THC exposure during adolescence does not modify nicotine reinforcing effects and relapse in adult male mice

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Acknowledgements

This work was supported by “Plan Nacional sobre Drogas” (#2014I019) and “Ministerio de Economía y Competitividad” (SAF2017-85299-R) to FB, the "Generalitat de Catalunya-AGAUR" (#2014-SGR-1547), ICREA-Acadèmia (2015) and “Ministerio de Economía y Competitividad” (#SAF2017-84060-R) to RM.

Conflict of interest

The authors declare that there are no conflicts of interest.

Abstract

Rationale: Cannabis use is typically initiated during adolescence, and different studies suggest that adolescent cannabinoid exposure may increase the risk for drug addiction in adulthood.

Objectives: This study investigated the effects of adolescent exposure to the main psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (THC), in the reinforcing properties of nicotine in adult male mice. Possible alterations in relapse to nicotine-seeking behaviour in adult animals due to THC adolescent exposure were also evaluated.

Methods: Adolescent mice were exposed to escalating doses of THC from PND35 to PND49. When mice reached adulthood (PND70), surgical procedures were applied for further behavioural evaluation. Nicotine self-administration sessions were conducted consecutively for 10 days. Following extinction, mice were tested for cue- and stress-induced reinstatement of nicotine-seeking behaviour.

Results: Adolescent THC treatment did not modify acquisition and extinction of nicotine self-administration in adulthood. Moreover, THC exposure did not alter relapse to nicotine seeking induced by stress or nicotine-associated cues.

Conclusions: These results suggest that a history of exposure to THC during adolescence under these particular conditions does not modify the reinforcing effects and seeking behaviour of nicotine in the adult period.

Keywords: Δ^9-tetrahydrocannabinol, nicotine, adolescence, mice, reward, relapse, self-administration

Introduction

Cannabis remains the most widely used illicit substance worldwide. The regular use of cannabis often begins during adolescence which is a vulnerable period of brain development. During adolescence, several neurotransmitter systems including the endocannabinoid system undergo extensive reorganization to promote the maturation of the central nervous system (Fernández-Ruiz et al. 2000; Harkany et al. 2007). Adolescent cannabis exposure might affect the development of the endocannabinoid system and induce neurobiological changes that affect adult brain function (Higuera-Matas et al. 2015). In agreement, preclinical and human clinical studies suggest that exposure to cannabinoids during adolescence may increase the risk for the appearance of neuropsychiatric diseases in adult life including emotional dysregulation, psychotic-like symptoms, cognitive deficits, and increased addiction vulnerability (Renard et al. 2016; Spear, 2016).

Taking into account these findings, a gateway drug hypothesis was postulated suggesting that adolescent cannabis use can increase the risk for subsequent use of addictive drugs later in life (Fergusson et al. 2006; Fergusson and Boden, 2008). However, it is controversial whether the facilitation of later drug abuse in humans could be due to adolescent use of cannabis or because non-pharmacological factors predispose certain individuals to addiction in general (Morral et al. 2002; MacCoun, 2006; Vanyukov et al. 2012). Indeed, the interaction between cannabis consumption and genetic or environmental factors during adolescence may have a crucial influence in the detrimental effects of this drug in adulthood (Rubino and Parolaro, 2016), since most psychiatric disorders involve multiple ethiopathological factors (Caspi and Moffitt, 2006). A direct effect of cannabis exposure can be tested in animal models by exposing rodents to Δ^9-tetrahydrocannabinol (THC) and later allowing them to self-administer another drug.

Changes in the self-administration behaviour of these rodents can be attributed to the effects of the prior drug exposure.

Some studies have shown increased opioid sensitivity in adult animals exposed to THC during the adolescence by using this experimental approach. Heroin self-administration was greater in THC-exposed rats as compared to control animals (Ellgren et al. 2007), although no changes were observed in a later study (Stopponi et al. 2014). Moreover, cue- (Tomasiewicz et al. 2012) and yohimbine-induced reinstatement of heroin-seeking behaviour (Stopponi et al. 2014) were stronger in THC treated animals that in controls. Adolescent THC treatment increased self-administration of the synthetic cannabinoid WIN55,212-2 in adulthood (Scherma et al. 2016), suggesting also an increased risk of subsequent cannabis use.

Tobacco consumption is one of the main public health problems worldwide and represents a leading cause of preventable deaths in most developed countries. In this context, an increased risk of tobacco dependence in adulthood could be a crucial detrimental consequence of cannabis adolescent use (Patton et al. 2005). While cannabis and tobacco are often co-use (Agrawal et al. 2008) epidemiological evidence suggests that cannabis consumption sometimes precedes regular tobacco use suggesting that THC consumption by teenagers could increase the risk for developing tobacco dependence when they reach young adulthood (Patton et al. 2005; Badiani et al. 2015). However, to the best of our knowledge, no animal study has evaluated possible modifications in the self-administration of nicotine in adulthood due to THC adolescent exposure.

Materials & Methods

Animals

Experiments were performed using adolescent male C57BL/6J mice (Charles River, France), housed 4 per cage in a room with controlled temperature (21 ± 1°C), and humidity (55 ± 10%) and with a normal 12 h light/dark cycle (lights on 8:00 AM). Food and water were available ad libitum. Before starting with the nicotine self-administration training sessions, mice were single-housed, switched to reversed cycle (lights off 8:00 AM) and habituated for 1 week to the new conditions. Animal procedures were conducted in accordance with the guidelines of the European Communities Directive 2010/63/EU regulating animal research, the local ethical committee (CEEA-IMAS-UPF), and the statement of compliance with standards for use of laboratory animals by foreign institutions no. A5388-01 approved by the National Institutes of Health (USA).

Drugs

THC stored at 100 mg/ml in ethanol (THC-Pharm-GmbH, Germany) was dissolved in 5% Tween-80 and physiological saline solution, and administered by subcutaneous (sc) route in a volume of 5 ml/kg of body weight at the doses of 3, 6 and 12 mg/kg. (-)-Nicotine hydrogen tartrate salt ((-)-1-methyl-2(3-pyridyl) pyrrolidine; Sigma) was dissolved in physiological saline (0.9% NaCl), adjusted to pH 7.4 and contingently administered by intravenous (iv) route at the dose of 30 μg/kg per infusion (free base). Ketamine hydrochloride (100 mg/kg; Imalgène 1000) and xylazine hydrochloride (20 mg/kg; Sigma) were mixed and diluted in ethanol (5%) and saline (95%). This anaesthetic mixture was administered intraperitoneally (ip) in a volume of 10 ml/kg body weight. Thiopental sodium (5 mg/ml; Braun Medical S.A.) was dissolved in distilled water and delivered in a volume of 0.05 ml through the iv catheter.

Adolescent THC treatment

We evaluated the long-term effects of adolescent THC exposure in nicotine self-administration, extinction and cue- or stress-induced reinstatement of nicotine-seeking behaviour during adulthood. The adolescence period, defined as the complete time span from childhood (shortly before puberty) to adulthood, was considered as previously reported (Rubino et al. 2008; Cadoni et al. 2015). Starting at PND 35, mice were administered with escalating doses of THC (PND 35–39: 3 mg/kg, PND 40–44: 6 mg/kg, and PND 45–49: 12 mg/kg) or vehicle during 15 days in order to counter the development of drug tolerance (Renard et al. 2017) (Fig. 1a). These doses were based on previous studies (Rubino et al. 2008; Llorente-Berzal et al. 2013; Scherma et al. 2016; Bruijnzeel et al. 2019). Moreover, similar to previous studies (Quinn et al. 2008; Llorente-Berzal et al. 2013; Saravia et al. 2019) animals remained undisturbed from PND 50 to 69, and they underwent surgical procedures at PND 70 for further behavioural evaluation (Fig. 1a).

Nicotine self-administration

Jugular vein catheterization. Mice were anesthetized with a ketamine/xylazine mixture and then implanted with indwelling iv silastic catheters in their right jugular vein as previously described (Soria et al. 2005). Briefly, silicone tubing of 6 cm long (0.3mm inner diameter, 0.6mm outer diameter; Silastic, Dow Corning) was fitted to a 22-gauge steel cannula (Semat) bent at a right angle and then embedded in a dental cement disk with an underlying nylon mesh. The catheter tubing was inserted 1.1 cm into the right jugular vein and anchored with suture. The remaining tubing ran subcutaneously to the cannula, which exited at the midscapular region. All incisions were sutured and coated with local antibiotic (Bactroban, GlaxoSmithKline). After surgery, mice were left to recover for 5-7 days before initiation of self-administration sessions.

Apparatus. The experiments were conducted in mouse operant chambers (model ENV-307A-CT; Med Associates Inc.) equipped with two holes, one randomly selected as the active hole and the other as the inactive hole. Pump noise and stimuli lights (cues), one located inside the active hole and the other above it, were paired with the delivery of the reinforcer. Nicotine (30 μg/kg/infusion) was delivered in a volume of 23.5 μl over 2 s via a syringe mounted on a microinfusion pump and connected via flexible polymer tubing to a single channel liquid swivel and to the mouse iv catheter.

Nicotine self-administration training. Operant model procedure was applied similarly to previous reports (Plaza-Zabala et al. 2013). Daily self-administration sessions of 1 h duration were conducted consecutively for 10 days. Mice were trained under a fixed ratio 1 schedule of reinforcement with a 10-s time-out. Each daily session started with a priming injection of the drug. The stimuli light together with the pump noise signalled delivery of nicotine infusion. During the 10-s time-out period, the cue light was off and no reward was provided after active nose-poking. The session was terminated after 50 reinforcers were delivered or after 1 h, whichever occurred first. The criteria for the acquisition of self-administration behaviour were achieved when in three consecutive sessions: (1) mice maintained a stable responding with <20% deviation from the mean of the total number of reinforcers earned (80% stability); (2) at least 75% responding on the active hole, and (3) a minimum of 6 reinforcers per session. The patency of iv catheters was evaluated at the end of nicotine self-administration training by an infusion of 0.05 ml of thiopental sodium through the catheter. If prominent signs of anaesthesia were not present immediately after, the mouse was removed from the experiment. Only mice with patent catheter that achieved all acquisition criteria were moved to the extinction phase.

Extinction. Animals were allowed to rest for 1 day after thiopental testing. During the extinction period, nicotine and environmental cues were not delivered after nose-poking

in the active hole. Mice were subjected to 1 h daily sessions 6 days per week for 20 days. During this period, mice achieved the extinction criterion when active responses were <35% of the mean responses obtained during the 3 last days of nicotine self-administration across 3 consecutive extinction sessions. Only mice that reached the extinction criterion were evaluated for reinstatement. These mice were equally distributed in two groups considering the time required for achieving the extinction criterion and the mean responses during acquisition: one group was tested for cue-induced reinstatement and the other for stress-induced reinstatement.

Cue-induced reinstatement of nicotine-seeking behaviour. One day after reaching the extinction criterion, a group of mice was tested in a single cue-induced reinstatement session that lasted for 1 h. At the beginning of the session, mice were re-exposed to the pump noise and stimuli lights (environmental cues) for 2 s, after which each active nose-poke led to the presentation of the same environmental cues. Nicotine was not available through the entire session.

Stress-induced reinstatement of nicotine-seeking behaviour. Another group of mice was exposed to acute stress, consisting on receiving intermittent footshock stimuli (5 footshocks separated by 1-min periods) at an intensity of 0.22 mA during 5 min immediately before the reinstatement session, as previously described (Martín-García et al. 2009). Test for reinstatement was conducted under the same conditions used in the extinction phase.

The total number of animals used in the different experimental phases is summarized in Fig. 1b.

Data Analysis

Two-way ANOVA with repeated measures was used to analyse the effect of THC treatment on body weight, with treatment as between-subject factor and day as within-

subject factor. Newman-Keuls comparisons were performed as *post hoc* analysis. For the evaluation of the acquisition of nicotine self-administration and extinction three-way ANOVA with repeated measures was employed (*treatment* as between-subject factor; *hole* and *day* as within-subject factors). Only within factors *hole* and *day* were considered in case that the interaction with *treatment* factor resulted non-significant. Subsequent *post hoc* comparisons (Newman-Keuls) were used for each day of training in the presence of significant interaction between factors. A similar analysis was applied to evaluate the reinstatement elicited by stress exposure or by presentation of nicotine-associated cues in mice extinguished from operant behaviour (with *experimental phase* and *hole* as within-subject factors, and *treatment* as between-subject factor). χ^2 test with Yates' correction for low observed frequencies was used to compare the percentage of acquisition and extinction between animals subjected to THC or vehicle during adolescence. All the animals reaching the reinstatement phase were included in this analysis. The level of significance was $p < 0.05$ in all the experiments.

Results

Reduced body weight after adolescent THC treatment is still present in adulthood

Adolescent male mice were treated with escalating doses of THC or vehicle for 15 days (PND 35–39: 3 mg/kg, PND 40–44: 6 mg/kg, and PND 45–49: 12 mg/kg) (Fig. 1a). Body weight was assessed daily throughout THC treatment and right before the surgical procedures required for behavioural testing in the adulthood (PND70). As displayed in Fig. 2a, the weight gain of mice exposed to THC was lower than that of vehicle-treated animals during adolescent treatment (two-way ANOVA with repeated measures: significant interaction of treatment × day factors: $F_{(14,980)}$=22.12, P<0.001), in agreement with previous reports employing similar protocols (Rubino et al. 2008; Stopponi et al. 2014; Saravia et al. 2019). *Post hoc* comparisons revealed significant differences in body weight from day 10 of treatment (days 10-12, P<0.05; days 13-15, P<0.01). These differences were still present when mice reached adulthood (PND70) (P<0.01), probably as a residual effect from the treatment period since weight gain was not significantly different between THC- and vehicle-exposed groups (Fig. 2b).

Adolescent THC treatment does not affect acquisition and extinction of nicotine self-administration in adulthood

Mice previously exposed to THC or vehicle during adolescence were trained to self-administer nicotine (30 μg/kg per infusion) during 10 consecutive days. An increase in responding on the active hole developed over sessions in both vehicle- and THC-treated mice, whereas responding on the inactive hole remained at low levels (Fig. 3a), indicating a progress in operant responding for nicotine (two-way ANOVA with repeated measures: significant interaction of hole × day factors ($F_{(9,414)}$=20.38, P<0.01). Vehicle- and THC-

exposed mice displayed similar responding across days, and the total amount of nicotine consumed by both groups resulted identical (6.3 ± 0.5 and 6.32 ± 0.5 mg/kg, respectively; Fig. 3b). Acquisition criteria were achieved by 81% of vehicle-exposed mice and 100% of THC-treated mice (Yates's χ^2=2.72, p=0.09), and both groups required the same time to acquire operant responding for nicotine (7.3 ± 0.4 days) (Fig. 3c and 3d). Mice that met the acquisition criteria for nicotine self-administration behaviour underwent an extinction schedule for 20 sessions (Fig. 3e). The extinction criterion was achieved by 90% of mice treated with vehicle in 7.3 ± 0.7 days and by 86% of THC-exposed mice in 7.5 ± 0.8 days, indicating that both groups displayed the same ability to extinguish nicotine self-administration (Fig. 3f and g).

THC exposure during adolescence does not influence cue- nor footshock-induced reinstatement of nicotine-seeking behaviour in adulthood

Animals that met the extinction criterion were tested for reinstatement of nicotine-seeking behaviour. Reinstatement studies were performed using a between-subjects design. Thus, different groups of mice were tested for reinstatement by exposure to either environmental cues or footshock stress. When allocating animals to different groups for reinstatement evaluation, we excluded any possible bias of nicotine acquisition levels by performing a two-way ANOVA with repeated-measures analysis. A significant effect of training day was revealed ($F_{(9,297)}$=15.12, P<0.001), but not of the different experimental groups selected ($F_{(3,33)}$=0.45, n.s.) nor interaction between both factors ($F_{(27,297)}$=0.57, n.s.), indicating that the different groups of mice tested for reinstatement were similar in terms of previous self-administration levels. Mice exposed to nicotine-associated cues reinstated a previously extinguished nicotine-seeking behaviour (Fig. 4a), as observed in previous studies (Plaza-Zabala et al. 2013). Importantly, no differences were observed

between reinstatement levels displayed by THC- and vehicle-treated mice, as revealed by three-way ANOVA with repeated measures showing a significant effect of experimental phase and hole and their interaction ($F_{(1,17)}$=61.30, P<0.001; $F_{(1,17)}$=57.23, P<0.001; $F_{(1,17)}$=68.80, P<0.001 respectively), but not of treatment ($F_{(1,17)}$=0.46, n.s.). Similarly, footshock-stress exposure induced reinstatement of nicotine-seeking behaviour in mice previously treated with either THC or vehicle (Fig. 4b). Three-way ANOVA confirmed that THC treatment exerted no effect in footshock-induced responding ($F_{(1,16)}$=3.79, n.s.), but a robust reinstatement of nicotine-seeking was further supported by significant effects of experimental phase, hole and their interaction ($F_{(1,16)}$=43.83, P<0.001; $F_{(1,16)}$=19.83, P<0.001; $F_{(1,16)}$=37.99, P<0.001 respectively). Notably, reinstatement levels were lower after footshock-stress exposure than those observed after nicotine-associated cue presentation, in agreement with previous reports (Plaza-Zabala et al. 2010). These data suggest that adolescent THC exposure does not alter relapse to nicotine-seeking in the adulthood induced by stress or nicotine-associated cues.

Discussion

This study evaluated the effects of adolescent THC exposure in the addictive properties of nicotine in adulthood. The reinforcing effects of nicotine were not modified as revealed the lack of changes in the nicotine self-administration paradigm. The reinstatement of nicotine-seeking behaviour induced by footshock-stress and nicotine-associated cues was also similar between adolescent THC treated and control mice. These data suggest that adolescent cannabinoid exposure would not be associated to increased tobacco consumption later in life.

Mice were treated with escalating doses of THC from PND35 to PND49. This adolescent THC exposure regimen was similar to that used in previous experiments carried out in rodents (Rubino et al. 2008; Llorente-Berzal et al. 2013; Cadoni et al. 2015; Saravia et al. 2019). The use of similar THC exposure regimens facilitates comparisons among different preclinical studies. However, doses usually used in preclinical studies are higher than those that would be consumed voluntarily, and greater than those observed in human smoking cannabis. Rodent models are limited with respect to modeling human substance abuse, including doses and duration of substance intake during adolescence (Bruijnzeel et al. 2019), and it would be important to address such limitations experimentally for instance by using cannabinoid self-administration models (Kirschmann et al. 2017).

The weight gain of mice treated with THC was lower than those exposed to vehicle, as previously reported (Rubino et al. 2008; Stopponi et al. 2014; Scherma et al. 2016; Saravia et al. 2019). An anxiogenic-like effect induced by a high dose of THC could explain the observed changes in body weight. Indeed, one week after the finishing of the treatment, a previous study demonstrated the existence of an anxiety-like behaviour that was higher in adolescent rodents exposed to THC compared to controls (Stopponi et al. 2014). Non-specific inhibition of ingestion, secondary to the sedative effects of THC

could also be involved in this effect on body weight. In addition, epidemiological studies show that the prevalence of obesity is lower in cannabis users than in nonusers (Le Strat and Le Foll, 2011). This difference was not accounted for by tobacco smoking status and was still present after adjusting for variables such as sex and age (Le Strat and Le Foll, 2011), suggesting a direct effect related to exposure to the THC present in cannabis smoke. In agreement, chronic THC treatment reduced energy intake and prevented high fat diet-induced increases in body weight and adiposity in adult male mice (Cluny et al. 2015), an effect possibly related to changes in gut microbiota. Intriguingly, these effects are paradoxical since blockade of cannabinoid CB1 receptors with rimonabant led to the development of a successful therapeutic approach for obesity (Le Foll et al. 2013) by inducing weight loss and improving metabolic profile.

Epidemiological evidence suggests that prior cannabis use increases the likelihood of becoming dependent on tobacco (Patton et al. 2005; Timberlake et al. 2007; Badiani et al. 2015). Numerous studies in adult rodents also demonstrate that treatment with cannabinoid receptor ligands can modulate the addictive properties of nicotine (Solinas et al. 2008; Maldonado and Berrendero, 2010). Cannabinoid antagonists reduce the rewarding effects of nicotine as shown by behavioural and neurochemical approaches (Cohen et al. 2002; Le Foll and Goldberg, 2004) while cannabinoid agonists enhance nicotine reward and reinstate nicotine-seeking behaviour (Gamaleddin et al. 2012). Congruent with this, prior exposure to THC increased the addictive properties of nicotine in adult rats (Panlilio et al. 2013). Thus, the percentage of rats that acquired the nicotine self-administration behaviour was 94% in THC-exposed animals and only 65% in vehicle-treated animals. In this study, THC was administered for 3 days in adult animals, and nicotine self-administration experiments started 1 week following the last THC

injection (Panlilio et al. 2013). However, whether adolescent THC affects nicotine reward and relapse later in life remains to be investigated.

Under our experimental conditions, adolescent THC exposure did not alter the performance of intravenous nicotine self-administration in adulthood. Control and THC treated mice showed similar number of infusions in the active hole and required the same time to acquire operant responding for nicotine. In agreement, THC treatment during adolescence did not modify behavioural responses induced by amphetamine in adult rats (Ellgren et al. 2004). On the contrary, there is evidence that adolescent THC exposure may increase the vulnerability and reward sensitivity to at least some psychoactive drugs when tested in adulthood. Thus, chronic THC exposure during adolescence increased opioid self-administration in adult male rats (Ellgren et al. 2007; Tomasiewicz et al. 2012). This effect was associated with increased μ-opioid receptor function in the ventral tegmental area (Ellgren et al. 2007) and enhanced proenkephalin peptide in the nucleus accumbens in the adult period (Tomasiewicz et al. 2012). In addition, exposure to THC in adolescent male rats increased the self-administration of the synthetic cannabinoid WIN55,212-2 (Scherma et al. 2016). In this study, THC-treated animals showed a reduced capacity for WIN55,212-2 to increase dopamine levels in the nucleus accumbens shell during adulthood (Scherma et al. 2016). As a whole, the above-described preclinical studies suggest that THC chronic exposure during adolescence may induce long-term alterations in reward neural pathways and increase sensitivity to certain drug classes, such as opioids, when tested later in adulthood. However, the effects of adolescent THC on addiction vulnerability could not be generalized to all drugs of abuse.

Stress and nicotine-associated cues exposure leading to nicotine relapse are crucial for the maintenance of tobacco addiction (Stoker and Markou, 2015; Mantsch et al. 2016). THC adolescent exposure did not modify cue- and footshock stress-induced reinstatement

of nicotine-seeking behaviour in adult mice. However, THC treatment during adolescence increased the reinstatement of heroin self-administration induced by cues but not stress (24 h food deprivation) in adult rats (Tomasiewicz et al. 2012). In a subsequent study, when a pharmacological stressor (yohimbine) was used to reinstate heroin self-administration behaviour, the response of THC-treated rats was stronger in comparison with controls (Stopponi et al. 2014). These results suggest that the mechanisms by which adolescent THC could influence the relapse to drug-seeking behaviours in adulthood are complex, and may depend on several factors including the type of stimulus use to trigger relapse, the specific drug evaluated and the animal species.

Similar to our results, other studies found no evidence of adverse affective or cognitive outcomes during adulthood due to adolescent THC treatment (Ballinger et al. 2015; Bruijnzeel et al. 2019; Saravia et al. 2019), suggesting that adverse effects associated with adolescent THC exposure might be also influenced by non-cannabinoid factors of cannabis use. Frequent concomitants of human cannabis use include psychiatric disorders, environmental stressors, and polysubstance use (Ketcherside and Filbey, 2015; Liu et al. 2018; Pinto et al. 2019). Indeed, the interaction between genetic or environmental events and cannabinoid exposure in the adolescent period can contribute to exacerbate behavioural deficits in adulthood (Rubino and Parolaro, 2016). Thus, adolescent mice treated with THC and exposed to stress exhibited impaired cued fear extinction in adulthood (Saravia et al. 2019), while no effect was observed in animals exposed to these two factors separately. Chronic adolescent treatment with THC induced a deficit in cued fear conditioning only in adult mice with a mutation in disrupted-in-schizophrenia 1 gene (Ballinger et al. 2015). A possible interaction between adolescent THC and genetic/environmental factors that potentially alters the addictive properties of nicotine is an important issue that needs to be addressed in future research.

The use only of male mice is a limitation of this study since several reports show evidences for sex differences in the central effects of THC (Rubino and Parolaro, 2015; Cooper and Craft, 2018), although different results have been found depending on the response evaluated. These effects may depend on the different pharmacokinetics described for THC between males and females (Craft et al. 2017), the existence of different neurodevelopmental trajectories between both sexes (Lenroot et al. 2007), and interactions between the endocannabinoid system and sex hormones that could affect the maturation of the adolescent brain (Rubino and Parolaro, 2015). Specifically, the effects of adolescent cannabinoid exposure on adult drug self-administration seem to present sex-dependency. Higher adult cocaine self-administration rates have been reported in adult female, but not male, rats treated with the synthetic cannabinoid CP55,940 (Higuera-Matas et al. 2008). An increase in morphine self-administration under the fixed ratio 1 schedule was also described in adult males, but not in females, exposed to the same synthetic agonist during the adolescent period (Biscaia et al. 2008). Therefore, the investigation about how males and females might differ in the effects of adolescent THC exposure in the addictive properties of nicotine will require special attention in the future. In summary, under our particular experimental conditions, these data did not reveal evidence for alterations in nicotine reward and relapse during adulthood due to adolescent THC exposure. These results do not support the gateway hypothesis in which prior cannabis facilitates a progression to tobacco dependence. Future research evaluating several aspects such as the interaction between THC and genetic/environmental factors, or the possible influence of sex will be necessary in order to better know the impact of THC during adolescence on nicotine dependence in adulthood.

Figure legends

Figure 1. **A,** Time schedule of THC exposure during adolescence, and nicotine self-administration, extinction and drug-seeking reinstatement during adulthood. **B,** Total number of animals included in each experimental phase. PND, post-natal day.

Figure 2. **A,** Body weight of mice during THC exposure (3, 6 and 12 mg/kg) and at the beginning of behavioural testing in adulthood (PND70). **B,** Body weight gain from PND49 (at the end of THC treatment) to PND70 (before surgery). Data are expressed as mean ± S.E.M. ★$p<0.05$, ★★$p<0.01$ between treatments (Newman-Keuls test) (n = 36 mice per group). VEH, vehicle.

Figure 3. Acquisition and extinction of nicotine self-administration behaviour in animals previously exposed to THC or vehicle during adolescence. **A,** Mean number of nose-poking responses displayed in the active and inactive holes during the acquisition period. Mice were trained daily to obtain 30 µg/kg/infusion under a FR1 schedule of reinforcement (n = 21-26 mice per group). **B,** Mean nicotine intake observed per animal for the whole acquisition period. **C,** Percentage of animals achieving all the acquisition criteria by the end of the acquisition period. **D,** Mean number of days required to reach all acquisition criteria. **E,** Mean number of nose-pokes displayed during extinction training (n = 21 mice per group). **F,** Percentage of animals reaching the extinction criterion after a 20-day extinction period. **G,** Mean days required to fulfil the extinction criterion. VEH, vehicle. Data are expressed as mean ± S.E.M. #$p<0.05$, ##$p<0.01$ between holes (Newman-Keuls test).

Figure 4. Reinstatement of nicotine self-administration behaviour in animals previously exposed to THC or vehicle during adolescence. **A,** Mean number of nose-poking responses displayed in the active and inactive holes during extinction and cue-induced reinstatement. Mice were re-exposed to nicotine-associated environmental cues at the beginning of the reinstatement session and after each active nose-poke, in the absence of nicotine (n = 9-10 mice per group). **B,** Mean number of nose-poking responses displayed in the active and inactive holes during extinction and stress-induced reinstatement. Mice were exposed to 5 footshocks delivered throughout 5 minutes immediately before the reinstatement session, which was performed in the same conditions than the extinction training (n = 9 mice per group). VEH, vehicle. Data are expressed as mean ± S.E.M. ★★$p<0.01$, between experimental phases or between holes (Newman-Keuls test).

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