Long-lasting recovery of psychotic-like symptoms in isolation-reared rats after chronic but not acute treatment with the cannabinoid antagonist AM251

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Abstract
In this work we investigated the ability of AM251 to reverse schizophrenia-like symptoms produced by a neurodevelopmental animal model based on a social isolation procedure. First, we assessed the validity of our isolation-rearing protocol and, as expected, isolation-reared rats showed hyperlocomotion in a novel environment, cognitive impairment in the novel object recognition (NOR) test and a significant increase in the number of aggressive behaviours in the social interaction test compared to group-housed controls. This behavioural picture was associated with a reduction in CB1 receptor/G protein coupling in specific brain areas as well as reduced c-Fos immunoreactivity in the prefrontal cortex and caudate putamen. In this model, chronic but not acute treatment with the CB1 receptor antagonist AM251 counteracted isolation-induced cognitive impairment in the NOR test and aggressive behaviours in the social interaction test. This behavioural recovery was accompanied by the rescue of CB1 receptor functionality and c-Fos levels in all brain regions altered in isolation-reared rats. Moreover, chronic AM251 also increased c-Fos immunoreactivity in the nucleus accumbens, as previously demonstrated for antipsychotic drugs. Interestingly, the behavioural recovery due to chronic AM251 administration persisted until 10 d after discontinuing the treatment, indicating a long-lasting effect of the cannabinoid antagonist on psychotic-like symptoms.

Key words: Aggressive behaviour, cannabinoid antagonist, CB1 receptor, isolation rearing, novel object recognition.

Introduction
Different theories have attempted to clarify the aetiology of schizophrenia but the exact causes of this complex and multifactorial mental disorder remain unknown.

The aetiology of schizophrenia has been largely demonstrated as an involvement of the dopaminergic and glutamatergic systems (Coyle, 2006; Howes & Kapur, 2009) but recent experimental evidence strongly suggest that alterations in the endocannabinoid system may also contribute to the pathogenesis of the disease (De Marchi et al. 2003; Giuffrida et al. 2004; Leweke et al. 1999; Lewis et al. 2005; Newell et al. 2006; Sundram et al. 2005; Ujike et al. 2002). Accordingly, a ‘cannabinoid hypothesis’ of schizophrenia has been suggested (Muller-Vahl & Emrich, 2008).

Based on this hypothesis, the pharmacological manipulation of the endocannabinoid system may represent a promising tool for improving symptoms of the disease; however, the experimental findings concerning the effects of CB1 receptor (CB1R) agonists and antagonists on schizophrenia-like symptoms are still controversial, often with different effects depending on the drug, the dose, the species and the model used.
for simulating positive or negative symptoms (for review see Parolaro et al. 2010). In general, acute administrations of CB₁R agonists reduce the positive symptoms induced by dopaminergic and glutamatergic agents (Gorriti et al. 1999; Marcellino et al. 2008; Przegalinski et al. 2005), whereas the ability of CB₁R antagonists to reverse schizophrenia-like positive symptoms is still under debate (for review see Parolaro et al. 2010; Roser et al. 2008).

With regard to the negative symptoms of schizophrenia, most studies have investigated the effects of a manipulation of the endocannabinoid system on the prepulse inhibition (PPI) of the acoustic startle reflex. CB₁R agonists cause disruption of the PPI (Martin et al. 2003; Nagai et al. 2006; Schneider & Koch, 2002) reversed by CB₁R antagonists, in contrast CB₁R antagonists show no effect or even an improvement in NMDA antagonist- or D₃ agonist-induced disruption of PPI (Ballmaier et al. 2007; Malone et al. 2004; Martin et al. 2003). Finally, genetic CB₁R disruption counteracted the social deficit induced by PCP in mice (Haller et al. 2005).

We recently demonstrated that the CB₁R antagonist, AM251, restored the cognitive impairment in the novel object recognition (NOR) test and reduced avolition in the forced swim test, a behavioural test commonly also used to assess depression, induced by chronic intermittent PCP treatment, a pharmacological model reproducing some schizophrenia-like symptoms (Guidali et al. 2010). This behavioural recovery was correlated with the restoration of CB₁R function in all brain areas altered by PCP administration. Moreover, chronic AM251 co-treatment antagonized the PCP-induced increase in c-Fos expression in the prefrontal cortex (PFC), a key region for the integration of cognitive and negative signs of schizophrenia. In the same brain region, chronic AM251 treatment counteracted the increase in 2-arachidonoylglycerol observed in PCP-treated rats and enhanced anandamide levels in the same cerebral area (Guidali et al. 2010). Recently, Seillier’s group, using a subchronic PCP model of aspects of schizophrenia in rats, reported that acute AM251 reversed the PCP-induced working-memory deficit but had no effect in the social interaction test both in PCP- and saline-treated rats. Moreover, they found no changes in CB₁R expression, although PCP-treated rats showed an increase in receptor-stimulated [³⁵S]GTPyS binding in the anterior cingulate cortex and nucleus accumbens (NAc), accompanied by a reduction in the CA2/3 fields of the hippocampus (Seillier et al. 2010). These findings partially disagree with those previously reported by our group (Viganò et al. 2009) and these discrepancies might be explained by differences in drug regimen and the age of the animals used in the studies.

In this work we investigated the ability of AM251 to reverse schizophrenia-like symptoms produced by a neurodevelopmental animal model based on the social isolation procedure. Rearing rats in social isolation from weaning produces persistent behavioural and neurochemical alterations compared to group-housed controls (Fone & Porkess, 2008; Lapiz et al. 2003). Behavioural changes observed in isolation-reared rats may have translation relevance to several core symptoms of schizophrenia such as locomotor hyperactivity in a novel environment, impaired sensorimotor gating, aggressive behaviour and cognitive impairment (Fone & Porkess, 2008). Interestingly, recent papers showed alterations in several components of the endocannabinoid system in different brain regions of isolation-reared rats, including important areas implicated in the pathophysiology of schizophrenia (Robinson et al. 2010; Sciolino et al. 2010).

On these bases, in this study we evaluated the effects of a pharmacological manipulation of the endocannabinoid system by acute and chronic AM251 treatment on isolation rearing-induced cognitive impairment and aggressive behaviour. Moreover, we analysed at different time points the effects of isolation rearing and chronic AM251 treatment on the endocannabinoid system in terms of CB₁R density and functionality.

**Materials and methods**

**Animals**

At weaning (PND 21), male Lister Hooded rats (Harlan, Italy) were randomly housed in groups of four (grouped) or alone (isolated). All animals were housed in the same room and had visual, auditory and olfactory contact with animals caged nearby, on a 12-h light/dark cycle (lights on 08:00 hours) and in a temperature- (24±2 °C) and humidity-controlled environment (50±10%) with food and water available ad libitum. The isolated animals were left undisturbed in their cages and received the minimal handling associated with husbandry (cage and bedding changed weekly).

All experiments took place during the light phase and were performed in 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.
**Drug administration**

AM251 (Tocris, Italy) was dissolved in DMSO, Tween-80 and saline (1:1:8). The drug was acutely or chronically administered at 0.5 mg/kg (with the injection volume of 5 ml/kg) i.p.

For acute treatment each animal received a single injection 80 min before the test session, whereas for chronic administration AM251 was given daily for 3 wk and animals underwent a series of behavioural tests 24 h, 72 h and 10 d after the last AM251 (or vehicle) administration. The different behavioural tests were performed on separate groups of animals (Fig. 1).

**Behavioural tests**

*Spontaneous locomotor activity*

Rats were placed in a computer-controlled infrared activity monitoring arena. The arena consisted of a clear acrylic box, 43 × 43 × 32 cm (Ugo Basile, Italy) placed in a sound-attenuating room. The cage was fitted with two parallel infrared beams, located 2 cm and 6 cm from the floor and cumulative horizontal and vertical movement counts were recorded for 1 h.

*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. Animals performed each test individually. A 10-min habituation session preceded the experimental trials. The experiment was performed and analysed as previously described in Vigano` et al. (2009). Briefly, after habituation each animal was placed in the arena and allowed to explore two identical previously unseen objects for 10 min (familiarization phase). After an inter-trial interval of 1 h one of the two familiar objects was replaced by a novel, previously unseen object and rats were returned to the arena for the 3-min test phase. During the test phase the time spent exploring the familiar object ($E_f$) and the new object ($E_n$) was videotaped and recorded separately by two observers blind to the treatment groups and the discrimination index was calculated as follows: $[(E_n - E_f)/(E_n + E_f)] \times 100$.

**Social interaction test**

The test was carried out in a room illuminated with a dim overhead light. On the day of testing, each animal was habituated for 10 min in the test arena (60 × 60 × 60 cm), an open-field box made of Plexiglas. During the test session, each animal was allowed to freely explore an unfamiliar congener in the arena for 10 min. The arena was cleaned with 0.1% acetic acid and dried after each trial. Social behaviours were defined as sniffing, following, grooming, mounting and nosing. Aggressive behaviours were defined as attacking, biting, tail rattling and aggressive grooming. The whole testing phase was videotaped, analysed by two observers blind to the treatment groups and we recorded the time spent in social behaviours and the number of aggressive behaviours.

**Biochemical assays**

For assessment of long-term effects of isolation rearing and AM251 treatment on CB$_1$R function, biochemical analyses were performed on separate groups of animals not tested for behaviour. Rats were decapitated and brains were rapidly removed, frozen in liquid nitrogen and stored at −80 °C until processing.

**Autoradiographic-binding assays**

Coronal sections (20-μm-thick) were cut on a cryostat and mounted on gelatin-coated slides. The sections were stored at −80 °C until processing.
CP-55,940 receptor autoradiographic binding was performed as described previously (Rubino et al. 2000; Vigano` et al. 2009).

CP-55,940-stimulated [35S]GTPγS binding in autoradiography

This was determined as described previously by our group (Rubino et al. 2000; Vigano` 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 (Vigano` et al. 2005).

c-Fos immunohistochemistry

c-Fos expression was assessed as described previously (Guidali et al. 2010). Briefly, sections were incubated with a primary antibody to c-Fos (Abcam, UK) diluted 1/50 for 48 h at 4 °C and with biotylated secondary antibody diluted 1/100 for 1 h at room temperature and finally incubated with avidin–biotin–peroxidase complex (Vector ABC kit, Vector Laboratories, USA) for 1 h at room temperature. Slides were then incubated in chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB) for 5 min. Some control sections were stained without the primary antibody. Positive neurons were counted as described previously (Guidali et al. 2010).

Statistical analysis

All analysis were performed using GraphPad Prism 3.0 software and data are reported as mean ± S.E.M. Results were analysed using unpaired Student’s t test or two-way ANOVA followed up by Bonferroni’s post-hoc test to examine group differences. The level of statistical significance was set at p < 0.05.

Results

Behavioural assessment of isolation-rearing protocol

Figure 2 shows the behavioural scene after 5 wk of isolation rearing.

Fig. 2. Behavioural phenotype after 5 wk of isolation rearing. (a) Horizontal (left) and vertical (right) activity assessed in the activity cage. (b) Exploration time of identical objects during the familiarization phase (left), exploration time of the familiar vs. novel object (centre) and the discrimination index (right) during the test phase in the novel object recognition test. (c) Number of aggressive behaviours (left) and time spent in social behaviours (right) during the social interaction test. Results are expressed as mean ± S.E.M. ** p < 0.01 vs. familiar object (t test), *** p < 0.001 vs. grouped (t test).
Isolation-reared rats were significantly more active in the novel environment than group-housed rats ($t = 8.820$, $p < 0.0001$) without any alteration in rearing activity between the two groups ($t = 1.876$, $p = 0.0832$) (Fig. 2a).

Moreover, the isolation-rearing protocol caused an impairment of cognitive functions as demonstrated by a significant reduction in the discrimination index during the test phase of the NOR test ($t = 10.25$, $p < 0.0001$) compared to group-reared controls. In both groups there was no difference in the time spent exploring the two identical objects during the familiarization phase, but isolated animals failed to discriminate between the new and familiar object in the test phase (Fig. 2b). The locomotor activity was not altered both in grouped and isolated rats (data not shown).

In the social interaction test, no differences were found in the time spent in active behaviours between isolation- and group-reared animals ($t = 0.1969$, $p = 0.8504$) but isolation rearing caused a significant increase in aggressive behaviours compared to group-reared controls ($t = 32.75$, $p < 0.0001$) (Fig. 2c).

**Effects of the isolation-rearing protocol on CB,R functionality**

After 5 wk of isolation rearing, we investigated the effects of housing condition on CB,R density and functionality.

Figure 3a shows the effects of isolation rearing on CB,R density. Isolation rearing had no effect on CB,R density in all the cerebral regions analysed.

We found significant changes in CB,R functionality as shown by the GTPyS binding assay (Fig. 3b). Particularly, the isolation-rearing protocol induced a significant reduction in CB,R functionality in the PFC ($t = 2.093$, $p = 0.0481$), NAc ($t = 3.572$, $p = 0.0017$), caudate putamen (CPu) ($t = 3.507$, $p = 0.0020$), hippocampus (Hippo) ($t = 2.546$, $p = 0.0216$) and ventral tegmental area (VTA) ($t = 3.438$, $p = 0.0044$).

**Effects of acute AM251 administration on cognitive impairment and aggressive behaviour induced by isolation rearing protocol**

In the NOR test two-way ANOVA found acute AM251 treatment did not alter the exploration time in the familiarization phase both in grouped and isolated rats and failed to improve the recognition memory disrupted by social isolation rearing (housing: $F_{1,12} = 75.24$, $p < 0.0001$; drug: $F_{1,12} = 0.0001214$, $p = 0.9914$; no interaction) (Fig. 4). Moreover, acute AM251 alone did not affect the discrimination index in socially reared rats and there were no differences in the locomotor activity between all the groups considered (data not shown).

In the social interaction test acute AM251 did not significantly reduce the number of aggressive events.
in isolation-reared rats (housing: $F_{1,12} = 6.834, p = 0.0226$; drug: $F_{1,12} = 4.755, p = 0.0498$; drug x housing interaction: $F_{1,12} = 19.43, p = 0.0023$) (Fig. 5a) and the recovery of this parameter was still evident at 72 h (housing: $F_{1,12} = 5.482, p = 0.0373$; drug: $F_{1,12} = 12.27, p = 0.0044$; no interaction) (Fig. 5b) and 10 d after the last AM251 administration (housing: $F_{1,12} = 10.48, p = 0.0071$; drug: $F_{1,12} = 6.403, p = 0.0264$; drug x housing interaction: $F_{1,12} = 10.39, p = 0.0073$) (Fig. 5c). Neither housing conditions nor AM251 treatment altered the time spent exploring the two identical objects during the familiarization phase and AM251 alone did not affect the recognition memory in socially reared rats. The locomotor activity was not altered in any of the groups analysed (data not shown).

Figure 6 shows the effects of chronic AM251 treatment on the aggressive behaviours in the social interaction test (Fig. 6a). We observed a reduction in the number of aggressive behaviours in isolation-reared rats 72 h and 10 d after the last AM251 administration compared to what was observed in the social interaction test performed 24 h after the last injection. However, the aggressive behaviours in isolation-reared rats were still significantly increased compared to group-reared controls at both time-points. Isolation-reared rats chronically administered with AM251 showed a significant reduction in the number of aggressive events in the social interaction test performed 24 h after discontinuing treatment (drug: $F_{1,12} = 13.71, p = 0.0030$; drug x housing interaction: $F_{1,12} = 13.71, p = 0.0030$) and this recovery was still evident at 72 h (drug: $F_{1,12} = 11.37, p = 0.0056$; drug x housing interaction: $F_{1,12} = 11.37, p = 0.0056$) and 10 d (drug: $F_{1,12} = 6.716, p = 0.0236$; drug x housing interaction: $F_{1,12} = 6.716, p = 0.0236$) after the last AM251 administration.
Chronic AM251 did not alter the time spent in social behaviours.

**Effects of AM251 chronic treatment on CB1R functionality**

After 3 wk of chronic AM251 (or vehicle) treatment we found no alterations in CB1R density in group-housed or isolation-reared rats, in all the brain regions analysed by two-way ANOVA (Fig. 7a).

Fig. 7b represents the results of CP-55,940-stimulated GTPyS autoradiographic-binding assay performed 24 h after the last chronic AM251 (or vehicle) administration.

AM251 had no effect on CB1R functionality in group-housed controls but counteracted the alterations observed in rats reared in isolation in the PFC, NAc, Hippo, and VTA. After 3 wk of chronic vehicle treatment, isolation-reared rats still showed a significant reduction in the CB1R functionality in the PFC, NAc, Hippo, and VTA compared to group-housed controls (PFC, housing: $F_{1,28} = 6.610$, $p = 0.0157$; NAc: housing: $F_{1,28} = 7.083$, $p = 0.0127$; Hippo, housing: $F_{1,28} = 8.549$, $p = 0.0068$; VTA, housing: $F_{1,28} = 8.549$, $p = 0.0068$), indicating that these alterations were not influenced by daily handling. The reduction reported in isolation-reared rats were counteracted by AM251 chronic administration (PFC, drug × housing interaction: $F_{1,28} = 8.881$, $p = 0.0059$; NAc, drug × housing interaction: $F_{1,28} = 5.848$, $p = 0.0223$; Hippo, drug × housing interaction: $F_{1,28} = 8.410$, $p = 0.0072$).
c-Fos immunohistochemistry

Figure 8a shows the effects on c-Fos immunoreactivity in rats reared in isolation for 5 wk in the PFC, CPu, and NAc. Isolation rearing significantly reduced c-Fos expression in the PFC ($t = 5.346, p < 0.0001$) and CPu ($t = 3.583, p = 0.0038$) compared to group-housed controls, but had no effect on c-Fos expression in the NAc.

Figure 8b represents the effects of isolation rearing and chronic AM251 (or vehicle) administration on c-Fos expression. Two-way ANOVA showed the reduction in c-Fos observed in the PFC was still evident in isolation-reared rats after 3 wk of chronic vehicle treatment (housing: $F_{1,28} = 4.927, p = 0.0357$). Moreover, chronic AM251 administration counteracted the reduction in c-Fos expression observed in the PFC of isolation-reared rats (drug $\times$ housing interaction: $F_{1,28} = 4.804, p = 0.0379$). Instead, chronic handling due to chronic vehicle treatment recovered the reduction in c-Fos expression observed in the CPu of rats reared in isolation and AM251 treatment did not show any further effect.

Chronic AM251 administration per se significantly increased c-Fos expression in NAc ($F_{1,28} = 20.52, p = 0.0001$) in isolation and group-reared rats.

Discussion

In the present study, using the social isolation paradigm as a model of psychotic-like behaviours, we demonstrated that alterations in CB$_1$R functionality...
represent one of the molecular mechanisms contributing to the behavioural phenotype observed in isolation-reared rats. In addition, we clearly highlighted that chronic AM251 exerted an apparent beneficial action in terms of reversing the behavioural phenotype and the alterations in CB₁R functionality as well as the reduction in neuronal activity induced by isolation.

Fig. 7. Effect of chronic AM251 on CB₁ receptor density and functionality. (a) [³H]CP-55,940 receptor autoradiographic binding. (b) CP-55,940-stimulated [³⁵S]GTPγS binding in autoradiography. Results are expressed as mean ± S.E.M. * p < 0.05, ** p < 0.01 vs. grouped + vehicle; †† p < 0.01, ††† p < 0.001 vs. isolated + vehicle (Bonferroni’s post-hoc test).

Fig. 8. Effect of (a) social isolation protocol and (b) chronic AM251 treatment on c-Fos immunoreactivity in the prefrontal cortex (PFC), nucleus accumbens (NAc) and caudate putamen (CPu). (a) c-Fos immunoreactivity evaluated after 5 wk of isolation rearing. (b) c-Fos immunoreactivity after 3 wk of chronic AM251 administration. Results are expressed as mean ± S.E.M. ** p < 0.01, *** p < 0.001 vs. grouped (t test); ** p < 0.01 vs. grouped + vehicle; † p < 0.05, †† p < 0.01 vs. isolated + vehicle (Bonferroni’s post-hoc test). For abbreviations see Fig. 3 legend.
First, we assessed the validity of the isolation-rearing protocol before performing other behavioural and neurochemical analyses. In fact, animals reared in isolation show a pattern of behavioural alterations such as hyperlocomotion in a novel environment (Bakshe & Geyer, 1999; Einon & Morgan, 1976) cognitive impairment (Bianchi et al. 2006; Fone & Porkess, 2008; Hellemans et al. 2004; Lapiz et al. 2000; Lu et al. 2003) and an increase in aggressive behaviours in the social interaction test (Toth et al. 2008; Vale & Montgomery, 1997; Wongwitdecha & Marsden, 1996). In our study, after 5 wk of isolation rearing, rats presented a marked increase in total horizontal locomotor activity, a cognitive impairment in the NOR test and a significant increase in the number of aggressive behaviours.

Recent studies have demonstrated a close association between the behavioural phenotype induced by isolation rearing and the presence of alterations in the endocannabinoid system (Malone et al. 2008; Robinson et al. 2010), thus in these animals, using autoradiographic techniques, we explored the levels of CB1 receptor and observed no changes in CB1 receptor binding sites in all brain regions considered. Our data appear in contrast to the results reported in recent papers that demonstrated a reduction in CB1 immunoreactivity restricted to CPU and amygdala (Malone et al. 2008) or, more recently, an increase of the CB1 receptor mRNA expression (Robinson et al. 2010). The discrepancies between our study and those of Malone and Robinson can be due to the different techniques used to evaluate CB1 receptor density (autoradiographic binding assay or immunohistochemistry), the different rat species (Lister Hooded or Sprague–Dawley) and the duration of the isolation-rearing protocol (5 wk or 8 wk), in fact it is possible that down-regulation in CB1 receptor could appear after longer periods of isolation.

Despite the results regarding CB1 receptor density, our data show widely diffused alterations in CB1/11 receptor protein coupling in isolation-reared rats. In particular, it was significantly reduced in the PFC (−35%), CPU (−45%), NAc (−52%), Hippo (−39%) and VTA (−58%).

We can speculate that an increase in the endocannabinoid levels could underlie the reduction in CB1 functionality observed in isolation-reared rats. In line with this, Sciolino et al. (2010) demonstrated alterations in anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels in socially isolated rats: they found an increase in 2-AG in the PFC and an increase of 2-AG and AEA in the piriform cortex. Moreover, Robinson et al. (2010) observed an increase in mRNA levels of the enzymes responsible for the synthesis of AEA and 2-AG, accompanied by a reduction in FAAH mRNA levels in rats reared in isolation (Robinson et al. 2010), thus suggesting the presence of alterations in endocannabinoid content. Taken together, all these observations suggest that the widely diffused desensitization of CB1 receptor we observed might be ascribed to elevation of endocannabinoid level induced by isolation.

It is well established that schizophrenia and other psychiatric conditions are associated with specific alterations in the dopaminergic (Howes & Kapur, 2009) and endocannabinoid (Parolaro et al. 2010) systems. Interestingly, an overlapping distribution of cannabinoid receptors with dopaminergic receptors has been demonstrated in most brain areas where we found alterations in CB1 receptor functionality (Hermann et al. 2002). Cannabinoid receptors have been shown to modulate dopaminergic transmission through trans-synaptic mechanisms, involving GABAergic and glutamatergic synapses (Chiu et al. 2010; van der Stelt & Di Marzo, 2003). In our model, the alteration in the endocannabinoid system observed in isolation-reared rats may reflect a homeostatic mechanism to hyper-dopaminergic transmission produced by isolation or, alternatively, be a direct cause of the psychosis through a reduction of the endocannabinoid inhibitory control on dopaminergic transmission (Pistis et al. 2002; Robbe et al. 2002). In line with this, the reduction in CB1 receptor functionality may enhance dopamine transmission which may, at least in part, account for the behavioural alterations observed in isolation-reared rats. It is worth noting that all brain regions showing altered CB1 receptor functionality in the CPu may account for the hyperlocomotion observed in isolation-reared rats (Marcellino et al. 2008) and the mesolimbic dopaminergic pathways, originating from the VTA and projecting to forebrain nuclei such as NAc may be involved in aggressive behaviours (Fone & Porkess, 2008) as well as psychotic symptoms (Laviolette & Grace, 2006; Watanabe et al. 1998).

Atypical antipsychotic drugs are effective in alleviating isolation rearing-induced cognitive impairment and social withdrawal (Feifel et al. 2004; Li et al. 2007a; Toua et al. 2010) and recent findings suggest that CB1 receptor antagonists possess a pharmacological profile reminiscent of atypical antipsychotics (Guidali et al. 2010). In fact, our previous work demonstrated the ability of
chronic treatment with the CB$_1$R antagonist AM251 to reverse psychotic-like symptoms in a pharmacological model of aspects of schizophrenia based on chronic-intermittent PCP injections (Guidali et al. 2010), suggesting a potential antipsychotic action of this compound.

In the present work, we further investigated the potential antipsychotic effect of acute and chronic AM251 treatment on isolation rearing-induced behavioural and neurochemical alterations. Acute AM251 administration did not reverse isolation-induced cognitive impairment and social withdrawal, suggesting that a single AM251 administration may not be sufficient to reach a beneficial effect. The results obtained with chronic AM251 treatment appear more interesting. To the best of our knowledge, this is the first study evaluating the effect of a chronic pharmacological treatment of the endocannabinoid system in rats reared in isolation. Rats were chronically administered with AM251 (or vehicle) daily for 3 wk, as reported previously, for the atypical antipsychotic clozapine (Li et al. 2007b). Interestingly, the alterations observed in rats reared in isolation have been reported to be particularly influenced by chronic handling (Scioli et al. 2010) and this might be taken into consideration when performing a chronic pharmacological treatment in socially isolated rats. In our model, chronic handling did not affect the cognitive impairment induced by isolation whereas the aggressive behaviours were reduced by handling but still remained significantly elevated when compared to group-housed controls. Intriguingly, chronic AM251 alone did not affect the cognitive functions in group housed controls. However, chronic treatment of AM251 to isolation-reared rats significantly improved the performance in the NOR test and reduced the aggressive behaviours in the social interaction test. It is worth noting that this recovery persisted up to 10 d after discontinuing the treatment, indicating a long-lasting effect of the cannabinoid antagonist on psychotic-like symptoms. This is an intriguing property of AM251 since, following treatment with antipsychotic drugs, patients have shown a relapse of psychotic symptoms when taken off the drug (Li et al. 2007a) and, moreover, antipsychotics have been associated with untoward effects upon withdrawal (Lee & Robertson, 1997).

The mechanisms underlying the beneficial effects of AM251 on psychotic-like symptoms are still unclear. We have clearly demonstrated that psychotic symptoms in isolation-reared rats are accompanied by alterations in the endocannabinoid system, thus we first investigated a direct effect of AM251 treatment on CB$_1$R functionality. Chronic AM251 had no effect on CB$_1$R density in grouped or isolated rats. The alterations in CB$_1$R/G protein coupling reported in isolated rats in the PFC, NAc, Hippo, and VTA were still evident after 3 wk of chronic vehicle treatment, except for the reduction in the CPu that seemed to have been counteracted by chronic handling. Interestingly, chronic AM251 completely restored CB$_1$R functionality in the PFC, NAc, Hippo, and VTA in isolated rats without having per se any effect in all brain areas analysed. Since a decreased CB$_1$R functionality in the PFC and Hippo has been associated with cognitive impairment (Bilkei-Gorzo et al. 2005; Eggen et al. 2010; Hill et al. 2005), we can speculate that the ability of AM251 to normalize the CB$_1$R functionality in these areas may underlie the restoration of cognitive functions in isolation-reared rats. Similarly, if a reduction in CB$_1$R functionality in the mesolimbic pathway could account for the increased dopamine in the NAc responsible for aggressive behaviours (van Erp & Miczek, 2000), the ability of AM251 to restore normal CB$_1$R functionality in this pathway may, at least in part, contribute to the recovery of aggressiveness.

Finally, we investigated c-Fos immunoreactivity in the PFC, CPu, and NAc of isolation-reared rats to identify activated neurons and extended circuits since c-Fos is the most widely used functional anatomical marker of activated neurons within the central nervous system (Kovacs, 2008). Isolation significantly reduced c-Fos immunoreactivity in the PFC and CPu without affecting it in the NAc. It has previously been demonstrated that a decrease in c-Fos expression in the PFC after social isolation (Levine et al. 2007) and reduced neuronal activity in this area may be associated with negative symptoms of schizophrenia such as social withdrawal observed after isolation (Perlstein et al. 2003; Weinberger & Berman, 1988). In this paper, we extended the analyses of c-Fos immunoreactivity to other forebrain regions and our results contribute to the improvement of knowledge on the effects of isolation rearing on neuronal activity. However, further investigations are needed to clarify the functional significance of the alterations observed.

To further support the hypothesized antipsychotic properties of AM251, we investigated its effects on c-Fos protein expression. Chronic AM251 treatment counteracted the reduction in c-Fos expression in the PFC and CPu of isolated rats without altering it in group-housed controls. In the NAc, AM251 per se significantly increased c-Fos expression in group- and isolation-reared rats. These areas are reported to be fundamental for determining the therapeutic outcome of antipsychotic drugs. As demonstrated previously, both typical and atypical antipsychotics increase c-Fos
levels in the NAc and this effect appears to be predictive of an antipsychotic action (Fujimura et al. 2000; Oka et al. 2004; Wan et al. 1995). Moreover, AM251 increased c-Fos in the NAc, thus suggesting its potential antipsychotic action. Additionally, a boost in c-Fos expression in the PFC is correlated to a superior efficacy against negative symptoms of schizophrenia (Deutch & Duman, 1996; Robertson & Fibiger, 1996), thus the increase in c-Fos immunoreactivity due to AM251 treatment in this area may account for the reduction of aggressiveness observed in isolated rats and further support the antipsychotic potential of this cannabinoid antagonist. Finally, several studies have shown that c-Fos expression in rat CPu is a reliable index of the extrapyramidal symptom liability of antipsychotic drugs (Marchese et al. 2008; Robertson et al. 1994; Wan et al. 1995). In our model, AM251 re-established a normal striatal neuronal activity without further enhancement of c-Fos over the control levels rather than as observed for typical antipsychotics (Marchese et al. 2008; Robertson et al. 1994; Wan et al. 1995). This might be suggestive of a drug nearly devoid of extrapyramidal side-effects, as already demonstrated for clozapine (Marchese et al. 2008; Robertson et al. 1994; Wan et al. 1995).

In conclusion, our results suggest a potential antipsychotic role of the cannabinoid antagonist AM251 in a neurodevelopmental model of psychotic-like symptoms. Acute AM251 administration is not sufficient to reach a therapeutic effect whereas chronic treatment counteracts both aggressive behaviour and cognitive impairment induced by isolation. The behavioural recovery appears to be mediated by the rescue of CB1R functionality altered by isolation in specific brain areas that may impact neuronal activation, as demonstrated by c-Fos immunoreactivity, as well as other neurotransmitter systems.

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Statement of Interest

None.

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