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Memory impairment following combined exposure to Δ^9-tetrahydrocannabinol and ethanol in rats

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Abstract

Cannabis derivatives and alcohol are widely co-abused, particularly among adolescents. Since both ethanol and cannabinoids are known to impair learning and memory, the present study investigated in rats the effects of combined exposure to ethanol and Δ^9-tetrahydrocannabinol (THC) in a memory task, the object recognition test. The results of the present study provide evidence that ethanol, voluntarily ingested in alcohol-preferring rats, and THC, given by intraperitoneal injection, have a synergic action to impair object recognition, when a 15-min interval was adopted between the sample phase and the choice phase of the test. Neither voluntary ethanol ingestion nor 2 or 5 mg/kg of THC were able per se to modify object recognition in these experimental conditions, but when voluntary ethanol ingestion was combined with administration of these doses of THC object recognition was markedly impaired. THC impaired object recognition only at the dose of 10 mg/kg, when its administration was not combined with that of ethanol. The selective cannabinoid CB_1 receptor antagonist SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2, 4-dichloro-phenyl)-4-methyl-1H-pyrazole carboxamide·HCl) at the dose of 1 mg/kg reversed the amnesic effect of THC, 10 mg/kg, suggesting that the effect is mediated by this receptor subtype. The synergism of ethanol and THC was not detected when an inter-trial interval of 1 min was adopted. The present findings are in keeping with the notion that Cannabis derivatives impair memory processes and provide evidence for a synergic action of THC and ethanol, thus emphasizing the risks consequent to their co-administration.

Keywords: Δ^9-Tetrahydrocannabinol; Ethanol intake; Cannabinoid CB_1 receptor; SR 141716A; Object recognition task; Alcohol-preferring rat

1. Introduction

A large body of evidence indicates that alcohol and Cannabis derivatives are widely co-abused, particularly among adolescents (Patton et al., 1995; Poulin and Elliott, 1997). The combined exposure to these drugs has been shown to markedly reduce the driving skill and has been proposed to be responsible for a number of driving accidents among young drivers (Augsburger and Rivier, 1997).

It is well known that ethanol abuse is associated with cognitive impairment and loss of memory in humans (Butters, 1985; Freund, 1973), as well as in experimental animal models (Arendt et al., 1989; Hodges et al., 1991). Moreover, naturally occurring cannabinoids, such as Δ^9-tetrahydrocannabinol (THC), endogenous cannabinoids, such as anandamide, or synthetic agonists at cannabinoid CB_1 receptors have been demonstrated to impair learning and memory in humans (Croft et al., 2001), in nonhuman primates (Evans and Wenger, 1992) and in rodents (Fehr et al., 1976; Stiglick and Kalant, 1983; Stiglick et al., 1984; Lichtman et al., 1995; Brodkin and Moerschbaecher, 1997; Jentsch et al., 1997; Stella et al., 1997; Mallet and Beninger, 1998; Nava et al., 2000).

These findings raise interest for the possible interactions between alcohol and cannabinoids on learning and memory processes when their consumption is combined. Thus, the present study was aimed at investigating in rats the effects of combined exposure to ethanol and THC in a memory task, the object recognition test.

The choice of this test was adopted because cannabinoids have been reported to affect the working memory in rats (Jentsch et al., 1997), and the object recognition test has

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been proposed to represent a "pure" working memory task, which does not involve the retention of a rule, but it is entirely based on the spontaneous exploratory behaviour of rats towards objects (Ennaceur and Delacour, 1988; Ennaceur and Meliani, 1992; Scali et al., 1997; Ennaceur et al., 1997; Bartolini et al., 1996). In addition, unlike the radial maze that represents the most popular working memory test in the rat (Olton, 1987), the object recognition task does not involve the use of a positive reward (usually a small food pellet). The use of food as a reward may render difficult to interpret the results obtained in a memory test with THC, which is known to affect both reward (Lepore et al., 1995; Cheer et al., 2000; Cossu et al., 2001; Braida et al., 2001) and feeding mechanisms (Colombo et al., 1998; Williams et al., 1998; Giuliani et al., 2000; Koch, 2001).

Moreover, to more closely mimic the effect of the spontaneous use of these drugs, the present study was carried out using genetically selected Marchigian Sardinian alcohol-preferring (msP) rats, which voluntarily ingest pharmacologically active doses of ethanol in order to experience positive psychotropic effects of ethanol (Ciccocioppo et al., 1999a,b). Indeed, under the experimental conditions adopted in this study ethanol consumption by msP rats may mimic the recreational consumption of alcoholic beverages in humans.

2. Materials and methods

2.1. Animals

Male genetically selected alcohol-preferring rats were employed in most of the experiments. They were bred in the Department of Pharmacological Sciences and Experimental Medicine of the University of Camerino (Marche, Italy) for 36–38 generations from Sardinian alcohol-preferring rats of the 13th generation, provided by the Department of Neurosciences of the University of Cagliari, Italy (Agabio et al., 1996; Colombo, 1997; Lobina et al., 1997). These animals are referred to as Marchigian Sardinian alcohol-preferring (msP) rats. At the time of the experiments, rats were between 2 and 3 months of age. This age was chosen because young msP rats perform much better than older rats in the object recognition task, but also because our study was aimed at providing an experimental model to study the effects of alcohol and cannabinoid abuse in adolescents.

In some experiments also male Wistar rats (Charles River, Calco, Co, Italy) were used, again at the age of 2–3 months.

Rats were kept in a room with a reverse 12:12 h light/dark cycle (lights off at 10:00 a.m.), temperature of 20–22 °C and humidity of 45–55%. Rats were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). The procedures were conducted in adherence to the European Community Council Directive for Care and Use of Laboratory Animals.

2.2. Drugs

THC was purchased from Sigma-Aldrich (St. Louis, MO, USA) in vials containing 10 mg of the drug in 1 ml of absolute ethanol. Vials were evaporated under nitrogen and the residue dissolved in 20% dimethylsulfoxide, 10% Tween 80 and distilled water according to Wu and French (2000). The cannabinoid CB_1 receptor antagonist SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1(2, 4-dichloro-phenyl)-4-methyl-1H-pyrazole carboxamide·HCl), was a generous gift of Sanofi-Synthélabo Recherche (Montpellier, France); it was dissolved in a few drops of dimethylsulfoxide and Tween 80 and then diluted in saline. THC and SR 141716A were administered by intraperitoneal injection in a volume of 1 ml/kg.

2.3. Voluntary ethanol intake

Most of the experiments were carried out in ethanol-experienced msP rats. At the age of 45–50 days they were selected for 10% ethanol (v/v) preference, offering them free choice between water and 10% ethanol 24 h/day for 15 days. Water and 10% ethanol were offered in graduated drinking tubes equipped with metallic drinking spouts. The rats employed in the following experiments had a 24-h ethanol intake between 5 and 6 g/kg with a percent ethanol preference [ml of ethanol solution/ml of total fluids (water + 10% ethanol) ingested in 24 h × 100] above 80. Before the experiments, for 2 weeks rats had water and food available during the entire day, while 10% ethanol was offered for 30 min/day at the beginning of the dark phase (10:00 a.m.) of the reverse light/dark cycle.

In some experiments, ethanol-naive msP rats were employed; they had never had access to ethanol before the experiments and their ethanol preference was assessed only after the object recognition test.

2.4. Object recognition

The object recognition test was carried out according to Ennaceur and Delacour (1988) and Bartolini et al. (1996). Briefly, rats were placed in a circular grey wooden box (diameter of 70 cm, height of 40 cm) illuminated with a 25 W lamp suspended 1 m above the box. The objects to be discriminated were made of plastic or metal and existed in duplicate. Their weight was such that they could not be displaced by rats.

For 5 days before testing, all the rats were submitted to daily habituation sessions, where they were allowed to explore the box for 15 min. On the day of testing, a session of two trials, separated by an inter-trial interval of either 1 or 15 min, was carried out. In the first trial (T1), also referred to as the sample trial, two identical objects (A1 and A2) were placed in two defined sites of the box, at approximately the same distance from the point where the rat was placed at the beginning of the trial. Animals were

allowed to explore them for 3 min. During the second trial (T2), the choice trial, one of the objects presented in T1 was replaced by a new object with different shape and colour, and of different material (plastic vs. metal). The other object (the familiar object) was a duplicate of that presented during T1, in order to avoid olfactory trails. Care was taken to avoid object and place preference by randomly changing the role of the objects (familiar or new object) and their position in the box during T2. Furthermore, in order to avoid olfactory cues the objects to be discriminated were carefully cleaned. The objects were unfamiliar to the animals, devoid of any natural significance for rats, and had never been associated with reinforcement. The duration of T2 was of 3 min.

The adopted basic measure was the time spent by rats in exploring objects. Exploration of an object was defined as directing the nose at a distance equal or inferior to 2 cm to the object, and/or touching it with the nose. Turning around the object was not considered as exploratory behaviour. The total time spent in exploring the two objects in T1 was indicated as *e1*, and the total time spent in exploring the new and the familiar object in T2 was defined as *e2*. The difference in time spent exploring the two objects in T2 was defined as discrimination index (*d1*); it was obtained by subtracting the time spent to explore the new object from that spent to explore the familiar object. A positive value of *d1* indicates that the rat spent more time exploring the new object than the familiar object. The negative value of *d1*, like that observed in response to some drug treatments, indicates that the rat devoted more time to the familiar object rather that to the new one, implying that drug treatment impaired the recognition of the new object.

The results of the present experiments were expressed as the discrimination ratio (*d2*), which represents the ratio between the discrimination index (*d1*) and the total time spent exploring the two objects in T2 (*e2*) (Ennaceur et al., 1997). The difference between *e1* and *e2* was also analysed to evaluate possible habituation of rats.

2.5. Experiment 1: effect of THC and ethanol on the object recognition test in ethanol-experienced msP rats

2.5.1. One-minute inter-trial interval

Two doses of THC were tested, 5 or 10 mg/kg. Six groups of rats were employed. Three groups of msP rats received intraperitoneal injection of either vehicle or of one of the two doses of THC, immediately after the 30-min access to 10% ethanol. Three other groups did not have access to ethanol on the testing day, and received intraperitoneal injection of either vehicle or of one of the two doses of THC. Different rats were used in different experimental groups.

Twenty minutes after the intraperitoneal administration, rats were placed in the testing box for the T1 session of object recognition. One minute later, the T2 session (the choice trial) took place.

2.5.2. Fifteen-minute inter-trial interval

Four doses of THC were tested, 0.2, 2, 5 or 10 mg/kg. Ten groups of rats were employed. Five groups of msP rats received intraperitoneal injection of either vehicle or of one of the four doses of THC, immediately after the 30-min access to 10% ethanol. Five other groups did not have access to ethanol on the testing day, and received intraperitoneal injection of either vehicle or of one of the four doses of THC. Different rats were used in different experimental groups.

Twenty minutes after the intraperitoneal administration rats were placed in the testing box for the T1 trial of object recognition. The T2 session (the choice trial) took place after an inter-trial interval of 15 min.

In another experiment, two groups of rats were given intraperitoneal injection of the selective cannabinoid CB_1 receptor antagonist SR 141716A, 1 mg/kg, 10 min before intraperitoneal administration of THC, 10 mg/kg, or vehicle.

2.6. Experiment 2: effect of THC on the object recognition test in ethanol-naive msP rats or in genetically unselected Wistar rats

Previous studies have shown that THC induces memory impairment in rats in the radial maze at doses of 2–3 mg/kg (Lichtman et al., 1995; Nava et al., 2000). To investigate whether the low amnesic potency of THC in the present study might be related to cross-tolerance between ethanol and cannabinoids (see, for review Hungund and Basavarajappa, 2000) in msP rats, the effect of intraperitoneal injections of THC was tested in ethanol-naive msP rats, as well as in genetically unselected Wistar rats.

Groups of rats that had never received access to ethanol were intraperitoneally injected with either vehicle or the two doses (2 or 5 mg/kg) of THC that did not evoke an amnesic effect in the previous experiment. Different rats were used in different experimental groups.

Twenty minutes after the intraperitoneal administration, rats were placed in the testing box for the T1 trial of object recognition. The experiments were carried out with an inter-trial interval of 15 min.

2.7. Experiment 3: effect of THC on locomotor activity of msP rats

The responsiveness of msP rats to THC was also assessed in regard to its effect on locomotor activity. Ethanol-naive msP rats received an intraperitoneal injection of THC during the dark phase of the light cycle. The open field, used to measure locomotor activity, consisted of a wooden chamber (40 cm high) with a circular base (70 cm diameter). The apparatus was placed in a soundproof room, illuminated by a white 25 W lamp placed 1 m over the centre of the arena.

Three groups of six msP rats were used: one received intraperitoneal vehicle, while the other two received either 0.2 or 2 mg/kg of THC. One day before the test, rats were

confined in the open arena for 20 min to allow them to habituate to the testing apparatus. After treatment, rats were placed for 1 h in the open field; the rat behaviour in the test session was videotaped, analysed and scored. The following parameters were measured: time spent in locomotor activity and number of rearing reactions.

2.8. Statistical analysis

Data concerning the object recognition test are expressed as *e1*, *e2* and *d2*, and reported as means ± S.E.M. The *e1* and *e2* data were analysed by means of a two-way analysis of variance with between group comparisons for treatment and within group comparison for trial (T1 or T2). The *d2* data, as well as the rearing or locomotor activity data, were analysed by one-way analysis of variance with between-group comparisons. Post-hoc comparisons were carried out by means of the Newman–Keuls test. Statistical significance was set at $P<0.05$.

3. Results

3.1. Experiment 1: effect of THC and ethanol on the object recognition test in ethanol-experienced msP rats

3.1.1. One-minute inter-trial interval

Comparisons of the total time spent exploring the test objects indicated that there were no group differences for either the sample (T1) or the choice trial (T2) ($P>0.05$).

The overall analysis of variance of the *d2* values revealed a statistically significant treatment effect [$F(5, 43)=4.34$; $P<0.01$].

In rats that did not receive access to ethanol, a statistically significant reduction of *d2* values was observed in response to THC, 10 mg/kg, but not 5 mg/kg.

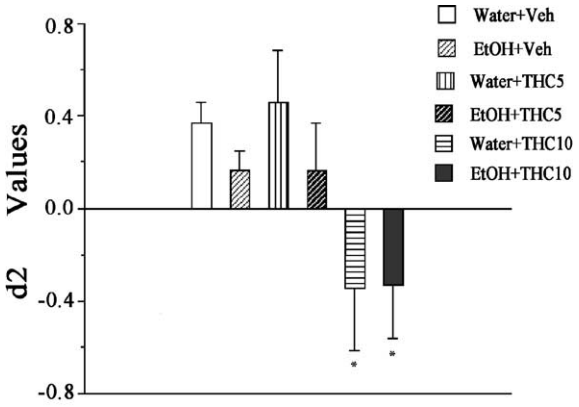

Fig. 1. Discrimination ratio (*d2*) in object recognition task (with 1-min inter-trial interval) following voluntary intake of 10% ethanol (EtOH) or water (Water), combined with intraperitoneal injection of Δ^9-tetrahydrocannabinol (THC), 5 or 10 mg/kg, or its vehicle (Veh). Values are means ± S.E.M. of 6–11 subjects/group. Difference from controls: $*P<0.05$; where not indicated, difference was not statistically significant.

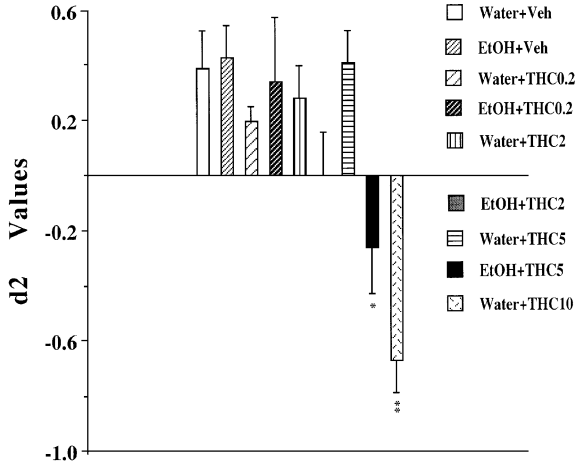

Fig. 2. Discrimination ratio (*d2*) in object recognition (with 15-min inter-trial interval) following voluntary intake of 10% ethanol (EtOH) or water (Water), combined with intraperitoneal injection of Δ^9-tetrahydrocannabinol (THC), 0.2, 2, 5 or 10 mg/kg, or its vehicle (Veh). Values are means ± S.E.M. of 6–13 subjects/group. Difference from controls: $*P<0.05$; $**P<0.01$; where not indicated, difference was not statistically significant.

On the other hand, no significant effect on *d2* measures was induced by voluntary ethanol intake, in comparison to rats which were not offered access to ethanol (Fig. 1).

In rats that received intraperitoneal injection of THC after access to ethanol, a statistically significant reduction in *d2* measures was observed in response 10, but not 5 mg/kg of THC (Fig. 1). The *d2* values following THC, 10 mg/kg, after access to ethanol were essentially identical to those observed following THC without access to ethanol.

3.1.2. Fifteen-minute inter-trial interval

Comparisons of the total time spent exploring the test objects indicated that there were no group differences for either the sample (T1) or the choice trial (T2) at doses of 2 or 5 mg/kg ($P>0.05$). On the other hand, the exploration time was significantly reduced by 0.2 or 10 mg/kg. Following combined administration of ethanol and 10 mg/kg of THC rats were completely immobile both during T1 and T2; therefore *d2* values following this treatment were not included in the statistical analysis.

The overall analysis of variance of the *d2* data revealed a statistically significant treatment effect [$F(8,77)=4.97$; $P<0.01$].

In rats that did not have access to ethanol, post hoc comparisons revealed a statistically significant reduction in *d2* values in response to THC, 10 mg/kg, but not at lower doses, 0.2, 2 or 5 mg/kg (Fig. 2). Thus, the discrimination ratio was markedly reduced only in response to the highest dose tested.

Moreover, no significant effect on *d2* values was detected following voluntary ethanol intake, in comparison to rats that were not offered access to ethanol (Fig. 2).

When access to ethanol was combined with intraperitoneal injection of THC, a statistically significant reduction in

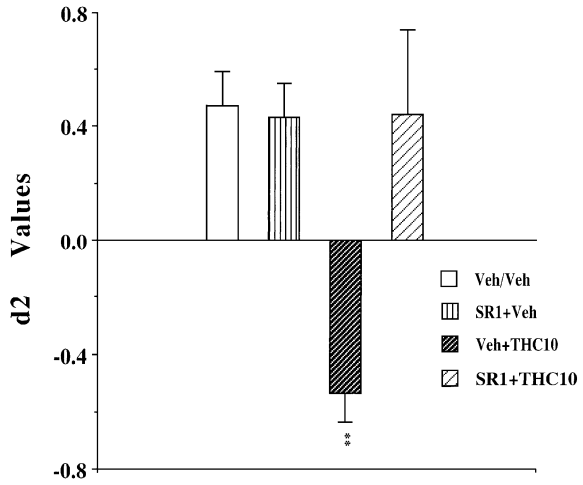

Fig. 3. Discrimination ratio (*d2*) in object recognition (with 15-min inter-trial interval) in response to pre-treatment with the cannabinoid CB_1 receptor antagonist SR 141716A followed by Δ^9-tetrahydrocannabinol (THC), 10 mg/kg, or its vehicle (Veh). Values are means ± S.E.M. of six subjects/group. Difference from controls: $**P<0.01$; where not indicated, difference was not statistically significant.

d2 values was observed also following administration of 5 mg/kg of THC (Fig. 2). The dose of 2 mg/kg, combined with ethanol, induced a pronounced reduction in *d2* values, even though the difference did not reach statistical significance. The dose of 0.2 mg/kg did not modify the discrimination ratio. The dose of 10 mg/kg of THC, combined with ethanol, resulted in pronounced immobility of msP rats; thus data from this treatment were not included in the statistical analysis.

For the experiment in which SR 141716A was tested vs. the amnesic effect of THC, 10 mg/kg, the analysis of variance revealed a statistically significant treatment effect [$F(3,20)=7.41$; $P<0.01$]. As shown in Fig. 3, pre-treatment with SR 141716A, 1 mg/kg, completely reversed the reduction of *d2* values induced by THC, 10 mg/kg. In fact, *d2* values in rats pre-treated with SR 141716A, 1 mg/kg, before THC, 10 mg/kg, were essentially identical to those measured in control rats receiving only vehicle administrations.

3.2. Experiment 2: effect of THC on the object recognition test in ethanol-naive msP rats or in genetically unselected Wistar rats

Comparisons of the total time spent exploring the test objects indicated that there were no group differences for either the sample (T1) or the choice trial (T2) ($P>0.05$).

The overall analysis of variance of the *d2* data in ethanol-naive msP rats revealed no statistically significant treatment effect following of THC, 2 or 5 mg/kg [$F(2,21)=0.35$; $P>0.05$] (Fig. 4A).

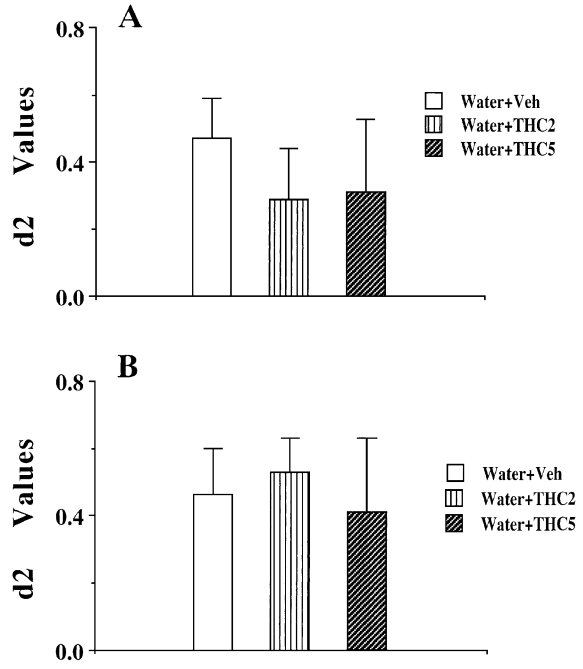

Fig. 4. Discrimination ratio (*d2*) in object recognition (with 15-min inter-trial interval) following intraperitoneal injection of Δ^9-tetrahydrocannabinol (THC), 2 or 5 mg/kg, or its vehicle (Veh) in ethanol-naive msP rats (Panel A) or in non selected Wistar rats (Panel B). Values are means ± S.E.M. of eight subjects/group for ethanol-naive msP rats and of nine subjects/group for Wistar rats. Difference from controls was not statistically significant.

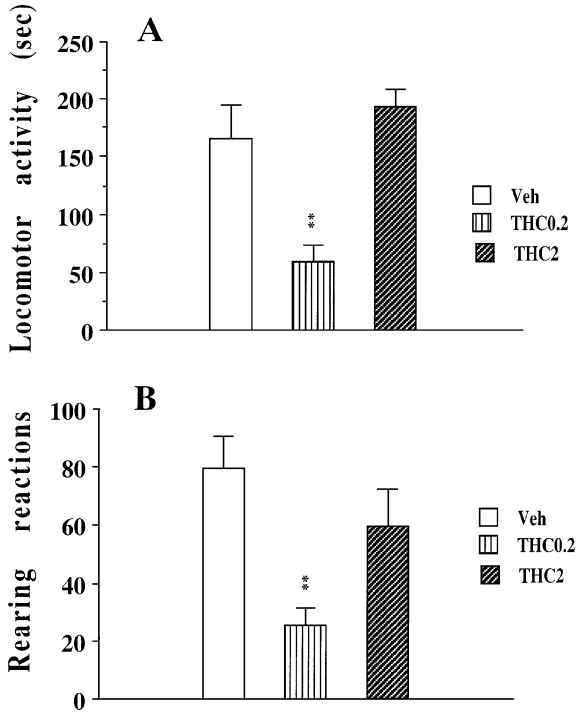

Fig. 5. Time spent in locomotor activity (Panel A) and number of rearing reactions (Panel B) following intraperitoneal injection of Δ^9-tetrahydrocannabinol (THC), 0.2 or 2 mg/kg, or its vehicle (Veh) in ethanol-naive msP rats. Values are means ± S.E.M. of 6 subjects/group. Difference from controls: $**P<0.01$; where not indicated, difference was not statistically significant.

Again, the overall analysis of variance of the *d2* data in unselected Wistar rats revealed no statistically significant treatment effect following THC, 2 or 5 mg/kg [$F(2,24) = 0.12$; $P > 0.05$] (Fig. 4B).

3.3. Experiment 3: effect of THC on locomotor activity of ethanol-naive msP rats

The overall analysis of variance revealed a statistically significant treatment effect both on locomotor activity [$F(2,15) = 11.96$; $P < 0.01$] (Fig. 5A) and on rearing reactions [$F(2,15) = 7.22$; $P < 0.01$] (Fig. 5B). Intraperitoneal administration of 0.2 mg/kg of THC markedly reduced both locomotor activity and rearing reactions ($P < 0.01$). On the other hand, the dose of 2 mg/kg did not significantly modify either locomotor activity or rearing reactions.

4. Discussion

The results of the present study provide evidence that ethanol, voluntarily ingested by alcohol-preferring rats, and THC have a synergistic action to impair memory in the object recognition task, when an interval of 15 min was adopted between the sample and the choice trial. In these experimental conditions, neither voluntary ethanol ingestion nor doses of 2 or 5 mg/kg of THC were able per se to modify object recognition; but when voluntary ethanol ingestion was combined with administration of these doses of THC object recognition was markedly impaired.

The selective cannabinoid CB_1 receptor antagonist SR 141716A completely reversed the amnesic effect of THC, 10 mg/kg, supporting the view that it is mediated by this receptor subtype.

The synergism of ethanol and THC, evident when the inter-trial interval in the object recognition task was of 15 min, was not detected following an inter-trial interval of 1 min. This finding suggests that the combined exposure to ethanol and THC may impair memory retention, during the longer inter-trial interval.

Several findings of the present study require some comments. First, the observation that voluntary ethanol ingestion did not modify object recognition is probably not surprising. It should be noted that msP rats show pronounced tolerance to the effects of ethanol or of other central nervous system depressant, such as anaesthetics. The alcohol-preferring rats employed in the present study promptly ingest 0.7–0.9 g/kg of ethanol, as soon as they are offered access to ethanol solution and usually they do not further increase their intake up to at least 60 min after access to ethanol. These amounts of ethanol raise blood ethanol levels to about 40–50 mg%, which are enough to evoke a rewarding experience and to exert an antidepressant-like effect (Ciccocioppo et al., 1999a,b), but they do not produce signs of behavioural impairment or of intoxication.

More surprising is the finding that memory impairment in msP rats in response to THC was only observed at 10 mg/kg, but not at doses of 2 or 5 mg/kg. Other authors have reported a marked amnesic effect in rats of THC in the radial maze test within this interval of doses (Lichtman et al., 1995; Nava et al., 2000). Therefore, experiments were carried out in ethanol-naive msP rats to investigate whether the low responsiveness to the amnesic effect of THC might be related to cross-tolerance between ethanol and THC. This possibility has been put forward by a variety of reports in the literature, reviewed by Hungund and Basavarajappa (2000). However, in ethanol-naive msP rats doses of THC of 2 or 5 mg/kg did not influence object recognition. Similar results were also obtained in genetically unselected Wistar rats. All these findings suggest that the low responsiveness to THC in the object recognition task might be linked to the nature of the test, rather than to the strain of rats employed in the present study.

On the other hand, the responsiveness of msP rats to the effect of THC on locomotion was strictly similar to that reported in the literature for other strains of rats; in fact, in accordance with data published by Sanudo-Pena et al. (2000), 0.2 mg/kg of THC produced a marked and statistically significant reduction in locomotor activity. These findings indicate that the compound used in the present study was fully active.

It is worth noticing that most of the studies concerning the amnesic effect of THC in rats have been carried out in the radial maze (Olton, 1987), not in the object recognition task. Ennaceur and Meliani (1992) reported lack of correlation between the results obtained in the object recognition and in the radial maze tests. They suggested that the differences among the two tests might be related to: (a) amount of information, probably larger in the radial maze than in the object recognition task; (b) the relative weight of distant vs. local cues, the former being probably more important in the radial maze; (c) the use of a reinforcer and significant reference memory component in the radial maze, completely absent in the object recognition task. In another article, Ennaceur et al. (1997) summarize information of several studies showing that the performance in the two tests might involve different neuronal substrates; the rat hippocampus is probably not required for judging the familiarity of complex objects, while the hippocampus is apparently an important site of action for the effect in the radial maze (Lichtman et al., 1995). This view is supported also by the following findings: (a) extensive hippocampal lesions can spare object recognition performance (Mumby et al., 1992, 1995; Steele and Rawlins, 1993); (b) electrophysiological studies have failed to identify neurons in the hippocampus that respond to novel objects (Zhu et al., 1995a); (c) induction of c-*fos* in rats following exposure to novel objects has been observed in a number of cortical regions, but not in the hippocampus (Zhu et al., 1995b).

The induction of long-term potentiation is largely recognized as a key cellular mechanism in learning processes and

memory formation. Inhibition of long-term potentiation has been shown to impair both learning and memory retrieval (for review, see Malenka and Nicoll, 1999). Induction of long-term potentiation is under the control of excitatory aminoacids, and increase in glutamate neurotransmission is critical for long-term potentiation. Conversely, stimulation of gamma-aminobutyric acid (GABA) neurotransmission exerts an opposite effect, lowering the ability of glutamate to evoke long-term potentiation. To our knowledge, no study has been so far published on the effect of simultaneous administration of ethanol and cannabinoid receptor agonists on long-term potentiation. However, both ethanol and THC exert a profound effect on long-term potentiation, by inhibiting the ability of glutamate to facilitate synaptic strength and/or by increasing the GABA inhibitory control on cell membrane potential (Auclair et al., 2000; Givens and McMahon, 1995; Hoffman and Lupica, 2000; Schummers et al., 1997; Schummers and Browning, 2001). Therefore, ethanol and THC could act synergically to impair memory processes, by a negative modulation of long-term potentiation through the interference with glutamate and/or GABA neurotransmission.

In conclusion, the main finding of the present study is that combined exposure to ethanol, voluntarily ingested at not intoxicating doses, and THC have a synergic action to impair object recognition in rats. These findings are in keeping with the notion that abuse of cannabis derivatives exerts negative influence on memory processes; they emphasize that this effect can be markedly increased as a consequence of co-administration of alcohol. This study provides also an interesting experimental model for further investigations on the interactions between cannabinoids and alcohol.

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