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Ethanol improves short-term social memory in rats. Involvement of opioid and muscarinic receptors

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Abstract

Some human and animal studies have demonstrated enhancement of memory processes when ethanol was administered immediately after training and subjects were later tested in the drug-free state. The aim of this study was to evaluate the effect of acute ethanol administration (0.5, 1.0 and 2.0 g/kg) by intraperitoneal (i.p.) and oral route on short-term memory, using the social recognition test in rats. The actions of scopolamine (0.06 and 0.5 mg/kg, i.p.) and naloxone (1.0 mg/kg, i.p.) and their interaction with ethanol in relation to short-term memory were also studied. The doses of ethanol used did not show any sedative effect, which was assessed by measuring locomotor activity. The results indicate that acute low doses of ethanol (0.5 and 1.0 g/kg, i.p.) improve the short-term olfactory memory in rats in a specific and time-dependent manner, and that this action is, at least in part, related to opioid, but not to muscarinic receptors. In addition, these findings confirm that the social recognition test in rats is a useful and reliable model to investigate short-term memory affected by ethanol.

Keywords: Ethanol; Social memory; Olfactory memory; Naloxone; Scopolamine; (Rat)

1. Introduction

Ethanol is one of the oldest and the most widely consumed psychoactive drugs. The traditional view is that ethanol presents negative effects on cognitive processes, promoting loss of concentration and judgement and lapses in memory (Goodwin et al., 1970; Birnbaum and Parker, 1977). Chronic exposure to ethanol, as well as acute ethanol administration prior to task acquisition, impairs memory in both humans (Parker et al., 1974; Birnbaum et al., 1978) and rodents (Freund, 1970; Freund and Walker, 1971). However, ethanol is a drug that has apparently opposite effects on retention depending on when it is given relative to the task, as well as the nature of the task under study (Ladner et al., 2001). Thus, an increasing number of human and animal studies have demonstrated enhancement of memory when alcohol was administered immediately after training and subjects were later tested in the drug-free state. In humans, post-training administration of ethanol improves verbal recall (Parker et al., 1980; Lamberty et al., 1990; Tyson and Schirmuly, 1994), visual recognition (Parker et al., 1981), verbal recognition (Bruce and Pihl, 1997) and kinesthetic memory (Hewitt et al., 1996). The immediate post-training intraperitoneal injection of ethanol (0.75–4.5 g/kg) enhances retention in a one-trial passive avoidance task in mice (Alkana and Parker, 1979), and intermediary doses (0.75 and 1.5 g/kg) improve the performance in a "water-finding task" in rats (Melia et al., 1986). In brief, all these studies suggest that ethanol impedes the acquisition or storage of new memory traces (Wickelgren, 1975; Parker et al., 1976), but does not significantly interfere with retrieval of established memories (Tamerin et al., 1971; Jones, 1973; Birnbaum et al., 1978).

As stated by Dantzer et al. (1987), a form of memory very similar to factual memory in humans that has received little attention in behavioural pharmacology is the so-called "social memory" or "recognition paradigm" which is mainly generated from olfactory cues (Sawyer et al., 1984). This short-term working memory model is based on the fact that rodents spend more time investigating unfamiliar juvenile conspecifics more intensely than familiar ones. When the same juvenile is presented twice, the duration of investigation is reduced on the second presenta-

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tion (Dantzer et al., 1987). Social memory is prolonged by repeated exposure to the stimulus of the juvenile rat and is impaired by retroactively interfering stimuli. It can be facilitated by memory-enhancing drugs and disrupted by pharmacological and pathophysiological models known to impair memory in rodents (Perio et al., 1989; Terranova et al., 1994, 1996; Mikolajczak et al., 2002).

Many studies support the idea that the cognitive impairment in man and the disruption of memory processes in various animal models by chronic ethanol intake is related to a depression of brain cholinergic activity (Antuono et al., 1980; Arendt et al., 1983, 1989; Lishman, 1990; Hodges et al., 1991). Furthermore, acute treatment with ethanol reduces the acetylcholine release in vitro (Kalant and Grose, 1967; Carmichael and Israel, 1975) and in vivo (Erickson and Graham, 1973; Morgan and Phillis, 1975). It has been demonstrated that both intrinsic cholinergic neurons and projections from other parts of the brain exist in the olfactory system of the rat (for review, see Kasa, 1986), and that cholinergic receptor antagonists are able to affect the activity of neurons in olfactory bulbs. Moreover, the muscarinic receptor antagonist scopolamine promotes a blockade of olfactory perception (Spector and Hull, 1972; Mollenauer et al., 1974) and discrimination (Cheal, 1981; Soffié and Lamberty, 1988), disrupting the working (Winters et al., 2000) and long-term (Aglade et al., 1999) olfactory memory in rats.

Besides this involvement of the cholinergic system, there is considerable evidence that the reinforcing effects of ethanol are mediated, at least in part, by the brain's endogenous opioid system, and that a number of behavioural and pharmacological effects of ethanol, such as hypothermia, euphoria, analgesia and motor activation, as well as the development of tolerance and dependence, are similar to those produced by opioids (Kalant, 1977). The participation of the opioid system in memory processes is still controversial. States of heightened emotion and arousal, such as those that may occur during crimes or traumatic accidents, can impair human memory. The blockade of opioid receptors with naltrexone reverses this memory impairment related to arousal (Katzen-Perez et al., 2001). Some reports indicate that the endogenous opioids are involved in memory storage, since opioid receptor antagonists given after training enhance memory retention, whereas opioid receptor agonists impair it (Izquierdo, 1979; Izquierdo and Netto, 1985; Netto et al., 1986). In the same way, the intracerebroventricular injection of endomorphin-1, an endogenous μ-opioid receptor agonist, increases scopolamine-induced impairment of passive avoidance learning in mice (Ukai et al., 2001), while the μ-opioid receptor antagonist naloxonazine inhibits this effect (Ukai and Lin, 2002). On the other hand, maternal memory in rats appears to be mediated by endogenous opioid activity around the time of parturition (Byrnes and Bridges, 2000). Moreover, the i.p. administration of the opioid receptor antagonist naloxone (1.0 mg/kg) impairs spatial performance on the T-maze in rats (Lukaszewska, 1997).

The present study was therefore designed to investigate whether ethanol would affect short-term olfactory memory in rats and whether this action is related to the muscarinic and/or opioid receptors.

2. Materials and methods

2.1. Animals

Male Wistar rats from our own colony were used. Adult rats (3 months old, 300–370 g) were housed individually in plastic cages (42 × 34 × 17 cm) and they were used only after at least 14 days of habituation to their new environment. Juvenile rats (25–30 days old, 100–150 g) were kept in groups of 10 per cage and served as social stimuli for the adult rats. Animals were maintained in a room with controlled temperature (23 ± 1 °C) and a 12-h light cycle (lights on 7:00 a.m.) with free access to food and water. All procedures used in the present study complied with the guidelines on animal care of the UFSC Ethics Committee on the Use of Animals which follows the "Principles of laboratory animal care" from NIH publication No. 85–23.

2.2. Drugs

Ethanol (Merck) was diluted in 0.9% NaCl (saline) to the concentration of 10% w/v (0.5 and 1.0 g/kg doses) or to the concentration of 20% w/v (2.0 g/kg dose). Scopolamine hydrobromide (SIGMA, 0.06 and 0.5 mg/kg) and naloxone (RBI, 1.0 mg/kg) were dissolved in 0.9% saline. The control solution consisted of an equivalent volume of NaCl solution. All drugs were administered intraperitoneally (i.p.), except in a set of experiments where the ethanol and saline were administered by oral route, i.e., gavage.

2.3. Social recognition test

The short-term memory was assessed with the social recognition or social investigation test. The test was scored by the same rater in an observation room, where the rats had been habituated for at least 1 h before the beginning of the test. The experiments were carried out in the light phase (between 1:00 and 6:00 p.m.). All juveniles were isolated in individual cages for 20 min prior to the beginning of the experiment.

The social recognition test consisted of two successive presentations (5 min each) separated by a delay period. During the first presentation, a juvenile rat was put in the home cage of the adult rat, and the time spent by the adult on investigating the juvenile was recorded. Nosing, sniffing, grooming, pawing or close following of the juvenile by the adult were considered as intents of social investigation behaviour (Thor and Holloway, 1982; Sawyer et al., 1984). At the end of the first presentation, the juvenile was removed and kept in an individual cage during the delay period.

The same juvenile was re-exposed to the adult rat after a delay period of 30 or 120 min. What generally takes place in this kind of test is that if the delay period is less than 40 min, adult male rats display recognition of this juvenile as indicated by a significant reduction in the social investigation time during the second presentation (Dantzer et al., 1987; Engelmann et al., 1995). However, when the same juvenile is re-exposed longer (more than 60 min) after the first presentation, the adult rat no longer recognizes this juvenile, i.e., the social investigation time in the second presentation is similar to that observed during the first. Thus, a 30-min interval between two presentations of the same conspecific juvenile was used to demonstrate possible "amnesic" effect consecutive to a given drug administration. In contrast, a 120-min interval was selected as a temporal window suitable for testing memory-enhancing treatments.

2.3.1. Dose-dependent effects of ethanol on social memory

Adult rats were treated with a single dose of ethanol (0.5, 1.0 or 2.0 g/kg, i.p.) or saline immediately after the end of the first presentation. The same juvenile was re-exposed after a delay period of 30 or 120 min. A second experiment was performed to detect nonspecific effects of ethanol, such as habituation or deficit in the motor activity, that might have been responsible for the reduced investigatory behaviour of the adult rat during the second presentation. In this experiment, a different juvenile to the one used in the first presentation was exposed to the adult rat during the second encounter, with a similar duration of social investigation time being expected.

To discard a possible aversive component promoted by i.p. administration of ethanol which in turn might affect the retention response, the same ethanol doses were administered by oral route immediately after the end of the first presentation.

2.3.2. Temporal properties of ethanol effects on social memory

To detect for how long the ethanol was able to extend the recognition ability of the adult rats, a selected dose of ethanol (1.0 g/kg, i.p.) or saline (i.p.) was administered immediately after the end of the first presentation, and the same juvenile was re-exposed to the adult rat after a delay period of 0.5, 2, 4, 6 or 24 h. Additionally, to investigate the influence of the injection time of ethanol, the same dose (1.0 g/kg) or saline (i.p.) was administered via i.p. route to the adult rats 0, 15, 30 or 60 min after the end of the first presentation and the same juvenile was re-exposed after an interval of 120 min.

2.3.3. Participation of muscarinic and opioid receptors in the effects of ethanol on social memory

The importance of muscarinic and/or opioid receptors in the effect of ethanol on social memory in adult rats was investigated through the co-administration of the central muscarinic receptor antagonist scopolamine (0.06 or 0.5 mg/kg, i.p.) or the opioid receptor antagonist naloxone (1.0 mg/kg, i.p.) with ethanol (1.0 g/kg, i.p.) or saline (i.p.) immediately after the end of the first encounter, and the same juvenile was re-exposed to the adult rat after a delay period of 30 or 120 min.

2.4. Locomotor activity

The locomotor activity of the adult rats treated with saline (i.p.) or ethanol (0.5, 1.0 or 2.0 g/kg, i.p.) was measured in activity cages. The activity cages (70 × 30 × 22 cm), with a steel grid floor, equipped with three parallel horizontal infrared beams positioned 3 cm above the floor and spaced evenly along the longitudinal axis, had a digital counter which recorded photocell beam interruptions. All experiments were conducted between 1:00 and 5:00 p.m. The items of data obtained were expressed as signals corresponding to spontaneous movements for 5 min. The basal locomotor activity was measured on day 1, without administration of any drug. On the second day, the locomotor activity of the adult rats was measured 30 and 120 min after a single dose of ethanol (0.5, 1.0 or 2.0 g/kg, i.p.) or saline (i.p.).

2.5. Statistical analysis

All values are expressed as means ± S.E.M. (n equals the number of rats included in each analysis). The statistical comparison of results was carried out using one- or two-way ANOVA. Following significant ANOVAs, means of investigation time and locomotor activity were compared by the Newman–Keuls test. The accepted level of significance for the tests was $P \leq 0.05$.

3. Results

3.1. Social recognition test

3.1.1. Dose-dependent effect of ethanol on social memory

The control group confirmed the previously described response pattern of short-term olfactory memory in rats after delay periods of 30 and 120 min (Fig. 1). Two-way ANOVA (time × stimuli) revealed significant effects for the time (30 or 120 min) [$F(1,23) = 8.65$; $P = 0.007$], for the stimuli (same or different juveniles) [$F(1,23) = 13.84$; $P = 0.001$], and for the interaction between these factors [$F(1,23) = 19.26$; $P = 0.0002$]. Subsequent Newman–Keuls test indicated that adult rats injected with control solution and exposed 30 min later to the same juvenile spent less time investigating it than on the first exposure. However, the animals exposed 120 min later to the same juvenile spent as much time investigating this juvenile as they did on the first exposure. When a different juvenile was used during the second presentation, no significant reduction of the investigation time was observed regardless of the time interval, 30 or 120 min (Fig. 1).

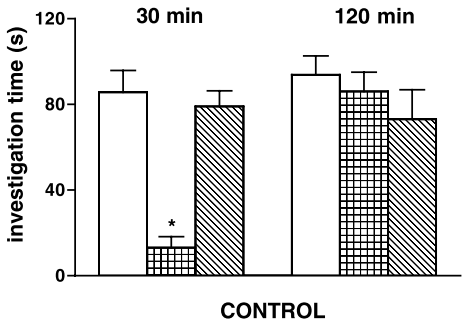

Fig. 1. Influence of the delay period between the first and the second presentation. Bars represent the social investigation ($n=6-8$ animals in each group) [sec (s); mean ± S.E.M.] during the first (white) and second presentation (squares: towards same juvenile; hatched: towards different juvenile). *$P \leq 0.05$ compared to the first presentation (Newman–Keuls test).

As shown in Fig. 2, animals injected with ethanol (0.5, 1.0 or 2.0 g/kg) via the i.p. route, immediately after the end of the initial exposure to the juvenile rat and exposed 30 min later to the same juvenile, behaved in a similar way to saline-treated rats [$F(3,25)=0.14$; $P=0.94$]. Fig. 3 summarises the dose-dependent effects of ethanol on the investigation time of the same juvenile (Fig. 3A) or the different juvenile (Fig. 3B) in the forgetting procedure, when exposed 120 min later to adult rats. Two-way ANOVA (treatment and stimuli) revealed significant effects for the main factors and their interactions [$F(3,53)=29.60$; $P<0.0001$; $F(1,53)=8.71$; $P=0.005$; $F(3,53)=3.91$; $P=0.01$, respectively]. Subsequent Newman–Keuls tests indicated that ethanol at doses of 0.5 and 1.0 g/kg (i.p.) did not change the time spent in exploring the unfamiliar juvenile during the second encounter (120 min), while the highest dose tested, 2.0 g/kg (i.p.), significantly reduced the investigation time in the different juvenile conditions (Fig. 3B). These results suggest that only low doses of ethanol (0.5 or 1.0 g/kg, i.p.) specifically enhance the recognition of an already familiar juvenile by adult rats.

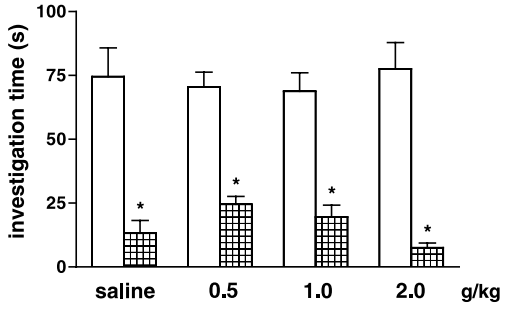

Fig. 2. The effects of ethanol (0.5, 1.0 or 2.0 g/kg, i.p.) on investigation time when the same juvenile was re-exposed at 30 min after the first presentation. Data are expressed as mean ± S.E.M. ($n=6-8$ animals in each group). The bars represent the investigation time in the first (white) and second presentation (squares). *$P \leq 0.05$ compared to the first presentation of the same group (Newman–Keuls test).

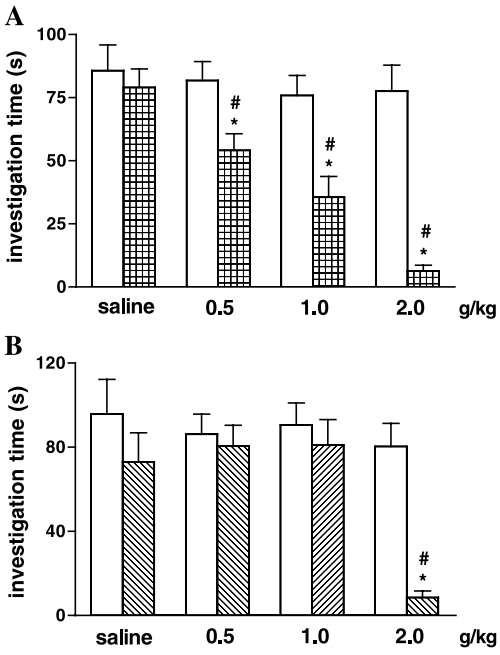

Fig. 3. The effects of ethanol (0.5, 1.0 or 2.0 g/kg, i.p.) on investigation time when the same (panel A) or a different juvenile (panel B) was exposed at 120 min after the first presentation. Data are expressed as mean ± S.E.M. ($n=7-9$ animals in each group). The bars represent the investigation time in the first (white) and second presentation (squares: towards same juvenile; hatched: towards different juvenile). *$P \leq 0.05$ compared to the first presentation of the same group. #$P \leq 0.05$ compared to the second presentation of the control group (Newman–Keuls test).

The results of ethanol administration (0.5, 1.0 or 2.0 g/kg, by oral route) on investigation time when the juvenile rats were re-exposed after a delay period of 120 min are given in Table 1. Two-way ANOVA indicated a significant effect for the treatment [$F(3,56)=28.07$; $P<0.0001$], for the stimuli [$F(1,56)=45.59$; $P<0.0001$] and for the interaction between treatment and stimuli [$F(3,56)=6.65$; $P=0.0006$]. In a similar manner to that found with the i.p. experiment, only the highest dose of ethanol tested (2.0 g/kg) acted in a nonspecific way, i.e., reducing the investigation time when a different juvenile was used during the second presentation

Table 1
The effects of ethanol administration (0.5, 1.0 or 2.0 g/kg, by oral route) on investigation time when the same or a different juvenile was exposed at 120 min

Treatment (g/kg)	Same juvenile		Different juvenile	
	T0	T120	T0	T120
Control	109.6 ± 6.0	93.2 ± 7.5	111.3 ± 9.6	102.1 ± 8.9
Ethanol 0.5	112.2 ± 6.9	31.9 ± 8.0[a,b]	112.0 ± 7.8	89.5 ± 15.5
Ethanol 1.0	111.7 ± 6.2	23.4 ± 6.4[a,b]	111.0 ± 13.1	104.6 ± 10.6
Ethanol 2.0	96.6 ± 5.9	5.1 ± 2.4[a,b]	88.5 ± 8.2	28.0 ± 8.1[a,b]

Data are expressed as the means ± S.E.M. of the investigation time (s) during the first (T0) and second exposure (T120) ($n=8-9$ animals in each group).

[a] $P \leq 0.05$ compared to the first presentation (T0) of the same group.

[b] $P \leq 0.05$ compared to the second presentation (T120) of the control group (Newman–Keuls test).

Table 2
Influence of different inter-exposure intervals with the same juvenile on investigation time of adult rats injected with ethanol (1.0 g/kg, i.p.) immediately after the end of the first presentation

Inter-exposure intervals (h)	0.5	2	4	6	24
Control					
1st	74.5 ± 11.3	85.7 ± 10.2	88.2 ± 10.7	89.7 ± 8.5	100.8 ± 9.6
2nd	13.2 ± 5.0[a]	79.0 ± 7.4	78.2 ± 8.2	88.7 ± 6.0	92.3 ± 6.2
ethanol					
1st	73.0 ± 6.7	75.9 ± 8.0	106.7 ± 8.1	94.3 ± 11.0	94.4 ± 6.9
2nd	17.4 ± 5.1[a]	35.6 ± 8.3[a,b]	44.3 ± 4.5[a,b]	78.5 ± 10.4	104.6 ± 7.1

Data are expressed as the means ± S.E.M. of the investigation time (s) for seven to eight animals in each group.
[a] $P \leq 0.05$ compared to the 1st presentation of the same group.
[b] $P \leq 0.05$ compared to the 2nd presentation of the control group (Newman–Keuls test).

(Table 1). These results discard the involvement of an aversive component caused by i.p. injection of ethanol.

3.1.2. Temporal properties of ethanol effects on social memory

Table 2 illustrates the effects of ethanol (1.0 g/kg, i.p.) injected immediately after the end of the first presentation in extending the recognition ability of the adult rats compared to the control group. Two-way ANOVA indicated significant effects for the treatment [$F(1,58) = 9.74$; $P = 0.003$], for the time [$F(4,58) = 39.39$; $P < 0.0001$], and for the interaction factor between treatment and time [$F(4,58) = 5.92$; $P = 0.0004$]. Confirming the preceding experiments, control rats presented similar investigation times in the second encounter 2 h after the first presentation. Post hoc comparisons indicated that ethanol facilitated the social memory when the same juvenile was re-exposed up to 4 h, but at not 6 or 24 h, after the initial presentation (Table 2).

Table 3
Influence of the injection time of ethanol (1.0 g/kg, i.p.) after 1st presentation on investigation time when the same juvenile was re-exposed at 120 min

Injection time after 1st presentation (min)	0	15	30	60
Control				
T0	85.7 ± 10.2	89.8 ± 9.0	86.0 ± 5.4	90.2 ± 6.5
T120	79.0 ± 7.4	79.5 ± 8.2	84.5 ± 2.4	84.0 ± 5.4
ethanol				
T0	75.9 ± 8.0	81.9 ± 4.2	87.0 ± 5.8	96.0 ± 7.1
T120	35.6 ± 8.3[a,b]	32.7 ± 5.4[a,b]	38.1 ± 9.6[a,b]	71.9 ± 10.9

Data are expressed as the means ± S.E.M. of the investigation time (s).
[a] $P \leq 0.05$ compared to the 1st presentation of the same group ($n = 7–8$ animals in each group).
[b] $P \leq 0.05$ compared to the 2nd presentation of the control group (Newman–Keuls test).

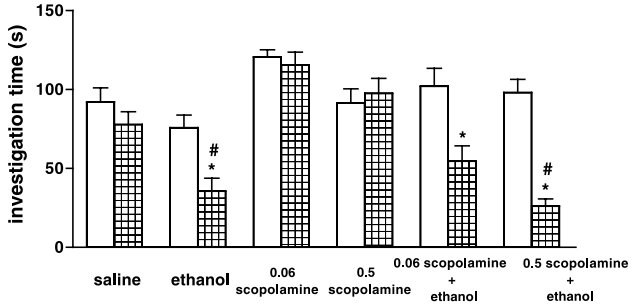

Fig. 4. Effects of scopolamine (0.06 or 0.5 mg/kg, i.p.) on investigation time when administered alone or co-administered with ethanol (1.0 g/kg, i.p.). The same juvenile was re-exposed at 120 min after the first presentation. Data are expressed as mean ± S.E.M. ($n = 6–8$ animals in each group). The bars represent the investigation time in the first (white) and second presentation (squares). *$P \leq 0.05$ compared to the first presentation of the same group. #$P \leq 0.05$ compared to the second presentation of the control group (Newman–Keuls test).

The influence of different injection times of ethanol on social memory can be seen in Table 3. Two-way ANOVA revealed significant effects for the treatment factor [$F(1,54) = 40.47$; $P < 0.0001$] and for the time factor [$F(3,54) = 2.85$; $P = 0.04$]. It seems clear from this data that the facilitatory effect of ethanol (1.0 g/kg) on olfactory memory lasted 30 min from the injection (Table 3).

3.1.3. Influence of muscarinic and opioid receptors on the effects of ethanol on social memory

The highest dose of scopolamine tested (0.5 mg/kg, i.p.) prolonged the investigation time when the same juvenile was re-exposed at 30 min, indicating a disruption caused by this dose to the recognition ability of the adult rat, while the lower dose (0.06 mg/kg, i.p.) did not promote any significant effect during the second encounter involving the same juvenile (data not shown). When the delay period was extended to 120 min, one-way ANOVA revealed a significant effect for the

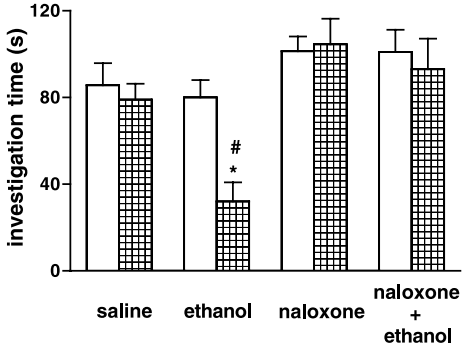

Fig. 5. Effects of naloxone (1.0 mg/kg, i.p.) on investigation time when administered alone or co-administered with ethanol (1.0 g/kg, i.p.). The same juvenile was re-exposed at 120 min after the first presentation. Data are expressed as mean ± S.E.M. ($n = 7–9$ animals in each group). The bars represent the investigation time in the first (white) and second presentation (squares). *$P \leq 0.05$ compared to the first presentation of the same group. #$P \leq 0.05$ compared to the second presentation of the control group (Newman–Keuls test).

Table 4
The effects of ethanol (0.5, 1.0 or 2.0 g/kg, i.p.) treatment on locomotor activity in rats

Treatment (g/kg)	Locomotor activity		
	Basal	30 min	120 min
Control	51.8 ± 5.0	33.2 ± 4.0	20.4 ± 3.6
ethanol 0.5	45.8 ± 4.3	20.7 ± 4.4	12.6 ± 3.8
ethanol 1.0	53.3 ± 5.7	27.4 ± 4.1	14.4 ± 2.7
ethanol 2.0	44.0 ± 4.4	19.4 ± 3.3[a]	10.5 ± 2.1[a]

Data are expressed as mean ± S.E.M. ($n = 8–9$ animals in each group) of locomotor activity measures during 5 min on previous day (baseline, without ethanol), 30 and 120 min after treatments.

[a] $P \leq 0.05$ compared to the respective control group (Newman–Keuls test).

treatment factor [$F(5,38) = 18.74$; $P < 0.0001$] (Fig. 4). At this time, the same doses of scopolamine (0.06 or 0.5 mg/kg, i.p.) did not promote any significant effect during the second encounter involving the same juvenile when administered alone. Moreover, the co-administration of scopolamine (0.06 or 0.5 mg/kg, i.p.) and ethanol (1.0 g/kg, i.p.) did not reverse the reduction of investigation time promoted by ethanol (Fig. 4).

Animals injected with naloxone (1.0 mg/kg, i.p.) immediately after the end of the initial exposure to the juvenile rat and exposed 30 min later to the same juvenile behaved in a similar way to saline-treated rats (data not shown).

The effects of co-administration of naloxone (1.0 mg/kg, i.p.) with ethanol (1.0 g/kg, i.p.) are illustrated in Fig. 5. Naloxone reversed the reduction of the investigation time promoted by ethanol when the same juvenile was re-exposed 120 min after the first presentation [$F(3,27) = 1.10$, $P = 0.001$]. However, when naloxone (1.0 mg/kg, i.p.) was injected alone, no significant alteration was recorded during the second encounter with the same juvenile (Fig. 5).

3.2. Locomotor activity

The effects of acute i.p. administration of control solution or ethanol (0.5, 1.0 or 2.0 g/kg) on rat locomotor activity are illustrated in Table 4. Two-way ANOVA with repeated measures revealed no significant effect for drug treatment [$F(3,38) = 2.47$, $P = 0.08$]. However, it indicated a significant effect for the repeated exposure to the activity cage [$F(2,76) = 93.97$, $P < 0.0001$]. The Newman–Keuls test indicated that all groups presented on the second day a significant reduction in locomotor activity compared to basal measures, i.e., habituation. Also, further comparisons between groups revealed that the 2 g/kg of ethanol significantly decrease the locomotor activity of rats compared to the control group (Table 4).

4. Discussion

Confirming previous studies, our results show that after a delay of 30 min between two distinct presentations of the same juvenile, the adult rat injected with control solution recognizes this juvenile, while a delay of 120 min induces forgetfulness in the adult rat. Thus, a 30-min interval between two presentations of the same conspecific juvenile was used to demonstrate possible "amnesic" effect consecutive to a given drug administration. In contrast, a 120-min interval was selected as a temporal window suitable for testing memory-enhancing treatments.

The present findings demonstrate that single administration of low doses of ethanol by oral (gavage) or intraperitoneal (i.p.) routes after the first presentation decreases the investigation time of the same juvenile in the forgetting procedure (exposure 120 min later to adult rats), suggesting that ethanol facilitates short-term memory.

These results might be surprising in the light of the preponderance of data in the literature suggesting that ethanol impairs memory in animal models (Givens, 1995; White et al., 1998; Matthews et al., 1999). However, it is important to note that ethanol is a drug that has apparently opposite effects on retention, depending on when it is given relative to the task as well as the nature of the task under study (Ladner et al., 2001). Post-training ethanol enhances retention in a one-trial passive avoidance task in mice (Alkana and Parker, 1979) and improves the performance in a "water-finding task" in rats (Melia et al., 1986), but seems to "impair" retention of an appetitive task (Babbini et al., 1991).

The social memory is a particular model of olfactory memory mediated by olfactory cues (Sawyer et al., 1984) and may involve a non-procedural form of a short-term working memory based upon the olfactory discriminative capacity of rats. The persistence with which an adult rat investigates a juvenile can be enhanced or decreased in a predictive manner by using inhibition and facilitation procedures (Dantzer et al., 1987). Moreover, the social memory in rats is sensitive to compounds which facilitate memory such as cholinomimetic drugs, nootropic drugs, benzodiazepine inverse receptor agonists (Perio et al., 1989) and the cannabinoid receptor antagonist SR141716 (Terranova et al., 1996).

There was no interference in the investigation time of the juvenile at 30 min by adult rats treated i.p. with ethanol. However, low doses of ethanol (0.5 and 1.0 g/kg, i.p.) administered after the first presentation reduced the investigation time of the same juvenile by adult rats when forgetfulness was fully developed after a 120-min delay. This response cannot be attributed to nonspecific effects of ethanol, since it was not observed when a different juvenile was used for the second exposure. Furthermore, facilitation cannot be attributed to direct effects of ethanol on trace acquisition such as altered perception, attention, or motivation, since training occurred in the drug-free state. Although post-training drug administration may induce state-dependent effects (Chute and Wright, 1973), a state-dependent explanation cannot apply to the present results since it would predict impaired rather than enhanced retention. Furthermore, the reduction of the investigation time by these doses

of ethanol cannot be explained by a locomotion deficit of the animals, since it was not observed in the locomotor activity test. Only the highest dose of ethanol tested (2.0 g/kg, i.p.) reduced the time spent in investigating a different juvenile, indicating an effect not related to memory.

Some authors (Colbern et al., 1986; Babbini et al., 1991) have reported that aversive effects of ethanol's injections or events which occur in the home cage during the inter-exposure period (such as attacks by their cagemates) may explain post-training ethanol facilitation performance. These explanations cannot be applied to the present results, since the administration of low doses of ethanol (0.5 and 1.0 g/kg) by oral route (which discard a possible pain stimulus related to ethanol's injection) promoted a facilitation of the social memory in a very similar manner when it was administered i.p. Moreover, attack by cagemates is an unlikely explanation for our results, since the adult rats were housed individually.

Consistent with the present data, Mikolajczak et al. (2001) recently demonstrated a facilitation of short-term olfactory memory in adult rats treated with ethanol and having a disturbed circadian cycle, although they failed to demonstrate the reduction of the investigation time when the same juvenile was re-exposed after a delay period of 120 min. A differential influence of circadian cycle upon the delay period of exposure, 30 or 120 min, may explain in part the discrepant results between our study and that of Mikolajczak et al. (2001).

In addition, while adult rats no longer recognized the juvenile after a delay period of 40 min (Dantzer et al., 1987; Engelmann et al., 1995); in our study, ethanol (1.0 g/kg, i.p.) extended the recognition ability of the adult rats for a period of 4 to 6 h. This enhancement of olfactory stimulus retention by ethanol is time-dependent, since it was only observed when ethanol was administered up to 30 min after the end of the first exposure to the juvenile rat. This time coincides with the limit period that the adult rat recognizes the juvenile. In other words, ethanol cannot enhance the olfactory memory information (the juvenile cue) that no longer exists. Time-dependent effects are well known for many memory-enhancing treatments, Perio et al. (1989), in a study of the effects of cholinergic drugs on the short-term memory, has already demonstrated the importance of administration time of arecoline on social memory. Arecoline was active only when injected immediately after the first presentation of the juvenile, but not when the injection was delayed by 5 min or more. The short period of time during which the injection of arecoline was efficacious led the authors to conclude that the drug-sensitive phase of this memory model is very brief. However, the present data demonstrate that the sensitive period for the pharmacological treatment in the social recognition test may be variable and dependent on the drug under evaluation.

The participation of the cholinergic system in olfactory perception and olfactory learning has been extensively studied in recent years. In the olfactory system of the rat, both intrinsic cholinergic neurons and projections from other parts of the brain exist (for review, see Kasa, 1986). The central muscarinic receptor antagonist scopolamine hydrobromide promotes a blockade of olfactory perception (Spector and Hull, 1972; Mollenauer et al., 1974) and discrimination (Cheal, 1981; Soffié and Lamberty, 1988), disrupting the working olfactory memory (Winters et al., 2000) in rats. In the present study, as previously demonstrated by Perio et al. (1989), the lower dose of scopolamine tested (0.06 mg/kg, i.p.) did not prevent the recognition ability of the adult rats after a delay period of 30 min when administered alone (data not shown). However, this dose of scopolamine that was able to antagonize the decrease in investigation time of the same juvenile after a delay period of 120 min by arecoline (Perio et al., 1989), did not antagonize the reduction of investigation time promoted by ethanol (1.0 g/kg, i.p.) under the same conditions. Moreover, reinforcing previous studies (Soffié and Lamberty, 1988), the highest dose of scopolamine tested (0.5 mg/kg, i.p.) impaired the social recognition ability of the adult rats when the same juvenile was re-exposed after an interval of 30 min (data not shown), demonstrating the importance of muscarinic receptor for the olfactory recognition. However, this "amnesic" dose of scopolamine did not affect the facilitatory action induced by ethanol (1.0 g/kg, i.p.) when the same juvenile was re-exposed after a delay period of 120 min.

There is considerable evidence that some pharmacological and behavioural effects of ethanol are mediated, at least in part, by the brain's endogenous opioid system (Kalant, 1977). On the other hand, it has been demonstrated that the release of endogenous opioid and the activation of opioid receptors improves memory processes in some contexts (Lukaszewska, 1997; Byrnes and Bridges, 2000). Our results indicate a participation of the opioid system in the facilitation of social olfactory memory by ethanol in rats. The co-administration of the opioid receptor antagonist naloxone (1.0 mg/kg, i.p.) reverses the memory-enhancing effect of ethanol (1.0 g/kg, i.p.) when the same juvenile is re-exposed at 120 min, while it has no effect when administered alone (at 30- or 120-min intervals). Recently, Olianas and Onali (1999) have demonstrated that activation of opioid receptors in the rat olfactory bulb enhances basal adenylyl cyclase activity and potentiates enzyme stimulation by G_s-coupled neurotransmitter receptors in a pertussis toxin-sensitive manner. However, further research is needed to clarify the mechanisms underlying the opioid receptor contribution to the enhancement of social olfactory memory by ethanol and the involvement of other neurotransmitters mediating this action.

In conclusion, these results demonstrate that acute low doses of ethanol improve the social memory in rats in a specific and time-dependent manner and that this action is, at least in part, related to opioid receptors. Furthermore, the present study confirms and extends the notion that the social recognition test is a useful and effective animal model to

investigate short-term memory affected by the post-acquisition administration of ethanol.

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