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European Journal of Pharmacology 444 (2002) 83–96

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Effects of acamprosate and some polyamine site ligands of NMDA receptor on short-term memory in rats

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Received 12 November 2001; received in revised form 8 January 2002; accepted 11 January 2002

Abstract

The aim of this study was to evaluate the effect of multiple acamprosate (500.0 mg/kg, p.o.) administration on short-term memory, using the social recognition test in rats. Ifenprodil (1.0 mg/kg, i.p.), arcaine (5.0 mg/kg, i.p.) and spermidine (20.0 mg/kg, i.p.) were chosen as polyamine ligands and their action or interaction with acamprosate was also studied. The doses used did not show any sedative activity, which was assessed by measuring locomotor activity and the hypnotic effect of ethanol. The findings suggest that acute acamprosate treatment did not impair short-term memory. Multiple acamprosate and a single spermidine or arcaine administration led to better performance in the memory test, whereas no significant difference was observed in ifenprodil-treated rats. Co-administration of a single arcaine or spermidine dose with multiple acamprosate produced worse results. This means that the effect of repeated acamprosate administration can be changed by the co-administration of other polyamine ligands, so that care should be taken in interpreting.

Keywords: Acamprosate; Memory, Social; Polyamines; Ifenprodil

1. Introduction

Acamprosate (calcium acetyl homotaurinate) has been found beneficial for the treatment of alcoholism, because it reduces relapse in abstinent alcohol-dependent patients (Johnson and Ait-Daoud, 2000; Lesch et al., 2001; for review), but many aspects of its basic and clinical pharmacological profile are still under investigation (Allgaier et al., 2000; Popp and Lovinger, 2000; Czachowski et al., 2001; Gual and Lehert, 2001; Rammes et al., 2001; Rubio et al., 2001; Stromberg et al., 2001; Wu et al., 2001).

There is some evidence suggesting that acamprosate may act as ''partial co-agonist'' directly on a spermidine-sensitive site of the *N*-methyl-D-aspartate (NMDA) receptor, and express excitatory or inhibitory effects, depending on the experimental conditions (Naassila et al., 1998; Al-Qatari et al., 1998), while a direct or indirect effect of acamprosate on other systems cannot be excluded (Allgaier et al., 2000; Dahchour and De Witte, 2000; Piorunska-Mikolajczak et al., 2001; Wu et al., 2001; Okulicz-Kozaryn et al., in press).

Because it is known that alcoholics exhibit numerous neuropsychological deficits, particularly in memory function (O'Mahoney and Doherty, 1996; Parsons, 1998), it seems important to evaluate different memory functions during acamprosate treatment. There are only few reports concerning acamprosate effects on memory in humans, and the published results often lead to contradictory conclusions (Schneider et al., 1998; Soyka et al., 1998; Schneider et al., 1999). In our previous studies using the passive avoidance test, we found that acamprosate amplified the long-term memory, especially in older rats (Okulicz-Kozaryn et al., 1996) and in chronically ethanol-treated rats (Mikolajczak et al., 1994). Similarly, acamprosate had a fairly positive effect on working memory tasks in most groups of chronically ethanol-treated rats that were investigated (Mikolajczak et al., 1997).

The fact that acamprosate has no negative effect on working memory either in humans or animals, but can express a marked reduction of delayed free recall in humans (Schneider et al., 1999), may indicate that the influence of

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01276-1

acamprosate on learning and memory probably depends on the assessment of different memory functions and/or the type of test used.

One of the tests used for research on memory processes and learning processes in experimental animals is the social memory test (Thor and Holloway, 1982; Dantzer et al., 1987; Perio et al., 1989; Hlinak and Krejci, 1994; Griffin and Taylor, 1995; Arletti et al., 1997; Argyriou et al., 1998; Popik and van Ree, 1998; Becker and Grecksch, 2000; Dluzen et al., 2000; Ferguson et al., 2000; Kogan et al., 2000; Mikolajczak et al., 2001). This test is based on the ability of animals to recognise other animals by following chemosensory cues which are primary stimuli permitting this distinction (Carr et al., 1976; Thor and Holloway, 1982). It can be assumed that this memory takes the form of short-term memory, strictly related to sensory memory (Dantzer et al., 1987). Therefore, introduction of the social memory test, which is completely void of external enhancing stimuli, in the research on the memory of acamprosate-treated animals, should allow for better assessment of acamprosate cognitive properties in experimental animals.

There are suggestions, based on use of the abovementioned test, that NMDA glutamate receptors may be involved in the processing of socially relevant olfactory information. However, the retention of olfactory memory can be limited, due to the very complex relationships between different binding sites on the NMDA receptors and their ligands (Hlinak and Krejci, 1994, 1995). Since acamprosate is able to act at the polyamine site of the NMDA receptor (Naassila et al., 1998), ifenprodil, arcaine and spermidine were chosen for comparative purposes as polyamine ligands possessing the various agonistic-antagonistic properties at the binding site (Yamamura and Shimoiji, 1999 for review). Their own action or interaction with acamprosate were assessed in this study using the social memory test.

Preliminary accounts of part of the present results were given at a congress (Mikolajczak et al., 2000).

2. Materials and methods

2.1. Animals

The experiments were performed on male Wistar rats housed individually in their home cages, kept on a reversed 12/12 h night/day cycle (light 19:00–7:00) under constant ambient conditions (20 ± 2 °C, relative humidity 65%). The rats were fed a standard laboratory diet (pellets—Labofeed B (LSM)—Feeds and Concentrates Production Plant, Poland, ISO 9001:1996) and had tap water freely available in their home cages. The animals weighed 269 ± 4 g at the beginning of the experiments ($n = 126$). Juvenile ~ 30-day-old male rats kept in standard plastic cages with seven animals in each cage were used as social stimulus.

All animal procedures were conducted in accordance with all applicable Polish and international ethical standards, including the European Community guidelines for the use of laboratory animals.

2.2. Drugs and polyamine site ligands

Acamprosate (Aotal, Meram, France) was suspended in 1% methylcellulose, ifenprodil (ifenprodil tartrate, RBI, USA), arcaine (arcaine sulfate, RBI) and spermidine (spermidine trihydrochloride, ICN, USA) were dissolved in water for injection (Aqua pro Injectione, Polfa, Poland). Control rats were treated with an appropriate volume of 1% methylcellulose (control animals for acamprosate) or of water for injection (control rats for ifenprodil, arcaine and spermidine).

2.3. Social memory test

A social memory test design based on olfactory recognition (Thor and Holloway, 1982; Sawyer et al., 1984), which allowed measurement of the short-term memory conditions with a short-term recognition procedure, was used (Thor and Holloway, 1982; Dantzer et al., 1987; Griffin and Taylor, 1995). Briefly, the adult test rat was presented to a juvenile (~ 30 days old) male rat (social stimulus) for 5 min, and its social-investigatory behaviour (defined as being proximally oriented to juvenile rat or as having a direct contact with the other rat by sniffing, following, grooming or generally inspecting any body surface of the juvenile) was measured with a hand-held cumulative timer to the nearest 0.1 s (T1—first encounter). Next, after 30 min, the same procedure was repeated with the same juvenile rat (known juvenile rat); (T2—second encounter). Between the two successive exposures, the juveniles were housed individually. For evaluation of non-specific effects, an unknown new juvenile was exposed to the rat during "second exposure" (T2). As in other reports, the test values were expressed either as time spent on investigation and/or as ratio (Ratio of Investigation Duration) of time spent on investigation during T2 divided by T1 (Hlinak and Krejci, 1994; Arletti et al., 1997; Argyriou et al., 1998).

All adult rats were first tested (before introduction of a drug treatment to adult rats) during 4 consecutive days preceding the experiment (pretest) using the procedure described by others (Dantzer et al., 1987). Briefly, during the first day of pretest, the inter-exposure interval was 5 min and the same juvenile rat (known juvenile rat) was used as social stimulus (day 1). In the next 2 days, the amount of time between exposures was 30 min (days 2 and 3) but on day 3, a different, unknown new juvenile was introduced during T2. In the last day of pretest (day 4), the inter-exposure interval was 120 min; however, the same juvenile rat was presented during T1 and T2. Generally, each adult rat was exposed to various juvenile rats and only the animals that reliably investigated the juvenile rats without displaying

aggressive or sexual behaviour were used for their social recognition characteristics ($n = 88$). All animals were randomised after pretest.

All social investigations were conducted in the cages of adult rats during the dark phase (between 9:00 and 15:00) in a dimly illuminated, soundproof room.

2.4. Drug treatment

2.4.1. Dose-dependent effect of acamprosate on social memory

Rats were treated with a single dose of acamprosate (50.0, 200.0, 500.0 and 750.0 mg/kg, p.o.) or 1% methylcellulose and 60 min after drug or vehicle administration, social memory was assessed as described above. The experiment was performed using five separate groups of rats ($n = 6$ in each acamprosate-treated group, $n = 11$ in vehicle-treated group). The animals ($n = 35$) weighed 234 ± 4 g and there were no significant differences between any of the groups of rats (analysis of variance-ANOVA [$F(4,30) = 1.37$; $P > 0.1$]). Test values were expressed as time spent on investigation and as ratio (ratio of investigation duration) of time spent on investigation during T2 divided by T1.

These animals were not used in the part of the study concerning the effect of multiple acamprosate treatment on social memory.

2.4.2. Locomotor activity

Locomotor activity was evaluated using a PAN-licensed activity meter, Poland by placing animals in the centre of the apparatus and recording their activity with electromechanical counters (Mikolajczak et al., 1999). The data obtained were expressed as signals corresponding to spontaneous movements for 5 min. Locomotor activity was measured: 60 min after a single dose of acamprosate (50.0, 200.0 and 500.0 mg/kg, p.o.), 15 min after a single dose of arcaine (1.0, 3.0 and 5.0 mg/kg, i.p.) or spermidine (5.0, 10.0, 20.0, 40.0 and 80 mg/kg, i.p.) and 30 min after a single dose of ifenprodil (0.3, 1.0 and 10.0 mg/kg, i.p.). Control groups were treated according to the appropriate drug treatment schedule.

Additionally, another two separate groups of rats were treated with acamprosate (500.0 mg/kg/day, p.o.) or 1% methylcellulose for 21 ($21 \times$) consecutive days and locomotor activity was measured 60 min after the last dose of acamprosate. These animals were not used for social memory testing.

2.4.3. Ethanol-induced sleeping time

The effect of drugs or polyamine site ligands on the hypnotic action of ethanol was measured using the same procedure of drug administration as for locomotor activity. After the appropriate time from the moment the animals were given the drug, ethanol was given at the dose of 3.0 g/kg (20% solution (w/w), i.p.) and sleep time was measured. It was expressed as the time from the loss until the first return of the righting reflex (Mikolajczak et al., 1999).

The two experiments assessing sedative activity were done with 126 rats (seven animals per group) during the dark phase (between 9:00 and 15:00) in a dimly illuminated, soundproof room.

2.4.4. Effect of multiple acamprosate treatment

Adult rats were treated with acamprosate (500.0 mg/kg/day, p.o.) or 1% methylcellulose for 21 ($21 \times$) consecutive days (Table 1). Additionally, ifenprodil (1.0 mg/kg, i.p.), arcaine (5.0 mg/kg, i.p.) or spermidine (20.0 mg/kg, i.p.) were given once to other $21 \times$ acamprosate groups during the last day of the treatment. The effect of a single injection of arcaine (5.0 mg/kg, i.p.), ifenprodil (1.0 mg/kg, i.p.) or spermidine (20.0 mg/kg, i.p.) was also evaluated after the first 21-fold treatment with 1% methylcellulose (p.o.) and a single injection of arcaine, ifenprodil, spermidine or water for injection (i.p.). This procedure allowed checking the effect of one additional factor, i.e. possible difference produced by acute or chronic stress, which could be obtained during single or multiple treatment. Social memory (T1) was assessed 60 min after the last dose of acamprosate and then spermidine, arcaine or the appropriate volume of water for injection was administered 15 min before T2 (acamprosate + spermidine, acamprosate + arcaine or acamprosate, respectively). In the acamprosate + ifenprodil group, the dose of ifenprodil was administered 30 min before T2. The controls for acamprosate-treated rats received the last dose of 1% methylcelulose 60 min before T1 and the appropriate volume of water for injection was administered 15 min before T2. The effects of single arcaine, spermidine or ifenprodil injection were measured when—during the day of social memory testing—the last dose of 1% methylcellulose was administered and the drug was injected immediately (ifenprodil) or 15 min (arcaine and spermidine) after removal of the juvenile rat at the end of the T1. Next day, the same procedure was repeated for all investigated substances but the known juvenile rat was replaced by an unknown juvenile rat during the second encounter (measuring non-specific effect).

Animals weighed 444 ± 5 g at the beginning of the social memory measurement ($n = 64$) and there were no significant differences between any of the groups (ANOVA [$F(7,56) = 0.48$; $P > 0.1$]). The experiment assessing the effect of multiple acamprosate treatment on social memory was done with 64 adult male rats (eight animals per group) during the dark phase in a dimly illuminated, soundproof room.

2.5. Statistical analysis

All values were expressed as means $\pm$ S.E.M. (n equals the number of rats included in each analysis). Statistical comparison of results was carried out using an ANOVA followed by a least significant difference post hoc test used to analyse the data for locomotor activity or hypnotic effects of ethanol. The Kruskal–Wallis nonparametric ANOVA followed by a Mann–Whitney test (unpaired data) or Fried-

Table 1
Scheme of acamprosate and polyamine ligands treatment during social memory test

Group	Specific effect				Non-specific effect			
	Substance treatment (p.o.)	Time between substance 21st dose treatment and first encounter (min)	Substance injection before second encounter (i.p.)	Time between substance injection and second encounter—known juvenile (min)	Substance treatment (p.o.)	Time between substance 22nd dose treatment and first encounter—known juvenile (min)	Substance injection before second encounter (i.p.)	Time between substance injection and second encounter—unknown juvenile (min)
Acamprosate	21 × acamprosate	60	water for injection	15	22 × acamprosate	60	water for injection	15
Control	21 × 1% methylcellulose	60	water for injection	15	22 × 1% methylcellulose	60	water for injection	15
Acamprosate + arcaine	21 × acamprosate	60	arcaine	15	22 × acamprosate	60	arcaine	15
Acamprosate + ifenprodil	21 × acamprosate	60	ifenprodil	30	22 × acamprosate	60	ifenprodil	30
Acamprosate + spermidine	21 × acamprosate	60	spermidine	15	22 × acamprosate	60	spermidine	15
Arcaine	21 × 1% methylcellulose	60	arcaine	15	22 × 1% methylcellulose	60	arcaine	15
Ifenprodil	21 × 1% methylcellulose	60	ifenprodil	30	22 × 1% methylcellulose	60	ifenprodil	30
Spermidine	21 × 1% methylcellulose	60	spermidine	15	22 × 1% methylcellulose	60	spermidine	15

man nonparametric ANOVA followed by a Wilcoxon test (paired data) was applied for memory task data analysis.

3. Results

3.1. Social memory test

3.1.1. Pretest

As shown in Table 2, there were significant differences between values for ratio of investigation duration obtained in pretest during four consecutive days, using different inter-exposure intervals with the same familiar juvenile rat (known juvenile rat), (day 1—inter-exposure interval = 5 min, day 2—inter-exposure interval = 30 min and day 4—inter-exposure interval = 120 min) or the different juvenile rat (unknown juvenile rat), (day 3—inter-exposure interval = 30 min) during the second encounter (T2)—(Friedman ANOVA [$H(3,88) = 59.9$; $P < 0.0001$]). Detailed analysis showed that values of ratio of investigation duration: for day 1 were different from days 2–4; for day 2 were different from days 1, 3–4; for day 3 were different from days 1–2, 4; for day 4 were different from days 1–3, ($P < 0.05$, Wilcoxon test). Therefore, social recognition was taken to have occurred when the inter-exposure interval was 5 or 30 min. If the inter-exposure interval was 120 min, no facilitation effect during second encounter (T2) was observed. Similarly, the presentation of an unknown juvenile rat during T2 when the inter-exposure interval was 30 min also interrupted the social recognition task.

3.1.2. Dose-dependent effect of acamprosate on social memory

In the experiment testing the acute acamprosate effect on social memory, a single administration of the drug at doses of 50.0, 200.0, 500.0 and 750.0 mg/kg (p.o.) was used (Table 3, Fig. 1). The overall analysis of the results (effects within the group), expressed as time spent on social investigation, did not reveal significant differences in acamprosate-treated animals at the dose of: 50.0 mg/kg (Friedman ANOVA [$H(3,6) = 1.81$; $P > 0.1$]); 200.0 mg/kg (Friedman ANOVA [$H(3,6) = 5.80$; $P > 0.1$]); 500.0 mg/kg (Friedman ANOVA [$H(3,6) = 5.40$; $P > 0.1$]); 750.0 mg/kg (Friedman ANOVA [$H(3,6) = 6.10$; $P > 0.1$]) and in control rats (Friedman ANOVA [$H(3,11) = 1.90$; $P > 0.1$]) (Table 3). Detailed analysis showed distinction of the known juvenile (T2-specific effect vs. T1-specific effect) in acamprosate-treated animals with the dose of 500.0 mg/kg and in control rats, but the effects did not reach significance ($P < 0.1$, Wilcoxon test) (Table 3). Also, when test values were expressed as ratio of investigation duration, there were no significant differences, whether known or unknown juvenile rats were introduced to adult animals during the second exposure (Fig. 1) (Kruskal–Wallis ANOVA: [$H(4,34) = 4.06$; $P > 0.1$] or [$H(4,34) = 2,77$; $P > 0.1$], respectively). After the dose of 200.0 mg/kg of acamprosate a non-significant low-

Table 2
Values of ratio of investigation duration obtained in pretest during 4 consecutive days using different inter-exposure intervals with the same familiar juvenile rat (known juvenile rat)—(days 1, 2, and 4) or a different juvenile rat (unknown juvenile rat)—(day 3) during the second encounter (T2)

Inter exposure intervals	Day 1 (5 min)	Day 2 (30 min)	Day 3 (30 min)	Day 4 (120 min)
Ratio of investigation duration	$0.74 \pm 0.04^{b,c,d}$	$0.85 \pm 0.03^{a,c,d}$	$1.15 \pm 0.06^{a,b,c}$	$0.99 \pm 0.04^{a,b,d}$

Values expressed as means ± S.E.M.
$n = 88$.
Friedman test: $H(3,88) = 59.9$; $P < 0.0001$.
a, b, c, d—statistically significant difference vs. days 1, 2, 3 or 4, respectively; $P < 0.05$, (Wilcoxon test).
For details—see Materials and methods.

Table 3
The dose-dependent effect of a single acamprosate administration (p.o.) on time of social investigation in rats

Group	Specific effect (s)		Non-specific effect (next day) (s)		Friedman test ($H(3,6)=$)
	First encounter (T1)	Second encounter—known juvenile (T2)	First encounter—known juvenile (T1)	Second encounter—unknown juvenile (T2)	
Control	62.7 ± 7.7	52.7 ± 7.3[a]	56.4 ± 7.3	69.0 ± 11.1	1.90; $P>0.1$
Acamprosate, 50.0 mg/kg	42.0 ± 11.0	40.2 ± 10.4	34.4 ± 9.9	38.7 ± 12.7	1.81; $P>0.1$
Acamprosate, 200.0 mg/kg	54.2 ± 9.9	51.3 ± 4.8	50.5 ± 11.8	64.6 ± 10.4	5.80; $P>0.1$
Acamprosate, 500.0 mg/kg	56.2 ± 7.8	42.0 ± 8.6[a]	54.1 ± 11.0	56.5 ± 16.0	5.4; $P>0.1$
Acamprosate, 750.0 mg/kg	62.7 ± 13.1	44.7 ± 10.3	61.1 ± 13.2	89.7 ± 19.2	6.1; $P>0.1$

Data are expressed as the means ± S.E.M. for six animals in each acamprosate-treated group, control ($n = 11$)—separate group receiving 1 × 1% methylcellulose (p.o.).
a—statistically significant difference for: T1 (specific effect) vs. T2 (specific effect), $P \leq 0.1$, (Wilcoxon test).
For details—see Materials and methods and Table 1.

ering of social memory was observed, whereas with higher doses (500.0 and 750.0 mg/kg), a tendency to better fulfillment of the short-term memory task was found but none of these results was significant ($P>0.1$, Mann–Whitney test). The observed effects appear specific, because when the effects of an unknown juvenile rat are compared to that of a known juvenile rat, the results were significant (Friedman ANOVA [$H(1,35) = 4.23$; $P < 0.05$]).

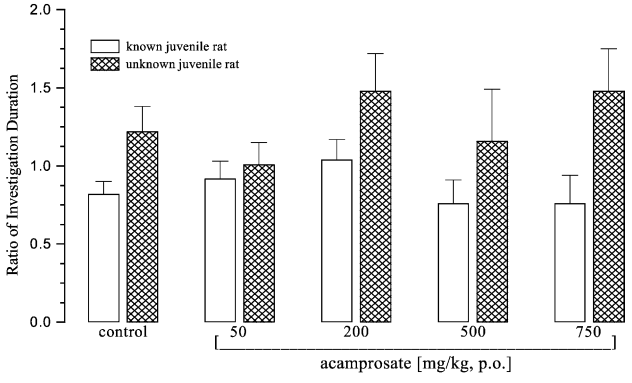

Fig. 1. The dose-dependent effect of a single acamprosate administration (p.o.) on social memory expressed as a ratio of investigation duration in rats. Ratio of investigation duration = time spent on social investigation during second exposure of the known or an unknown juvenile divided by time spent during first exposure of a juvenile. Data are expressed as the means ± S.E.M. for six animals in each acamprosate-treated group; control ($n = 11$)—separate group receiving 1 × 1% methylcellulose (p.o.); Kruskal–Wallis test for known juvenile rat or unknown juvenile rat, [$H(4,34) = 3.22$, $P>0.1$] or [$H(4,34) = 3.02$, $P>0.1$], respectively. Assessment of test specificity–comparison of ratio of investigation durations for known vs. unknown juvenile rat–Friedman test $H(1,35) = 4.23$; $P < 0.05$.

3.2. Locomotor activity

In the experiment testing the sedative action of acamprosate treatment on the basis of the assessment of locomotor activity, it was found that a single administration of acamprosate (p.o.) produced a dose-dependent lowering of locomotor activity (Fig. 2A), (one-way ANOVA [$F(3,24) = 6.19$; $P < 0.01$]). A single oral administration of the drug in a dose of 50.0 mg/kg significantly increased activity ($P < 0.01$), whereas the effect of 200.0 and 500.0 mg/kg was no different from that reported for the control group receiving 1% methylcellulose.

After a single administration of ifenprodil (0.3, 1.0, 10.0 mg/kg, i.p.), it was found that ifenprodil did not produce a significant dose-dependent change in locomotor activity (Fig. 2B), (ANOVA [($F(3,24) = 1.35$; $P>0.1$]). However, with the highest dose of ifenprodil, this behaviour tended to be reduced compared to that of control rats.

The results of the experiment testing the influence of a single administration of spermidine on locomotor activity showed that this polyamine did not affect this activity significantly at the doses investigated (5.0, 10.0, 20.0, 40.0, 80.0 mg/kg, i.p.)—(ANOVA [$F(5,36) = 0.41$; $P>0.1$]), (Fig. 2C). However, spermidine at a dose of 40.0 mg/kg and higher showed a tendency to depress such behaviour.

A single administration of arcaine (i.p.) at doses of 1.0, 3.0 and 5.0 mg/kg did not significantly alter locomotor activity (Fig. 2D), (ANOVA [$F(3,24) = 1.09$; $P>0.1$]) when compared to that of vehicle-treated animals.

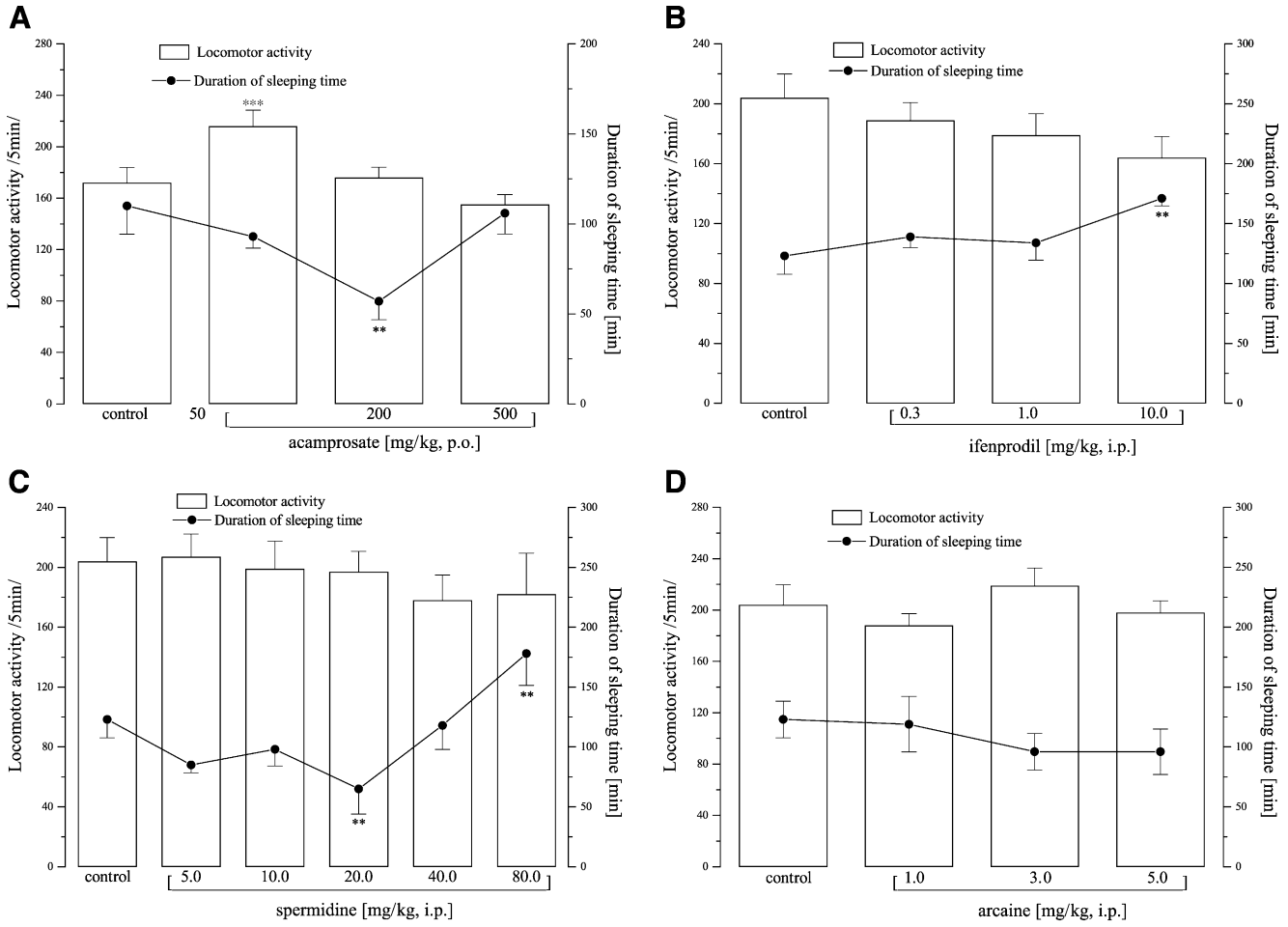

Fig. 2. The dose-dependent effect of a single acamprosate (panel A), ifenprodil (panel B), spermidine (panel C) or arcaine (panel D) treatment on locomotor activity and duration of ethanol (3.0 g/kg, i.p.) induced sleeping time in rats. Data are expressed as the means ± S.E.M. for seven animals. One-way ANOVA for locomotor activity or duration of sleeping time: for acamprosate: [$F(3,24)=6.19$, $P<0.01$] or [$F(3,24)=4.50$; $P<0.05$], respectively; for ifenprodil: [$F(3,24)=1.35$, $P>0.1$] or [$F(3,24)=3.10$; $P<0.05$], respectively; for spermidine: [$F(5,36)=0.41$, $P>0.1$] or [$F(5,36)=4.55$; $P<0.01$], respectively; for arcaine: [$F(3,24)=1.09$, $P>0.1$] or [$F(3,24)=0.61$; $P>0.1$], respectively. (***,**) Statistically significant difference vs. "control" group receiving 1% methylcellulose, $P<0.01$ or 0.05, respectively (least significant difference post hoc test) (panel A); (**) statistically significant difference vs. "control" group receiving water for injection, $P<0.05$ (least significant difference post hoc test) (panel B or C).

Comparison of the effects of single and multiple acamprosate administration on locomotor activity revealed significant differences between all groups (ANOVA $F(3,24)=6.70$; $P<0.01$), (Fig. 3). However, further analysis of the results of 21 × acamprosate treatment showed that, as for the single acamprosate effect on this behaviour (the data for 1 × acamprosate treatment was taken from Fig. 2A), there was no significant difference between the multiple acamprosate-treated and control animals (Fig. 3). Therefore, the statistical differences are due to the influence of prolonged treatment per se (21 × acamprosate vs. 1 × acamprosate, $P<0.01$ or 21 × control vs. 1 × control, $P<0.05$).

3.3. Ethanol-induced sleeping time

In this experiment, acamprosate shortened the ethanol-induced (3.0 g/kg, i.p.) sleeping time (ANOVA [$F(3,24)=4.50$; $P<0.05$]), but a significant effect after administration of a dose of 200.0 mg/kg was only found when compared to that in 1% methylcellulose-treated rats ($P<0.05$), (Fig. 2A).

Concerning the effect of ifenprodil on ethanol-induced sleeping time, a significant prolongation of dose-dependent (0.3, 1.0, 10.0 mg/kg, i.p.) relationship was found (ANOVA [($F(3,24)=3.22$; $P<0.05$]) after the highest dose of ifenprodil ($P<0.05$), (Fig. 2B).

Spermidine at doses of 5.0–20.0 mg/kg lowered the duration of ethanol-induced sleeping time (ANOVA [$F(5,36)=4.55$; $P<0.01$]) (Fig. 2C), but the effect was significant for a dose of 20.0 mg/kg only ($P<0.05$). At the highest dose (80.0 mg/kg) of spermidine, the opposite effect was measurable when compared to that in vehicle-treated animals ($P<0.05$).

There was no effect of arcaine at doses 1.0, 3.0 and 5.0 mg/kg (i.p.) on ethanol-induced sleeping time (ANOVA [$F(3,24)=0.61$; $P>0.1$]) (Fig. 2D).

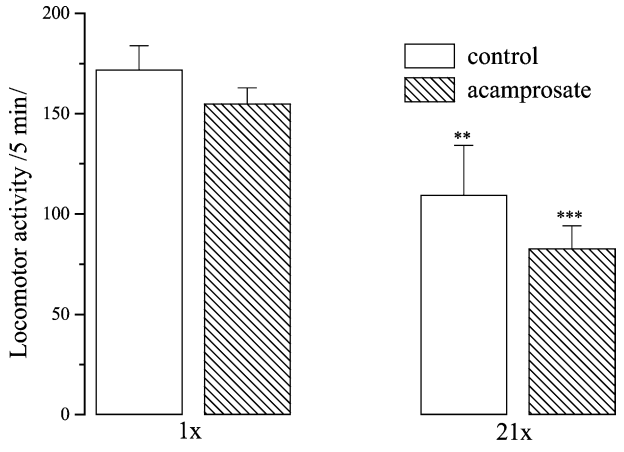

Fig. 3. Comparison of a single (1 ×) and multiple (21 ×) acamprosate (500.0 mg/kg, p.o.) administration on locomotor activity in rats. Experiment was conducted with four separate groups of rats (the data of 1 × acamprosate treatment was inserted from Fig. 2A). Data are expressed as the means ± S.E.M. for seven animals. Control—separate groups receiving 1 × or 21 × 1% methylcellulose (p.o.), respectively. One-way ANOVA: $F(3,24)=6.70$; $P<0.01$]; (***,**) statistically significant difference vs. appropriate 1 × treated group, $P<0.01$ or 0.05, respectively (least significant difference post hoc test).

3.4. Effect of multiple acamprosate treatment on social memory test

The effects of multiple (21 ×) acamprosate (500.0 mg/kg, p.o.) treatment and ifenprodil (1.0 mg/kg, i.p.), arcaine (5.0 mg/kg, i.p.) or spermidine (20.0 mg/kg, i.p.) given once to other 21 × acamprosate groups or administered alone on olfactory social memory test performance in adult rats, are shown in Table 4 and Fig. 4. Because compounds produced a pharmacologically measurable effect after 15 min (arcaine or spermidine) or 30 min (ifenprodil) after a single administration, an inter-exposure interval = 30 min was chosen for further investigation.

The overall analysis of the results (effects within the group) expressed as time of investigation revealed significant differences for acamprosate (Friedman ANOVA [$H(3,8)=16.6$; $P<0.001$]), acamprosate + ifenprodil (Friedman ANOVA [$H(3,8)=9.68$; $P<0.05$]), acamprosate + spermidine (Friedman ANOVA [$H(3,8)=12.7$; $P<0.01$]), arcaine (Friedman ANOVA [$H(3,8)=12.9$; $P<0.01$]), spermidine (Friedman ANOVA [$H(3,8)=9.45$; $P<0.05$]) and control (Friedman ANOVA [$H(3,8)=16.3$; $P<0.001$]), whereas for acamprosate + arcaine or ifenprodil, the results were non-significant (Friedman ANOVA: [$H(3,8)=3.15$; $P>0.1$] or [$H(3,8)=4.95$; $P>0.1$], respectively) (Table 4). Distinction of the known juvenile (T2-specific effect vs. T1-specific effect, Wilcoxon test) was found in acamprosate ($P<0.05$), acamprosate + ifenprodil ($P<0.05$), acamprosate + spermidine ($P<0.05$), arcaine ($P<0.05$), spermidine-treated ($P<0.05$) and control animals ($P<0.05$) (Table 4). In contrast, investigation time after acamprosate + arcaine, acamprosate + ifenprodil, acamprosate + spermidine, arcaine and spermidine treatment for rats re-exposed to a novel (unknown) juvenile as well as for the control animals (T2 non-specific) did not differ from the time obtained during the first exposure (T1 non-specific) (Table 3). However, results for acamprosate- and ifenprodil-treated rats were significant ($P<0.05$) (Table 4). There were no significant differences between any times of investigation in drug-treated and control animals when a first encounter (T1) was presented to a resident adult rat during a measurement of specific and non-specific effect (Table 4). As the observed values for time of investigation for T1 (specific effect), T2 (specific effect) and T2 (non-specific effect) showed a significant variability (Kruskal–Wallis ANOVA: [$H(3,63)=19.4$; $P<0.01$] or [$H(3,63)=15.0$; $P<0.05$] or [$H(3,63)=12.0$; $P<0.01$], respectively) (Table 4), therefore, for further analysis of drug effects on social memory after multiple treatment, the data were expressed as ratio of investigation duration as did other authors (Arletti et al., 1997; Argyriou et al., 1998).

Table 4
Effect of multiple (21 ×) acamprosate (500.0 mg/kg, p.o.) treatment and ifenprodil (1.0 mg/kg, i.p.), arcaine (5.0 mg/kg, i.p.) and spermidine (20.0 mg/kg, i.p.) given once to other multiple acamprosate-treated groups or administered alone, on time of social investigation in rats

Group	Specific effect (s)		Non-specific effect (next day) (s)		Friedman test ($H(3,8)$=)
	First encounter (T1)	Second encounter—known juvenile (T2)	First encounter—known juvenile (T1)	Second encounter—unknown juvenile (T2)	
Control	135.5 ± 11.8	114.0 ± 11.0[a]	145.6 ± 13.6[b]	147.6 ± 14.0	16.3; $P<0.001$
Acamprosate	135.7 ± 18.2	95.1 ± 20.1[a]	138.4 ± 12.3[b]	173.9 ± 17.4[c]	16.6; $P<0.001$
Acamprosate + arcaine	85.0 ± 13.2	81.4 ± 11.3	86.2 ± 14.6	105.9 ± 21.0	3.15; $P>0.1$
Acamprosate + ifenprodil	116.6 ± 10.7	85.6 ± 11.4[a]	108.7 ± 8.7[b]	131.4 ± 14.6	9.68; $P<0.05$
Acamprosate + spermidine	89.6 ± 16.1	83.4 ± 11.2	113.6 ± 13.3[b]	121.7 ± 15.7	12.7; $P<0.01$
Arcaine	117.6 ± 15.2	71.1 ± 15.5[a]	117.7 ± 17.2[b]	118.6 ± 22.8	12.9; $P<0.01$
Ifenprodil	93.0 ± 12.2	88.6 ± 15.3	95.0 ± 9.2	119.6 ± 9.9[c]	4.95; $P>0.1$
Spermidine	147.5 ± 6.0	109.7 ± 10.1[a]	143.9 ± 9.7[b]	146.7 ± 15.7	9.45; $P<0.05$
Kruskal–Wallis test $H(7,63)$=	19.4; $P<0.01$	15.0; $P<0.05$	20.0; $P<0.01$	11.0; $P>0.1$	

Data are expressed as the means ± S.E.M. for eight animals in each group.
a, b, c—statistically significant difference for: T1 (specific effect) vs. T2 (specific effect), T1 (non-specific effect) vs. T2 (specific effect) or T2 (non-specific effect) vs. T1 (non-specific effect), respectively; $P<0.05$, (Wilcoxon test).
For details—see Materials and methods and Table 1.

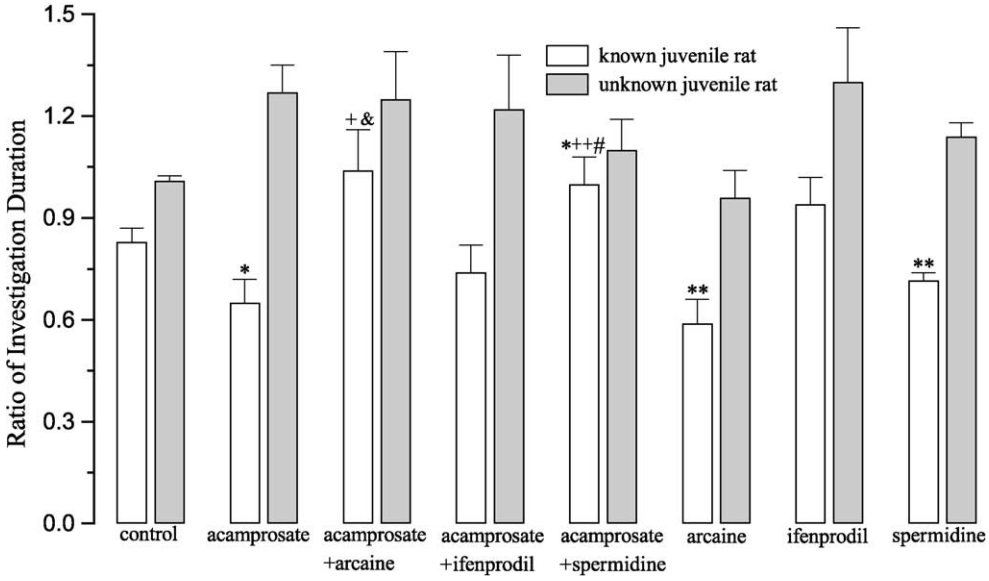

Fig. 4. Effect of multiple (21 ×) acamprosate (500.0 mg/kg, p.o.) treatment and ifenprodil (1.0 mg/kg, i.p.), arcaine (5.0 mg/kg, i.p.) and spermidine (20.0 mg/kg, i.p.) given once to other multiple acamprosate-treated groups or administered alone, on social memory expressed as a ratio of investigation duration in rats. Ratio of investigation duration = time spent on social investigation during second exposure of the known or an unknown juvenile divided by time spent during first exposure of a juvenile. Data are expressed as the means ± S.E.M. for eight animals, Kruskal–Wallis test for known juvenile rat or unknown juvenile rat, [$H(7,63) = 16.3$; $P < 0.05$] or [$H(7,63) = 18.4$; $P < 0.01$], respectively. (**,*) Statistically significant difference vs. 1% methylcellulose-treated group (control), $P < 0.05$ or 0.1, respectively (Mann–Whitney test); (++,+) statistically significant difference vs. acamprosate, $P < 0.01$ or 0.05, respectively (Mann–Whitney test); (&, #) statistically significant difference vs. arcaine or spermidine, respectively, $P < 0.05$ (Mann–Whitney test). Assessment of test specificity—comparison of ratio of investigation duration for known vs. unknown juvenile rat—Friedman test $H(1,64) = 35.1$; $P < 0.0001$.

It follows from the results expressed as ratio of investigation duration (Fig. 4) that these substances produced significantly different effects whether known or unknown juvenile rats were introduced to adult animals during the second exposure (Kruskal–Wallis ANOVA [$H(7,63) = 16.3$; $P < 0.05$] or [$H(7,63) = 18.4$; $P < 0.01$], respectively). The observed effects appear specific, since when the effects of an unknown juvenile rat are compared to that of a known juvenile rat, the values from the Friedman test are large [$H(1,64) = 35.1$; $P < 0.0001$].

Detailed analysis showed that multiple acamprosate administration led to improved performance in the social memory recognition task, however, the effect was not significant ($P < 0.1$, Mann–Whitney test), (Fig. 4).

The single and multiple effects of acamprosate (500.0 mg/kg, p.o.) on social memory were not significantly different when a known juvenile rat was introduced to adult animals during the second exposure (Kruskal–Wallis ANOVA [$H(3,32) = 4.73$; $P > 0.1$]) (the data of 1 × and 21 × acamprosate treatment were taken from Figs. 1 and 4). Further detailed analysis showed that there were no significant differences between either single or multiple acamprosate-treated rats (0.76 ± 0.15 vs. 0.66 ± 0.07, $P > 0.1$, Mann–Whitney test) and two control groups (0.82 ± 0.08 vs. 0.83 ± 0.04, $P > 0.1$, Mann–Whitney test).

Single spermidine (20.0 mg/kg) administration yielded better results for the task of social memory compared to the performance of vehicle-treated animals ($P < 0.05$, Mann–Whitney test) (Fig. 4). Similarly, after a single arcaine (5.0 mg/kg) treatment, enhancement of short-term memory was observed ($P < 0.05$, Mann–Whitney test) (Fig. 4). However, there was no significant effect of a single ifenprodil (1.0 mg/kg) administration on social memory, but only a tendency to impair performance of the memory task.

In the experiment concerning interaction between acamprosate and polyamine ligands, co-administration of acamprosate and arcaine (acamprosate + arcaine) or spermidine (acamprosate + spermidine) produced worse results for social memory than in the control group (for acamprosate + arcaine or acamprosate + spermidine, $P < 0.01$ and $P < 0.05$, respectively, Mann–Whitney test) (Fig. 4). These results were also the opposite of those obtained after single administration of spermidine or arcaine (acamprosate + spermidine vs. spermidine, $P < 0.05$; acamprosate + arcaine vs. arcaine, $P < 0.05$; Mann–Whitney test), (Fig. 4). The combined acamprosate and ifenprodil treatment slightly lowered the values for ratio of investigation duration compared to those for the control or the single administration of ifenprodil, but these effects were not significant (Fig. 4).

4. Discussion

The olfactory recognition social memory test used in this study is based on the tendency of rodents to investigate unfamiliar conspecifics more intensely than familiar ones (Thor and Holloway, 1982; Dantzer et al., 1987; Griffin and Taylor, 1995; Popik and van Ree, 1998). The values for

ratio of investigation duration obtained in the pretest for different inter-exposure intervals showed typical characteristics and were in agreement with data presented by others (Dantzer et al., 1987; Griffin and Taylor, 1995; Popik and van Ree, 1998). Therefore, we felt that this procedure could be used for studying the effects of acamprosate and polyamine ligands on short-term memory.

A single acamprosate treatment at the doses 50.0, 200.0, 500.0 and 750.0 mg/kg did not alter social memory. The next step was to assess the eventual influence of the sedative component concerning the effects of acamprosate and the other polyamine ligands on social memory in rats.

On the basis of the assessment of rats' locomotor activity and ethanol-induced sleeping time after the single administration, and as there was no significant difference in locomotor activity between the single and multiple acamprosate-treated rats at the dose of 500.0 mg/kg and the related control animals, in further investigations, this oral dose of acamprosate was considered appropriate. This dose of the drug did not exhibit sedative or stimulatory properties and was closest to that given by others (400 mg/kg, p.o.) concerning route of administration and pharmacological effects on ethanol drinking expressed, for example, by reduction of the preference for alcohol, modification of brain γ-aminobutyric acid (GABA) transmission in ethanol-treated animals or lowered motility and glutamate microdialysate content during ethanol withdrawal in rats (Gewiss et al., 1991; Daoust et al., 1992; Dahchour and De Witte, 1999). Moreover, we found that acamprosate at this dose produced a distinct alteration of the response in tests of learning and memory (Mikolajczak et al., 1994, 1997; Okulicz-Kozaryn et al., 1996; Okulicz-Kozaryn et al., in press).

Ifenprodil produced a non-significant dose-dependent change in the locomotor activity of rats, but a significant effect on ethanol-induced sleeping time. The highest dose of ifenprodil tended to reduce locomotor activity or the prolongation of ethanol-induced sleeping time, in agreement with the observations of other investigators (Sanger and Joly, 1991; Parada et al., 1992; Ginski and Witkin, 1994; Doyle and Shaw, 1998; Doyle et al., 1998; Malinowska et al., 1999). Therefore, for further investigation, the 1.0 mg/kg dose of ifenprodil was chosen because it involves no sedative activity and possibly excludes the adrenolytic component of its action (Malinowska et al., 1999).

Generally, the locomotor effect was not affected by spermidine, although a dose above 40.0 mg/kg tended to depress this behaviour. This was in agreement with the results of others who found that spermidine in higher doses (e.g. 100.0 mg/kg, i.p.) produced a marked motor disturbance (Shimada et al., 1994). In the second test, spermidine produced a U-shaped relationship with a minimum at 20.0 mg/kg. It is possible that the spermidine effect on ethanol action at doses lower than 20.0 mg/kg shows its agonistic activity, whereas at higher doses, the inhibitory effect of spermidine predominates, as is sometimes postulated by others (Daniell, 1992; Hundt et al., 1998). For further investigations, the dose of 20.0 mg/kg of spermidine was chosen due to its lack of sedative activity.

It needs to be mentioned that relatively low doses of arcaine (5.0 mg/kg, i.p.) were used in the present study, as arcaine in higher doses (>10.0 mg/kg, i.p.) can produce a strong inhibition in rats trained to discriminate ethanol from saline (Hundt et al., 1998).

Checking for non-specific effects in social interaction tests with a locomotor activity test and ethanol-induced sleeping does not give direct information. However, assuming that acamprosate and the polyamine ligands act at NMDA receptor sites, the involvement of doses which can cause sedation and motor impairment must be evaluated, because these effects can interfere with processes of learning and memory, as was shown for the higher doses of dizocilpine (the non-competitive antagonist of NMDA receptor) (Robinson et al., 1989; Heale and Harley, 1990; Fraser et al., 1997). Moreover, it is postulated that the effects of administration of higher doses of many investigated drugs on such a behavioural effect as aggression (measured by, e.g. placing a group-housed intruder male into the resident's homecage) are affected by the lowering of locomotor activity (Weerts et al., 1992; Miczek et al., 1994, 1998; Fish et al., 2000). Also, measuring another kind of behavioural spontaneous activity of rats, such as sniffing the floor or rearing, allows one to differentiate between specific and non-specific drug effects using a social memory test (Hlinak and Krejci, 1991, 1994). Although the factors which can characterise spontaneous activity of rats are sometimes differentiated into three independent variables "amount of activity" (roughly corresponds to locomotor activity as applied in our study), "sequential response organisation" and "exploratory activity" (Paulus and Geyer, 1993), we think that preliminary evaluation of locomotor activity is helpful in the assessment of effects of drug doses affecting motor performance.

It is known that ethanol has a very non-specific action because it alters the activity of many of the systems and that multiple neurotransmitter receptor systems mediate the effects of ethanol (Samson and Harris, 1992). It is known that the acute effects of ethanol disrupt glutamatergic activity via the NMDA receptor leading to inhibition of calcium influx (antagonistic activity) (Hoffman et al., 1989). On the other hand, acute ethanol administration enhances GABA neurotransmission, showing an agonistic property (Lovinger, 1997). Therefore, synergistic ethanol effects via the glutamatergic and GABAergic systems have been discussed (Davis and Wu, 2001) and depend on many factors such as difference in pharmacological activity of their own ligands, doses, route and schedule of treatment or species on which experiments were performed (Wessinger and Balster, 1987; Balster et al., 1992; Shelton and Balster, 1994, 1997; Gatto et al., 1995; Dildy-Mayfield et al., 1996; Wang et al., 1999). It is also known that ethanol at anaesthetic concentrations (50–400 mM) prevents movement in response to a noxious stimulus and that this general anaesthesia symptom is due to a spinal anaesthetic action (Antognini, 1997; Wang

et al., 1999). The enhancement of $GABA_A$- or glycine-induced inhibition has been proposed as a common mechanism of general anaesthesia (Franks and Lieb, 1993; Tanelian et al., 1993). Lately, it has been demonstrated that the anaesthetic action of *n*-alcohols is due to binding at a single site of $GABA_A$ or strychnine-sensitive glycine receptor (Mascia et al., 2000). However, a study of the depressant effect of ethanol (50–200 mM) on glutamate responses in spinal cord motor neurons showed that the direct depression of glutamate excitatory responses (especially via NMDA receptor system) plays a role independent of $GABA_A$- or glycine-induced inhibition (Wang et al., 1999). Since the dose of ethanol (3.0 g/kg, i.p.) used in our study produced a loss of righting reflex, leading to hypnosis and anaesthesia, it is an open question whether the relationships between acamprosate or polyamine ligands and acute treatment with an anaesthesia-inducing dose are due to participation of NMDA or GABAergic mechanisms.

Multiple acamprosate treatment and ifenprodil, arcaine or spermidine, given once to other 21 × acamprosate-treated groups or administered alone, produced specific effects on social recognition, because there was usually no impairment of alertness during a second contact (unknown juvenile rat). As there were no significant differences between the results expressed as time of investigation during the presentation of known juvenile on day 1 (T1-specific effect) and on the next day (T1 non-specific effect), it seems that the treatment with acamprosate or polyamines did not affect the acquisition/attentional processes. Therefore, the lowering of time of investigation during the second contact (the lower values of ratio of investigation duration) with a known juvenile (T2-specific effect) and the similar or even higher time of investigation during the presentation of an unknown juvenile (T2 non-specific effect) shows that the non-specific effect was eliminated and the values for ratio of investigation duration reflect short-time memory (Perio et al., 1989; Hlinak and Krejci, 1994).

Our results make it possible to claim that acamprosate has no negative effect on social memory in rats. They are in agreement with our previous results obtained with a working memory test of rats in a three-panel runway task (Okulicz-Kozaryn et al., in press). Moreover, similar results were obtained in humans (healthy volunteers), in whom it was shown that the short-term working memory and some type of long-term memory (recognising task) after acamprosate administration (during 7 consecutive days) were not altered, although there was an impairment in delayed free recall (Schneider et al., 1999). It thus seems that acamprosate does not show typical antagonistic activity of NMDA receptors, as was found in many studies on the effects of various NMDA receptor antagonists on learning and memory (Danysz and Wroblewski, 1989; Carey et al., 1998; Doyle et al., 1998; Aura and Riekkinen, 1999; Kretschmer and Fink, 1999; Newcomer and Krystal, 2001). Moreover, there is a report that, using the same social recognition test as used in the present study, some non-competitive NMDA antagonists (phencyclidine or dizocilpine) or competitive NMDA antagonists (*cis*-4-phosponomethyl-2-piperidine-carboxylic acid or 3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid) at doses not affecting motor performance, disrupted recognition capacity in adult male rats (Hlinak and Krejci, 1994). Because there are some results suggesting that acamprosate may act as ''partial co-agonist'' directly at the polyamine site of the NMDA receptor, and express excitatory or inhibitory effects, depending on the experimental conditions (Naassila et al., 1998; Al-Qatari et al., 1998), the next step of our study was to assess the influence of some polyamine ligands after their single or acamprosate-combined administration on social memory.

Single spermidine-treated rats showed facilitation of social memory. Because it is well known that polyamines act as regulators of NMDA receptor function (Yamamura and Shimoji, 1999) and are part of the glutamatergic receptor system, it is possible to accept that they could be involved in the processes of learning and memory (Izquiredo and Medina, 1995, 1997). Moreover, low levels of the spermidine and spermine were found in the frontal cortex of postmortem brain of patients with Down's syndrome and Alzheimer's disease, which may be relevant to the understanding of the pathogenesis of cognitive features of these diseases (Seidl et al., 1996). However, to our knowledge, only a few studies concerning the direct effect of polyamines on learning and memory are available. For example, in transgenic mice with putrescine (showing some antagonistic activity (Johnson, 1996)) overproduction in brain, impairment of spatial learning was found, using a Morris water maze (Halonen et al., 1993). Also, systemic administration of a high dose of spermidine (80.0 mg/kg, i.p.) potentiates dizocilpine-induced impairment of a learning task in rats, whereas spermidine without dizocilpine does not influence learning (Shimada et al., 1994). However, there are also contradictory results, because after spermidine administration in a relatively low dose (2.5 and 5.0 mg/kg, i.p.) there was an attenuation of 3-(carboxypiperazine-4-yl)-propyl-1-phosphonic acid-induced learning deficits (Meyer et al., 1998). Moreover, the intrahippocampal or intra-amygdala microinjection of spermidine caused a memory-enhancing effect in the inhibitory avoidance learning test (Rubin et al., 2000, 2001). Similar effects were found after low doses of intracerebroventricular (i.c.v.) spermine using the Morris water maze (Conway, 1998). The enhancement of social memory observed in our study after spermidine treatment may thus be due to the expression of spermidine agonistic activity.

However, the effect of arcaine now shown is rather surprising. It is known that arcaine, in a dose of 1.35 μg (i.c.v.), which does not alter locomotor activity, did not affect conditioned avoidance responses or passive avoidance behaviour in rats (Artemowicz and Wisniewski, 1998; Rubin et al., 2000). Yet, to our knowledge, there are no data available concerning the effect of arcaine on short-term memory, but it is known that arcaine is a competitive polyamine-site antagonist (Reynolds, 1990; Sacaan and Johnson,

1990), and it can be speculated that—in analogy to high doses of spermidine—this compound should instead disrupt social memory performance. However, there is another possibility, based on relatively new data, namely that arcaine characteristics are different from those of spermidine or spermine (Doyle and Shaw, 1998), because arcaine can interact with the NMDA channel at a binding site (site 3) different from that of endogenous polyamines (Sharma and Reynolds, 1999; Yang and Reis, 1999). Therefore, the observed enhancement of social memory after arcaine treatment may be due an effect on this different binding site.

The relatively low, negative and statistically non-significant effect of ifenprodil on memory is in agreement with results obtained by others. It was found that ifenprodil in relative high doses (10.0–30.0 mg/kg, i.p.) did not impair performance of many learning and memory tasks in animals (Sanger and Joly, 1991; Parada et al., 1992; Erakovic et al., 1997; Napiorkowska-Pawlak et al., 2000), even at doses which can lower locomotor activity of rats (Doyle et al., 1998). However, there is a report that ifenprodil produces an impairment of passive avoidance behaviour, but after i.c.v. (1000 nmol/rat) administration (Murata and Kawasaki, 1993).

It is possible that the differences between the effects of a single administration of spermidine, arcaine and ifenprodil on social memory are due to their different characteristics at the polyamine site of NMDA receptor complex (Carter et al., 1990; Schoemaker et al., 1990; Williams, 1993; Rao et al., 1991; Williams et al., 1994; Kew and Kemp, 1998; Masuko et al., 1999; Sharma and Reynolds, 1999; Hofner and Wanner, 2000; Coughenour and Barr, 2000) or, for example, to the ifenprodil (in the dose used here) effect on the 5-HT_3 receptor channel (Napiorkowska-Pawlak et al., 2000). Of course, it is difficult to explain such phenomena directly on the basis of our data.

It can be inferred from the abovementioned results of our study that multiple acamprosate administration did not impair short-term memory, but that its interaction with arcaine or spermidine after their single injection produced negative effects on this kind of memory. This is similar to the results concerning the interaction between spermidine and arcaine when co-administration of spermidine and arcaine completely reversed the spermidine-induced improvement in inhibitory avoidance (Rubin et al., 2000, 2001). Based on our data, it is difficult to explain the nature of the acamprosate + spermidine or acamprosate + arcaine interaction directly.

To our knowledge, there are no published data available concerning an interaction between acamprosate and polyamine ligands after acute as well as chronic treatment from the pharmacokinetic point of view. However, it seems that the interaction is not affected by pharmacokinetic changes due to the fact that acamprosate does not appear to be metabolised (Wilde and Wagstaff, 1997; Saivin et al., 1998) and there were no adverse pharmacokinetic interactions in patients treated with acamprosate in combinations with such compounds as diazepam, oxazepam, phenobarbital during an alcohol withdrawal period (Aubin et al., 1995). Therefore, we think that the probability of a pharmacokinetic interaction between acamprosate and compounds investigated here, including any influence on distribution, elimination or metabolism processes, is rather weak.

Considering the possibility of eventual changes in the properties of the polyamine site after chronic acamprosate administration, which may modify the interaction of the other ligands with the site, especially on the molecular level, again, to our knowledge, there are no data available for resolving this question. It is known that spermidine (significantly) or arcaine (non-significantly) can decrease specific [^{3}H]acamprosate binding to rat brain membranes and/or in the presence of acamprosate, lower of affinity and number of binding sites for [^{14}C]spermidine (Naassila et al., 1998). These authors postulated that acamprosate as a partial co-agonist should produce enhancement of NMDA receptor activity when this receptor is at low levels of physiological stimulation, but that inhibition can be observed when the receptor is strongly stimulated, particularly when the NMDA receptor complex is highly activated by polyamines. Moreover, one of electrophysiological studies showed that acamprosate reversed the potentiating effects of spermine on the function of naive NMDA receptors expressed in a subpopulation of striatal neurons, whereas acamprosate alone did not alter receptor function (Popp and Lovinger, 2000). It is also an open question whether the multiple acamprosate treatment alters properties of the polyamine sites. The consequence of the abovementioned acamprosate actions are rather difficult to extrapolate in vivo. However, it is possible to say that the differences found in our study, between the effects of the acamprosate alone or its combination with spermidine or arcaine on short-term memory are due to this kind of interaction on the molecular level.

Additionally, because almost no effect of ifenprodil on acamprosate activity was expressed in the social memory test, it can be said, that in this case, acamprosate probably acts via a polyamine site other than ifenprodil-sensitive. However, to confirm this hypothesis, additional studies have to be carried out to elucidate the exact nature of the acamprosate and ifenprodil interaction on the molecular level.

In conclusion, our findings suggest that multiple acamprosate treatment does not impair short-term memory. However, this effect can easily be reversed if other polyamine ligands are present, so the results obtained should be approached carefully. Acamprosate could be helpful in the treatment of cognitively impaired alcoholics, but more detailed studies concerning the functional interactions between acamprosate and polyamine-site ligands in ethanol-treated animals are necessary to test this hypothesis.

Acknowledgements

We wish to thank Prof. Susanna Genedani from Universita degli Studi di Modena, Dipartimento di Scienze

Biomediche, Sezione di Farmacologia, Italy, for her fruitful help in the introduction of the social memory test at our laboratory.

This work was supported by grant 501-3-10-02 from K. Marcinkowski University of Medical Sciences in Poznan, Poland.

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