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# Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters

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**Abstract**

The aim of the present study was to examine the effects of the cannabinoid agonist CP 55,940 and the antagonist SR 141716A, alone and in combination, on rat exploratory and anxiety-like behaviour in the holeboard and elevated plus-maze tests. A further aim was to evaluate the effects of these treatments on hypothalamic neurotransmitters. Animals treated with CP 55,940 doses of 0.125 and 0.1 mg/kg exhibited less exploration and an increase in anxiety-like behaviour accompanied by great motor inhibition. No hypoactivity was seen at 0.075 mg/kg dosage, but anxiety and neophobic responses persisted, indicating independent and specific effects. Motor activity effects induced by CP 55,940 were reversed by pretreatment with SR 141716A (3 mg/kg). Surprisingly, when administered on its own, the antagonist also induced a reduction in exploratory parameters and an increase in anxiety-like responses. These apparently similar effects might be caused by different neural mechanisms. Finally, CP 55,940 increased hypothalamic dopamine and serotonin levels. These increases might be involved in the activation of the hypothalamic–pituitary–adrenal axis described for cannabinoids. 

*Keywords:* Rat; Cannabinoids; Exploratory behaviour; Motor activity; Anxiety; Plus-maze; Holeboard; Serotonin; Dopamine; GABA; Hypothalamus

## 1. Introduction

In rodents, both $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), the major psychoactive component of the plant, *Cannabis sativa* L., and other synthetic compounds alter specific types of behaviour, including nociception, motor activity, memory and feeding (Chaperon and Thiébot, 1999). It has been reported that cannabinoids modify rodent behavioural responses to novelty in different tests (Giuliani et al., 2000; Hernández-Tristán et al., 2000; Navarro et al., 1993; Onaivi et al., 1990). One of the most widely used experimental models for the study of anxiety is the elevated plus-maze, which exploits the aversion of rodents for novel, high and open spaces (Pellow et al., 1985). Acute and subacute doses of $\Delta^9$-THC increase the aversion of rats and mice to the open-arm structures of the test apparatus. They also induce a reduction in the number of entries into the closed arms, showing deterioration in their motor activity (Onaivi et al., 1990). This effect could explain the anxiogenic reactions reported in humans acutely exposed to *Cannabis* derivatives (Hall and Solowj, 1998). A similar effect is seen with rats subchronically treated with the potent cannabinoid agonist HU-210 in the X-maze test. However, the marked hypoactivity produced by acute treatment with this compound impairs the evaluation of its anxiogenic effects (Giuliani et al., 2000).

In the holeboard test, a paradigm involving novelty and uncertainly, $\Delta^9$-THC is also able to produce a decrease of the site-direct exploratory behaviour of rats. This reduction is accompanied by a decrease in motor activity (general ambulation and rearing) (Hernández-Tristán et al., 2000). Evaluation of the anxiogenic or emotional responses to cannabinoids is, therefore, complicated since there could be interaction between motor activity and these effects.

Paradoxically, the selective CB1 receptor antagonist SR 141716A (3 mg/kg) induces the complete abolition of open-arm entries in the elevated plus-maze in rats. SR 141716A is also able to produce dose-dependent anxiogenic-like responses in the defensive withdrawal test (Navarro et al., 1997). Surprisingly, there are no studies of combined agonist/antagonist treatments to clarify these apparently very similar

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effects of cannabinoid agonist and antagonist on anxiety-related behaviours.

It has been demonstrated that $\Delta^9$-THC and other cannabinoids stimulate adrenocorticotropic hormone (ACTH) secretion (Murphy et al., 1998), and that central corticotropin-releasing factor (CRF) systems play an important role in the mediation of cannabinoid-induced anxiogenic patterns (Rodríguez de Fonseca et al., 1996). The exact mechanisms underlying the effects of cannabinoids on the hypothalamic–pituitary–adrenal (HPA) axis are unclear, but could be due to possible alterations in neurotransmitter modulation of CRF secretion. Previous studies have reported that cannabinoids are able to alter the activity of hypothalamic dopaminergic (Rodríguez de Fonseca et al., 1992) and serotoninergic neurones (Kramer and Ben-David, 1978). These systems are involved in the regulation of CRF release. In this way, there are only a few studies which have studied the cannabinoid effects on hypothalamic neurotransmitters. These studies were carried out only at the level of the medial basal hypothalamus (MBH) (Murphy et al., 1990; Fernández-Ruiz et al., 1992), excluding important serotoninergic terminals projecting from the midbrain raphe nuclei to the paraventricular hypothalamic nucleus (PVN), which controls the synthesis and release of CRF.

The first aim of this investigation was to study the effects of different doses of the cannabinoid agonist CP 55,940 and the antagonist SR 141716A, alone or in combination, on exploratory behaviour and anxiety in the rat. The holeboard and the elevated plus-maze tests were used to independently measure locomotion, exploration and anxiety. The second purpose was to evaluate the effects of these treatments on hypothalamic neurotransmitters involved in CRF release.

## 2. Method

### 2.1. Animals

Male Wistar rats (ANUC, Madrid, Spain) weighing 250–300 g were housed in groups of five and maintained at 22±2°C while exposed to a reversed 12:12 h light–dark photoperiod. They had free access to food and water. All experiments were conducted according to European Animal Care Guidelines.

### 2.2. Drugs

CP 55,940 (Tocris, Madrid, Spain) and SR 141716A (Sanofi Recherche, Montpellier, France) were freshly prepared and dissolved in a Tween 80–saline solution. All drugs were administered by intraperitoneal injection.

### 2.3. Treatments

Animals were randomly assigned to various groups and intraperitoneally injected with either SR 141716A (0.4 ml — 3 mg/kg body weight) or Tween–saline vehicle (equal volume). Fifteen minutes later, both SR 141716 and vehicle-injected animals were also injected with CP 55,940. Three different doses (0.125, 0.1 and 0.075 mg/kg body weight) were tested.

### 2.4. Behavioural testing

Animals were tested 30 min after the second injection. They first performed the holeboard and then the elevated plus-maze test. All behavioural procedures took place in the same room under red light.

### 2.5. Holeboard test

The holeboard is a box (60 × 60 × 45) with matt-painted metallic walls and a plastic-covered wooden floor, which bears four equally spaced holes (3.8 cm in diameter). It is divided into 36 squares (10 × 10 cm). The holeboard provokes neophobia, leading to exploratory and escape behaviour, and allows the estimation of motor activity. The 5-min duration of the test was based on previous studies

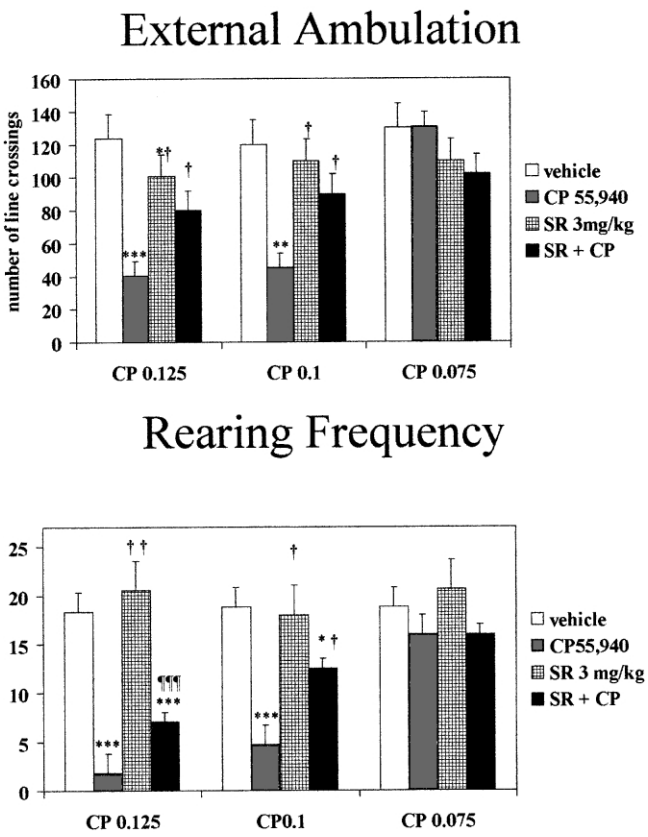


Fig. 1. Effects of CP 55,940 and SR 141716A on motor activity parameters measured by the holeboard test (external ambulation and rearing frequency). Each histogram is the means ± S.E.M., $n = 10$ for each group. Significantly different from the respective control group: * $P < .05$, ** $P < .01$, *** $P < .001$. Significantly different from the CP 55,940 group: † $P < .05$, †† $P < .01$. Significantly different from the SR 141716 A group: ¶¶¶ $P < .001$.

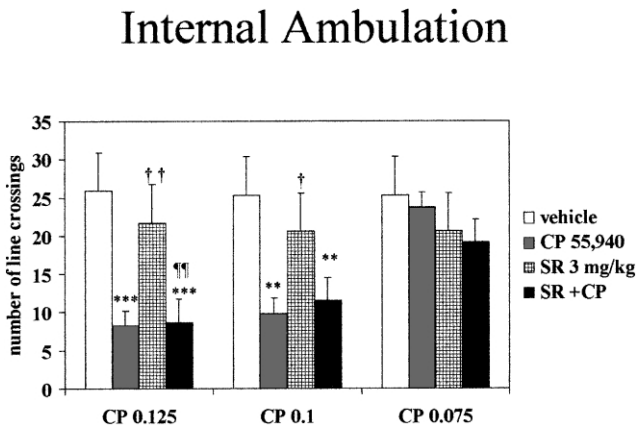


Fig. 2. Effects on internal ambulation measured by the holeboard test. Results are presented as means ± S.E.M., $n = 10$ for each group. Significantly different from the respective control group: * $P < .05$, ** $P < .01$, *** $P < .001$. Significantly different from the CP 55,940 group: † $P < .05$, †† $P < .01$. Significantly different from the SR 141716 A group: ¶¶ $P < .01$.

which revealed habituation after this time (File and Wardill, 1975). The following parameters were recorded: frequency of rearing (number of times the rat stood on its hindlimbs), frequency of external ambulation (number of line crossings in peripheral areas close to the wall), frequency of internal ambulation (number of line crossings in the central area), frequency and duration of head-dipping and frequency and duration of stereotypic behaviour (grooming, scratching).

### 2.6. Elevated plus-maze

The plus-maze consisted of two open arms (50 × 10 cm) and two enclosed arms of the same size, and 40-cm high walls arranged so that the arms of the same type were opposite each other. The junction of the four arms formed a central, square arena (10 × 10 cm). The maze was elevated 62 cm above the ground. At the beginning of the test, each rat was placed gently into the central arena, facing an enclosed arm to avoid artificially inducing a significant pattern, and to increase maze exploration (Pellow et al., 1985). All tests lasted for 5 min. The number of open- and closed-arm entries and the time spent in each arm were recorded. A rat was taken to have entered an arm when all four paws were in it. These data were used to calculate anxiety-related behavioural indices, defined as the percentage of time spent in open arms ([open time/total time × 100]) and the percentage of open-arm entries ([open entries/total entries × 100]). The smaller this ratio scores, the more "anxious" the rat.

### 2.7. Sampling

Immediately after finishing the elevated plus-maze, the animals were sacrificed. The brain was removed and the hypothalamus (including the paraventricular area) dissected and quickly weighed for the analysis of γ-aminobutyric acid (GABA), dopamine (DA), L-3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine or serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA).

### 2.8. Neurochemical determinations

All neurochemical determinations were carried out by HPLC with electrochemical detection. Tissues were homogenized in 10 vol of ice-cold perchloric acid containing 0.2 mM sodium disulphite and 0.43 mM EDTA. Dihydroxybenzylamine (25 ng/ml), *N*-methyl-5-HT (200 ng/ml) and 5-aminopentanoic acid (5 μg/ml) were added as internal standards for analysis of catecholamines, indolamines and GABA, respectively. Samples were then centrifuged for 3 min (15,000 × *g*) and the supernatants removed and divided into three parts for the different analyses. Details on the method of DA and 5-HT analysis have been reported previously (Fernández-Ruiz et al., 1989). GABA concentration was determined according to Smith and Sharp (1994). Briefly, 25 μl of each sample was neutralised with 50 μl of 0.1 N NaOH and stored at −40°C until analysis. This was done by derivation of GABA and 5-aminopenta-

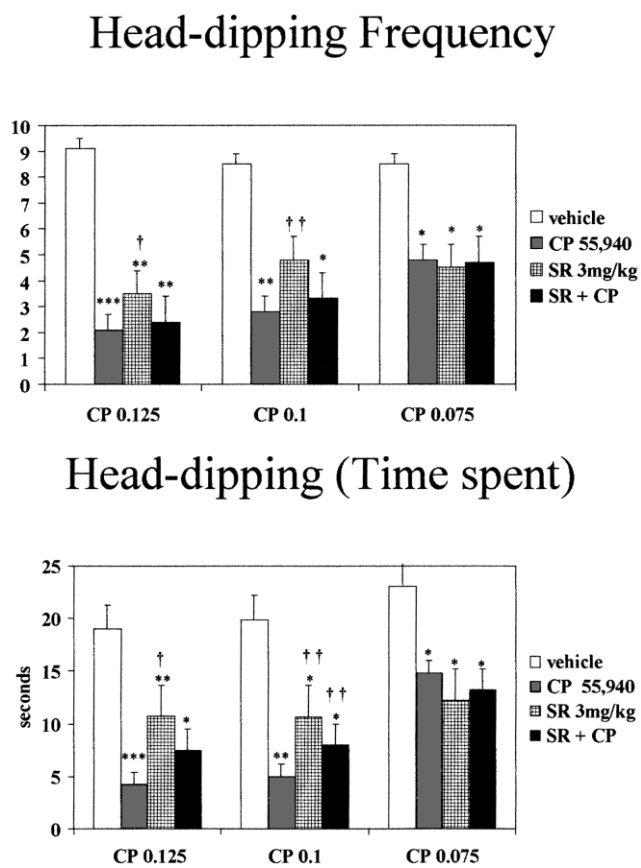


Fig. 3. Site-direct exploration parameters of rats treated with CP 55,940, SR 141716A, or both. Values are means ± S.E.M., $n = 10$ for each group. Significantly different from the respective control group: * $P < .05$, ** $P < .01$, *** $P < .001$. Significantly different from the CP 55,940 group: † $P < .05$, †† $P < .01$.

noic acid through the addition of 5 μl of *o*-phthaldehyde (OPA)-sulphite solution.

### 2.9. Statistical analysis

When appropriate, parametric (one-way ANOVA followed by Student–Newman–Keuls test) or nonparametric (Kruskal–Wallis followed by Mann–Whitney *U* test) methods were used. Significance was set at $P < .05$.

## 3. Results

### 3.1. Holeboard test

The 0.125 and 0.1 mg/kg CP 55,940 doses significantly affected both external ambulation and rearing frequency ($P < .001$ and $P < .01$, respectively) (Fig. 1). At 0.075 mg/kg, these parameters did not vary from normal. Post hoc analysis revealed differences between

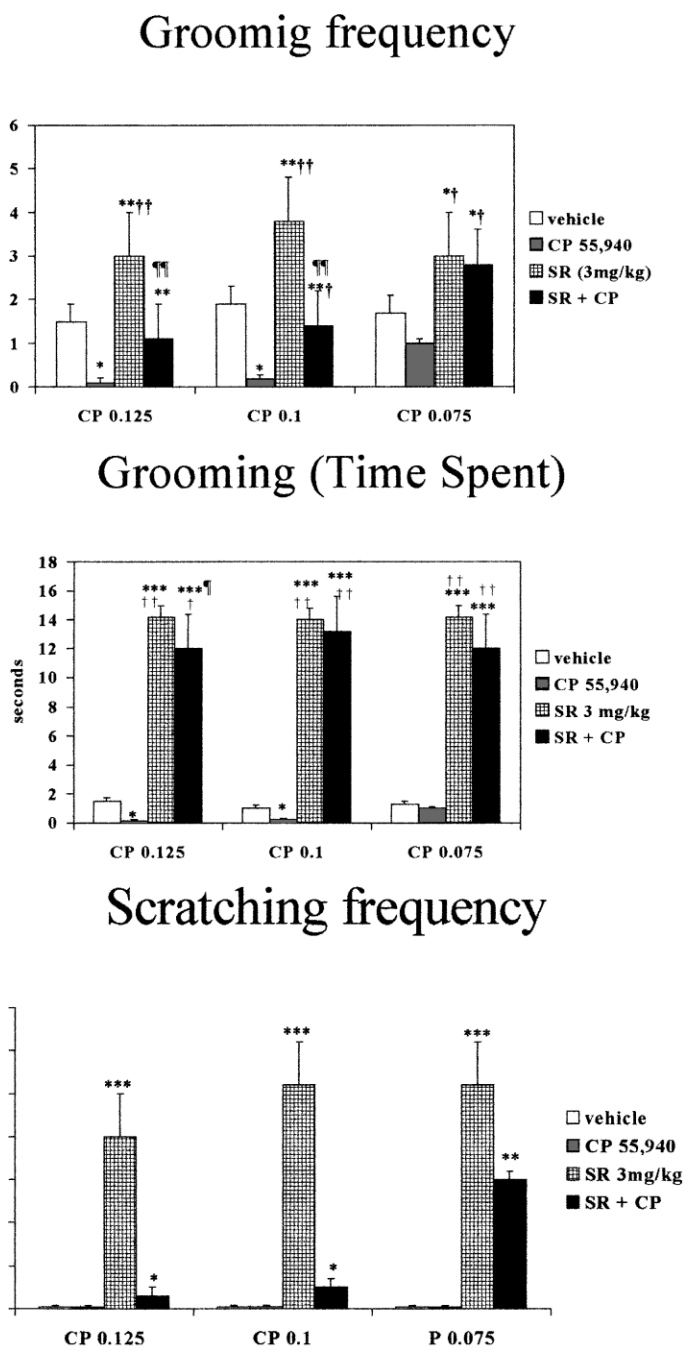


Fig. 4. Effect of CP 55,940 and SR 141716A on stereotyped behaviour (frequency and time spent on grooming and scratching frequency), $n = 10$ for each group. Significantly different from the respective control group: * $P < .05$, ** $P < .01$, *** $P < .001$. Significantly different from the CP 55,940 group: † $P < .05$, †† $P < .01$. Significantly different from SR 141716A group: ¶ $P < .05$, ¶¶ $P < .01$.

CP 55,940-treated animals and the other groups. The two higher doses induced a significant decrease in external ambulation and rearing frequency. Pretreatment with the antagonist reversed the effect on external ambulation: partially at the higher CP 55,940 dose ($P<.05$) and totally at the intermediate dose ($P=.32$). With respect to rearing activity, the inhibition induced by CP 55,940 was only partially reversed by either dose. Treatment with SR 141716A alone did not affect these parameters.

Internal ambulation was altered in a way similar to external ambulation (Fig. 2). There were significant changes at the two higher doses of CP 55,940 ($P<.001$ and $P<.01$), but no effects were seen at the lower dose ($P=.459$). Pretreatment with SR 141716A only partially reversed the effect of the 0.1 mg/kg dose. All doses of CP 55,940 decreased frequency and duration of head-dipping (site-direct exploration) (Fig. 3) in a dose-dependent manner. The coadministration of the antagonist did not attenuate these effects. However, the administration of SR 141716A alone also reduced both these parameters with respect to controls ($P<.01$ for frequency, $P>.05$ for duration).

At the higher doses, CP 55,940 completely abolished frequency and time spent grooming (Fig. 4). Pretreatment with the antagonist annulled this effect. Animals treated solely with SR 141716A exhibit a great enhancement of this pattern ($P<.01$). Treatment with SR 141716A induced intense scratching on its own ($P<.001$) and in combined treatment ($P<.05$ for the two higher doses, $P<.01$ for the lowest dose of CP 55,940).

### 3.2. Elevated plus-maze test

Fig. 5 shows the differences between groups with respect to the percentage of open-arm entries and the time spent there at all three dosage levels. Post hoc analysis revealed significant differences between CP 55,940-treated groups compared to controls. Regarding the parameter "percentage of open-arm entries," we have to indicate a low control score with respect other similar studies. In this way, we have to point out that generally, the frequency of open-arm entries is two to three, but this number depends on the rat strain and other experimental conditions. Neither the effect on open-arm entries nor the time spent in open arms was reversed by the administration of SR 141716A. This is due to an effect of the antagonist on these patterns since, by itself, it reduced open-arm visits and time spent there ($P<.01$ in both parameters with respect to controls). There were also differences between groups in the number of closed-arm visits at the first and second dosage levels. Doses of 0.125 and 0.1 mg/kg of CP 55,940 significantly reduced the number of entries to closed arms ($P=.008$ and .02, respectively), indicating locomotor inhibition. Coadministration with SR 141716A only attenuated the effect of the agonist on the number of entries to closed arms ($P=.015$ for 0.125 mg/kg and $P=.03$ for 0.1 mg/kg).

### 3.3. Neurochemical determinations

DA contents in hypothalamus were affected by the CP 55,940 treatments [$F(7,40)=3.84$, $P=.003$] (Table 1). Post hoc analysis revealed that DA increased dose-dependently in animals treated with the cannabinoid. SR 141716A reversed this effect at all doses. SR 141716A on its own did not cause DA levels to vary in the hypothalamus ($P=.42$). There was

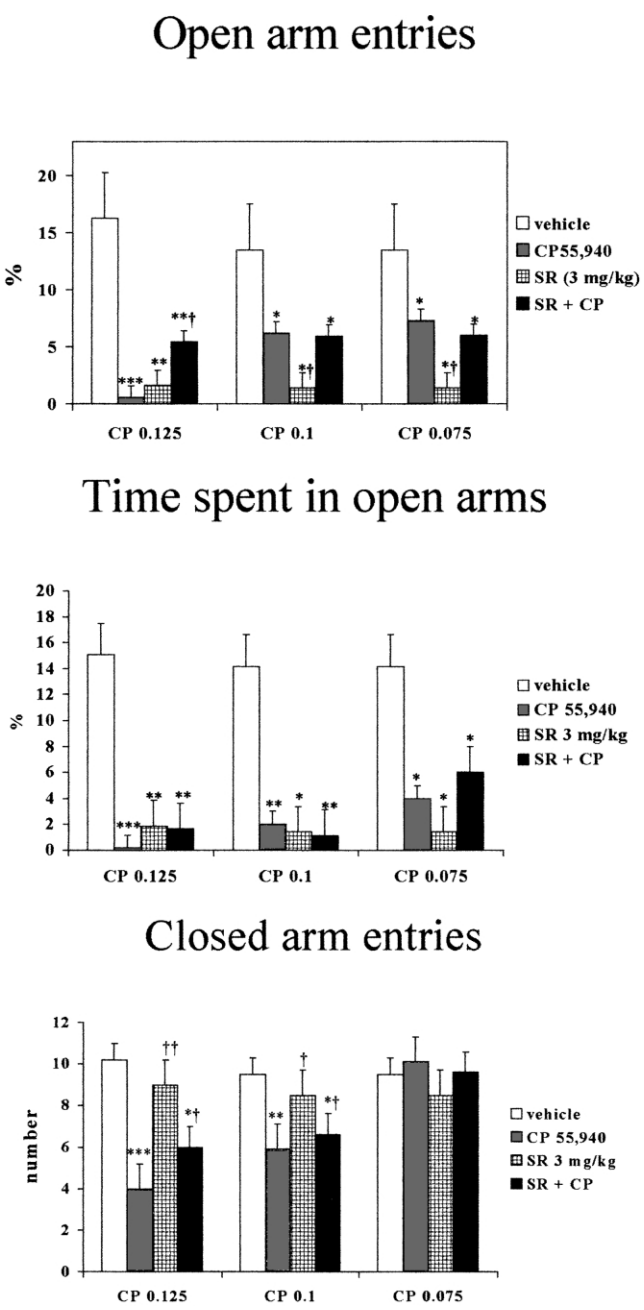


Fig. 5. Effects of CP 55,940 and SR 141716A on elevated plus-maze test behaviour in rats. Each histogram represents the means ± S.E.M. of the percentage of open-arm entries, the percentage time spent there and number of closed arm visits, $n=10$ per group. Significantly different from the respective control group: $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$. Significantly different from the CP 55,940 group: $^{\dagger}P<.05$, $^{\dagger\dagger}P<.01$.

Table 1
Neurotransmitters and metabolite contents measured in the hypothalamus

| | Control | SR | CP 0.125 | SR + CP 0.125 | CP 0.1 | SR + CP 0.1 | CP 0.075 | SR + CP 0.075 |
|---|---|---|---|---|---|---|---|---|
| DA (ng/g) | 243.7 ± 9.7 | 314.3 ± 18.1† | 541.3 ± 17.8** | 320.1 ± 13.6 | 379.1 ± 23.3* | 344.5 ± 26.8 | 380.01 ± 22.8* | 330.3 ± 26.3† |
| DOPAC (ng/g) | 47.5 ± 1.3 | 42.9 ± 5.4 | 60.8 ± 4.9 | 62.8 ± 10.8 | 53.95 ± 3.2 | 60.5 ± 10.8 | 59.7 ± 8.23 | 50.2 ± 4.4 |
| DOPAC/DA | 0.16 ± 0.09 | 0.16 ± 0.08 | 0.22 ± 0.04 | 0.2 ± 0.02 | 0.19 ± 0.02 | 0.17 ± 0.01 | 0.23 ± 0.01 | 0.23 ± 0.02 |
| 5-HT (ng/g) | 978.4 ± 125.4 | 930.8 ± 151.7 | 2104.3 ± 137.3**,¶ | 1247.1 ± 136.6 | 1362.2 ± 68.21* | 1246.4 ± 71.53 | 1408.65 ± 147.6* | 971.7 ± 107.3† |
| 5-HIAA (ng/g) | 624.3 ± 113.2 | 480.1 ± 56.8 | 820.2 ± 130.2 | 672.3 ± 105.5 | 746.4 ± 94.2 | 765.6 ± 108.1 | 800.2 ± 131.3 | 748.2 ± 55.92 |
| 5-HIAA/5-HT | 0.46 ± 0.04 | 0.54 ± 0.05 | 0.57 ± 0.07 | 0.47 ± 0.05 | 0.55 ± 0.07 | 0.6 ± 0.03 | 0.59 ± 0.03 | 0.65 ± 0.2 |
| GABA (μg/g) | 401.5 ± 30.9 | 402.9 ± 25.4 | 409.3 ± 19.6 | 497.7 ± 58.9 | 506.75 ± 50.5 | 509.6 ± 125.8 | 464.67 ± 36.2 | 376.4 ± 53 |

GABA, DA, serotonin and its metabolites contents measured in the hypothalamus. Values represent means ± S.E.M. of groups of six.
* Significantly different from controls: $P < .05$.
** Significantly different from controls: $P < .01$.
† $P < .05$ with respect CP 0.125 group.
¶ Significantly different from the SR 141716 A group: $P < .05$.

no difference between groups in hypothalamic DOPAC concentrations [$F(7,40) = 1.63$, $P = .15$] or on DA turnover DOPAC/DA [$F(7,40) = 1.66$, $P = .146$]. 5-HT levels were also affected by the CP 55,940 treatments [$F(7,40) = 3.083$, $P = .011$]. CP 55,940 increased the concentration of this neurotransmitter in the hypothalamus at all the doses tested. In all cases, this was totally reversed by pretreatment with SR 141716A. HIAA levels did not vary [$F(7,40) = 1.089$, $P = .38$]. The 5-HIAA/5-HT turnover remained unchanged after treatments [$F(7,40) = 1.4918$, $P = .19$].

Finally, GABA hypothalamic GABA concentrations remained unchanged after treatments with CP or SR [$F(7,40) = 0.004$, $P = .9$].

## 4. Discussion

These results show that acute administration of CP 55,940 independently alters exploratory behaviour and motor activity in the rat, and leads to anxiety-like responses. Paradoxically, the selective antagonist of the CB1 receptor, SR 141716A, shares some of these effects.

As with other cannabinoids (Hernández-Tristán et al., 2000; Navarro et al., 1993), CP 55,940 altered general motor activity at higher doses. External ambulation and rearing frequency in the holeboard test decreased, as did the number of closed-arm visits in the elevated plus-maze test, a reliable index of motor activity (Cruz et al., 1994). Pretreatment with the antagonist SR 141716A reversed the hypoactivity induced by the agonist. This result corroborates those obtained by other authors who have observed reversion of catalepsy induced by CP 55,940 and WIN 55,212-2 (Rinaldi-Carmona et al., 1994), indicating that the effects of cannabinoids on motor activity are mediated by CB1 receptors. SR 141716A induced no effect on general activity parameters but increased some stereotyped behavioural patterns such as frequency of, and time spent, grooming, a pattern completely abolished by the agonist at the higher doses. SR 141716A also augmented the frequency of scratching. This pattern has been described in studies of cannabinoid withdrawal syndrome and is associated with the administration of this antagonist, indicating an intrinsic effect of this drug (Aceto et al., 1996).

Treatment with CP 55,940 also led to a great decrease in internal ambulation. This parameter has both a motor component and an exploratory drive related to thigmotaxis, a natural reaction in which rats remain close to vertical surfaces and avoid open spaces (Albonnetti and Farabollini, 1992). A decrease in internal ambulation could indicate reduced exploration related to higher emotional reactivity or fear. However, the great motor inhibition displayed by the animals at these doses could mask this effect. It is interesting that acute treatment with CP 55,940 induced a dose-dependent decrease in the frequency and duration of head-dipping, and site-direct exploration. This appears to be independent of motor effects since the low dose induced a decrease in exploration with the absence of motor effects.

Similarly, acute treatment with CP 55,940 reduced anxiety-related indices in a dose-dependent manner and increased the rats' natural aversion to the open arms. Though there could be some motor inhibition interference leading to unspecific responses, the absence of hypoactivity at the low dose indicates separate effects.

Together, these results indicate that CP 55,940 increases neophobic and anxiety responses in the rat in the elevated plus-maze and holeboard tests, and agree with those obtained in other studies with other cannabinoid agonists. The natural cannabinoid agonist $\Delta^9$-THC increases the aversion to open arms in the elevated plus-maze in rats and mice in a manner similar to other anxiogenic drugs (Onaivi et al., 1990). This cannabinoid also increases the neophobic response in the holeboard test and emotional reactivity in the dark–light emergence test (Hernández-Tristán et al., 2000; Navarro et al., 1993).

Moreover, habituated rats treated with low doses of the potent cannabinoid agonist HU-210 (4–20 μg/kg) display anxiogenic responses in the withdrawal defensive test. At higher doses, HU-210 induces similar but probably unspecific effects due to the great motor inhibition the animals experience (Rodríguez de Fonseca et al., 1996). Therefore,

it seems that cannabinoids increase specific emotional response towards new environments.

The effects of CP 55,940 on anxiety and exploration were not reversed by pretreatment with SR 141716A at any of the doses tested. This could indicate unspecificity of these effects but, as demonstrated in this study, the antagonist has an effect on its own, reducing the frequency and duration of head-dipping in the holeboard test and reducing open-arm visits on the elevated plus-maze test. Other authors have described similar responses after acute treatment with SR 141716A. Some authors report it to practically abolish open-arm entries in the elevated plus-maze in a manner similar to the present study (Navarro et al., 1997). It has been observed that the antagonist gives rise to anxiogenic responses in the defensive withdrawal test (Rodríguez de Fonseca et al., 1994). The antagonist not only affects this behaviour; when administered alone, it alters memory consolidation, incentive learning, arousal and other patterns (Sañudo-Peña et al., 1997; Terranova et al., 1996; Santucci et al., 1996). This indicates that SR 141716 A could act as an inverse agonist or that the endogenous cannabinoid system could be tonically active under certain conditions regulating emotional states. To try to demonstrate the latter possibility, some studies have examined whether SR 141716A stimulates immediate early expression of the c-*fos* gene in rat brain (the induction of c-*fos* is indicative of neuronal activation). SR 141716A induces c-*fos* expression in mesocorticolimbic structures (Alonso et al., 1999).

SR 141716A produces a different pattern of neuronal activation than HU-210 (100 μg/kg). One difference is the powerful activation of the PVN by HU-210. The PVN controls the synthesis and release of CRF (Rodríguez de Fonseca et al., 1994). Recent studies have demonstrated that the administration of different cannabinoid agonists dose-dependently increases plasma levels of ACTH and CORT. This is reversed by SR 141716 A (Murphy et al., 1998). Furthermore, acute treatment with SR 141716A induces anxiety responses without exerting any action on CORT levels, suggesting that hypothetical cannabinoid tone may have a role in behavioural functions without affecting HPA axis activity (Rodríguez de Fonseca et al., 1994). Therefore, cannabinoid agonist or antagonist could exert their actions on anxiety by different neural mechanisms.

As mentioned above, there is some evidence that cannabinoids activate the HPA axis. The CRF antagonist CRF 12-41 attenuates the anxiogenic responses induced by HU-210 (Rodríguez de Fonseca et al., 1996). CRF has been shown to act as a very potent anxiogenic agent (Song et al., 1997). The administration of cannabinoids increases plasma ACTH and CORT levels. In addition, cannabinoid agonists administered acutely in rats increase the expression of the immediate early gene c-*fos* in the PVN (Herkenham and Brady, 1994). Therefore, either directly or indirectly via neurotransmitters, cannabinoids cause neuronal activation within the PVN, a predominant site of CRH neuronal cell bodies. Some of these neurotransmitters are 5-HT (Fuller, 1992; Feldman and Weidenfeld, 1998), DA (Eaton et al., 1996) and, in lesser proportion, GABA (Grossman et al., 1993).

There are few studies on the effects of cannabinoids on the serotoninergic system. It has been observed that $\Delta^9$-THC raises extracellular levels of 5-HT, and possibly increases its synthesis in the mouse brain (Johnson et al., 1976, 1981). A recent study shows that cannabinoids could presynaptically inhibit the release of 5-HT through CB1 receptors in mice brain cortices (Nakazi et al., 2000). No changes in 5-HT or its metabolite 5-HIAA have been found in the MBH (Fernández-Ruiz et al., 1992). The present results indicate that CP 55,940 dose-dependently increases 5-HT throughout the hypothalamus (including the PVN), with no changes in either 5-HIAA or serotonin turnover. This could indicate an increase in serotonin synthesis. The increase in 5-HT levels was reversed by pretreatment with SR 141716A, revealing mediation by CB1 receptors. The increase of hypothalamic 5-HT has been related to the regulation of the HPA axis. Conversely, an activation of CFR neurons induces an increase in hypothalamic biogenic amine concentration, including serotonin (Song et al., 1997). CRF-containing neurones projecting from the PVN into the median eminence receive synaptic input from 5-HT neurones projecting from the midbrain raphe nuclei. Serotonin stimulates the release of CRF from isolated rat hypothalamus in vitro. Drugs that enhance serotonin function (serotonin precursors, uptake inhibitors and agonists increases) increase ACTH and CRF in vivo. Moreover, the release of CRF is inhibited in rats when 5-HT is depleted by serotonin neurotoxins (Fuller, 1992). Thus, the raised serotonin levels detected in the hypothalamus in rats treated with CP 55,940 could mediate the activation of HPA axis induced by cannabinoids.

Treatment with CP 55,940 also produced a dose-dependent increase in DA concentration in the hypothalamus, an effect reversed by SR 141716A. Several studies have demonstrated that cannabinoids can increase DA levels in MBH (Rodríguez de Fonseca et al., 1992). This was related to the decrease in plasma prolactin seen in animals treated with cannabinoids, since dopaminergic neurones of the hypothalamic tuberoinfundibular system inhibit the secretion of prolactine (Murphy et al., 1998). However, DA also stimulates CRF release in the PVN (Eaton et al., 1996) and could be involved in the activation of the HPA axis by cannabinoids. Finally, other studies have found changes in GABA levels in the MBH after cannabinoid treatment (DeMiguel et al., 1998). In the present study, GABA concentration did not vary with CP 55,940 treatment; these different results are probably due to the use of complete hypothalamus.

In conclusion, the results of the present study indicate that acute treatment with the synthetic cannabinoid CP 55,940 increases the neophobic response, reducing exploratory drive in the holeboard test, and increases anxiety-like responses in the elevated plus-maze test. At high doses,

these effects are masked by profound hypoactivity. At low doses, hypoactivity disappears, but anxiety and neophobic responses persist. Both effects are dose-dependent. The CB1 selective antagonist SR 141716A did not reverse these effects since it induced similar behavioural responses in both tests. This could be due to different mechanisms of action. In the case of the agonist, the activation of the HPA axis might be involved, probably through the activation of serotoninergic and dopaminergic transmission.

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