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Abstract: The endocannabinoid CB1 receptor has been implicated in the inhibitory control of learning and memory. In the present experiment, we compared the behavioral response of CB1 receptor knockout mice (CB1R-/-) with animals administered CB1 receptor antagonist/inverse agonist SR141716A (rimonabant; 3 mg/kg IP, 30 min pre-trial) in terms of acquisition and retention of a habituation task and changes in cerebral monoamines. The results can be summarized as follows: (i.) The acute and chronic invalidation of the CB1 receptor resulted in an increase of behavioral habituation during the first exposure to an open field, indicative of enhanced acquisition of the task; (ii.) CB1R-/- mice, but not rimonabant-treated animals, showed enhanced long-term retention of the habituation task when tested 48 hours and 1 week subsequent to the first exposure to the open field, respectively; (iii.) The facilitation of long-term retention of the

habituation task in CB1R-/- mice was accompanied by a selective and site-specific increase in serotonin activity in hippocampus; and (iv.) Rimonabant-treated animals displayed 'antidepressant-like' alterations of cerebral monoamines, that is, most parameters of monoaminergic activity were increased especially in dorsal striatum and hippocampus. Taken together, the present findings demonstrate that the genetic disruption of the CB1 receptor gene can cause an improvement of behavioral habituation, which is considered to represent a form of 'non-associative' learning. Furthermore, the data support our assumption of a rimonabant-sensitive cannabinoid receptive site that is different from the 'classical' CB1 receptor and which, under physiological conditions, might be involved in the inhibitory control of the acquisition but not retention of non-associative learning tasks.

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21/05/07

Prof. P.E. Gold
Editor-in-Chief
Neurobiology of Learning and Memory

Dear Prof. Gold,

Attached is a copy of a manuscript by Thiemann et al. on "*The genetic versus pharmacological invalidation of the cannabinoid CB_1 receptor results in differential effects on 'non-associative' memory and forebrain monoamine concentrations in mice*".

Please consider publication of the manuscript as a regular article in *Neurobiology of Learning and Memory*.

Yours sincerely,

Dr. Rüdiger Hasenöhrl
Reader in Neuroscience

The genetic versus pharmacological invalidation of the cannabinoid CB_1 receptor results in differential effects on 'non-associative' memory and forebrain monoamine concentrations in mice

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Running title: The CB_1 cannabinoid receptor and its role in habituation learning

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Abstract - The endocannabinoid CB_1 receptor has been implicated in the *inhibitory* control of learning and memory. In the present experiment, we compared the behavioral response of CB_1 receptor knockout mice ($CB_1R^{-/-}$) with animals administered CB_1 receptor antagonist/inverse agonist SR141716A (Rimonabant; 3 mg/kg IP, 30 min pre-trial) in terms of acquisition and retention of a habituation task and changes in cerebral monoamines. The results can be summarized as follows: (i.) The acute and chronic invalidation of the CB_1 receptor resulted in an increase of behavioral habituation during the first exposure to an open field, indicative of enhanced acquisition of the task; (ii.) $CB_1R^{-/-}$ mice, but not rimonabant-treated animals, showed enhanced long-term retention of the habituation task when tested 48 hours and 1 week subsequent to the first exposure to the open field, respectively; (iii.) The facilitation of long-term retention of the habituation task in $CB_1R^{-/-}$ mice was accompanied by a selective and site-specific increase in serotonin activity in hippocampus; and (iv.) Rimonabant-treated animals displayed 'antidepressant-like' alterations of cerebral monoamines, that is, most parameters of monoaminergic activity were increased especially in dorsal striatum and hippocampus. Taken together, the present findings demonstrate that the genetic disruption of the CB_1 receptor gene can cause an improvement of behavioral habituation, which is considered to represent a form of 'non-associative' learning. Furthermore, the data support our assumption of a rimonabant-sensitive cannabinoid receptive site that is different from the 'classical' CB_1 receptor and which, under physiological conditions, might be involved in the *inhibitory* control of the acquisition but not retention of non-associative learning tasks.

Keywords: Rimonabant; CB_1-knockout; Hippocampus; Habituation; Biogenic amines

Introduction

The endocannabinoid system is an important neuromodulatory system in the brain. Several neuropsychological functions are under control of the cannabinoid receptor subtype 1 (CB_1 receptor) and of its endogenous lipid ligands anandamide and 2-arachidonoylglycerol, respectively. CB_1 receptors are present at different levels in several brain regions like frontal cortex, dorsal/ventral (nucleus accumbens) striatum, hippocampal formation, septum, and amygdala complex (Fride, 2005; Marsicano & Lutz, 2006). At physiological and pathophysiological level, endocannabinoids have been shown to play regulatory roles in several 'integrative' behavioral responses including locomotion, anxiety- and depressive-like states as well as cardiovascular, feeding and pain control (Pacher, Batkai & Kunos, 2006). Of special interest is the potential role of endocannabinoids in 'cognitive' functioning, that is, neural processes related to learning as well as memory consolidation and retention. A follow-up of behavioral experiments using animals with a genetic or pharmacological blockade of the CB_1 receptor indicates that under physiological conditions endocannabinoids might exert an *inhibitory* control over mnemonic processes. Thus, reducing the endocannabinoid tone by either destruction of the CB_1 receptor gene or by blocking endocannabinoid transmission at CB_1-receptive sites resulted in a facilitation of learning and memory processing (for review see Lichtman, Varvel & Martin, 2002).

Up to now, almost all experiments aimed at investigating the functional role of (endo)cannabinoids in mnemonic functions were conducted with animal models of 'associative' learning and memory, that is, examining cannabinoid effects on acquisition, retention and extinction of tasks with explicitly defined stimulus-response contingencies

like the radial-arm (Lichtman, 2000) and Morris water maze (Varvel & Lichtman, 2002), inhibitory avoidance (Barros, Carlis, Maidana, Silva, Baisch, Ramirez & Izquierdo, 2004), and fear conditioning (Marsicano, Wotjak, Azad, Bisogno, Rammes, Cascio, Hermann, Tang, Hofmann, Zieglgansberger, Di Marzo & Lutz, 2002). The role of the endocannabinoid tone in 'non-associative' learning function remains to be elucidated. Consequently, the aim of the present study was to examine the effects of genetic and pharmacological blockade of the CB_1 receptor with rimonabant on behavioral habituation, a form of non-associative learning. In rodents, habituation can be measured by examining exploratory behavior in a novel environment and is reflected by a decrease in vertical activity (rearing) over time. With regard to the work cited above, we held it possible that the genetic and pharmacological blockade of the CB_1 receptor could amplify the rate of habituation acquisition and retention. However since previous studies from our lab provided evidence for differential effects of a CB_1 receptor knockout versus rimonabant treatment in various behavioral essays (Thiemann, van der Stelt, Petrosino, Molleman, Di Marzo & Hasenöhrl, 2007a; Thiemann, Ledent, Molleman & Hasenöhrl, 2007b), this assumption needed to be tested. Furthermore, monoaminergic neurons are crucially involved in the control of behavioral processes related to exploration, anxiety, learning, and memory (Graeff, 2002; Rolls, 2000) and our recent studies showed that the genetic and pharmacological manipulation of the endocannabinoid system influenced cerebral monoamine concentrations and turnover rates (Thiemann et al., 2007b). Therefore, we expected to find changes in the concentrations of dopamine (DA), serotonin (5-HT), and their metabolites in the brain of $CB_1R^{-/-}$ and rimonabant-treated mice.

Materials and methods

Subjects

The experiments were carried out in accordance with the Animals Scientific Procedures Act 1986 and were approved by the U.K. Home Office. CB_1 receptor knockout ($CB_1R^{-/-}$) mice were used which were bred in-house from CD1 backcrossed mice (Ledent, Valverde, Cossu, Petitet, Aubert, Beslot, Böhme, Imperato, Pedrazzini, Roques, Vassart, Fratta & Parmentier, 1999). The $CB_1R^{-/-}$ mice and their littermate controls ($CB_1R^{+/+}$) were obtained by intercrossing heterozygous ($CB_1R^{+/-}$) breeding pairs; they were 3 month old and weighed 30-35 g. Genotyping was performed by a PCR-based assay using DNA extracted from the tail. Subjects were drug- and test-naive, and were used only once. The animals were maintained on a normal 12 hr light/dark cycle (lights on at 7.30 am) and were tested during the light phase. They were housed 4-5 per cage and were handled and weighed daily for 7 days before the start of the experiments.

Drugs

Rimonabant (SR141716A; Sanofi-Synthelabo, France; Rinaldi-Carmona, Barth, Heaulme, Alonso, Shire, Congy, Soubrie, Breliere & Le Fur 1995) was dissolved in 0.9% saline containing 2% ethanol. Rimonabant was administered at a dosage of 3 mg/kg which proved effective in previous studies investigating the effects of the compound in terms of locomotor activity (Compton, Aceto, Lowe & Martin, 1996), fear/anxiety (Haller, Bakos, Szirmay, Ledent & Freund, 2002), and psychostimulant-induced behavioral sensitization (Thiemann et al., 2007a). The animals of the control

group received the vehicle and all injections were IP in a volume of 5.0 ml/kg body weight.

Apparatus and Behavioral Procedure

Habituation was measured in square open-field compartments (40 x 40 x 50 cm; black floor and walls) which were set up in a sound-protected experimental chamber adjacent to the animal holding facility. The open-field compartments were placed on top of an under light that provided infrared illumination to a closed-circuit video camera mounted 2 m above the apparatus. The digitized image of the path taken by each animal was stored and analyzed *post hoc* with a video tracking system (EthoVision; Noldus, The Netherlands) which determined the position of the animal in the open field 5 times per second. Thirty minutes before the first exposure to the open field (acquisition), wild-type mice were administered either rimonabant (3 mg/kg; IP) or vehicle; the $CB_1R^{-/-}$ mice received vehicle only. After the first exposure, the mice were re-exposed to the open field after three time intervals: at 4 hours after acquisition, in order to assess short-term memory (STM), and at 48 hours and 1 week after the first exposure, in order to assess long-term memory (LTM) of the task, respectively (Izquierdo, Medina, Vianna, Izquierdo & Barros, 1999). Thirty min prior to the retention tests, animals were administered vehicle ($CB_1R^{-/-}$ mice) or 3 mg/kg rimonabant IP ($CB_1R^{+/+}$ mice). Two behavioral parameters, *rearing* (i.e. standing on the hind legs with the forelegs in the air or against the wall) and *locomotion* (i.e. horizontal movements in m), were registered during each 5-min session (see Gerhardt, Hasenöhrl, Hock & Huston, 1993, for details). To determine the amount of habituation during the 4 hour, 48 hour and 1-week follow-up

sessions, the amount of rearing behavior was expressed as percentages of the corresponding values of the control group. Furthermore, the *sojourn time* in the central zone (20 x 20 cm) of the open field was measured to assess possible anxioactive properties of the genetic manipulation and pharmacological treatment, respectively.

Neurochemical analysis

After the end of behavioral testing the animals of the different treatment groups underwent *post-mortem* neurochemical analysis. The animals were decapitated, their brains were quickly removed, and the medial frontal cortex (cortical tissue anterior to the genus of the corpus callosum), ventral striatum (nucleus accumbens, olfactory tubercle, anterior parts of ventral pallidum), neostriatum (anterior parts of caudate-putamen with globus pallidus as posterior border) and the hippocampus (anterior parts of the hippocampal formation with CA1, CA3 and dentate gyrus), were dissected out bilaterally on ice. Following dissection, the samples of brain tissue were weighed, placed in plastic tubes containing 0.5 ml of 0.1 M perchloric acid, and then homogenized and centrifuged. The resulting supernatant was filtered through 0.2 µm syringe filters (Chromacol, UK) and the extracts were stored at -70°C until HPLC-EC analysis. The tissue samples were analyzed for norepinephrine (NA), serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), dopamine (DA), dihydrophenylacetic acid (DOPAC) and homovanillic acid (HVA) levels. The HPLC system consisted out of a Waters 1525 Binary HPLC pump, a Waters 717 plus autosampler, a Waters 2465 Electrochemical Detector and a spherisorb 5 µm analytical column ODS2 (4.6 x 250 mm; Waters, U.K.) set at 29°C. The flow cell consisted out of a glassy carbon working electrode, 2.0 mm in

diameter, and an ‘in situ’ silver reference electrode. The mobile phase consisted out of 920 ml double distilled water, 6.599 mg sodium dihydrogen phosphate (NaH2PO4; Sigma, U.K.), 197.2 mg Pic B8 (containing water, octane sulfonic acid, methyl alcohol and acetic acid; Waters, U.K.), 8 ml acetonitrile (CH3CN; Sigma, U.K.) adjusted to a pH of 2.9 with 0-Phosphoric acid. The mobile phase was filtered using 0.2 µm disc filters (Sigma-Aldrich, UK) and degassed using nitric oxygen (NO). The mobile phase flow rate was 0.9 ml/min and a Waters 2465 EC detector was set at 0.7 mV. To quantify the sample peaks each chemical (NA, DA, DOPAC, HVA, 5-HT, 5-HIAA) was compared with external standards (Sigma, U.K.) that were prepared freshly and injected before and after each sample run. The tissue samples were analyzed for 5-HT (serotonin), 5-hydroxyindole acetic acid (5-HIAA), dopamine, dihydrophenylacetic acid (DOPAC), and homovanillic acid (HVA) levels using HPLC with electrochemical detection (see Thiemann et al., 2007b for technical details).

Statistical analysis

For the analysis of behavioral and neurochemical data, Mann-Whitney *U* tests (two-tailed) for independent measures were used.

Results

Habituation to novelty

Figure 1 depicts the amount of locomotor activity and the number of rearing measured for each group during the first 5-min exposure to the open field (acquisition) expressed as successive 1 min intervals. The three treatment groups showed a gradual decrease

in horizontal locomotor activity across time (Fig. 1A.), which was accompanied by a steady increase of vertical activity during the first 3 min of the observation period (Fig. 1B.). However, during the last two minutes $CB_1R^{-/-}$ mice as well as animals administered rimonabant showed less rearing compared with vehicle controls indicative of enhanced acquisition of the habituation task ($0.03 < P$-values < 0.05). The three groups did not differ from each other in the time spent in the centre of the open field (range: 40.6 ± 6.9 to 55.4 ± 8.0 s; P-values > 0.05). The effects of the acute and chronic blockade of the CB_1 receptor on short and long-term retention of the habituation task are shown in Figure 2A. The mice administered rimonabant in a dosage of 3 mg/kg did not differ from controls in the amount of rearing when re-exposed to the open field after each of the three different inter-trial intervals (corresponding P-values > 0.05). No significant change in vertical activity was observed in $CB_1R^{-/-}$ mice when tested 4 hours after the first exposure ($P > 0.05$); however a marked decrease in rearing was apparent after 48 hours ($P = 0.01$ vs. Controls; $P = 0.04$ vs. rimonabant treated mice) as well as after 1 week following the acquisition trial ($P = 0.04$ vs. Controls), suggestive of a CB_1 receptor disruption induced amplification of long-term retention performance. During the three different retention test trials, the groups of animals did not significantly differ from each other in gross locomotor activity (Fig. 2B) as well as in sojourn time in the centre of the open field (Fig. 2C; corresponding P-values > 0.05).

Brain monoamines

In $CB_1R^{-/-}$ mice, a decrease in serotonin concentrations was observed in hippocampus ($P = 0.05$) which was accompanied by an increase in 5-HT (5-HIAA/5-HT) turnover ($P =$

0.046). On the other hand, in animals administered rimonabant concentrations of dopamine, DOPAC and 5-HIAA were enhanced in hippocampus (DA: $P = 0.02$; DOPAC: $P = 0.005$; 5-HIAA: $P = 0.012$) and dorsal striatum (DA: $P = 0.003$; DOPAC: $P = 0.02$; 5-HIAA: $P = 0.001$); serotonin concentrations were increased in the dorsal striatum ($P = 0.005$). Apparent differences between rimonabant-treated and $CB_1R^{-/-}$ mice were evident with regard to frontal cortex 5-HT turnover, which was decreased in the latter group ($P = 0.05$); furthermore, $CB_1R^{-/-}$ mice had lower concentrations of DA ($P = 0.026$), 5-HT ($P = 0.046$) and 5-HIAA ($P = 0.038$) in hippocampus and reduced levels of DA ($P = 0.015$), HVA ($P = 0.016$), 5-HT ($P = 0.009$) and 5-HIAA levels ($P = 0.015$) in dorsal striatum compared with the rimonabant treated group.

Discussion

Our data are in line with the results of a recent study showing that the deletion of the CB_1 receptor can improve non-associative learning (Degroot, Salhoff, Davis & Nomikos, 2005) and support the general idea that endocannabinoids are involved in the *inhibitory* control of learning and mnemonic functions. Accordingly, we found that the functional invalidation of the CB_1 receptor gene resulted in a facilitation of both acquisition and long-term retention of habituation. On the other hand, the acute blockade of the CB_1 receptor with rimonabant enhanced acquisition of habituation but failed to amplify long-term retention of the task. The differential effect of a genetic invalidation versus pharmacological blockade of the CB_1 receptor with rimonabant on habituation is not surprising in the light of our previous studies demonstrating similar differences with regard to behavioral sensitization (Thiemann et al., 2007a) and emotional reactivity

(Thiemann et al., 2007b). Here, rimonabant potentiated psychostimulant-sensitization and increased anxiety whereas the genetic invalidation of the CB_1 receptor had opposite effects and reduced behavioral sensitization and failed to influence fear and anxiety-related behaviors. Thus, our data supports the assumption of a specific rimonabant-sensitive cannabinoid receptive site that is different from the 'classical' CB_1 receptor (Di Marzo, Breivogel, Tao, Bridgen, Razdan, Zimmer, Zimmer & Martin, 2000) which, under physiological conditions, seems to be involved in the inhibitory control of the acquisition but not retention of non-associative learning.

It is significant to note that the results observed in $CB_1R^{-/-}$ mice and rimonabant-treated animals were not confounded by non-specific effects on general locomotor activity and emotional reactivity, as the two groups of mice had the same activity level and central sojourn time during the different habituation sessions compared to vehicle controls. Thus, enhanced habituation learning and retention rather than motoric deficiency and/or changes in the emotional state may account for the observed decrease in exploratory behavior. The fact that habituation was improved is of general importance for the evaluation of the role of the endocannabinoid tone in associative learning and memory processes (Barros et al., 2004; Lichtman, 2000; Marsicano et al., 2002), since for one habituation does not involve application of conventional reinforcers, such as food or escape from or avoidance of aversive stimulation and secondly since endocannabinoids are known to play a pivotal role in stress, fear/anxiety and pain control (Pacher et al., 2006 for review), which are important factors in aversive conditioning, but not for habituation. This makes an interpretation of the performance enhancement following CB_1 receptor invalidation by genetic or pharmacological means

simply in terms of an interaction between the genetic/pharmacological manipulation and physiological processes induced by a punishing/aversive stimulus, unlikely.

Brain monoaminergic systems are known to play important roles in processes underlying learning and memory. In our $CB_1R^{-/-}$ mice, the concentration of serotonin was decreased in the hippocampus while 5-HIAA/5-HT turnover rates were increased in this brain region suggestive of an enhanced indolamine tone. It is accepted that exploration of an unfamiliar environment (Vanderwolf's (1969) Type I, 'theta' behavior, including walking, sniffing, and rearing) is associated with increased dentate field excitatory postsynaptic potentials (fEPSPs) to perforant path stimulation (Weiler, Hasenöhrl, van Landeghem, van Landeghem, Brankack, Huston & Haas, 1998). Furthermore, it was found recently that exposure to a novel environment is paralleled by increased hippocampal 5-HT turnover (Storey, Robertson, Beattie, Reid, Mitchell & Balfour, 2006). Based on the results of only these few studies, detailed considerations regarding the relationship between CB_1 receptor function, monoamines, and behavioral habituation would be premature. However, because CB_1 receptor gene disruption led to enhanced habituation learning/retention and increased serotonin turnover in the hippocampus, one could speculate that the behavioral effects of the CB_1 receptor gene disruption might be partly related to changes in monoaminergic correlates involved in the process of suppressing the biological significance of and attention to a repeatedly presented stimulus configurations. Rimonabant is known to augment the synaptic concentration of biogenic amines similarly to antidepressant drugs, that is, it enhances the synaptic availability of serotonin and, to a lesser extent, dopamine in the brain (Witkin, Tzavara & Nomikos, 2005). Our post-mortem neurochemistry results are in line with the proposed

'antidepressant-like' neurochemical profile of the compound. The injection of rimonabant resulted in a marked increase in monoamine concentrations in the hippocampus and dorsal striatum, respectively, which might be instrumental to the observed facilitation of the acquisition of the habituation task (Thiel, Müller, Huston & Schwarting, 1999). Furthermore, a clear distinction in the neurochemical profile between $CB_1R^{-/-}$ mice and rimonabant-treated animals was apparent providing additional neurochemical evidence for the existence of a rimonabant-sensitive receptive site in the brain, which is different to the classical CB_1 receptor.

What are the functional implications of the present results in terms of learning and memory retention? Our findings provide supportive evidence for the hypothesis that the endocannabinoid system is involved in the *inhibitory* control of associative processing in a way similar to endogenous opiates (Izquierdo, 1982) and neuronal histamine (Huston, Wagner & Hasenöhrl, 1997). $CB_1R^{-/-}$ mice displayed enhanced acquisition and prolonged retention of the habituation task compared to wild-type controls and this inability to forget may result in behavioral perseveration evidenced in more complex learning paradigms like the Morris water maze (Varvel & Lichtman, 2002). Thus, it is feasible that cannabinoids disrupt memory through a CB_1 receptor mechanism of action, and that under physiological conditions the endocannabinoid system may have a paramount role in facilitating extinction and/or forgetting processes (Marsicano et al., 2002).

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FIGURE LEGENDS

Figure 1. Temporal effect of genetic and pharmacological disruption of the CB_1 receptor on A. the mean (+SEM) distance moved and B. the mean (±SEM) number of rearing during the first exposure to the open field (acquisition trial). $CB_1R^{+/+}$ mice were administered rimonabant (3 mg/kg) or vehicle (controls; 0.5 ml/kg; IP) 30 min pre-trial. $CB_1R^{-/-}$ animals received vehicle only. The 5-min test period is depicted as successive 1-min intervals. Sample size was 8 per group. Measure of effect: $^{*}P < 0.05$, indicative of superior acquisition of the habituation task.

Figure 2. Effect of genetic and pharmacological disruption of CB_1 receptor on A. the number of rearing expressed as mean (-SEM) percentage of $CB_1^{+/+}$ controls, B. mean (+SEM) horizontal locomotor activity, and C. mean (+SEM) sojourn time in the centre of the open field. Measurements were taken 4 hours, 48 hours, and 1 week after the first exposure to the open field, respectively. Raw data of rearing for controls was: 4 hours = 29.87 ± 3.54; 48 hours = 41.52 ± 4.05; 1 week = 39.49 ± 3.54. Sample size was 8 per group. Measure of effect based on statistical analysis of raw data: $^{*}P < 0.05$ vs. controls and $^{\#}P < 0.05$ vs. rimonabant-treated animals, indicative of superior long-term retention of the habituation task.

Figure 1

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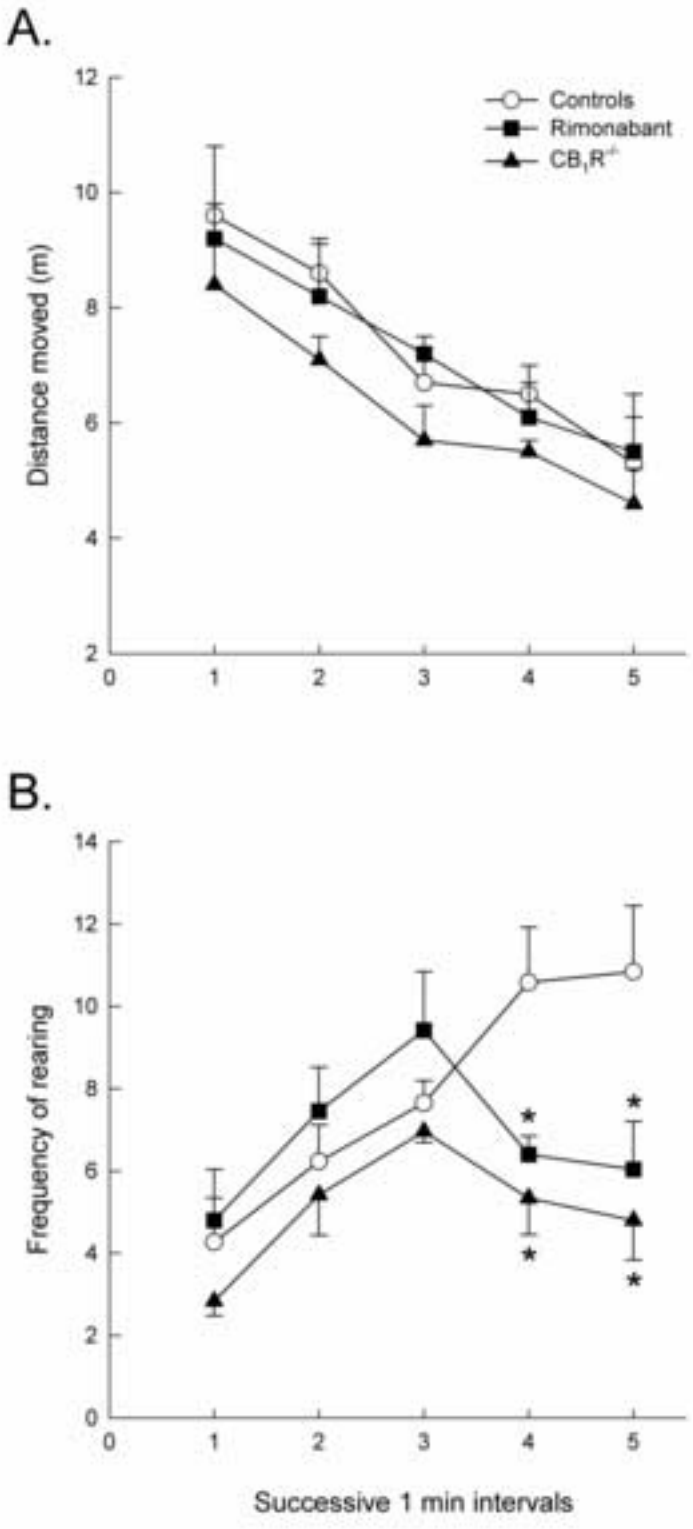

Figure 2

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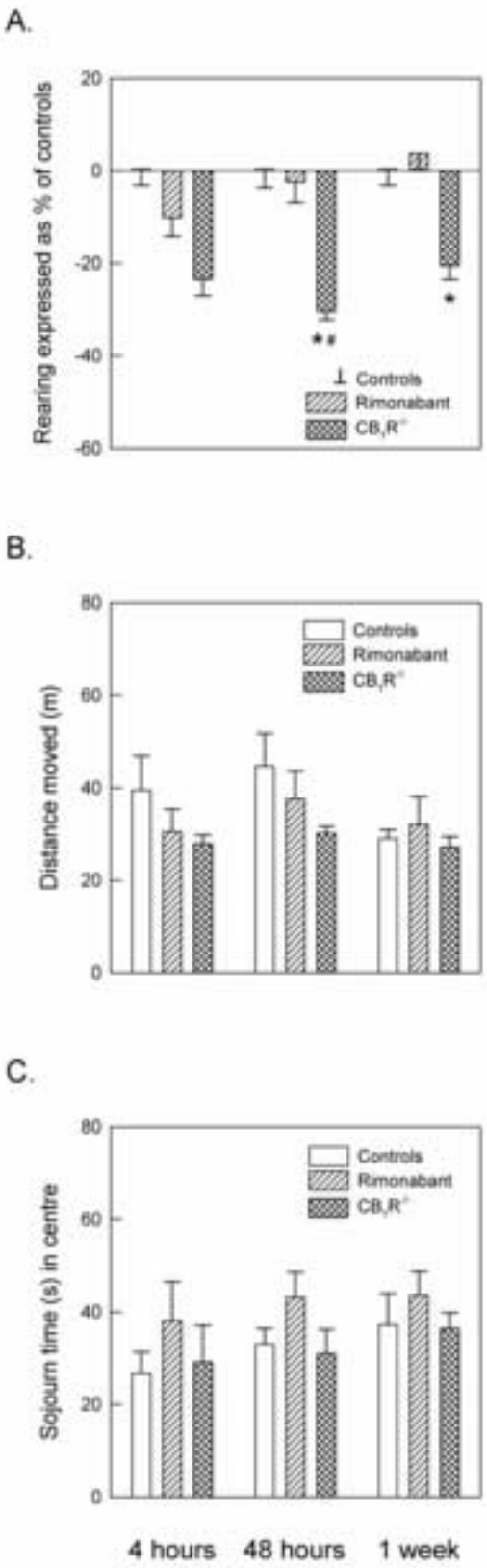

Table 1. Means and SEMs for the *ex vivo* measurements (in nanograms per milligram) and turnover quotients obtained upon animals sacrificed after the last 5-min test trial in the habituation task

Treatment/ brain region	DA	DOPAC	HVA	5-HT	5-HIAA	DOPAC/ DA	HVA/ DA	5-HIAA/ 5-HT
Frontal cortex								
Controls	1.01±0.50	0.41±0.17	*n.d.*	1.29±0.25	0.56±0.11	0.50±0.06	*n.d.*	0.33±0.06
Rimonabant 3 mg/kg	1.31±0.64	0.42±0.08	*n.d.*	1.79±0.47	0.73±0.19	0.73±0.19	*n.d.*	0.41±0.02
$CB_1R^{-/-}$	2.57±1.93	0.57±0.31	*n.d.*	1.67±0.35	0.63±0.12	0.42±0.07	*n.d.*	0.34±0.01$^{\#}$
Hippocampus								
Controls	0.25±0.06	0.12±0.03	*n.d.*	1.01±0.17	1.38±0.16	0.44±0.08	*n.d.*	1.35±0.10
Rimonabant 3 mg/kg	0.60±0.11*	0.65±0.38**	*n.d.*	2.10±0.63	3.31±0.91*	1.51±1.06	*n.d.*	1.19±0.31
$CB_1R^{-/-}$	0.26±0.07$^{\#}$	0.28±0.10	*n.d.*	0.68±0.14*$^{\#}$	1.39±0.24$^{\#}$	0.54±0.09	*n.d.*	2.01±0.11*
Dorsal striatum								
Controls	16.81±1.36	3.46±0.56	2.00±0.42	0.75±0.09	0.42±0.06	0.20±0.03	0.12±0.02	0.55±0.03
Rimonabant 3 mg/kg	47.81±6.39**	10.49±2.27*	6.05±1.27*	2.05±0.26**	1.12±0.17**	0.21±0.04	0.10±0.03	0.55±0.04
$CB_1R^{-/-}$	19.07±1.25$^{\#}$	3.48±0.22	2.42±0.12$^{\#}$	0.89±0.12$^{\#\#}$	0.55±0.11$^{\#}$	0.18±0.01	0.14±0.01	0.60±0.04
Ventral striatum								
Controls	21.81±1.79	3.93±0.35	0.95±0.12	1.47±0.33	*n.d.*	0.18±0.01	0.43±0.03	*n.d.*
Rimonabant 3 mg/kg	20.93±2.12	3.90±0.43	0.90±0.11	1.23±0.29	*n.d.*	0.19±0.01	0.42±0.01	*n.d.*
$CB_1R^{-/-}$	18.90±4.44	4.06±0.90	1.12±0.13	1.45±0.11	*n.d.*	0.27±0.06	0.52±0.01	*n.d.*

Note: Measure of effect; $^{**}P \leq 0.01$, $^{*}P \leq 0.05$ vs. $CB_1R^{+/+}$ Controls; $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ vs. Rimonabant-treated animals; number of animals per group = 6 to 8; *n.d.*, not determined.