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Cannabinoid CB_1 receptor in the modulation of stress coping behavior in mice: The role of serotonin and different forebrain neuronal subpopulations

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ABSTRACT

The endocannabinoid system (ECS) may either enhance or inhibit responses to aversive stimuli, possibly caused by its modulatory activity on diverse neurotransmitters. The aim of this work was to investigate the involvement of serotonin (5-HT) and catecholamines, as well as the role of glutamatergic and GABAergic cannabinoid type 1 (CB_1) receptor, in responses to the antidepressant-like doses of the CB_1 receptor agonist Δ^9-tetrahydrocannabinol (THC) and the antagonist rimonabant in the forced swim test (FST). Mice received acute injections of low doses of THC (0.1 or 0.5 mg/kg) or high dose of rimonabant (3 or 10 mg/kg) after treatment with the 5-HT synthesis inhibitor pCPA (100 mg/kg, 4 days), the 5-HT_{1A} receptor antagonist WAY100635 (1 mg/kg, acute) or the non-selective blocker of catecholamine synthesis, AMPT (20 mg/kg, acute). THC and rimonabant were also tested in mutant mice lacking CB_1 receptor in specific forebrain neuronal subpopulations.

Both THC and rimonabant induced antidepressant-like effects, quantified as immobility in the FST. However, only THC effects were reversed by pCPA or WAY100635. In contrast, only AMPT could attenuate the rimonabant effect. We also found decreased immobility in mice lacking the CB_1 receptor in glutamatergic cortical neurons, but not in forebrain GABAergic neurons, as compared with wild-type controls. The effect of THC persisted in mutant mice with CB_1 receptor inactivation in GABAergic neurons, whereas rimonabant effects were alleviated in these mutants. Thus, employing both pharmacological and genetic tools, we could show that the ECS regulates stress responses by influencing GABAergic, glutamatergic and monoaminergic transmission. The antidepressant-like action of THC depends on serotonergic neurotransmission, whereas rimonabant effects are mediated by CB_1 receptor on GABAergic neurons and by catecholamine signaling.

1. Introduction

The herb *Cannabis sativa* induces a diversity of emotional responses ranging from anxiolytic and relaxing effects to the induction of acute panic attacks (Hall and Solowij, 1998). Similarly, divergent emotional responses have been observed in both humans and rodents after the administration of Δ^9-tetrahydrocannabinol (THC), the main psychoactive compound from this plant (Berrendero and Maldonado, 2002; Patel and Hillard, 2006; Zuardi et al., 1982). Postsynaptically produced endocannabinoids, the endogenous counterparts of THC, including anandamide and 2-arachidonoyl glycerol, function as retrograde modulators of synaptic activity, which, through activation of presynaptic CB_1 receptor, restrain neurotransmitter release from presynaptic terminals. As the CB_1 receptor is present on both GABAergic and glutamatergic terminals (Kano et al., 2009), the endocannabinoid system (ECS) is able to control the activation of both inhibitory and excitatory neurotransmission. Therefore, depending on its specific spatio-temporal activation within neuronal circuits, this system can act as a major "bi-directional" neuromodulator (for a review, see Moreira and Lutz, 2008).

This "dual" role of endocannabinoid signaling has likely been the reason for a number of contradictory results in rodent models of anxiety and depression (Hill and Gorzalka, 2005; Viveros et al., 2005). This is supported by recent studies, using conditional mutant mice lacking the CB_1 receptor either on GABAergic or glutamatergic neurons, supporting the notion that the two populations might be important for the biphasic effect (Lafenêtre et al., 2009; Jacob et al., 2009; Häring et al., 2011). Another explanation might be a variation in the initial baseline stress level of an animal, which depends on a multitude of genetic, environmental and experimental factors. This baseline might alter the activity of the

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Abbreviations

AMPT	α-methyl para-tyrosine
CB1	receptor, cannabinoid type 1 receptor
DMSO	dimethylsulfoxid
ECS	endocannabinoid system
FST	forced swim test
GABA	γ-amino buryric acid
Glu-$CB_1^{-/-}$ mice	$CB_1^{flox/flox;Nex\text{-}cre}$ mice
GABA-$CB_1^{-/-}$ mice	$CB_1^{flox/flox;Dlx5/6\text{-}cre}$ mice
i.p.	intraperitoneally
pCPA	parachlorophenylalanine
PFC	prefrontal cortex
Rim	rimonabant; 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (also called SR141716)
THC	Δ^9-tetrahydrocannabinol
WAY	WAY100635; N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide

ECS, thus, resulting in the same behavioral effect induced by opposite pharmacological interventions (e.g., CB_1 receptor signaling blockade or enhancement) (Wotjak, 2005; Viveros et al., 2005). Such a dose dependent cannabinoid-induced biphasic effect on the behavioral performance can also be seen in the forced swim test (FST). One of the most widely used behavioral paradigms to detect antidepressant-like activities of drugs (Lucki et al., 2001; Cryan and Mombereau, 2004). It is based on the observation that rodents, when exposed to an inescapable situation (immersion in a beaker filled with water), will cease over several minutes to engage in escape-oriented movements and adopt an immobile passive "floating" posture. Acquired immobility is often interpreted as "behavioral despair", mimicking psychomotor impairments experienced by depressed patients (Cryan and Mombereau, 2004). A reduction of immobility time in the FST is especially observed after treatment with a broad range of antidepressants, which increase serotonergic and/or noradrenergic neurotransmission (Cryan and Mombereau, 2004). In this model, CB_1 receptor activation can lead to a decrease or increase of immobility (Bambico et al., 2007, 2012; Egashira et al., 2008; El-Alfy et al., 2010). Blocking the CB_1 receptor with SR141716 (rimonabant) can also induce either an antidepressant-like effect (Griebel et al., 2005; Steiner et al., 2008a) or increase immobility behavior, depending on the dose (Steiner et al., 2008b; Beyer et al., 2010).

Thus, before we consider the ECS as a valid strategy for developing new drugs for the treatment of mood disorders (Gobbi et al., 2005; Bambico et al., 2010; Gorzalka and Hill, 2011), we should understand the reasons for these complex responses (Häring et al., 2012). Surprisingly, even though marihuana has been used for recreational purposes since centuries, studies on antidepressive potentials of its major component, THC, are still sparse.

One possible mechanism through which cannabinoids interfere with stress-related responses might be through monoaminergic mechanisms. Several studies connected the ECS with serotonergic transmission. Indeed, the CB_1 receptor antagonist rimonabant was shown to increase the efflux of 5-HT and noradrenaline in the rat prefrontal cortex (Tzavara et al., 2003). CB_1 receptor is expressed in mouse serotonergic raphe neurons (Häring et al., 2007) and in noradrenergic nerve terminals in the rat frontal cortex (Oropeza et al., 2007). In addition, CB_1 receptor signaling influences the firing rate of serotonergic and noradrenergic neurons in the rat raphe nuclei and locus coeruleus, respectively (Gobbi et al., 2005; Muntoni et al., 2006). Altogether, accumulating evidence supports the involvement of CB_1 receptor signaling in the regulation of monoaminergic neurotransmission, which could, in turn, mediate endocannabinoid effects in the FST.

Using a combination of pharmacological and genetic approaches, we aimed at investigating the contradictory findings of ECS modulation in emotion and how serotonergic and catecholamine transmission might be involved in. To address this issue, we studied the antidepressant-like effects of the CB_1 receptor agonist THC, and the antagonist/inverse agonist rimonabant in combination with drugs disrupting serotonergic (with parachlorophenylalanine, pCPA; Weissman and Koe, 1965) and catecholamine (with α-methyl-para-tyrosine, AMPT; Corrodi and Hanson, 1966) transmission. Both drugs were also shown to block the antidepressant-like effects of 5-HT or dopamine reuptake inhibitors (O'Leary et al., 2007). Furthermore, we included mice lacking the CB_1 receptor in specific neuronal subpopulations, namely in GABAergic forebrain neurons (GABA-CB_1 mouse line) and glutamatergic cortical neurons (Glu-CB_1 mouse line) to investigate the role of these cells in THC and rimonabant effects.

2. Methods

2.1. Animals

This study was performed on adult (3–5 months old) male C57BL/6N mice, as well as mutants and littermate controls in a predominant C57BL/6N background. Animals were housed in a temperature- and humidity-controlled room with a 12 h light–dark cycle (lights on at 1 am) and had access to food and water *ad libitum*. The experimental protocols were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ethical Committee on animal care and use of Rhineland-Palatinate, Germany. Generation, breeding and genotyping of the mutant lines were performed according to previous publications: $CB_1^{flox/flox;Nex\text{-}cre}$ mice (referred to as Glu-$CB_1^{-/-}$ mice; Monory et al., 2006), $CB_1^{flox/flox;Dlx5/6\text{-}cre}$ mice (referred to as GABA-$CB_1^{-/-}$ mice; Monory et al., 2006; Massa et al., 2010). Animals were in a predominant C57BL/6N background (at least 7 backcrosses) and were group housed (3–5 animals per cage) until one week before behavioral testing, when they were single housed to avoid behavioral differences between dominant and subordinate animals. All experiments were performed during the second half of the light phase.

2.2. Drug treatments

Injections were given intraperitoneally (i.p.) in a volume of 10 ml/kg body weight. Stock solution of rimonabant (SR141716; NIMH Chemical Synthesis and Drug Supply Program) was prepared by solving the lyophilized drug in DMSO (Sigma Aldrich). Working solution contained the respective rimonabant concentration dissolved in a 0.9 w/v % NaCl solution containing 2 vol % DMSO and 2.5 vol % polyoxyethylenesorbitan monooleate (Tween-80; Sigma Aldrich). THC (THC Pharm, Frankfurt, Germany) was warmed and dissolved in 100% ethanol. Working solution contained the respective THC concentration dissolved in a 0.9 w/v % NaCl solution containing 0.5 vol % ethanol and 2.5 vol % Tween-80. WAY100635 (Sigma–Aldrich) was diluted in 0.9 w/v % NaCl solution. pCPA (Sigma–Aldrich) was suspended in a 0.9 w/v % NaCl solution containing 2.5 vol % Tween-80. AMPT (Sigma–Aldrich) was suspended in a 0.9 w/v % NaCl solution containing 10 vol % Tween-80. All vehicle controls contained the respective concentration of Tween-80, DMSO and/or ethanol dissolved in a 0.9 w/v % NaCl solution.

Single drug injections were given 30 min prior to the experiment. If the mice were exposed to two different drugs, first drug was applied 45 min and the second drug 30 min before the experiment. pCPA was injected every 24 h for 4 days with the last injection on the day of the FST. For each experiment, vehicle treatment was given as control in the same injection schedule as the respective drug treated mice. The doses were selected based on previous works, WAY100635 (Braida et al., 2007; Egashira et al., 2008); AMPT (Jesse et al., 2010); pCPA (Kaster et al., 2005); SR141716 (Tzavara et al., 2003; Griebel et al., 2005).

2.3. Open field

To evaluate potential effects by the drugs on locomotor activity we performed an open field test. The open field was an H 40 cm × W 40 cm × L 40 cm box illuminated at 200 lux, in which the animal was placed for 5 min to allowed free exploration. The animal movement was recorded, and the distance moved was scored by the SMART program (PanLab, Spain).

2.4. Forced swim test (FST)

The paradigm was performed in a round glass beaker (18 cm in diameter and 30 cm in height) filled with tap water at 25 ± 0.5 °C. The water level was approximately 20 cm to prevent the animal from touching the bottom of the glass. The mouse was also unable to climb out off the beaker. The animal was carefully lowered into the water and recorded on DVD for 6 min. The first 2 min were not evaluated; however, floating behavior was scored for the following 4 min by an experimenter blind to genotype and treatment. Floating was defined by immobility of the animal and minimal movements to keep the body's balance. The functionality of the paradigm was successfully tested by acute i.p. injection of the antidepressant drug imipramine (30 mg/kg), which resulted in a significant decrease in floating behavior as compared to saline treated animals ($t_{16} = 10.45$; $p < 0.001$; $n = 9$).

2.5. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). All behavioral endpoints of the open field and FST were analyzed using Student *t*-test, one-way ANOVA or two-way ANOVA followed by the Newman–Keuls Multiple Comparison post-test depending on the combination of genotype and treatment factors. Graphs and statistics were generated by GraphPad Prism 4.03 Software. Results were considered to be significant at $p < 0.05$.

3. Results

3.1. Locomotor activity

To avoid potential disturbing factors related to locomotor activity, all drugs were tested in the open field test. In fact none of the drugs and doses applied altered the distance moved as compared to respective control groups (Table 1).

3.2. Antidepressant-like effects

Animals treated with a low dose of THC (0.1 and 0.5 mg/kg) showed a significant reduction in floating behavior ($F_{2,23} = 4.17$; $p < 0.05$; Fig. 1A). The THC effect of 0.1 mg/kg was prevented by a pretreatment with the 5-HT synthesis inhibitor pCPA 100 mg/kg (Pretreatment factor: $F_{1,68} = 1.77$; $p > 0.05$; THC factor: $F_{1,68} = 10.12$; $p < 0.01$; Interaction$_{[\text{Pretreatment} \times \text{THC}]}$: $F_{1,68} = 7.33$; $p < 0.01$; Fig. 1B) and the 5-HT$_{1A}$ receptor antagonist WAY100635 1 mg/kg (Pretreatment factor: $F_{1,35} = 3.41$; $p > 0.05$; THC factor: $F_{1,35} = 3.07$; $p > 0.05$; Interaction$_{[\text{Pretreatment} \times \text{THC}]}$: $F_{1,35} = 3.79$; $p = 0.0596$; Column comparison$_{[\text{Veh+THC vs WAY+THC}]}$ with Newman–Keuls Multiple Comparison post-test $q = 3.846$; $p < 0.05$; Fig. 3B; Fig. 1C). In addition, the effect of THC (0.1 mg/kg) was prevented by a pre-treatment with a low dose (0.5 mg/kg) of rimonabant (Pretreatment factor: $F_{1,90} = 5.619$; $p < 0.05$; THC factor; $F_{1,90} = 1.638$; $p > 0.05$; Interaction$_{[\text{Pretreatment} \times \text{THC}]}$: $F_{1,90} = 4.669$; $p < 0.05$; see Fig. 1D). Applying rimonabant alone in higher dose (3 and 10 mg/kg) also resulted in a decreased immobility ($F_{2,19} = 10.74$; $p < 0.001$; Fig. 2A). Co-administration of pCPA 100 mg/kg (Pretreatment factor: $F_{1,36} = 0.57$; $p > 0.05$; rimonabant factor: $F_{1,36} = 25.97$; $p < 0.0001$; Interaction: $F_{1,36} = 0.15$; $p > 0.05$; Fig. 2B) and WAY100635 (Pretreatment factor: $F_{1,33} = 0.38$; $p > 0.05$; rimonabant factor: $F_{1,33} = 52.32$; $p < 0.0001$; Interaction$_{[\text{Pretreatment} \times \text{rimonabant}]}$: $F_{1,33} = 0.29$; $p > 0.05$; Fig. 2C), respectively, failed to block the effect of 10 mg/kg rimonabant. In contrast, a *per se* ineffective dose of 20 mg/kg AMPT attenuated the effect of rimonabant (Pretreatment factor: $F_{1,44} = 14.18$; $p > 0.05$; Rimonabant factor: $F_{1,44} = 30.49$; $p < 0.01$; Interaction$_{[\text{Pretreatment} \times \text{rimonabant}]}$: $F_{1,44} = 13.43$; $p < 0.001$; Fig. 2D).

In order to test whether THC and rimonabant effects depend on CB_1 receptor activation on specific glutamatergic or GABAergic neuronal population, we tested these drugs in conditional mutant mice lacking CB_1 receptor specifically in these neuronal subpopulations. However, we first characterized the phenotype of these animals in the FST, without any treatment. Analyzing the floating behavior in the conditional CB_1 receptor knock-out mice revealed a significant decrease in floating time for Glu-$CB_1^{-/-}$ mutants (mean ± SEM: WT = 82.9 ± 16 s and Glu-$CB_1^{-/-}$ = 27.8 ± 8.6 s; $t_{10} = 3.02$; $p < 0.01$; $n = 6$; Pre-test not shown but is similar as depicted in Fig. 3A), without changes in open field activity (mean ± SEM: WT = 1331 ± 202 cm and Glu-$CB_1^{-/-}$ = 1544 ± 498 cm; $t_{16} = 0.39$, ns; $n = 9$). The difference in phenotype in these animals was annulled by the pretreatment with pCPA (Genotype factor: $F_{1,46} = 1.47$; ns; Treatment factor: $F_{1,46} = 1.72$; ns; Interaction$_{[\text{Genotype} \times \text{Treatment}]}$: $F_{1,46} = 4.33$; $p < 0.05$; Fig. 3A). On the other hand, CB_1 receptor deletion from

Table 1
Locomotor activity (cm moved in 5 min) in the open field after different pharmacological treatments.

Experimental groups	Distance moved, mean ± SEM	Statistics
Effects of THC and rimonabant		**One-way ANOVA**
Vehicle	1642 ± 182.6	$F_{4,38} = 0.59$; ns
THC [0.1 mg/kg]	1574 ± 178.2	
THC [0.5 mg/kg]	1675 ± 189.1	
Rim [3 mg/kg]	1784 ± 190.9	
Rim [10 mg/kg]	1577 ± 135.9	
Interaction of THC with serotonin release		**Two-way ANOVA**
Vehicle + Vehicle	1752 ± 227.0	Interaction (Rim/pCPA): $F_{1,36} = 0.001$; ns
Vehicle + THC [0.1 mg/kg]	1891 ± 171.0	Vehicle × Rim: $F_{1,36} = 0.56$; ns
pCPA [100 mg/kg] + Vehicle	1697 ± 207.5	Vehicle × pCPA: $F_{1,36} = 0.06$; ns
pCPA [100 mg/kg] + THC [0.1 mg/kg]	1849 ± 161.4	Interaction (Rim/WAY): $F_{1,36} = 0.01$; ns
WAY [1 mg/kg] + Vehicle	1535 ± 116.3	Vehicle × Rim: $F_{1,36} = 0.46$; ns
WAY [1 mg/kg] + THC [0.1 mg/kg]	1637 ± 176.2	Vehicle × WAY: $F_{1,36} = 1.77$; ns
Interaction of rimonabant with serotonin release		**Two-way ANOVA**
Vehicle + Vehicle	1668 ± 142.9	Interaction (Rim/pCPA): $F_{1,36} = 0.93$; ns
Vehicle + Rim [10 mg/kg]	1740 ± 220.0	Vehicle × Rim: $F_{1,36} = 0.03$; ns
pCPA [100 mg/kg] + Vehicle	1847 ± 115.0	Vehicle × pCPA: $F_{1,36} = 0.26$; ns
pCPA [100 mg/kg] + Rim [10 mg/kg]	1615 ± 128.5	Interaction (Rim/WAY): $F_{1,36} = 3.63$; ns
WAY [1 mg/kg] + Vehicle	1955 ± 102.1	Vehicle × Rim: $F_{1,36} = 0.003$; ns
WAY [1 mg/kg] + Rim [10 mg/kg]	1435 ± 129.5	Vehicle × WAY: $F_{1,36} = 2.09$; ns
Interaction of rimonabant with catecholamine release		**Two-way ANOVA**
Vehicle + Vehicle	1873 ± 174.2	Interaction: $F_{1,41} = 0.103$; ns
Vehicle + Rim [10 mg/kg]	1789 ± 120.1	Vehicle × Rim: $F_{1,41} = 0.097$; ns
AMPT [20 mg/kg] + Vehicle	1710 ± 62.5	Vehicle × AMPT: $F_{1,41} = 1.202$; ns
AMPT [20 mg/kg] + Rim [10 mg/kg]	1716 ± 116.8	

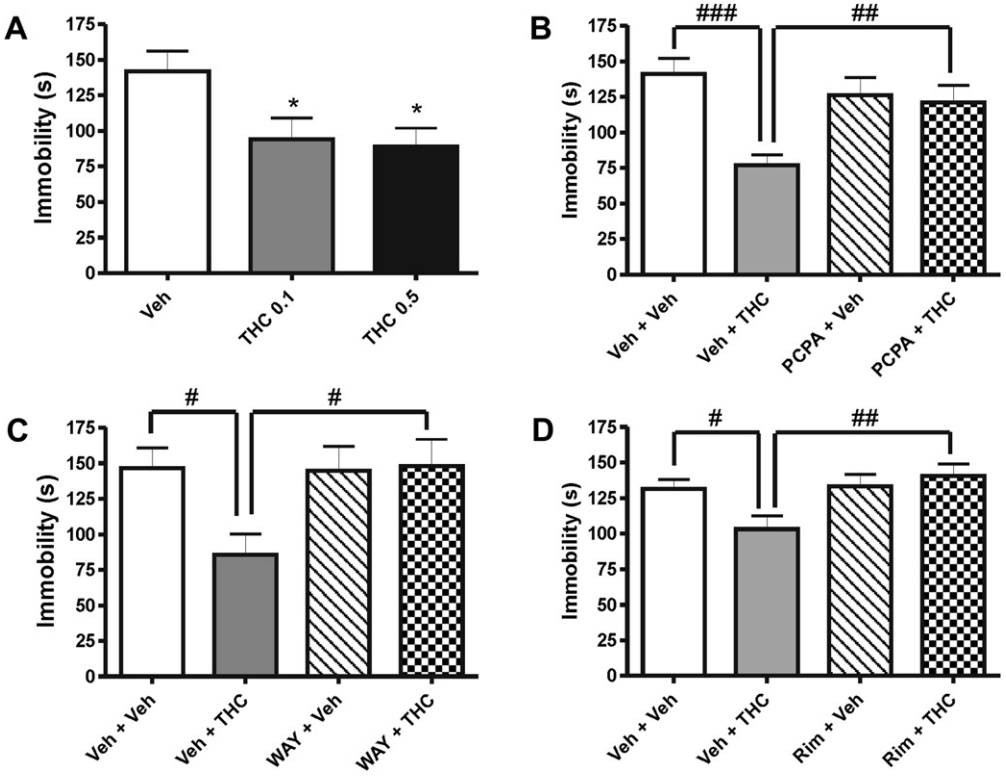

Fig. 1. Antidepressant-like effects of THC and the role of serotonin. Treatment with (A) THC (0.1 mg/kg and 0.5 mg/kg) decreased immobility in the forced swim test. The effect of THC (0.1 mg/kg) was attenuated when combined (B) with the serotonin synthesis inhibitor pCPA (100 mg/kg), (C) with the 5-HT_{1A} receptor antagonist WAY100635 (WAY; 1 mg/kg), and (D) with a *per se* non-effective dose of rimonabant (Rim; 0.5 mg/kg). Data are expressed as mean ± SEM. $n = 9–24$; $^*p < 0.05$ (students *t*-test); $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.001$ (Newman–Keuls Multiple Comparison post-test following two-way ANOVA).

forebrain GABAergic neurons had no effect on the performance in the FST (mean ± SEM: WT = 90.8 ± 26.3 and GABA-$CB_1^{-/-}$ = 65.3 ± 17.2; $t_{11} = 0.83$, ns; $n = 6–7$; Pre-test not shown but is similar as depicted in Fig. 3B and C). By testing drugs in these animals, we were able to show that the THC effect was still present in GABA-$CB_1^{-/-}$ mutants (Genotype factor: $F_{1,67} = 0.622$; ns; Treatment factor: $F_{1,67} = 10.89$; $p < 0.01$; Interaction$_{[\text{Genotype} \times \text{Treatment}]}$: $F_{1,68} = 0.371$; ns; Fig. 3B). On the contrary, the decrease in floating induced by a dose of 10 mg/kg rimonabant was not detectable in GABA-$CB_1^{-/-}$ animals (Genotype factor: $F_{1,33} = 6.97$; $p < 0.05$; Treatment factor: $F_{1,33} = 14.64$; $p < 0.001$; Interaction$_{[\text{Genotype} \times \text{Treatment}]}$: $F_{1,33} = 5.17$; $p < 0.05$; Fig. 3C).

4. Discussion

Our results confirm previous findings on the contradictory roles of the ECS activation and inhibition regarding stress coping. We could show that low dose of THC (0.1 and 0.5 mg/kg) or high dose of the CB_1 receptor antagonist rimonabant (3 and 10 mg/kg) led to a decrease of immobility, indicating an antidepressant-like behavior. THC effects were prevented by a *per se* ineffective dose of rimonabant (0.5 mg/kg; Fig. 1D), proving the CB_1 receptor dependence of the THC effect. Remarkably, inhibition of 5-HT synthesis, and 5-HT_{1A} receptor blockade, respectively, was also able to prevent the effects of THC, but not of rimonabant. On the other hand, using a genetic approach, we could show that the antidepressant-like effects of rimonabant, but not of THC for the doses used in this study, seem to depend exclusively on CB_1 receptor in GABAergic neurons. Low doses of THC might act mainly via other CB_1 receptor populations, potentially on glutamatergic neurons, even though there is no final proof for this notion, due to the situation that Glu-$CB_1^{-/-}$ mice showed already an antidepressant-like behavior which cannot be further enhanced by THC.

These findings suggest that, even though both drugs have antidepressant-like properties, they seem to interfere with different circuits, serotonergic transmission being important for the behavioral response to low doses of THC, and catecholamines for the rimonabant effects. The antidepressant-like effect of THC is in line with previous data obtained with other cannabinoids (Jiang et al., 2005; Hill and Gorzalka, 2005; Bambico et al., 2007). In addition, the role of 5-HT was also proposed previously for this class of substances. For instance, Bambico et al. (2007) demonstrated that the antidepressant-like effects of WIN-55,212-2, a synthetic cannabinoid agonist, was blocked by pCPA in rats. Likewise, WAY100635 blocked the effect of cannabidiol, a non-psychotomimetic phytocannabinoid, in mice in the FST (Zanelati et al., 2010). Finally, the same 5-HT_{1A} receptor antagonist also blocked the anxiolytic-like effects of THC in rats (Braida et al., 2007). The latter result is highly congruent with our findings, as anxiolytic drugs can also have antidepressent-like effects and *vice versa* (Jiang et al., 2005; Höschl and Svestka, 2008).

A relevant neuronal circuit in respect to our finding might be the projection between prefrontal cortex (PFC) and serotonergic neurons in the raphe nuclei, which is modulated by cannabinoids, as proposed by Bambico et al. (2007). The PFC, a region highly involved in the processing and evaluation of a stressful situation, has strong glutamatergic connections with the raphe nuclei (Jankowski and Sesack, 2004). Interestingly, the connection seems to be indirect, as decrease in excitatory drive leads to an increased 5-HT transmission. Thus, the local CB_1 receptor activation on glutamatergic terminals in the PFC by the synthetic cannabinoid receptor agonist WIN55,212 resulted in an increased firing of serotonergic neurons

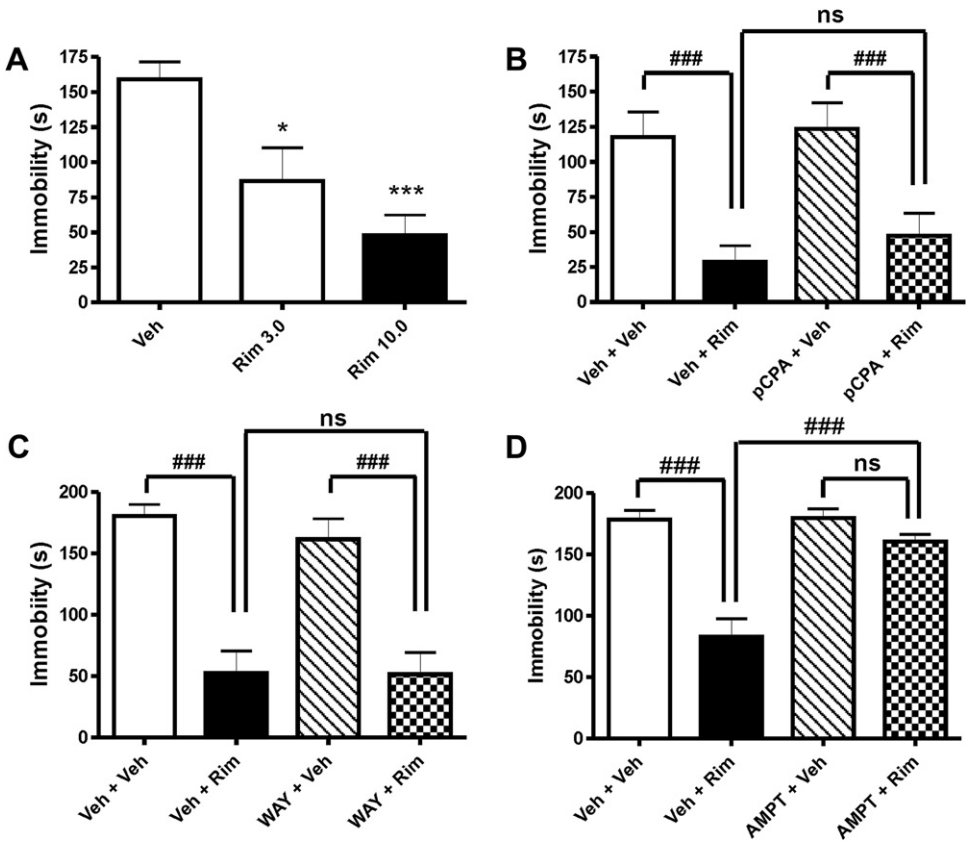

Fig. 2. Antidepressant-like effects of rimonabant and the role of serotonin and catecholamines. Treatment with (A) rimonabant (Rim; 3 and 10 mg/kg) both decreased immobility in the forced swim test. The decrease in immobility induced by rimonabant (10 mg/kg) was not altered by (B) the serotonin synthesis inhibitor pCPA (100 mg/kg) or (C) the 5-HT_{1A} receptor antagonist WAY100635 (WAY; 1 mg/kg), however, by (D) the catecholamine synthesis inhibitor AMPT (20 mg/kg). Data are expressed as mean ± SEM. $n = 9–12$; $^{*}p < 0.05$, $^{***}p < 0.001$ (students *t*-test); $\#\#\#p < 0.001$ (Newman–Keuls Multiple Comparison post-test following two-way ANOVA); ns, non significant.

(Bambico et al., 2007). Earlier studies already suggested that an reduced excitatory input from the PFC is followed by a decreased activation of inhibitory neurons in the raphe nuclei, leading to an increased 5-HT transmission and subsequently to decreased anxiety and depressive-like behavior (Celada et al., 2001).

Regarding the effects of high doses of rimonabant, this seems to be in contrast with the clinical effects of this drug, which may induce anxiety and depression in patients (for reviews, see Moreira and Crippa, 2009; Moreira et al., 2009). However, the acute/sub-chronic antidepressant-like effect of this CB_1 receptor antagonist was shown previously in rodents exposed to the FST (Griebel et al., 2005; Steiner et al., 2008a). One explanation could be the chronic use in clinical applications, resulting in the negative side effects. Also one should keep in mind the clinical intent to reduce obesity using rimonabant. Obesity might sensitize the body to an increased susceptibility toward depressive behavior. Nevertheless, our data

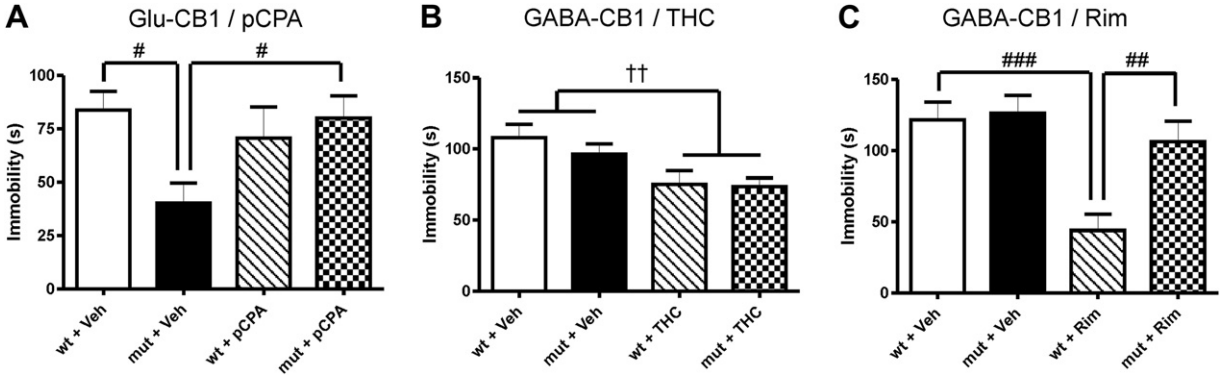

Fig. 3. Behavioral phenotype after cell type-specific CB_1 receptor deletion, and pharmacological effects with THC and rimonabant. (A) Inactivation of CB_1 receptor in cortical glutamatergic neurons led to a decrease in immobility (black bar), which was blocked by the treatment with pCPA (100 mg/kg), comparable as in wild-type controls. (B) Inactivation of CB_1 receptor in forebrain GABAergic neurons did not alter immobility (black bar), and the effect of THC (0.1 mg/kg) on immobility is still detectable in mutant mice. (C) The effect of rimonabant (Rim; 10 mg/kg), however, was not present in GABA-$CB_1^{-/-}$ mice. Data are expressed as mean ± SEM; $n = 10–15$; $\#p < 0.05$; $\#\#p < 0.01$; $\#\#\#p < 0.001$ (Newman–Keuls Multiple Comparison post-test following two-way ANOVA); $\dagger\dagger p<0.01$ (two-way ANOVA treatment factor). ns, non significant. Abbreviations: mut, mutant, i.e. Glu-$CB_1^{-/-}$ or GABA-$CB_1^{-/-}$; wt, wild-type littermate control, i.e. Glu-$CB_1^{+/+}$ or GABA-$CB_1^{+/+}$, respectively.

strongly suggest that this antagonist/inverse agonist acts via the inhibition of CB_1 receptor on GABAergic terminals, since the decrease in floating induced by rimonabant was abolished when injected into GABA-$CB_1^{-/-}$ mutant mice. Why inhibiting 5-HT transmission had no effect on the action of rimonabant is not clear. This seems to be in contrast with neurochemical data, showing that similar doses of rimonabant increased 5-HT in the prefrontal cortex (Tzavara et al., 2003). One possibility could be the systemic increase in GABAergic transmission as the result of the blockade of CB_1 receptor, which could attenuate the effect of an increased serotonergic transmission downstream in the stress circuit. Also possible would be an assisting role of 5-HT transmission after rimonabant treatment. In this respect, Tzavara and colleagues also showed an increased release of catecholamines as response to rimonabant treatment, thus potentially covering the behavioral effect of blocking 5-HT transmission (Tzavara et al., 2003). In consistence with this finding, we were able to attenuate the antidepressant-like effect of rimonabant by applying a *per se* ineffective dose of AMPT, a blocker of catecholamine synthesis. The additional absence of the behavioral effects of rimonabant in the GABA-$CB_1^{-/-}$ mice suggests a mechanism for the action of this drug, mediated by catecholamine signaling and controlled by GABA release. Recent finding also suggest an important role for the opioid system regarding the antidepressant-like effects of rimonabant (Lockie et al., 2011). Thus, they were able to block the rimonabant-induced decrease in immobility by interfering with opioid signaling, in particular by blocking the κ-opioid receptor (Lockie et al., 2011). Interestingly, the activation of this receptor was also suggested to increase the noradrenergic drive in the hypothalamic paraventricular nucleus (Laorden et al., 2000).

Contrasting with the rimonabant treatment, which seems to depend on CB_1 receptors on GABAergic neurons, specific deletion of the receptor in GABAergic neurons (GABA-$CB_1^{-/-}$ mice) did not induce an antidepressant-like response. However, GABA-$CB_1^{-/-}$ mice are still responsive to THC, suggesting that GABAergic CB_1 receptor is unlikely to be the target of low doses of THC. Therefore, we suggest a possible connection to CB_1 receptor on other neuronal populations. Recent findings in our group have highlighted the importance of glutamatergic CB_1 receptors, showing that comparable doses of CB_1 receptor agonist failed to induce an anxiolytic effect in Glu-$CB_1^{-/-}$ mutants tested in the elevated plus maze (Rey et al., 2012). Due to the fact that Glu-$CB_1^{-/-}$ mice showed a decrease in floating behavior without treatment, it was not possible to test the equivalent hypothesis in the FST. A similar decrease in immobility was previously observed in Glu-$CB_1^{-/-}$ mice (Steiner et al., 2008c). This increased stress-coping behavior in Glu-$CB_1^{-/-}$ mice is in contrast with other studies in which these mutants actually showed increased anxiety-like responses (Lafenêtre et al., 2009; Jacob et al., 2009; Häring et al., 2011). Thus, the decrease in floating behavior may rather be an inadequate fear response than a positive stress coping behavior. This situation would prevent a stringent interpretation of a possible antidepressant-like of THC effect in Glu-$CB_1^{-/-}$ mice.

The present data also suggest that the behavioral changes in the FST in Glu-$CB_1^{-/-}$ may depend on serotonergic transmission, as it was blocked by pCPA. Why the inhibition of 5-HT transmission blocks this effect is not clear. One possibility might be a continuous over-excitation of the serotonergic neurons via a different pathway (independent of inhibitory interneurons in the raphe) as suggested above, caused by a general elevated excitatory drive.

In summary, by using both pharmacological and genetic approaches, we could provide new insights into how to reconcile the contradictory findings on antidepressant-like effect of CB_1 receptor agonist and antagonist/inverse agonist. Low doses of agonist clearly depended on serotonergic transmission. High doses of antagonist/inverse agonist, on the other hand, dominantly acted via CB_1 receptor on GABAergic neurons and depended at least not mainly on serotonergic transmission, but on catecholamine transmission. Our data further suggest a two-neuronal subpopulation model in which glutamatergic and GABAergic neurons, under the control of the CB_1 receptor, seem to be differently sensitive to cannabinergic drugs.

Statement of conflicts of interest

The authors declare to have no conflicts of interest.

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