BDNF Overexpression Prevents Cognitive Deficit Elicited by Adolescent Cannabis Exposure and Host Susceptibility Interaction

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Abstract

Cannabis abuse in adolescence is associated with increased risk of psychotic disorders. Δ-9-tetrahydrocannabinol (THC) is the primary psychoactive component of cannabis. Disrupted-In-Schizophrenia-1 (DISC1) protein is a driver for major mental illness by influencing neurodevelopmental processes. Here, utilizing a unique mouse model based on host (DISC1) X environment (THC administration) interaction, we aimed at studying the pathobiological basis through which THC exposure elicits psychiatric manifestations. Wild-Type and dominant-negative-DISC1 (DN-DISC1) mice were injected with THC (10 mg/kg) or vehicle for 10 days during mid-adolescence-equivalent period. Behavioral tests were conducted to assess exploratory activity (open field test, light-dark box test) and cognitive function (novel object recognition test). Electrophysiological effect of THC was evaluated using acute hippocampal slices, and hippocampal cannabinoid receptor type 1 and brain-derived neurotrophic factor (BDNF) protein levels were measured. Our results indicate that THC exposure elicits deficits in exploratory activity and recognition memory, together with reduced short-term synaptic facilitation and loss of BDNF surge in the hippocampus of DN-DISC mice, but not in wild-type mice. Over-expression of BDNF in the hippocampus of THC-treated DN-DISC1 mice prevented the impairment in recognition memory. The results of this study imply that induction of BDNF following adolescence THC exposure may serve as a homeostatic response geared to maintain proper cognitive function against exogenous insult. The BDNF surge in response to THC is perturbed in the presence of mutant DISC1, suggesting DISC1 may be a useful probe to identify biological cascades involved in the neurochemical, electrophysiological, and behavioral effects of cannabis related psychiatric manifestations.

Introduction

Psychotic disorders are increasingly considered as disorders of brain development (1). While it is widely accepted that host-environment interactions contribute to the pathogenesis of psychotic disorders (2, 3), the biological mechanisms that mediate such interactions are unclear. Thus, utilizing animal models for investigating synergistic effects between a candidate gene and an environmental factor may provide better understanding of the complicated mechanisms underlying genetic susceptibility for different environmental insults.

Cannabis is the most commonly used illicit drug among adolescents in the U.S. (4) and in Europe (5), and is increasingly becoming legalized for recreational use (6). The consequences of cannabis use during adolescence is of increased concern due to its deleterious effects on brain development (7). Several studies have shown that cannabis use during adolescence increases the risk of developing first episode psychosis as well as various psychotic disorders later in life (8–12). Recent studies suggest that the primary psychoactive component of cannabis, Δ9-tetrahydrocannabinol (THC), interferes with maturational and developmental processes in the brain of adolescents and causes psychopathological effects, such as disinhibition and neurocognitive abnormalities, especially in learning and memory processes (13–15). Nevertheless, merely a small fraction of adolescent cannabis users develop a psychotic disorder. One possible explanation is that certain individuals are genetically susceptible to mental illness. This explanation is supported by a recent study reporting that cannabis use in early adolescence moderates the association between the genetic risk for schizophrenia and cortical maturation among male individuals (16).

The *Disrupted-In-Schizophrenia-1 (DISC1)* gene was discovered through a unique Scottish pedigree in which a balanced translocation in this gene segregated with severe mental illness including schizophrenia (17). Although abundant research has been conducted since its discovery, currently it is still unclear whether genetic variation in *DISC1* is involved in sporadic cases of schizophrenia in the general population.

DISC1 protein is a molecular driver for major mental illness by influencing biological processes that mediate neurodevelopment (18), including progenitor cell proliferation, neurite outgrowth, neuronal migration and synapse formation and maintenance (19, 20). DISC1 is highly expressed in the developing brain, both prenatally and postnatally from birth through adulthood, including the olfactory bulb and hippocampus, regions of adult neurogenesis (21). DISC1 is an intracellular scaffold protein which interacts with several proteins, thus serving as a hub protein for a variety of cellular processes (22). Therefore, DISC1 is an attractive target for studying the biological mechanisms that underpin host susceptibility to mental illness induced by environmental insults during brain development.

In a previous work from our team, we studied the long lasting effect of adolescence THC use in adult mice (23). In the current study, we aimed to investigate the effect of robust THC administration during adolescence in the post adolescent-young adulthood period in order to model the effect of cannabis on susceptible host in the context of first episode psychosis. Furthermore, we investigated the biological mechanism of the interaction between perturbed DISC1 signaling and THC exposure during adolescence. We employed a transgenic mouse model expressing a putative dominant-negative mutant form of DISC1 (DN-DISC1) under the control of the αCaMKII promoter, thereby

restricting its expression to forebrain pyramidal neurons (24). We exposed adolescent DN-DISC1 mice to sub-chronic THC administration and evaluated the behavioral, biochemical and electrophysiological effects a few days after THC administration. We aimed to recapitulate the clinical presentation of adolescent/early adulthood patients presenting to psychiatry clinics with first clinical signs of a psychotic disorder after robust use of cannabis. This host (DN-DISC1 mice) X environment (THC administration) experimental paradigm enabled us to study the biological mechanisms through which DISC1 pathway perturbation interacts with adolescence THC exposure to exert behavioral deficits. Specifically, we investigated the role of BDNF signaling as it was previously suggested to be aberrant in this mouse model (25).

Results

Adolescent THC exposure induces aberrant exploratory activity in DN-DISC1 mice, but not in WT mice

We first wanted to see whether interaction of THC exposure with genetic aberrations can affect behavioral parameters. WT and DN-DISC1 mice exposed sub-chronically to THC did not show a decrease in locomotor activity during a ten-minute period of exploration in the open field arena (Fig. 1A). Vehicle-treated DN-DISC1 mice did not differ from vehicle-treated WT mice in terms of time spent in the anxiety-provoking center of the open field arena. Nevertheless, following sub-chronic THC administration, DN-DISC1 mice spent more time exploring the arena center compared to vehicle-treated DN-DISC1 mice. This effect was not evident in WT mice exposed to THC. Two-way ANOVA revealed a significant genotype X exposure interaction (Fig. 1B).

In the light-dark box test, THC-treated DN-DISC1 mice spent the longest period of time exploring the anxiety-provoking light chamber compared to the other experimental groups. Two-way ANOVA revealed a significant main effect of exposure and genotype, though genotype X exposure interaction was nonsignificant (Fig. 1D). Evaluation of the latency to enter the dark compartment of the light-dark box, showed that THC-treated DN-DISC1 mice demonstrated the longest latency to enter the non-anxiety-provoking dark chamber compared to other experimental groups (Fig. 1E). Main effects of genotype and exposure were significant, however genotype X exposure interaction was nonsignificant.

THC interacts with DISC1 to induce impaired object recognition memory

We examined whether THC would affect cognitive function in our genetically susceptible DN-DISC mice (Fig. 2A). Vehicle-treated WT mice, as well as THC-treated WT mice and vehicle-treated DN-DISC1 mice- displayed intact object recognition memory, manifested by preference to explore the novel object (exploring the novel object more than the familiar object), following a retention period of 1 hour. However, DN-DISC1 mice exposed sub-chronically to THC did not show preference for the novel object, indicating disrupted short-term object recognition memory (Fig. 2B). Two-way ANOVA revealed a significant genotype X exposure interaction (Fig. 2B).

Since object recognition is considered to be a hippocampus-dependent task (26), we aimed to investigate the effect of THC on synaptic function in the CA3-CA1 hippocampal pathway using acute hippocampal slices of an additional cohort of DN-DISC1 mice that were treated sub-chronically with THC. First, we measured the effect of THC on input-output relationship in the CA3-CA1 synaptic connections. For this, we recorded field excitatory postsynaptic potential (fEPSP) in CA1 area evoked by low frequency stimulation (0.1 Hz) of CA3 axons at gradually increased stimulation intensities (Fig 2C). The slope of input (amplitude of fiber volley) / output (amplitude of fEPSP) curve was similar between THC-treated and vehicle-treated DN-DISC1 mice ($p = 0.58$), indicating that basal CA3-CA1 synaptic transmission was preserved following THC exposure. To examine the influence of THC on short-term synaptic plasticity, a critical determinant of synaptic computation and memory function,(26, 27) we recorded fEPSP in CA1 stratum radiatum, while stimulating the CA3 axons in the Shaffer Collateral pathway by bursts composed of 5 stimuli at 50 Hz (Fig. 2D).

THC significantly reduced synaptic facilitation as estimated by a decrease in the relative fEPSP slope within the burst in DN-DISC1 mice, while demonstrating no interaction between THC and stimulus number during the burst. This indicates that for THC-treated DN-DISC1 mice, facilitation in CA3-CA1 connections is decreased equally throughout the burst relative to vehicle-treated controls. Thus, sub-chronic administration of THC reduces high-pass filter properties of CA3-CA1

hippocampal synapses that may contribute to the observed behavioral deficits.

THC administration induces an increase in hippocampal CB1R and BDNF levels in WT mice, but not in DN-DISC1 mice

Next, we evaluated CB1R expression levels in the hippocampi of the mice using western blot analysis. We found a significant genotype X exposure interaction (Fig. 3A), as CB1R expression was down regulated following THC administration in the DN-DISC1 mice whereas in the WT mice it was slightly upregulated.

Taking into account that THC is known to upregulate BDNF expression (28–30), we measured BDNF protein levels in the hippocampus using ELISA. In WT mice, sub-chronic administration of THC resulted in a significant increase of 42% in hippocampal BDNF (Fig. 3B). However, in DN-DISC1 treated sub-chronically with THC, this increase was not evident, revealing a significant genotype X exposure interaction.

Hippocampal over-expression of BDNF prior to THC administration specifically prevented the deficit in object recognition memory in DN-DISC1 mice

Since WT mice responded to sub-chronic THC administration with a surge in hippocampal BDNF, which was not observed in the DN-DISC1 mice, we

postulated that BDNF upsurge prevents the WT mice from developing the defective phenotype. Therefore, we over-expressed BDNF-EGFP in the hippocampus of DN-DISC1 mice using a lentiviral vector, while the control group received a lentivirus carrying an EGFP vector. Both experimental groups were later treated sub-chronically with THC, according to the protocol conducted on the previous cohorts of mice. Therefore, both groups are referred to as DN-DISC1-THC mice. Thereafter, mice underwent behavioral tests as conducted in the first cohort of mice (Fig. 4A).

After completion of behavioral tests, mice were sacrificed and several assays were conducted in order to validate over-expression of BDNF in the hippocampus. First, we verified EGFP expression in the dorsal hippocampus (Fig. 4B). In addition, hippocampal BDNF levels were determined using ELISA, and hippocampal p-TrkB, indicating BDNF signaling, were measured using western blot analysis. We detected a significant increase of 27% in hippocampal BDNF levels and a significant increase of 74% in hippocampal p-TrkB levels in DN-DISC1-THC mice injected with the BDNF-EGFP vector (BDNF-treated DN-DISC1-THC mice) compared with DN-DISC1 mice injected only with the EGFP vector (sham-treated DN-DISC1-THC mice) (Fig. 4C-D).

Behavioral analysis revealed that, in the hippocampus-dependent novel object recognition test, BDNF-treated DN-DISC1-THC mice showed intact preference to the novel object, which was not displayed by the sham-treated DN-DISC1-THC mice (Fig. 4I). Importantly, BDNF treatment did not induce alterations in behavior in the open field test (Fig. 4E-F), or in the light-dark box test (Fig. 4G-H), as compared with the sham treatment.

Discussion

Our results suggest that sub-chronic exposure to THC in adolescence-equivalent period results in significant aberrations in behavior only in mice harboring host susceptibility (DN-DISC1 mice). This finding is consistent with the accepted view that genetic background interacts with environmental insults to induce psychopathology (gene X environment interaction) (31–33). Our study reveals a strong connection between DISC1 X THC interactions and cognitive function. DN-DISC1 mice exposed to THC showed impairments in exploratory activity, reduced performance in object recognition, a hippocampal dependent memory test, together with reduced hippocampal short-term synaptic facilitation.

As DISC1 protein is considered a hub protein that interacts with numerous intracellular proteins for multiple neural signaling cascades, likely relevant to the pathobiology of mental illness (18), it is plausible that THC-elicited cellular cascades interact with DISC1. We observed that DN-DISC1 mice fail to respond to THC administration with a BDNF surge in the hippocampus, as compared to WT mice. Furthermore, we have shown that an intervention that increases the levels of hippocampal BDNF levels ameliorates the cognitive deficit, indicating that the interaction of DISC1 with the cascade starting in THC exposure and leading eventually to BDNF induction, is involved in the cognitive deficits at the mechanistic level. The fact that BDNF introduction did not affect the behavior of the mice in the open field and light-dark box tests suggests that the effect of hippocampal BDNF over expression is more specific to the cognitive aspects investigated in this study.

BDNF has been reportedly elevated in multiple brain areas following exposure to THC (28, 29), in particular in the hippocampus (30) where BDNF is well

characterized as a key modulator of hippocampal synaptic plasticity (34). In addition, cannabinoid signaling induces BDNF mediated neuroprotection of striatal neurons against excitotoxicity (29). Nonetheless, the *in vivo* role for BDNF induction in response to THC exposure was unclear. This study has provided an answer to this important question. The interaction of DISC1 with the cascade from THC to BDNF leads to the loss of hippocampal BDNF surge and cognitive deficits, which is prevented by the exogenous supply of BDNF. Therefore, these results imply that the induction of BDNF after THC exposure is a homeostatic response of the host to maintain proper cognitive function against the exogenous insult. Any host factors (e.g., genetic variations) that provide vulnerability to this homeostatic and compensatory mechanism may underlie the risk for cognitive deficits and mental dysfunction elicited by THC or cannabis.

Cannabis abuse during adolescence has long been known to be a risk factor for psychosis (3), with evidence for increased psychotogenic effect of cannabis with high THC concentration (35). A recent study reported that cannabis imposes an even greater risk for first-episode psychosis than previously thought (12). Mechanistically, it is suggested that THC disturbs the physiological control of the endogenous cannabinoid system over glutamate and GABA release. One hypothesis is that transient blockade of the CB1 receptor, by exposure to THC, leads to an excess of glutamate and consequently too great an influx of calcium in the postsynaptic neuron, thereby causing excitotoxicity (13). Alternatively, cannabinoid signaling dysregulation in CCK-positive CB1R-expressing GABAergic interneurons, induced by the administration of THC, may in turn result in desynchronization of neural oscillations in the gamma range (36).

Nonetheless, it was unclear how this strong environmental stressor (adolescent cannabis exposure) interacts with host factors. To address this question, some

groups have exposed THC to genetic animal models that by themselves display behavioral deficits, relevant to schizophrenia and related disorders (23, 32, 33). Encouraged by the findings from Ballinger et al. (23), that focused on the long lasting effects in adulthood of childhood-adolescent THC use, we sought to investigate the effect of robust THC use during adolescence in the context of first episode psychosis in post adolescent-young adulthood period. Importantly, these distinct paradigms provided divergent results in terms of cognitive domains affected, as Ballinger et al. did not describe long lasting deficits in object recognition memory, but rather reported deficits in emotional memory. Importantly, the current study reveals a novel mechanism through which cannabis exerts its detrimental effect on the susceptible DN-DISC1 mutant mice.

The mechanism proposed in the present study aids in understadning of such preclinical studies. We believe that the strength of this study lies in providing an important mechanism downstream of cannabis exposure by using a host-environmental factor interaction model. Future studies may test whether BDNF-mediated improvement of THC-induced cognitive deficits, is underpinned by rescue of short-term synaptic plasticity impairments.

Our study has some limitations. The behavioral results, demonstrating significant gene X exposure interactions in some, but not all the behavioral parameters evaluated, indicate the complexity of the interaction. Specifically, we found that THC-treated DN-DISC1 mice spent more time exploring the anxiety provoking - center of the open field arena and light chamber of the light-dark box. This could be conceptualized as a result of anxiolytic effect of THC on the DN-DISC1 susceptible mice, similar to the effect seen following benzodiazepines treatment in these paradigms (37). Alternatively, it can also be interpreted as "poor judgment" or behavioral disinhibition (38) and thus as a possible indication for

psychosis in the DN-DISC1 susceptible mice. Notably, animal models cannot recapitulate the complexity of schizophrenia or first episode psychosis (39), but rather serve as an efficient tool to test genetic predisposition (DISC1) to pathological states in the context of an environmental insult (THC). Therefore, we focused on the evident cognitive effect that was observed in our study. The other behavioral measures and their possible meaning in the context of psychosis- should be interpreted with caution.

To conclude, we present a mechanism mediating host susceptibility to environmental insult using a translational mouse model with possible relevance to schizophrenia onset. The development of a host-environment interaction model that utilizes perturbed DISC1 pathway as host vulnerability and THC exposure as an environmental insult, could be helpful in elucidating the underpinning pathobiology of psychotic disorders, in discovering novel pharmacological targets, and in testing therapeutic potential of novel treatment modalities. We suggest that future studies should use the signaling pathway proposed here as a possible target for treating patients at the early stage of mental illness, and that enhancement of BDNF signaling may be a viable approach to prevent deterioration of symptoms towards full blown severe mental disease such as schizophrenia.

Materials and Methods

Animals

We used a homozygous line of dominant-negative disrupted in schizophrenia 1 (DN-DISC1) mice developed on a C57BL/6J background (24, 40–42). DN-DISC1 mice express a putative dominant-negative C-terminal truncated DISC1 under the control of the αCaMKII promoter. Using this line created thorough homozygous inbreeding, we do not have littermate controls. Importantly, it was shown that the control non-littermate wild-type mice (offspring of the heterozygote DN-DISC1 mice that do not express the truncated DISC1), do not differ from C57BL/6J in various behavioral tests (24). Thus, as controls we used Wild-Type C57BL/6 (WT) mice from Harlan (Harlan Laboratories, Jerusalem, Israel).

Mice were housed in a group of 3-5 per cage. All mice were kept in a 12 h light/dark cycle and had access to food and water ad libitum. Behavioral training and testing were completed in the light cycle between 8:00 A.M and 5:00 P.M. All animal experiments and protocols were approved by the Committee for Animal Research at Tel Aviv University, Israel.

Experimental design

In the first cohort, six weeks old (+/- 5 days) male WT (n=22) and DN-DISC1 (n=19) mice were injected intraperitoneally with THC (10 mg/kg) or vehicle for 10 days during adolescence-equivalent period (postnatal 42–51d). Three days after the last THC administration, the behavioral effects of sub-chronic THC administration were evaluated utilizing behavioral tests to assess exploratory

activity (open field, light-dark box test) and cognitive function (novel object recognition test).

For electrophysiological analysis, we used another cohort of six weeks old (+/- 5 days) male DN-DISC1 mice. The mice were injected intraperitoneally with THC (10 mg/kg) (n=5) or vehicle (n=5) for 10 days during adolescence-equivalent period (postnatal 42–51d). Three-four days after the last THC administration the effects of sub-chronic THC injections on hippocampal short term facilitation were evaluated using field EPSP recordings in the Schaffer Collateral (SC) of acute hippocampal slices.

In another mice cohort, five weeks old (+/- 5 days) DN-DISC1 mice were injected with lentiviral vectors carrying BDNF-EGFP (n=15) or EGFP (n=14) into the dorsal hippocampus. One week later (six weeks old) mice were injected intraperitoneally with 10 mg/kg THC for 10 days. Three days after the last THC administration, mice underwent behavioral tests to assess exploratory activity (open field, light-dark box test) and recognition memory (novel object recognition test).

Behavioral tests

Analysis of the behavioral testing was conducted using the EthoVision XT 10 software platform (Noldus, Wageningen, Netherlands).

Open field test

In the open field test, mice were placed in a 47x47x51cm arena for 10 minutes. Total distance travelled and duration of time exploring the periphery and the center of the arena were measured using the EthoVision software.

Light-dark box test

The light-dark apparatus is a rectangular box divided into a small (27x18x30cm) and a large (27x27x30cm) chambers with a small entrance (6x10cm) located in

the center of the partition at floor level. The small chamber is a closed compartment painted in black (dark chamber), whereas the large chamber is transparent, open-topped and brightly illuminated (light chamber). Mice are placed in the brightly lit chamber and are allowed to freely explore both chambers for 10 minutes. Latency of the first crossing from the light chamber to the dark chamber and time spent in the light chamber were recorded and analyzed by EthoVision software.

Novel object recognition test

The test was conducted as reported previously (40). Briefly, mice were individually habituated to the open-field arena for three consecutive days. During the training session, two identical objects were placed in the arena and the mice were free to explore for 10 minutes. Following a retention period of 1 hour, mice were placed back to the same arena for another 10 minutes while one of the objects has been replaced with a novel object different in shape and size. Duration of time spent sniffing each object was quantified by EthoVision software. Novel object preference was calculated as the percentage of time spent sniffing the novel object relative to the total time spent sniffing both objects. Mice that did not sniff any of the objects were excluded from analysis.

Electrophysiology

Coronal hippocampal slices (400 μm) were prepared in a cold (4 °C) storage buffer containing (in mM): sucrose, 206; KCl, 2; $MgSO_4$, 2; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $CaCl_2$, 1; $MgCl_2$, 1; glucose, 10. The slicing procedure was performed using a Leica VT1200 vibrating microtome as described.(43) Slices were transferred to a submerged recovery chamber at room temperature containing oxygenated (95% O2 and 5% CO2) artificial cerebrospinal fluid (ACSF) for 1 h before recording.

Experiments were performed at room temperature in a recording chamber on the stage of an Olympus BX51WI microscope equipped with IR DIC optics. fEPSPs were recorded with a glass pipette containing Tyrode solution (1 – 2 MΩ) from proximal synapses in the CA1 stratum radiatum using a MultiClamp700B amplifier (Molecular Devices). Stimulation of the Schaffer Collateral pathway was delivered through a glass suction electrode (10 – 20 μm tip) filled with Tyrode solution. fEPSPs were induced by single stimuli at 0.1 Hz or by bursts (5 stimuli at 50 Hz, inter-burst-interval of 30 sec) as described before (44). Electrophysiological data were analyzed using pClamp10 (Molecular Devices).

Tissue analysis

Dissection and tissue collection

For western blot and enzyme-linked immunosorbent assay (ELISA), brains of all mice were removed and the hippocampi of each mouse were dissected, divided into left and right hemispheres and cryopreserved at -80˚C. Dissection of the mice brains was conducted using the Stainless Steel Zivic Adult Mouse Brain Slicer Matrix with 1.0 mm coronal section slice intervals (Zivic Instruments, PA, USA).

ELISA for BDNF Quantification

For protein extraction, dissected hippocampi of one hemisphere were thawed, and total protein was produced as previously described (45). Protein concentration was determined using the bicinchoninic acid (BCA) kit (Thermo Scientific, Waltham, MA, USA). Quantification of BDNF levels was conducted using a BDNF specific ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions (50 μg of each sample). The absorbance at 450 and 570 nm was recorded on a Microplate Reader (LabSystems Multiskan MS).

Synaptic protein extraction and Western Blot Analysis

Syn-PER™ Synaptic Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used for extraction of proteins expressed in the synapses of the hippocampal tissues according to the manufacturer's instructions. The protein concentration of the synaptosomal fractions was quantified utilizing the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Proteins were separated by 4–20% Mini-PROTEAN® TGX™ precast polyacrylamide gel (Bio-Rad Laboratories, CA, USA) electrophoresis and transferred to nitrocellulose membranes. The membranes were probed overnight at 4°C with the following primary antibodies: rabbit Anti-Cannabinoid Receptor 1 (1:1000, 17978-1-AP, proteintech, Chicago, IL, USA), rabbit Anti-TrkB (phospho Y515) (1 μg/ml, ab109684, Abcam, Cambridge, United Kingdom) and as a loading control mouse anti-Actin (1:500, MAB1501, EMD Millipore, Darmstadt, Germany). Thereafter, membranes were incubated with secondary antibodies: goat anti-mouse IRDye 680CW or 800CW and goat anti-rabbit IRDye 680CW or 800CW (1:5,000, infra-red dye, LI-COR Biosciences, NE, USA) for 1 hour at room temperature. The membranes were then scanned with Odyssey Infrared Imager (model 9120, LI-COR Biosciences). Densitometric analysis of Western blots was performed using Image Studio™ Lite software (LI-COR Biosciences) to measure the area and density of protein bands

Histology

Mice were transcardially perfused, under ketamine/xylazine anesthesia, with cold phosphate-buffered saline (PBS), followed by paraformaldehyde 4% in

phosphate buffer. The brains were immersed in 4% paraformaldehyde for 24 h at 4ºC followed by cryoprotection in 30% sucrose for an additional 48 h. The brains were frozen in chilled 2-methylbutane (Sigma-Aldrich) and subsequently sectioned into slices measuring 10μm. In order to demonstrate the location of the stereotaxic injection of the viral vectors carrying the EGFP gene, slides were stained with DAPI (1:500; Sigma-Aldrich) and sections were mounted with fluorescent mounting solution (Fluoromount-G, SouthernBiotech), covered with a cover slide, and sealed with nail polish. Images were taken using the Axio Imager.Z2 microscope (Zeiss, Thornwood, NY).

Preparation of viral vectors

High-titer lentiviral stocks pseudotyped with the vesicular stomatitis virus G-protein (VSV-G) were produced in HEK-293T cells as previously described.(46) In brief, HEK-293T cells were transfected with lentiviral transfer construct pLL3.7.hSyn.BDNF.IRES.EGFP.WPRE or, pLL3.7.hSyn.EGFP.WPRE and packaging constructs pMDLg-pRRE, pRSV-REV and pMD2.G envelope protein construct, by means of calcium phosphate transfection. Titers were determined by transducing HEK-293T cells with serial dilutions of concentrated lentivirus. EGFP fluorescence was evaluated by flow cytometry (FACSCalibur, Becton Dickinson Immunocytometry Systems) at 72 h after transduction. The final titer of lentiviruses for administration to the mice was determined by ELISA for p24, an abundant HIV-1 virus capsid protein (Lenti-XTM p24 Rapid Titer Kit, Clontech, Mountain View, CA, USA).

Administration of viral vectors

Under ketamine/xylazine anesthesia, mice were placed in a stereotactic frame (Kopf, Tujunga, CA), and 10^8 viral vectors (either BDNF-EGFP or EGFP alone)

in 1-μl volume were injected into each hippocampus bilaterally at 0.25 μL/min (Hamilton 701N syringe) to the following coordinates (relative to the bregma): anterior-posterior, -2mm; medial-lateral, ±1.6mm; dorsal-ventral, -2.5mm. The needle was withdrawn after 3 minutes.

Statistical analysis

All data are expressed as the mean + SEM. Statistical analyses were performed using a commercial software (GraphPad Prism 6). Comparisons between four treatment groups (WT+Vehicle, WT+THC, DN-DISC1+Vehicle, DN-DISC1+THC) were conducted using a two-way ANOVA, followed by Tukey's post-hoc test. Corrections for multiple comparisons were made when appropriate. Comparison between two groups were conducted using two-tailed *t*-test. For the electrophysiological study, two-way ANOVA with repeated measures was conducted. Statistical significance was considered for $P<0.05$ in all statistical analyses.

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Conflict of Interest

All authors declare that there are no financial disclosures relevant to this work.

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Figure legends

Figure 1. THC interacts with DISC1 to promote aberrant exploratory activity. **(A-C)** Ten-minute period of exploration in the open field arena. **(A)** Wild-type (WT) and dominant negative DISC1 (DN-DISC1) mice did not demonstrate decreased locomotor activity following sub-chronic exposure to THC. Main effect of genotype was statistically significant ($F_{1,37}$=45.47, P < .0001), but main effect of exposure and genotype X exposure interaction were not significant ($F_{1,37}$=1.43, P = .24 and $F_{1,37}$=0.02, P = .89, respectively). **(B)** THC-treated DN-DISC1 mice spent more time exploring the arena center compared to vehicle-treated DN-DISC1 mice, but this effect was not evident in WT mice exposed to THC. Genotype X exposure interaction reached significance ($F_{1,37}$=4.41, P = .04), but main effects of genotype and exposure were both nonsignificant ($F_{1,37}$=0.05, P = .83 and $F_{1,37}$=1.59, P = .22, respectively). **(C)** Illustration of the exploration path in the open field arena tracked by the EthoVision software of a representative mouse from each experimental group. **(D-E)** Light-dark box test. **(D)** Time spent in the light chamber. THC-treated DN-DISC1 mice spent the longest period of time exploring the anxiety-provoking light chamber compared with the other experimental groups. Genotype and exposure, both had significant main effects on time spent in the light chamber ($F_{1,37}$=12.88, P = .001, $F_{1,37}$=6.86, P = .013, respectively), however, genotype X exposure interaction was nonsignificant ($F_{1,37}$=0.07, P = .8). **(E)** Latency to enter the dark chamber. THC-treated DN-DISC1 mice demonstrated the longest latency to enter the dark chamber compared with other experimental groups. Main effects of genotype and exposure were significant ($F_{1,37}$=40.43, P < .0001, $F_{1,37}$=6.375, P = .016), but genotype X exposure interaction was nonsignificant ($F_{1,37}$=2.67, P = .11). Data

(Mean+SEM) were obtained from 19 DN-DISC1 males (9 Vehicle and 10 THC) and 22 WT males (11 Vehicle and 11 THC). Statistical significance was determined using two-way ANOVA followed by Tukey's post-hoc test. *$P < .05$.

Figure 2. THC interacts with DISC1 to produce impaired object recognition memory. **(A)** An illustration of the novel object recognition test. **(B)** Percentage of time spent exploring the novel object relative to the total time spent exploring both objects. Dashed line represents 50% preference indicating no preference to a specific object. A significant genotype X exposure interaction was noted ($F_{1,36}$=5.79, P = .021). Post-hoc analysis indicated that dominant negative DISC1 (DN-DISC1) mice exposed to THC spent significantly less time sniffing the novel object compared to vehicle-treated DN-DISC1 mice (P = .046). Data were obtained from 19 DN-DISC1 males (9 Vehicle and 10 THC) and 22 wild-type (WT) males (11 Vehicle and 11 THC), and presented as Mean+SEM. Statistical significance was determined using two-way ANOVA followed by Tukey's post-hoc test. **(C-D)** fEPSP recordings evoked by low frequency **(C)** and high-frequency **(D)** stimulation in CA3-CA1 connections in acute hippocampal slices. **(C)** Input-output relationship between the amplitude of fiber volley and the amplitude of fEPSP for gradually increasing stimulation intensities was not different between THC- and Vehicle- treated DN-DISC1 mice (nVehicle = 10, NVehicle = 5; nTHC = 10, NTHC = 5; P = 0.58, unpaired, two-tailed student t-test between the slopes). Insert: Representative fEPSP recordings at 50 μA stimulation amplitude (black – Vehicle, grey – THC). **(D)** Left: Representative recordings of fEPSPs in vehicle- (black) and THC-treated (grey) DN-DISC1 mice evoked by a burst: each burst contains 5 stimuli; inter-spike interval, 20 ms; inter-burst interval was 30 s. Right: Evaluation of synaptic facilitation in CA3-CA1 connections. Relative amplitude of fEPSPs within a burst, normalized to

the first stimuli in the burst. Facilitation is lower in THC-treated DN-DISC1 mice (10 slices from 5 mice of each group; $F_{1,36}$ = 11.96, P = 0.0014). Interaction with stimulus number is non-significant ($F_{3,36}$ = 0.39, P = .76). Data were averaged from 10 DN-DISC1 males (5 Vehicle and 5 THC). Data are presented as Mean±SEM. Statistical analysis was performed using two-way ANOVA with repeated measurements. *P < .05. **P < .01.

Figure 3. DISC1 interacts with THC in terms of hippocampal expression of CB1R and BDNF. **(A)** Synaptic CB1R levels were measured using Western blot analysis. The densitometry measurement of CB1 receptor signal was normalized to Actin. A significant genotype X exposure interaction was observed ($F_{1,33}$=6.41, P = .02), but main effects of both genotype and exposure were nonsignificant ($F_{1,33}$=1.14, P =.29 and $F_{1,33}$=0.19, P =.67, respectively). Data were obtained from 16 dominant-negative DISC1 (DN-DISC1) males (7 Vehicle and 9 THC) and 21 wild-type (WT) males (11 Vehicle and 10 THC). **(B)** BDNF levels in the hippocampus were measured using ELISA. A significant genotype X exposure interaction was observed ($F_{1,35}$=18.08, P = .0001). Post-hoc analysis showed that WT mice exposed to THC exhibit increased levels of hippocampal BDNF compared to vehicle-treated WT mice (P = .0003), whereas THC-treated DN-DISC1 mice did not differ from vehicle-treated DN-DISC1 mice (P = .47). Data were obtained from 19 DN-DISC1 males (9 Vehicle and 10 THC) and 20 WT males (11 Vehicle and 9 THC). Statistical significance was determined using two-way ANOVA followed by Tukey's post-hoc test. Data are presented as Mean+SEM. *p < .05; ***p = .0001; ns=not significant.

Figure 4. Hippocampal over-expression of BDNF prior to THC administration prevented the THC-induced deficit in object recognition memory in DN-DISC1 mice. **(A)** Experimental timeline for BDNF over-

expression in the hippocampus prior to sub-chronic adolescent THC administration. **(B-D)** Validation of BDNF over-expression in the hippocampus. **(B)** Representative image of EGFP expression demonstrating the location of the stereotaxic injection of the viral vectors. EGFP positive cells under control of the neuron-specific synapsin promoter (green) DAPI (4′,6-diamidino-2-phenylindole) (blue). **(C)** ELISA measurement identified a significant increase of 27% in hippocampal BDNF levels (t_{19}= 3.36, P = .0033) in DN-DISC1-THC mice injected with the BDNF-EGFP vector (BDNF) compared with DN-DISC1-THC mice injected only with the EGFP vector (EGFP). **(D)** Western blot analysis detected a significant increase of 74% in hippocampal p-TrkB levels (t_{19}=3.83, P = .0011), as an indication for BDNF signaling, in the BDNF mice compared with the EGFP mice. **(C-D)** Data were obtained from 21 DN-DISC1 males (10 EGFP and 11 BDNF). **(E-F)** Open field test measured for 10 minutes. **(E)** BDNF treatment did not induce alterations in locomotor activity or in **(F)** time spent in the center of the open field arena. **(G-H)** Light-dark box test. **(G)** BDNF treatment did not affect the time spent in the light chamber or the **(H)** latency to enter the dark chamber. Data were obtained from 29 DN-DISC1 males (14 EGFP and 15 BDNF). **(I)** Novel object recognition test. Percentage of time spent exploring the novel object relative to the total time spent exploring both objects. Dashed line represents 50% preference indicating no preference to a specific object. BDNF mice show intact preference towards the novel object compared with the EGFP mice (t_{26}=2.35, P = .026). Data were obtained from 28 DN-DISC1 males (13 EGFP and 15 BDNF). Statistical significance was determined using two-tailed Student's *t*-test. Data are presented as Mean+SEM. *P < .05. **P < .01. DN-DISC1-THC, dominant-negative DISC1 mice injected with Δ9-tetrahydrocannabinol.

Abbreviations

Brain-derived neurotrophic factor (BDNF); Cannabinoid receptor type 1 (CB1R); Disrupted-In-Schizophrenia-1 (DISC1); phosphorylated-Tropomyosin receptor kinase B (p-TrkB); α calcium/calmodulin dependent kinase II (αCaMKII); Δ9-tetrahydrocannabinol (THC).

We have addressed all comments by the reviewers (please see below a detailed response to the reviewers). These changes have further improved our manuscript, and we wish to thank the referees for their important comments and helpful advice.

REVIEWERS' COMMENTS

Reviewer: 1

Comments to the Author
Using a transgenic mouse model that selectively affects expression of DISC1 protein in forebrain pyramidal neurons, the authors present evidence consistent with a gene (i.e., the dominant negative DISC1 mutation) x environment (i.e., exposure during the critical adolescent period to THC, the active component of marijuana) effect on exploratory behavior, novel object recognition, an electrophysiological measure of hippocampal plasticity, and expression of the endogenous cannabinoid type 1 receptor, and both BDNF and the tyrosine receptor kinase that transduces the BDNF signal. Moreover, in the transgenic mice treated with a viral vector that leads to over-expression of BDNF, effects of sub-chronic THC exposure during the critical adolescent period can be attenuated. The mouse model suggests that an inability of adolescent carriers of DISC1 “risk” alleles to mount a heightened “BDNF response (i.e., increased expression of BDNF and its receptor)” during a critical period (e.g., younger than age 15 years) may be a mechanism to account for their susceptibility to disruptive “pro-psychotic” effects of THC. The submission has very clear translational implications: activation of signaling pathways that are not dependent on DISC1 for the induction of expression of BDNF.

1) The authors may wish to comment on the fact the THC-treated DN-DISC1 mice spent more time in the center of the open field and more time in the “anxiety-provoking” light chamber (and had longer latencies to enter the dark-side of the box), which is usually ascribed to anxiolytic effects. Thus, the data suggest that mere anxiolysis during the critical period of THC exposure may not be protective against a psychotic outcome in these susceptible persons (i.e., carriers of DISC1 risk alleles.

Following the reviewer's comment, this paragraph was added to the discussion section:

Our study has some limitations. The behavioral results, demonstrating significant gene X exposure interactions in some, but not all the behavioral parameters evaluated, indicate the complexity of the interaction. Specifically, we found that THC-treated DN-DISC1 mice spent more time exploring the anxiety provoking - center of the open field arena and light chamber of the light-dark box. This could be conceptualized as a result of anxiolytic effect of THC on the DN-DISC1 susceptible mice, similar to the effect seen following benzodiazepines treatment in these paradigms (37). Alternatively, it can also be interpreted as "poor judgment" or behavioral disinhibition (38) and thus as a possible indication for psychosis in the DN-DISC1 susceptible

mice. Notably, animal models cannot recapitulate the complexity of schizophrenia or first episode psychosis (39), but rather serve as an efficient tool to test genetic predisposition (DISC1) to pathological states in the context of an environmental insult (THC). Therefore, we focused on the evident cognitive effect that was observed in our study. The other behavioral measures and their possible meaning in the context of psychosis- should be interpreted with caution.

2) The authors may also wish to note that THC exposure may affect other dimensions of psychosis and "biomarkers" of psychosis risk. For example, THC exposure may disrupt the spatially and temporally-selective modulatory effects of the CCK-expressing GABAergic interneurons on the microcircuit between the fast-spiking, parvalbumin-expressing GABAergic interneurons projecting onto assemblies of pyramidal output neurons, especially in layer 3 of the cerebral cortex. The CCK-expressing GABAergic interneurons express CB1 (endogenous cannabinoid receptor type 1). The disruptive effects of THC may be manifest as a lowered power spectrum of synchronous gamma oscillatory activity.

We thank the reviewer for this comment. We added the following paragraph to the discussion section:

Mechanistically, it is suggested that THC disturbs the physiological control of the endogenous cannabinoid system over glutamate and GABA release. One hypothesis is that transient blockade of the CB1 receptor, by exposure to THC, leads to an excess of glutamate and consequently too great an influx of calcium in the postsynaptic neuron, thereby causing excitotoxicity (13). Alternatively, cannabinoid signaling dysregulation in CCK-positive CB1R-expressing GABAergic interneurons, induced by the administration of THC, may in turn result in desynchronization of neural oscillations in the gamma range (36).

Reviewer: 2

Comments to the Author
This manuscript by Segal-Gavish et al. presents a novel approach of studying a geneXenvironment interaction of DISC1 and THC exposure in a mouse model of schizophrenia. The paper is well written, the introduction leads to the hypothesis. Methods are precisely described in detail and the results support the assumptions of the research.
Nevertheless, the study results do not perfectly match the hypothesis and are more complex than portrayed in the abstract and text. Interaction of genotype and "treatment" (probably better defined as "exposure") was found in only 2 out of 4 behavioral measures (time exploring the center in the open field and novel object recognition). In the other two (time spent in light chamber and latency to enter the dark chamber) the interaction was not significant, although a trend is apparent in the graphs. In these measures a "treatment" effect was found also in the control group. Also there was no effect of BDNF introduction on open field and light/dark box measures.
This complex picture should be described more precisely in the Results section of the text and discussed as a limitation of the study.

Following the reviewer's comment, we changed the word "treatment" into "exposure" in the results section and rephrased the description of the light/dark box results in a more precise manner. Please see the following paragraph:

In the light-dark box test, THC-treated DN-DISC1 mice spent the longest period of time exploring the anxiety-provoking light chamber compared to the other experimental groups. Two-way ANOVA revealed a significant main effect of exposure and genotype, though genotype X exposure interaction was nonsignificant (Fig. 1D). Evaluation of the latency to enter the dark compartment of the light-dark box, showed that THC-treated DN-DISC1 mice demonstrated the longest latency to enter the non-anxiety-provoking dark chamber compared to other experimental groups (Fig. 1E). Main effects of genotype and exposure were significant, however, genotype X exposure interaction was nonsignificant.

In addition, we added a limitations paragraph to the discussion section discussing the points raised by the reviewer:

Our study has some limitations. The behavioral results, demonstrating significant gene X exposure interactions in some, but not all the behavioral parameters evaluated,

indicate the complexity of the interaction. Specifically, we found that THC-treated DN-DISC1 mice spent more time exploring the anxiety provoking - center of the open field arena and light chamber of the light-dark box. This could be conceptualized as a result of anxiolytic effect of THC on the DN-DISC1 susceptible mice, similar to the effect seen following benzodiazepines treatment in these paradigms (37). Alternatively, it can also be interpreted as "poor judgment" or behavioral disinhibition (38) and thus as a possible indication for psychosis in the DN-DISC1 susceptible mice. Notably, animal models cannot recapitulate the complexity of schizophrenia or first episode psychosis (39), but rather serve as an efficient tool to test genetic predisposition (DISC1) to pathological states in the context of an environmental insult (THC). Therefore, we focused on the evident cognitive effect that was observed in our study. The other behavioral measures and their possible meaning in the context of psychosis- should be interpreted with caution.

As for the comment concerning BDNF, this sentence was added to the discussion:

The fact that BDNF introduction did not affect the behavior of the mice in the open field and light-dark box tests suggests that the effect of hippocampal BDNF over expression is more specific to the cognitive aspects investigated in this study.

Other suggested changes:

Introduction:

1) Cannabis is defined as an illegal drug. It should be mentioned that this is changing in many places.

Following the reviewer's suggestion, we added this information to the introduction:

Cannabis is the most commonly used illicit drug among adolescents in the U.S. (4) and in Europe (5), and is increasingly becoming legalized for recreational use (6).

2) The interest in DISC1 as a schizophrenia gene started after a genetic study in one Scottish pedigree. Its role in the genetics of schizophrenia in the general population is less clear. This should be stated.

This paragraph was added to the introduction according to the reviewer's request: The *Disrupted-In-Schizophrenia-1* (*DISC1*) gene was discovered through a unique Scottish pedigree in which a balanced translocation in this gene segregated with severe mental illness including schizophrenia (17). Although abundant research has been conducted since its discovery, currently it is still unclear whether genetic variation in *DISC1* is involved in sporadic cases of schizophrenia in the general population.

Discussion:

1) The validity of the behavioral measures as a model for schizophrenia should be discussed as a limitation of the study.

As mentioned above, this point was added as a limitation as part of a limitations paragraph in the discussion section (Page 12, line 17).

2) Results show that DN-DISC1 mice exposed to THC were less "anxious". How does this translate to the clinical features of schizophrenia?

Following the reviewer's comment, the complexity of the behavioral results including those of the open field, with the possible "anti-anxiogenic" effect of THC on the susceptible DN-DISC1 mice, are now discussed in the paper as a new paragraph of study limitations in the discussion part (Page 12, line 17).

Yet, this point raises a key challenge in working with animal models and trying to interpret behavioral abnormalities of a model in the context of a complex brain disorder like schizophrenia. This is the reason for which the current study focused on the cognitive (novel object recognition) abnormalities and the electrophysiological

(hippocampal plasticity) and biochemical (disruption in hippocampal BDNF induction) in the animal model, rather than on the other non-specific behavioral findings.

Minor points:

Results:
Page 6, line 7 delete Moreover
End of first paragraph Change 1B to 1b
Page 7, change "2C" to "2c".

Discussion
Page 11, line 3 delete the word "first"
Page 11, Line 6 change "complemented" to "prevented"
Page 12, Line 4 change "is to provide" to "lies in providing"

All minor points were addressed accordingly in the revised version of the manuscript.

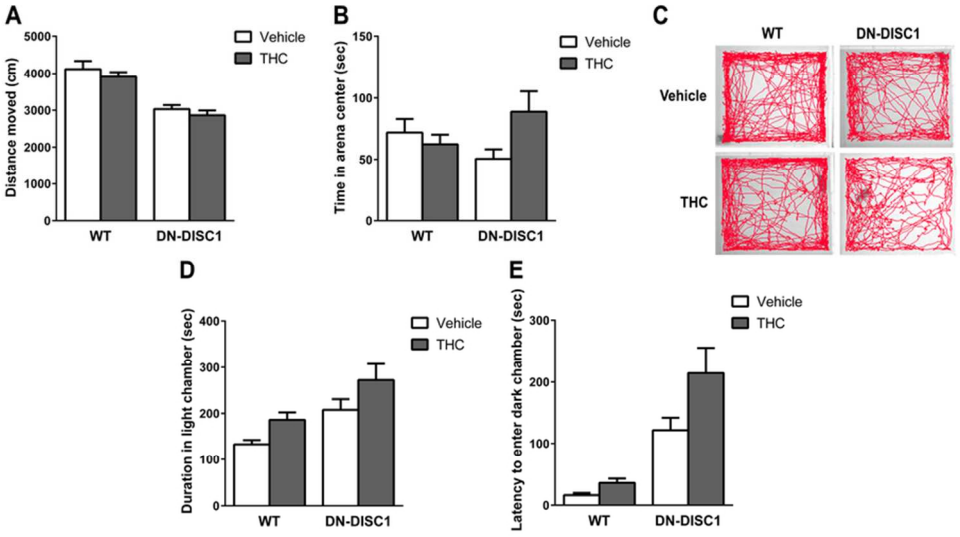

Figure 1. THC interacts with DISC1 to promote aberrant exploratory activity. (A-C) Ten-minute period of exploration in the open field arena. (A) Wild-type (WT) and dominant negative DISC1 (DN-DISC1) mice did not demonstrate decreased locomotor activity following sub-chronic exposure to THC. Main effect of genotype was statistically significant ($F_{1,37}=45.47$, $P < .0001$), but main effect of treatment and genotype X treatment interaction were not significant ($F_{1,37}=1.43$, $P = .24$ and $F_{1,37}=0.02$, $P = .89$, respectively). (B) THC-treated DN-DISC1 mice spent more time exploring the arena center compared to vehicle-treated DN-DISC1 mice, but this effect was not evident in WT mice exposed to THC. Genotype X treatment interaction reached significance ($F_{1,37}=4.41$, $P = .04$), but main effects of genotype and treatment were both nonsignificant ($F_{1,37}=0.05$, $P = .83$ and $F_{1,37}=1.59$, $P = .22$, respectively). (C) Illustration of the exploration path in the open field arena tracked by the EthoVision software of a representative mouse from each experimental group. (D-E) Light-dark box test. (D) Time spent in the light chamber. THC-treated DN-DISC1 mice spent the longest period of time exploring the anxiety-provoking light chamber compared with the other experimental groups. Genotype and treatment, both had significant main effects on time spent in the light chamber ($F_{1,37}=12.88$, $P = .001$, $F_{1,37}=6.86$, $P = .013$, respectively), however, genotype X treatment interaction was nonsignificant ($F_{1,37}=0.07$, $P = .8$). (E) Latency to enter the dark chamber. THC-treated DN-DISC1 mice demonstrated the longest latency to enter the dark chamber compared with other experimental groups. Main effects of genotype and treatment were significant ($F_{1,37}=40.43$, $P < .0001$, $F_{1,37}=6.375$, $P = .016$), but genotype X treatment interaction was nonsignificant ($F_{1,37}=2.67$, $P = .11$). Data (Mean+SEM) were obtained from 19 DN-DISC1 males (9 Vehicle and 10 THC) and 22 WT males (11 Vehicle and 11 THC). Statistical significance was determined using two-way ANOVA followed by Tukey's post-hoc test. *$P < .05$.

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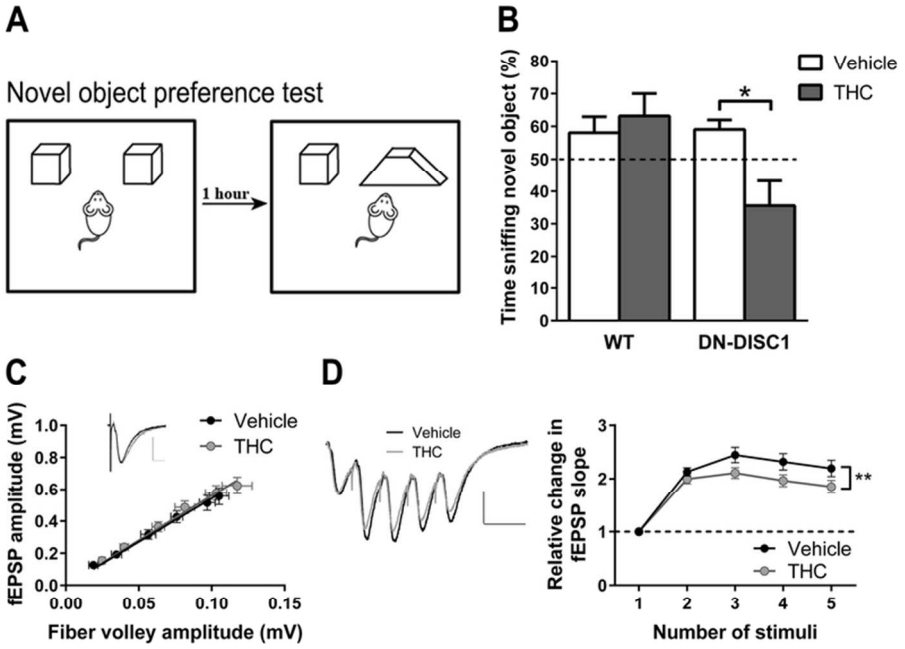

Figure 2. THC interacts with DISC1 to produce impaired object recognition memory. (A) An illustration of the novel object recognition test. (B) Percentage of time spent exploring the novel object relative to the total time spent exploring both objects. Dashed line represents 50% preference indicating no preference to a specific object. A significant genotype X treatment interaction was noted ($F_{1,36}=5.79$, $P = .021$). Post-hoc analysis indicated that dominant negative DISC1 (DN-DISC1) mice exposed to THC spent significantly less time sniffing the novel object compared to vehicle-treated DN-DISC1 mice ($P = .046$). Data were obtained from 19 DN-DISC1 males (9 Vehicle and 10 THC) and 22 wild-type (WT) males (11 Vehicle and 11 THC), and presented as Mean+SEM. Statistical significance was determined using two-way ANOVA followed by Tukey's post-hoc test. (C-D) fEPSP recordings evoked by low frequency (C) and high-frequency (D) stimulation in CA3-CA1 connections in acute hippocampal slices. (C) Input-output relationship between the amplitude of fiber volley and the amplitude of fEPSP for gradually increasing stimulation intensities was not different between THC- and Vehicle- treated DN-DISC1 mice (nVehicle = 10, NVehicle = 5; nTHC = 10, NTHC = 5; $P = 0.58$, unpaired, two-tailed student t-test between the slopes). Insert: Representative fEPSP recordings at 50 μA stimulation amplitude (black – Vehicle, grey – THC). (D) Left: Representative recordings of fEPSPs in vehicle- (black) and THC-treated (grey) DN-DISC1 mice evoked by a burst: each burst contains 5 stimuli; inter-spike interval, 20 ms; inter-burst interval was 30 s. Right: Evaluation of synaptic facilitation in CA3-CA1 connections. Relative amplitude of fEPSPs within a burst, normalized to the first stimuli in the burst. Facilitation is lower in THC-treated DN-DISC1 mice (10 slices from 5 mice of each group; $F_{1,36} = 11.96$, $P = 0.0014$). Interaction with stimulus number is non-significant ($F_{3,36} = 0.39$, $P = .76$). Data were averaged from 10 DN-DISC1 males (5 Vehicle and 5 THC). Data are presented as Mean±SEM. Statistical analysis was performed using two-way ANOVA with repeated measurements. *$P < .05$. **$P < .01$.

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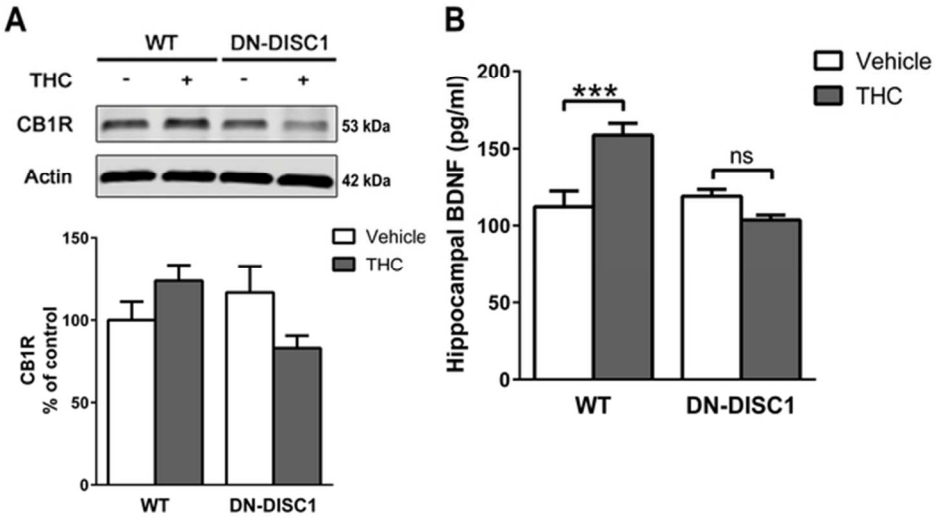

Figure 3. DISC1 interacts with THC in terms of hippocampal expression of CB1R and BDNF. (A) Synaptic CB1R levels were measured using Western blot analysis. The densitometry measurement of CB1 receptor signal was normalized to Actin. A significant genotype X treatment interaction was observed ($F_{1,33}=6.41$, $P = .02$), but main effects of both genotype and treatment were nonsignificant ($F_{1,33}=1.14$, $P = .29$ and $F_{1,33}=0.19$, $P = .67$, respectively). Data were obtained from 16 dominant-negative DISC1 (DN-DISC1) males (7 Vehicle and 9 THC) and 21 wild-type (WT) males (11 Vehicle and 10 THC). (B) BDNF levels in the hippocampus were measured using ELISA. A significant genotype X treatment interaction was observed ($F_{1,35}=18.08$, $P = .0001$). Post-hoc analysis showed that WT mice exposed to THC exhibit increased levels of hippocampal BDNF compared to vehicle-treated WT mice ($P = .0003$), whereas THC-treated DN-DISC1 mice did not differ from vehicle-treated DN-DISC1 mice ($P = .47$). Data were obtained from 19 DN-DISC1 males (9 Vehicle and 10 THC) and 20 WT males (11 Vehicle and 9 THC). Statistical significance was determined using two-way ANOVA followed by Tukey's post-hoc test. Data are presented as Mean+SEM. *$p < .05$; ***$p = .0001$; ns=not significant.

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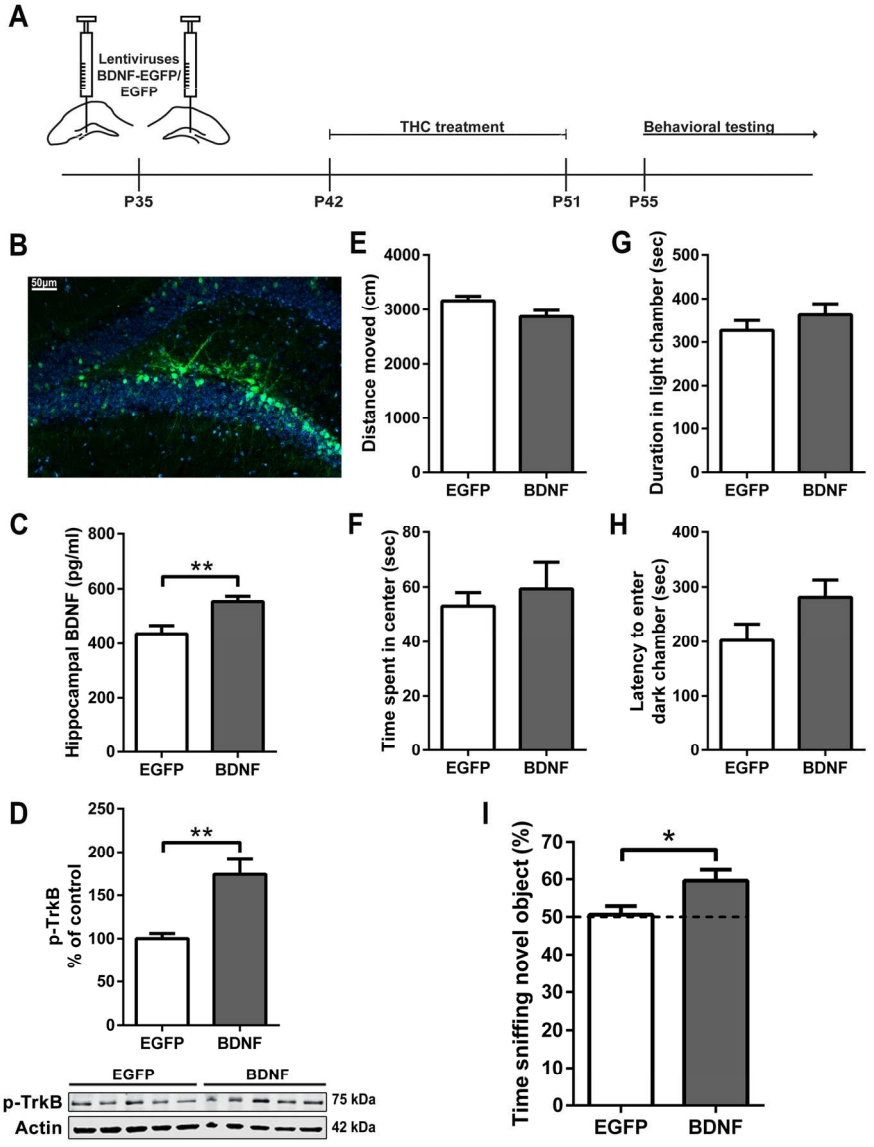

Figure 4. Hippocampal over-expression of BDNF prior to THC administration prevented the THC-induced deficit in object recognition memory in DN-DISC1 mice. (A) Experimental timeline for BDNF over-expression in the hippocampus prior to sub-chronic adolescent THC administration. (B-D) Validation of BDNF over-expression in the hippocampus. (B) Representative image of EGFP expression demonstrating the location of the stereotaxic injection of the viral vectors. EGFP positive cells under control of the neuron-specific synapsin promoter (green) DAPI (4',6-diamidino-2-phenylindole) (blue). (C) ELISA measurement identified a significant increase of 27% in hippocampal BDNF levels (t_{19}= 3.36, P = .0033) in DN-DISC1-THC mice injected with the BDNF-EGFP vector (BDNF) compared with DN-DISC1-THC mice injected only with the EGFP vector (EGFP). (D) Western blot analysis detected a significant increase of 74% in hippocampal p-TrkB levels (t_{19}=3.83, P = .0011), as an indication for BDNF signaling, in the BDNF mice compared with the EGFP mice. (C-D) Data were obtained from 21 DN-DISC1 males (10 EGFP and 11 BDNF). (E-F) Open field test measured for 10 minutes. (E) BDNF treatment did not induce alterations in locomotor activity or in (F) time spent in the center of the open field arena. (G-H) Light-dark box test. (G) BDNF treatment did not affect the

time spent in the light chamber or the (H) latency to enter the dark chamber. Data were obtained from 29 DN-DISC1 males (14 EGFP and 15 BDNF). (I) Novel object recognition test. Percentage of time spent exploring the novel object relative to the total time spent exploring both objects. Dashed line represents 50% preference indicating no preference to a specific object. BDNF mice show intact preference towards the novel object compared with the EGFP mice ($t_{26}=2.35$, $P = .026$). Data were obtained from 28 DN-DISC1 males (13 EGFP and 15 BDNF). Statistical significance was determined using two-tailed Student's t-test. Data are presented as Mean+SEM. *P < .05. **P < .01. DN-DISC1-THC, dominant-negative DISC1 mice injected with Δ9-tetrahydrocannabinol.

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