

# Cannabinoid CB<sub>1</sub> receptor antagonism prevents neurochemical and behavioural deficits induced by chronic phencyclidine

Cinzia Guidali<sup>1\*</sup>, Daniela Viganò<sup>1,2\*</sup>, Stefania Petrosino<sup>3</sup>, Erica Zamberletti<sup>1</sup>,  
Natalia Realini<sup>1</sup>, Giorgio Binelli<sup>4</sup>, Tiziana Rubino<sup>1</sup>, Vincenzo Di Marzo<sup>3</sup>  
and Daniela Parolaro<sup>1,2</sup>

<sup>1</sup> DBSF and Neuroscience Center, University of Insubria, Varese, Italy

<sup>2</sup> Zardi-Gori Foundation, Milan, Italy

<sup>3</sup> Endocannabinoid Research Group, Institute of Biomolecular Chemistry, CNR, Naples, Italy

<sup>4</sup> DBSM, University of Insubria, Varese, Italy

## Abstract

Clinical and laboratory studies suggest that the endocannabinoid system is involved in schizophrenia disorders. Recent evidence indicates that cannabinoid receptor (CB<sub>1</sub>) antagonists have a pharmacological profile similar to antipsychotic drugs. We investigated the behavioural and biochemical effects of the CB<sub>1</sub> antagonist AM251 in a phencyclidine (PCP) animal paradigm modelling the cognitive deficit and some negative symptoms of schizophrenia. Chronic AM251 (0.5 mg/kg for 3 wk) improved the PCP-altered recognition memory, as indicated by a significant amelioration of the discrimination index compared to chronic PCP alone (2.58 mg/kg for 1 month). AM251 also reversed the PCP-induced increase in immobility in the forced swim test resembling avolition, a negative sign of schizophrenia. In order to analyse the mechanisms underlying these behaviours, we studied the effects of AM251 on the endocannabinoid system (in terms of CB<sub>1</sub> receptor density and functional activity and endocannabinoid levels) and c-Fos protein expression. The antagonist counteracted the alterations in CB<sub>1</sub> receptor function induced by PCP in selected cerebral regions involved in schizophrenia. In addition, in the prefrontal cortex, the key region in the integration of cognitive and negative functions, AM251 markedly raised anandamide levels and reversed the PCP-induced increase of 2-arachidonoylglycerol concentrations. Finally, chronic AM251 fully reversed the PCP-elicited expression of c-Fos protein in the prefrontal cortical region. These findings suggest an antipsychotic-like profile of the CB<sub>1</sub> cannabinoid receptor antagonist which, by restoring the function of the endocannabinoid system, might directly or indirectly normalize some of the neurochemical maladaptations present in this schizophrenia-like animal model.

Received 15 September 2009; Reviewed 16 October 2009; Revised 26 January 2010; Accepted 29 January 2010;  
First published online 3 March 2010

**Key words:** AM251 antipsychotic activity, chronic phencyclidine, endocannabinoid system, rat, schizophrenia.

## Introduction

Alterations in cannabinoid signalling in the brain may contribute to the pathophysiological findings of schizophrenia. For instance, the levels of endocannabinoids in the cerebrospinal fluid and blood, and of the cannabinoid receptor (CB<sub>1</sub>) in the brain are altered in

schizophrenia patients (De Marchi *et al.* 2003; Dean *et al.* 2001; Giuffrida *et al.* 2004; Leweke *et al.* 1999, 2007; Newell *et al.* 2006; Potvin *et al.* 2008; Zavitsanou *et al.* 2004). In addition, cannabis use might constitute a substantial environmental risk factor for schizophrenia, increasing the severity of symptoms, especially cognitive impairment (D'Souza *et al.* 2005; Green, 2005; Pencer *et al.* 2005).

We recently studied the influence of delta-9-tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana, on the endocannabinoid system in cognitive deficits produced by chronic phencyclidine (PCP) (Viganò *et al.* 2009). Chronic intermittent

Address for correspondence: Professor D. Parolaro, DBSF and Neuroscience Center, University of Insubria, Via A. da Giussano 10, 21052 Busto Arsizio, Varese, Italy.

Tel.: 0039 0331 339417 Fax: 0039 0331 339459

Email: daniela.parolaro@uninsubria.it

\* These authors contributed equally to this work.

PCP injections induced cognitive impairment, with alterations in CB<sub>1</sub> receptor function and in endocannabinoid levels, mainly in the prefrontal cortex (PFC). This cognitive deficit was worsened by chronic co-administration of THC, consistent with the theory that marijuana may be a risk factor for the development or worsening of schizophrenia. THC co-exposure led to a further de-regulation of the endocannabinoid system which might be one cause of the cognitive impairment.

Recent findings suggest that CB<sub>1</sub> receptor antagonists have a pharmacological profile similar to anti-psychotic drugs, although their ability to reverse schizophrenia-like positive symptoms is still debated, with reports of a reduction, an increase or no effect on hyperlocomotion and stereotypies produced by dopaminergic agents (for a review see Roser *et al.* 2008). The same differences were found when testing CB<sub>1</sub> antagonists on sensorimotor gating of startle responses to strong exteroceptive stimuli [prepulse inhibition of the acoustic startle response (PPI)]. Ballmaier *et al.* (2007) and Malone *et al.* (2004) showed that SR141716 significantly counteracted PPI produced by PCP, dizocilpine and apomorphine in rodents, whereas SR141716 alone did not affect PPI, indicating effects similar to those observed with the atypical anti-psychotic clozapine. In contrast, Martin *et al.* (2003) found that SR141716A alone did not reverse the disruption of PPI produced by the dopamine D<sub>2</sub> receptor agonist or the NMDA antagonist MK-801.

Apart from PPI experiments, Haller *et al.* (2005) reported that genetic CB<sub>1</sub> disruption in mice counteracted the PCP-induced social deficit. Disappointingly, no clinical improvement, as indicated by the Positive and Negative Syndrome Scale (PANSS), was found for SR141716A compared to placebo in patients with schizophrenia disorders (Meltzer *et al.* 2004). These differences in SR141716's effects in animals and humans might be explained by the possibly inadequate dose, the route of administration and the failure to penetrate the blood-brain barrier at a sufficiently high concentration (Meltzer *et al.* 2004).

There are no experimental studies of the effects of chronic CB<sub>1</sub> antagonists on schizophrenia-related cognitive processes and negative behaviours in animal models. We therefore studied the effects of chronic co-exposure to a CB<sub>1</sub> receptor antagonist, AM251, on cognitive impairment and negative schizophrenia-like behaviours induced by chronic intermittent PCP injections. In brief, 72 h after the last PCP + AM251 co-injection, we ran a novel object recognition (NOR) test to verify the integrity of recognition memory, and the forced swim test (FST) to model the negative

symptoms of schizophrenia resembling avolition (Mouri *et al.* 2007; Noda *et al.* 1995, 1997, 2000). Furthermore, in view of the previously reported alteration of the endocannabinoid system in animals chronically treated with PCP (Viganò *et al.* 2009), we assessed the effects of AM251 on CB<sub>1</sub> receptor density ([<sup>3</sup>H]CP-55,940 receptor binding), functionality (CP-55,940-stimulated [<sup>35</sup>S]GTPγS binding) and the levels of the main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and of two cannabinoid receptor-inactive anandamide congeners, oleylethanolamide (OEA) and palmitoylethanolamide (PEA). Finally, we evaluated how co-administration of AM251 affected the expression of the proto-oncogene c-Fos as a useful method of mapping the distribution of neurons activated by pharmacological stimuli.

## Material and methods

### Animals

Adolescent male Lister hooded rats (Harlan, Italy) weighing 126–150 g upon arrival were used. Rats were grouped 3–4 per cage and maintained on a 12-h light/dark cycle (lights on 08:00 hours) with food/water available *ad libitum*. All experiments were conducted during the light phase and performed in strict accordance with the guidelines released by the Italian Ministry of Health (D.L.116/92) and (D.L.111/94-B), and the European Community directives regulating animal research (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

### Drugs

Drugs used were phencyclidine hydrochloride (PCP, Sigma-Aldrich, UK), dissolved in saline. AM251 (Tocris, Italy) dissolved in DMSO, Tween-80 and saline (1:1:8). Clozapine (Sigma, Italy) was dissolved in a minimum volume of acetic acid and pH adjusted (pH 6).

### Treatment regimens

The rats were allowed to acclimate in their new environment for 1 wk before the start of the treatment.

Chronic-intermittent PCP exposure: i.p. injections of PCP (2.58 mg/kg) or saline (1 ml/kg) once daily according to the procedure of Cochran *et al.* (2003) were performed at the time intervals indicated in Fig. 1.

PCP + AM251 chronic treatment: chronic treatment with AM251 (0.5 mg/kg i.p.) or vehicle was associated with PCP (Fig. 1). As positive control, chronic

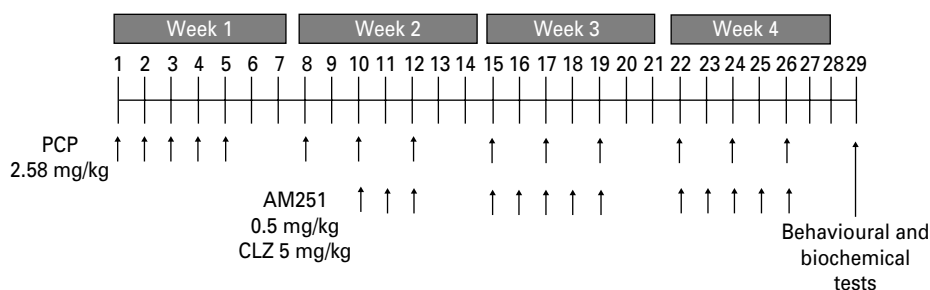


Fig. 1. Treatment schedule. Male Lister hooded rats were randomly assigned to the different groups.

treatment with clozapine (5 mg/kg i.p.) was associated with PCP (Fig. 1). This protocol did not induce any significant alterations in the body weight/growth curve (data not shown).

### Behavioural tests

All the behavioural tests were performed 72 h after the end of chronic treatment (day 29).

### NOR test

The experimental apparatus used for the object recognition test was an open-field box (60 × 60 × 60 cm) made of Plexiglas, placed in a dimly illuminated room. On the day of testing, the animals were habituated in a quiet laboratory for 1 h before the experimental procedure began. Animals performed each test individually. A 10-min habituation session preceded the experimental trials. The experiment was performed and analysed as described previously (Viganò *et al.* 2009).

### FST

The increased immobility produced by PCP in the FST is considered a validated model of avolition, which is a negative symptom of schizophrenia (Mouri *et al.* 2007; Noda *et al.* 2000). Rats were placed into the testing room at least 1 h prior to the start of the test. A separate group of animals was tested in the FST using a modification of the protocol originally described by Porsolt *et al.* (1978). Rats were forced to swim inside glass cylinders (height 50 cm, diameter 20 cm, containing water to a depth of 25 cm, maintained at 23–25 °C) for 15 min. The session was videotaped for later analysis. Analysis of videotape was done by an experimenter who was blind to the rats' treatment. The duration of behaviours was monitored every 5 min of the 15-min test. Immobility was defined as the time spent by the animal floating in the water making

only those movements necessary to keep its head above the water.

### Biochemical assays

All the biochemical analyses were performed after the NOR test. Rats were decapitated and brains were rapidly removed, frozen in liquid nitrogen and stored at –80 °C until processing.

### Autoradiographic-binding studies

Coronal sections (20- $\mu$ m thick) were cut on a cryostat and thaw-mounted on gelatin-coated slides. The sections were stored at –80 °C until processing.

### [<sup>3</sup>H]CP-55,940 receptor autoradiographic binding

The [<sup>3</sup>H]CP-55,940 receptor autoradiographic binding was performed as described previously (Rubino *et al.* 1997, 2000; Viganò *et al.* 2009).

### CP-55,940-stimulated [<sup>35</sup>S]GTP $\gamma$ S binding in autoradiography

This was determined as described previously by our group (Rubino *et al.* 2000; Viganò *et al.* 2009).

### Image analysis

The intensity of the autoradiographic films was assessed by measuring the grey levels with an image analysis system consisting of a scanner connected to a PC running Microsoft Windows. The images were analysed using Image-Pro Plus 5.0 (MediaCybernetics, USA) as described previously (Viganò *et al.* 2005).

### Immunohistochemistry

#### c-Fos staining

Coronal sections (12- $\mu$ m thick) were cut on a cryostat and thaw-mounted on gelatin-coated slides. The expression of c-Fos protein was assessed as described

previously (Filipkowski *et al.* 2000) with slight modifications. In brief, sections were fixed with 4% PFA for 5 min at  $-20^{\circ}\text{C}$ , rinsed with 0.01 M PBS and incubated for 30 min in 0.3%  $\text{H}_2\text{O}_2$ -methanol to inactivate endogenous peroxidase activity and reduce non-specific staining. Then the sections were washed three times in 0.01 M PBS buffer containing 0.5% Triton X-100. For intracellular staining, sections were fixed with 100% cold methanol at  $-20^{\circ}\text{C}$  for 30 min. The sections were washed as above and incubated for 1 h with 1% BSA in 0.01 M PBS buffer containing 0.5% Triton X-100, and then for 48 h at  $4^{\circ}\text{C}$  with a primary antibody to c-Fos (Abcam, UK) diluted 1/50. The sections were washed and incubated with biotinylated secondary antibody diluted 1/100 for 1 h at room temperature. Next, the sections were washed as above and incubated with avidin-biotin-peroxidase complex (Vector ABC kit, Vector Laboratories, USA) for 1 h at room temperature. The sections were then incubated in chromogen 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 4–5 min. The reaction was stopped in PBS, and the slides were dried prior to being dehydrated in gradual concentrated ethanol, cleared in xylene and cover-slipped with mounting medium. To control for specificity of immunostaining, some sections were processed without the primary antibody as negative control.

#### Counting of c-Fos-labelled neurons

Tissue sections were examined at low power ( $40\times$ ) by light microscopy in order to determine the number of c-Fos-positive neurons, indicated by the brown colour of their nuclei. Positive nuclei were counted only when structure of appropriate size and shape demonstrated clear increases in immunoreactivity when compared to the background level. The total number of positive cells for ten similar sections through the PFC area was counted in every animal. The number (mean  $\pm$  S.E.M.) of positive cells in the area for each animal in each group (10 sections/animal) was calculated.

#### Measurement of endocannabinoids

Cerebral areas (PFC and hippocampus) were dissected from animals belonging to all experimental groups. They were obtained by regional dissection on ice according to the method of Heffner *et al.* (1980), frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until use.

Tissues were homogenized in 5 vol. of chloroform/methanol/Tris-HCl 50 mM (2:1:1) containing 20 pmol of d8-AEA, d4-OEA, d4-PEA and d5-2-AG as internal standard (Bisogno *et al.* 1997; Devane *et al.* 1992).

Homogenates were centrifuged at 13 000 g for 16 min ( $4^{\circ}\text{C}$ ), the aqueous phase plus debris was collected and extracted again twice with 1 vol. of chloroform. The organic phases from the three extractions were pooled and the organic solvents evaporated in a rotating evaporator. Lyophilized samples were stored frozen at  $-80^{\circ}\text{C}$  until analysed.

Lyophilized extracts were purified and analysed by isotope dilution liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry (LC-APCI-MS) as reported previously (Bisogno *et al.* 1997; Marsicano *et al.* 2002). Mass spectrometric (MS) detection was performed in the selected ion monitoring mode using  $m/z$  values of 356 and 348 (molecular ions +1 for deuterated and undeuterated AEA), 304 and 300 (molecular ions +1 for deuterated and undeuterated PEA), 330 and 326 (molecular ions +1 for deuterated and undeuterated OEA), and 384.35 and 379.35 (molecular ions +1 for deuterated and undeuterated 2-AG). AEA, PEA, OEA and 2-AG levels in unknown samples were calculated on the basis of their area ratios with the internal deuterated standard signal areas. For 2-AG, the areas of the peaks corresponding to 1(3)- and 2-isomers were added together. The amounts of endocannabinoids were expressed as pmol/wet tissue weight.

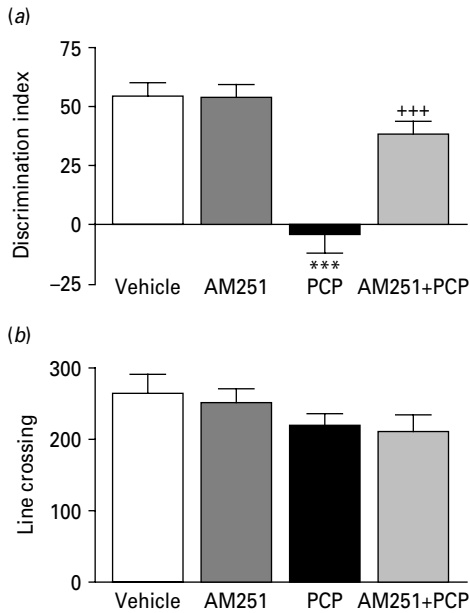
#### Statistical analysis

Data are expressed as mean  $\pm$  S.E.M. Behavioural and biochemical data were analysed using two-way analysis of variance (ANOVA) with drug (saline, PCP) and treatment (vehicle, AM251) as factors followed by Duncan's *post-hoc* test when required.

## Results

#### NOR test

Figure 2a shows the effect of chronic AM251 on the cognitive impairment provoked by PCP in the NOR test. As demonstrated previously (Viganò *et al.* 2009), repeated PCP caused a significant reduction in the discrimination index (two-way ANOVA for drug:  $F_{1,25}=30.51$ ,  $p=9.66\times 10^{-6}$ , Duncan's *post-hoc* test  $p<0.0005$ ), indicating a cognitive deficit 72 h after the last injection. Chronic co-treatment with AM251 significantly counteracted the impairment in the NOR paradigm, with significant improvement in the discrimination index compared to PCP alone (two-way ANOVA for drug  $\times$  treatment interaction:  $F_{1,25}=10.04$ ,  $p=0.004$ , Duncan's *post-hoc* test  $p<0.0005$ ), similarly to what we had previously found for clozapine (Viganò *et al.* 2009). AM251 alone had no notable effect on NOR



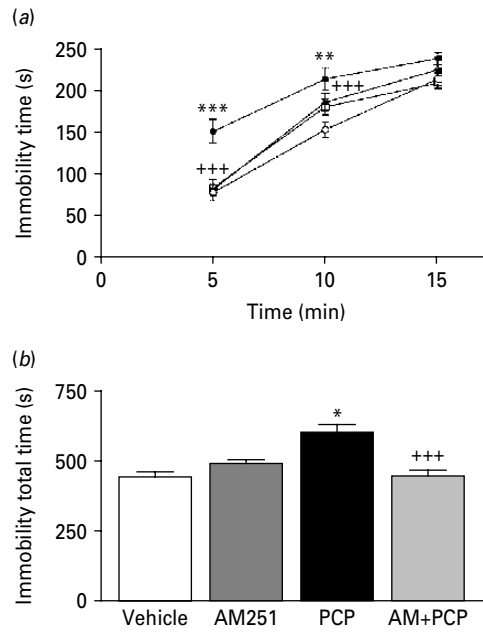
**Fig. 2.** Effect of chronic AM251 treatment (0.5 mg/kg i.p.) on PCP-induced deficit in the novel object recognition (NOR) test. (a) Represents the discrimination index during the test phase. (b) Represents the number of line crossings. The NOR test was performed 72 h after the last drug injection. Data are expressed as the mean  $\pm$  S.E.M. of at least six animals per group. \*\*\*  $p < 0.0005$  vs. vehicle; +++  $p < 0.0005$  vs. PCP (Duncan's *post-hoc* test).

(Fig. 2a). All the animals spent equal time exploring the familiar objects (right and left objects) in the familiarization phase of the NOR test (vehicle: right object  $55.8 \pm 5$ , left object  $63.7 \pm 7.4$ ; PCP: right object  $50.3 \pm 7.2$ , left object  $62.6 \pm 7.3$ ; AM251: right object  $58.6 \pm 7.4$ , left object  $59.6 \pm 6.2$ ; AM251 + PCP: right object  $44.6 \pm 6.4$ , left object  $46.8 \pm 7.4$ ).

Neither treatment affected locomotor activity, as demonstrated by the Any Maze program analysis of the number of line crossings (Fig. 2b).

#### Avolition in the FST

Due to the fact that our PCP model did not induce reduction in social interaction (Egerton *et al.* 2008), as a negative symptom of schizophrenia we decided to evaluate avolition in the FST. The increased immobility produced by PCP in the FST can, in fact, be considered a validated experimental model of avolition (Mouri *et al.* 2007). It is sensitive to atypical antipsychotic treatment but not to haloperidol and tricyclic antidepressant treatment (Noda *et al.* 1995, 1997). Since the effects of these compounds in this model are consistent with the clinical effects, this model could be used as a behavioural model of



**Fig. 3.** Effect of chronic AM251 treatment (0.5 mg/kg i.p.) on PCP-induced immobility in the forced swim test (FST). (a) Time spent immobile every 5 min during the 15-min FST period. (b) Time spent immobile during the 15-min total session. Data are expressed as the mean  $\pm$  S.E.M. of at least five animals per group. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$  vs. vehicle; +++  $p < 0.0005$  vs. PCP (Duncan's *post-hoc* test).

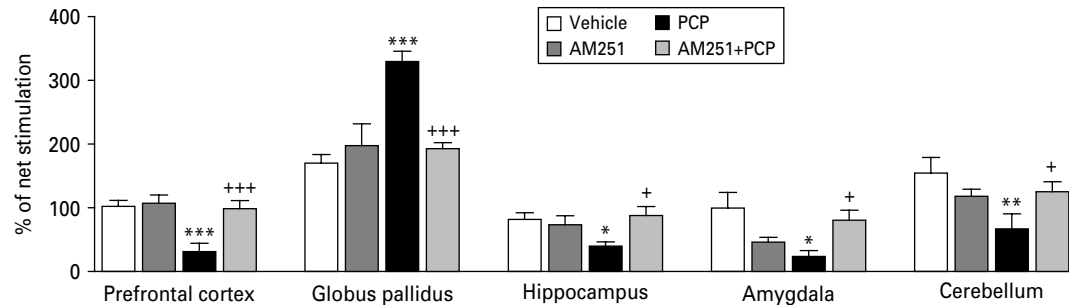
negative symptoms (Mouri *et al.* 2007). Based on these considerations, we tested the effect of AM251 on the increased immobility produced by PCP in the FST. Figure 3 shows the effects of chronic AM251 and PCP alone or together, in the FST. Chronic PCP significantly prolonged the time spent immobile at 5-min (Fig. 3a, two-way ANOVA for drug:  $F_{1,19} = 14.3$ ,  $p = 0.00126$ , Duncan's *post-hoc* test  $p < 0.0005$ ) and 10-min (Fig. 3a; two-way ANOVA for drug:  $F_{1,19} = 6.52$ ,  $p = 0.019$ , Duncan's *post-hoc* test  $p < 0.005$ ) as well as during the whole 15-min session (two-way ANOVA for drug:  $F_{1,19} = 7.81$ ,  $p = 0.0115$ , Duncan's *post-hoc* test  $p < 0.05$ ) (Fig. 3b). When AM251 was administered together with PCP it significantly counteracted the increase in immobility in the first 5-min session (two-way ANOVA for drug  $\times$  treatment interaction:  $F_{1,19} = 12.93$ ,  $p = 0.00192$ , Duncan's *post-hoc* test  $p < 0.0005$ ) and during the whole 15-min session (two-way ANOVA for drug  $\times$  treatment interaction:  $F_{1,19} = 25.48$ ,  $p = 7.13 \times 10^{-5}$ , Duncan's *post-hoc* test  $p < 0.0005$ ). AM251 alone had no effect in the FST. Its protective effect was similar to that of clozapine, which in the present study reduced the increased total time (s) spent in immobility provoked by PCP by  $\sim 15\%$

**Table 1.** Effect of chronic PCP and AM251 co-treatment on CB<sub>1</sub> receptor binding

	<sup>3</sup> H]CP-55,940 receptor binding (fmol/mg of tissue)			
	Vehicle	AM251	PCP	AM251 + PCP
Prefrontal cortex	144.6 ± 4.67	171.6 ± 5.11	138.4 ± 1.74	165.9 ± 12.1
Nucleus accumbens	143.7 ± 4.09	162.9 ± 9.81	168 ± 0.631	155.3 ± 13.25
Caudate putamen	242.1 ± 10.21	263.6 ± 7.47	261.1 ± 11.77	240.3 ± 13.49
Globus pallidus	492.6 ± 6.56	497.5 ± 10.62	512.2 ± 24.41	527.9 ± 20.34
Hypothalamus	124.6 ± 10.16	101.5 ± 5.14	133.2 ± 1.926	143.4 ± 10.94
Thalamus	137.4 ± 13.79	109 ± 1.71	140.1 ± 6.337	126.9 ± 10.41
Amygdala	121.5 ± 5.91	126.2 ± 6.84	134.1 ± 4.336	141.5 ± 9.05
Hippocampus	239.6 ± 16.87	206.8 ± 11.56	235 ± 7.56	235 ± 13.63
Substantia nigra	526.8 ± 16.38	557 ± 21.36	550 ± 31.1	544.2 ± 19.15
Ventral tegmental area	76.01 ± 9.77	95.61 ± 7.56	113.2 ± 8.818**	91.37 ± 5.49 <sup>+</sup>
Cerebellum	306.4 ± 7.94	301.8 ± 21.78	362.4 ± 19.82*	370.6 ± 10.31

Data are expressed in fmol/mg of tissue as the mean ± S.E.M. of at least four animals.

\*  $p < 0.05$ , \*\*  $p < 0.005$  vs. vehicle, <sup>+</sup>  $p < 0.05$  vs. PCP (Duncan's *post-hoc* test).



**Fig. 4.** Effect of chronic PCP and AM251 co-treatment on net CP-55,940-stimulated [<sup>35</sup>S]GTP $\gamma$ S binding. Bars indicate the mean ± S.E.M. of at least four animals except for globus pallidus. \*  $p < 0.05$ , \*\*  $p < 0.005$ , \*\*\*  $p < 0.0005$  vs. vehicle; <sup>+</sup>  $p < 0.05$ , +++  $p < 0.005$  vs. PCP (Duncan's *post-hoc* test).

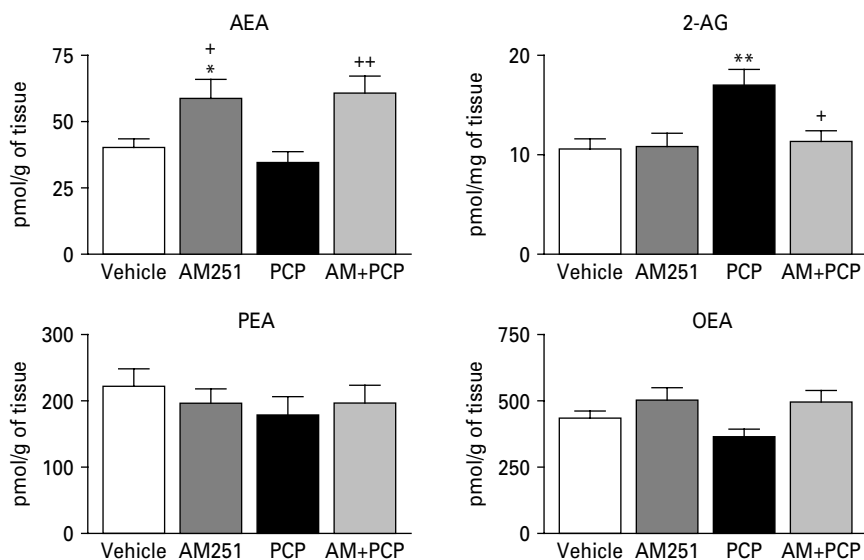
(vehicle  $447 \pm 18.3$ , PCP  $604.8 \pm 27.3$ , clozapine + PCP  $511.2 \pm 5.3$ ).

### CB<sub>1</sub> receptor function

To verify the function of the CB<sub>1</sub> receptor after PCP + AM251 co-administration, we measured CB<sub>1</sub> receptor density and functional activity using [<sup>3</sup>H]CP-55,940 receptor binding and CP-55,940-stimulated GTP $\gamma$ S binding, respectively. Table 1 summarizes the results of [<sup>3</sup>H]CP-55,940 receptor-binding studies in rats treated with PCP alone or in combination with AM251. PCP *per se* up-regulated CB<sub>1</sub> in the ventral tegmental area (VTA) and cerebellum, respectively, of about 49% and 18% compared to control (two-way ANOVA for drug: VTA:  $F_{1,26} = 13.13$ ,  $p = 0.0012$ , Duncan's *post-hoc* test  $p < 0.05$ ; cerebellum:  $F_{1,26} = 4.41$ ,  $p = 0.0454$ , Duncan's *post-hoc* test  $p < 0.005$ ), thus

confirming our previous findings (Viganò *et al.* 2009). AM251 co-exposure reversed this effect (two-way ANOVA for drug × treatment interaction:  $F_{1,26} = 6.98$ ,  $p = 0.0137$ , Duncan's *post-hoc* test  $p < 0.05$ ) in the VTA but not in the cerebellum. Concerning the PFC, two-way ANOVA revealed a significant treatment effect ( $F_{1,12} = 8.29$ ,  $p = 0.0138$ ); however, *post-hoc* test did not show a significant difference between either AM251 vs. vehicle ( $p = 0.07$ ) or AM251 + PCP vs. PCP ( $p = 0.07$ ).

The results of GTP $\gamma$ S binding are shown in Fig 4. Among all the cerebral areas analysed PCP alone reduced CB<sub>1</sub> receptor coupling to G proteins in the PFC (61%; two-way ANOVA for drug:  $F_{1,24} = 8.14$ ,  $p = 0.00876$ , Duncan's *post-hoc* test  $p < 0.005$ ) and cerebellum (57%; two-way ANOVA for drug:  $F_{1,14} = 4.64$ ,  $p = 0.049$ , Duncan's *post-hoc* test  $p < 0.01$ ), but



**Fig. 5.** Effect of chronic PCP and AM251 co-treatment on endocannabinoid (AEA, 2-AG, PEA, OEA) contents in the prefrontal cortex. Data are expressed as the mean  $\pm$  S.E.M. of at least four animals per group. \*  $p < 0.05$ , \*\*  $p < 0.005$  vs. vehicle; +  $p < 0.05$ , ++  $p < 0.01$  vs. PCP (Duncan's *post-hoc* test).

increased it by  $\sim 93\%$  in the globus pallidus (two-way ANOVA for drug:  $F_{1,11} = 12.28$ ,  $p = 0.0049$ , Duncan's *post-hoc* test  $p < 0.005$ ). Regarding the hippocampus and amygdala, two-way ANOVA did not show a significant effect of drug; however, *post-hoc* analysis revealed a significant reduction of 56% ( $p < 0.05$ ) and 77% ( $p < 0.005$ ), respectively, compared to control. These data confirm our previous results using the same PCP paradigm (Viganò *et al.* 2009). When AM251 was co-administered with PCP, the desensitization of CB<sub>1</sub> receptors was significantly counteracted, restoring the control values in the PFC (two-way ANOVA for drug  $\times$  treatment:  $F_{1,24} = 5.20$ ,  $p = 0.031$ , Duncan's *post-hoc* test  $p < 0.005$ ), hippocampus (two-way ANOVA for drug  $\times$  treatment:  $F_{1,24} = 4.35$ ,  $p = 0.047$ , Duncan's *post-hoc* test  $p < 0.05$ ), amygdala (two-way ANOVA for drug  $\times$  treatment:  $F_{1,25} = 14.51$ ,  $p = 0.0008$ , Duncan's *post-hoc* test  $p < 0.05$ ), and cerebellum (two-way ANOVA for drug  $\times$  treatment:  $F_{1,14} = 6.17$ ,  $p = 0.0262$ , Duncan's *post-hoc* test  $p < 0.05$ ). Concomitantly, AM251 reversed the increase in GTP $\gamma$ S binding observed in the globus pallidus of PCP-treated rats (two-way ANOVA for drug  $\times$  treatment:  $F_{1,11} = 13.6$ ,  $p = 0.0035$ , Duncan's *post-hoc* test  $p < 0.005$ ).

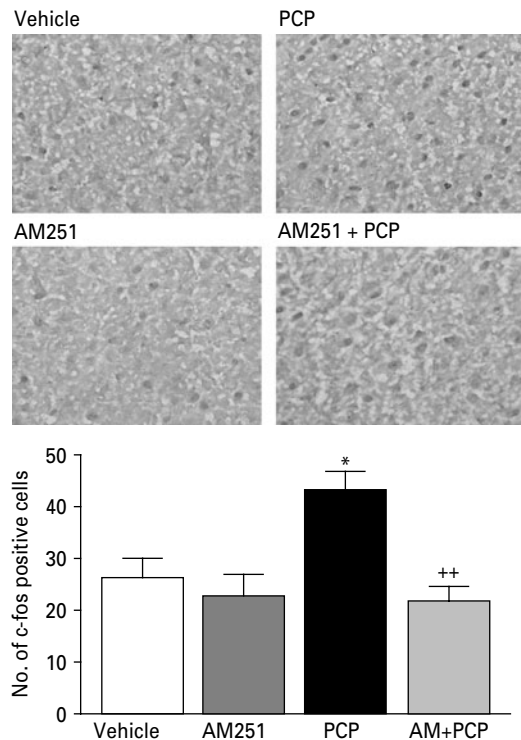
### Endocannabinoids

Given the key role of the PFC and hippocampus in integrating the cognitive and negative functions altered in this PCP model of schizophrenia (Pratt

*et al.* 2008; Viganò *et al.* 2009), we analysed the endocannabinoid content in these two cerebral regions. Figure 5 shows the effect of AM251 co-treatment on PCP-induced changes in endocannabinoid content in the PFC. As reported previously (Viganò *et al.* 2009), PCP alone did not significantly affect AEA levels, although it significantly raised 2-AG levels (two-way ANOVA for drug:  $F_{1,12} = 8.04$ ,  $p = 0.015$ , Duncan's *post-hoc* test  $p < 0.005$ ). Chronic AM251 alone raised AEA levels by 45% (two-way ANOVA for treatment:  $F_{1,12} = 16.93$ ,  $p = 0.0014$ , Duncan's *post-hoc* test  $p < 0.05$ ) without changing 2-AG content. The significant increase in AEA levels was also evident in PCP and AM251 co-treated rats compared to either vehicle or PCP alone ( $p < 0.01$ ). Finally, AM251 co-treatment reversed the PCP-induced rise in 2-AG levels (two-way ANOVA for drug  $\times$  treatment:  $F_{1,12} = 5.84$ ,  $p = 0.0324$ , Duncan's *post-hoc* test  $p < 0.01$ ). No differences in PEA and OEA levels were found in the PFC in any of the treated groups (Fig. 5). Endocannabinoid content did not change in the hippocampus of treated rats (data not shown).

### c-Fos protein expression

c-Fos protein expression was analysed in the PFC to study neuronal activation in response to treatment with PCP and AM251. As shown in the representative photomicrographs and histograms presented in Fig. 6, chronic PCP significantly raised c-Fos protein



**Fig. 6.** Effect of chronic PCP and AM251 treatment on c-Fos protein expression in the rat prefrontal cortex. (a) Representative photomicrograph of c-Fos protein expression (magnification 400 $\times$ ). (b) Count of c-Fos-positive cells. The data are expressed as the mean  $\pm$  S.E.M. of at least four animals per group. \*  $p < 0.01$  vs. vehicle; ++  $p < 0.005$  vs. PCP (Duncan's *post-hoc* test).

expression (64%) compared to control (two-way ANOVA for drug:  $F_{1,14} = 4.69$ ,  $p = 0.0479$ , Duncan's *post-hoc* test  $p < 0.01$ ). Co-treatment with AM251 significantly antagonized the increased c-Fos expression induced by PCP (two-way ANOVA for drug  $\times$  treatment:  $F_{1,14} = 6.08$ ,  $p = 0.0271$ , Duncan's *post-hoc* test  $p < 0.005$ ) but had no effect on its own.

## Discussion

### Behavioural results

The main finding of the present study was that chronic treatment with the specific CB<sub>1</sub> receptor antagonist AM251 significantly counteracts the impairment in recognition memory and avolition induced by chronic PCP, two signs often considered reminiscent of schizophrenia-like symptoms.

In the NOR test, AM251 counteracted the reduction in the discrimination index, thus improving recognition memory without affecting locomotor activity.

This is the first paper reporting a beneficial effect of CB<sub>1</sub> antagonist chronic treatment on cognitive dysfunctions induced by PCP. Our data agree with the recent paper of Seillier *et al.* (2009) showing that acute administration of AM251 ameliorates working-memory deficit in PCP-treated rats.

It is well known that acute SR141716A (Gessa *et al.* 1998; Tzavara *et al.* 2003) increases acetylcholine (ACh) efflux in some cerebral areas relevant for cognition (medial PFC and hippocampus) at doses improving cognitive performance, suggesting pro-cholinergic properties, a supposed mechanism of action of drugs that improve cognition. Moreover, atypical antipsychotics such as clozapine and olanzapine boost ACh efflux in the cortex and hippocampus (Gray & Roth, 2007; Ichikawa *et al.* 2002; Shirazi-Southall *et al.* 2002), thus explaining their beneficial effect on cognitive function in schizophrenia. On these bases it could be postulated that the improvement in cognitive performance in our model might be due to the chronic blockade of CB<sub>1</sub> receptors, possibly leading to an enhancement in cholinergic transmission in the PFC.

In addition to its beneficial effect on cognitive performance, we found here that chronic AM251 antagonizes the PCP-induced increase in immobility in the FST, thus improving avolition, a negative schizophrenia-like symptom. As stated in the Introduction, there are controversial results on the efficacy of CB<sub>1</sub> antagonists in reversing negative schizophrenia-like signs, but our data seem to support the idea that chronic AM251 might ameliorate at least this negative sign. Controversially, acute AM251 administration did not affect the deficit in social interaction induced by chronic PCP (Seillier *et al.* 2009). The discrepancy between this and our data may be attributed to differences in the experimental design (chronic *vs.* intermittent PCP, acute *vs.* chronic AM251, 5–10 d *vs.* 72 h withdrawal) and/or to the presence of a different sensitivity of different negative signs to AM251 treatment. In the present work, AM251 showed the same efficacy as clozapine, the one antipsychotic exerting at best modest therapeutic efficacy on negative symptoms (see O'Tuathaigh *et al.* 2009) in the FST, thus suggesting a possible beneficial effect of this compound when given chronically.

Concluding the low dose of chronic AM251 that we used in our study antagonized the PCP-induced cognitive impairment and avolition, but had no such effect administered alone, suggesting that the endocannabinoid system might be involved in triggering these PCP-induced deficits; therefore we surveyed the different components of the endocannabinoid system.



### Endocannabinoid system

Another intriguing finding of the present study is the effect of AM251 on CB<sub>1</sub> receptor density and coupling in the different brain regions. As we have previously observed (Viganò *et al.* 2009) PCP induced a significant and localized increase in receptor-binding sites in the VTA and cerebellum whereas alterations in the CB<sub>1</sub> receptor efficiency were widely distributed throughout the brain. AM251 reversed the PCP-induced alteration in CB<sub>1</sub> receptor density and efficiency. Interestingly, this effect was observed in the brain regions that are implicated in the modulation of emotionality and cognition (PFC, hippocampus, amygdala, VTA, globus pallidus and cerebellum), two behavioural dimensions disrupted in schizophrenia.

Since the PFC and hippocampus are of particular interest in view of their potentially important role in the modulation of both emotionality and cognition (Barker *et al.* 2007; Bast & Feldon, 2003; Dere *et al.* 2007; Jenkins *et al.* 2008; Noda *et al.* 2000) we then measured the levels of the main endocannabinoids (AEA, 2-AG), and of AEA-related compounds with no activity at CB<sub>1</sub> receptors (OEA, PEA) in these areas. These results were also needed because a possible maladaptation of endocannabinoid tone was observed previously in animal models of schizophrenia (Seillier *et al.* 2009; Tzavara *et al.* 2006; Viganò *et al.* 2009) and in schizophrenia patients (De Marchi *et al.* 2003; Giuffrida *et al.* 2004; Leweke *et al.* 2007; Potvin *et al.* 2008). AM251 significantly increased AEA levels, but not OEA or PEA levels, in the PFC, while simultaneously reversing the PCP-induced increase in 2-AG in this area, but produced no effects in the hippocampus. Interestingly, our previous study analysing the effect of chronic THC in the same experimental model showed worse cognitive performance in THC + PCP-treated rats, coupled with lack of normalization (or even worsening) of CB<sub>1</sub> signalling throughout the brain, and with a reduction of AEA levels in the PFC. Therefore, it appears that the opposite effects on the cognitive performance of PCP-treated rats for THC (worsening in Viganò *et al.* 2009) and AM251 (improvement in the present study) are accompanied by different actions on CB<sub>1</sub> receptor functional activity in various brain regions, and by opposite effects on anandamide levels in the PFC. This suggests that the effects of THC and AM251 on endocannabinoid tone might be partly responsible for their opposite effects on PCP-induced cognitive impairment. Therefore, our findings might be interpreted as giving support to the hypothesis that recovery of CB<sub>1</sub> receptor function throughout the brain, together with enhanced brain

levels of AEA, plays some beneficial effect as suggested by some authors (Giuffrida *et al.* 2004; Leweke *et al.* 2007). More generally, these results strengthen the hypothesis that impairment of the endocannabinoid system is involved in cognitive deficits and some psychotic-like symptoms induced by PCP.

Finally chronic AM251 produced an inhibitory effect in the PFC, on PCP-induced increased levels of 2-AG, the most abundant and selective endogenous CB<sub>1</sub> agonist involved in the regulation of synaptic plasticity. One possibility is that PCP-induced increase of 2-AG levels in the PFC could be a causal factor in the cognitive deficits observed after PCP administration. The bulk of evidence identifies 2-AG as the most probable retrograde mediator at glutamatergic synapses (Gerdeman *et al.* 2002; Robbe *et al.* 2002; Sjöström *et al.* 2003; Straiker & Mackie, 2005), thought to suppress glutamate release through activation of CB<sub>1</sub> receptors in presynaptic axon terminals. Reduction in extracellular glutamate is observed in frontal areas of the brain of rodents treated chronically with NMDA receptor antagonists (Kondziella *et al.* 2006; Zuo *et al.* 2006), as well as in schizophrenia patients (Bartha *et al.* 1997; Bubeníková-Valesová *et al.* 2008; Théberge *et al.* 2002). In line with this, we suggest that chronic inhibition of CB<sub>1</sub> receptors might contribute to restoring a glutamatergic tone in the PFC by antagonizing the CB<sub>1</sub>-mediated inhibition of glutamate release. On these bases CB<sub>1</sub> receptor blockade lowering the effect of high 2-AG levels ameliorates recognition memory, a cognitive performance specifically dependent upon the integrity of the PFC.

### c-Fos activation

Finally, we investigated the effect of chronic PCP on the expression of the c-Fos protein, a marker of neuronal activation (Herrera & Robertson, 1996). PCP and related drugs (ketamine and dizocilpine) induce c-Fos protein in several brain areas and this may be involved in their psychotomimetic effects (Abdel-Naby Sayed *et al.* 2001; Dragunow & Faull, 1990; Duncan *et al.* 1998; Sugita *et al.* 1996; Zuo *et al.* 2009). Chronic administration of NMDA antagonists activates many brain regions, but we analysed c-Fos expression in the PFC region since it is the most important area regarding the behavioural and biochemical alterations observed in this study. Chronic PCP significantly boosted the expression of c-Fos protein in the PFC, in accord with Zuo *et al.* (2009), who reported an elevation in c-Fos induced by chronic MK-801 in the same region. Moreover, for the first time, we demonstrated that PCP-elicited expression of c-Fos protein was fully

reversed by chronic AM251, resembling the effects of the atypical antipsychotic drug clozapine, as also reported by Zuo *et al.* (2009). The mechanisms underlying the alteration of c-Fos expression are not completely understood, but Zuo *et al.* (2009) proposed that MK-801-induced c-Fos protein expression might be primarily due to reduced glutamatergic neurotransmission. In this scenario, AM251 antagonism of c-Fos induction might reflect its ability to reinstate glutamate transmission. Besides the blockade of NMDA receptors, multiple receptors and different pathways may be involved in the effects of PCP and AM251 on c-Fos protein.

In conclusion, the endocannabinoid system appears to have an important role in the cognitive and psychotic-like symptoms induced by PCP, and the CB<sub>1</sub> receptor antagonist AM251 exhibits pharmacological features reminiscent of atypical antipsychotics. Further investigations are needed to clarify the exact mechanisms of action of CB<sub>1</sub> antagonists with regard to their influence on the endocannabinoid system and their potential effects on psychosis.

#### Acknowledgments

This work was supported by the Ministero dell'Istruzione, dell'Università e della Ricerca PRIN2005, by the University of Insubria FAR2006 and by the Zardi-Gori Foundation. We are grateful to Judy Baggott for language editing of the manuscript.

#### Statement of Interest

None.

#### References

- Abdel-Naby Sayed M, Noda Y, Mahmoud Hamdy M, Mamiya T, *et al.* (2001). Enhancement of immobility induced by repeated phencyclidine injection: association with c-Fos protein in the mouse brain. *Behavioral Brain Reserach* **124**, 71–76.
- Ballmaier M, Bortolato M, Rizzetti C, Zoli M, *et al.* (2007). Cannabinoid receptor antagonists counteract sensorimotor gating deficits in the phencyclidine model of psychosis. *Neuropsychopharmacology* **32**, 2098–2107.
- Barker GR, Bird F, Alexander V, Warburton EC (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *Journal of Neuroscience* **27**, 2948–2957.
- Bartha R, Williamson PC, Drost DJ, Malla A, *et al.* (1997). Measurement of glutamate and glutamine in the medial prefrontal cortex of never-treated schizophrenic patients and healthy controls by proton magnetic resonance spectroscopy. *Archives for General Psychiatry* **54**, 959–965.
- Bast T, Feldon J (2003). Hippocampal modulation of sensorimotor processes. *Progress in Neurobiology* **70**, 319–345.
- Bisogno T, Sepe N, Melck D, Maurelli S, *et al.* (1997). Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. *Biochemical Journal* **322**, 671–677.
- Bubeniková-Valesová V, Horáček J, Vrajová M, Höschl C (2008). Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. *Neuroscience and Biobehavioral Review* **32**, 1014–1023.
- Cochran SM, Kennedy M, McKerchar CE, Steward LJ, *et al.* (2003). Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. *Neuropsychopharmacology* **28**, 265–275.
- De Marchi N, De Petrocellis L, Orlando P, Daniele F, *et al.* (2003). Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids in Health and Disease* **2**, 5–14.
- Dean B, Sundram S, Bradbury R, Scarr E, *et al.* (2001). Studies on [<sup>3</sup>H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience* **103**, 9–15.
- Dere E, Huston JP, De Souza Silva MA (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience and Biobehavioural Review* **31**, 673–704.
- Devane WA, Hanus L, Breuer A, Pertwee RG, *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949.
- D'Souza DC, Abi-Saab WM, Madonick S, Forselius-Bielen K, *et al.* (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry* **57**, 594–608.
- Dragunow M, Faull RL (1990). MK801 induces c-fos protein in thalamic and neocortical neurons of rat brain. *Neuroscience Letters* **113**, 144–150.
- Duncan GE, Moy SS, Knapp DJ, Mueller RA, *et al.* (1998). Metabolic mapping of the rat brain after subanesthetic doses of ketamine: potential relevance to schizophrenia. *Brain Research* **787**, 181–190.
- Egerton A, Reid L, McGregor S, Cochran SM, *et al.* (2008). Subchronic and chronic PCP treatment produces temporally distinct deficits in attentional set shifting and prepulse inhibition in rats. *Psychopharmacology* **198**, 37–49.
- Filipkowski RK, Rydz M, Berdel B, Morys J, *et al.* (2000). Tactile experience induces c-fos expression in rat barrel cortex. *Learning and Memory* **7**, 116–122.
- Gerdeman GL, Ronesi J, Lovinger DM (2002). Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nature Neuroscience* **5**, 446–451.
- Gessa GL, Casu MA, Carta G, Mascia MS (1998). Cannabinoids decrease acetylcholine release in the

- medial-prefrontal cortex and hippocampus, reversal by SR 141716A. *European Journal of Pharmacology* **355**, 119–124.
- Giuffrida A, Leweke FM, Gerth CW, Schreiber D, et al.** (2004). Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology* **29**, 2108–2114.
- Gray JA, Roth BL** (2007). Molecular targets for treating cognitive dysfunction in schizophrenia. *Schizophrenia Bulletin* **33**, 1100–1119.
- Green AI** (2005). Schizophrenia and comorbid substance use disorder: effects of antipsychotics. *Journal of Clinical Psychiatry* **6**, 21–26.
- Haller J, Szirmai M, Varga B, Ledent C, et al.** (2005). Cannabinoid CB1 receptor dependent effects of the NMDA antagonist phencyclidine in the social withdrawal model of schizophrenia. *Behavioural Pharmacology* **16**, 415–422.
- Heffner TG, Hartman JA, Seiden LS** (1980). A rapid method for the regional dissection of the rat brain. *Pharmacology Biochemistry and Behavior* **13**, 453–456.
- Herrera DG, Robertson HA** (1996). Activation of c-fos in the brain. *Progress in Neurobiology* **50**, 83–107.
- Ichikawa J, Li Z, Dai J, Meltzer HY** (2002). Atypical antipsychotic drugs, quetiapine, iloperidone, and melperone, preferentially increase dopamine and acetylcholine release in rat medial prefrontal cortex: role of 5-HT1A receptor agonism. *Brain Research* **956**, 349–357.
- Jenkins TA, Harte MK, McKibben CE, Elliott JJ, et al.** (2008). Disturbances in social interaction occur along with pathophysiological deficits following sub-chronic phencyclidine administration in the rat. *Behavioral Brain Research* **194**, 230–235.
- Kondziella D, Brenner E, Eyjolfsson EM, Markinhuhta KR, et al.** (2006). Glial-neuronal interactions are impaired in the schizophrenia model of repeated MK801 exposure. *Neuropsychopharmacology* **31**, 1880–1887.
- Leweke FM, Giuffrida A, Koethe D, Schreiber D, et al.** (2007). Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophrenia Research* **94**, 29–36.
- Leweke FM, Giuffrida A, Wurster U, Emrich HM, et al.** (1999). Elevated endogenous cannabinoids in schizophrenia. *Neuroreport* **10**, 1665–1669.
- Malone DT, Long LE, Taylor DA** (2004). The effect of SR 141716 and apomorphine on sensorimotor gating in Swiss mice. *Pharmacology Biochemistry and Behavior* **77**, 839–845.
- Marsicano G, Wotjak CT, Azad SC, Bisogno T, et al.** (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**, 530–534.
- Martin SD, Libretto SE, Pratt DJ, Brewin JS, et al.** (2003). Clinical experience with the long-acting injectable formulation of the atypical antipsychotic, risperidone. *Current Medical Research and Opinion* **19**, 298–305.
- Meltzer HY, Arvanitis L, Bauer D, Rein W** (2004). Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. *American Journal of Psychiatry* **161**, 975–984.
- Mouri A, Noda Y, Enomoto T, Nabeshima T** (2007). Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. *Neurochemistry International* **51**, 173–184.
- Newell KA, Deng C, Huang XF** (2006). Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. *Experimental Brain Research* **172**, 556–560.
- Noda Y, Kamei H, Mamiya T, Furukawa H, et al.** (2000). Repeated phencyclidine treatment induces negative symptom-like behavior in forced swimming test in mice: imbalance of prefrontal serotonergic and dopaminergic functions. *Neuropsychopharmacology* **23**, 375–387.
- Noda Y, Mamiya T, Furukawa H, Nabeshima T** (1997). Effects of antidepressants on phencyclidine-induced enhancement of immobility in a forced swimming test in mice. *European Journal of Pharmacology* **324**, 135–140.
- Noda Y, Yamada K, Furukawa H, Nabeshima T** (1995). Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. *British Journal of Pharmacology* **116**, 2531–2537.
- O’Tuathaigh CM, Kirby BP, Moran PM, Waddington JL** (2009). Mutant mouse models: genotype-phenotype relationships to negative symptoms in schizophrenia. *Schizophrenia Bulletin*. Published online: 24 November 2009. doi:10.1093/schbul/sbp125.
- Pencer A, Addington J, Addington D** (2005). Outcome of a first episode of psychosis in adolescence: a 2-year follow-up. *Psychiatry Research* **133**, 35–43.
- Porsolt RD, Anton G, Blavet N, Jalfre M** (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. *European Journal of Pharmacology* **47**, 379–391.
- Potvin S, Kouassi E, Lipp O, Bouchard RH, et al.** (2008). Endogenous cannabinoids in patients with schizophrenia and substance use disorder during quetiapine therapy. *Journal of Psychopharmacology* **22**, 262–269.
- Pratt JA, Winchester C, Egerton A, Cochran SM, et al.** (2008). Modelling prefrontal cortex deficits in schizophrenia: implications for treatment. *British Journal of Pharmacology* **153** (Suppl. 1), 465–470.
- Robbe D, Kopf M, Remaury A, Bockaert J, et al.** (2002). Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proceedings of the National Academy of Sciences USA* **99**, 8384–8388.
- Roser P, Vollenweider FX, Kawohl W** (2008). Potential antipsychotic properties of central cannabinoid (CB1) receptor antagonists. *World Journal of Biological Psychiatry* **7**, 1–12.
- Rubino T, Patrini G, Parenti M, Massi P, et al.** (1997). Chronic treatment with a synthetic cannabinoid CP-55,940 alters G-protein expression in the rat central nervous system. *Molecular Brain Research* **44**, 191–197.
- Rubino T, Viganò D, Costa B, Colleoni M, et al.** (2000). Loss of cannabinoid-stimulated guanosine 5’-(O-(3-[(35)S]thiotriphosphate) binding without receptor down-regulation in brain regions of

- anandamide-tolerant rats. *Journal of Neurochemistry* **75**, 2478–2484.
- Seillier A, Advani T, Cassano T, Hensler JG, et al.** (2009). Inhibition of fatty-acid amide hydrolase and CB1 receptor antagonism differentially affect behavioural responses in normal and PCP-treated rats. *International Journal of Neuropsychopharmacology* **17**, 1–14.
- Shirazi-Southall S, Rodriguez DE, Nomikos GG** (2002). Effects of typical and atypical antipsychotics and receptor selective compounds on acetylcholine efflux in the hippocampus of the rat. *Neuropsychopharmacology* **26**, 583–594.
- Sjöström PJ, Turrigiano GG, Nelson SB** (2003). Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. *Neuron* **39**, 641–654.
- Straiker A, Mackie K** (2005). Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. *Journal of Physiology* **569**, 501–517.
- Sugita S, Namima M, Nabeshima T, Okamoto K, et al.** (1996). Phencyclidine-induced expression of c-Fos-like immunoreactivity in mouse brain regions. *Neurochemistry International* **28**, 545–550.
- Théberge J, Bartha R, Drost DJ, Menon RS, et al.** (2002). Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. *American Journal of Psychiatry* **159**, 1944–1946.
- Tzavara ET, Davis RJ, Perry KW, Li X, et al.** (2003). The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. *British Journal of Pharmacology* **138**, 544–553.
- Tzavara ET, Li DL, Moutsimilli L, Bisogno T, et al.** (2006). Endocannabinoids activate transient receptor potential vanilloid 1 receptors to reduce hyperdopaminergia-related hyperactivity: therapeutic implications. *Biological Psychiatry* **59**, 508–515.
- Viganò D, Guidali C, Petrosino S, Realini N, et al.** (2009). Involvement of the endocannabinoid system in phencyclidine-induced cognitive deficits modelling schizophrenia. *International Journal of Neuropsychopharmacology* **12**, 599–614.
- Viganò D, Rubino T, Vaccani A, Bianchessi S, et al.** (2005). Molecular mechanisms involved in the asymmetric interaction between cannabinoid and opioid systems. *Psychopharmacology (Berlin)* **182**, 527–536.
- Zavitsanou K, Garrick T, Huang XF** (2004). Selective antagonist [<sup>3</sup>H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **28**, 355–360.
- Zuo DY, Cao Y, Zhang L, Wang HF, et al.** (2009). Effects of acute and chronic administration of MK-801 on c-Fos protein expression in mice brain regions implicated in schizophrenia with or without clozapine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **33**, 290–295.
- Zuo DY, Zhang YH, Cao Y, Wu CF, et al.** (2006). Effect of acute and chronic MK-801 administration on extracellular glutamate and ascorbic acid release in the prefrontal cortex of freely moving mice on line with open-field behaviour. *Life Science* **78**, 2172–2178.