Altered cannabinoid CB_1 receptor mRNA expression in mesencephalon from mice exposed to repeated methamphetamine and methanandamide treatments

Leos Landa [1], Michal Jurajda [2], Alexandra Sulcova [3]

1 University of Veterinary and Pharmaceutical Sciences Brno, Department of Pharmacology and Pharmacy, Czech Republic
2 Masaryk University Brno, Department of Pathological Physiology, Czech Republic
3 CEITEC – Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Correspondence to: Prof. Alexandra Sulcova, MD., PhD.
CEITEC – Central European Institute of Technology, Masaryk University
Kamenice 5/A19, 625 00 Brno, Czech Republic.
TEL: +420 5 4949 7610; FAX: +420 5 494 2364; E-MAIL: sulcova@med.muni.cz

Submitted: 2011-10-02 Accepted: 2011-11-10 Published online: 2012-01-15

Key words: **behavioural sensitization; methamphetamine; methanandamide; CB_1 receptor mRNA expression; mouse mesencephalon**

Neuroendocrinol Lett 2011; **32**(6):841–846 **PMID:** 22286781 NEL320611A07

Abstract

OBJECTIVES: Since among others also our previous studies suggested an interaction between the endocannabinoid system and methamphetamine brain mechanisms we focused on possible changes in relative expression of cannabinoid CB_1 receptor mRNA in mesencephalon from mice sensitized by repeated treatments to methamphetamine stimulatory effects and cross-sensitized by cannabinoid CB_1 receptor agonist methanandamide pre-treatment.

METHODS: The Open Field Test was used to measure changes in terms of behavioural sensitization or cross-sensitization to drug effects on locomotion in male mice treated repeatedly with either methamphetamine or methamphetamine after pre-treatment with methanandamide. After each measurement one third of animals were sacrificed and the brain was stored. RNA was isolated from the midbrain and used for reverse transcription and subsequent real-time PCR.

RESULTS AND CONCLUSION: The evaluation of behavioural drug effects showed both development of sensitization to methamphetamine stimulatory effects after repeated treatment and cross-sensitization to them by pre-treatment with cannabinoid receptor CB_1 agonist methanandamide. Real-time PCR analyses revealed an increase in CB_1 receptor mRNA expression after the first dose of methanandamide followed by decrease after the combined treatment with methamphetamine challenge dose. Our findings suggest that particularly repeated pre-treatment with CB_1 agonist methanandamide can elicit increase in the mRNA expression level at least in the mouse mesencephalon neurons associated with cross-sensitization to methamphetamine stimulatory effects.

ORIGINAL ARTICLE

Abbreviations:

Bmax	- maximal binding capacity
CAN	- mice after the 1st dose of methanandamide
CAN/M	- mice sensitized with methanandamide after the challenge dose of methamphetamine
DA	- dopamine
GAPDH	- glyceraldehyde-3-phosphate dehydrogenase
M	- mice after the 1st dose of methamphetamine
M/M	- mice sensitized with methamphetamine after the challenge dose of methamphetamine
V	- mice after the dose of vehicle
VTA	- ventral tegmental area

INTRODUCTION

Repeated administration of various psychotropic drugs can elicit behavioural sensitization – a phenomenon characterised by gradually increasing response to the drug (Robinson & Berridge 1993). This phenomenon has been well described for majority of addictive substances including amphetamines (Kameda *et al.* 2011) and cannabinoids (Rubino *et al.* 2003). An increased response to the tested drug may be also elicited by previous repeated administration of a drug different from the drug tested, which is termed as cross-sensitization. Cross-sensitization was observed, for example, after repeated treatment with tetrahydrocannabinol to heroin (Singh *et al.* 2005).

It has been identified, that the crucial neuronal circuits essential for the development of sensitization involve namely dopaminergic, glutamatergic, GABAergic and serotonergic projections between VTA, nucleus accumbens, prefrontal cortex, hippocampus and amygdala (Ago *et al.* 2008). Particularly, the mesolimbic dopaminergic projection from the VTA to nucleus accumbens is considered as the most important for effects associated with reward properties of abused drugs (Kalivas *et al.* 1993). Stimulation of cannabinoid CB_1 receptors present on GABAergic and glutamatergic nerve terminals negatively regulates the release of GABA and glutamate and that way influence the mesolimbic DA functions (Chiang & Chen 2007). The endocannabinoid system consists of cannabinoid receptors (CB_1, CB_2), their endogenous ligands (endocannabinoids), and enzymes for their biosynthesis and degradation. It is known, that CB_1 receptors located in VTA on presynaptic glutamatergic and GABAergic neurons act as retrograde inhibiting modulators and influence their input to VTA dopaminergic neurons which is believed to activate the reward pathway of addictive substances (Maldonado *et al.* 2006).

The first results from our laboratory suggesting an interaction between the endocannabinoid system and methamphetamine brain mechanisms were obtained in the rat I.V. drug self-administration model (Vinklerova *et al.* 2002). Later we have created an original experimental paradigm showing development of behavioural sensitization to psychostimulant methamphetamine effects and also cross-sensitization elicited by cannabinoid CB_1 receptor agonist methanandamide pretreatment (Landa *et al.* 2006a;b) confirming that there exists some relationship between the endocannabinoid system and methamphetamine effect processing.

The present study was designed with respect to results obtained in our previous behavioural studies as well as in the preliminary pilot studies focusing on CB_1 receptor expression (Landa & Jurajda 2007a;b) and density (Sulcova *et al.* 2007) in rodent mesencephalon, and to data confirming that structures responsible for the development of behavioural sensitization to psychostimulants (including methamphetamine) are parts of mesencephalon (namely VTA) with high CB_1 receptor density (Ago *et al.* 2008). The attention was focused on possible changes revealed by quantitative polymerase chain reaction (qPCR) in relative expression of CB_1 receptor mRNA in mouse mesencephalon during a) sensitization to methamphetamine and b) cross-sensitization to methamphetamine induced by repeated pretreatment with CB_1 receptor agonist methanandamide.

MATERIAL AND METHODS

Animals

Male mice (strain ICR, TOP-VELAZ s. r. o., Prague, Czech Republic) with an initial weight of 18–21 g were used. They were randomly allocated into two treatment groups. Experimental sessions in the behavioural part of the experiment were always performed in the same light period between 1:00 p.m. and 3:00 p.m. in order to minimise possible variability due to circadian rhythms.

Apparatus

Locomotor activity was measured using an open-field equipped with Actitrack (Panlab, S.L., Spain). This device consists of two square-shaped frames that deliver beams of infrared rays into the space inside the square. A plastic box is placed in this square to act as an open-field arena (base 30 × 30 cm, height 20 cm), in which the animal can move freely. The apparatus software records locomotor activity of the animal by registering the beam interruptions caused by movements of the body. Using this equipment we have determined the Distance Travelled (trajectory in cm per 3 minutes).

Drugs

Vehicle and all drugs were always given in a volume adequate to drug solutions (10 ml/kg).

(+)-Methamphetamine, (d-N,α-Dimethylphenylethylamine; d-Desoxyephedrine), (Sigma Chemical Co.) dissolved in saline.

(R)-(+)-Methanandamide, (R)-N-(2-hydroxy-1-methylethyl)-5Z, 8Z, 11Z-eicosotetraenamide) supplied pre-dissolved in anhydrous ethanol 5 mg/ml (Tocris Cookson Ltd., UK) was diluted in saline to the concentration giving the chosen dose to be administered to animals in a volume of 10 ml/kg; vehicle therefore contained an adequate part of ethanol (a final concen-

tration in the injection below 1%) to make effects of placebo and the drug comparable.

Procedure

Mice were randomly divided into 2 groups (n_1=24, n_2=24) and all were given vehicle on Day 1 (10 ml/kg). There were no applications from Days 2 to 6. For the next seven days animals were daily treated intraperitoneally as follows: a) n_1: methamphetamine at the dose of 2.5 mg/kg/day, b) n_2: methanandamide at the dose of 0.5 mg/kg. On Day 14 all animals were given intraperitoneally methamphetamine at the dose of 2.5 mg/kg (challenge dose).

Changes in locomotion were measured for the period of 3 minutes in the open field on Days 1 (1st record), 7 (2nd record) and 14 (3rd record) 15 minutes after drug application to assess sensitizing phenomenon. After each measurement one third of both groups was decapitated (75 minutes after drug administration) and the brain was stored in RNAlater (Ambion). For RNA isolation we used excised mesencephalon only. The total RNA was isolated by means of RNAEasy Mini Kit (Qiagene) and the subsequent reverse transcription was performed with Omniscript RT Kit (Qiagene) and RNAse OUT Ribonuclease Inhibitor (Invitrogen). Relative expression of CB_1 receptor (assay Mn00432621_s, Life Technologies) was compared to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (assay Mn99999915_1g, Life Technologies) using real time cycler ABI SDS 7000 (AppliedBiosystems). All real time PCR reactions were performed using TaqMan Gene Expression Master Mix (Life Technologies).

Data analysis

As the data was not normally distributed (according to the Kolmogorov-Smirnov test of normality), non-parametric statistics were used: Mann-Whitney U test, two-tailed (statistical analysis package STATISTICA – StatSoft, Inc., Tulsa, USA).

RESULTS

In the behavioural part of the study (Figure 1), the treatments in the group n_1 caused significant increase (p<0.01) in locomotion after the 1st application of methamphetamine (M) compared to the application of vehicle (V1) (see Figure 1; V1 versus M). The challenge dose of M produced a significant increase in Distance Travelled (p<0.05) in animals pre-treated repeatedly with M when compared to the animals after the 1st application of M (see Figure 1; M versus M/M).

The 1st applications of methanandamide (CAN) compared to the application of vehicle (V2) evoked in the group n_2 significant decrease (p<0.01) in locomotion (see Figure 1, V2 versus CAN). The challenge dose of M produced a significant increase in Distance Travelled (p<0.01) in animals pre-treated repeatedly with

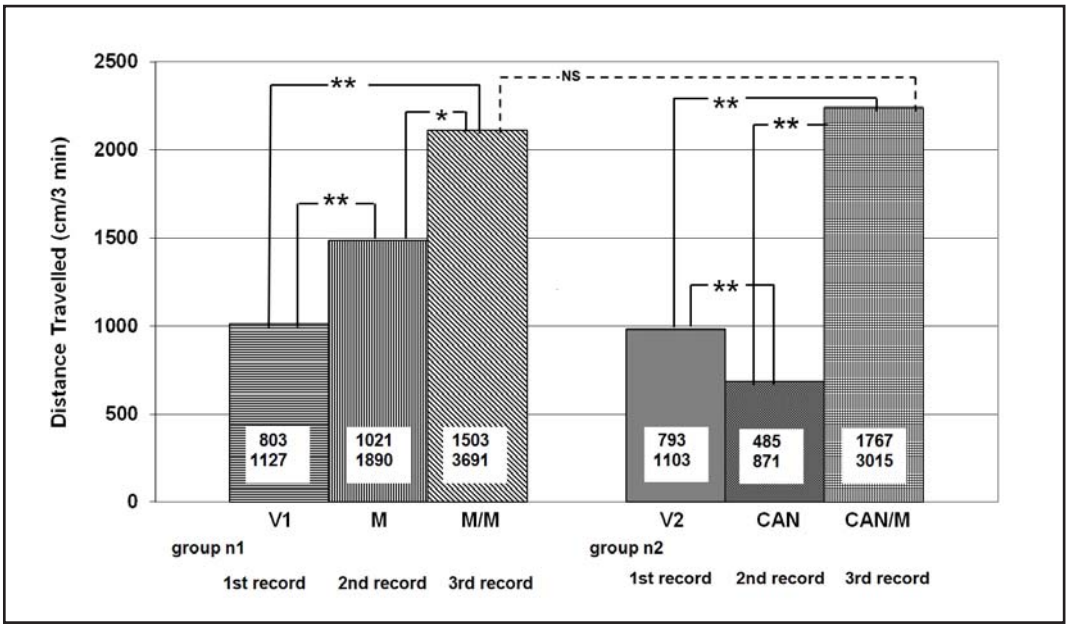

Fig. 1. Effects of drug treatments on Distance Travelled (cm/3 min) in the mouse open field test shown as median (interquartile range Q1 to Q3):
V1 = mice after the dose of vehicle in the group n_1, V2 = mice after the dose of vehicle in the group n_2, M = mice after the 1st dose of methamphetamine (2.5 mg/kg), M/M = mice sensitized with methamphetamine after the challenge dose of methamphetamine (2.5 mg/kg), CAN = mice after the 1st dose of methanandamide (0.5 mg/kg), CAN/M = mice sensitized with methanandamide after the challenge dose of methamphetamine (2.5 mg/kg)
*p<0.05, **p<0.01, NS = non-significant, the nonparametric Mann-Whitney U test, two tailed.

CAN when compared to the animals after the 1st application of CAN (see Figure 1; CAN versus CAN/M).

Real-time PCR results showed no significant changes after various treatments in the group n_1 (see Figure 2; V1 and M versus M/M). The treatments in the group n_2 caused significant increase ($p<0.01$) in relative expression of CB_1 receptor mRNA after the 1st application of CAN compared to the application of vehicle (V2) (see Figure 2; V2 versus CAN). The challenge dose of M produced a significant decrease in relative expression of CB_1 receptor mRNA ($p<0.05$) in animals pre-treated repeatedly with CAN when compared to the animals after the 1st application of CAN (see Figure 2; CAN versus CAN/M).

There was no significant change in relative expression of CB_1 receptor mRNA between animals after the MET challenge dose (those were pre-treated with MET) and animals after the MET challenge dose (those were pre-treated with CAN) – see Figure 2; M/M versus CAN/M.

DISCUSSION

The behavioural part of this study confirmed both development of sensitization to methamphetamine stimulatory effects on mouse locomotor behaviour during its repeated administration and cross-sensitization to such effects caused by pre-treatment with cannabinoid CB_1 receptor agonist methanandamide prior to methamphetamine challenge dose administration. Both these findings are in accordance with our previous experimental experiences (Landa *et al.* 2006a;b) as well as suggestions of some others (e.g.: Cadoni et. al. 2001; Wolf *et al.* 2002; Tanda & Goldberg 2003; Chiang & Chen 2007; Wiskerke *et al.* 2008; Panlilio *et al.* 2010).

Neurobiological mechanisms underlying phenomenon of behavioural cross-sensitization are believed to increase vulnerability for use of other drugs of abuse (Steketee & Kalivas 2011). In the case of psychostimulants (including methamphetamine) and cannabinoids it is believed that they induce increase in dopamine activation in the mesolimbic reward pathway. The stimulation of specific cannabinoid CB_1 receptor relieves suppression upon dopaminergic neurons, leading to dopamine release and thus facilitates responses to administration of psychostimulants. However, all outcomes of studies oriented towards involvement of CB_1 receptor in effects of amphetamines have not been consistent (e.g.: Ellgren *et al.* 2004; Solinas *et al.* 2007; Thiemann *et al.* 2008; Panlilio *et al.* 2010).

The part of the present study dealing with relationship between cannabinoid CB_1 receptor agonist methanandamide and methamphetamine effects on the level of CB_1 receptor mRNA expression brought rather controversial results, too. Neither single nor repeated methamphetamine dose of 2.5 mg/kg caused significant increase in relative expression of CB_1 receptor mRNA in the mouse mesencephalon (just a trend to stimulation

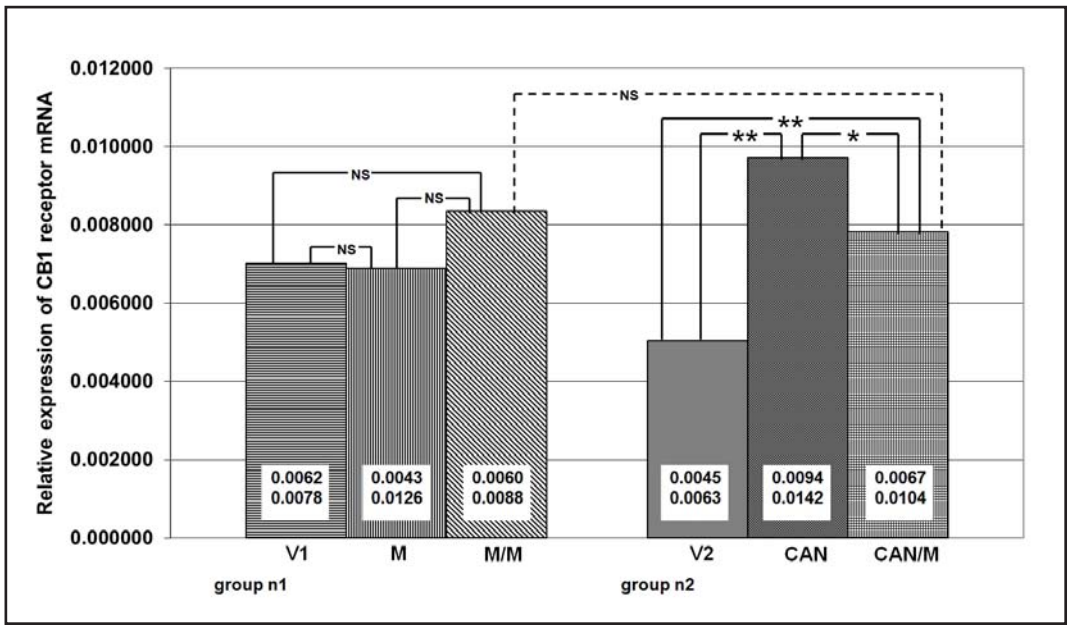

Fig. 2. Effects of drug treatments on relative expression of CB_1 receptor mRNA when compared to GAPDH mRNA shown as median (interquartile range Q1 to Q3):
V1 = mice after the dose of vehicle in the group n_1, V2 = mice after the dose of vehicle in the group n_2, M = mice after the 1st dose of methamphetamine (2.5 mg/kg), M/M = mice sensitized with methamphetamine after the challenge dose of methamphetamine (2.5 mg/kg), CAN = mice after the 1st dose of methanandamide (0.5 mg/kg), CAN/M = mice sensitized with methanandamide after the challenge dose of methamphetamine (2.5 mg/kg).
*$p<0.05$, **$p<0.01$, NS = non-significant, the nonparametric Mann-Whitney U test, two tailed.

of expression was registered after the repeated treatment). Increased CB_1 receptor expression across rat brain regions including medial prefrontal cortex, striatum, amygdaloid complex and hippocampal formation was reported after the exposure to methamphetamine treatment, however, with the dosing regimen (4 mg/kg, subcutaneously × 4 injections, 2 h apart), inducing neurotoxic effects (Bortolato *et al.* 2010).

On the contrary, there was measured a decrease in numbers of CB_1 receptor (both Bmax and mRNA) in the mouse nucleus accumbens after the repeated chronic methamphetamine administration (4 mg/kg/day) developing behavioural sensitization while microinjection of CB_1 antagonist into the nucleus accumbens suppressed the behavioural sensitization to methamphetamine (Chiang & Chen 2007). The activation of the CB_1 receptor was evaluated as a cause facilitating adaptive responses to psychostimulants, such as reduction of dopamine and serotonin turnovers resulting in sensitization (Thiemann *et al.* 2008). However, the density of cannabinoid CB_1 receptor mRNA-positive neurons was significantly lower in Cannabis sativa users (Villares 2007).

Thus the mechanisms that regulate CB_1 receptor modifications are far from being completely understood. Moreover adaptations vary by brain region (Sim-Selley 2003) and the results of studies dealing with CB_1 receptor density are dependent also on the method used (e.g. receptor binding, mRNA expression, immunofluorescence). There is also evidence that internalization of CB_1 receptors following agonist treatment can occur (Coutts *et al.* 2001). This could be a reason for discrepant results we have obtained in the present study using PCR evaluation of the relative expression of CB_1 mRNA comparing to another one with immunofluorescent detection of receptors the intensity of which was assayed by image analysis (Sulcova *et al.* 2007). The latter one showed on the surface of VTA neuronal membranes in rats sensitized to methamphetamine I.V. self-administration decreased density of cannabinoid CB_1 receptors while in the present study a trend to the increase in expression of CB_1 receptors in methamphetamine sensitized mice was found.

In spite that the increased expression of CB_1 receptor was associated in the present study with methanadamide cross-sensitization to methamphetamine effects on mouse locomotion, there was measured after the drug challenge dose significantly lower expression of CB_1 receptor but still significantly higher than under the influence of vehicle treatment and with no difference from the level in mice pretreated repeatedly with methamphetamine. Nevertheless, increased expression of CB_1 receptor in mesencephalon was associated with higher sensitivity to methamphetamine psychostimulatory effects.

This is in agreement with findings that CB_1 knockout mice as well as wild type mice pre-treated with CB_1 receptor inverse agonist AM 251 were less sensitive to the psychomotor stimulant as well as locomotor sensitizing effects of amphetamine (Thiemann 2008), and to some extent are also consistent with our earlier study (Vinklerova *et al.* 2002) in which self-administration of methamphetamine was reduced by AM 251, and increased by methanandamide.

In conclusion, the results of the present study brought further evidence that modulation of CB_1 receptor expression may play an important role in behavioural responses to methamphetamine. Pharmacological support of CB_1 receptor activity may increase expression of CB_1 receptor mRNA associated with sensitization to methamphetamine stimulatory effects what supports the hypothesis on increased vulnerability to methamphetamine abuse after neuroplastic changes induced by cannabinoid CB_1 receptor agonists including delta-9-tetrahydrocannabinol from marijuana.

ACKNOWLEDGEMENTS

This work was supported by the project "CEITEC – Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

REFERENCES

1 Ago Y, Nakamura S, Baba A, Matsuda T (2008). Neuropsychotoxicity of Abused Drugs: Effects of Serotonin Receptor Ligands on Methamphetamine- and Cocaine-Induced Behavioral Sensitization in Mice. J Pharmacol Sci. **106:** 15–21.

2 Bortolato M, Frau R, Bini V, Luesu W, Loriga R, Collu M, Gessa GL, Ennas MG, Castelli MP (2010). Methamphetamine neurotoxicity increases brain expression and alters behavioral functions of CB1 cannabinoid receptors. J Psychiatr Res. **44:** 944–955.

3 Cadoni C, Pisanu A, Solinas M, Acquas E, Di Chiara G (2001). Behavioural sensitization after repeated exposure to Delta 9-tetrahydrocannabinol and cross-sensitization with morphine. Psychopharmacology (Berl) **158:** 259–266.

4 Chiang YC, Chen JC (2007). The role of the cannabinoid type 1 receptor and down-stream cAMP/DARPP-32 signal in the nucleus accumbens of methamphetamine-sensitized rats. J Neurochem. **103:** 2505–2517.

5 Coutts AA, Anavi-Goffer S, Ross RA, MacEwan DJ, Mackie K, Pertwee RG, Irving AJ (2001). Agonist-Induced Internalization and Trafficking of Cannabinoid CB1 Receptors in Hippocampal Neurons, J Neurosci. **21:** 2425–2433.

6 Ellgren M, Hurd YL, Franck J (2004). Amphetamine effects on dopamine levels and behavior following cannabinoid exposure during adolescence. Eur J Pharmacol. **497:** 205–213.

7 Kalivas PW, Sorg BA, Holka MS (1993). The pharmacology and neural circuitry of sensitization to psychostimulants. Behav Pharmacol. **4:** 315–334.

8 Kameda SR, Fukushiro DF, Trombin TF, Procopio-Souza R, Patti CL, Hollais AW, Calzavara MB, Abilio VC, Ribeiro RA, Tufik S, D'Almeida V, Frussa R (2011). Adolescent mice are more vulnerable than adults to single injection-induced behavioral sensitization to amphetamine. Pharmacol Biochem Be. **98:** 320–324.

9 Landa L, Slais K, Sulcova A (2006a). Involvement of cannabinoid CB1 and CB2 receptor activity in the development of behavioural sensitization to methamphetamine effects in mice. Neuroendocrinol Lett. **27:** 63–69.

10 Landa L, Slais K, Sulcova, A (2006b). Impact of cannabinoid receptor ligands on behavioural sensitization to antiaggressive methamphetamine effects in the model of mouse agonistic behaviour. Neuroendocrinol Lett. **27:** 703–710.

11 Landa L, Jurajda M (2007a). Acute and repeated exposure to methamphetamine changes expression of CB1 receptor in mesencephalon of the mouse brain. Eur Neuropsychopharm. **17:** S557–S557.
12 Landa L, Jurajda M (2007b). Behavioural sensitization to methamphetamine affects expression of cannabinoid CB1 receptors in mouse midbrain. Behav Pharmacol. **18:** S27–S28.
13 Maldonado R, Valverde O, Berrendero F (2006). Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. **29:** 225–232.
14 Panlilio LV, Justinova Z, Goldberg SR (2010). Animal models of cannabinoid reward. Br J Pharmacol. **160:** 499–510.
15 Robinson TE, Berridge KC (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev. **18:** 247–291.
16 Rubino T, Vigano D, Massi P, Parolaro D (2003). Cellular mechanisms of Delta(9)-tetrahydrocannabinol behavioural sensitization. Eur J Neurosci. **17:** 325–330.
17 Sim-Selley LJ (2003). Regulation of Cannabinoid CB1 Receptors in the Central Nervous System by Chronic Cannabinoids. Crit Rev Neurobiol. **15:** 91–119.
18 Singh ME, McGregor IS, Naplet PE (2005). Repeated exposure to Δ9-tetrahydrocannabinol alters heroin-induced locomotor sensitisation and Fos-immunoreactivity. Neuropharmacology **49:** 1189–1200.
19 Solinas M, Yasar S, Goldberg SR (2007). Endocannabinoid system involvement in brain reward processes related to drug abuse. Pharmacol Res. **56:** 393–405.
20 Steketee JD, Kalivas PW (2011). Drug Wanting: Behavioral Sensitization and Relapse to Drug-Seeking Behavior. Pharmacol Rev. **63:** 348–365.
21 Sulcova A, Dubovy P, Kucerova J (2007). Behavioural sensitization to methamphetamine is associated with cannabinoid CB1 receptors downregulation in the rat brain VTA. Behav Pharmacol. **18:** S44–S44.
22 Tanda G, Goldberg SR (2003). Cannabinoids: reward, dependence, and underlying neurochemical mechanisms – review of recent preclinical data. Psychopharmacology **169:** 115–134.
23 Thiemann G, Di Marzo V, Molleman A, Hasenöhrl RU (2008). The CB1 cannabinoid receptor antagonist AM251 attenuates amphetamine-induced behavioural sensitization while causing monoamine changes in nucleus accumbens and hippocampus. Pharmacol Biochem Behav. **89:** 384–391.
24 Villares J (2007). Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. Neuroscience **145:** 323–334.
25 Vinklerova J, Novakova J, Sulcova A (2002). Inhibition of methamphetamine self-administration in rats by cannabinoid receptor antagonist AM 251. J Psychopharmacol. **16:** 139–143.
26 Wiskerke J, Pattij T, Schoffelmeer AN, De Vries TJ (2008). The role of CB1 receptors in psychostimulant addiction. Addict Biol. **13:** 225–238.
27 Wolf ME (2002). Addiction: making the connection between behavioral changes and neuronal plasticity in specific pathways. Mol Interv. **2:** 146–157.