Permanent alterations of hippocampal GABAergic neurotransmission in adult rats following perinatal Δ^9-THC exposure

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Abstract

The long-lasting effects of gestational cannabinoids exposure on the adult brain of the offspring are still controversial. It has already been shown that pre- or perinatal cannabinoids exposure induces learning and memory disruption in rat adult offspring, associated with permanent alterations of cortical glutamatergic neurotransmission and cognitive deficits.

In the present study, the risk of long-term consequences induced by perinatal exposure to cannabinoids on rat hippocampal GABAergic system of the offspring, has been explored. To this purpose, pregnant rats were treated daily with Delta^9–tetrahydrocannabinol (Δ^9-THC; 5 mg/kg) or its vehicle.

Perinatal exposure to Δ^9-THC induced a significant reduction ($p < 0.05$) in basal and K^+-evoked [^{3}H]-GABA outflow of 90-day-old rat hippocampal slices. These effects were associated with a reduction of hippocampal [^{3}H]-GABA uptake compared to vehicle exposed group. Perinatal exposure to Δ^9-THC induced a significant reduction of CB1 receptor binding (B_{max}) in the hippocampus of 90-day-old rats. However, a pharmacological challenge with either Δ^9-THC (0.1 μM) or WIN55,212-2 (2 μM), similarly reduced K^+-evoked [^{3}H]-GABA outflow in both experimental groups. These reductions were significantly blocked by adding the selective CB1 receptor antagonist SR141716A.

These findings suggest that maternal exposure to cannabinoids induces long-term alterations of hippocampal GABAergic system. Interestingly, previous behavioural studies demonstrated that, under the same experimental conditions as in the present study, perinatal cannabinoids exposure induced cognitive impairments in adult rats, thus resembling some effects observed in humans. Although it is difficult and sometimes misleading to extrapolate findings obtained from animal models to humans, the possibility that an alteration of hippocampus aminoacidergic transmission might underlie, at least in part, some of the cognitive deficits affecting the offspring of marijuana users, is supported.

Keywords: Maternal marijuana consumption; GABA outflow; SR141716A; WIN55,212-2; Slices; CB1 receptor binding.

1. Introduction

Marijuana remains one of the world's most widely used illicit drug during pregnancy [1,2]. Despite its wide use, it is poorly understood and still controversial to which extent cannabis exposure during gestation and/or lactation may have long-lasting effects in the adult brain of the progeny [3-7]. Potential reasons that make difficult to identify the existence of a possible correlation between cannabis exposure and its long-lasting effects on the adult brain, may depend on the amount, the pattern and timing of exposure relative to fetal development. Most of this information derives from three major prospective longitudinal studies, with follow-up data on the offspring beyond the early neonatal period, the Ottawa Prenatal Prospective Study (OPPS), the Maternal Health Practices and Child Development study (MHPCD) and the Generation R study. These studies reported the long-term effects of marijuana on the executive functions in the offspring either at childhood, adolescence or adulthood [8].

Accumulating evidence from animal studies indicate that the endocannabinoid system (eCBs) and cannabinoid receptors (either CB1 or CB2 subtypes) emerge during fetal brain development supporting their potential participation in the regulation of specific processes related to neuronal progenitor differentiation, axonal migration and neurite outgrowth [7, 9-10]. Thus, the use of marijuana during pregnancy, by influencing the delicate development of neuronal networks, can have long-lasting consequences on brain maturation. Based on this evidence, research programs have been developed to evaluate weather pre- and perinatal marijuana exposure, by altering both the eCBs and CB1 receptor functions, may lead to subtle long-term changes in the signaling and the functional activity of different neurotransmitters systems. Despite the numerous studies, it is still difficult to understand which neurobiological system(s) is(are) the most vulnerable to early cannabis exposure for brain development and how cannabis-induced alterations are then maintained in the adulthood. Specifically, human and animal studies have shown that cannabis exposure during critical periods of brain development influences the expression of key genes involved in neuronal growth, an effect that may lead to long-term changes in the functionality of certain neurotransmitters in adulthood [11]. For instance, *in utero* marijuana exposure is associated with an abnormal regulation of D2 gene expression in the amygdala of human fetus, leading then to a possible impairment of mesocorticolimbic neural systems [4, 12-13], contributing later in life to increased risk of illicit substance abuse and behavioral disorders. Furthermore, previous studies in rodents have also

reported that perinatal Δ^9-tetrahydrocannabinol (Δ^9-THC) exposure causes a long-lasting impairment to the opioid system at different levels, including gene expression, receptor binding and opioidergic neurotransmission [14-18]. Additional studies performed in rats prenatally exposed to Δ^9-THC or WIN55,212-2 (a synthetic CB1/CB2 receptor agonist) have also demonstrated an alteration of glutamatergic gene expression, impairment of hippocampal long-term potentiation (LTP) along with alteration in cortical and hippocampal glutamate release and cognitive deficits. Moreover it has also been observed a reduction of cortical NMDA receptor responsiveness and dysfunction in neurite outgrowth [19-21].

Considering the important role played by glutamate in neuronal proliferation, differentiation and synaptic plasticity [22], it has been suggested that these changes might lead to an inappropriate neuronal development and function, causing the observed learning deficits, atypical locomotor activity and altered emotional behavior in rodents prenatally exposed to Δ^9-THC or WIN55,212-2 [19, 23, 24].

Among the numerous extrinsic factors that guide the maturation of embryonic cortical neurons, GABA has been proposed to influence proliferation, migration of developing neurons and promote synaptic growth and formation [25]. To our knowledge, just few studies are present in literature, demonstrating that prenatal exposure to Δ^9-THC or WIN55,212-2 affects the GABAergic system [26, 27] inducing an alteration on tangential and radial migration of post-mitotic neurons in the dorsal pallidum. Furthermore, it has been shown *in vivo* that perinatal Δ^9-THC exposure increases in adult rats the motor inhibition induced by $GABA_B$ receptor agonist baclofen, without affecting GABA content and GAD activity in limbic motor areas [28].

In the attempt to contribute to the understanding of the possible vulnerability of GABAergic system to early cannabinoid exposure, we exposed pregnant rats to a low dose of Δ^9-THC from gestational day (GD) 15 to postnatal day (PD) 9. We then explored the possible long-term impact on hippocampal GABAergic neurotransmission in 90-days male rats. The results provide evidence on long-term effects of prenatal Δ^9-THC exposure on GABAergic function in the hippocampus of male adult rats.

2. Methods

2.1. Animal care

The study was carried out in accordance with current Italian legislation (D.L. 116, 1992) that allows experimentation on laboratory animals only after submission and approval of a Research Project to the Ministry of Health (Rome, Italy), and in strict accordance with the European Council Directives on animal use in research (n. 2010/63/EU). Accordingly to the ARRIVE guidelines, all possible efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

2.2. Animals and exposure conditions

Perinatal animal treatments were performed as previously described [29]. Primiparous Wistar female rats (Harlan SRC, Milan, Italy), weighing 250-280 g were housed for one week before exposure to males, in a temperature-controlled animal facility. The rats were kept on 12 h/12 h light/dark cycle with free access to food and water. For mating, pairs of females were placed with single male rat in the late afternoon. Vaginal smears were taken the following morning at 09: 00 h. The day on which sperm was present was defined as Gestation Day 0 (GD 0). Treatment was performed daily with Δ^9-THC (5 mg/kg), dissolved in sesame oil and prepared as previously described [30], and administered by gavage, from GD 15 to postnatal day (PD) 9. This dose is considered to be equivalent to current estimates of moderate human exposure to Δ^9-THC after correction for route of administration and body surface area [31].

Control dams continued to receive the vehicle (sesame oil). Litters were reduced to a standard size of six male pups (when possible) within 24 h after birth and pups were weaned at 21 days of age. All experiments were performed in male offspring of Δ^9-THC and vehicle-treated dams, at the age of 90 days. One male pup per litter per treatment group was used in each experiment. The pups were bred in the University's animal housing facility; they were fed a normal diet and received no other drug treatment until age 90 days.

2.3. Reproduction data

Body weights of each dam were taken on GD 0, GD 20 and PD 9. The number of dams giving birth and the length of pregnancy were determined. Litter size at birth, postnatal mortality (the number of male pups that died before weaning) and pup weight gain were recorded.

2.4. Substances

Δ^9-tetrahydrocannabinol (Δ^9-THC) and (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinyl-methyl)-pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-1-naphthalenyl)methanone) mesylate (WIN55,212-2) were obtained from Sigma, Italy. *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride (SR1414176A, selective CB1 receptor antagonist) was purchased from Tocris Bioscience, UK.

2.5. [^{3}H]-GABA outflow experiments

2.5.1. Hippocampal slice preparation

On the day of the release experiment, 90-day-old rats perinatally exposed to Δ^9-THC or to vehicle (i.e. control rats) were euthanized under light ether anesthesia. The brains were rapidly removed, both hippocampus isolated and cut in 400-μm-thick slices with a fresh tissue vibratome (Technical Products International, Inc., USA). The slices were then allowed to equilibrate for 20 min at room temperature in a Krebs solution both containing in (mM) NaCl 118; KCl 4.4; $CaCl_2$ 1.2; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; glucose 10, and gassed with a mixture of 95% O_2 plus 5% CO_2.

2.5.2. Spontaneous [^{3}H]-GABA outflow from hippocampal slices

After the preparation, the two groups of slices were incubated at 37º C in the medium containing [^{3}H]-GABA 40 nM (specific activity: 32.9 Ci/mmol, Amersham Biosciences, Milan, Italy). Thereafter, 2-3 slices of each groups, were transferred to oxygenated superfusion chambers (0.6 ml/min each; 37º C) and superfused at a constant flow rate (0.35 ml/min) with an oxygenated Krebs solution containing 30 μM SKF89976A (an inhibitor of the high-affinity GABA carrier).

After a 30-min perfusion to equilibrate the system, from each chamber 5 min fractions of perfusate were collected for a total of 9 samples. In the experiments where the challenge was evaluated, the first three samples were used to assess spontaneous [^{3}H]-GABA outflow. After these samples were collected, the drugs under investigation (Δ^9-THC, 0.1 μM; WIN55,212-2, 2 μM) were added to the superfusion medium and maintained until the end of the experiment. When required, the CB1 receptor antagonist SR141716A was added to the superfusion medium 5

min before Δ^9-THC or WIN55,212-2. At the end of the experiment hippocampal slices were weighed out, dissociated in 1 ml of NaOH 1N and then mixed with 7.5 ml of scintillation liquid. The collected superfusate fractions were mixed with 5 ml of scintillation liquid and the tritium content was determined by scintillation counting (Beckmann LS 1800).

2.5.3. K^+-evoked [^{3}H]-GABA outflow from hippocampal slices

To investigate the effect of perinatal Δ^9-THC treatment on K^+-evoked [^{3}H]-GABA outflow, 5-min fractions of perfusate were collected for a total of 14 samples. During this period, the slices were stimulated by pulses (2 min duration) of high potassium (KCl, 30 mM) Krebs solution (corrected for osmolarity by replacing KCl for NaCl), delivered 45 (St_1) and 70 (St_2) min after the onset of superfusion. In the set of experiments where pharmacological challenges were required, the effects of Δ^9-THC (0.1 μM) or WIN55,212-2 (2 μM) were evaluated by adding the compound under investigation to the superfusion medium 5 min before St_2 until the end of the experiment. When required, the CB1 receptor antagonist SR141716A was added to the superfusion medium 5 min before Δ^9-THC or WIN55,212-2. The tritium content in each sample was determined as previously described.

2.5.4. Data analysis

As previously demonstrated by Tanganelli et al. [32], [^{3}H]-GABA accounts for 90% of the radioactivity released under the present superfusion conditions and for 80% of the radioactivity stored in the slices at the end of the superfusion. Thus, the radioactivity stored within the tissue or measured in collected samples will be referred to [^{3}H]-GABA.

The amount of spontaneous tritium released per 5-min sample was calculated either as pmol/min/g of wet tissue or as a percentage of the total tissue tritium content (fractions collected plus slice content) at the onset of the fraction considered (fractional rate %; FR%). The effects of pharmacological treatments were calculated as percent changes of basal values (mean of the first three samples).

The K^+-evoked outflow in St_1 and St_2 was calculated as Net Extra-Release (NER) by subtracting the spontaneous [^{3}H]-outflow (determined by interpolation of the outflow measured 5 min before and 10 min after the onset of the stimulation) from the total tritium measured during the 2 min stimulation and the subsequent 8 min (i.e., two collected samples). This difference was

also calculated as a percentage of the total tissue tritium content at the onset of stimulation (NER%) [33]. When the effects of treatments on K^+-evoked [^{3}H]-GABA efflux were studied, the St_2/St_1 ratio for treated slices was calculated and compared with the corresponding St_2/St_1 value obtained from control slices assayed in parallel.

Statistical analyses were performed by using the Student's t-test or one-way ANOVA followed by post hoc Newman Keuls (GraphPad Prism Program, San Diego, CA) as appropriate. Significance was set up at $p < 0.05$ for all comparison.

2.6. [^{3}H]-GABA uptake experiments

Hippocampal slices, prepared as described above, were incubated for 10 min at 37°C in Krebs solution containing [^{3}H]-GABA 40 nM (specific activity: 32.9 Ci/mmol, Amersham Biosciences, Milan, Italy). Thereafter, the uptake was halted by replacing the incubation medium with ice-cold Krebs solution. After weighing, the slices were incubated in 0.5 ml of acidic ethanol (95% ethanol/5% 0.1 M HCl) for 30 min at 37°C, to extract the radioactivity that had accumulated, which was quantified by liquid scintillation spectrometry. Non-specific uptake was measured by following the same procedure at 0°C, and was less than 7 ± 4% of the total.

2.6.1. Data analysis

[^{3}H]-GABA uptake was expressed as dpm/g of fresh tissue. Statistical analysis was performed by using the Student's t-test.

2.7. Binding experiments

Male rats at approximately 90 days of age were euthanized by decapitation, their brains rapidly removed and the hippocampi were rapidly dissected on ice and disrupted in a Polytron (setting 5) in 10 volumes (v/w) of 50 mM Tris HCl, pH 7.4. The homogenate was centrifuged at 50,000 x g for 10 min and the pellet was resuspended twice in the same buffer and centrifuged. After the last centrifugation the pellet (30-60 μg of protein) was used for binding experiments. Saturation experiments were carried out using 10 concentrations (ranging from 0.3-30 nM) of the selective CB1 receptor ligand [^{3}H]-SR141716A (specific activity 50.0 Ci/mmol; Amersham, UK) for an incubation time of 30 min at 37°C. Non-specific binding was estimated in the presence of 1 μM of the unlabeled ligand. Separation of bound from free radioligand was

performed by rapid filtration through Whatman GF/B filters which were washed three times with ice-cold buffer. Filter bound radioactivity was measured by scintillation spectrometry. ~~The~~ Bradford protein assay [34] was used for protein determination using bovine serum albumin as a standard according to the supplier protocol (Bio-Rad, Milan, Italy).

2.7.1. Data analysis

Radioligand concentration-binding isotherms were calculated using computerized non-linear regression analysis of a rectangular hyperbola (GraphPad Prism Program, San Diego, CA). The maximal number of binding sites (B_{max}) and the apparent equilibrium dissociation constant (K_d) values of the radioligand were derived from the curve fitting.

3. Results

3.1. Reproduction data.

As previously reported [11], perinatal treatment with Δ^9-THC had no significant effects with regard to the dam gain weight during gestation or lactation, pregnancy length, litter size at birth, pup weight gain and post-natal survival *(data not shown)*.

3.2. Effect of perinatal Δ^9-THC exposure on spontaneous and K^+-evoked [^{3}H]-GABA outflow.

In hippocampal slices obtained from 90 day-old male rats perinatally exposed to Δ^9-THC (5 mg/kg, by gavage), spontaneous [^{3}H]-GABA outflow, evaluated as the mean of the first three samples collected every 5 min, was significantly lower as compared to the vehicle-treated group (Fig. 1A).

The application of a chemical depolarizing stimulus (KCl 30 mM; 2 min), applied at the 45th min of perfusion (St_1), increased [^{3}H]-GABA outflow in both groups under investigation. However, the enhancement found in hippocampal slices obtained from rats born to mothers exposed to Δ^9-THC was lower than that observed in hippocampal slices obtained from vehicle-treated dams. This was evident by evaluating either the NER (0.031 ± 0.0017 pmol and 0.045 ± 0.0011 pmol, respectively; $p < 0.05$) or the NER% (Fig. 1B).

It is worth noting that, in both groups of animals, the increases of [^{3}H]-GABA outflow induced by two consecutive K^+-depolarizing stimuli, delivered at the 45th (St_1) and 75th (St_2),

were similar, with the St_2/St_1 close to the unit (perinatally vehicle-treated group = 0.92 ± 0.06, n = 18; perinatally Δ^9-THC-treated group = 1.03 ± 0.04, n = 14; $p > 0.05$).

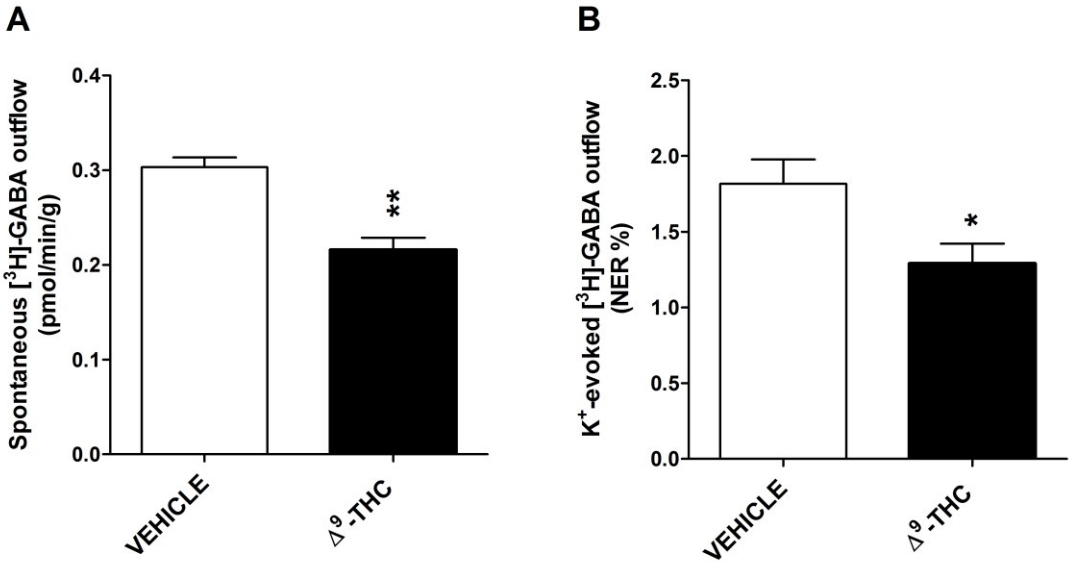

Figure 1. Effects of perinatal Δ^9-THC exposure on spontaneous (panel A) and K^+-evoked [^{3}H]-GABA outflow (panel B) from hippocampal slices of 90 day-old male adult rats. Spontaneous [^{3}H]-GABA outflow was expressed as pmol/min/g of wet tissue (panel A), while K^+-evoked release was expressed as percent Net Extra-Release (NER%; panel B), see Methods section for details. Control groups were perinatally treated with Δ^9-THC vehicle (sesame oil). Values represent the means ± S.E.M (n = 7 - 10, panel A; n = 7 - 8, panel B). * $p < 0.05$, ** $p < 0.01$ significantly different from the respective vehicle group based on Student's t-test.

3.3. Effect of perinatal Δ^9-THC exposure on [^{3}H]-GABA uptake.

In view of the above results, a set of experiments has been performed in order to evaluate [^{3}H]-GABA uptake in hippocampal slices obtained from 90-days old male rats perinatally exposed to vehicle or Δ^9-THC. As shown in figure 2, a significant reduction in [^{3}H]-GABA uptake was observed ($p < 0.01$; n = 5) in hippocampal slices obtained from 90-days old male rats perinatally exposed to Δ^9-THC compared to vehicle-exposed group (n = 5).

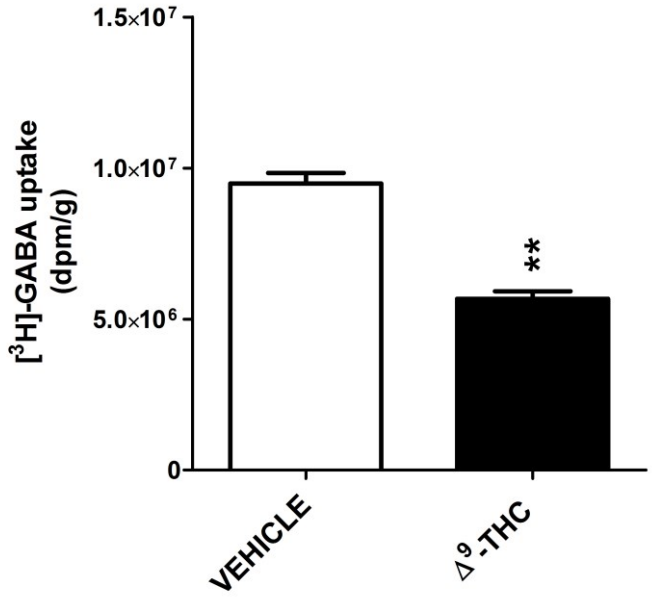

Figure 2. Effect of perinatal Δ^9-THC exposure on [^{3}H]-GABA uptake from hippocampal slices of 90 day-old male adult rats. [^{3}H]-GABA uptake was expressed in dpm/g of wet tissue, see Methods section for details. Control groups were prenatally treated with Δ^9-THC vehicle (sesame oil). Values represent the means ± S.E.M. (n = 6 each group). ** $p < 0.01$ significantly different from vehicle group based on Student's t-test.

3.4. Effect of perinatal Δ^9-THC exposure on CB1 receptor binding in hippocampus of 90 days-old rats.

As during fetal life the cannabinoid CB1 receptor plays a major role in brain development [35], binding experiments were performed by using the selective CB1 receptor ligand [^{3}H]-SR141716A to verify whether [^{3}H]-GABA outflow reduction observed in perinatally Δ^9-THC rats was associated with changes in CB1 receptor number and/or affinity. Specific binding was higher than 75% and [^{3}H]-SR141716A ligand labels a single high affinity site of cannabinoid CB1 receptor in both groups. Notably, 90-days-old male rats perinatally exposed to Δ^9-THC displayed a significant reduction ($p < 0.01$) of CB1 receptor B_{max} compared to vehicle-treated animals (Fig. 3A), while no differences were found in Kd value (Fig. 3B).

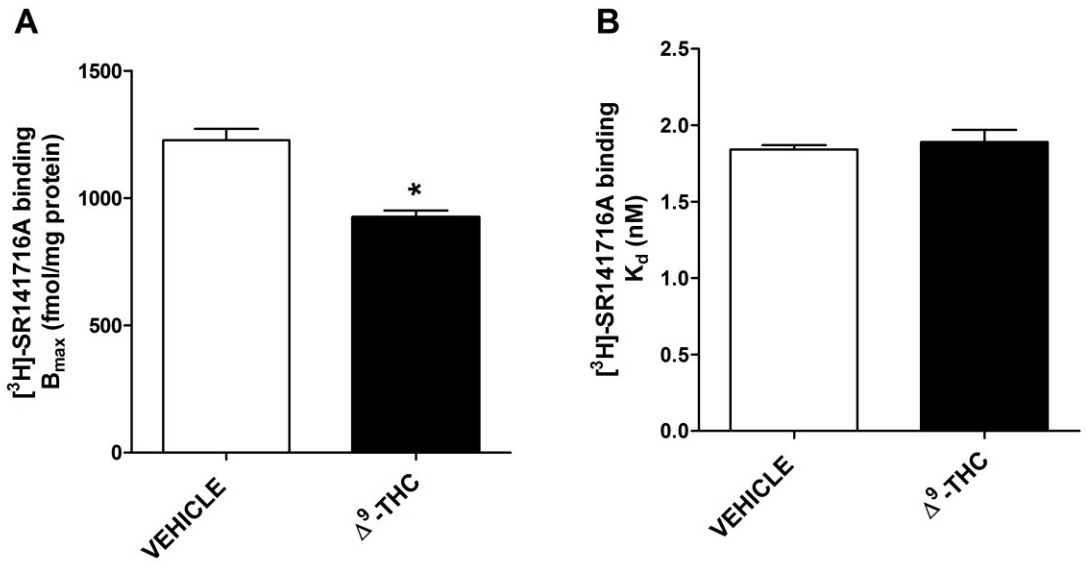

Figure 3. B_{max} (panel A) and K_d (panel B) of [^{3}H]-SR141716A binding to membranes prepared from hippocampus of 90 day-old rats prenatally treated with Δ^9-THC or its vehicle (sesame oil). [^{3}H]-SR141716A binding was determined by saturation analysis (0.3-30 nM). Non-specific binding was defined with 1 μM unlabeled SR141716A. Values represent the means ± S.E.M (n = 7) * $p < 0.05$ as compared with the vehicle group based on Student's t-test.

3.5. Effect of pharmacological challenges with Δ^9-THC or WIN55,212-2 on K^+-evoked [^{3}H]-GABA outflow.

In hippocampal slices obtained from 90 days-old male rats perinatally exposed to vehicle or Δ^9-THC, the addiction of Δ^9-THC (0.1 μM) or WIN55,212-2 (2 μM) to the perfusion medium did not alter spontaneous [^{3}H]-GABA outflow (Fig. 4A and 4B). Conversely, the addition Δ^9-THC (0.1 μM) or WIN55,212-2 (2 μM) to the perfusion medium, 5 min before St_2 similarly and significantly reduced K^+-evoked [^{3}H]-GABA efflux from hippocampal slices obtained from 90 day-old male rats perinatally exposed to vehicle (Fig. 5A and 5C). These reductions were blocked by adding to the perfusion medium, 5 min before Δ^9-THC or WIN55,212-2, the CB1 receptor antagonist SR141716A (100 nM), by itself ineffective on spontaneous (*data not shown*) and K^+-evoked [^{3}H]-GABA outflow (Fig. 5).

Unexpectedly, in hippocampal slices obtained from 90-days old male rats perinatally exposed to Δ^9-THC, the pharmacological challenges with Δ^9-THC or WIN55,212-2 induced the same effects as observed in the vehicle group (Fig. 5B and 5D).

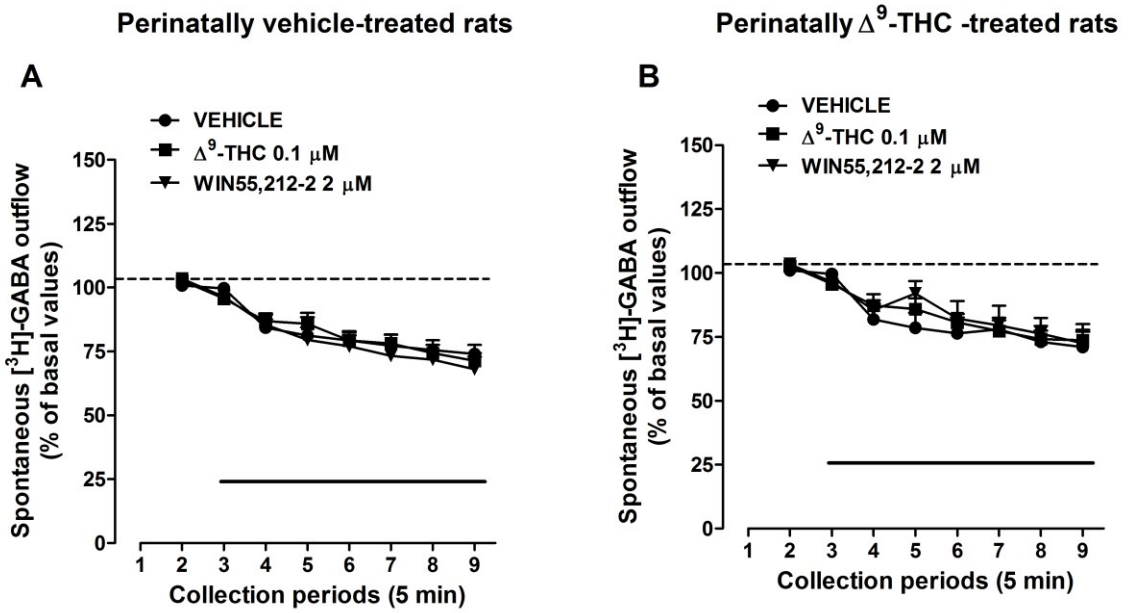

Figure 4. Effects of Δ^9-THC (1 μM) or WIN55,212-2 (2 μM) on spontaneous [^{3}H]-GABA outflow from hippocampal slices prepared from 90 day-old rats perinatally treated with Δ^9-THC (panel B) or its vehicle (sesame oil; panel A). The black bars indicate the period of perfusion with Δ^9-THC or WIN55,212-2. Data are expressed as percentage of basal values and represent the mean ± S.E.M. of 5-6 animals for each treatment.

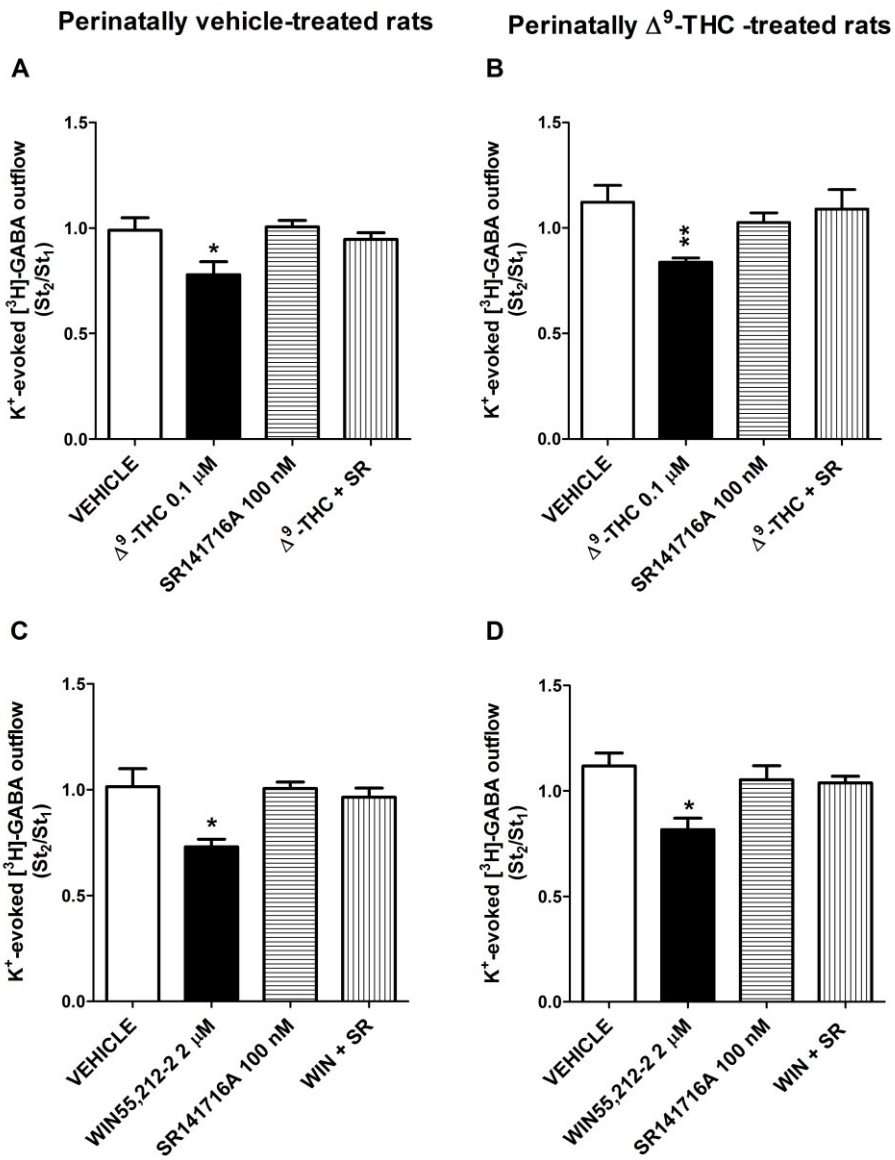

Figure 5. Effects of Δ^9-THC (1 μM, panel A, B) or WIN55,212-2 (2 μM, panel C, D), alone or in combination with the selective CB1 receptor antagonist SR141716A (100 nM), on K^+-evoked [^{3}H]-GABA outflow from hippocampal slices prepared from 90 day-old rats perinatally treated with Δ^9-THC (panel B, D) or its vehicle (sesame oil; panel A, C). Δ^9-THC or WIN55,212-2 were added 5 min before St_2 and maintained until the end of the experiment, while SR141716A was added to the medium 5 min before the agonist. Data are expressed as St_2/St_1 ratio and represent the mean ± S.E.M. of 5-8 animals for each treatment. * $p < 0.05$, ** $p < 0.01$ statistically different from the respective vehicle group as well as from SR141716A and Δ^9-THC + SR groups, based on ANOVA followed by Newman-Keuls test for multiple comparisons.

4. Discussion

The present study, by combining different methodological approaches, provides evidence that perinatal exposure to Δ^9-THC disrupts GABAergic neuronal function in the hippocampus of 90-day-old male offspring. In particular, basal and K^+-evoked [^{3}H]-GABA outflow is significantly reduced in hippocampal slices from perinatally Δ^9-THC-exposed rats as compared to vehicle, along with a reduction of [^{3}H]-GABA uptake. Furthermore, CB1 receptors density in the hippocampus of Δ^9-THC-exposed group is statistically decreased in respect to the vehicle group. Notably, maternal and neonate body weight and other reproductive or pregnancy outcomes were unaffected by Δ^9-THC treatment, thus indicating that the dose used did not induce deleterious effects on general physical status.

It is unclear whether any functional relationship exists between the decreased [^{3}H]-GABA uptake seen in Δ^9-THC-exposed rats and their impaired hippocampal GABAergic neurotransmission. Interestingly, preliminary data from our group seem to indicate that in the hippocampus of 90 day-old rats perinatally treated with Δ^9-THC there is a reduction in GABA transporter-1 (GAT-1) in respect to the control group. As the GAT-1 is mostly neuronal and removes GABA from the synaptic cleft [36], a reduction in its expression could underlie the impaired [^{3}H]-GABA uptake observed in Δ^9-THC group. Due to this impairment, it seems reasonable that, during the incubation period, slices obtained by rats perinatally exposed to Δ^9-THC took up less [^{3}H]-GABA than slices prepared from vehicle-exposed animals. Thus, the reduction in spontaneous [^{3}H]-GABA observed in slices obtained from rats perinatally exposed to Δ^9-THC could be merely ascribed to their reduced [^{3}H]-GABA content compared to vehicle group. On the contrary, the decreased [^{3}H]-GABA uptake, and the consequent reduction in [^{3}H]-GABA content, found in Δ^9-THC-exposed rats do not seem to justify the impairment in K^+-evoked [^{3}H]-GABA outflow observed in this experimental group. In fact, this impairment was also evident by evaluating the NER as the percentage of the total tissue tritium content at the onset of stimulation (NER%). Furthermore, a reduction in the uptake mechanism(s) should lead to an increase, and not to a decrease, in stimulation-evoked amino acid outflow from tissue slices [37]. The evidence that similar amount of [^{3}H]-GABA was released after either K^+ pulse (St_1 and St_2) suggest that a desensitization of the release machinery did not occur. Overall, these findings

suggest that perinatal exposure to Δ^9-THC leads to a permanent impairment in stimulus-secretory coupling of hippocampal GABAergic neurons. While the mechanisms discussed could per se cause a reduction in spontaneous K^+-evoked [^{3}H]-GABA outflow from the hippocampal slices of Δ^9-THC-rats, we cannot exclude that in these animals the anomalies in GABAergic neurotransmission are, instead, the consequence of a disturbance in the maturation of the GABAergic system. In fact, previous studies demonstrated that gestational cannabinoids exposure might induce permanent alterations on GABAergic systems in some brain areas, including the hippocampus. For instance, slight increases in the intensity of the positive immunoreactions to both GAD-65/67 and GABA have been reported in the cerebellar cortex of adult (120-150 days) rats prenatally exposed to the cannabinoid receptor agonist WIN55,212-2 [38]. Prenatal exposure to WIN55,212-2 also increased the number of migrating GABAergic interneurons through the cortical marginal zone [26], while perinatal Δ^9-THC exposure increased in adult rats the motor inhibition induced by $GABA_B$ receptor agonist baclofen, without affecting GABA content and GAD activity in limbic motor areas [28]. Finally, Δ^9-THC administration to pregnant mice in a restricted time window induced an increase in seizure susceptibility that was mediated by its interference with CB1-dependent regulation of both glutamatergic and GABAergic neuron development [27].

The CB1 receptor plays a fundamental neurodevelopmental role by transducing information carried on by ECs into the coordination of the intrinsic developmental program of developing neurons [27, 39]. Recent data demonstrated that embryonic Δ^9-THC exposure induced a transient disruption of CB1 receptor signaling that impedes the adequate temporally and spatially confined function of the receptor in cortical neuron development [27]. In the hippocampus, CB1 receptors are expressed by GABAergic interneurons [40-42] and their activation functions specifically to depress GABA release [43-45]. Thus, in the present study, the possibility that the permanent alterations in GABAergic transmission observed in perinatally Δ^9-THC rats were associated with changes in CB1 receptor binding was also explored. A significant reduction in the B_{max} value was found in hippocampal membranes obtained from 90-day-old rats perinatally exposed to Δ^9-THC as compared to ~~the~~ vehicle treated groups. This finding suggests that perinatal cannabinoid exposure alters the density of CB1 receptors in the hippocampus of adult rats, and is in line with previous data obtained by evaluating the long-term effects of perinatal Δ^9-THC treatment on hippocampal glutamate transmission. In fact, it has been

demonstrated that perinatal Δ^9-THC treatment induced a selective loss of K^+-evoked glutamate outflow responsivity to an acute CB1 receptor stimulation, an effect possibly due to a reduction of CB1 receptor density [29]. Notably, these findings have been obtained under the same experimental conditions as in the present study. On the other hand, the observed reduction in the CB1 receptor B_{max} value in Δ^9-THC prenatally-exposed rats is in contrast with the results reported by Garcia-Gil et al. [28] demonstrating that perinatal cannabinoid exposure does not appear to significantly alter cannabinoid receptor binding and mRNA expression in the brain, including the hippocampus of adult rats. At present the reason of this discrepancy is unclear, although this may be due to the different exposure period adopted in that study.

The existence of an inhibitory tone mediated by CB1 receptors on hippocampal GABAergic transmission in vehicle adult offspring, has been proven by the evidence that a challenge with Δ^9-THC or WIN55,212-2 significantly reduces K^+-evoked [^{3}H]-GABA outflow. This reduction is fully counteracted by treatment with the selective CB1 receptor antagonist SR141716A, thus confirming that it is exclusively mediated by CB1 receptor activation. Notably, perinatal exposure to Δ^9-THC did not induce any loss of the inhibitory CB1-mediated action on hippocampal GABAergic neurons of adult offspring. In fact, the addition to the perfusate medium of Δ^9-THC or WIN55,212-2, reduced K^+-evoked [^{3}H]-GABA outflow to a similar extent in both experimental groups. This finding is quite surprising in view of the observed reduction in CB1 receptor B_{max} value (present study), and is in contrast to what previously observed by investigating hippocampal glutamate transmission [29]. At present, the basis of these results is unclear. However, some hypotheses, to be validated in further studies, can be raised to explain these apparent discrepancies. The most conservative explanation is that the reduction of CB1 receptor number on hippocampal GABAergic neurons in adult rats perinatally exposed to Δ^9-THC might be compensated by an increase in the CB1 receptor transduction mechanism efficiency. Notably, an increased affinity to the agonist seems unlikely in view of the results of binding experiments, i.e. no differences in the K_d value between the two experimental groups. Another possibility is that perinatal Δ^9-THC exposure leads to a selective disturbance in the maturation of the glutamatergic system, thus leading to a permanent decrease in hippocampal glutamate levels and to a reduced CB1 receptor population only on glutamatergic neurons [29]. On the other hand, recent data demonstrated that in the hippocampus only a subpopulation of CB1 receptors, within nanometre distances from their target Cav2.2 channels,

are responsible for endocannabinoid-mediated modulation of GABA release [46]. Thus, it could be speculated that perinatal Δ^9-THC exposure selectively affects this subpopulation, without changing the responsivity of GABA outflow to CB1 receptor stimulation.

Overall, the present results suggest that perinatal exposure to Δ^9-THC alters hippocampal GABAergic transmission in adulthood. Notably, the exposure to cannabinoids in another critical period of central nervous system development, such as the adolescence, has been reported to lead to similar consequences. In fact, recent data have demonstrated that the exposure to the cannabinoid receptor agonist WIN55,212-2 during early (PD 35-40) or mid (PD 40-45) adolescence, markedly reduces prefrontal GABAergic transmission. In addition, those data indicate that repeated CB1 receptor stimulation in critical periods of development elicits an enduring impairment of hippocampal and prefrontal GABAergic neuronal function [47]. Furthermore, Δ^9-THC exposure between PD 35 and PD 45 in rodents reduces GAD67 expression in both parvalbumin and CCK-containing interneurons coincident with decreased basal GABA levels within the prefrontal cortex, which perpetuate negative symptoms and cognitive deficits [48]. While in the mature brain GABA acts as inhibitory neurotransmitter, in the course of embryonic development and first postnatal week, the GABAergic system is crucially involved in basic processes like cell survival, migration, neurite growth, synapse formation and maturation [49-51]. In immature hippocampal neurons, the activation of $GABA_A$ receptors depolarizes targeted cells and generates an enhancement of intracellular calcium that may be associated with the trophic action of GABA, fundamental in neuronal networks development [52-54]. Considering these multifaceted activities of GABA system during critical prenatal and neonatal periods, it is not surprising that early disturbance of GABAergic signaling can lead to permanent anomalous information processing, alterations in neuronal signaling, brain architecture and behavioral consequences. In view of the abundance of CB1 receptors, the endocannabinoid-mediated control of GABAergic neurotransmission and the relevance of the GABAergic system during development might have an enduring negative impact on GABAergic function in adult offspring.

The observed reduction in GABAergic transmission is in accordance with previous *in vivo* and *in vitro* studies carried out by evaluating the glutamatergic neurotransmission. In fact, by *in vivo* microdialysis it has been shown that prenatal exposure to WIN55,212-2 induced a significant decrease of basal and K^+-evoked glutamate release in the hippocampus of 40- and 80-day-old

male offspring [20], similar to the reduction observed in hippocampal slices of 40-day-old male offspring [29] perinatally exposed to Δ^9-THC. Furthermore, the impairment of glutamatergic transmission has been also detected *in vivo* in the cerebral cortex of adult male rats born from WIN55,212-2 treated dams [20] as well as *in vitro* on primary hippocampal cell cultures of early (PD 0 – PD 1) rats born from WIN55,212-2 treated dams [19].

5. Conclusions

Based on all these data it may be speculated that disturbance of the delicate balance between inhibitory and excitatory neurotransmitters in the hippocampus, may contribute to the long-lasting neurobiological and neurobehavioral alterations observed in adult rats pre- and/or perinatally exposed to cannabinoids [11, 14, 23, 55-57]. From a translational point of view, such alterations may, at least in part, account for the long-lasting and subtle disturbances in learning and memory processes as well as in emotional reactivity reported in offspring whose mother had consumed cannabis during pregnancy and lactation.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] Substance Abuse and Mental Health Services Administration. Results from the 2012 National Survey on Drug Use and Health: Detailed Tables. SAMHSA, Center for Behavioral Health Statistics and Quality, Rockville, MD (2013).

[2] A.J. Porath-Waller, Clearing the Smoke on Cannabis: Maternal Cannabis Use during Pregnancy, Canadian Centre on Substance Abuse, Ottawa, ON (2015).

[3] P.A. Fried, Conceptual issues in behavioral teratology and their application in determining long-term sequelae of prenatal marihuana exposure, J. Child Psychol. Psychiatry 43 (2002) 81-102.

[4] D. Jutras-Aswad, J.A. DiNieri, T. Harkany, Y.L. Hurd, Neurobiological consequences of maternal cannabis on human fetal development and its neuropsychiatric outcome, Eur. Arch. Psychiatry Clin. Neurosci. 259 (2009) 395-412.

[5] C.V. Morris, J.A. DiNieri, H. Szutorisz, Y.L. Hurd, Molecular mechanisms of maternal cannabis and cigarette use on human neurodevelopment, Eur. J. Neurosci. 34 (2011) 1574-83.

[6] A.C. Huizink, Prenatal cannabis exposure and infant outcomes: overview of studies Prog. Neuropsychopharmacol. Biol. Psychiatry 52 (2014) 45-52.

[7] A. Alpár, V. Di Marzo, T. Harkany, At the Tip of an Iceberg: Prenatal Marijuana and Its Possible Relation to Neuropsychiatric Outcome in the Offspring, Biol. Psychiatry 79 (2016) e33-45.

[8] D. Calvigioni, Y.L. Hurd, T. Harkany, E. Keimpema, Neuronal substrates and functional consequences of prenatal cannabis exposure, Eur. Child Adolesc. Psychiatry 23 (2014) 931-41.

[9] J.J. Fernández-Ruiz, F. Berrendero, M.L. Hernández, J. Romero, J.A. Ramos, Role of endocannabinoids in brain development, Life Sci. 65 (1999) 725-36.

[10] T. Harkany, E. Keimpema, K. Barabás, J. Mulder, Endocannabinoid functions controlling neuronal specification during brain development, Mol. Cell. Endocrinol. 286 (2008) S84-90.

[11] P. Campolongo, V. Trezza, T. Cassano, S. Gaetani, M.G. Morgese, M. Ubaldi, L. Soverchia, T. Antonelli, L. Ferraro, M. Massi, R. Ciccocioppo, V. Cuomo, Perinatal exposure to delta-9-tetrahydrocannabinol causes enduring cognitive deficits associated with alteration of cortical gene expression and neurotransmission in rats, Addict Biol. 12 (2007) 485-95.

[12] X. Wang, D. Dow-Edwards, V. Anderson, H. Minkoff, Y.L. Hurd, In utero marijuana exposure associated with abnormal amygdala dopamine D2 gene expression in the human fetus, Biol. Psychiatry. 56 (2004) 909-15.

[13] J.A. DiNieri, X. Wang, H. Szutorisz, S.M. Spano, J. Kaur, P. Casaccia, D. Dow-Edwards, Y.L. Hurd, Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring, Biol. Psychiatry 70 (2011) 763-9.

[14] M. Navarro, P. Rubio, F.R. de Fonseca, Behavioural consequences of maternal exposure to natural cannabinoids in rats, Psychopharmacology (Berl). 122 (1995) 1-14.

[15] J. Corchero, L. García-Gil, J. Manzanares, J.J. Fernández-Ruiz, J.A. Fuentes, J.A. Ramos, Perinatal delta9-tetrahydrocannabinol exposure reduces proenkephalin gene expression in the caudate-putamen of adult female rats, Life Sci. 63 (1998) 843-50.

[16] G. Vela, S. Martín, L. García-Gil, J.A. Crespo, M. Ruiz-Gayo, J.J, Fernández-Ruiz, C. García-Lecumberri, D. Pélaprat, J.A. Fuentes, J.A. Ramos, E. Ambrosio, Maternal exposure to delta9-tetrahydrocannabinol facilitates morphine self-administration behavior and changes regional binding to central mu opioid receptors in adult offspring female rats, Brain Res. 807 (1998) 101-9.

[17] O. Valverde, F. Noble, F. Beslot, V. Daugé, M.C. Fournié-Zaluski, B.P. Roques, Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect, Eur. J. Neurosci. 13 (2001) 1816-24.

[18] B. González, R. de Miguel, S. Martín, A. Pérez-Rosado, J. Romero, C. García-Lecumberri, J.J. Fernández-Ruiz, J.A. Ramos, E. Ambrosio, Effects of perinatal exposure to delta 9-tetrahydrocannabinol on operant morphine-reinforced behavior, Pharmacol. Biochem. Behav. 75 (2003) 577-84.

[19] G. Mereu, M. Fa`, L. Ferraro, R. Cagiano, T. Antonelli, M. Tattoli, V. Ghiglieri, S. Tanganelli, G.L. Gessa, V. Cuomo, Prenatal exposure to a cannabinoid agonist produces memory deficits linked to dysfunction in hippocampal long-term potentiation and glutamate release, Proc. Natl. Acad. Sci. 100 (2003) 4915-4920.

[20] T. Antonelli, S. Tanganelli, M.C. Tomasini, S. Finetti, L. Trabace, L. Steardo, V. Sabino, M.R. Carratu, V. Cuomo, L. Ferraro, Long-term effects on cortical glutamate release induced by prenatal exposure to the cannabinoid receptor agonist (R)-(+)-[2,3-dihydro-5-methyl-3-(4-

morpholinyl-methyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone: an in vivo microdialysis study in the awake rat, Neurosci. 124 (2004) 367-75.

[21] P. Castaldo, S. Magi, S. Gaetani, T. Cassano, L. Ferraro, T. Antonelli, S. Amoroso, V. Cuomo, Prenatal exposure to the cannabinoid receptor agonist WIN55,212–2 increases glutamate uptake through overexpression of GLT1 and EAAC1 glutamate transporter subtypes in rat frontal cerebral cortex, Neuropharmacology 53 (2007) 369–378.

[22] K.C. Luk, T.E. Kennedy, A.F. Sadikot, Glutamate promotes proliferation of striatal neuronal progenitors by an NMDA receptor-mediated mechanism, J. Neurosci. 23 (2003) 2239-50.

[23] L. Ferraro, M.C. Tomasini, S. Beggiato, S. Gaetani, T. Cassano, V. Cuomo, S. Amoroso, S. Tanganelli, T. Antonelli, Short- and long-term consequences of prenatal exposure to the cannabinoid agonist WIN55,212-2 on rat glutamate transmission and cognitive functions, J. Neural. Transm. (Vienna) 116 (2009) 1017-27.

[24] P. Campolongo, V. Trezza, P. Ratano, M. Palmery, V. Cuomo, Developmental consequences of perinatal cannabis exposure: behavioral and neuroendocrine effects in adult rodents, Psychopharmacology (Berl) 214 (2011) 5-15.

[25] D.D Wang, A.R. Kriegstein, Defining the role of GABA in cortical development, J. Physiol. 587 (2009) 1873-9.

[26] T.M. Saez, M.P. Aronne, L. Caltana, A.H. Brusco, Prenatal exposure to the CB1 and CB2 cannabinoid receptor agonist WIN 55,212-2 alters migration of early-born glutamatergic neurons and GABAergic interneurons in the rat cerebral cortex, J. Neurochem. 129 (2014) 637-48.

[27] A. de Salas-Quiroga, J. Díaz-Alonso, D. García-Rincón, F. Remmers, D. Vega, M. Gómez-Cañas, B. Lutz, M. Guzmán, I. Galve-Roperh, Prenatal exposure to cannabinoids evokes long-lasting functional alterations by targeting CB1 receptors on developing cortical neurons, Proc. Natl. Acad. Sci. 112 (2015) 13693-8.

[28] L. Garcia-Gil, R. de Miguel, J. Romero, A. Perez, J.A. Ramos, J.J. Fernández-Ruiz, Perinatal delta9-tetrahydrocannabinol exposure augmented the magnitude of motor inhibition caused by GABA(B), but not GABA(A), receptor agonists in adult rats, Neurotoxicol. Teratol. 21 (1999) 277-83.

[29] P. Castaldo, S. Magi, M. Cataldi, S. Arcangeli, V. Lariccia, A.A. Nasti, L. Ferraro, M.C. Tomasini, T. Antonelli, T. Cassano, V. Cuomo, S. Amoroso, Altered regulation of glutamate

release and decreased functional activity and expression of GLT1 and GLAST glutamate transporters in the hippocampus of adolescent rats perinatally exposed to Delta(9)-THC, Pharmacol. Res. 61 (2010) 334-41.

[30] F. Molina-Holgado, E. Molina-Holgado, M.L. Leret, M.I. González, T.A. Reader, Distribution of indoleamines and [3H]paroxetine binding in rat brain regions following acute or perinatal delta 9-tetrahydrocannabinol treatments, Neurochem. Res. 18 (1993), 1183-1191.

[31] F. Molina-Holgado, A. Amaro, M.I. González, F.J. Alvarez, M.L. Leret, Effect of maternal delta 9-tetrahydrocannabinol on developing serotonergic system, Eur. J. Pharmacol. 316 (1996), 39-42.

[32] S. Tanganelli, M. Pérez de la Mora, L. Ferraro, J. Méndez-Franco, L. Beani, F.A. Rambert, K. Fuxe, Modafinil and cortical gamma-aminobutyric acid outflow. Modulation by 5-hydroxytryptamine neurotoxins, Eur. J. Pharmacol. 273 (1995) 63-71.

[33] L. Ferraro, S. Beggiato, D. Marcellino, M. Frankowska, M. Filip, L.F. Agnati, T. Antonelli, M.C. Tomasini, S. Tanganelli, K. Fuxe, Nanomolar concentrations of cocaine enhance D2-like agonist-induced inhibition of the K+-evoked [3H]-dopamine efflux from rat striatal synaptosomes: a novel action of cocaine J. Neural. Transm. (Vienna), 117 (2010) 593-7.

[34] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248-54.

[35] E. Fride. Multiple roles for the endocannabinoid system during the earliest stages of life: pre- and postnatal development. J. Neuroendocrinol. Suppl. 1 (2008) 75-81.

[36] Y. Zhou, N.C. Danbolt, GABA and Glutamate Transporters in Brain. Front. Endocrinol. (Lausanne) 4 (2013) 165.

[37] P. Saransaari, S.S. Oja, Release of GABA and taurine from brain slices. Prog. Neurobiol. 38 (1992) 455-82.

[38] V. Benagiano, L. Lorusso, P. Flace, F. Girolamo, A. Rizzi, R. Sabatini, P. Auteri, L. Bosco, R. Cagiano, G. Ambrosi, Effects of prenatal exposure to the CB-1 receptor agonist WIN 55212-2 or CO on the GABAergic neuronal systems of rat cerebellar cortex, Neurosci. 149 (2007) 592-601.

[39] D. Psychoyos, K.Y. Vinod, J. Cao, S. Xie, R.L. Hyson, B. Wlodarczyk, W. He, T.B. Cooper, B.L. Hungund, R.H. Finnell, Cannabinoid receptor 1 signaling in embryo neurodevelopment, Birth Defects Res. B. Dev. Reprod. Toxicol. 95 (2012) 137-50.

[40] I. Katona, B. Sperlágh, A. Sík, A. Käfalvi, E.S. Vizi, K. Mackie, T.F. Freund, Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons, J. Neurosci. 19 (1999) 4544-58.

[41] I. Katona, B. Sperlágh, Z. Maglóczky, E. Sántha, A. Köfalvi, S. Czirják, K. Mackie, E.S. Vizi, T.F. Freund, GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus, Neurosci. 100 (2000), 797-804.

[42] K. Tsou, K. Mackie, M.C. Sañudo-Peña, J.M. Walker, Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation, Neurosci. 93 (1999) 969-975.

[43] A.F. Hoffman, C.R. Lupica, Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus, J. Neurosci. 20 (2000) 2470-9.

[44] R.I. Wilson, G. Kunos, R.A. Nicoll, Presynaptic specificity of endocannabinoid signaling in the hippocampus, Neuron 31 (2001) 453-62.

[45] G.G. Szabó, N. Lenkey, N. Holderith, T. Andrási, Z. Nusser, N. Hájos, Presynaptic calcium channel inhibition underlies CB_1 cannabinoid receptor-mediated suppression of GABA release, J. Neurosci. 34 (2014) 7958-63.

[46] N. Lenkey, T. Kirizs, N. Holderith, Z. Máté, G. Szabó, E.S. Vizi, N. Hájos, Z. Nusser. Tonic endocannabinoid-mediated modulation of GABA release is independent of the CB1 content of axon terminals. Nat. Commun. 6 (2015) 6557.

[47] D.K. Cass, E. Flores-Barrera, D.R. Thomases, W.F. Vital, A. Caballero, K.Y. Tseng, CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex, Mol. Psychiatry 19 (2014) 536-43.

[48] E. Zamberletti, S. Beggiato, L.Jr. Steardo, P. Prini, T. Antonelli, L. Ferraro, T. Rubino, D. Parolaro, Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats, Neurobiol. Dis. 63 (2014) 35-47.

[49] D.F. Owens, A.R. Kriegstein, Is there more to GABA than synaptic inhibition?, Nat. Rev. Neurosci. 3 (2002) 715-27.

[50] Y. Ben-Ari, GABA excites and sculpts immature neurons well before delivery: modulation by GABA of the development of ventricular progenitor cells, Epilepsy Curr. 7 (2007) 167-9.

[51] E. Sernagor, F. Chabrol, G. Bony, L. Cancedda, GABAergic control of neurite outgrowth and remodeling during development and adult neurogenesis: general rules and differences in diverse systems, Front. Cell. Neurosci. 14 (2010) 4:11.
[52] T.N. Behar, Y.X. Li, H.T. Tran, W. Ma, V. Dunlap, C. Scott, J.L. Barker, GABA stimulates chemotaxis and chemokinesis of embryonic cortical neurons via calcium-dependent mechanisms, J. Neurosci. 16 (1996) 1808-18.
[53] I. Colin-Le Brun, N. Ferrand, O. Caillard, P.Tosetti, Y. Ben-Ari, J.L. Gaïarsa, Spontaneous synaptic activity is required for the formation of functional GABAergic synapses in the developing rat hippocampus, J. Physiol. 559 (2004) 129-39.
[54] W. Kilb, S. Kirischuk, H.J. Luhmann, Role of tonic GABAergic currents during pre- and early postnatal rodent development, Front. Neural. Circuits, 3 (2013) 7:139.
[55] A. Higuera-Matas, F. Botreau, M. Miguéns, N. Del Olmo, E. Borcel, L. Pérez-Alvarez, C. García-Lecumberri, E. Ambrosio, Chronic periadolescent cannabinoid treatment enhances adult hippocampal PSA-NCAM expression in male Wistar rats but only has marginal effects on anxiety, learning and memory, Pharmacol. Biochem. Behav. 93 (2009) 482-90.
[56] H.R. Quinn, I. Matsumoto, P.D. Callaghan, L.E. Long, J.C. Arnold, N. Gunasekaran, M.R. Thompson, B. Dawson, P.E. Mallet, M.A. Kashem, H. Matsuda-Matsumoto, T. Iwazaki, I.S. McGregor, Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure, Neuropsychopharmacology 33 (2008) 1113-26.
[57] M. O'Shea, I.S. McGregor, P.E. Mallet, Repeated cannabinoid exposure during perinatal, adolescent or early adult ages produces similar longlasting deficits in object recognition and reduced social interaction in rats, J. Psychopharmacol. 20 (2006) 611-21.