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Chronic Adolescent Cannabis Exposure Leads to Schizophrenia-Related Phenotypes Through Pathological Alterations of the Hippocampal Formation

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1. Abstract

Chronic adolescent exposure to Δ9-tetrahydrocannabinol (THC), the psychoactive component in marijuana, induces a schizophrenia-resembling phenotype in adulthood. Substantial evidence has suggested that schizophrenia is associated with a dysregulation of distinct brain regions, among which one of them is that of the hippocampal formation. In the present study, we used an integrative combination of behavioural assays and molecular analyses in a neurodevelopmental rodent model to demonstrate that hippocampal dysfunction, and a functional dissociation between the dorsal and ventral subregions, contributes to the emergence of psychotic-like symptoms of adolescent THC-induced schizophrenia. We report that adolescent exposure to THC induces cognitive and affective disturbances characterized by impairments in spatial memory formation, onset of anxiety-like behaviours and alterations in receptor and neurotransmitter levels. These results implicate dysfunction in hippocampal signalling as a critical nexus point mediating THC-induced schizophrenic phenotypes. We anticipate that our results will pave the way for finding new neurochemical targets that therapeutical interventions can potentially alter to revert the impairments.

Keywords: Schizophrenia; Adolescence; Δ9-tetrahydrocannabinol; Dorsal hippocampus; Ventral hippocampus

Word count: 5199

2. Introduction

Schizophrenia is a severe neuropsychiatric disease characterized by three categories of symptoms: positive symptoms (delusions, hallucinations, disorganized speech), negative symptoms (alogia, anhedonia, social withdrawal), and cognitive deficits (American Psychiatric Association, 2013). Schizophrenia has a multifactorial etiology, with both genetic determinants and environmental contributors, such as substance abuse, increasing susceptibility to the disease. Marijuana is the most commonly used illicit drug for recreational purposes (Radhakrishnan *et al*., 2014). This has especially critical implications for adolescents between 12–17 years of age given that their brains are especially pliable and susceptible to extrinsic insult. In fact, this period incurs extensive remodelling of various neurotransmitter pathways, such as the endocannabinoid, mesocorticolimbic, and glutamatergic systems, whose reorganization is susceptible to insult by extrinsic stressors. Converging lines of evidence suggest that sustained and heavy exposure to cannabis during this critical neurodevelopmental period increases the risk of neuropsychiatric diseases in adulthood (Andreasson *et al*., 1987; Kuepper *et al*., 2011).

The endocannabinoid (eCB) system is a neuromodulator of the central nervous system and is critical for adolescent neurodevelopment, maintenance of synaptic plasticity, neuronal migration, and response to intrinsic and extrinsic agents. The eCB system is comprised primarily of CB_1 (CB1R) and CB_2 cannabinoid receptors and the endogenous cannabinoids that engage them (Lu and Mackie, 2016). In humans, CB1R expression increases throughout adolescence, with the most robust increases occurring in the prefrontal and hippocampal regions (Mato *et al*., 2003). Δ9-tetrahydrocannabinol (THC), the psychoactive component in marijuana, is an efficacious CB_1 receptor partial agonist (Herkenham *et al.* 1990). Given the importance of the eCB system in adolescent neurodevelopment, it is likely that environmental modulation of eCB signalling during this susceptible period can cause long-lasting neurobiological changes that would ultimately impair the functioning of the mature adult brain. For instance, persistent exposure to THC could induce

sustained activation of CB1Rs in the prefrontal and hippocampal regions, which would modulate dopaminergic transmission in the mesocorticolimbic system, and ultimately result in neurodevelopmental impairments resembling schizophrenia.

Substantial evidence has implicated aberrant activity of the mesocorticolimbic dopamine pathway during adolescent neurodevelopment as a crucial contributor to the onset of schizophrenia. This pathway consists of two distinct sets of dopaminergic neurons projecting from the ventral tegmental area (VTA): the mesolimbic pathway which projects to the nucleus accumbens (NAc) of the ventral striatum, and the mesocortical pathway which projects to the prefrontal cortex (PFC). According to the dopamine hypothesis, excessive dopaminergic output to the striatum, along with dopaminergic deficits in the frontal cortices, herald the emergence of the associated psychopathological symptoms (Howes and Kapur, 2009). However, dopaminergic neurons projecting from the midbrain of the VTA have been shown to form a functional loop with the hippocampus, which prompted researchers to propose that dopaminergic dysfunction in schizophrenia may actually be secondary to the disruption of upstream hippocampal regulation. The hippocampal formation is a temporal lobe structure essential for memory consolidation and is both structurally and functionally heterogeneous. Its anterior and posterior portions, corresponding respectively to the ventral (vHipp) and dorsal (dHipp) hippocampus in rodents, are responsible for different cognitive functions as they have different afferent and efferent neural networks (Benetti *et al*., 2009). With respect to the connectivity between the prefrontal and hippocampal regions, the hippocampus receives indirect projections from the ipsilateral medial prefrontal cortex (Roberts *et al*., 2006). Previous studies analyzing cognitive models of schizophrenia have observed perturbed connectivity between the prefrontal cortex and anterior and posterior hippocampal regions in first episode schizophrenic patients (Benetti *et al.,* 2009). Moreover, Ragland *et al*. (2016) demonstrated that the posterior hippocampus is hypoactive and the anterior hippocampus is hyperactive in patients with schizophrenia. Additionally, the size of the posterior hippocampal pyramidal neurons

has been found to be reduced in schizophrenic subjects (Benes *et al*., 1991). Thus, substantial evidence has evidently suggested that schizophrenia is associated with a dysregulation of distinct brain regions, among which one of them is that of the hippocampal formation.

Animal models are valuable tools for examining the long-term molecular, neuronal and behavioural effects of sustained cannabis exposure during adolescence. The laboratory of Dr. XXX (***supervisor name has been redacted for anonymity***) has previously demonstrated in a neurodevelopmental rodent model that chronic adolescent exposure to THC induces a schizophrenia-resembling behavioural phenotype characterized by long-lasting cognitive impairments. Moreover, the neuropathological alterations include a THC-induced hyperactivity of the PFC pyramidal neurons, accompanied by hyperactive DAergic neuronal activity in the VTA, concomitant with a loss of GABAergic inhibitory control within the PFC (***this citation has been redacted for anonymity***).

Additional relevant findings, previously established in Dr. XXX's (***supervisor name has been redacted for anonymity***) laboratory, demonstrated that cannabinoid modulation, directly in the hippocampus, elicited neuropsychiatric symptoms resembling a schizophrenia phenotype. X *et al.* (2019) demonstrated that direct bilateral intra-vHipp local injections of THC induced anxiety-like behaviour, in otherwise healthy rats, using the light/dark box anxiety test, based on a rat's natural aversion to bright environments. Specifically, drug-treated rats exhibited a significantly longer latency to emerge into the light compartment relative to their VEH-control counterparts. The latency time to re-enter from the dark to the light environment is considered to be the most important parameter to test anxiety. Moreover, X *et al.* (2015) demonstrated that local injection of WIN55, a synthetic CB1R agonist, directly into the vHipp was able to induce hyperactivation of the VTA-dopamine system in rodents. These findings implicate the hippocampus as a brain region that cannabinoids can manipulate to produce the associated neuropsychiatric phenotypes of schizophrenia. Moreover, a previous study states that the dHipp forms connections with the frontal

cortical structures and is responsible for cognitive processes such as memory recall and spatial learning. Conversely, the vHipp is responsible for emotional processing, such as anxiety (Fanselow and Dong, 2010). The vHipp regulates dopaminergic (DAergic) activity via a polysynaptic projection: vHipp glutamatergic inputs excite neurons in the NAc that, in turn, release γ-aminobutyric acid (GABA) at the ventral pallidum (VP), inhibiting its activity. The VP provides an inhibitory tone to the dopaminergic neurons of the VTA, therefore activation of the vHipp will increase dopaminergic activity at the VTA. Several lines of evidence have suggested hippocampal dysregulation as a contributor to schizophrenia-related DA dysfunction (Lodge and Grace, 2011).

Therefore, in this present study, our goal was to combine the established findings that (i) marijuana abuse during adolescence increases the risk of schizophrenia and, (ii) hippocampal dysfunction contributes to the disease burden. Using a validated rodent model, we hypothesized that long-term alteration of the brain's hippocampal formation, and functional dissociation between dHipp and vHipp, underlie the pathological dysregulation of the mesocorticolimbic system, leading to the psychotic-like symptoms of THC-induced schizophrenia.

3. Materials and methods

3.1. Subjects

Adolescent male Sprague-Dawley rats from Charles River Laboratories (Quebec, Canada) were obtained at postnatal day (PND) 28. Upon arrival, rats were housed in pairs under controlled conditions (12:12 light/dark cycle, constant temperature and humidity) and received food and water *ad libitum*. The total number of animals used for this study was 64: 16 vehicle and 16 THC rats were used for behavioral tests, 7 vehicles and 9 THC rats for protein analysis as well as 8 vehicles and 8 THC rats for MALDI studies. All experimental procedures were conducted in accordance with Governmental and Institutional guidelines for appropriate laboratory animal care and experimentation.

3.2. Drug Preparation and Administration

Male Sprague-Dawley rats received a dosing regimen of THC (Tocris, UK) or vehicle (VEH) intraperitoneal injections twice daily between postnatal days (PND) 35–45, a window comparable with adolescence in humans. The drug solution was prepared by dissolving THC in an ethanol, cremophor and saline (1:1:18) solution. Ethanol was subsequently evaporated using nitrogen gas. Dosages were administered at increasing concentrations (2.5 mg/kg; Days 1–3; 5 mg/kg; Days 4–7; 10 mg/kg, Days 8–11) to counteract drug tolerance development, by desensitization of the CB1 receptors, and to mimic a heavy smoking regimen. The volumes were adjusted per body weight. This protocol has been performed as previously described (Renard *et al*., 2016; 2017). Behavioural tests were initiated following a 30-day drug-free period at PND 75, a window comparable with adulthood in humans.

3.3. Behavioural experiments

Light-Dark Box Anxiety Test

The light-dark box behaviour task took place in a test apparatus consisting of two 50 × 25 × 37 cm compartments connected by a 10 × 10 cm opening. The 'dark box' was black and enclosed by a black lid, while the 'light box' was white, left uncovered, and illuminated by a lamp, providing 1500 lux, located 120 cm above the apparatus floor. The door allowed the rat to enter either compartment. The experiment commenced when a rat was placed at the center of the light box, facing the wall opposite the door, and allowed to explore both compartments freely for a subsequent 8 minutes. A rat's entry into a zone was considered to have occurred when it placed all 4 paws in that zones compartment. Experiments were recorded and subsequently analyzed offline with a video-tracking system (BehaView). The behaviors analyzed included: i) latency time to first enter the dark box; ii) latency time to re-enter the light box, and iii) total number of transitions between compartments (Renard *et al*., 2016).

Elevated Plus Maze Anxiety Test

The elevated plus maze (EPM) apparatus consists of 4 arms (10 x 50 cm) branching from a platform (10 x 10 cm) to form a plus shape. It was mounted 50 cm above the floor and dimly illuminated (40 lux). Two opposing arms were enclosed by 40 cm high walls (closed arms) while the other two arms were open with the exception of a 1cm high ledge (open arms). The experiment commenced when a rat was placed on the central platform, facing the closed arm, and allowed to explore the maze freely for a subsequent 10 minutes. A rat's entry into a respective arm was considered to have occurred when it placed all 4 paws in that arm. Experiments were recorded and subsequently analyzed offline with a video-tracking system (BehaView). The behaviors analyzed included: i) number of entries and ii) time spent in either the closed or open arms (Szkudlarek *et al*., 2019).

Object Location Memory Task

The object location apparatus consisted of an 80 x 80 cm black acrylic arena enclosed by 50 cm high walls with a distinctive orientation cue on one wall. The task included three phases: i) habituation, ii) training and iii) test. During the habituation phase, rats were individually allowed to explore the arena devoid of objects for 20 minutes to acclimate themselves to the environment. 24 hours later, during the training phase, the rats were re-introduced to the same arena, in the presence of a pair of identical objects, made of either glass or plastic of similar dimensions, placed 15 cm away from either corner of the arena. The experiment commenced when a rat was placed at the center of the arena, facing the objects, and allowed to explore them freely for a subsequent 3 minutes. 1 hour later, during the 1st condition of the test phase, rats were re-introduced to the same context for 3 minutes, with the same objects, but with the exception of one of the object's position being changed to another corner of the arena. The delay of 1 hour was chosen to investigate short-term memory for object location recognition. The following day, during the training phase, the rats were introduced to the same context for 3 minutes, with a pair of identical objects, but different of those used on the previous

day. 5 hours later, during the 2nd condition of the test phase, rats were tested for 3 minutes in the same context and with the same objects, with the exception of one of the object's position being changed to another corner of the arena. The delay of 5 hours was chosen to investigate long-term memory for object location recognition. After each test, the apparatus and objects were wiped down with 70% ethanol solution to avoid olfactory cue bias. The training and test phases were recorded and subsequently analyzed offline using a video-tracking system (BehaView). Object position was counterbalanced between rats. The recognition of a change in object location was defined by whether the subject spent more time exploring the object in the novel location. Exploration was defined when rats were sniffing an object. The preference for a novel object was conveyed using an object location recognition index (the time spent with a novel object/total time exploring both objects) expressed as a percentage.

3.4. Protein Extraction and Western Blotting

Rats were overdosed with intraperitoneal injections of sodium pentobarbital (240 mg/kg, i.p., EuthanylTM) and decapitated. Brains were removed and flash frozen. Bilateral micropunches of the ventral and dorsal hippocampus were isolated for protein isolation. A standard Western blotting procedure was performed as previously described (Lyons *et al*., 2013). Antibody dilutions were as follows: α-tubulin (mouse, 1: 7500; Sigma; rabbit, 1:5000; Santa Cruz Biotechnology), phosphorylated mTOR ser2448 (p-mTOR; rabbit, 1:1000; Cell Signaling Technology), total mTOR (t-mTOR; rabbit, 1:1000; Cell Signaling Technology), NMDAR2B (NR2B; rabbit, 1:750; Cell Signaling Technology), mGluR2/3 (rabbit, 1:750; Millipore), GAD67 (mouse, 1:5000; Millipore). Species-appropriate secondary antibodies used for detection were fluorophore-conjugated (LI-COR IRDye680RD and IRDye 800CW) (1:10000; Thermo Scientific). Membranes were scanned using a LI-COR Odyssey Infrared Imaging System and densitometry measurement were obtain using Image Studio Lite software and normalized to the signal intensity obtained for the α-tubulin.

3.5. Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry (MALDI-IMS)

Rats were overdosed with intraperitoneal injections of sodium pentobarbital (240 mg/kg, i.p., EuthanylTM) and brains were removed and flash frozen. Brains from VEH and THC-treated rats were randomly paired, sectioned to a thickness of 10 μm at -20°C using a cryostat (CM 1850, Leica Biosystems, Wetzlar, Germany) to collect ventral and dorsal hippocampus (-6 from bregma), and mounted onto the indium-tin-oxide (ITO) coated glass slides (Hudson Surface Technology, Old Tappan, NJ). Prior to matrix deposition, slides were dried in a desiccator under vacuum for half an hour. ZnO NP dispersion (Sigma-Aldrich, St. Louis, MO) was sprayed onto tissue sections using a TM-Sprayer (HTX Technologies, Chapel Hill, NC) at the following conditions: 1.0 mg/mL of ZnO NP dispersion in ACN/H2O (50/50%, vol), flow rate of 0.05 mL/min, nozzle temperature at 65 °C, moving nozzle velocity of 1200 mm/min, 32 passes, 3 mm line spacing and pressure of 10 psi. A Sciex 5800 MALDI TOF/TOF mass spectrometer (Framingham, MA) equipped with a 349 nm Nd: YLF laser with a pulse rate at 400 Hz was used and images was acquired with Sciex TOF-TOF Series Explorer and TOF-TOF Imaging. For MS mass calibration, 0.75 μL of 5.5 mg/mL α-cyano-4-hydroxycinnamic acid (CHCA, Sigma-Aldrich, St. Louis, MO) solution containing ACN/H2O/TFA (50/50/0.1%, v/v/v) was spotted, and the calibration was based on the following ions with m/z: [CHCA-H2O+H]+ at 170.04 Da, [CHCA+H]+ at 190.05 Da, [CHCA+Na]+ at 212.03 Da, [CHCA-H+2Na]+ at 234.01 Da and [2CHCA+H]+ at 379.09 Da. Mass spectra were acquired from m/z range of 50 to 500 Da in the positive mode and the spatial resolution for IMS was at 80 μm. For each pixel, the mass spectra were acquired by averaging signals from 40 to 60 shots. The laser intensity for IMS was optimized based on the number of signals from m/z range of 50 to 500 Da, peak resolution, and signal to noise ratio. MSiReader (developed by North Carolina State University) was used to display ion distributions within the tissue sections and to export the data from regions of interest for quantitation. For relative quantitation, the areas of the peaks corresponding to [GABA+K]+ at m/z 142 and [glutamic acid+K]+

at m/z 186 were integrated, and the peak area ratios between THC and VEH were obtained for subsequent statistical analysis (***this citation has been redacted for anonymity***).

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 24 (IBM). Sampled data were tested for statistical comparisons between groups using unpaired or one-sample Student's *t* test. The significance level was established at $P < 0.05$.

4. Results

4.1. Behavioural Tests

Adolescent THC Exposure Induces Anxiety-Like Behaviour

We investigated the effects of adolescent THC exposure on anxiety-like behaviours using the light-dark box test. The light-dark box is based on the natural aversion of rats to bright environments. The latency time to re-enter the light box is thought to be the most important parameter to test anxiety-like behaviour. As such, statistical analysis revealed that THC-treated rats exhibited a significantly longer latency to emerge into the light compartment relative to their VEH-control counterparts (t_{30}= -2.12, $P < 0.05$; Fig. 1B). To further examine the potential effects of adolescent THC exposure on anxiety-like behaviors, we used the elevated plus maze test. An increase in open arm duration and/or entries is reflective of anti-anxiety behavior. There was no significant effect of THC treatment on time spent in the open arms (t_{27} = 0.845, $P > 0.05$; Fig. 1D) or on the number of open arm entries (t_{27} = 1.248, $P > 0.05$; Fig. 1E) relative to the VEH-treated control rats.

Adolescent THC Exposures Induces Deficits in Long-Term Memory Measures

We next compared the effects of adolescent THC exposure on short-term or long-term spatial memory deficits for object location recognition using the object-location memory task. Statistical analysis revealed no significant differences in time spent exploring the novel location/object bias following 1 hour delay (t_{30}= 1.18, P > 0.05; Fig. 1G). However, adolescent THC treatment impaired the formation of object location recognition relative to vehicle controls in the 5h retention test (t_{30} = 2.33, P < 0.05; Fig. 1H).

4.2. Dorsal Hippocampus Western Blot Analysis

Adolescent THC Exposure Induces Significant Decreases in GAD67 and NR2B Protein Levels

We next analyzed protein expression levels of selected molecular signaling pathways relevant for both schizophrenia and THC exposure. Western blot analysis of protein expression levels of GAD67, the primary GABA-synthesizing enzyme in the dorsal hippocampus, revealed a decrease in adolescent THC versus VEH-treated rats (t_{12} = 2.01, P < 0.05; Fig. 2A). Moreover, a significant decrease was detected in protein levels of NMDAR2B (NR2B) (t_{13} = -2.17, P < 0.05; Fig. 2B) between groups, while no significant difference was detected in levels of mGluR2/3 (t_{11} = 0.709, P > 0.05; Fig. 2C). Analysis of p-mTOR revealed a trend of significance induced by THC treatment (t_{12} = 1.75, P = 0.05; Fig. 2D). However, THC exposure did not affect the expression of t-mTOR protein levels (t_{12} = 0.67, P > 0.05; Fig. 2D) or the ratio of p-mTOR/t-mTOR (t_{12} = 1.40, P > 0.05; Fig. 2D).

4.3. Ventral Hippocampus Western Blot Analysis

Adolescent THC Exposure Induces Significant Increases in mGluR2/3 and NR2B Protein Levels

In contrast to the results observed in the dorsal hippocampus, western blot analysis of ventral hippocampal protein expression revealed a significant increase in NMDAR2B (NR2B) (t_{13} = -2.17, P < 0.05; Fig. 3B) and mGluR2/3(t_{14} = -2.16, P < 0.05; Fig. 3C) induced by adolescent THC exposure, while

no differences were detected in protein expression levels of GAD67 (t_{10} = -0.84, P > 0.05; Fig. 3A) between groups. Moreover, THC treatment did not affect the levels of p-mTOR (t_{10} = -0.81, P > 0.05; Fig. 3D), the expression of t-mTOR (t_{10} = -1.15; P > 0.05; Fig 3D) as well as the ratio of p-mTOR/t-mTOR (t_{10} = -0.14, P > 0.05; Fig. 3D) when compared with VEH-treated rats.

4.4. Dorsal and Ventral Hippocampus MALDI Analysis of GABA and Glutamate

Adolescent THC Exposure Induces Significant Decreases in GABA Neurotransmitter Levels

MALDI mass spectrometric analysis successfully obtained quantitative information on the relative abundance and spatial distribution of [GABA+K] and [glutamic acid+K] within the dorsal and ventral hippocampus between THC and VEH treated rats. Statistical analysis revealed a significant decrease in the peak area ratios (THC/VEH) of GABA (t_7 = -9.95, P < 0.001; Fig. 4B), and a trend of significance in glutamate (t_7 = -1.89, P = 0.05; Fig. 4B), in the dorsal hippocampus. Accordingly, statistical analysis detected a significant decrease in the peak area ratios (THC/VEH) of GABA (t_7 = -2.13, P < 0.05; Fig.4C), and no significant difference in glutamate (t_7 = -1.18, P > 0.05; Fig. 4C), in the ventral hippocampus.

5. Discussion

Numerous studies have implicated adolescent cannabis consumption as a risk factor for developing schizophrenia later in life (Andréasson *et al.,* 1987). Moreover, substantial evidence has shown that aberrant activity during adolescent neurodevelopment of the endocannabinoid and mesocorticolimbic system, and specifically the cortical-hippocampal pathway, is a crucial contributor to the onset of schizophrenia in adulthood (Lu and Mackie, 2016; Ragland *et al.,* 2016; Howes and Kapur, 2009; Benetti *et al.,* 2009; Benes *et al.*, 1991). Given these previously established findings, here we demonstrate that long-term alteration of the brain's hippocampal formation, through chronic exposure to THC during a window comparable to adolescence, can induce molecular and behavioural

phenotypes resembling schizophrenia. Our present findings reveal that adolescent exposure to THC induces cognitive and affective disturbances characterized by impairments in spatial memory formation, onset of anxiety-like behaviours and alterations in receptor and neurotransmitter levels.

Using light-dark box behavioural testing, we found that rats exposed to THC during adolescence displayed a significant increase in anxiety-like behaviour at adulthood, consistent with our previous reports (X *et al*. 2016; 2017). This finding is corroborated by the fact that adolescent THC-treated rats demonstrated a longer latency to re-emerge into the light compartment following entrance into the dark environment, indicative of anxiety. Anxiety-related symptoms are a phenomenon commonly reported in schizophrenia. However, we observed no significant difference in anxiety levels between THC and VEH treated rats during elevated plus maze (EPM) paradigms. Several possible reasons may account for this statistical insignificance. For instance, the data obtained from anxiety assays, such as the EPM test, are typically less consistent and can be impacted by external factors and/or the genetic background of experimental animals (Renard *et al*., 2006). In addition to anxiety measures, we observed that rats receiving adolescent THC exposure displayed deficits in recognizing a change in object location, indicative of disturbances in spatial memory consolidation. The recognition of a change in object location was defined by whether the subject spent more time exploring the object in the novel location. Significant deficits in location recognition memory, demonstrated by their inability to discriminate between the familiarity of recently encountered object locations to the foreignness of novel object locations, were observed in the 5h retention test but not in the 1h. These findings suggest that sustained adolescent THC exposure leads to impairments in *long-term* spatial memory formation as opposed to *short-term*. Memory-related impairments represents another core cognitive feature of schizophrenia (Elvevag and Goldberg, 2000). Specifically, impairments in spatial memory is among one of the modalities that a schizophrenic patient demonstrates working memory deficits for (Park and Holzman, 1992). Consequently, the delayed-response task of object location recognition was chosen for this study as

it is a measure of spatial memory and it is predominantly associated with the hippocampus. In fact, the hippocampus is mainly implicated in regulating spatial memory and anxiety responses (Moser *et al*., 1995; Henke, 1990), with the dorsal and ventral portions taking on different roles. The dHipp is responsible for cognitive processes such as memory recall and spatial learning, whereas the vHipp is responsible for emotional processes, such as anxiety (Fanselow and Dong, 2010).

Upon characterizing the behavioural phenotypes of adolescent THC-induced hippocampal dysfunction, we sought out to identify the molecular underpinnings of the resulting schizophrenic psychopathology. We performed a series of molecular analyses within the dorsal and ventral hippocampus. Using western blotting, we observed that adolescent THC exposure induces significant decreases in GAD67 and NR2B protein levels in the dHipp, and significant increases in mGluR2/3 and NR2B protein levels in the vHipp. The NMDA receptor 2B subunit (NR2B) is a member of the ionotropic glutamate receptor family (Cohen *et al.,* 2015). We report here for the first time that adolescent THC exposure induces an imbalance in glutamate transmission through an opposing increase/decrease of NR2B expression levels among hippocampal subregions. This could potentially reflect a compensatory effect of one subregion to counteract the other in order to maintain the balance in the system. Our finding is supported by a previous study that used fMRI testing to analyze the impact of schizophrenia on the anterior and posterior hippocampus during spatial memory retrieval. Ragland *et al.* (2016) observed a reduced posterior and an increased anterior hippocampal activation during recognition of spatial changes. While Ragland *et al*. (2016) obtained these results on the behavioural level at the clinical stage, our study was able to observe a similar dissociation on the molecular level at the pre-clinical stage, thus confirming that this imbalance is a consistent finding among publications. Moreover, the hippocampal hyperactivity observed in human schizophrenia patients corresponds with other studies that have found a hyperactivity in the ventral hippocampus of schizophrenia rodent models as well (Lodge and Grace, 2011).

Furthermore, in order to investigate whether this imbalance is confirmed or related to neurotransmitter quantification, we analyzed the relative abundance and spatial distribution of [GABA+K] and [glutamic acid+K], two cardinal neurotransmitters that are altered in schizophrenia, using MALDI-IMS mass spectrometry. To our knowledge, this is the first study using MALDI to report that adolescent THC exposure induces significant decreases in GABA neurotransmitter levels in both the ventral and dorsal hippocampus. This was an unexpected finding considering that, during detection of GABA decreases, receptor expression levels in the ventral hippocampus were concertedly increasing. Although this finding was not anticipated, it can be accounted for by the fact that receptor expression levels do not always coincide with the neurotransmitter distribution detected by MALDI in that area. It is known that receptors can be desensitized by means of internalization and downregulation in response to sustained and chronic exposure to agonists. For instance, Renard *et al*. (2016) previously demonstrated that adolescent THC exposure induces a hyperactivity of the PFC pyramidal neurons, which are known to form connections with the dHipp neuronal populations. The subsequent influx of glutamate in the dHipp might overwhelm the NR2B receptors of that area, causing them to desensitize and decrease in expression levels to protect against overstimulation. To compensate, the vHipp would increase its respective NR2B receptor levels to restore homeostasis. Thus, one could speculate that GABA neurotransmitter levels decreasing in both subregions may reflect the fact that it did not necessitate any form of balance-restoring adaptations.

A number of limitations in our experimental approach may have restricted the outcomes of this study. Among the conventional imaging techniques and mass spectrometry-based platforms, MALDI is a relatively novel profiling strategy. Though it offers the unique advantage of directly visualizing the distribution of neurotransmitters from a tissue specimen, it lacks a database of publications from which we could compare, validate and rationalize our data to. Owing to this limited data availability, future studies are required to more fully investigate its implications. For instance,

MALDI can be used in tandem with alternative neuroimaging approaches, such as proton magnetic resonance spectroscopy (H-MRS), positron emission tomography (PET) and single photon emission computed tomography (SPECT) (Egerton *et al*., 2017). Another limitation is the use of animal models as a framework for studying neuropsychiatric diseases. It is impossible to fully reproduce schizophrenia in its entirety due to the nature and complexity of human symptoms (Viveros *et al.,* 2011).

In summary, the present findings demonstrate that hippocampal dysfunction, and a functional dissociation between the dHipp and vHipp, contribute to the emergence of psychotic-like symptoms of adolescent THC-induced schizophrenia. Though future studies are required to more fully characterize the mechanisms underlying the alteration, our study may pave the way for finding new neurochemical targets that therapeutical strategies and interventions can potentially alter to revert the impairments. Given that adolescence represents a vulnerable neurodevelopmental period, and the exponential rise in cannabis use among youth, these findings have critical implications for public health.

6. Figures

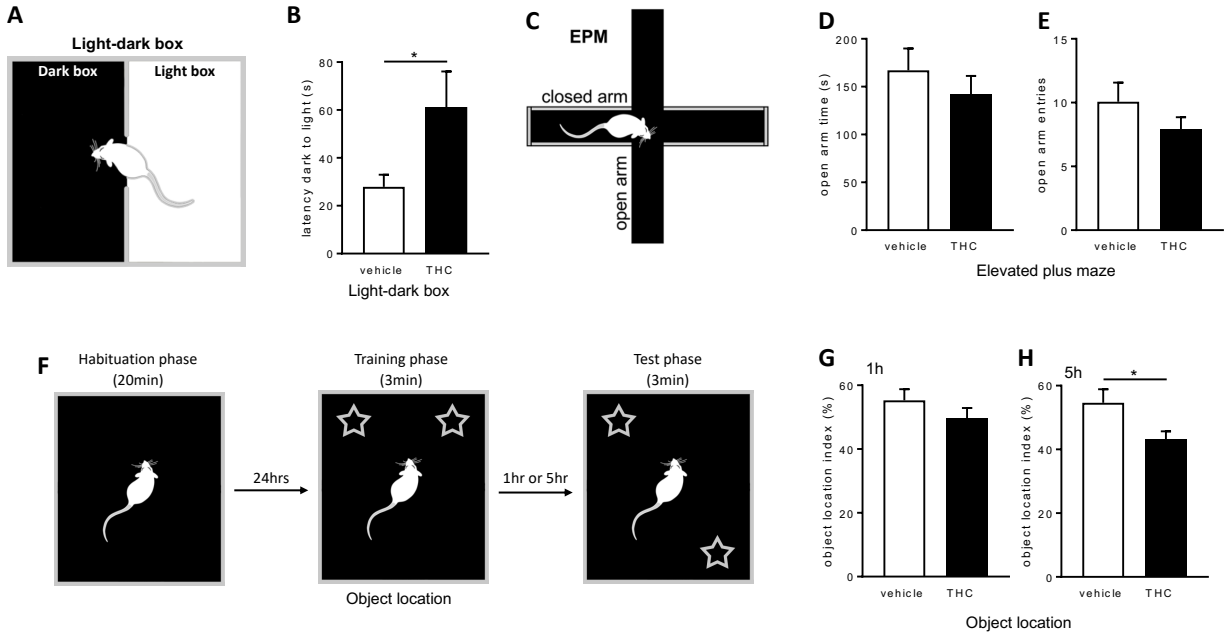

Figure 1. Effects of adolescent THC exposure on measures of anxiety and memory. (A) Schematic apparatus of the light-dark task. Group sizes (n): VEH (16), THC (16). **(B)** THC exposure during adolescence induced anxiety-like behaviours as increased latency to emerge into the light compartment compared to their VEH-control counterparts. **(C)** Schematic summary of the elevated plus maze task. Group sizes (n): VEH (14), THC (15). Anxiety-like behaviours were not observed as there was no significant effect of THC treatment on time spent in the open arms **(D)** or on the number of open arm entries **(E)** relative to the VEH-treated control rats. **(F)** Schematic summary of the object location task. Group sizes (n): VEH (16), THC (16). THC exposure during adolescence did not induce short-term **(G)** but did induce long-term **(H)** spatial memory deficits in the object location recognition task. Respective treatment groups were compared using unpaired Student's *t* test. * indicated $p < 0.05$. Error bars represent the standard error of the means (SEMs).

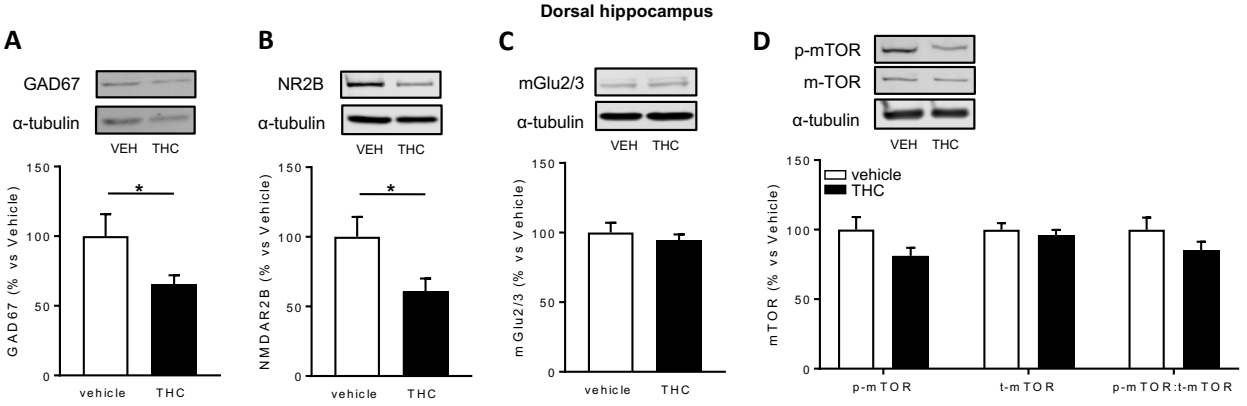

Figure 2. Effects of adolescent THC exposure on dorsal hippocampal protein biomarkers. (A) Representative western blot for GAD67 expression in the dHipp (top). A significant decrease in GAD67 expression is observed between adolescent THC (n=7) versus VEH-treated (n=7) rats. **(B)** Representative western blot for NR2B expression in the dHipp (top). A significant decrease in NR2B expression is observed between adolescent THC (n=8) versus VEH-treated (n=7) rats. **(C)** Representative western blot for mGluR2/3 expression in the dHipp (top). No significant changes in mGluR2/3 were found between groups. Group sizes (n): VEH (6), THC (7). **(D)** Representative western

blot for p-mTOR and t-mTOR expression in the dHipp (top). No significant changes in p-mTOR, t-mTOR and the ratio of phosphorylated to total mTOR were found between groups. Group sizes (n): VEH (7), THC (7). Respective treatment groups were compared using unpaired Student's *t* test. * indicated $p < 0.05$. Error bars represent the standard error of the means (SEMs).

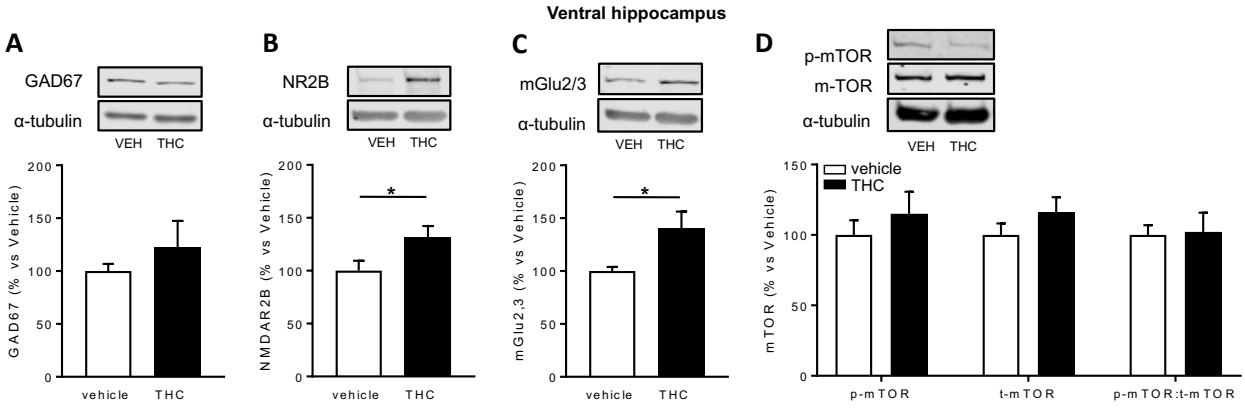

Figure 3. Effects of adolescent THC exposure on ventral hippocampal protein biomarkers. (A) Representative western blot for GAD67 expression in the vHipp (top). No significant changes in GAD67 were found between groups. Group sizes (n): VEH (6), THC (6). **(B)** Representative western blot for NR2B expression in the vHipp (top). A significant increase in NR2B expression is observed between adolescent THC (n=8) versus VEH-treated (n=7) rats. **(C)** Representative western blot for mGluR2/3 expression in the vHipp (top). No significant changes in mGluR2/3 were found between groups. Group sizes (n): VEH (7), THC (9). **(D)** Representative western blot for p-mTOR and t-mTOR expression in the vHipp (top). No significant changes in p-mTOR, t-mTOR and the ratio of phosphorylated to total mTOR were found between groups. Group sizes (n): VEH (6), THC (6). Respective treatment groups were compared using unpaired Student's *t* test. * indicated $p < 0.05$. Error bars represent the standard error of the means (SEMs).

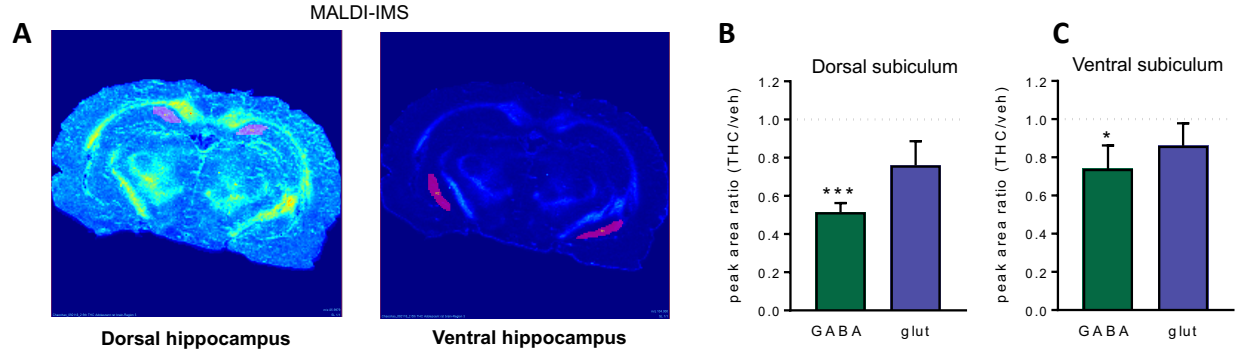

Figure 4. Effects of adolescent THC exposure on the relative abundance and spatial distribution of neurotransmitters GABA and glutamate. (A) Representative MALDI scans, taken at a bregma of -6.00 mm, showing *in situ* distribution of neurotransmitters in the dorsal and ventral hippocampus. **(B)** MALDI mass spectrometric analysis revealed a significant decrease in the peak area ratios of GABA and no significant difference in glutamate in the dorsal hippocampus. **(C)** Similarly, a significant decrease in the peak area ratios of GABA and no significant difference in glutamate was detected in the ventral hippocampus. Group sizes (n): THC/VEH (8). Respective treatment groups were compared using one-sample Student's *t* test. * indicated $p < 0.05$; *** $p < 0.001$. Error bars represent the standard error of the means (SEMs).

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