmed that AAV transduction was present in astrocytes of the CA2-CA3 areas (Figure S10 in Supplement 1). Thus, expression of aDN-DISC1 or *Disc1* KD in hippocampal astrocytes synergistically exacerbated the adverse cognitive effects of adolescent Δ^9-THC in adult mice.

Δ^9-THC Activates Proinflammatory Signaling in aDN-DISC1

To gain an unbiased insight in the mechanisms of G×E interaction, we performed RNA-seq analyses of hippocampal tissue samples derived from control and aDN-DISC1 mice treated with vehicle or Δ^9-THC as above. Our analyses focused on the 56 genes differentially expressed in both the aDN-DISC1 and Δ^9-THC conditions, but not in either condition alone (Figure 3A). This analysis revealed a significant upregulation of genes involved in the inflammatory pathways, including NF-κB signaling (Figure 3B). Full

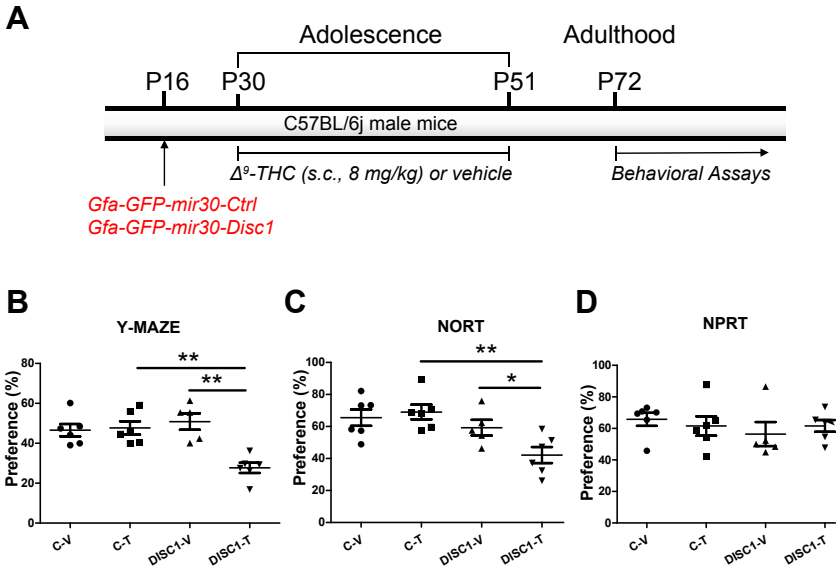

Figure 2. *Disc1* knockdown (KD) in hippocampal astrocytes. **(A)** Schematic diagram of the adeno-associated viral (AAV) vector injections and treatment protocol. **(B–D)** On all data panels, the y-axis depicts the preference (%); the x-axis depicts the experimental groups: mice injected with *Gfa*-green fluorescent protein (GFP)-*mir30*-control (*Ctrl*) AAV and treated with the vehicle (C-V) (n = 6), mice injected with *Gfa*-GFP-*mir30-Ctrl* AAV and treated with Δ9-tetrahydrocannabinol (Δ^9-THC) (C-T) (n = 6), mice injected with *Gfa*-GFP-*mir30-Disc1* AAV and treated with vehicle (DISC1-V) (n = 5), and mice injected with *Gfa*-GFP-*mir30-Disc1* AAV and treated with Δ^9-THC (DISC1-T) (n = 6). **(B)** Spatial recognition memory in the Y-maze. Compared with other groups, the DISC1-T group exhibited the significantly decreased preference for the previously blocked arm. Two-way analysis of variance of the preference data revealed a significant effect of *Disc1* KD ($F_{1,22}$ = 5.77, p = .027), a significant effect of Δ^9-THC ($F_{1,22}$ = 11.43, p = .003), and a significant *Disc1* KD × Δ^9-THC interaction ($F_{1,22}$ = 13.90, p = .001). Fisher's least significant difference post hoc test showed that the DISC1-T group was different from the C-T (p < .001) and C-V (p < .001) groups. **p < .001. **(C)** Novel object recognition test (NORT). Compared with other groups, the DISC1-T group exhibited a significantly decreased preference for the novel object. Two-way analysis of variance of the preference data revealed a significant effect of *Disc1* KD ($F_{1,22}$ = 11.20, p = .003) and borderline significance for the Δ^9-THC × *Disc1* KD interaction ($F_{1,22}$ = 4.35, p = .051); Fisher's least significant difference post hoc test showed that the DISC1-T group was different from the C-T (p < .001) and DISC1-V (p = .027) groups. *p < .05, **p < .01. **(D)** Novel place recognition test (NPRT). No significant effects of *Disc1* KD were found in the NPRT. P, postnatal day; s.c., subcutaneous.

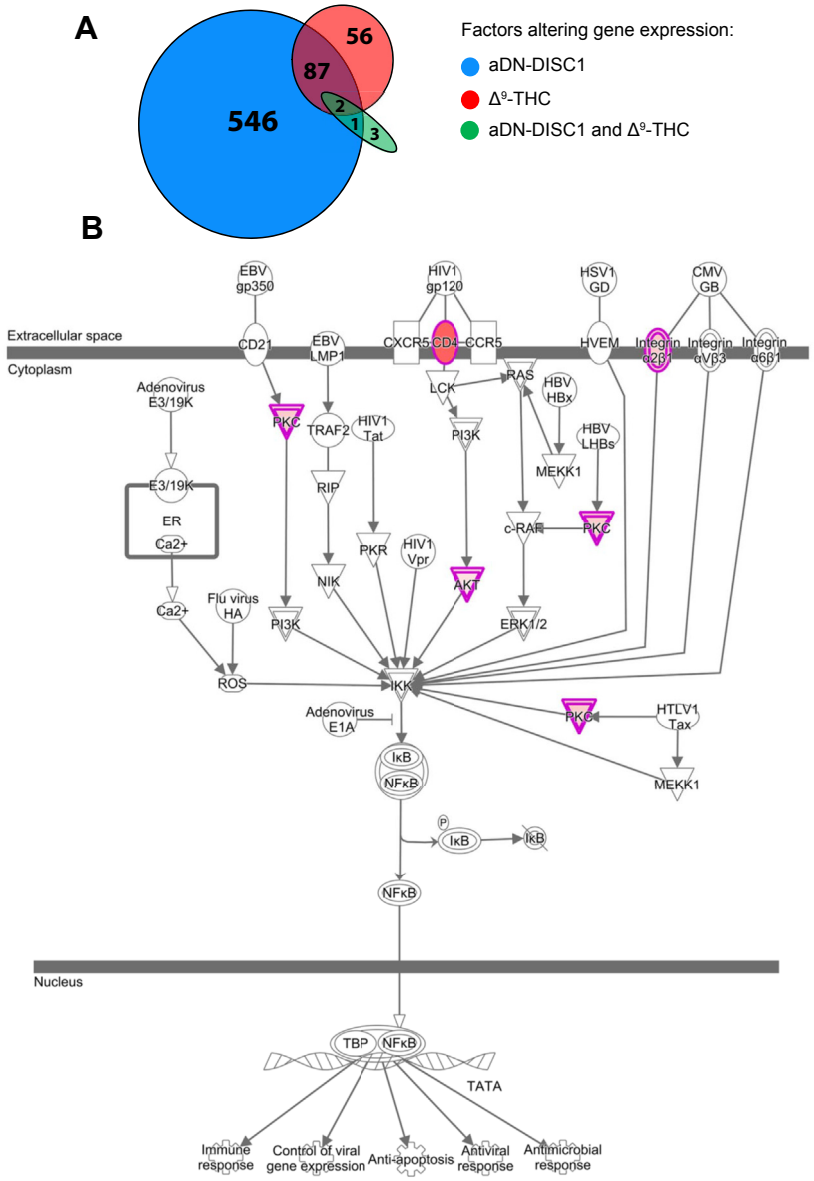

Figure 3. RNA sequencing (RNA-seq) identifies synergistic (gene × environment interaction) genes, which are enriched in the nuclear factor-κB (NF-κB) signaling pathway. **(A)** In astrocyte dominant-negative disrupted in schizophrenia 1 (aDN-DISC1) mice exposed to Δ9-tetrahydrocannabinol (Δ^9-THC), there were 145 differentially expressed genes (false discovery rate < .20) (see circle at upper right). Over a third of the 145 genes (n = 56, 38.6%) (see red shaded portion of circle) were synergistic (gene × environment interaction) genes because they were not found to be differentially expressed by aDN-DISC1 or Δ^9-THC alone. Two of the genes (*Ddit4*, *Sgk1*) were also differentially expressed by aDN-DISC1 or Δ^9-THC alone, and 87 genes were differentially expressed by aDN-DISC1 alone. The oval represents six genes differentially expressed after Δ^9-THC treatment: three were differentially expressed in wild-type mice and three were differentially expressed in the aDN-DISC1 mice, of which two were in aDN-DISC1 mice treated with Δ^9-THC. **(B)** Gene × environment interaction genes are enriched for membership in the NF-κB activation by viruses pathway (Z score = 2.00; $p = 1.74\times10^{-03}$; Benjamini-Hochberg–adjusted p value [false discovery rate] = .0253; four genes, all upregulated: *Akt2*, *Cd4*, *Itga5*, and *Prkch*).

lists of differentially expressed genes and pathways are presented in Tables S2 and S3 in Supplement 2, respectively (the National Center for Biotechnology Information accession number is GSE116813). Together with prior studies demonstrating that both Δ^9-THC and DISC1 influence NF-κB signaling (10), our results suggest that this inflammatory pathway may be a convergent target of DN-DISC1 and Δ^9-THC in astrocytes.

DISC1 Regulates Activation of NF-κB–COX-2 Signaling in Astrocytes

We focused on evaluation of expression of *Ptgs2* (i.e., *Cox-2*), which encodes for a constitutively expressed and inducible enzyme, COX-2, which converts arachidonic acid to prostaglandins (55,56). We chose to assess altered expression of *Ptgs2* in our G×E interaction model because Δ^9-THC induces a robust increase in activity and expression of COX-2 in astrocytes and mediates synaptic and cognitive effects of Δ^9-THC (10,57). Because tissue astrocytes and cultured cells exhibit significant transcriptome differences (58), we assessed *Ptgs2* expression in astrocytes acutely isolated from the hippocampus followed by Δ^9-THC treatment. We found a synergistic upregulation of *Ptgs2* in astrocytes of aDN-DISC1 mice treated with Δ^9-THC compared with other groups (significant DN-DISC1 × Δ^9-THC treatment interaction [$F_{1,8}$ = 87.43, p = .001]) (Figure 4A).

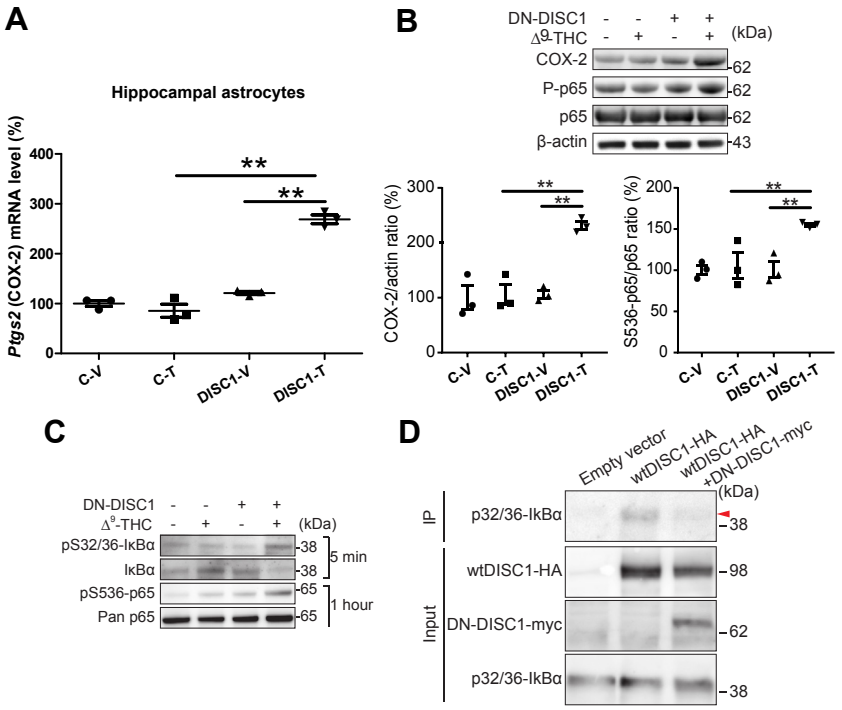

Figure 4. Synergistic effects of dominant-negative disrupted in schizophrenia 1 (DN-DISC1) and Δ9-tetrahydrocannabinol (Δ^9-THC) on the nuclear factor-κB–cyclooxygenase-2 (NF-κB-COX-2) signaling in astrocytes. **(A)** DISC1 and Δ^9-THC synergistically increased *Ptgs2* (gene encoding for COX-2 protein) expression in hippocampal tissue astrocytes. The graph plots the individual data points and superimposes the mean and error bars. Each point represents an independent sample from a single animal assayed in triplicates. Two-way analysis of variance revealed the significant effects of DN-DISC1 ($F_{1,8}$ = 139.52, $p < .001$), Δ^9-THC ($F_{1,8}$ = 59.15, $p < .001$), and a significant DN-DISC1 × Δ^9-THC treatment interaction ($F_{1,8}$ = 87.43, $p = .001$). Fisher's least significant difference (LSD) post hoc analysis showed that *Ptgs2* expression in the DN-DISC1 mice treated with Δ^9-THC (DISC1-T) group was significantly greater compared with the DN-DISC1 mice treated with vehicle (DISC1-V) group ($p < .001$) or the control mice treated with Δ^9-THC (C-T) group ($p < .001$). $^{**}p < .01$. **(B)** Representative Western blot images and densitometric analysis showing that DISC1 and Δ^9-THC treatment synergistically increased expression of COX-2 protein and phosphorylation of p65 (S536-p65/p65) in hippocampal astrocytes. Two-way analysis of variance revealed significant effects on COX-2 protein level (main effect: DISC1 [$F_{1,8}$ = 18.76, $p = .003$] and Δ^9-THC [$F_{1,8}$ = 18.79, $p = .002$]; significant DN-DISC1 × Δ^9-THC interaction [$F_{1,8}$ = 15.52, $p = .004$]) and phosphorylation of NF-κB p65 (main effect: DISC1 [$F_{1,8}$ = 9.29, $p = .016$] and Δ^9-THC [$F_{1,8}$ = 6.32, $p = .036$]; significant DN-DISC1 × Δ^9-THC interaction [$F_{1,8}$ = 6.03, $p = .040$]); Fisher's LSD post hoc analysis showed that the protein level of COX-2 (protein encoded by *Ptgs2* gene) in the DISC1-T group was significantly greater compared with the C-T group ($p < .001$) and DISC1-V ($p < .001$). Fisher's LSD post hoc analysis showed that the protein level of phosphorylated NF-κB p65 in the DISC1-T group was significantly greater compared with the C-T ($p = .005$) or DISC1-V ($p = .008$) groups. No other significant differences were detected in expression of COX-2 or phosphorylated NF-κB p65. Data are presented as mean ± SEM; $n = 3$ independent samples in each group. $^{**}p < .01$. **(C)** Δ^9-THC treatment of primary DN-DISC1 astrocytes upregulated phosphorylation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IκBα) and decreased IκBα expression (5-minute interval) and increased phosphorylation of NF-κB p65 (1-hour interval). **(D)** Protein interaction of DISC1 with phosphorylated IκBα (p32/36-IκBα) was assessed by co-immunoprecipitation in human embryonic kidney 293 cells. Hemagglutinin-tagged wild-type DISC1 (wtDISC1-HA) interacts with endogenous phosphorylated IκBα. Overexpression of myc-tagged DN-DISC1 (DN-DISC1-myc) reduced wtDISC1-phosphorylated IκBα interaction. Input for each protein is presented. mRNA, messenger RNA.

We then assessed the protein levels of COX-2 and phosphorylated NF-κB p65 in astrocytes isolated from the hippocampus 1 day after in vivo Δ^9-THC or vehicle treatment. Consistent with messenger RNA data, we found a significant and synergistic increase in COX-2 level (significant DN-DISC1 × Δ^9-THC interaction [$F_{1,8}$ = 15.52, $p = .004$]) and phosphorylation of NF-κB p65 compared with other groups (significant DN-DISC1 × Δ^9-THC interaction [$F_{1,8}$ = 6.03, p = .040]) (Figure 4B).

To further evaluate activation of NF-κB p65 signaling in astrocytes, we measured the phosphorylation levels of NF-κB p65 and IκBα, an upstream signaling protein of NF-κB p65, in primary DN-DISC1 or control astrocytes treated with Δ^9-THC (5 μM for 5 minutes or 1 hour) or vehicle. Consistent with in vivo data, aDN-DISC1 treated with Δ^9-THC had synergistically increased phosphorylation of NF-κB p65 (Δ^9-THC treatment for an hour) and enhanced phosphorylation of IκBα (Δ^9-THC treatment for 5 minutes), compared with other conditions (Figure 4C).

Given that phosphorylation of IκBα leads to its dissociation from p65 and degradation, an event required before nuclear translocation of the liberated p65 (59,60), DISC1 may interact with phospho-IκBα for stabilization of the cytoplasmic IκBα:p65 complex. Indeed, co-immunoprecipitation experiments confirmed protein interaction between wild-type DISC1 and phospho-IκBα. Importantly, this interaction was disrupted by overexpression of DN-DISC1 (Figure 4D). Our results suggest that binding of DISC1 to phospho-IκBα may stabilize IκBα activity, supporting inhibitory action of IκBα on NF-κB. Expression of aDN-DISC1 leads to decreased levels of endogenous DISC1 (26) that may facilitate degradation of phospho-IκBα and activation of NF-κB as a result of its release from binding to IκBα.

As upregulation of COX-2 could increase glutamate secretion by astrocytes (11), we examined the effects of Δ^9-THC on levels of glutamate in the hippocampus and culture medium collected from primary astrocytes. Compared with other groups, there was a significant increase in glutamate levels in the hippocampus of aDN-DISC1 mice treated with Δ^9-THC ($p < .05$) (Figure S11A in Supplement 1). In addition, we found a synergistically increased secretion of glutamate by primary aDN-DISC1 following stimulation of Δ^9-THC (significant aDN-DISC1 × Δ^9-THC interaction [$F_{1,18}$ = 6.74, $p = .02$]) (Figure S11B in Supplement 1). Collectively, these results suggest that DN-DISC1 and Δ^9-THC interact to activate the proinflammatory NF-κB-COX-2

signaling and increase secretion of glutamate by astrocytes.

Decreased GAD$^+$PV$^+$ Immunoreactivity in the CA3 Area of the Hippocampus

Chronic exposure to Δ^9-THC during adolescence was associated with decreased GAD67 expression in PV$^+$ interneurons (61–65). Given enhanced vulnerability of gamma-aminobutyric acid–positive (GABA$^+$) PV$^+$ neurons to adverse effects of chronic excitotoxicity that may be a result of increased secretion of glutamate by aDN-DISC1 (66–69), we assessed the integrity of presynaptic GAD$^+$PV$^+$ boutons in control and aDN-DISC1 mice. Because our AAV results strongly suggested that the CA areas of the hippocampus could be critically involved in producing deficient recognition memory, we focused on examination of presynaptic GAD$^+$PV$^+$ boutons in these areas (Figure S12 in Supplement 1). Compared with aDN-DISC1 mice treated with vehicle or control mice treated with Δ^9-THC, we found significantly decreased intensity of GAD$^+$PV$^+$ boutons on the surface of pyramidal neurons of the CA3 but not the CA1 area in aDN-DISC1 mice treated with Δ^9-THC ($p < .05$) (Figure 5). No significant changes of GAD$^+$PV$^+$ bouton size or density were found (Figure S13 in Supplement 1). Reduced immunoreactivity of GAD$^+$PV$^+$ boutons was not associated with a general decrease in GAD$^+$ immunoreactivity (Figure S14 in Supplement 1), suggesting that PV$^+$ GABA neurons could be selectively and synergistically affected in aDN-DISC1 mice treated with Δ^9-THC during adolescence.

Blockade of NF-κB–COX-2 Activation Prevents Cognitive Impairment

Because our results had suggested synergistic elevation of the proinflammatory NF-κB–COX-2 signaling in aDN-DISC1 treated with Δ^9-THC, we hypothesized that inhibition of COX-2 activity concurrently with Δ^9-THC injections during adolescence may prevent the development of cognitive impairment in adult mice. To test this prediction, we assessed the effects of COX-2 inhibition (NS398, 10 mg/kg, daily subcutaneous injections 30 minutes before Δ^9-THC injections) on different types of recognition memory in control and aDN-DISC1 mice treated with Δ^9-THC at adolescence (Figure 6). We found that NS398 prevented the development of memory deficits in aDN-DISC1 mice treated with Δ^9-THC (significant aDN-DISC1 × NS398 interaction for Y-maze [$F_{1,29} = 5.03$, $p = .033$], NORT [$F_{1,29} = 8.99$, $p = .006$], and NPRT [$F_{1,29} = 19.81$, $p < .001$]). No effects of NS398

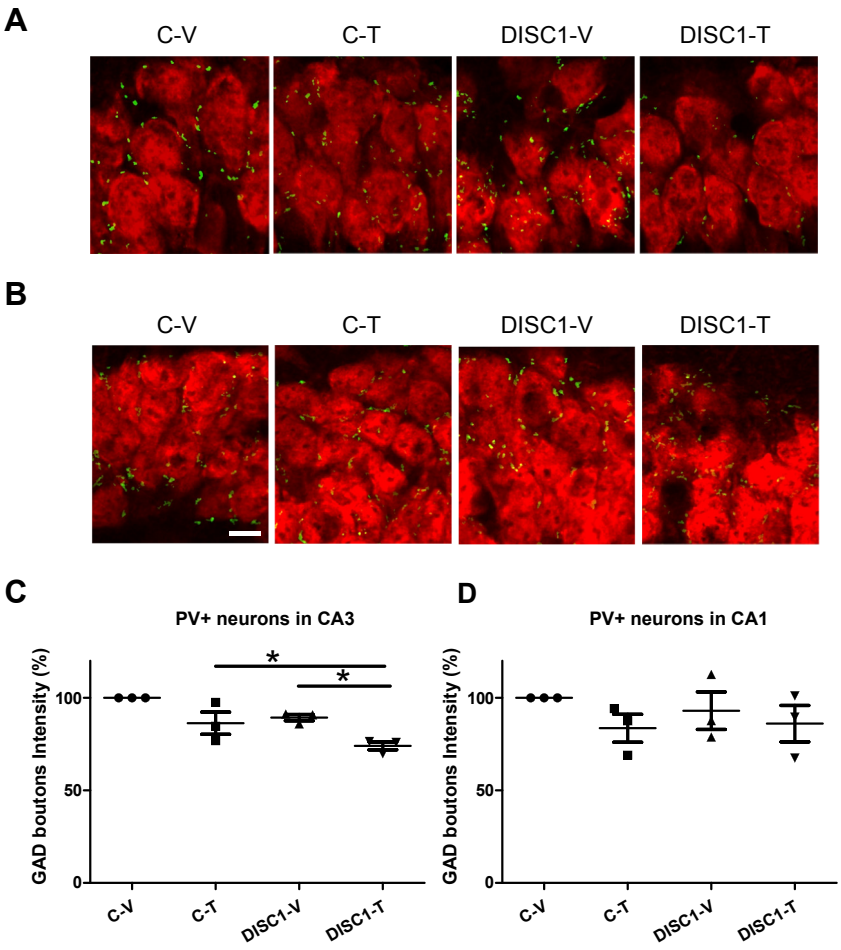

Figure 5. Astrocyte dominant-negative disrupted in schizophrenia 1 (aDN-DISC1) and Δ9-tetrahydrocannabinol (Δ^9-THC) treatment synergistically decreases glutamate decarboxylase–positive (GAD$^+$) and parvalbumin-positive (PV$^+$) immunoreactivity in the CA3 area of the hippocampus. **(A)** Representative images of GAD boutons intensity in pyramidal neurons of the CA3 area of the hippocampus; note the decreased intensity in the aDN-DISC1 mice treated with Δ^9-THC (DISC1-T) group. **(B)** Representative images of GAD boutons intensity in pyramidal neurons of the CA1 area of the hippocampus; note the comparable intensity in all groups. **(C, D)** The y-axis depicts the percentage of GAD boutons intensity in CA3 or CA1 PV neurons in relation to the level for the control vehicle group; the x-axis depicts the experimental groups: control mice treated with vehicle (C-V), control mice treated with Δ^9-THC (C-T), aDN-DISC1 mice treated with vehicle (DISC1-V), and DISC1-T. **(C)** Quantitative analyses of the intensity of GAD$^+$PV$^+$ presynaptic boutons around pyramidal neurons of the CA3 area of the hippocampus. $n = 3$ sections per mouse, 3 mice per group; each data point represents 1 mouse. Two-way analysis of variance of the intensity data revealed significant effect of group ($F_{1,8} = 12.06$, $p = .008$) and Δ^9-THC ($F_{1,8} = 19.16$, $p = .002$); Fisher's least significant difference post hoc test showed that the DISC1-T group was significantly different from the C-T and DISC1-V groups. *$p < .05$. **(D)** Quantitative analyses of the intensity of GAD$^+$PV$^+$ presynaptic boutons around pyramidal neurons of the CA1 area of the hippocampus. Two-way analysis of variance of the intensity data revealed no significant effects of DN-DISC1 ($F_{1,8} = 0.075$, $p = .791$), no significant effects of Δ^9-THC ($F_{1,8} = 2.129$, $p = .183$), and no DN-DISC1 × Δ^9-THC treatment interaction ($F_{1,8} = 0.347$, $p = .572$). $n = 3$ sections per mouse, 3 mice per group; each data point represents 1 mouse.

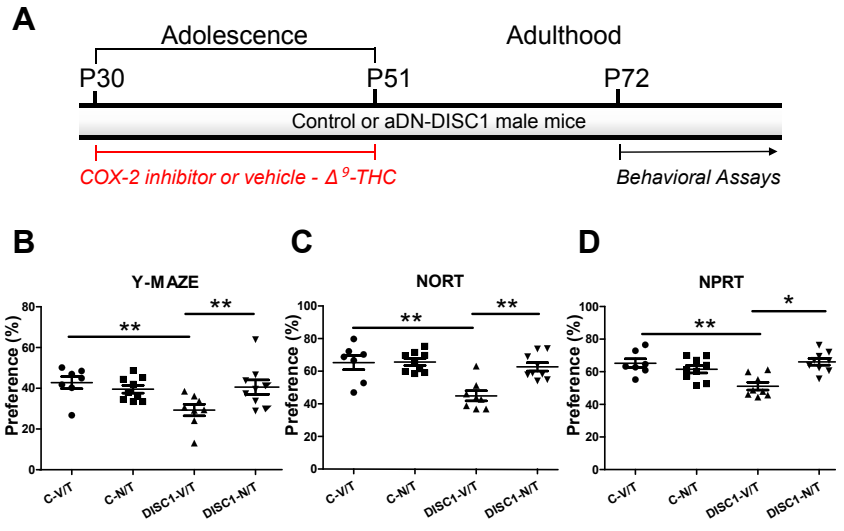

Figure 6. Rescuing the memory deficits with the cyclooxygenase-2 (COX-2) inhibitor. **(A)** Schematic diagram of the treatment protocol. **(B–D)** The y-axis depicts the preference (%); the x-axis depicts the experimental groups: control mice treated with vehicle and Δ9-tetrahydrocannabinol (Δ^9-THC) (C-V/T) (n = 7), control mice treated with the selective COX-2 inhibitor (NS398) and Δ^9-THC (C-N/T) (n = 9), astrocyte dominant-negative disrupted in schizophrenia 1 (aDN-DISC1) mice treated with vehicle and Δ^9-THC (DISC1-V/T) (n = 8), and aDN-DISC1 mice treated with the NS398 and Δ^9-THC (DISC1-N/T) (n = 9). NS398 (10 mg/kg, subcutaneous) was administrated daily 30 minutes before Δ^9-THC injection (10 mg/kg, subcutaneous). **(B)** Spatial recognition memory in the Y-maze. Significantly decreased preference for the previously blocked arm in aDN-DISC1 mice treated with Δ^9-THC was significantly restored by NS398 co-treatment. Two-way analysis of variance of the preference data revealed a significant effect of aDN-DISC1 ($F_{1,29}$ = 4.68, p = .039), no effect of NS398 ($F_{1,29}$ = 9.26, p = .205), and a significant aDN-DISC1 × NS398 interaction ($F_{1,29}$ = 5.03, p = .033); Fisher's least significant difference (LSD) post hoc test showed that aDN-DISC1 significantly reduced preference for the previously blocked arm in Δ^9-THC–treated mice (DISC1-V/T vs. C-V/T, p = .004) and NS398 co-treatment significantly increased this preference (DISC1-V/T vs. DISC1-N/T, p = .007). n = 7–9 mice per group. $^{**}p < .01$. **(C)** Novel object recognition test (NORT). Significantly decreased preference for the novel object in aDN-DISC1 mice treated with Δ^9-THC was significantly restored by NS398 co-treatment. Two-way analysis of variance of the preference data revealed a significant effect of aDN-DISC1 ($F_{1,29}$ = 11.64, p = .002) and NS398 ($F_{1,29}$ = 8.61, p = .006) and a significant aDN-DISC1 × NS398 interaction ($F_{1,29}$ = 8.99, p = .006); Fisher LSD post hoc test showed that aDN-DISC1 significantly reduced preference for the novel object in Δ^9-THC–treated mice (DISC1-V/T vs. C-V/T, $p < .001$) and NS398 co-treatment significantly increased this preference (DISC1-V/T vs. DISC1-N/T, $p < .001$). n = 7–9 mice per group. $^{**}p < .01$. **(D)** Novel place recognition test (NPRT). Significantly decreased preference for the novel place of one of two identical objects in aDN-DISC1 mice treated with Δ^9-THC was significantly restored by NS398 co-treatment. Two-way analysis of variance of the preference data revealed no effect of aDN-DISC1 ($F_{1,29}$ = 2.05, p = .163), no effect of NS398 ($F_{1,29}$ = 2.89, p = .100), and the significant aDN-DISC1 × NS398 interaction ($F_{1,29}$ = 19.81, $p < .001$); Fisher's LSD post hoc test showed that aDN-DISC1 significantly reduced preference for the novel object in Δ^9-THC–treated mice (DISC1-V/T vs. C-V/T, $p < .001$) and NS398 co-treatment significantly increased this preference (DISC1-V/T vs. DISC1-N/T, p = .043). n = 7–9 mice per group. $^{*}p < .05$, $^{**}p < .01$. P, postnatal day.

were found on locomotor activity in mice in the Y-maze (Figure S15 in Supplement 1).

We also evaluated whether the COX-2 inhibition would prevent elevated secretion of glutamate by primary aDN-DISC1 following stimulation with Δ^9-THC. We found that NS398 reversed increased secretion of glutamate by primary aDN-DISC1 treated with Δ^9-THC (Figure S16 in Supplement 1). These results suggest convergence of effects of DN-DISC1 and Δ^9-THC on NF-κB–COX-2 signaling in astrocytes, leading to increased production of glutamate by astrocytes.

DISCUSSION

We report that inducible expression of aDN-DISC1, but not neurons or KD of endogenous *Disc1* in hippocampal astrocytes, interacts with adolescent Δ^9-THC exposure to impair recognition memory in adult mice. The present findings suggest that DN-DISC1 and Δ^9-THC synergistically activate the NF-κB–COX-2 pathway in astrocytes, leading to increased secretion of glutamate and decreased immunoreactivity of PV^+ presynaptic boutons around pyramidal neurons of the CA3 area of the hippocampus. Deficient recognition memory could be prevented with the COX-2 inhibitor. Our data demonstrate that astrocyte genetic risk factors can exacerbate cognitive effects of adolescent cannabis use and indicate a putative target for preventive treatment.

Adolescent, not adult, exposure to Δ^9-THC was required for the development of deficient recognition memory in adult mice with expression of aDN-DISC1. These results are consistent with other preclinical reports on effects of adolescent exposure to cannabinoids and resulting cognitive impairments (3,70–72). Lack of effects of Δ^9-THC in aDN-DISC1 mice on fear conditioning is in line with the unaltered performance in the Morris water maze (73–75) or aversive memory tasks in mice following adolescent treatment with cannabinoids (2,70,76–78). This selectivity in cognitive effects of cannabinoids could be related to differential distribution of CNR1 in the neural circuits underlying various cognitive tasks.

The current work is congruent with human studies that demonstrate that cannabis use during adolescence could have lasting effects on cognition (2,70,78–80) that is likely related to continuing maturation of the brain in general (81–84) and cannabinoid receptors in particular (85–87). Our results are consistent with human studies that adolescent cannabis use tends to affect working memory in adulthood (88,89), particularly spatial processing that is dependent on the integrity of the hippocampus (90,91). While human studies suggest an association (92–94), animal models enable us to establish a causal relationship and neurobiological mechanisms. In this context, our study significantly extends the existing literature on effects of cannabinoids on spatial working memory as evaluated in rodents with spatial recognition tests (95).

Our data clearly demonstrate that expression of the same risk factor in different brain cell types produces differential neurobehavioral outcomes in mice treated with Δ^9-THC. Astrocyte but not neuronal expression of DN-DISC1 interacts with adolescent Δ^9-THC to lead to recognition memory impairment in adult mice. In contrast, neuronal expression of DN-DISC1 and Δ^9-THC treatment seem to have greater effects on fear conditioning, consistent with our prior studies with a constitutive DN-DISC1 model (18).

The effects on recognition memory in aDN-DISC1 mice are unlikely dependent on early developmental effects of DN-DISC1, as turning off expression of DN-DISC1 after P21 completely eliminates the cognitive effects observed in our model. This appears in line with our prior reports on differential effects of DN-DISC1 on various behaviors depending on the time this risk factor was expressed in neurons or astrocytes (96,97). In addition, our data with DOX manipulation suggests that expression of DN-DISC1 during adolescent exposure to Δ^9-THC is critical for the cognitive effects observed in our model. However, one cannot completely rule out the potential effects of DOX itself on neuroinflammatory processes in astrocytes that may have contributed to the preventive effects of DOX treatment.

The glial fibrillary acidic protein (GFAP)-tetracycline-controlled transactivator;DN-DISC1 model has some limitations that make identification of underlying neural circuits mechanisms challenging. The *GFAP* promoter is active in the hippocampus and subcortical regions (98), and in addition to astrocytes, it is active in progenitor cells of the dentate gyrus of the hippocampus and the olfactory bulbs (99–101). Our findings with the viral KD of *Disc1* in the CA areas were designed to address these limitations and suggest that the bulk of cognitive effects observed in DN-DISC1 mice treated with Δ^9-THC are related to altered expression of *Disc1* in hippocampal astrocytes. Additionally, the similar outcomes of *Disc1* KD and DN-DISC1 suggest that the observed behavioral outcomes are likely due to altered expression of endogenous *Disc1* rather than "off-target" or so-called gain-of-function effects of DN-DISC1 (30).

Although previous research and our current work clearly indicate that adolescent cannabis exposure can produce long-lasting behavioral and cognitive problems, there has been no direct comparison made between cognitive effects of cannabis and other psychoactive drugs. Indeed, there are numerous reports on long-term effects of psychostimulants used during adolescence (102–105). We found that chronic treatment with amphetamine of DN-DISC1 mice did not replicate the phenotypes produced by Δ^9-THC, suggesting some selectivity in behavioral outcomes of adolescence exposure to cannabinoids versus psychostimulants. Future studies will need to perform a more comprehensive dose-dependent comparative analysis.

The majority of preclinical research on cannabis has focused on GABA or glutamatergic neurons (106–111). However, there is a growing appreciation that glial cells also contribute to the detrimental behavioral effects associated with cannabis (2–4), as glial cells also express CNR1 and other factors of the endocannabinoid system (112). A recent study has shown that deletion of *Cnr1* in mouse astrocytes prevents acute effects of Δ^9-THC on spatial working memory and long-term depression at hippocampal CA3-CA1 synapses. Critically, abolition of the same receptor on GABA or glutamate neurons does not lead to the same rescue phenomenon, suggesting that deficits in working memory triggered by acute administration of Δ^9-THC could be due to the activation of CNR1 signaling in astrocytes (9). To further support the major role of astrocytes in the mechanisms of cognitive impairment following Δ^9-THC exposure, another study has demonstrated that chronic Δ^9-THC triggers a sustained activation of COX-2 and increased production of prostaglandin E2 in the brain. The activation of this signaling mechanism is initiated via CNR1-coupled G protein $\beta\gamma$ subunits (10). However, astrocytes also express CNR2 (113,114). Thus, it is conceivable that at least some of the cognitive effects of Δ^9-THC may have been mediated by CNR2. Future research will address this critical question.

There are significant variations in response to cannabis among users, suggesting genetic disposition (76,115–117). Consistent with human findings, preclinical studies with mouse models carrying mutations in *NRG1*, *COMT*, or *DISC1* have shown that the effects of Δ^9-THC on adolescent or adult mutant mice can dramatically differ from those on control littermates (15,118). However, the neurobiological and molecular underpinnings of how genetic variants could moderate effects of Δ^9-THC remain poorly understood (16,119,120). Moreover, there are very few, if any, studies of the molecular mechanism of cell type–specific G×E interactions that mediate adverse effects of environmental risk factors, including cannabis. We have studied the neurobiological mechanisms of G×E interaction relevant to major psychiatric conditions using a rare mutation of a neurodevelopmental risk factor, DN-DISC1 (121), as an experimental genetic tool to identify the molecular mechanisms whereby aDN-DISC1 influences the signaling pathways activated by Δ^9-THC. Based on the results of an unbiased RNA-seq analysis and prior studies (9), we identified the neuroinflammatory signaling in astrocytes that appears to be a convergent target for DN-DISC1 and inflammatory factors upregulated by Δ^9-THC. Specifically, we found that DN-DISC1 and Δ^9-THC synergistically activate NF-κB–COX-2 signaling that might lead to increased secretion of glutamate by astrocytes. To test this molecular hypothesis, we inhibited activation of COX-2 with the selective inhibitor and were able to prevent the development of cognitive deficits in aDN-DISC1 mice treated with Δ^9-THC. We believe this pharmacological approach could be applied to other G×E rodent models with the goal to use COX-2 inhibitors to counteract and/or ameliorate psychosis-like behavioral alterations associated with neuroinflammatory conditions produced by several environmental factors, including chronic Δ^9-THC exposure during adolescence. This would be congruent with several studies that demonstrated that add-on treatment with COX-2 inhibitors had some beneficial antipsychotic and cognitive effects (122–127). However, given cell and regional heterogeneity of the hippocampus, future studies will need to validate the above molecular events using isolated tissue astrocytes from different areas of the hippocampus.

Consistent with prior rodent studies (106–111), we also found synergetic adverse effects of Δ^9-THC on the integrity of GABA

neurons. Our findings indicate that the intensity of PV^+ presynaptic boutons around pyramidal neurons of the CA3 area are predominantly affected, suggesting that inhibitory influence of PV^+ cells in the hippocampus could be compromised in aDN-DISC1 mice treated with Δ^9-THC, potentially leading to altered excitatory-inhibitory balance underlying aspects of cognitive dysfunction. In addition, in line with a recent publication, decreased PV^+ could also lead to abnormal long-term depression at hippocampal CA3-CA1 synapses (10). Future studies will address these possibilities in detail.

In conclusion, our work for the first time demonstrates that a genetic predisposition and adolescent Δ^9-THC exposure could synergistically produce a sustained activation of NF-κB–COX-2 signaling in astrocytes. This leads to elevated secretion of glutamate, reduced immunoreactivity of parvalbumin-positive presynaptic boutons around pyramidal neurons of the CA3 area of the hippocampus, and deficient memory. The observed cognitive deficits can be prevented with the COX-2 inhibitor, suggesting future targets for therapeutic interventions.

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YJ and BA performed the behavioral experiments, YJ and XZ analyzed the data, XZ and AS performed the biochemical studies, AVS and YH performed morphological experiments, JP supervised RNA sequencing analyses, YJ and MVP wrote the manuscript, and AK and MVP jointly supervised the entire project.

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ARTICLE INFORMATION

From the Department of Psychiatry and Behavioral Sciences (YJ, XZ, AVS, YH, BA, AS, JP, AK, MVP) and Solomon H. Snyder Department of Neuroscience (MVP), Johns Hopkins University School of Medicine; and the Kennedy Krieger Institute (JP), Baltimore, Maryland.

YJ and XZ contributed equally to this work.

Address correspondence to Mikhail V. Pletnikov, M.D., Ph.D., Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 600 North Wolfe Street, CMSC 8-121, Baltimore, MD 21287; E-mail: mpletni1@jhu.edu; or Atsushi Kamiya, M.D., Ph.D., Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Meyer 3-146, Baltimore, MD 21287; E-mail: akamiya1@jhmi.edu.

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REFERENCES

1. Volkow ND, Swanson JM, Evins AE, DeLisi LE, Meier MH, Gonzalez R, *et al.* (2016): Effects of cannabis use on human behavior, including cognition, motivation, and psychosis: A review. JAMA Psychiatry 73:292–297.
2. Chadwick B, Miller ML, Hurd YL (2013): Cannabis use during adolescent development: Susceptibility to psychiatric illness. Front Psychiatry 4:129.
3. Szutorisz H, Hurd YL (2018): High times for cannabis: Epigenetic imprint and its legacy on brain and behavior. Neurosci Biobehav Rev 85:93–101.
4. Broyd SJ, van Hell HH, Beale C, Yucel M, Solowij N (2016): Acute and chronic effects of cannabinoids on human cognition-A systematic review. Biol Psychiatry 79:557–567.
5. Atakan Z (2012): Cannabis, a complex plant: Different compounds and different effects on individuals. Ther Adv Psychopharmacol 2:241–254.
6. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990): Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564.
7. Navarrete M, Araque A (2010): Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron 68:113–126.
8. Stella N (2010): Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia 58:1017–1030.
9. Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F, *et al.* (2012): Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Cell 148:1039–1050.
10. Chen R, Zhang J, Fan N, Teng ZQ, Wu Y, Yang H, *et al.* (2013): Delta9-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. Cell 155:1154–1165.
11. Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, *et al.* (1998): Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 391:281–285.
12. Hasin DS (2018): US epidemiology of cannabis use and associated problems. Neuropsychopharmacology 43:195–212.
13. Blanco C, Hasin DS, Wall MM, Florez-Salamanca L, Hoertel N, Wang S, *et al.* (2016): Cannabis use and risk of psychiatric disorders: Prospective evidence from a US national longitudinal study. JAMA Psychiatry 73:388–395.
14. Levine A, Clemenza K, Rynn M, Lieberman J (2017): Evidence for the risks and consequences of adolescent cannabis exposure. J Am Acad Child Adolesc Psychiatry 56:214–225.
15. O'Tuathaigh CM, Hryniewiecka M, Behan A, Tighe O, Coughlan C, Desbonnet L, *et al.* (2010): Chronic adolescent exposure to delta-9-tetrahydrocannabinol in COMT mutant mice: Impact on psychosis-related and other phenotypes. Neuropsychopharmacology 35:2262–2273.
16. Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T (2013): Transmembrane domain Nrg1 mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence. Int J Neuropsychopharmacol 16:163–175.
17. Tantra M, Krocher T, Papiol S, Winkler D, Rockle I, Jatho J, *et al.* (2014): St8sia2 deficiency plus juvenile cannabis exposure in mice synergistically affect higher cognition in adulthood. Behav Brain Res 275:166–175.
18. Ballinger MD, Saito A, Abazyan B, Taniguchi Y, Huang CH, Ito K, *et al.* (2015): Adolescent cannabis exposure interacts with mutant DISC1 to produce impaired adult emotional memory. Neurobiol Dis 82:176–184.
19. Segal-Gavish H, Gazit N, Barhum Y, Ben-Zur T, Taler M, Hornfeld SH, *et al.* (2017): BDNF overexpression prevents cognitive deficit elicited by adolescent cannabis exposure and host susceptibility interaction. Hum Mol Genet 26:2462–2471.
20. Kamiya A, Kubo K, Tomoda T, Takaki M, Youn R, Ozeki Y, *et al.* (2005): A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. Nat Cell Biol 7:1167–1178.
21. Kamiya A, Tan PL, Kubo K, Engelhard C, Ishizuka K, Kubo A, *et al.* (2008): Recruitment of PCM1 to the centrosome by the cooperative action of DISC1 and BBS4: A candidate for psychiatric illnesses. Arch Gen Psychiatry 65:996–1006.

22. Niwa M, Kamiya A, Murai R, Kubo K, Gruber AJ, Tomita K, *et al.* (2010): Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. Neuron 65:480–489.
23. Pletnikov MV, Ayhan Y, Nikolskaia O, Xu Y, Ovanesov MV, Huang H, *et al.* (2008): Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. Mol Psychiatry 13:173–186, 115.
24. Ayhan Y, Abazyan B, Nomura J, Kim R, Ladenheim B, Krasnova IN, *et al.* (2011): Differential effects of prenatal and postnatal expressions of mutant human DISC1 on neurobehavioral phenotypes in transgenic mice: Evidence for neurodevelopmental origin of major psychiatric disorders. Mol Psychiatry 16:293–306.
25. Abazyan B, Nomura J, Kannan G, Ishizuka K, Tamashiro KL, Nucifora F, *et al.* (2010): Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology. Biol Psychiatry 68:1172–1181.
26. Ma TM, Abazyan S, Abazyan B, Nomura J, Yang C, Seshadri S, *et al.* (2013): Pathogenic disruption of DISC1-serine racemase binding elicits schizophrenia-like behavior via D-serine depletion. Mol Psychiatry 18:557–567.
27. Pogorelov VM, Nomura J, Kim J, Kannan G, Ayhan Y, Yang C, *et al.* (2012): Mutant DISC1 affects methamphetamine-induced sensitization and conditioned place preference: A comorbidity model. Neuropharmacology 62:1242–1251.
28. Terrillion CE, Abazyan B, Yang Z, Crawford J, Shevelkin AV, Jouroukhin Y, *et al.* (2017): DISC1 in astrocytes influences adult neurogenesis and hippocampus-dependent behaviors in mice. Neuropsychopharmacology 42:2242–2251.
29. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, *et al.* (2000): Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet 9:1415–1423.
30. Brandon NJ, Sawa A (2011): Linking neurodevelopmental and synaptic theories of mental illness through DISC1. Nat Rev Neurosci 12:707–722.
31. Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014): Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427.
32. Farrell MS, Werge T, Sklar P, Owen MJ, Ophoff RA, O'Donovan MC, *et al.* (2015): Evaluating historical candidate genes for schizophrenia. Mol Psychiatry 20:555–562.
33. Sullivan PF, Daly MJ, O'Donovan M (2012): Genetic architectures of psychiatric disorders: The emerging picture and its implications. Nat Rev Genet 13:537–551.
34. Geschwind DH, Flint J (2015): Genetics and genomics of psychiatric disease. Science 349:1489–1494.
35. Abazyan B, Dziedzic J, Hua K, Abazyan S, Yang C, Mori S, *et al.* (2014): Chronic exposure of mutant DISC1 mice to lead produces sex-dependent abnormalities consistent with schizophrenia and related mental disorders: A gene-environment interaction study. Schizophr Bull 40:575–584.
36. Saito A, Taniguchi Y, Kim SH, Selvakumar B, Perez G, Ballinger MD, *et al.* (2017): Developmental alcohol exposure impairs activity-dependent s-nitrosylation of NDEL1 for neuronal maturation. Cereb Cortex 27:3918–3929.
37. Koga M, Serritella AV, Messmer MM, Hayashi-Takagi A, Hester LD, Snyder SH, *et al.* (2011): Glutathione is a physiologic reservoir of neuronal glutamate. Biochem Biophys Res Commun 409:596–602.
38. Spear L (2000): Modeling adolescent development and alcohol use in animals. Alcohol Res Health 24:115–123.
39. Laviola G, Macri S, Morley-Fletcher S, Adriani W (2003): Risk-taking behavior in adolescent mice: Psychobiological determinants and early epigenetic influence. Neurosci Biobehav Rev 27:19–31.
40. Casey BJ, Pattwell SS, Glatt CE, Lee FS (2013): Treating the developing brain: Implications from human imaging and mouse genetics. Annu Rev Med 64:427–439.
41. Dutta S, Sengupta P (2016): Men and mice: Relating their ages. Life Sci 152:244–248.
42. Ge WP, Miyawaki A, Gage FH, Jan YN, Jan LY (2012): Local generation of glia is a major astrocyte source in postnatal cortex. Nature 484:376–380.
43. Kanski R, van Strien ME, van Tijn P, Hol EM (2014): A star is born: New insights into the mechanism of astrogenesis. Cell Mol Life Sci 71:433–447.
44. Lundqvist T (2010): Imaging cognitive deficits in drug abuse. Curr Top Behav Neurosci 3:247–275.
45. Barkus E, Murray RM (2010): Substance use in adolescence and psychosis: Clarifying the relationship. Annu Rev Clin Psychol 6:365–389.
46. Barbizet J (1963): Defect of memorizing of hippocampal-mammillary origin: A review. J Neurol Neurosurg Psychiatry 26:127–135.
47. Meissner WW (1967): Hippocampus and learning. Int J Neuropsychiatry 3:298–310.
48. Nakazawa K, McHugh TJ, Wilson MA, Tonegawa S (2004): NMDA receptors, place cells and hippocampal spatial memory. Nat Rev Neurosci 5:361–372.
49. Miller LL, Branconnier RJ (1983): Cannabis: Effects on memory and the cholinergic limbic system. Psychol Bull 93:441–456.
50. Schulz S (2011): MDMA & cannabis: A mini-review of cognitive, behavioral, and neurobiological effects of co-consumption. Curr Drug Abuse Rev 4:81–86.
51. Kutlu MG, Gould TJ (2016): Effects of drugs of abuse on hippocampal plasticity and hippocampus-dependent learning and memory: Contributions to development and maintenance of addiction. Learn Mem 23:515–533.
52. Hudson R, Rushlow W, Laviolette SR (2018): Phytocannabinoids modulate emotional memory processing through interactions with the ventral hippocampus and mesolimbic dopamine system: Implications for neuropsychiatric pathology. Psychopharmacology (Berl) 235:447–458.
53. Chye Y, Suo C, Yucel M, den Ouden L, Solowij N, Lorenzetti V (2017): Cannabis-related hippocampal volumetric abnormalities specific to subregions in dependent users. Psychopharmacology (Berl) 234:2149–2157.
54. Ghaderi M, Rezayof A, Vousooghi N, Zarrindast MR (2016): Dorsal hippocampal NMDA receptors mediate the interactive effects of arachidonylcyclopropylamide and MDMA/ecstasy on memory retrieval in rats. Prog Neuropsychopharmacol Biol Psychiatry 66:41–47.
55. Kirkby NS, Chan MV, Zaiss AK, Garcia-Vaz E, Jiao J, Berglund LM, *et al.* (2016): Systematic study of constitutive cyclooxygenase-2 expression: Role of NF-kappaB and NFAT transcriptional pathways. Proc Natl Acad Sci U S A 113:434–439.
56. Ricciotti E, FitzGerald GA (2011): Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 31:986–1000.
57. Zamberletti E, Gabaglio M, Grilli M, Prini P, Catanese A, Pittaluga A, *et al.* (2016): Long-term hippocampal glutamate synapse and astrocyte dysfunctions underlying the altered phenotype induced by adolescent THC treatment in male rats. Pharmacol Res 111:459–470.
58. LoVerso PR, Cui F (2016): Cell type-specific transcriptome profiling in mammalian brains. Front Biosci 21:973–985.
59. Perkins ND (2006): Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 25:6717–6730.
60. Christian F, Smith EL, Carmody RJ (2016): The regulation of NF-kappaB subunits by phosphorylation. Cells 5:E12.
61. Behan AT, Hryniewiecka M, O'Tuathaigh CM, Kinsella A, Cannon M, Karayiorgou M, *et al.* (2012): Chronic adolescent exposure to delta-9-tetrahydrocannabinol in COMT mutant mice: Impact on indices of dopaminergic, endocannabinoid and GABAergic pathways. Neuropsychopharmacology 37:1773–1783.
62. 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.

63. Gomes FV, Issy AC, Ferreira FR, Viveros MP, Del Bel EA, Guimaraes FS (2014): Cannabidiol attenuates sensorimotor gating disruption and molecular changes induced by chronic antagonism of NMDA receptors in mice. Int J Neuropsychopharmacol 18:pyu041.
64. Friend L, Weed J, Sandoval P, Nufer T, Ostlund I, Edwards JG (2017): CB1-dependent long-term depression in ventral tegmental area GABA neurons: A novel target for marijuana. J Neurosci 37:10943–10954.
65. 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.
66. Huang L, Zhao S, Lu W, Guan S, Zhu Y, Wang JH (2015): Acidosis-induced dysfunction of cortical GABAergic neurons through astrocyte-related excitotoxicity. PLoS One 10:e0140324.
67. Zhao YL, Xiang Q, Shi QY, Li SY, Tan L, Wang JT, *et al.* (2011): GABAergic excitotoxicity injury of the immature hippocampal pyramidal neurons' exposure to isoflurane. Anesth Analg 113:1152–1160.
68. Nakazawa K, Zsiros V, Jiang Z, Nakao K, Kolata S, Zhang S, *et al.* (2012): GABAergic interneuron origin of schizophrenia pathophysiology. Neuropharmacology 62:1574–1583.
69. Desfeux A, El Ghazi F, Jegou S, Legros H, Marret S, Laudenbach V, *et al.* (2010): Dual effect of glutamate on GABAergic interneuron survival during cerebral cortex development in mice neonates. Cereb Cortex 20:1092–1108.
70. Rubino T, Parolaro D (2016): The impact of exposure to cannabinoids in adolescence: Insights from animal models. Biol Psychiatry 79:578–585.
71. Rubino T, Parolaro D (2008): Long lasting consequences of cannabis exposure in adolescence. Mol Cell Endocrinol 286:S108–S113.
72. Davies SN, Pertwee RG, Riedel G (2002): Functions of cannabinoid receptors in the hippocampus. Neuropharmacology 42:993–1007.
73. Varvel SA, Hamm RJ, Martin BR, Lichtman AH (2001): Differential effects of delta 9-THC on spatial reference and working memory in mice. Psychopharmacology (Berl) 157:142–150.
74. Cha YM, White AM, Kuhn CM, Wilson WA, Swartzwelder HS (2006): Differential effects of delta9-THC on learning in adolescent and adult rats. Pharmacol Biochem Behav 83:448–455.
75. Wise LE, Long KA, Abdullah RA, Long JZ, Cravatt BF, Lichtman AH (2012): Dual fatty acid amide hydrolase and monoacylglycerol lipase blockade produces THC-like Morris water maze deficits in mice. ACS Chem Neurosci 3:369–378.
76. Silveira MM, Arnold JC, Laviolette SR, Hillard CJ, Celorrio M, Aymerich MS, *et al.* (2017): Seeing through the smoke: Human and animal studies of cannabis use and endocannabinoid signalling in corticolimbic networks. Neurosci Biobehav Rev 76:380–395.
77. Jutras-Aswad D, Jacobs MM, Yiannoulos G, Roussos P, Bitsios P, Nomura Y, *et al.* (2012): Cannabis-dependence risk relates to synergism between neuroticism and proenkephalin SNPs associated with amygdala gene expression: Case-control study. PLoS One 7:e39243.
78. Morris CV, DiNieri JA, Szutorisz H, Hurd YL (2011): Molecular mechanisms of maternal cannabis and cigarette use on human neurodevelopment. Eur J Neurosci 34:1574–1583.
79. Barthelemy OJ, Richardson MA, Cabral HJ, Frank DA (2016): Prenatal, perinatal, and adolescent exposure to marijuana: Relationships with aggressive behavior. Neurotoxicol Teratol 58:60–77.
80. Alegria AA, Hasin DS, Nunes EV, Liu SM, Davies C, Grant BF, *et al.* (2010): Comorbidity of generalized anxiety disorder and substance use disorders: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. J Clin Psychiatry 71:1187–1195; quiz 1252-1183.
81. Ehrenreich H, Rinn T, Kunert HJ, Moeller MR, Poser W, Schilling L, *et al.* (1999): Specific attentional dysfunction in adults following early start of cannabis use. Psychopharmacology (Berl) 142:295–301.
82. Pistis M, Perra S, Pillolla G, Melis M, Muntoni AL, Gessa GL (2004): Adolescent exposure to cannabinoids induces long-lasting changes in the response to drugs of abuse of rat midbrain dopamine neurons. Biol Psychiatry 56:86–94.
83. Pope HG Jr, Gruber AJ, Hudson JI, Cohane G, Huestis MA, Yurgelun-Todd D (2003): Early-onset cannabis use and cognitive deficits: What is the nature of the association? Drug Alcohol Depend 69:303–310.
84. 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.
85. Belue RC, Howlett AC, Westlake TM, Hutchings DE (1995): The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. Neurotoxicol Teratol 17:25–30.
86. McLaughlin CR, Martin BR, Compton DR, Abood ME (1994): Cannabinoid receptors in developing rats: Detection of mRNA and receptor binding. Drug Alcohol Depend 36:27–31.
87. Seeman P (1999): Images in neuroscience. Brain development, X: Pruning during development. Am J Psychiatry 156:168.
88. Luna B, Garver KE, Urban TA, Lazar NA, Sweeney JA (2004): Maturation of cognitive processes from late childhood to adulthood. Child Dev 75:1357–1372.
89. Luna B, Padmanabhan A, O'Hearn K (2010): What has fMRI told us about the development of cognitive control through adolescence? Brain Cogn 72:101–113.
90. Hassabis D, Kumaran D, Vann SD, Maguire EA (2007): Patients with hippocampal amnesia cannot imagine new experiences. Proc Natl Acad Sci U S A 104:1726–1731.
91. Kesner RP (2007): Behavioral functions of the CA3 subregion of the hippocampus. Learn Mem 14:771–781.
92. 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.
93. Padula CB, Schweinsburg AD, Tapert SF (2007): Spatial working memory performance and fMRI activation interaction in abstinent adolescent marijuana users. Psychol Addict Behav 21:478–487.
94. Cousijn J, Wiers RW, Ridderinkhof KR, van den Brink W, Veltman DJ, Goudriaan AE (2014): Effect of baseline cannabis use and working-memory network function on changes in cannabis use in heavy cannabis users: A prospective fMRI study. Hum Brain Mapp 35:2470–2482.
95. Realini N, Rubino T, Parolaro D (2009): Neurobiological alterations at adult age triggered by adolescent exposure to cannabinoids. Pharmacol Res 60:132–138.
96. Xia M, Broek JA, Jouroukhin Y, Schoenfelder J, Abazyan S, Jaaro-Peled H, *et al.* (2016): Cell type-specific effects of mutant DISC1: A proteomics study. Mol Neuropsychiatry 2:28–36.
97. Xia M, Zhu S, Shevelkin A, Ross CA, Pletnikov M (2016): DISC1, astrocytes and neuronal maturation: A possible mechanistic link with implications for mental disorders. J Neurochem 138:518–524.
98. Lee Y, Messing A, Su M, Brenner M (2008): GFAP promoter elements required for region-specific and astrocyte-specific expression. Glia 56:481–493.
99. Weber T, Baier V, Pauly R, Sahay A, Baur M, Herrmann E, *et al.* (2011): Inducible gene expression in GFAP+ progenitor cells of the SGZ and the dorsal wall of the SVZ-A novel tool to manipulate and trace adult neurogenesis. Glia 59:615–626.
100. Casper KB, McCarthy KD (2006): GFAP-positive progenitor cells produce neurons and oligodendrocytes throughout the CNS. Mol Cell Neurosci 31:676–684.
101. Namba T, Mochizuki H, Suzuki R, Onodera M, Yamaguchi M, Namiki H, *et al.* (2011): Time-lapse imaging reveals symmetric neurogenic cell division of GFAP-expressing progenitors for expansion of postnatal dentate granule neurons. PLoS One 6:e25303.
102. Berman SM, Kuczenski R, McCracken JT, London ED (2009): Potential adverse effects of amphetamine treatment on brain and behavior: A review. Mol Psychiatry 14:123–142.
103. Park TM, Haning WF 3rd (2016): Stimulant use disorders. Child Adolesc Psychiatr Clin North Am 25:461–471.

104. Marshall BD, Werb D (2010): Health outcomes associated with methamphetamine use among young people: A systematic review. Addiction 105:991–1002.
105. Levine DA (2007): "Pharming": The abuse of prescription and over-the-counter drugs in teens. Curr Opin Pediatr 19:270–274.
106. Cortes-Briones JA, Cahill JD, Skosnik PD, Mathalon DH, Williams A, Sewell RA, *et al.* (2015): The psychosis-like effects of Delta(9)-tetrahydrocannabinol are associated with increased cortical noise in healthy humans. Biol Psychiatry 78:805–813.
107. Radhakrishnan R, Skosnik PD, Cortes-Briones J, Sewell RA, Carbuto M, Schnakenberg A, *et al.* (2015): GABA deficits enhance the psychotomimetic effects of Delta9-THC. Neuropsychopharmacology 40:2047–2056.
108. Cortes-Briones J, Skosnik PD, Mathalon D, Cahill J, Pittman B, Williams A, *et al.* (2015): Delta9-THC disrupts gamma (gamma)-band neural oscillations in humans. Neuropsychopharmacology 40:2124–2134.
109. Skosnik PD, Krishnan GP, D'Souza DC, Hetrick WP, O'Donnell BF (2014): Disrupted gamma-band neural oscillations during coherent motion perception in heavy cannabis users. Neuropsychopharmacology 39:3087–3099.
110. Zamberletti E, Beggiato S, Steardo L Jr, Prini P, Antonelli T, Ferraro L, *et al.* (2014): Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. Neurobiol Dis 63:35–47.
111. Colizzi M, McGuire P, Pertwee RG, Bhattacharyya S (2016): Effect of cannabis on glutamate signalling in the brain: A systematic review of human and animal evidence. Neurosci Biobehav Rev 64:359–381.
112. Stella N (2004): Cannabinoid signaling in glial cells. Glia 48:267–277.
113. Fernandez-Trapero M, Espejo-Porras F, Rodriguez-Cueto C, Coates JR, Perez-Diaz C, de Lago E, *et al.* (2017): Upregulation of CB2 receptors in reactive astrocytes in canine degenerative myelopathy, a disease model of amyotrophic lateral sclerosis. Dis Model Mech 10:551–558.
114. Cassano T, Calcagnini S, Pace L, De Marco F, Romano A, Gaetani S (2017): Cannabinoid receptor 2 signaling in neurodegenerative disorders: From pathogenesis to a promising therapeutic target. Front Neurosci 11:30.
115. Benyamina A, Bonhomme-Faivre L, Picard V, Sabbagh A, Richard D, Blecha L, *et al.* (2009): Association between ABCB1 C3435T polymorphism and increased risk of cannabis dependence. Prog Neuropsychopharmacol Biol Psychiatry 33:1270–1274.
116. Spiro AS, Wong A, Boucher AA, Arnold JC (2012): Enhanced brain disposition and effects of Delta9-tetrahydrocannabinol in P-glycoprotein and breast cancer resistance protein knockout mice. PLoS One 7:e35937.
117. Saito A, Ballinger MD, Pletnikov MV, Wong DF, Kamiya A (2013): Endocannabinoid system: Potential novel targets for treatment of schizophrenia. Neurobiol Dis 53:10–17.
118. O'Tuathaigh CM, Clarke G, Walsh J, Desbonnet L, Petit E, O'Leary C, *et al.* (2012): Genetic vs. pharmacological inactivation of COMT influences cannabinoid-induced expression of schizophrenia-related phenotypes. Int J Neuropsychopharmacol 15:1331–1342.
119. Calvignac-Spencer S, Merkel K, Kutzner N, Kuhl H, Boesch C, Kappeler PM, *et al.* (2013): Carrion fly-derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. Mol Ecol 22:915–924.
120. Du H, Kwon IK, Kim J (2013): Neuregulin-1 impairs the long-term depression of hippocampal inhibitory synapses by facilitating the degradation of endocannabinoid 2-AG. J Neurosci 33:15022–15031.
121. Ayhan Y, McFarland R, Pletnikov MV (2016): Animal models of gene-environment interaction in schizophrenia: A dimensional perspective. Prog Neurobiol 136:1–27.
122. Zheng W, Cai DB, Yang XH, Ungvari GS, Ng CH, Muller N, *et al.* (2017): Adjunctive celecoxib for schizophrenia: A meta-analysis of randomized, double-blind, placebo-controlled trials. J Psychiatr Res 92:139–146.
123. Muller N, Riedel M, Scheppach C, Brandstatter B, Sokullu S, Krampe K, *et al.* (2002): Beneficial antipsychotic effects of celecoxib add-on therapy compared to risperidone alone in schizophrenia. Am J Psychiatry 159:1029–1034.
124. Marini S, De Berardis D, Vellante F, Santacroce R, Orsolini L, Valchera A, *et al.* (2016): Celecoxib adjunctive treatment to antipsychotics in schizophrenia: A review of randomized clinical add-on trials. Mediators Inflamm 2016:3476240.
125. Nitta M, Kishimoto T, Muller N, Weiser M, Davidson M, Kane JM, *et al.* (2013): Adjunctive use of nonsteroidal anti-inflammatory drugs for schizophrenia: A meta-analytic investigation of randomized controlled trials. Schcizophr Bull 39:1230–1241.
126. Akhondzadeh S, Tabatabaee M, Amini H, Ahmadi Abhari SA, Abbasi SH, Behnam B (2007): Celecoxib as adjunctive therapy in schizophrenia: A double-blind, randomized and placebo-controlled trial. Schcizophr Res 90:179–185.
127. Rapaport MH, Delrahim KK, Bresee CJ, Maddux RE, Ahmadpour O, Dolnak D (2005): Celecoxib augmentation of continuously ill patients with schizophrenia. Biol Psychiatry 57:1594–1596.