Role of cyclin-dependent kinase 5 in psychosis and the modulatory effects of cannabinoids

Marta Barrera-Conde\textsuperscript{a,b}, Emma Veza-estévez\textsuperscript{a}, Maria Gomis-gonzalez\textsuperscript{a}, Jordi Garcia-Quintana\textsuperscript{a}, Amira Trabsa\textsuperscript{c}, Laura Martínez-sadurní\textsuperscript{c}, Mitona Pujades\textsuperscript{a}, Víctor Perez\textsuperscript{c,d,e}, Rafael de la Torre\textsuperscript{a,b,f}, Daniel Bergé\textsuperscript{c,d,g}, Patricia Robledo\textsuperscript{a,b,*}

\textsuperscript{a} Integrative Pharmacology and Systems Neuroscience, Neuroscience Research Program, IMIM-Hospital del Mar Research Institute, Barcelona, Spain
\textsuperscript{b} Department of Medicine and Life Sciences, University Pompeu Fabra, Barcelona, Spain
\textsuperscript{c} Neuropsychiatry and Addictions Institute (INAD) of Parc de Salut Mar, Barcelona, Spain
\textsuperscript{d} Department of Psychiatry and Forensic Medicine, Autonomous University of Barcelona, Bellaterra, Spain
\textsuperscript{e} Centro de Investigación Biomédica en Red de Salud Mental G21. Instituto de Salud Carlos III, Madrid, Spain
\textsuperscript{f} Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain
\textsuperscript{g} Neuroimaging Group, Neuroscience Research Program, IMIM-Hospital del Mar Research Institute, Barcelona, Spain

\textbf{A B S T R A C T}

Cyclin-dependent kinase 5 (CDK5) is a serine/threonine kinase that has emerged as a key regulator of neurotransmission in complex cognitive processes. Its expression is altered in treated schizophrenia patients, and cannabinoids modulate CDK5 levels in the brain of rodents. However, the role of this kinase, and its interaction with cannabis use in first-episode psychosis (FEP) patients is still not known. Hence, we studied the expression changes of CDK5 and its signaling partner, postsynaptic density protein 95 (PSD95) in olfactory neuroepithelial (ON) cells of FEP patients with (FEP/c) and without (FEP/nc) prior cannabis use, and in a dual-hit mouse model of psychosis. In this model, adolescent mice were exposed to the cannabinoid receptor 1 agonist (CB1R) WIN-55,212-2 (WIN: 1 mg/kg) during 21 days, and to the N-methyl-D-aspartate receptor (NMDAR) blocker phencyclidine (PCP: 10 mg/kg) during 10 days. FEP/c showed less social functioning deficits, lower CDK5 and higher PSD95 levels than FEP/nc. These changes correlated with social skills, but not cognitive deficits. Consistently, exposure of ON cells from FEP/nc patients to WIN in vitro reduced CDK5 levels. Convergent results were obtained in mice, where PCP by itself induced more sociability deficits, and PSD95/CDK5 alterations in the prefrontal cortex and hippocampus than exposure to PCP-WIN. In addition, central blockade of CDK5 activity with roscovitine in PCP-treated mice restored both sociability impairments and PSD95 levels. We provide translational evidence that increased CDK5 could be an early indicator of psychosis associated with social deficits, and that this biomarker is modulated by prior cannabis use.

1. Introduction

\textit{Cannabis sativa} is the most widely consumed recreational drug, and the overall number of users is estimated to increase every year, mostly due to a reduction in its perceived harm. The prevalence rates are especially high in adolescents, which raises public health concerns (UNODC. United Nations Office on Drugs and Crime, 2021) since cannabis can have an impact on neural development mediated by the endocannabinoid system, and can induce enduring consequences on cognitive, social and emotional processing (Harkany et al., 2007; Miller et al., 2019; Parolaro et al., 2010; Albaugh et al., 2021; Hurd et al., 2019). In addition, the association between cannabis and the risk of suffering a psychotic condition, such as schizophrenia, has been extensively described (Large et al., 2014; Di Forti et al., 2019). Schizophrenia is a multidimensional disorder consisting of positive and negative symptoms and cognitive alterations. Negative symptoms and cognitive deficits are the main determinants of loss of functionality in schizophrenia patients (Giuliani et al., 2021). However, there is still conflicting data regarding the neurobiological consequences of cannabis use and its impact on these symptoms, possibly due to the heterogeneity of

---

\textsuperscript{a} Corresponding author at: IMIM-Hospital del Mar Research Institute, PRBB, Calle Dr. Aiguader 88, Barcelona 08003, Spain.
E-mail address: probledo@imim.es (P. Robledo).

https://doi.org/10.1016/j.nbd.2022.105942
Received 6 October 2022; Received in revised form 21 November 2022; Accepted 2 December 2022
Available online 5 December 2022
0969-9961/© 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
the disease, the time since illness onset, or the effects of antipsychotic medication. Studies in first-episode psychosis (FEP) patients, avoiding confounding variables like long-term antipsychotic treatment and illness chronicity, have found intriguing differences in the symptom profile between cannabis users and non-users. Indeed, it has been shown that FEP non-cannabis users exhibit greater cognitive disabilities (short- and long-term memory deficits, increased impulsivity or low IQ), a lower degree of dissociation and positive symptoms (Ricci et al., 2021) and more negative symptoms (anhedonia, apathy, asociality, social withdrawal) than FEP cannabis users (de Vos et al., 2020; Pope et al., 2021; Cunha et al., 2013). In addition, a recent study found a specific positive correlation between the frequency of cannabis use and the severity of diminished expression, one of the two dimensions of negative symptoms consisting of blunted affect, poor rapport and aloxia (Ihler et al., 2021). Studies aimed at unraveling the brain changes associated with cannabis use in FEP patients have also yielded conflicting results, with some studies showing brain volume loss in the hippocampus (HPC) and prefrontal cortex (PFC) of cannabis users as compared to non-users (see (Rapp et al., 2012) for review), while others describing the opposite (Cunha et al., 2013). In addition, very little data is available associating cannabis use, brain alterations and distinct psychotic symptoms. Studies on changes in specific molecular biomarkers of early-onset psychosis and cannabis use in relation to symptom profile are lacking. Some studies have reported an association between severe cannabis use and larger peripheral alterations in endocannabinoids (Bioque et al., 2013), and reductions in plasmatic concentrations of the brain-derived neurotrophic factor in FEP cannabis users (Toll et al., 2020) with respect to non-users. Yet, whether these molecular differences are relevant for the symptomatology of psychotic disorders or not, is still not clear.

One of the leading theories put forward to explain the wide clinical features in psychosis is related to alterations in the glutamatergic system, especially in the N-methyl-D-aspartate receptor (NMDAR) (Egeron et al., 2020; Hu et al., 2015; Schwartz et al., 2012; Uno and Coyle, 2019). Indeed, chronic administration of phencyclidine (PCP), an NMDAR blocker, induces long-term receptor hypofunction, and a pro-psychotic phenotype in animal models and humans (Jentsch and Roth, 1999). Previous studies using the subchronic PCP model of psychosis have investigated the impact of cannabinoids on behavioral alterations, such as memory and social behavior. Thus, after neonatal PCP exposure in mice, chronic delta 9-tetrahydrocannabinol (THC) administration worsened memory performance (Rodriguez et al., 2017). THC is known to produce its psychoactive effects via cannabinoid receptor 1 (CB1R). Contrastingly, self-administration of the synthetic cannabinoid WIN 55,212–2 (WIN), which also binds to CB1R, attenuated PCP-induced cognitive alterations in adult rats (Spano et al., 2010; Spano et al., 2013), while chronic exposure to WIN or acute exposure to low doses of THC, ameliorated social interaction (Spano et al., 2010; Seillier et al., 2020). Additionally, alterations in the endocannabinoid system have been involved in PCP-induced social withdrawal (Seillier et al., 2013). These studies support an interaction between the endocannabinoid and glutamatergic systems in psychotic symptoms. Still, evidence for intermediary molecular participants is lacking.

Notably, cyclin-dependent kinase 5 (CDK5), has been consistently implicated in brain development and synaptic plasticity (Lagace et al., 2008; Cheung et al., 2006; Posada-Duque et al., 2017; Dhavan and Tsai, 2001), and it is at the crossroads between NMDAR and CB1R function. CDK5 phosphorylates NMDARs (Li et al., 2001), and modulates their anchoring to the membrane through postsynaptic density protein 95 (PSD95) (Bianchetta et al., 2011; Morabito et al., 2004; Zhang et al., 2008). In addition, chronic administration of cannabinoids alters the expression of CDK5 in the prefrontal (PFC) cortex of mice (Lazenka et al., 2017), and the levels of PSD95 in rats (Renard et al., 2016). Interestingly, PSD95 knock-out mice show behavioral alterations that resemble psychosis endophenotypes (Coley and Gao, 2019), and changes in CDK5 expression/signaling have been observed in post-mortem brains (Engmann et al., 2011; Ramos-Miguel et al., 2013), and olfactory neuroepithelial (ON) cells (Barrera-Conde et al., 2021) of treated schizophrenia patients. However, it is still unknown whether PSD95/CDK5 expression is modulated by cannabis use in FEP patients.

Given these data, we evaluated differences in negative symptoms and cognitive alterations, as well as expression changes in CDK5/PSD95 in ON cells of recently diagnosed FEP patients, with or without a history of cannabis use. The ON has been widely used as a surrogate model of the central nervous system function to study biomarkers of psychiatric conditions (Borgmann-Winter et al., 2009; Borgmann-Winter et al., 2015; Hortuchi et al., 2016; Sawa et al., 2017; Leung et al., 2007; Jaaro-Peled et al., 2022). In addition, we used a dual-hit mouse model of psychosis (Guerin et al., 2021), where adolescent mice received an initial exposure to the CB1 agonist WIN (1 mg/kg during 21 days) to mimic cannabis use in patients before the psychotic episode, and then combined this treatment with a sub-chronic administration of the glutamate receptor antagonist PCP (10 mg/kg during 10 days) to emulate continued cannabis use in patients around the time of the psychotic episode. Similar dosing regimens for WIN and PCP, administered separately in adolescent mice, have been shown to induce persistent cognitive and social deficits (Mourzi et al., 2019; Mourzi et al., 2007; Hashimoto et al., 2005; Tsivion-Visbord et al., 2020). We then assessed in adult mice, behavioral alterations and PSD95/CDK5 levels in the HPC and the PFC, two structures rich in CB1R (Tao et al., 2020) that have been related to cognitive and affective processing (Rubin et al., 2014; Dixon et al., 2017). Finally, the specific involvement of CDK5 in behavioral outcomes was tested by central administration of the CDK5 inhibitor roscovitine (Li et al., 2001; Ryder et al., 2003; Taylor et al., 2007).

2. Materials and methods

2.1. Subject recruitment, clinical and neuropsychological evaluation

A cross-sectional study was conducted in FEP patients without a history of cannabis use (FEP/c), with a history of cannabis use (FEP/c), control subjects without any prior cannabis use (C/n), and control subjects with a history of cannabis use (C/c). A total of 38 subjects (13 FEP/c, 15 FEP/c, 5C/n and 5C/c) were newly recruited over the extent on one year at the Psychiatry Unit in the Hospital del Mar (Barcelona, Spain). Neuropsychological examinations and extraction of the ON were performed at the IMIM-Hospital del Mar Research Institute (Barcelona, Spain). All FEP patients met psychosis criteria from the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Control subjects were excluded if they or their first-degree relatives had any severe neurological disorders. Other exclusion criteria included: showing medical conditions with nasal repercussions (rhinitis or severe bleeding) or use of drugs of abuse. In the case of cannabis users, control subjects were included in the study if they reported regular use of cannabis (5 or more joints per week), and FEP patients had to report regular consumption of cannabis (5 or more joints per week) when the psychotic outbreak occurred. Following hospitalization, FEP cannabis users were instructed to refrain from taking any drugs, including cannabis. Cognitive function was assessed by the short and long-term memory tests from the Complutense Verbal Learning Test (TAVEC) (de Benedet and Alejandre, 2014), working memory was evaluated by the digit span test from the Wechsler Adult Intelligence Scale (WAIS III) (Maharani et al., 2021). Impulsivity was evaluated by the STROOP test (Sharma and McKenna, 1996). Premorbid IQ was calculated based on the vocabulary task from WAIS III (Maharani et al., 2021). The Positive and Negative Symptoms Scale (PANSS) was used to evaluate negative symptoms severity (Kay and Fiszbein, 1987). We used the Premorbid Adjustment Scale (PAS) to assess the lifetime social development skills of FEP patients during their childhood, early adolescence, late adolescence and adulthood (Premorbid adjustment Scale Struktured Interview n.d.; Cannon-Spoor et al., 1982). Four different aspects were evaluated for childhood (sociability and introversion, collaboration with others,
school performance, and school adaptation skills); five different items for early adolescence (sociability and introversion, collaboration with others, school performance, school adaptation skills and sociosexual relations); five different items for late adolescence (sociability and introversion, collaboration with others, school performance, school adaptation skills, sociosexual relations), and three different items for adults (sociability and introversion, collaboration with others and sociosexual relations). Each item is given a score from 0 (very good) to 6 (very bad). The score for each age period is the mean of all individual items divided by 6, and the total score is the mean of all age period scores. All subjects gave their written consent to participate in the study, and the entire procedure was approved by the local institutional ethics committee (CEIM-PSMAR).

2.2. Nasal exfoliation and cell culture

Nasal brushing was performed directly in the middle and upper turbinate avoiding the respiratory epithelium. Samples were cultured in Dulbecco’s Modified Eagle Medium/Ham F-12 (DMEM/F12) containing 10% FBS, 2% glutamine and 1% streptomycin penicillin (GibcoBRL) to obtain immature olfactory neurons as previously described (Barrera-Conde et al., 2021; Delgado-Sequera et al., 2021; Guinart et al., 2020; Galindo et al., 2018). Aliquots of ON cells at passage 3 were used in subsequent biochemical studies.

2.3. Detection of delta-9-tetrahydrocannabinol in hair by LC-MS/MS

Before nasal exfoliation, the first 3 cm of the hair sample from the proximal region of the scalp, representing hair growth in the last three months, were collected in all cannabis users (C/c = 5; FEP/c = 13; n total = 18) to quantify delta-9-tetrahydrocannabinol. Hair samples were washed, finely cut in minimal portions, and 25 mg were weighed. Standard solutions of delta-9-tetrahydrocannabinol (THC) and its deuterated analogue delta-9-tetrahydrocannabinol-d3 (Cerilliant®) were used for the calibration and quality control samples. Four replicates with a concentration of 1 ng/mg of THC and four quality control samples (0.04 ng/mg) were prepared daily for each analytical batch by adding suitable amounts of methanol working solutions to 25 mg drug free hair samples. An external quality control sample CTRL-H-20 (TripCheck®, Comedical s.a.s, Trento, Italy) at a concentration range between 0.33 and 0.61 ng/mg was included at each analytical batch. Briefly, all samples were digested by 0.5 ml of M3 reagent (acidic acetonitrile 95:15%) by vigorous vortex mixing and transferred into LC/MS injection vials. Aliquots (3 μl) were analyzed following a previously validated method with an ultra-high-performance liquid chromatography system (Waters Acuity UPLC, Waters Corporation, Milford, USA) coupled with a triple quadrupole mass spectrometer (LC-MS/MS) (Waters Xevo TQ, Waters® Corporation). The chromatographic separation was carried out on an Acquity UPLC HSS C18 Waters® column (2.1 mm × 150 mm, 1.8 μm) using a linear gradient elution consisted of MilliQ water, 1 mM ammonium formate, and 0.01% formic acid (Solvent A). Solvent B was composed of methanol, 1 mM ammonium formate and 0.01% formic acid. Analytes were detected with a triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) with positive electrospray ionization (ESI). MRM transitions for Δ9-THC were 315 > 193 and 315 > 259 for quantitative and qualitative identification, respectively. The internal standard transition monitored was 318 > 262. For each validation assay, the within run precision (relative standard deviation, Sw %) and trueness (systematic error %) were ≤ 15%. The limit of detection (LOD) and quantification (LOQ) were 0.005 and 0.04 ng/mg, respectively.

2.4. In vitro pharmacological study

To examine the specific effects of in vitro CB1 activation on the expression of CDK5, ON cells from FEP/c and C/c at passage four were treated with 100 nM of (R)-(−)-WIN 55,212–2 mesylate salt (WIN) (Sigma Aldrich, USA) diluted in enriched DMEM/F12 medium (0.001% DMSO) or its vehicle for 21 days. The medium was changed every 72 h. Before protein extraction, flasks were rinsed with enriched DMEM/F12 without WIN to avoid the onboard effects of WIN. Samples were then processed in western blot analysis.

2.5. Animals and in vivo drug treatments

A total of 72 adolescent male C57BL/6J mice (Charles River, France) at postnatal day (PD) 30 (18–21 g) at the beginning of experiments were used. Mice were group-housed in a room with a reversed 12-h light/dark cycle (lights off at 8 am), with temperature (21 ± 2°C) and humidity (50 ± 10%) kept constant, and food and water provided ad libitum. The dual-hit mouse model of psychosis consisted in an intraperitoneal (i.p.) chronic administration of (R)-(−)-WIN-55,212–2 mesylate salt (WIN; Sigma Aldrich, USA) at a dose of 1 mg/kg daily for 21 days (PD30-PD51), and phencyclidine hydrochloride (PCP; NATA, Australia) (10 mg/kg; i.p.) for 10 days (PD37-PD49), 5 days a week. The dose of WIN was chosen to mimic heavy cannabis use in patients before the first psychotic episode. Indeed, FEP cannabis users in our study report smoking >5 joints a week, and it has been described that the odds of suffering a psychotic disorder among daily cannabis users are 3.2 times higher than for non-cannabis users (Di Forti et al., 2019). Also, we administered WIN to mice for seven days before concomitant exposure to PCP + WIN to emulate the history of cannabis use in FEP patients, who reported cannabis consumption before and during the psychotic outbreak. Accordingly, this dosing regimen of WIN administered in adolescent C57BL/6J male mice, induces persistent memory deficits (Mouro et al., 2019), providing face validity, since it replicates the altered phenotype in human cannabis users (Becker et al., 2018). The doses and duration of PCP administration were selected as they induce long-lasting latent learning (Mouri et al., 2007), memory (Hashimoto et al., 2005), social behavior and pre-pulse inhibition (Tsivion-Visbord et al., 2020) deficits in mice. Since FEP patients were not using cannabis when neuropsychological and clinical evaluations were performed, we evaluated behavioral outcomes in mice 30 days after the end of treatments, when drugs were no longer on-board. Thus, surgeries (PD58–62) and behavioral assessments (PD70–75) were performed in adult mice during the dark phase of the light/dark cycle.

2.6. Surgeries and intracerebroventricular administration of roscovitine

The specific involvement of CDK5 in behavioral outcomes was analyzed by intracerebroventricular (ICV) administration of the CDK5 inhibitor roscovitine (Sigma Aldrich, USA). To choose the dose of roscovitine that did not induce unwanted motor effects, 25 C57BL/6J male naive mice at PD58 were anesthetized with ketamine/xylazine (2:1; 0.10 ml/10 g, i.p.) and placed in a stereotaxic apparatus. Meloxicam (Boehringer Engelman, Germany) (2 mg/kg, 0.10 ml, subcutaneously) was injected 30 min before surgery and once a day for three days after surgery to facilitate recovery. Unilateral cannulas were implanted vertically in the left lateral ventricle (AP, –0.22 ML, +1.00 DV, –2.50 mm from bregma) and fixed to the skull with dental cement. At PD75, mice received roscovitine or vehicle ICV (3, 10 and 30 nM). The ICV infusions were performed at a constant rate of 1 μl/min using a micro-infusion pump (Harvard Apparatus) attached to a 10 μl Hamilton microsyringe (Hamilton, Nevada, USA). To connect the cannula and the microsyringe a polyethylene tube was used. To prevent reflux the tube
was removed from the cannula 2 min after the infusion. Immediately after the injections, locomotor activity was evaluated in individual locomotor activity cages (see below). The different doses of roscovitine and vehicle were administered according to a within-subjects design. Locomotor activity was evaluated 10 min after administration, and horizontal activity and general small movements were evaluated for 60 min. The results showed that roscovitine significantly reduced locomotor activity at the doses of 3 and 10 nM, but not at 30 nM (significant effect of roscovitine \( F(3,18)=3.37; p < 0.05 \)) (Supplementary Fig. 1). No significant effects of roscovitine were observed on stereotypic behavior at any of the doses tested. Therefore, the dose of 30 nM of roscovitine was chosen for further experiments (see below).

2.7. Behavioral assessments

Locomotion and stereotypic behavior alterations induced by PCP administration (Castañé et al., 2015) were evaluated by using individual locomotor activity boxes as previously described (Vinals et al., 2015).

Long-term memory was evaluated in a novel object recognition (NOR) paradigm, using a V-maze, as previously described (Vinals et al., 2015). Briefly, mice were first habituated to the empty maze for 5 min. Then, two identical objects were placed on each of the maze’s arms and mice could explore them for 9 min (training phase). Twenty-four hours later, one of the familiar objects was replaced arbitrarily with a novel object (test phase), and mice were again allowed to explore the maze during 9 min. A discrimination index (DI) was extracted as the difference in exploration between the novel object (TN) and the familiar object (TF) divided by the total time exploring (TN + TF) in the test phase.

To evaluate social interaction, a three chamber maze was used as previously described (Coley and Gao, 2019; Kaidanovich-Beilin et al., 2010). Briefly, prior to the beginning of the experiment mice were allowed to habituate to the maze for 5 min. First, sociability was assessed by placing an unfamiliar mouse (M) (adolescent C57BL/6J mouse at PD 30–35) in a wire containment box located in one of the lateral chambers of the maze. In the other lateral chamber, an identical empty wire containment box (E) was placed. Subsequently, the walls separating the center from the lateral chambers were removed, allowing exploration of the maze for 10 min. The sociability index was calculated by measuring the difference between the time exploring the M (TM) and the time exploring the empty box (TE), divided by the total time exploring (TM + TE) (SI = [TM-TE]/(TM + TE)). Second, social novelty was assessed by placing a different novel mouse (M’) (adolescent C57BL/6J mouse at PD 30–35) in the wire containment box that was previously empty. Mice were allowed to freely explore for 10 min. The social novelty index was calculated by the difference between the time exploring the familiar mouse (TM) and the time exploring the novel mouse (TM’) (SNI = [(TM’-TM)/TM + TM’]).

To assess the behavioral effects of roscovitine on sociability deficits induced by PCP and their modulation by WIN treatment, a naive batch of 24 male mice were used. Mice received WIN for 21 days (PD30-PD51) and PCP for 10 days (PD37-PD49). At PDS8-PD62, mice underwent ICV cannulae placement, and at PD75-PD85 they underwent behavioral testing. Mice received roscovitine (30 nM) or vehicle 10 min after the training phase in the NOR test, and 10 min after the habitation phase in the sociability test. Long-term memory in the NOR test was evaluated 24 h after the infusions. Sociability performance was evaluated 20 min after roscovitine administration, followed by social novelty assessment. Roscovitine and vehicle were administered in both tests according to a within-subjects design, with a 72 h washout period between administrations.

2.8. Western blots

Harvested ON cells in passage 3 were processed for western blot analysis as previously described (Galindo et al., 2018). PSD95 and CDK5 protein levels were quantified in ON cells from FEP/nC, FEP/c, C/nC and C/n (n = 5 per group). In mice, the HPC and the PFC were dissected 24 h after the last behavioral evaluation, and brain tissue was frozen in dry ice and stored at −80°C until processing. Primary antibodies were incubated overnight at 4°C. Physical protein extraction was performed using sterile scrapers before adding a lysis buffer. Lysis buffer was added at 30 μl per mg of wet weight to homogenize the tissue and extract proteins. Protein concentration was quantified, and equal amounts of protein from tissue (30 μg) and ON cells (30 μg) were loaded into 10% polyacrylamide gels and transferred to PVDF sheets (Immobilon, MERCK Millipore, USA). Blocking was done using 5% powdered milk diluted in Tris-buffered saline +0.1% Tween-20 (T-TBSX) for 45 min. PSD95 was detected using sc-32,290 (1:250) and CDKS5 was detected using sc-6247 (1:500) (Santa Cruz Biotechnology, USA). The housekeeping gene β-III-Tubulin 66,240–1-lg (1:5000) (ProteinTech, USA) allowed protein normalization. Primary antibodies were incubated at 4°C overnight. Secondary antibodies DyLight 800 anti-mouse (1:5000) and DyLight 680 anti-rabbit (1:5000) (Thermo Fisher, USA) were incubated at room temperature for 2 h to label the primary antibodies. Amersham typhoon 5 was used to quantify protein expression and protein densities were expressed as fold-change of the control group after normalization. To confirm that protein expression was normalized correctly with β-III-Tubulin in the pharmacological studies, we performed separate experiments where different amounts of lysates (5, 10, 15, 20, 30, 35 and 40 μg) were loaded from human ON cells and mouse HPC and PFC tissue. The results corroborate that with the amount of protein loaded (30 μg), β-III-Tubulin expression fell within the linear range of detection (Supplementary Fig. 2).

To evaluate the effects of roscovitine on PSD95 and CDK5 expression in the HPC and PFC, following behavioral evaluations, adult mice (PD88) received one roscovitine infusion (30 nM) 20 min before tissue extraction.

2.9. Statistical analysis

Student’s t-tests were used to evaluate the differences between FEP/nc and FEP/c in negative and cognitive symptoms. Two-way analysis of variance (ANOVA) was used to assess the main effects of cannabis use and psychosis, and their interaction on CDK5/PSD95 expression in ON cells, with cannabis use and group as between-subject factors. In vitro effects of WIN in ON cells of control and FEP/nc patients were analyzed by a two-way ANOVA, with WIN or vehicle as a within-subject factor, and group as a between-subject factor. In animal studies, the exploration index data for the sociability and novel object recognition tests were analyzed using three-way ANOVAs with novel/familiar or empty/mouse as within-subject factors, and WIN or PCP as between-subject factors. The memory and social discrimination index data were assessed with two-way ANOVAs (WIN ad PCP treatments as between-subject factors). The main effects of roscovitine (within-subjects factor) and of WIN and PCP treatments (between-subject factors), and their interaction were evaluated with three-way ANOVAs. When significant interactions were observed in the ANOVAs, individual comparisons were performed with the Bonferroni post-hoc test (Abdi, 2007). All the statistical values for these comparisons are shown in Supplementary Table 1. The statistical significance was set at \( p < 0.05 \). Grubb’s test was used to identify potential outliers. Additionally, Cohen’s d was calculated to measure the effect size (Supplementary Table 2). The relationship between variables was addressed by Pearson correlations and linear regression fit calculations. Statistical calculations were made using SPSS statistics for Windows and graphs were designed using GraphPad Prism 8 (La Jolla, USA).

3. Results

3.1. Demographics and clinical assessments

Demographic, tobacco use, cannabis use, and clinical data are shown
Table 1
Demographic and clinical data.

<table>
<thead>
<tr>
<th></th>
<th>FEP/nc</th>
<th>FEP/c</th>
<th>C/nc</th>
<th>C/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>23.7 (19–31)</td>
<td>27.8 (19–35)#</td>
<td>28.1 (23–35)</td>
<td>24.7 (18–32)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>7/8</td>
<td>9/4</td>
<td>3/2</td>
<td>3/2</td>
</tr>
<tr>
<td>BMI</td>
<td>22.3 (18.2–32.4)</td>
<td>24.4 (15.2–44.80)</td>
<td>24 (18.6–33.1)</td>
<td>21.6 (18.8–24)</td>
</tr>
<tr>
<td>Tobacco use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Users: n (%)</td>
<td>3/15 20%</td>
<td>13/13100%</td>
<td>1/5 20%</td>
<td>5/5100%</td>
</tr>
<tr>
<td>Units per week</td>
<td>47 (2–140)</td>
<td>66.3 (24–140)</td>
<td>20</td>
<td>22 (7–35)</td>
</tr>
<tr>
<td>Cannabis use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular users: n (%)</td>
<td></td>
<td>13/13100%</td>
<td>–</td>
<td>5/5100%</td>
</tr>
<tr>
<td>Age of onset</td>
<td>–</td>
<td>17.7 (12–27)</td>
<td>–</td>
<td>17.4 (14–23)</td>
</tr>
<tr>
<td>Length of use</td>
<td>–</td>
<td>9 (2–19)</td>
<td>–</td>
<td>6.2 (1–15)</td>
</tr>
<tr>
<td>Joints per week</td>
<td>–</td>
<td>25 (5–70)</td>
<td>–</td>
<td>33.2 (7–70)</td>
</tr>
<tr>
<td>THC in hair (ng/mg)</td>
<td>0.01 (0–0.02)</td>
<td>0.01 (0–0.02)</td>
<td>0.0</td>
<td>0.48 (0.08–1.29)</td>
</tr>
<tr>
<td>Clinical data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since first episode psychosis (months)</td>
<td>6.4 (1.2–17)</td>
<td>5.6 (1.1–11.3)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Time untreated (months)</td>
<td>1.9 (0.1–8.2)</td>
<td>2.5 (0.2–10.1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Time since hospitalization (months)</td>
<td>4.5 (0.8–8.8)</td>
<td>3.1 (0.5–9.4)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PANSS total</td>
<td>63 (39–83)</td>
<td>62.1 (36–90)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Antipsychotic treatment (n)</td>
<td>7</td>
<td>6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>7</td>
<td>6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clozapine</td>
<td>0</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cariprazine</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Paliperidone</td>
<td>1</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lurasidone</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Asenapine</td>
<td>1</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Risperidone</td>
<td>1</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Unretrated</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are shown as mean and minimum to maximum values. FEP/c were slightly older than FEP/nc (F(3,42) = 2.89, p > 0.05), but no other significant differences were observed between groups in the rest of the variables. Number of samples for delta-9-tetrahydrocannabinol (THC) quantification in hair: FEP/nc n = 11; FEP/c n = 11; C/nc n = 5; C/c n = 4. (n) number of patients treated with each antipsychotic. (m) months. (#) p < 0.05 vs. FEP/nc.

in Table 1. Age was the only parameter that differed significantly between groups, with FEP/c being older than FEP/nc (p < 0.05). Although not significant, a higher number of cannabis users reported more tobacco use than non-cannabis users. Age of onset of cannabis use, length of use and joints per week were similar in FEP/c and C/c, but relevant concentrations of THC in hair (<0.05 ng/mg) were only detected in C/c. No other significant differences were observed between groups regarding clinical data or number of patients treated with different antipsychotics.

3.2. FEP non-cannabis users show more negative symptoms and similar cognitive deficits than FEP cannabis users

No significant differences were observed in premorbid IQ (Fig. 1A), short-term memory (Fig. 1B), long-term memory (Fig. 1C), working memory (Fig. 1D) or impulsivity (Fig. 1E) between FEP/nc and FEP/c. However, FEP/c showed less asociality (t = 2.29; p < 0.05), lower social withdrawal (t = 2.35; p < 0.05) and less conversational deficits (t = 2.57; p < 0.05) (Fig. 1F) than FEP/nc, along with a lower score in the PANSS negative subscale (NI-N7) (t = 2.24; p < 0.05) (Fig. 1G). In addition, FEP/c presented better premorbid social skills than FEP/nc as measured with the PAS questionnaire (t = 2.31; p < 0.05) (Fig. 1H-I). See Supplementary Table 1 for extended statistical data. For negative symptoms in the PANSS scale and social skills in the PAS, differences between the means showed large effect sizes (Supplementary Table 2).

3.3. FEP non-cannabis users show higher CDK5 and lower PSD95 levels than FEP cannabis users

Subsequently, we evaluated the expression of PSD95 and CDK5 in ON cells of control subjects and FEP patients. Fig. 2A shows an exemplifying photomicrograph of ON cells at passage four. We found an overall effect of cannabis use in the expression of PSD95 in ON cells of both control and FEP (significant main effect of cannabis F(1,16) = 4.54; p < 0.05) (Fig. 2B and D). On the other hand, a significant interaction was observed between cannabis use and FEP (F(1,16) = 5.15; p < 0.05), and post-hoc comparisons revealed that the expression of CDK5 in ON cells of FEP/nc was higher than in C/nc, while FEP/c showed similar levels to C/c (Fig. 2C and D). See Supplementary Table 1 for extended statistical data. The differences between the means for PSD95 and CDK5 in FEP showed large effect sizes (Supplementary Table 2).

A significant negative association between the expression of PSD95 and CDK5 was observed (r = −0.79, p < 0.01) (Fig. 2E), as well as a significant linear regression (R = 0.62, p < 0.01) (Fig. 2F). Moreover, PSD95 was negatively correlated with social behavior during childhood (r = −0.73, p < 0.05) (Fig. 2E), and showed a significant linear regression (R² = 0.53, p < 0.05) (Fig. 2G), whereas CDK5 was positively correlated (r = 0.82, p < 0.01) (Fig. 2E), and showed a significant linear regression (R² = 0.68, p < 0.01) (Fig. 2H) with this parameter also. High CDK5 levels also correlated with worse total PAS score (r = 0.71, p < 0.05) and both parameters were linearly related (R² = 0.49, p < 0.05) (Fig. 2I), suggesting that an increase in CDK5 could be associated with social deficits in FEP.

3.4. Chronic stimulation of CB1R in ON cells in vitro decreases CDK5 in FEP non-cannabis users

To evaluate whether chronic administration of the CB1R agonist WIN would emulate the effects of cannabis use on CDK5, we exposed ON cells from C/nc and FEP/nc to WIN for 21 days in vitro (Fig. 2J). We found that WIN treatment in ON cells significantly reduced CDK5 expression in FEP/nc, and had no effect on C/nc (significant main effect of WIN F(1,16) = 5.17; p < 0.05) and significant interaction F(1,16) = 5.82; p < 0.05) (Fig. 2K-L). See Supplementary Table 1 for extended data.
statistical data. Differences between the means of FEP cells exposed to WIN or VEH showed large effect sizes (Supplementary Table 2).

3.5. **Chronic activation of CB1R specifically abrogates sociability in PCP-treated mice**

In parallel, we evaluated at PD70 the effects of CB1R activation on the psychotic phenotype induced by subchronic administration of PCP (Fig. 3A). The assessment of positive symptoms (increased locomotor activity and stereotyped behavior) revealed no significant alterations in mice treated with PCP or WIN alone or in combination, with respect to vehicle-treated mice (Supplementary Fig. 3A-B).

In addition, during the training session in the NOR test, no significant differences were observed in exploration time in PCP, WIN or PCP + WIN treated mice (Supplementary Fig. 3C), indicating that the effects observed during the test session are not confounded by unwanted motor effects. Thus, during the test phase we found that both PCP and WIN administered alone or together impaired long-term memory with respect to vehicle-treated mice (Supplementary Fig. 3A-B).

On the other hand, sociability was only impaired in PCP-VEH mice, and when WIN was co-administered with PCP, this deficit was abolished, both in terms of exploration time of the stimulus mouse (significant main effect of exploration time F(1,36)=20.27; p < 0.001 and significant interaction F(1,36)=10.54; p < 0.01) (Fig. 3B). See Supplementary Table 1 for extended statistical data. Differences between the means for sociability in PCP-VEH and PCP-WIN treated mice showed large effect sizes (Supplementary Table 2). In contrast, social novelty was reduced both in PCP-VEH and PCP-WIN mice (significant main effect of PCP treatment F(1,36)=19.37; p < 0.001) (Supplementary Fig. 4A).

After the behavioral evaluations, we extracted brain tissue at PD75 and quantified the expression of PSD95 and CDK5 in the HPC and the PFC. In the HPC, PSD95 was reduced in PCP-VEH treated mice as compared to VEH-VEH, and this effect was abrogated in mice treated concomitantly with PCP and WIN (significant main effects of PCP F(1,16)=24.10; p < 0.001; of WIN F(1,36)=13.59; p < 0.01 and significant interaction F(1,36)=12.51; p < 0.01) (Fig. 4A-B). The expression of CDK5 was increased in PCP-VEH treated mice, and this effect was also abolished when mice were treated with both PCP and WIN (significant interaction F(1,36)=16.20; p < 0.01) (Fig. 4A-C). See Supplementary Table 1 for extended statistical data. The differences between the means for PSD95 and CDK5 in the HPC of PCP-VEH and PCP-WIN treated mice showed large effect sizes (Supplementary Table 2).

In addition, the expression levels of PSD95 and CDK5 in the HPC were associated with sociability performance (PSD95 $r = 0.48$, p < 0.05).
Fig. 2. PSD95 and CDK5 expression in olfactory neuroepithelial (ON) cells in first-episode psychosis patients (FEP) and controls. (A) Photomicrograph of ON cells at passage four. (B) A significant main effect of cannabis was observed for PSD95 expression, where both control subjects (C/c) and patients that use cannabis (FEP/c) showed higher levels with respect to non-users ($N = 5$ per group). (C) Significantly lower levels of CDK5 were observed in FEP/c with respect to FEP/nc ($N = 5$ per group). (D) Representative Western blot showing the expression of CDK5 and PSD95 in ON cells of the different groups. (E) Pearson correlation analysis between clinical measures in FEP/nc and FEP/c and expression levels of PSD95 and CDK5 in the ON ($N = 10$). Significant correlations are marked with a black dot. PSD95 negatively correlated with CDK5 expression (F) and with PAS during childhood (G), whereas CDK5 was positively associated with PAS during childhood (H) and with PAS total score (I). The correlation plots show the Pearson’s $r$ coefficients and the $R^2$ square fit calculation. (J) Timeline for the drug incubation protocol in in vitro studies in ON cells from controls non-cannabis users (C/nc) and FEP/nc treated with WIN 55-212-2 (WIN) 100 nM for 21 days ($N = 20$). (K) Representative Western blot showing the expression of CDK5 in ON cells after incubation with WIN. (L) CDK5 expression in FEP/nc was significantly reduced when the cell culture medium was enriched with WIN. Violin plots show minimum to maximum values. Data are mean ± SEM. Two-way ANOVA followed by Bonferroni post-hoc test. *$p < 0.05$. **$p < 0.05$ significant cannabis effect.
3.6. Inhibition of CDK5 reverses sociability deficits observed in PCP-treated mice and normalizes PSD95 expression

To assess the involvement of CDK5 on PCP-driven sociability deficits, we administered the CDK5 inhibitor roscovitine to mice treated chronically with PCP and WIN. The protocol for roscovitine administration during behavior (PD75-PD85) and before tissue extraction (PD88) is shown in Fig. 5A. We found that roscovitine abrogated the sociability deficits observed in the PCP-VEH treated group (Fig. 5B) (significant main effects of PCP F(1,20)=13.93; p < 0.01; of roscovitine F(1,20)=5.34; p < 0.05 and significant interaction F(1,20)=23.68; p < 0.01). The differences between the means for sociability in PCP-VEH treated mice receiving or not roscovitine showed large effect sizes (Supplementary Table 2). Notably, this result was specific to sociability deficits, since no significant effects of roscovitine were observed on social novelty in PCP-treated mice (Supplementary Fig. 4B). See Supplementary Tables 1 and 2 for extended statistical data.

Regarding cognitive effects, roscovitine slightly enhanced long-term memory performance in control mice, but it did not modulate the deficits induced by the administration of PCP or WIN alone or together (significant main effects of PCP F(1,20)=30.86; p < 0.0001; of WIN F(1,20)=32.17; p < 0.0001 and significant interaction F(1,20)=12.68; p < 0.01) (Fig. 5C).

Western blot analysis showed that roscovitine restored PSD95 downregulation in PCP-VEH treated mice to control levels in the HPC.
(Fig. 5 D-E) and the PFC (Fig. 5 F-G). As expected, the changes in CDK5 expression induced by PCP alone or in combination with WIN were not modulated by roscovitine administration in the HPC (significant interaction F(1,16) = 7.93; p < 0.05) nor in the PFC (significant interaction F(1,16) = 10.04; p < 0.01) (Supplementary Fig. 5A-B). See Supplementary Table 1 for extended statistical data.

Fig. 4. Hippocampal (HPC) and prefrontal (PFC) expression changes in CDK5 and PSD95 in mice. (A) Representative Western blot showing the expression of CDK5 and PSD95 in the HPC. (B) PCP exposure reduced PSD95 expression, and (C) increased CDK5 levels. Both effects were reversed in mice treated with PCP plus WIN. N = 5 per group. (D) Pearson correlation analysis between PSD95/CDK5 expression in the HPC and sociability and novel object (NOR) discrimination indexes (N = 20). Significant correlations are marked with a black dot. PSD95 (E) was positively correlated and CDK5 (F) was negatively associated with sociability. PSD95 (G) and CDK5 (H) were positively correlated in both structures. (I) Representative Western blot showing the expression of CDK5 and PSD95 in the PFC. PSD95 (J) expression was decreased and CDK5 (K) levels were increased in PCP-HEH treated mice and both of these effects were reversed in mice exposed to PCP plus WIN. N = 5 per group. (L) Pearson correlation analysis between PSD95/CDK5 expression in the PFC and sociability and novel object (NOR) discrimination indexes (N = 20). Significant correlations are marked with a black dot. PSD95 levels (M) did not significantly correlate with sociability, while CDK5 in the PFC was significantly associated with lower sociability performance (N). In addition, CDK5 in the PFC was associated with lower levels of PSD95 in the HPC (O). The correlation plots show the Pearson’s r coefficients the R square fit calculation. Data are mean ± SEM. Two-way ANOVA followed by Bonferroni post-hoc test. *p < 0.05; **p < 0.01.

Fig. 5. Effects of the CDK5 inhibitor roscovitine in mice. (A) Adolescent mice (PD30) were treated with 1 mg/kg of WIN 55–212,2 (WIN) for 21 days (PD30–PD51), and with 10 mg/kg of PCP for 10 days from PD37 to PD49. Mice were implanted with intracerebroventricular (ICV) cannulas at PD58–PD62. Roscovitine was administered ICV and mice were tested in behavioral paradigms at PD75–PD85. Tissue extraction for biochemical studies was performed in adult mice at PD88. (B) Mice treated with PCP-VEH showed deficits in sociability, and roscovitine reversed these effects. (C) Roscovitine increased novel object recognition memory in VEH-VEH treated mice but did not reverse the memory alterations induced by PCP administration. N = 5–7 per group. Representative Western blots showing the expression of PSD95 in the hippocampus (D) and the prefrontal cortex (F) following ICV administration of roscovitine. Inhibition of CDK5 activity restored PSD95 expression in the PCP-VEH group to control levels in both the hippocampus (E) and the prefrontal cortex (G). N = 4–5 per group. Data are mean ± SEM. Two- and three-way ANOVAs followed by Bonferroni post-hoc test. *p < 0.05; ***p < 0.001.
4. Discussion

In this study, we provide evidence that CDK5 overexpression and lower levels of PSD95 in ON cells of FEP/nc are associated with alterations in social functioning, but not cognitive deficits. Notably, we found that FEP/c showed better sociability than non-users, and normalized CDK5 and PSD95 expression. In vitro exposure to WIN in ON cells reduced CDK5 levels in FEP/nc but had no effect in C/nc, confirming that stimulation of CB1R by cannabinoids specifically modulates CDK5 expression in FEP. Similar findings were obtained in a dual-hit mouse model of psychosis, where sociability deficits were paralleled by alterations in CDK5 and PSD95 levels in the PFC and HPC of PCP-VEH, but not in PCP-WIN treated mice. Moreover, the administration of roscovitine abrogated PCP-driven sociability deficits in mice, confirming the involvement of CDK5 on this psychotic trait.

In agreement with previous data (de Vos et al., 2020; Pope et al., 2021), our results indicate that FEP/nc present more negative symptoms (more asociality, social withdrawal, conversational problems, and higher premorbid social deficits) than FEP/c. In contrast, a recent study associated frequent cannabis use in FEP with blunted speech, rather than with the apathy dimension of negative symptoms (anhedonia, asociality, social withdrawal) (Cunha et al., 2013). Yet, our results are consistent with other studies showing no differences in cognitive performance between both populations (de Vos et al., 2020; Pope et al., 2021), although one report finds better cognitive performance in FEP/c than in FEP/nc (Rapp et al., 2012). Therefore, our data supports the existence of phenotypical differences between FEP/c and FEP/nc, which could be due to higher premorbid functioning and lower baseline levels of negative symptoms in FEP/c (Ferraro et al., 2013; Ferraro et al., 2019) or by intrinsic biological disparities in these two populations.

To gain insight into the molecular mechanisms involved in the differences between FEP/nc and FEP/c, we investigated the expression of PSD95 and CDK5 in the ON. These proteins have been associated with both CB1R and NMDAR functions and are strongly interrelated (Bianchetta et al., 2011; Morabito et al., 2004). Congruently, we found a correlation between CDK5 overexpression and PSD95 downregulation in ON cells from FEP patients. Notably, we observed that only social but not cognitive deficits were correlated with higher CDK5 and lower PSD95 levels. Previous studies in post-mortem brains have shown that CDK5 expression is decreased in schizophrenia patients chronically treated with antipsychotics, while antipsychotic-free schizophrenia patients did not show any alterations vs. controls (Ramos-Miguel et al., 2013). These results are in agreement with those obtained by our group showing a similar decrease in CDK5 protein expression in the ON of schizophrenia patients (Barrera-Conde et al., 2021). Together, these data reveal that long-term treatment with antipsychotics may be an important factor contributing to the modulation of CDK5 expression in the brain and in the ON cells in schizophrenia.

Another factor that may regulate these proteins is age. Indeed, CDK5 overexpression and PSD95 downregulation have been associated with aging in neurodegenerative disorders (Gupta and Singh, 2019). However, in our study the effect of age does not seem to be playing a crucial role, since FEP/c are older than FEP/nc and show lower CDK5 and higher PSD95 levels. Altogether, our data suggest that increased CDK5 expression in ON cells may be an indicator of social deficits in early psychosis.

Importantly, sociability deficits observed in FEP/nc patients were mimicked in mice chronically exposed to PCP during adolescence, and CDK5/PSD95 alterations observed in ON cells of FEP/nc patients were also present in the HPC and PFC of these mice. Chronic PCP administration is known to produce social deficits (Seillier et al., 2013) and reduce PSD95 expression in the PFC and the ventral HPC (Gigg et al., 2020). On the other hand, genetic alterations of CDK5 in the HPC cause behavioral alterations that resemble a psychotic phenotype (Rudenko et al., 2015), and the activity and expression of this kinase has been linked to social defeat models (Yin et al., 2021; Zhang et al., 2018).

However, to the extent of our knowledge, this is the first study associating CDK5 overexpression and social deficits in psychosis. This relationship was further confirmed by a pharmacological approach using the CDK5 inhibitor roscovitine, which reversed sociability deficits and restored PSD95 expression to control levels in PCP-treated mice but had no effect on long-term recognition memory or social novelty deficits.

Moreover, we showed that cannabinoids modulate CDK5/PSD95 and social behavior. FEP/c exhibited better social skills than FEP/nc and normalized CDK5/PSD95 levels in the ON. We confirmed the role of cannabis on CDK5 in in vitro studies, where ON cells of FEP and controls non-cannabis users were exposed to WIN. Hence, CDK5 expression was decreased in FEP/nc, but was not modulated in C/nc, indicating that there is a distinct effect of cannabinoids on CDK5 via CB1R activation only in certain vulnerable populations. Our preclinical data is also consistent with these results, since WIN exposure restored expression changes to control levels in PCP-treated mice but did not modulate CDK5/PSD95 levels in VEH-treated mice. Thus, these translational findings in both FEP patients and in a psychosis-like mouse model reveal an interaction between CB1R and NMDAR signaling via CDK5/PSD95 that has an impact on social behavior. This association could be directly mediated by pathways activated by G-protein signaling in CB1R and calcium entrance in NMDAR, or by a potential physical interaction of CB1R and NMDAR (Sánchez-Blázquez et al., 2014).

These findings, however, need to be interpreted considering their limitations. First, our cross-sectional approach in humans impedes knowing whether the link between sociability deficits and PSD95/CDK5 expression changes is persistent over time. Hence, longitudinal measurements would help to validate the importance of these proteins for the prognosis of psychosis. Second, further studies should be carried out with a higher number of human subjects to corroborate our findings. Third, there is an inherent limitation in the use of animal models to study psychosis. Although the PCP model has been largely validated (Jentsch and Roth, 1999; Seillier et al., 2020; Seillier et al., 2013) and represents a useful tool to study causality, behavioral alterations in animals will never be an exact representation of human symptomatology.

5. Conclusions

This translational data in FEP patients and in a mouse model of psychosis shows that sociability deficits are associated with higher expression of CDK5, suggesting that this protein could be an early biomarker of social deficits in psychosis. In addition, the results in FEP/c and mice treated with PCP-WIN during adolescence reveal an interplay between CB1R and NMDAR function through changes in CDK5/PSD95 expression specifically associated with social functioning.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2022.105942.

CRediT authorship contribution statement

Marta Barrera-Conde: Conceptualization, Data curation, Writing – original draft, Formal analysis, Writing – review & editing, Visualization. Emma Veza-Estévez: Investigation, Methodology. Maria Gomez-Gonzalez: Investigation, Methodology. Jordi Garcia-Quintana: Investigation, Methodology. Laura Martinez-Sadurní: Investigation, Methodology. Amira Trabsa: Investigation, Methodology. Rafael de la Torre: Writing – review & editing. Daniel Berge: Writing – review & editing, Funding acquisition. Patricia Robledo: Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Conflict of interest

The authors declare no competing interests.
Data availability
Data will be made available on request.

Acknowledgements
This work was supported by grants from Instituto de Salud Carlos III to PR and DB (PI18/00053) and Co-funded by the European Union, and DUEE de la Generalitat de Catalunya 2017 SGR 138 (RT) from the Departament d’Economia i Coneixement de la Generalitat de Catalunya (Spain). The authors would like to acknowledge the Clinical Research Unit at IMIM-Hospital del Mar Medical Research Institute for their help in the human studies, and Dr. Arnau Busquets for interesting discussions on the behavioral tests in mice.

References
Jentsch, J.D., Roth, R.H., 1999. The neuregulopharmacology of phenylcycline: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology. 20 (3), 201–225.