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INVITED REVIEW

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Sex-specific susceptibility to psychotic-like states provoked by prenatal THC exposure: Reversal by pregnenolone

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Abstract

Sociocultural attitudes towards cannabis legalization contribute to the common misconception that it is a relatively safe drug and its use during pregnancy poses no risk to the fetus. However, longitudinal studies demonstrate that maternal cannabis exposure results in adverse outcomes in the offspring, with a heightened risk for developing psychopathology. One of the most reported psychiatric outcomes is the proneness to psychotic-like experiences during childhood. How exposure to cannabis during gestation increases psychosis susceptibility in children and adolescents remains elusive. Preclinical research has indicated that in utero exposure to the major psychoactive component of cannabis, delta-9-tetrahydrocannabinol (THC), deranges brain developmental trajectories towards vulnerable psychotic-like endophenotypes later in life. Here, we present how prenatal THC exposure (PCE) deregulates mesolimbic dopamine development predisposing the offspring to schizophrenia-relevant phenotypes, exclusively when exposed to environmental challenges, such as stress or THC. Detrimental effects of PCE are sex-specific because female offspring do not display psychotic-like outcomes upon exposure to these challenges. Moreover, we present how pregnenolone, a neurosteroid that showed beneficial properties on the effects elicited by cannabis intoxication, normalizes mesolimbic dopamine function and rescues psychotic-like phenotypes. We, therefore, suggest this neurosteroid as a safe "disease-modifying" aid to prevent the onset of psychoses in vulnerable individuals. Our findings corroborate clinical evidence and highlight the relevance of early diagnostic screening and preventative strategies for young individuals at risk for mental diseases, such as male PCE offspring.

KEYWORDS
dopamine, introduction, neurosteroids, prenatal cannabis, schizophrenia, sensorimotor gating

Schizophrenia is a severe and debilitating psychiatric disorder characterized by disorganized thinking and perception, lack of motivation and emotional responsiveness. Although it is not as common as other mental illnesses, schizophrenia affects one in 300 people (0.32%) worldwide (World Health Organization, WHO, 2022) and its prevalence has risen from 13 million in the nineties to approximately 21 million in 2016.[1]

The symptomatology of the disease is classified into positive, negative and disorganization symptoms. Positive symptoms are extremely

common and include hallucinations, featuring perceptual experiences in the absence of corresponding stimuli (i.e., hearing voices or seeing things) and delusions, typically characterized by the manifestation of illogical, bizarre ideas and delusions of persecution. Although negative symptoms consist of flattened emotion, loss of motivation, social withdrawal, alogia and anhedonia, disorganization symptoms comprise deficits in attention, memory and problem-solving. Negative symptoms often manifest alongside the disruption of cognitive function, thereby resulting in the deterioration of social and occupational functioning with a considerable worsening of patient quality of life.[2,3] Although benchmark antipsychotic medications allow to efficiently manage positive symptoms, therapeutic options for negative symptoms are an unmet medical need.[4] As a result, this mental disease is one of the top ten leading causes of disability around the globe, with mortality and economic burden being strikingly higher than other most prevalent mental diseases.[1,5]

Over the past decade, huge efforts have been made to implement both prediction and prevention programs of this disease and attain a major decrease in its incidence. Because of its chronic clinical course and neurodevelopmental origin,[6,7] schizophrenia is one of the first mental diseases in which predictive and preventive intervention strategies have been applied.[6–10] However, these preventive programs were based on predictive basic symptoms without taking into consideration relevant aetiological risk factors for this disorder, such as individual biological make-up, maternal infections and complications during pregnancy and delivery, substance abuse, and stress, to name a few. Importantly, the pathogenesis of schizophrenia cannot be explained by alterations of a single genetic mutation, neurodevelopmental teratogen or environmental risk factor. Schizophrenia is a complex and heterogeneous condition with multiple neuronal pathways involved, which are differently affected by the interplay of genetic components and environmental risk factors.[11,12] Each risk factor, per se or in combination, may negatively impact diverse molecular and cellular pathways and affect nervous system with different modalities, sometimes in an opposite and time-dependent manner.[13,14] Accordingly, the neurodevelopmental hypothesis of schizophrenia[11,15] posits that a single component (i.e., genetic, biological, chemical, psychosocial) may be not sufficient to elicit disease onset. An insult may alter brain developmental trajectories and pathologically "*prime*" the nervous system to a second risk factor, such as psychosocial stress or drug abuse, thereby leading to the onset of a full clinical syndrome. According to this hypothesis, it would be possible to prevent the onset of the disease either by removing predictive modifiable risk factors associated with psychosis or by introducing therapeutic options.

A substantial body of animal and human research documents that prenatal cannabis exposure (PCE) is likely one of the environmental risk factors endowed with all the features of the multiple hits hypothesis of schizophrenia. In humans, PCE increases the risk of developing psychosis, especially during (pre)adolescence.[16–18] In rodents, PCE produces a spectrum of molecular, epigenetic, neurophysiological and brain circuit alterations predisposing the offspring to behavioral phenotypes showing high isomorphism with schizophrenia.[19,20] According to human findings, a PCE offspring does not display psychotic-like phenotypes unless exposed to an environmental challenge, such as Δ9-tetrahydrocannabinol (THC) or stress, thereby acting as a "second hit" during preadolescence. As described below, these "silent" psychotic-like phenotypes reveal a critical role of sex as a biological variable for susceptibility and resilience.[19,20]

We highlight some of our findings demonstrating how PCE sex-dependently deregulates mesolimbic dopamine development thereby priming to psychotic-like states upon exposure to environmental challenges, such as stress or THC. This experimental model serves the purpose of not only deciphering the pathological trajectories of circuit development induced by PCE, but also potentially aiding in the design of preventive interventions prior to exposure to subsequent challenges. Accordingly, our findings show a potential correcting activity of pregnenolone (PREG) and extend its therapeutic properties from preventing the effects of acute cannabis intoxication[21–23] to comprising a safe "disease-modifying" aid for primary intervention programs aiming to prevent the onset of psychoses in a vulnerable segment of the population.

1 | MATERNAL THC EXPOSURE PRODUCES PSYCHOPATHOLOGICAL SEQUELAE IN THE OFFSPRING

Sociocultural attitudes toward cannabis legalization contribute to the common misconception that marijuana is a relatively safe drug even during pregnancy because of its natural origin.[24–26] In addition, childbearing women are encouraged to use cannabis for the treatment of symptoms associated with the first trimester of pregnancy, such as nausea, vomiting and anxiety states,[24] with the overall perception of safety or slight risk from its use.[25,27–30] As a result, rates of cannabis use in the year before gestation, as well as during gestation and lactation, are increasing over time in North America as well as in Europe.[31–33]

In sharp contrast, increasing and alarming clinical evidence shows how cannabis use during pregnancy is associated with detrimental effects on the progeny. First, maternal cannabis use results in overt adverse outcomes in newborn, including preterm and low-weight birth, exaggerated startle responses and poor startle habituation to novel stimuli, and increased neonatal morbidity.[34–37] Second, prenatal cannabis exposure, by negatively affecting cognitive and neurobehavioral domains, heightens the risk for psychopathology on the progeny, especially during childhood,[16,17,38,39] thereby leading to the manifestation of neuropsychiatric disorders. To date, four large longitudinal studies (Ottawa Prenatal Prospective [OPPS], Maternal Health Practices and Child Development Project [MHPCD], Generation R and Adolescent Brain Cognitive Development [ABCD]), and about 20 reports have analyzed the impact of PCE on multiple behavioral and cognitive outcomes.

According to these studies, PCE children and adolescents display a series of cognitive and behavioral problems, including hyperactivity, inattention, impulsivity, heightened sensitivity to drugs of abuse and externalizing behaviors.[16,40–52] Finally, the most recent and largest

cross-sectional study ABCD reports a proneness to psychotic-like experiences, depression and anxiety.[17,38,53,54] Overall, these epidemiological findings clearly indicate that in utero cannabis exposure impacts the developing brain, resulting in a heightened risk for psychopathology during childhood. As outlined elsewhere,[55] an analysis of sex as a biological variable is seldom investigated in the outcome of PCE offspring.

2 | PRENATAL CANNABIS EXPOSURE ELICITS SEX- AND AGE- SPECIFIC PSYCHOTIC-LIKE (ENDO)PHENOTYPES

By contrast to the general belief, mental disorders are highly prevalent during childhood. A recent meta-analysis estimated that prevalence of mental disorders in children and adolescents reaches approximately 15% worldwide, with 10%–20% of children experiencing one or more psychological problems.[56] Although schizophrenia rarely manifests before puberty, its incidence strikingly increases from age 13 years.[57] Moreover, early-onset schizophrenia typically shows worse long-term outcomes, including more resistance to antipsychotic medications, more severe cognitive deficits, and a higher dropout from school or work compared to individuals characterized by adult-onset disease.[58] Thus, early detection and intervention become essential to avoid these psychopathological manifestations evolve into severe mental illness later in life.

Cannabis-induced neurocognitive and behavioral sequelae on the offspring rely on the impact of the main psychoactive ingredient of cannabis, THC, on brain development. Generally, phytocannabinoids, as a result of their lipophilic nature, easily cross the placenta,[59,60] enter the bloodstream and reach the fetal nervous system, where they interfere with the activity of the endogenous cannabinoid system (ECB).[61–65] CB1 receptors, key components of ECB, are widely expressed in the brain, including the basal ganglia, prefrontal cortex and hippocampus, where they influence the release and function of neurotransmitters implicated in the etiopathogenesis of neuropsychiatric disorders.[64,66–71] Among neurotransmitter systems, dopamine is one of the earliest to develop[72] and its early dysregulation has been proposed to be one important etiological factor of several dopamine-dependent neuropsychiatric conditions, including schizophrenia and substance use disorders.[72,73]

According to the "dual hits hypothesis of schizophrenia",[74] we discovered that PCE acts as a "first hit" and biases mesolimbic dopamine system by deranging its developmental trajectories exclusively in male preadolescent offspring.[19] PCE male progeny exhibit multiple molecular and synaptic changes in dopaminergic neurons of the ventral tegmental area (VTA), including disruption of excitatory-to-inhibitory balance, enhanced excitability and a switch in synaptic plasticity, from long-term depression to long-term potentiation. This phenotype is associated with a hyper-responsiveness to acute THC as exemplified by a larger increase in dopamine release, firing frequency and robust deficits in sensorimotor gating function, as indexed by prepulse inhibition (PPI) deficits (Figure 1).[19,75,76] PPI of the acoustic startle reflex is an operational measure of sensorimotor gating and provides an endophenotypic signature with a high degree of homology between experimental animals and humans.[77,78] As such, this cross-species neurophysiological measure is deficient in schizophrenia patients, as well as in other dopamine-dependent neuropsychiatric conditions, such as obsessive–compulsive disorder,[79] Tourette's syndrome,[80] and mania.[81] Interestingly, this neurophysiological index has been proposed as a biomarker for the study of the prodrome and first acute states of psychosis, thereby enabling the identification of individuals at the greatest risk for developing schizophrenia.[82,83] Indeed, during prodromal stages of late onset disorders, patients experience a heightened sensory and perceptual awareness that may result from deficient PPI processing.[84] Notably, as observed in preadolescent PCE offspring,[20] schizophrenia patients exhibit a greater vulnerability to environmental stressors throughout early stages of neurodevelopment.[85–87]

In rodents, PPI is widely used to study neurobiological mechanisms of psychiatric disorders characterized by sensorimotor gating dysfunctions because it satisfies all the criteria of an animal model in psychiatric research: (i) face validity, with parallel changes of PPI in patients and rodents under pharmacological (i.e., dopaminergic agonists and other psychotomimetic drugs) and environmental manipulation (social isolation, sleep deprivation)[75,88–91]; (ii) predictive validity, such that antipsychotic drugs restore PPI in both patients and rodents[77]; and (iii) construct validity, such that there is an overlap between humans and rodents with regards to pathological underpinnings.[92–94] Importantly, PPI is regulated by dopamine signaling within forebrain circuits.[95–98] Accordingly, we observed that THC-induced deterioration of PPI positively correlates with dopamine levels in the nucleus accumbens shell whose new synthesis is required for such PPI deficits to manifest.[19]

Remarkably, PCE female offspring do not display either spontaneous or THC-induced psychotic-like phenotypes. In addition, they exhibit adaptive coping strategies to acute stress[76] and are protected from stress-induced detrimental effects on PPI (M. Melis, personal communication; February 21, 2022). Such a sex-specific effect of PCE is in line with previous investigations[52,99–106] suggesting how female sex often provides a protective factor in response to the same intrauterine environmental insults.

Although the mechanism(s) by which PCE females appear to be protected is (are) a matter of investigation, our findings show that they exhibit a normal transmission and function of the mesolimbic dopamine system.[76] PCE females do not display altered firing frequency of VTA dopamine cells and their responsiveness to acute THC administration does not differ from control counterparts.[76] Accordingly, in utero THC exposure does not affect synaptic properties of VTA dopamine neurons.[76] One could therefore speculate that female sex protects mesolimbic dopamine system from detrimental perturbations of in utero THC. One explanation might be that sex steroids determine a "resilient" phenotype through their organizational effects. Sex hormone receptors are present from early stages of development,[107] and both androgen and estrogen(α) receptors are widely expressed in midbrain dopamine neurons,[108,109] where they

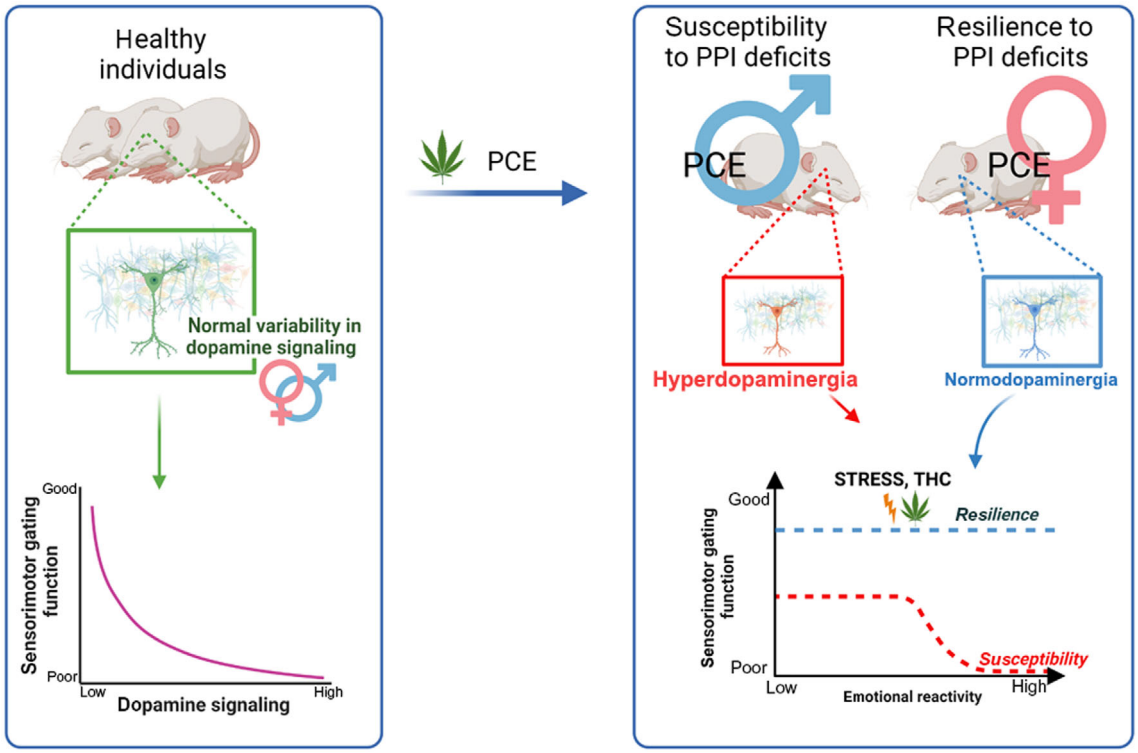

FIGURE 1 Prenatal cannabis exposure confers preadolescent rats (postnatal days 15–28) a psychiatric endophenotype susceptible to subsequent environmental challenges (i.e., delta-9-tetrahydrocannabinol [THC] or stress). This endophenotype (impairments in sensorimotor gating function, as indexed by prepulse inhibition [PPI] deficits) is sex-specific and reflects multiscale alterations of dopamine transmission, leading to vulnerability to discrete dopamine-dependent psychiatric disorders. PCE, prenatal THC exposure

contribute to neurophysiological and behavioral redouts under the control of the dopamine system.[108,109] Testosterone, but not estrogen, increases dopamine synthesis and metabolism in the adolescent rat brain, changing these neurons toward hyperdopaminergic phenotypes.[109] Interestingly, the strain of rats used in our studies (i.e., Sprague–Dawley) feature higher plasma testosterone concentrations than females across development, especially during the first postnatal days and juvenile period.[110–112]

Besides the influence of sex hormones, mounting evidence points to significant sex differences in brain transport systems during development, including the efficiency of the blood–brain barrier function in protecting the central nervous system from xenobiotics.[113,114] For example, the multidrug resistance-related protein 1 (P-glycoprotein, Abcb1) and the breast cancer resistance protein (Abcg2) play a key role in limiting the brain distribution of several xenobiotics, including cannabinoids.[115,116] The expression of these transporters is sex-dependent as females feature a heightened expression of *Abcg2* mRNA in the placenta,[117] thereby resulting more protected against the effects of xenobiotics during pregnancy. Additionally, because THC inhibits the proper function of Abcg2,[116] it is likely that brain THC disposition might be higher in males than females, leading to a more pronounced teratogenic impact on neurodevelopment.

Notwithstanding the molecular mechanisms, our findings support the growing evidence that male sex is a risk factor for PCE outcome as well as for other neuropsychiatric disorders characterized by neurodevelopmental origin and dopamine signaling deregulations.[118,119]

3 | PREGNENOLONE RESTORES PCE MALE DOPAMINE FUNCTION AND RESCUES FROM PSYCHOTIC-LIKE (ENDO)PHENOTYPES

The endophenotype induced by PCE in preadolescent male rats is instrumental for testing potential therapeutic interventions aimed at preventing the phenoconversion into late-onset psychiatric disorders, especially those in the psychotic spectrum. Notably, the most used antipsychotic agents exert neurological or cardiometabolic adverse effects also in pediatric patients.[120–122] Hence, both prevention and treatment of this condition represent an unmet medical need. In this framework, it is noteworthy that PREG, a precursor of all steroid hormones, has therapeutic properties in schizophrenia symptoms, including cognitive impairments, negative symptoms and psychotic-like states induced by cannabis intoxication.[21,22,123–128] PREG, marketed as a dietary supplement in the US and other European Union Countries, has been tested in patients for various brain diseases, ranging from neurological to psychiatric disorders, showing no remarkable adverse effects.[129–132] Unlike second-generation antipsychotics, this neurosteroid is safe and well-tolerated in humans, including no effects on body weight, intermediate metabolism, heart rate, blood

pressure.[23,126] Interestingly, clinical trials have reported that PREG supplementation (daily dosages ranging from 30 to 500 mg) ameliorated overall psychopathology severity and functioning of schizophrenia patients, improving both positive symptom scores, as well as attention and working memory performance.[123,133] Conversely, schizophrenic patients exhibited decreased levels of this neurosteroid[134,135] and, in rodents, atypical antipsychotics treatment increased its brain levels.[134,136,137] Accordingly, PREG counteracted schizophrenia-like phenotypes, including PPI deficits, in an animal model of this disease, namely dopamine transporter knockout (DAT-KO) mice.[125] In addition, acute administration of this neurosteroid rescues a series of THC-induced behavioral phenotypes in mice with relevance to schizophrenia, including psychomotor agitation, sensorimotor gating deficits and disruption of cognitive function and social interaction.[21]

Building on these findings, we investigated its potential restorative effects in male preadolescent PCE rats. Our experimental design was aimed at resuming dopaminergic signaling to prevent PPI deficits and at providing a potential therapeutic use. Thus, we repeatedly administered PREG (postnatal days 15–23) to PCE offspring and performed a multiscale analysis of PCE male progeny in the absence of the drug (i.e., 24–48 h after the last administration).[19] PREG resumed not only the normal intrinsic and synaptic properties of dopamine neurons, but also their synaptic properties and plasticity. PREG also restored normal responsiveness of mesolimbic transmission to acute THC and prevented acute THC-induced deficits of PPI.[19] Notably, PREG protective properties also extend to stress-induced disruption of PPI in male PCE progeny (M. Melis, personal communication; February 21, 2022). These actions are ascribed to PREG itself and not its downstream neuroactive metabolites (e.g., progesterone, allopregnanolone) because pharmacological inhibition of the enzyme implicated in its metabolism (3 beta-hydroxysteroid dehydrogenase) does not modify its protective effect.

Although there is no clear mechanistic evidence as to how PREG reverses psychotic-like (endo)phenotypes provoked by PCE, several molecular targets may account for its rescue properties. For example, PREG binds the sigma-1 (σ1) receptor, an endoplasmic reticulum-associated chaperone highly expressed in the brain, including key dopaminergic regions.[138] This chaperon protein is localized in midbrain dopaminergic neurons and in the striatum,[139–141] where it modulates dopamine function and release.[142] Although the effects of σ1 receptor activation on dopamine system are conflicting, their ligands (including PREG) have been shown to attenuate dopamine signaling deregulation in response to psychostimulant drugs.[142–144] Remarkably, chronic treatment with supraphysiological doses of PREG reduces both stress- and cocaine cue-induced craving in individuals with cocaine use disorder.[145] PREG also acts as a potent negative allosteric modulator of CB1 receptor signaling[22] and blocks all the phenotypic signatures of cannabinoid intoxication.[22] However, at this stage, we could speculate that the rescue properties of PREG in PCE progeny cannot be ascribed to a negative modulation of CB1 receptor, but rather to its intracellular signaling.

Besides PREG biological targets, endogenous serum levels of PREG are reduced in patients with schizophrenia.[135] Additionally, the benchmark antipsychotic clozapine robustly elevates PREG both in schizophrenia individuals and rodents,[123,126,146,147] suggesting that changes in this neurosteroid may play a role in the neurobiology of this disorder as well as in response to its medication. Interestingly, Brittany et al.[148] evaluated baseline neurosteroid levels, including PREG, in bipolar depressed participants with a history of a cannabis use disorders. These patients showed a significant decrease in pregnenolone/pregnanolone ratio, and PREG supplementation results in greater depression remission rates than placebo.[124,148] However, to the best of our knowledge, no study has ever reported alterations of endogenous PREG levels in individuals exposed to cannabis either during pregnancy/adolescence and/or at risk of developing psychosis. The abovementioned findings and preclinical evidence[19,21,22] warrant further investigations on PREG endogenous levels in PCE offspring per se and in response to environmental challenges, as well as after PREG administration.

Collectively, our data support that PREG may be a valuable aid to mitigate PCE impact ("first-hit") on dopamine system function, and to promote resilience towards "second hits" such as acute exposure to THC or stress, which often precipitate dopamine-dependent psychiatric conditions including schizophrenia. Finally, our observations support the role of stress as a key factor in the onset of several dopamine-related psychiatric conditions,[149–151] and of sensitive windows of vulnerability of mesolimbic dopamine system for pathological development.[152–154]

4 | PREGNENOLONE AS A NEUROSTEROID-BASED TOOL FOR THE TREATMENT OF PSYCHOTIC-LIKE CONDITIONS: APPLICABILITY AND LIMITATIONS

Psychotic spectrum disorders (including those induce by cannabis exposure) are mainly treated using first- and second-generation antipsychotics, which were introduced in the market in 1950 s and 1990 s, respectively. Although these drugs are helpful for many psychotic manifestations, they do not relieve symptoms (mainly cognitive and negative symptoms) in around one-quarter of patients.[155,156] In addition, they are associated with a variety of severe side effects, such as sedation, metabolic disturbance, sexual dysfunction and motor complications, which can affect patient subjective well-being and result in poor adherence to the treatments. Thus, novel therapeutic approaches to treat psychotic-like conditions, with comparable efficacy and lower side effects, represent an unmet clinical need. In this framework, PREG has shown beneficial effects on cardinal symptoms of psychotic spectrum disorders, including cognitive and negative symptoms that are often refractory to these medications.[123,127] For example, in randomized controlled clinical trials, PREG supplementation significantly reduced the scores of SANS (Scale for the Assessment of Negative Symptoms), and its serum levels positively correlated with improvement in cognitive functions, as indexed by the BACS (Brief Assessment of Cognition in Schizophrenia) assessment

battery[123,127]; adjunctive PREG also ameliorated visual attention deficits in patients with recent-onset schizophrenia and schizoaffective disorders compared to placebo.[157] In rodent models, its exogenous administration enhances learning and memory in several behavioral tasks, such as object recognition memory and social transmission of food preference tests.[22,125,158,159] Moreover, acute and chronic administration of this neurosteroid reversed psychotic-like phenotypes with face validity to positive symptoms in humans.[125]

Although the abovementioned studies provide substantial evidence that PREG may serve as a novel therapeutic tool in schizophrenia-related conditions, several factors may lower its clinical applicability. First, PREG binds to multiple molecular targets. Its pleiotropic activities and lack of selectivity therefore complicate its clinical applicability. Second, as for other steroids, PREG exhibits poor bioavailability, irrespective of the routes of administration.[160–162] Its rapid metabolism results in a short biological half-life that requires multiple administrations to maintain sufficient bioavailability. Third, PREG is the precursor for the synthesis of different classes of endogenous steroids, many of which are endowed with activity in the central nervous system. Thus, once administered, brain and peripheral metabolism may affect its actions by converting PREG into unintended metabolites, which can elicit synergistic, additive and antagonistic effects, significantly influencing its activity in the brain. Four, PREG might exert its antipsychotic-like effects with the contribution of downstream brain active metabolites, such as allopregnanolone. Irrespective of its mechanisms of action, a number of synthetic derivatives of PREG have been designed to have higher bioavailability and selectivity compared to their parent steroid PREG. Some of them have been recently shown to display a better pharmacokinetic profile, comparable therapeutic effects and less toxicity.[23,163,164]

5 | CONCLUSIONS

In the present study, we have presented the current state of our research showing how PCE affects dopamine developmental trajectories and their behavioral readout exclusively in male progeny, which can be efficiently rescued by preventive PREG administration. We would like to emphasize that (1) more attention should be given to early detection and intervention strategies for mental diseases. There is now a broad consensus that a prompt diagnosis and early intervention can have tremendous life-changing consequences on patient outcomes, with beneficial repercussions on social acceptance, scholar performance, productivity and wellness later in life. The major challenge is to discover reliable and quantifiable signs, such as deficits in sensorimotor processing, which enable intervention in the prodrome phases of the mental disease. As abovementioned, animal models are pivotal for introducing novel tools for detection and pharmacological intervention that may correct illness trajectory as close to disorder onset as possible. Here, we showed PCE as a sex- and age- specific psychopathological endophenotype, a trait shared by a number of childhood-onset psychiatric diseases characterized by alterations in mesolimbic dopamine transmission, such as schizophrenia, attention deficit hyperactivity disorder and substance use disorders. (2) Children and adolescents should be the main target population for primary prevention of mental disease. More attention should be given to the influence of environmental factors on sensitive neurodevelopmental windows for the onset of psychopathologies, especially in males.

We have documented for the first time that PCE elicits a sex-specific psychopathological endophenotype susceptible to subsequent challenges (i.e., cannabis use, acute stress) during a sensitive period of neurodevelopment (i.e., preadolescence). By contrast to other etiological risk factors related to schizophrenia and other mental disorders (i.e. prenatal infections or obstetric complications), PCE is a predictive modifiable risk factor. Policies should be implemented so that healthcare providers, obstetricians and gynecologists inform child-bearing women of the harms of cannabis on the fetus. (3) Therapeutic interventions before the onset of overt neurological and behavioral symptoms might prevent or significantly correct the development of mental illnesses. Preventive treatment with PREG can resume a "normodopaminergic state" and rescue from PCE related pathological (endo)phenotypes, suggesting a novel therapeutic approach for offspring exposed to cannabis during pregnancy. Notably, here, we document for the first time that PREG is able to counteract PPI deficits provoked not only by THC challenge, but also stress manipulations. This is of particular importance as growing evidence shows a functional cross-talk between neurosteroids and endocannabinoids in psychiatric diseases strictly connected to stress exposures, such as post-traumatic stress disorder.[165] These systems are deeply involved in stress response and emotional processes, both fundamental components in the pathophysiology of psychiatric conditions, including psychosis.[128] Stress events, such as childhood trauma, and cannabis use are environmental factors that are highly prevalent during (pre)adolescence.[166] Previous studies show that cannabis and childhood trauma interact with each other, thereby heightening the susceptibility of manifesting psychosis,[167–169] resulting in additive effects.[170,171] PREG, being the unique neurosteroid negatively modulating CB1 receptors, might act as key player in the homeostatic mechanisms involving both neurosteroid and endocannabinoid signaling, which can be concomitantly affected by cannabis use and stress events during the vulnerable period of adolescence.

Although the use of psychotropic drugs is on the rise among children with mental health, in the US, half of children with mental disorders do not receive medication, and about one in seven have at least one treatable mental health disorder. If unmedicated, these patients exhibit worse mental health outcomes across the lifespan and less response to psychiatric treatment later in life. Future studies are warranted to assess whether PREG therapeutic potential might be extended to other neurobehavioral domains associated with PCE across childhood/adolescence, given its well-established protective actions of acute THC intoxication in rodents and lack of major side effects in patients.

AUTHOR CONTRIBUTIONS

Roberto Frau: Conceptualization; writing – original draft; writing – review and editing. **Miriam Melis:** Conceptualization; writing – review and editing.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interests.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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