ELSEVIER

European Journal of Pharmacology 347 (1998) 29–40

ejp

# Facilitation of cognitive functions by a specific $\alpha_2$-adrenoceptor antagonist, atipamezole

Antti Haapalinna [a,*], Jouni Sirviö [b], Risto Lammintausta [a]

[a] *Orion, Orion Pharma, Preclinical R&D, P.O. Box 425, FIN-20101 Turku, Finland*
[b] *A.I. Virtanen Institute, University of Kuopio, Kuopio, Finland*

Received 19 January 1998; accepted 27 January 1998

**Abstract**

The present experiments investigated the effects of a specific and potent $\alpha_2$-adrenoceptor antagonist, atipamezole (as a stimulator of the noradrenergic system) on cognitive performance in rats. Atipamezole enhanced the acquisition of a linear-arm maze test and also improved the choice accuracy of poorly performing rats in a delayed (20 min) three-choice maze test. Furthermore, atipamezole improved the achievement of a one-trial appetite-maze when injected immediately after teaching, thus having an effect on consolidation. Atipamezole clearly impaired the acquisition of the active avoidance test. The present results indicate that stimulation of noradrenergic system by atipamezole improves the performance of animals in tasks assessing relational learning and memory, possibly affecting attention, short-term memory and the speed of information processing. It has also an effect on a consolidation process unrelated to attentional or motivational mechanisms. In a stressful test, stimulation of noradrenaline release leads to impairment of performance.
© 1998 Elsevier Science B.V.

*Keywords:* $\alpha_2$-Adrenoceptor; Atipamezole; Consolidation; Learning; Memory; Noradrenergic system

## 1. Introduction

The importance of the forebrain projections of the noradrenergic nucleus, locus coeruleus, in the mediation of attention, learning and memory is supported by many investigations (Anlezark et al., 1973; Robbins et al., 1985). The neurones in the locus coeruleus are activated by novel and salient external stimuli (Aston-Jones et al., 1991; Sara et al., 1994; Simson and Weiss, 1988). In the terminal fields (e.g., cortical areas, hippocampus and amygdala), noradrenaline modulates the excitability of neurones leading to an enhanced 'signal-to-noise' ratio, thought to support focusing of attention (Berridge et al., 1993; Dahl and Winson, 1985; Harley, 1987). Furthermore, noradrenaline is also known to play an important modulatory role in a form of synaptic plasticity, long term potentiation, considered to be associated with memory processes (Bliss et al., 1983; Dahl and Winson, 1985; Harley, 1987). At the behavioural level, noradrenaline can facilitate memory consolidation and retrieval. Blockade of $\beta$-adrenoceptors has been reported to impair consolidation (McGaugh, 1989; McGaugh et al., 1990), whereas the electrical stimulation of the noradrenergic neurones of the locus coeruleus has been shown to improve memory retrieval (Sara and Devauges, 1988). However, the results from several lesion studies have been conflicting (Connor et al., 1992; Selden et al., 1990; Valjakka et al., 1990).

The activity of the noradrenergic neurones in the locus coeruleus is regulated by $\alpha_2$-adrenergic autoreceptors (Cedarbaum and Aghajanian, 1977; Simson and Weiss, 1988). Blockade of these receptors by an $\alpha_2$-adrenoceptor antagonist can increase the firing rate of the neurones and increase the release of noradrenaline in the brain (Freedman and Aghajanian, 1984; Pettibone et al., 1985; Scheinin et al., 1988). It has been postulated that an appropriate level of stimulation of the central noradrenergic system can improve cognitive functions. Interestingly, some reports have shown that the $\alpha_2$-adrenoceptor antagonists, yohimbine and idazoxan, improve performance in some learning and memory tests (Bunsey and Strupp, 1995; Devauges and Sara, 1990; Sara and Devauges, 1989; Sara et al., 1994). Unfortunately, the specificity and selectivity

* Corresponding author. Tel.: +358-2-2727670; fax: +358-2-2727432; e-mail: antti.haapalinna@orion.fi

0014-2999/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.

of those compounds have been questioned. Yohimbine has affinity to many receptors other than noradrenergic receptors, e.g., dopaminergic, 5-hydroxytryptaminergic and benzodiazepine receptors (Lal et al., 1983; Van Oene et al., 1984; Winter and Rabin, 1992). Even though, idazoxan is a more specific $\alpha_2$-adrenoceptor antagonist than yohimbine (Freedman and Aghajanian, 1984), it has a high affinity for noradrenergic imidazoline binding sites (Miralles et al., 1993). Yohimbine, idazoxan and also some more novel $\alpha_2$-adrenoceptor antagonists such as RX821002, (2-methoxy idazoxan), BRL 44408 and ARC 239 have affinity for 5-hydroxytryptamine (5-HT) 5-$HT_{1A}$ receptors (Meana et al., 1996; Sanger and Schoemaker, 1992; Winter and Rabin, 1992). Furthermore, yohimbine, idazoxan and ethoxy idazoxan have been demonstrated to have 5-$HT_{1A}$-receptor agonistic properties in vivo (Jordan et al., 1995; Llado et al., 1996; Pettibone et al., 1985; Sanger and Schoemaker, 1992; Winter and Rabin, 1992) and 5-$HT_{1A}$-receptor agonists have been reported to clearly modulate cognitive function (Carli et al., 1992; Mendelson et al., 1993). While these possible direct effects of the $\alpha_2$-adrenoceptor antagonists on other receptors should not be exaggerated, they should be taken into consideration when the effects of these drugs are assessed.

Even if the behavioral effects of yohimbine and idazoxan are exclusively mediated via $\alpha_2$-adrenoceptors, it has been speculated that their action is due to behavioural arousal rather than to any specific enhancement of the various processes involved in learning and memory (Dickinson et al., 1989a,b; Huang et al., 1987). With post-training administration of an agent it is possible to influence only the memory consolidation phase; whereas with the pretraining administration paradigm which allows the drug to influence the acquisition and consolidation phase, it becomes involved with other non-cognitive influences including arousal and motivation. Idazoxan has been ineffective when administered post-training in learning tasks (Dickinson et al., 1989a,b).

Atipamezole is a specific and potent $\alpha_2$-adrenoceptor antagonist, which has minimal effects on other receptors. It can thus be considered as a specific tool with which to evaluate the effects of $\alpha_2$-adrenoceptor blockade in vivo (Haapalinna et al., 1997; Scheinin et al., 1988; Virtanen et al., 1989; Winter and Rabin, 1992). Atipamezole does differ from the other commonly used $\alpha_2$-adrenoceptor antagonists in brain neurochemical, in vivo electrophysiological and behavioural experiments (Haapalinna et al., 1997; Jordan et al., 1995; Winter and Rabin, 1992; Yavich et al., 1994). In the evaluation of the effects of atipamezole on cognitive functions, it has been found that atipamezole stabilizes age-associated electroencephalogram changes, improves passive avoidance retention in aged rats (Riekkinen et al., 1992) and improves intermediate-term memory retention in a radial-arm maze task (Ylinen et al., 1996). Depending on the testing conditions and the dose used, atipamezole has been reported to have no effect or to improve the performance of rats in an attentional task (Jäkälä et al., 1992; Sirviö et al., 1993). It has also been reported to impair spatial learning of rats in a Morris water maze test (Sirviö et al., 1992).

The aim of the present experiment was to elucidate further the effects of atipamezole on different learning and memory processes. Previous studies had revealed that atipamezole has some beneficial effects on attentional processes and memory recall, but impaired acquisition. Therefore we studied the effects of atipamezole on short-term memory in a three-choice maze task as well as on acquisition of long-term memory using a linear-arm maze and active avoidance tasks. The linear-arm maze task assesses the spatial relational memory of rats and is thought to be analogous to human declarative memory whereas active avoidance test assesses emotionally meaningful stimulus–response learning, a form of non-declarative memory. With respect to the different phases of long-term memory (acquisition, consolidation, and retrieval) a lighted-arm maze task was used to assess the effect of atipamezole on the consolidation phase.

## 2. Materials and methods

### 2.1. Animals

Male rats of the Sprague–Dawley strain (Bantin and Kingman, Sweden) were used in all the tests. In the linear-arm maze test, 30 approximately 7-month-old rats, weighing 466–657 g at the beginning of the study, were used. In the three-choice maze task, 40 rats and in the lighted-arm maze tests 58 rats, in the active avoidance test, 20 rats and in the conditioned avoidance response test, 15 rats, weighing 222–286 g at the beginning of the study, were used. The animals were housed in groups of five in the same cage, under standard conditions ($21 \pm 1$°C, light–dark cycle with lights between 0600 and 1800). Softwood granulated aspen was used as bedding. The animals had free access to water and food until behavioural testing began. All experimentation was approved by the local laboratory animal care committee.

### 2.2. Test substances

Atipamezole (hydrochloride, Orion, Orion Pharma, Finland) is a potent $\alpha_2$-adrenoceptor antagonist, which in receptor binding studies and functional in vitro and in vivo studies is reported to have over 200 times higher $\alpha_2$-adrenoceptor vs. $\alpha_1$-adrenoceptor selectivity than idazoxan and yohimbine (Haapalinna et al., 1997; Virtanen et al., 1989). It does not display differential affinity for $\alpha_2$-adrenoceptor subtypes (Haapalinna et al., 1997; Sjöholm et al., 1992). In studies with isolated organs or in binding studies, atipamezole had no effects on adrenergic $\beta$, histamine $H_1$, histamine $H_2$, muscarine, dopamine $D_2$, $\gamma$-aminobutyric acid,

opiate, benzodiazepine, 5-hydroxytryptamine 5-$HT_2$ (Virtanen et al., 1989) or 5-$HT_{1A}$ receptors (Winter and Rabin, 1992). Atipamezole has only low affinity for non-adrenoceptor [$^3$H]idazoxan binding sites (imidazoline $I_2$ sites), but has affinity for a pharmacologically uncharacterized non-adrenergic binding site in neonatal rat lung (Savontaus et al., 1997; Sjöholm et al., 1992). Atipamezole penetrates rapidly into the brain after its subcutaneous (s.c.) injection (Biegon et al., 1992) and its elimination half-life is approximately 2 h in rats (material on file). Atipamezole antagonizes central $\alpha_2$-adrenoceptors and potentiates the effects on novelty already at a dose of 30 $\mu$g/kg s.c. in rats (Haapalinna et al., 1997). A dose of 300 $\mu$g/kg s.c. was used in the present learning and memory experiments, since this has been reported to be an optimum dose in previous studies (Jäkälä et al., 1992; Sirviö et al., 1993; Ylinen et al., 1996). The dose is known to block central $\alpha_2$-adrenoceptors within the time schedules used and to increase central noradrenaline release (Haapalinna et al., 1997). The dose selection for the consolidation test was based on a pilot study, with a smaller number of animals, where atipamezole at doses 0.3 and 1 mg/kg s.c. did not differentiate from control, but animals that had received 0.1 mg/kg s.c. made fewer errors than control animals. Atipamezole was dissolved in distilled water, which was also used as control treatment. Haloperidol (base, Orion, Medipolar, Finland) was dissolved in distilled water with a few drops of 1 M hydrochloric acid. The route of administration was s.c. and the injection volume was 1 ml/kg in all the experiments. Commercial 45 mg pellets (Bio Serve, USA) were used as reward food in the maze tasks.

### 2.3. Apparatuses

#### 2.3.1. The linear-arm maze

The linear-arm maze has been described (including its illustration) earlier by Liljequist et al. (1997). The maze is a wooden platform in the shape of two crosses joined by a bridge. The stem (starting arm) was 90 cm long and 20 cm wide. The other five arms (goal arms) were 50 cm long and 12 cm wide. Four goal arms were situated perpendicularly to the stem and to the fifth arm which was located opposite to the stem. Edges, 2.0 cm high, surrounded all sides of the stem and the arms. At the end of each goal arm, was a hole 1 cm deep and 3 cm in diameter, which served as a food container. The starting platform (20 cm $\times$ 20 cm) was separated from the stem by a guillotine door (12 cm high and 7 cm wide). The frame was 20 cm high and 20 cm wide. The holes at the end of the goal arms were baited with three pellets of reward food. The maze was elevated 31 cm above the floor in a test room that contained other objects, as well as the test apparatus (e.g., table, shelves and door) providing extramaze cues for spatial navigation.

#### 2.3.2. The three-choice maze

The three-choice maze is a wooden platform in the shape of a cross. The stem (starting arm) is 90 cm long and 20 cm wide. The other three arms (goal arms) are 50 cm long and 12 cm wide. On all sides of the stem and the arms are edges, 2.0 cm high. At the end of each goal arm, there is a hole 1 cm deep and 3 cm in diameter, which serves as a food container. The starting platform (20 cm $\times$ 20 cm) is separated from the stem by a raisable door. The door is 12 cm high and 7 cm wide. The frame is 20 cm high and 20 cm wide. The maze was elevated 31 cm above the floor in a test room that contained other objects as well as the test apparatus.

In the lighted-arm consolidation test, the three-choice maze was situated in a dimly lit test room. One of the goal arms was lit by a fluorescent desk lamp, and only the hole in this arm was filled with reward food. The fluorescent desk lamp also served as the only source of illumination in the experimental room.

#### 2.3.3. Two-way shuttle boxes

In the active avoidance learning test, two automated two-way shuttle boxes (Ugo Basile, Italy) were used. The cage (21 cm $\times$ 49 cm $\times$ 21 cm) is divided into two sections by a partition with an opening (9 cm in diameter) at floor level. The floor consists of 40 bars which are 3 mm in diameter. The effect on conditioned responses was studied with the same apparatus.

### 2.4. Experimental protocols

#### 2.4.1. Linear-arm maze performance

In the linear-arm maze test, the habituation of the rats to handling (and administration of water), test room and reward food was started four days before training. Two days before training, the animals were placed on a food deprivation schedule that reduced their body weights to 90–95% of their initial weights. One day before training they were also habituated to the unbaited maze; five animals from the same cage at the same time for 10 min. The next day, the goal arms were baited, and the teaching trial, with one rat at a time, carried out. The rat received atipamezole (0.3 mg/kg) or distilled water and 60 min later it was placed in the starting platform. After 10 s, the door was opened and the rat was allowed to explore the maze until all the baits were found. The time to find all baits (time), re-entries made into already visited arms (errors) and correct choices made before the first error (corrects) were recorded. To evaluate behavioural activation, the total arm entries made per time (speed) was later calculated. At this first time (teaching), every rat was allowed to stay in the maze for at least 5 min. On the next day, the proper memory and learning testing began and these continued for 4 days. The rats were given a total of eight trials, two trials per day. The intertrial interval was

50 min. Atipamezole or distilled water was administered 30 min before the first trial of the day. Otherwise testing trials were identical to the teaching trial. There were 15 rats in both groups. The test solutions were kept in coded bottles, so the investigator was blind to the treatment. The sequence of the treatments was randomised in the teaching trial and maintained thereafter constant for each individual rat during the experiment.

*2.4.2. Three-choice maze performance*

In the short-term memory test, 40 rats were fasted to 85% of their initial normal weight and maintained at this weight plus 5 g per week for growth throughout the experiment. First the subjects were habituated to handling (weighing and subcutaneous injection of water), test room, reward food and the maze for one week. At the beginning, there were food pellets also on the starting platform and on the stem, but at the end of the week pellets were only in the food cups (4 pellets/cup) at the end of the arms. During training and testing, there was always a forced trial and one of the three baited arms was blocked by a piece of wood (the block). The rat was put into the starting platform and the door was opened 10 s later. The animal was allowed to eat pellets from unblocked arms and was returned to the starting platform. Then the block was removed from the maze and the door was opened (= choice trial). If the animal entered the previously blocked arm, it was allowed to eat the reward food and then returned to the home cage. If the animal entered the already visited, empty arm, it was returned to the starting platform and the door was opened 5 s later. This was repeated until the animal entered the correct unvisited arm, which still had its bait. After three trainings, the delay from the forced trial to the choice trial was lengthened to 30 s, so that the animal was 20 s in a separate waiting cage and 10 s on the starting platform. After the delay, the door was opened. A correct choice was recorded if the animal entered the previously blocked arm where it was allowed eat the food. After that, the animal was returned to the home cage. If the animal entered either of the previously unblocked and visited arms it was returned to the home cage without the reward. The blocked arm was changed randomly and the animals were trained repeatedly until every rat had made only correct choices in six consecutive trials with 30 s delay. After that, the delay was lengthened to 1 min, 5 min, 10 min and 20 min and two trials per delay per day, with a 1 h interval were performed. All of the 40 rats made totally correct choices in all trials with the delay 10 min or shorter. In the two trials with the 20 min delay, 19 rats made an incorrect choice in both trials (poor performers) and 21 rats made two correct choices (good performers). Only the poor performers were selected for further testing. The poor performers were subjected again to two 30 s delay and two 20 min delay trials three days before the drug test. All the rats made two correct choices with 30 s delay tests and none of the rats made more than one correct choice in the 20 min delay tests. The drug test was carried out with a crossover design consisting of two trials per day with a 20 min delay and a 1 h intertrial interval. In the first test day, 10 animals (group A) were injected with atipamezole (0.3 mg/kg s.c.) and nine (group B) with distilled water 20 min before the first forced trial of day. The first choice run was 40 min from the injection. The second forced trial was 80 min after the injection and the second choice run was 100 min after the injection. Twenty-four hours later the animals in group A were injected with water and the animals in group B with atipamezole (0.3 mg/kg s.c.) and the same test procedure was carried out.

*2.4.3. Evaluation of consolidation*

The animals were placed on a food deprivation schedule, two days before training, to reduce their body weights to 90% of their initial weight. During these days the rats were habituated to handling (three times/day), the test room and the reward food. On the training day, the rat was placed in the starting platform and 10 s later the door was opened and the rat was allowed to explore the maze until it found food in the lighted arm of the maze and consumed it. After that, the rat was allowed to stay in the maze for an additional 2 min. The rat was then gently taken out of the maze and received a subcutaneous injection of distilled water or test solution (atipamezole, 0.03, 0.1 or 2 mg/kg). The sequence of different treatments was randomised and the experimenter was blind to the doses. During this teaching trial, if a rat went into goal arm directly or within 2 min (i.e., it did not avoid the lighted-arm) or it did not enter the goal arm within 8 min (i.e., it avoided the lighted-arm too extensively), it was rejected. Training continued until there were 10 animals in each treatment group. The food deprivation schedule stopped at the evening of the training day and started again 2 days before the retention test. The retention test was performed 1 week after training. The retention test procedures were identical to those used in the teaching trial. The arm entries made before eating and the latency to eating, after the door was opened, were recorded. The arm choice was recorded when the animal placed all four paws on the floor of the arm. If the animal entered either the dark arm, re-entered the starting arm or entered the lit arm but did not eat, the response was recorded as an error.

To verify the variables monitored in the test, the effect of habituation and the significance of the reward food in the lighted arm on the performance of rats was studied in a separate test. In this study eight rats were handled as normal controls (reward food in the lighted-arm) and five rats, that entered the lighted-arm according to the rules, were allowed to spend a total of 6 min in the maze, which at this time did not contain the reward food. Otherwise the protocol was similar to that used in the drug test. All animals were injected with water immediately after training and were tested one week after that.

*2.4.4. Evaluation of active avoidance learning and conditioned avoidance responses*

The animals were injected with water or atipamezole (0.3 mg/kg) and 10 min later they were placed singly into the shuttle box. After a 10 min habituation period (20 min from the injection) in the apparatus, the animals were subjected to 10 avoidance trials per day for 5 days. During the first 3 s of each trial, a light signal was presented, warning the animal to avoid the shock by moving to the other compartment. If the animal did not respond within this period, the light remained on and a 0.7 mA shock (3 s duration) was applied. Moving to the other compartment during the signal, before the shock, was considered as the correct avoidance. If the animal changed compartment during the shock, the current flow was discontinued and the response was considered as an escape. If no response occurred during the shock period, the shock and the light were terminated after 3 s and this was considered as a failure. If the animal changed the compartment between trials (i.e., no light or shock present) it was considered as an inter trial crossing. There were 10 animals in both treatment groups.

The possible effect of atipamezole on conditioned avoidance responses was studied, due to the findings in the active avoidance learning test. Haloperidol was used as a positive control. Fifteen rats were trained, without any treatment, to avoid the shock with a similar protocol as above, but the test consisted of 20 trials per day. The animals were trained once a day for one week and had reached an average level of 16 correct avoidances per day. In the baseline test all the animals received an injection of water and were immediately placed individually into the shuttle box. Twenty minutes later they were subjected to 20 trials. On the next day the animals received an injection of either water, atipamezole (0.3 mg/kg s.c.) or haloperidol (0.1 mg/kg s.c.) 20 min before the 20 trials test. There were five animals in each treatment group.

*2.5. Statistical analysis*

All the results are expressed as means ± S.E.M.. In the three-choice maze test, the paired analysis was made by the nonparametric Wilcoxon signed-rank test (two-tailed). In the lighted-arm test, the group differences were analysed by the Kruskal–Wallis nonparametric one-way analysis of variance and comparison between the control and the other groups were done by the Mann–Whitney *U* test (two-tailed). In the linear-arm maze test and in the active avoidance learning test, the normality assumption was not reached, and therefore the rank transformation of the data was applied (Conover and Iman, 1981). In the case of ties among the observations, midranks were applied. After rank transformation, the two-factor analysis of variance for repeated measurements (RM ANOVA) was used to analyse the ranked data. In the linear-arm maze test, there were two separate analyses: (1) difference between teaching and the first trial (treatment group as factor and teaching time and trial 1 as repetition) and in the case of interaction further analysis within the treatment group was made by the nonparametric Wilcoxon signed-rank test (two-tailed); (2) the effect of treatments in repeated trials (trials 1–8; treatment group as factor and trial as repetition). The RM ANOVA was used in the active avoidance learning test (treatment and trials as factors). In the conditioned avoidance response test, the paired analysis (baseline-test) was made by the nonparametric Wilcoxon signed-rank test (two-tailed). The criterion for statistical significance was $P < 0.05$ in all statistical evaluations.

## 3. Results

*3.1. Effect of atipamezole on linear-arm maze performance*

The effects of atipamezole (0.3 mg/kg s.c.) on the performance of rats in the linear-arm maze are presented in Fig. 1A–D. There was a clear decrease in the number of errors from the teaching trial to trial 1 (Fig. 1A). There was no significant overall treatment effect, but a clear repetition effect and an interaction between treatment and repetition (treatment: $F(1,28) = 0.0$, $P = 1$; trial: $F(1,28) = 35.49$, $P < 0.001$; interaction: $F(1,28) = 5.54$, $P < 0.05$). The change from the teaching trial to trial 1 was not statistically significant in the control group ($P > 0.1$), but was highly statistically significant in the atipamezole-treated group ($P < 0.0001$). The RM ANOVA analysis revealed that there is only a slight difference between the groups in the number of errors also during the repeated trials (1–8) and the number of errors clearly decreased trial by trial in both groups (treatment: $F(1,28) = 2.84$, $P = 0.10$; trial: $F(7.196) = 6.18$, $P = 0.0001$; interaction: $F(7.196) = 1.15$, $P > 0.2$). There was also a clear increase in the number of correct choices from the teaching trial to trial 1 (Fig. 1B). In the RM ANOVA analysis, there was no treatment effect, but a clear repetition effect and a significant interaction between treatment and repetition (treatment: $F(1,28) = 0.27$, $P > 0.6$; trial: $F(1,28) = 27.62$, $P = 0.0001$; interaction: $F(1,28) = 9.07$, $P < 0.01$). The difference between teaching trial and trial 1 in the control group did not reach significance ($P = 0.07$), but was clearly statistically significant in the atipamezole-treated group ($P < 0.001$). Although, the number of correct choices made before the first error was slightly higher in the atipamezole-treated group than in the control group in repeated trials, the difference was not significant (treatment: $F(1,28) = 2.13$, $P > 0.1$; trial: $F(7,196) = 10.83$, $P = 0.0001$; interaction: $F(7,196) = 1.59$, $P > 0.1$). The time used to complete the task diminished from the teaching trial to trial 1 equally in both groups (treatment: $F(1,28) = 1.73$, $P > 0.1$; trial: $F(1.28) = 33.90$, $P = 0.0001$; interaction: $F(1,28) = 0.43$, $P > 0.5$). During the repeated trials, the atipamezole-treated animals used

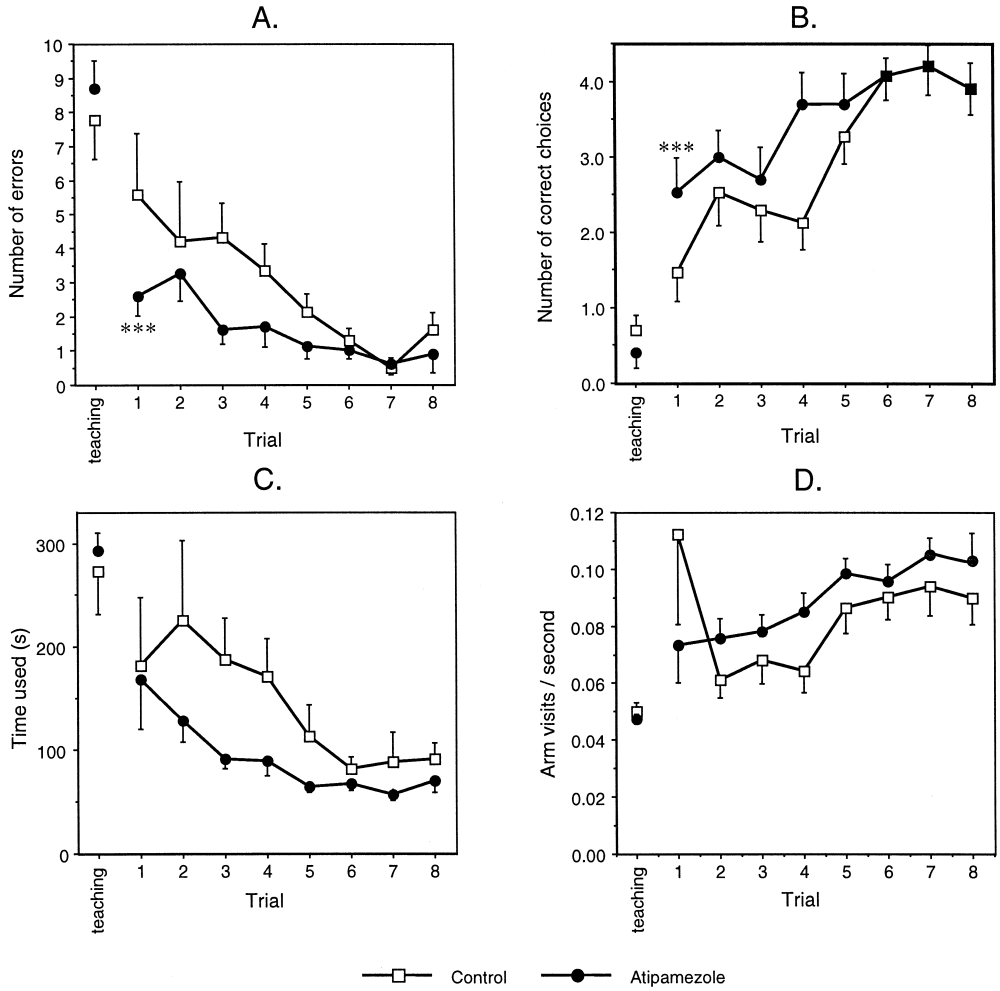


Fig. 1. The performance of control (○) and atipamezole (0.3 mg/kg s.c.) (●) treated rats in the linear-arm maze. The results are expressed as mean ± S.E.M. (A) The number of errors/trial. (B) The number of correct choices/trial. (C) The mean time used to complete the task/trial in seconds (Time). (D) The total number of arms visited/seconds in trial (Speed). The RM ANOVA revealed a significant interaction between treatment and repetition in the number of errors ($P < 0.05$) and in correct choices ($P < 0.01$) from the teaching time to the trial 1 and further analyses were made by the Wilcoxon test (* * * $P < 0.001$, two-tailed). The change in the Time and the Speed from the teaching trial to trial 1 was significant ($P < 001$) in both groups without interaction. In the repeated trials (1–8), RM ANOVA revealed a significant trial effect, but there was no significant treatment effect or interaction between treatment and trials in the performance (all variables). Both groups consisted of 15 animals.

slightly less time per trial to complete the task than the control animals. The time also decreased trial by trial in both groups, but there were no interactions between treatments and trial (treatment: $F(1,28) = 3.51$, $P < 0.1$; trial: $F(7,196) = 15.45$, $P = 0.0001$; interaction: $F(7,196) = 0.90$, $P > 0.50$). The speed in arm visits from teaching trial to trial 1 increased in both groups (Fig. 1D) with no interaction being present (treatment: $F(1,28) = 0.92$, $P > 0.3$; trial: $F(1.28) = 3.97$, $P < 0.01$; interaction: $F(1,28) = 0.10$, $P > 0.7$). The increase in speed from teaching trial to trial 1 was slightly greater in the control group than in the atipamezole group (Fig. 1D). The analysis of the speed in the repeated trials by RM ANOVA revealed no overall treatment effect, a significant repetition effect and no

Table 1
213The effect of atipamezole (0.3 mg/kg s.c.) on the three-choice maze achievement with 20- min delay in the poorly performing rats

| Treatment | The number of animals that made: | | | Correct choices (mean ± S.E.M.) |
|---|---|---|---|---|
| | Two correct choices | One correct choice | No correct choice | |
| Control | 4 | 7 | 8 | 0.79 ± 0.18 |
| Atipamezole | 11 | 4 | 4 | 1.37 ± 0.19[a] |

The rats ($n = 19$) were tested crossover in two parts (10 and 9 in group).
[a] $P < 0.05$, Wilcoxon test (two-tailed).

interaction between treatment and repetition (treatment: $F(1,28) = 2.50$, $P > 0.1$; trial: $F(7,196) = 7.38$, $P = 0.0001$; interaction: $F(7,196) = 1.13$, $P > 0.3$).

### 3.2. Effect of atipamezole on short-term memory

Atipamezole improved the choice accuracy of the poorly performing rats in the three-choice maze with 20-min delay (Table 1). In the first testing day, one of the nine control animals and five rats from the ten atipamezole-treated animals performed perfectly, i.e., made two correct choices. In the second testing day, six of the nine atipamezole-treated and two of the ten control animals performed perfectly. The difference between treatments in the total number of correct choices was statistically significant ($P < 0.05$).

### 3.3. Effect on consolidation

In the lighted-arm test, five rats were rejected during the teaching trial. One rat entered the lighted-arm directly and four did not enter it within 8 min. In the teaching trial (before any treatment), the mean numbers of entries before eating were 14.0, 14.9, 15.6 and 12.5 in the control, atipamezole 0.03, 0.1 and 2 mg/kg groups, respectively. The corresponding mean latencies before eating in the lighted-arm were 289.9 s, 305.4 s, 326.8 s and 288.0 s. Analysis of variance revealed no differences between the groups in the teaching trial (errors: $H = 2.32$; $P > 0.5$, latency: $H = 2.75$; $P > 0.4$). One week later, the groups that had received post-training injection of low doses of atipamezole made fewer errors and had shorter latencies than the control group (Fig. 2). In the testing trial, analysis of variance also revealed a significant difference between

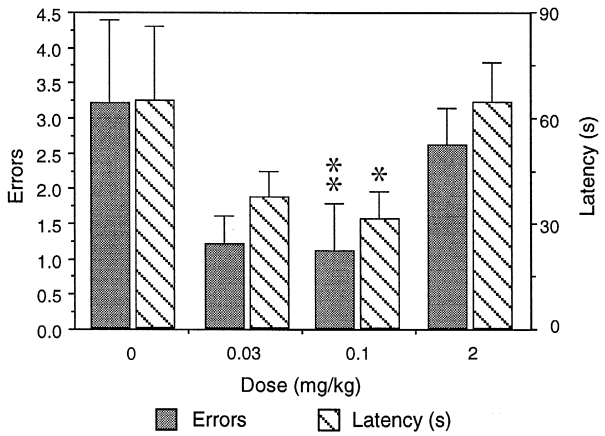


Fig. 2. The effect of posttrial administered atipamezole on memory storage in rats. The retention test was performed 1 week after training. The results are expressed as means ± S.E.M. of the groups. There were 10 animals in each group. The results were analysed by the Kruskal–Wallis nonparametric analysis of variance followed by the Mann–Whitney *U*-test (two-tailed). The left ordinate axis: the number of errors (stippled columns). The right ordinate axis: the latencies (hatched columns). $*\ P < 0.05$ and $**\ P < 0.01$, when compared with the control.

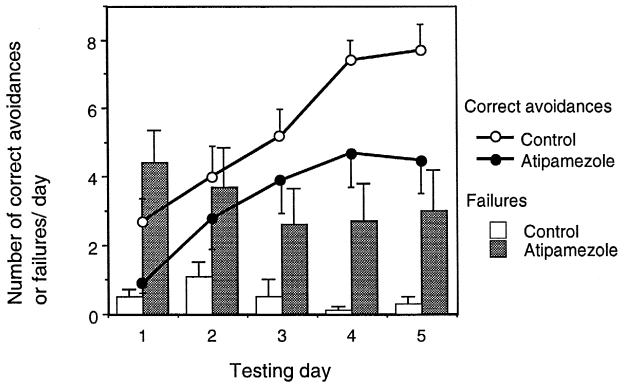


Fig. 3. The effect of atipamezole on active avoidance learning in a shuttle box. There were 10 animals/group and each day consisted of 10 trials. The number of correct avoidances and fails per day are expressed as mean ± S.E.M. The failures indicate the number of shocks not avoided during the conditioned stimulus or escaped during the shock. The effects of treatment and training on performance were analysed by RM ANOVA. Correct avoidances: control (○) and atipamezole (●) (treatment: $P < 0.05$; day: $P = 0.0001$; interaction: $P = 1$)]. Fails: control (open columns) and atipamezole (stippled columns) [treatment: $P < 0.001$; day: $P < 0.001$; interaction: $P < 0.1$).

the groups (errors: $H = 9.07$; $P < 0.05$, latency: $H = 8.46$; $P < 0.05$). At the dose of 0.1 mg/kg, the difference from the control group was statistically significant. The control group made $3.2 \pm 1.1$ errors and the latency was $64.6 \pm 21.0$ s, the group receiving 0.03 mg/kg made $1.2 \pm 0.4$ errors ($P = 0.1$) and used time $37.2 \pm 7.2$ ($P = 0.1$), at 0.1 mg/kg they made $1.1 \pm 0.7$ errors ($P < 0{,}01$) and latency was $31.2 \pm 7.4$ s ($P < 0{,}05$). In the 2 mg/kg group, the number of errors was $2.6 \pm 0.5$ and the latency was $64.5 \pm 10.2$. In the test that evaluated the effect of habituation, the animals that were given a reward in the lighted-arm made an average of 13.4 entries into dark arms before entering the lighted-arm and the average latency was 356 s in the teaching trial. The corresponding values for the rats that visited the unbaited maze were 15.6 and they were allowed to stay on the maze at least for 360 s. One week after training, the rats that had obtained a reward in the maze made slightly fewer errors and had clearly shorter latency than the rats that had visited the unbaited maze (errors: $3.4 \pm 1.3$ and $6.4 \pm 1.5$, $P = 0.10$; latency: $55.9 \pm 14.2$ and $131.4 \pm 33.0$ s, $P < 0.05$, respectively).

### 3.4. Effect on active avoidance learning and conditioned avoidance responses

The atipamezole (0.3 mg/kg s.c.)-treated animals made clearly less correct avoidances than control animals in the active avoidance learning task (Fig. 3) (treatment: $F(1,18) = 6.36$, $P < 0.05$; day: $F(4,72) = 16.98$, $P = 0.0001$; interaction: $F(4,72) = 0.98$, $P = 1$). There was no treatment effect on escapes. The number of escapes decreased from day 1 to day 5 in both groups (treatment: $F(1,18) = 1.30$, $P > 0.2$; trial: $F(4,72) = 9.94$, $P = 0.0001$; interaction:

Table 2
The effects of atipamezole and haloperidol on conditioned avoidance responses in fully trained rats

| Treatment | Correct avoidances | | Escapes | | Intertrial crossings | |
|---|---|---|---|---|---|---|
| | Baseline | Test | Baseline | Test | Baseline | Test |
| Control | 16.3 ± 3.1 | 17.8 ± 0.9 | 2.0 ± 1.4 | 1.8 ± 0.6 | 6.5 ± 4.6 | 4.5 ± 3.2 |
| Atipamezole[a] | 16.8 ± 1.7 | 17.4 ± 1.4 | 3.0 ± 1.5 | 2.2 ± 2.2 | 8.2 ± 7.0 | 6.6 ± 4.5 |
| Haloperidol[b] | 16.8 ± 1.2 | 1.8 ± 1.1[c] | 2.8 ± 1.2 | 6.6 ± 1.6[c] | 1.4 ± 0.4 | 2.0 ± 0.4 |

[a] 0.3 mg/kg s.c.
[b] 0.1 mg/kg s.c.
Values are mean ± S.E.M., $n = 5$ per group.
[c] = $P < 0.05$, Wilcoxon signed-rank test (two-tailed).

$F(4,72) = 1.11$, $P > 0.3$). The atipamezole-treated animals failed clearly more frequently to avoid or escape the shock than control animals (treatment: $F(1,18) = 8.48$, $P < 0.01$; trial: $F(4,72) = 5.74$, $P < 0.001$; interaction: $F(4,72) = 2.06$, $P < 0.1$) (Fig. 3). Atipamezole did not have any effect on spontaneous motor activity during the test and there was no difference between the atipamezole-treated and the control animals in intertrial crossings (treatment: $F(1,18) = 0.12$, $P > 0.7$; trial: $F(4,72) = 0.86$, $P > 0.5$; interaction: $F(4,72) = 0.73$, $P > 0.5$). The average number of intertrial crossings in control and atipamezole group during the whole test was 6.4 and 7.1, respectively.

Atipamezole did not have any effect on conditioned avoidance responses in the fully trained animals. Haloperidol clearly disturbed conditioned avoidance responses and there was a significant decrease in avoidances and increase in shock-induced escapes with no effect on intertrial crossings (Table 2).

## 4. Discussion

The present study elucidated the effects of the specific $\alpha_2$-adrenoceptor antagonist, atipamezole, as a stimulator of noradrenaline release, on cognitive functions. The particular objective of this study was to investigate the effects of atipamezole on (acquisition) relational learning and memory, conditioned stimulus learning and on consolidation processes involved in relational memory.

In the present study, atipamezole improved performance of adult rats trained on a linear-arm maze test. The clearest difference in performance was obtained between the teaching trial and the first proper test trial. In the teaching trial, the animals were in the maze for the first time with reward food at the end of the arms and there were no differences in what was mostly spontaneous behaviour between the groups. In the first trial, since the animals had experienced the maze, their behaviour was more goal-oriented than in the teaching trial. Although there was a decrease in the number of errors and an increase in the correct choices from the teaching trial to trial 1 in both groups, the differences were clearly greater in the atipamezole-treated group. It is worth noticing that the time used to complete the task, from the teaching trial to trial 1, decreased similarly in both groups. Furthermore, the speed in arm visits increased slightly more in the control group. Thus it is unlikely that the better performance of atipamezole-treated animals is simply due to nonspecific behavioural arousal. Locus coeruleus neurones respond to novelty or change in incoming information and idazoxan treatment has been shown to enhance such a response to novelty (Sara et al., 1994). In the present study, in contrast to the habituation procedure, the external cues outside the maze obtained a relevant role in the teaching trial, when there was reward food for the first time in the maze. An intriguing interpretation of the remarkable improvement in the performance between the teaching trial and the first proper test trial in the atipamezole-treated animals is that the drug selectively enhances the response to the change in the significance of information leading to a new behavioural strategy.

In the repeated trials, atipamezole-treated animals maintained their good performance level in the number of errors and correct choices and the control animals only reached the same level of performance in the final trials. Undoubtedly, learning took place during testing in both groups. Thus, it is conceivable that the effect of atipamezole is partially also due to improved learning and the atipamezole-treated animals achieved the maximum level earlier than the control animals. Similarly, it has been reported that idazoxan-treated rats needed fewer trials than control animals to reach a criterion of good performance in a linear maze test (Devauges and Sara, 1990).

The three-choice maze was developed from a traditional T-maze by adding one arm into it. This was done because we found in our previous pilot studies that adult rats performed too well in the T-maze, even after a 30-min delay. The traditional T-maze is possibly too simple and also the chance level is 50%. In the present study, when the animals were fully trained to the test with short delays, about half of the group showed performance deficits when the delay was prolonged to 20 min. Atipamezole improved the achievement of these poorly performing animals in the maze. Previously, atipamezole had similar effects in a five serial reaction time test where in a subpopulation of rats with poor choice accuracy, seven of eight rats slightly

improved their performance after atipamezole treatment (Jäkälä et al., 1992). Release of noradrenaline is thought to have a role in distractibility and a lesion of dorsal noradrenergic bundle was reported not to affect performance in the five serial reaction time tests in normal conditions, but when the animals were distracted with a white noise, the choice accuracy of lesioned animals became impaired (Cole and Robbins, 1992). Interestingly, in a recent study, with a three-choice visual discrimination apparatus, idazoxan did not have any effect on the performance of rats in a normal vigilance task. However, when irrelevant odour cues were presented during part of the trials, a subpopulation of the animals were clearly distracted and idazoxan (1 mg/kg s.c.) improved the performance of those animals (Bunsey and Strupp, 1995). In the present study, only half of the animals made incorrect choices after the 20 min delay. The reason for the delay dependent poor performance in this subpopulation is unknown. Although it was not intended to disturb the animals in the test, the very act of handling of the animals during the delay could be disruptive. Thus, also in this study the poorly performing animals could be more easily distracted than the well-performing animals and stimulation of noradrenergic system by atipamezole could reduce this phenomenon.

The possible effect on consolidation is easier to interpret with an uncomplicated test than it is with a more complex maze that measures also nonlearned functions such as working memory. The lighted-arm test is a modified version of the one-trial appetitive Y-maze discrimination task used by Sternberg et al. (1985a) in consolidation tests. In the lighted-arm test, habituation already affected behaviour, thus the animals that had stayed previously in the maze had shorter latency and made fewer errors 1 week later in their second visit. The number of errors and the latency were clearly further decreased if the animals had also received a food reward in the maze. Thus, it is clear that the rats normally shunned the lighted-arm, but also learned to associate it with food. Consequently, it was possible to measure a level of learning after a single trial in the test. In the present study, atipamezole when injected immediately after training, was able to improve memory retrieval one week later in the retention test. It is also important to note that there were no drug administrations before either training or testing trials. This indicates that atipamezole facilitates memory storage processes rather than attentional or motivational mechanisms. This is especially interesting since idazoxan is reported to be effective in a passive avoidance task (Dickinson et al., 1989a) or in a radial arm maze task (Dickinson et al., 1989b) only when injected before training, but not when given immediately after training and idazoxan has been proposed to exert its effects on arousal, attention and/or perception rather than learning and consolidation per se (Dickinson et al., 1989a,b). On the other hand, appropriate stimulation of the noradrenergic system has been established to improve consolidation (McGaugh, 1989). Furthermore, several types of compounds that have effects on the adrenergic system are reported to have effects on the memory consolidation process (Gold and Buskirk, 1978b; Introini-Collison et al., 1992, 1996; Introini-Collison and McGaugh, 1989; Sternberg et al., 1985a,b; Strupp et al., 1991). A U-shaped dose–response curve is typically obtained in posttrial studies (McGaugh, 1989) and that was seen also in the present study, the dose of 0.1 mg/kg s.c. of atipamezole being the most effective. Although the atipamezole dose of 2 mg/kg was inferior in this test, it did not cause any impairment in memory retrieval as previously reported with a peripheral injection of noradrenaline at a high dose (Gold and Buskirk, 1978a).

The timing of the injection is important and drug treatment usually affects consolidation only immediately after training and not necessarily any longer if drug administration is delayed for example, by 15 min (Benloucif et al., 1990; Gold et al., 1982; Strupp et al., 1991). That, together with the conventional U-shaped dose–response curve, suggests that the negative results with idazoxan on consolidation should be viewed with caution, because the studies were carried out only in a narrow dose range (Dickinson et al., 1989a,b). It is also quite plausible that atipamezole, as an $\alpha_2$-adrenoceptor antagonist, has an influence on memory consolidation process due to enhanced release of noradrenaline, which then interacts with other central neurotransmitter systems.

In contrast to the other results, atipamezole evidently impaired the performance in the two-way active avoidance learning test. However, neurones in the locus coeruleus are reported to respond in a characteristic manner to a conditioned stimulus that predicts a shock (Jacobs et al., 1991). Furthermore, lesion of the central noradrenergic system has been reported to impair both passive (Crow and Wendlandt, 1976) and active (Bennet et al., 1990) avoidance learning, indicating that an intact noradrenergic system is important in learning to avoid an aversive stimulus. Thus the stimulation of noradrenergic system by atipamezole would be anticipated to cause an improvement in avoidance learning. The most notable effect was that the number of failures was significantly higher in the atipamezole-treated group than in the control group, i.e., atipamezole-treated animals did not even escape the shock. Usually, such an impairment in active avoidance learning and performance is seen after treatment with neuroleptics, which impair the ability to initiate a response, causing failure to avoid the shock, but not necessarily affecting the ability to escape the shock (Sanger, 1986). In agreement with previous studies, haloperidol in the present study caused a failure to avoid the shocks and there was a compensatory increase in the number of shock-induced escapes. It should be pointed out, that atipamezole did not disturb the avoidance performance of the fully trained rats. Thus, it is unlikely that the impairment in avoidance learning by atipamezole is caused by a direct disturbance of motor processes.

The test situation (for example fear of electric shock or forced swimming) itself may be so stressful that it could interfere with the performance of animals in the test. It has been reported that stress-sensitive rat strains exhibited floating behaviour in a water T-maze or in a Morris water maze, without motivation to solve the task, but low stress responders quickly mastered the task (Grauer and Kapon, 1993; Vogel and Harris, 1991). Repeated uncontrollable stress has been reported to impair learning (Luine et al., 1994) and the relationship between stress and central noradrenaline is well-known (Glavin, 1985). The increase in the number of failures in the atipamezole-treated group, observed in the present study, resembles the behavioural depression state produced by uncontrollable stress which is clearly associated with a major increase of central noradrenaline release (Weiss et al., 1981). The most obvious explanation for the present findings is that they are due to the combination of the stimulation of the noradrenergic system by atipamezole and the slightly stressful nature of the test. It is possible that this could account for the previous negative results obtained with atipamezole in a Morris water maze test, where some of the atipamezole-treated animals exhibited floating behaviour (Sirviö et al., 1992).

According to the theory of the role of noradrenergic system in cognitive functions (Aston-Jones et al., 1991; Harley, 1987; Robbins et al., 1985; Sara et al., 1994), a specific $\alpha_2$-adrenoceptor antagonist, like atipamezole, by stimulating endogenous noradrenaline release, should increase alertness, improve selective attention, decrease distractibility, improve learning and consolidation. However, it has been proposed that the learning improvement noted after yohimbine treatment is caused mainly through behavioural arousal, because the improvement was seen only at doses that stimulated also nonconditioned behaviour (Huang et al., 1987). This is probably explained by effects other than $\alpha_2$-adrenoceptor antagonism (Lal et al., 1983; Van Oene et al., 1984; Winter and Rabin, 1992). For example, yohimbine (1 and 3 mg/kg s.c.), but not atipamezole (0.1–10 mg/kg s.c.), has been found to stimulate spontaneous motor activity, reflecting presumably the extensive stimulation of dopamine release by yohimbine, an effect which is only marginal with atipamezole (Haapalinna et al., 1997). Although, in the linear-arm maze test, atipamezole slightly shortened also the time to solve the task, it did not have any significant effect on speed in arm visits. This also suggests that the decrease in time to solve the task is mainly due to the better choice accuracy and not to stimulation of behaviour. Furthermore, the present results with atipamezole are in general agreement with previous studies with idazoxan (Bunsey and Strupp, 1995; Devauges and Sara, 1990; Dickinson et al., 1989a,b; Sara and Devauges, 1989; Sara et al., 1994). However, atipamezole and idazoxan also block postsynaptic $\alpha_2$-adrenoceptors that are thought to participate in the improvement of working memory seen with $\alpha_2$-adrenoceptor agonists (Arnsten and Cai, 1993). Nevertheless, this has been described only in old animals with impaired working memory. The present studies were performed with adult animals, thus providing information about the effects of this $\alpha_2$-adrenoceptor antagonist on cognitive functions in the normally functioning rat brain. Moreover, at low doses, $\alpha_2$-adrenoceptor antagonists are considered to act predominantly at presynaptical receptors and increase noradrenaline release (Arnsten and Cai, 1993).

In conclusion, the present results with atipamezole, when supplemented with earlier results (Jäkälä et al., 1992; Sirviö et al., 1993; Ylinen et al., 1996), revealed that atipamezole improved performance of adult rats in maze tests measuring relational learning and short-term memory with a possible effect on attention, distractibility, working memory and information processing speed. Furthermore, atipamezole improved learning also when administered after training, indicating an effect on storage of representational memory in addition to the above mechanisms. Even though the present effects on cognitive functions are thought to be mediated via the stimulation of central noradrenaline release, there was no evidence that the effects were due to nonspecific behavioural arousal. The effect on the active avoidance learning test was somewhat surprisingly at odds with the findings in the maze tests, and atipamezole clearly impaired the acquisition of this test. This is proposed to be due to an interaction of the evaluation in noradrenaline release induced by atipamezole and the stressful nature of the test, interfering with the performance of rats.

## Acknowledgements

Dr. E. MacDonald is acknowledged for revising the language of the manuscript, Dr. P. Hakulinen is acknowledged for professional advice with statistical analyses and Ms. K. Svärd is acknowledged for preparing the mazes used in the tests.

## References

Anlezark, G.M., Crow, T.J., Greenway, A.P., 1973. Impaired learning and decreased cortical norepinephrine after bilateral locus coeruleus lesions. Science 181, 682–684.

Arnsten, A.F.T., Cai, J.X., 1993. Postsynaptic alpha-2 receptor stimulation improves memory in aged monkeys: indirect effects of yohimbine versus direct effects of clonidine. Neurobiol. Aging 14, 597–603.

Aston-Jones, G., Chiang, C., Alexinsky, T., 1991, Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. In: Barnes, C.D., Pompeiano, O. (Eds.), Neurobiology of the Locus Coeruleus, Vol. 88. Elsevier, Amsterdam, pp. 501–520.

Benloucif, S., Mortimer, R.B., Bennett, E.L., Rosenzweig, M.R., 1990. The timing of an injection procedure affects pharmacological actions on memory. Pharmacol. Biochem. Behav. 37, 295–298.

Bennet, M.C., Kaleta-Michaels, S., Arnold, M., McGaugh, J.L., 1990. Impairment of active avoidance by the noradrenergic neurotoxin,

DSP4: attenuation by post-training epinephrine. Psychopharmacology 101, 505–510.

Berridge, C.W., Arnsten, A.F.T., Foote, S.L., 1993. Noradrenergic modulation of cognitive function—clinical implications of anatomical, electrophysiological and behavioural studies in animal models. Psychol. Med. 23, 557–564.

Biegon, A., Mathis, C.A., Budinger, T.F., 1992. Quantitative in vitro and ex vivo autoradiography of the $\alpha$2-adrenoceptor antagonist [$^3$H]atipamezole. Eur. J. Pharmacol. 224, 27–38.

Bliss, T.V.P., Goddard, G.V., Riives, M., 1983. Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. J. Physiol. 334, 475–491.

Bunsey, M.D., Strupp, B.J., 1995. Specific effects of idazoxan in a distraction task: evidence that endogenous norepinephrine plays a role in selective attention in rats. Behav. Neurosci. 109, 903–911.

Carli, M., Tranchina, S., Samanin, R., 1992. 8-Hydroxy-2-(di-*n*-propylamino)tetralin, a 5-HT$_{1A}$ receptor agonist, impairs performance in a passive avoidance task. Eur. J. Pharmacol. 211, 227–234.

Cedarbaum, J.M., Aghajanian, G.K., 1977. Catecholamine receptors on locus coeruleus neurons: pharmacological characterization. Eur. J. Pharmacol. 44, 375–385.

Cole, B.J., Robbins, T.W., 1992. Forebrain norepinephrine: role in controlled information processing in the rat. Neuropsychopharmacology 7, 129–142.

Connor, D.J., Dietz, S., Langlais, P.J., Thal, L.J., 1992. Behavioral effects of concurrent lesions of the dorsal noradrenergic bundle. Exp. Neurol. 116, 69–75.

Conover, W., Iman, R., 1981. Rank transformations as a bridge between parametric and nonparametric statistics. Am. Stat. 35, 124–128.

Crow, T.J., Wendlandt, S., 1976. Impaired acquisition of a passive avoidance response after lesions induced in the locus coeruleus by 6-OH-dopamine. Nature 259, 42–44.

Dahl, D., Winson, J., 1985. Action of norepinephrine in the dentate gyrus: I. Stimulation of locus coeruleus. Exp. Brain Res. 59, 491–496.

Devauges, V., Sara, S.J., 1990. Activation of the noradrenergic system facilitates an attentional shift in the rat. Behav. Brain Res. 39, 19–28.

Dickinson, S.L., Gadie, B., Tulloch, I.F., 1989a. Effect of idazoxan on passive avoidance behaviour in adult and in aged rats. Br. J. Pharmacol. 96, 14.

Dickinson, S.L., Gadie, B., Woodmansey, N.R., 1989b. Effect of repeated idazoxan treatment on radial arm maze performance in adult and in aged rats. Br. J. Pharmacol. 97, 384.

Freedman, J.E., Aghajanian, G.K., 1984. Idazoxan (RX 781094) selectively antagonizes $\alpha$2-adrenoceptors on rat central neurons. Eur. J. Pharmacol. 105, 265–272.

Glavin, G.B., 1985. Stress and brain noradrenaline: a review. Neurosci. Biobehav. Res. 9, 233–243.

Gold, P.E., Buskirk, R.V., 1978a. Posttraining brain norepinephrine concentrations: correlation with retention performance of avoidance training and with peripheral epinephrine modulation of memory processing. Behav. Biol. 23, 509–520.

Gold, P.E., Buskirk, R.V., 1978b. Effects of $\alpha$- and $\beta$-adrenergic receptor antagonists on post-trial epinephrine modulation of memory: relationship to post-training brain norepinephrine concentrations. Behav. Biol. 24, 168–184.

Gold, P.E., McCarty, R., Sternberg, D.B., 1982. Peripheral catecholamines and memory modulation. In: Marsan, C.A., Matties, H. (Eds.), Neuronal Plasticity and Memory Formation. Raven Press, New York, pp. 327–338.

Grauer, E., Kapon, Y., 1993. Wistar–Kyoto rats in the Morris water maze —impaired working memory and hyper-reactivity to stress. Behav. Brain Res. 59, 147–151.

Haapalinna, A., Viitamaa, T., MacDonald, E., Savola, J.-M., Tuomisto, L., Virtanen, R., Heinonen, E., 1997. Evaluation of the effects of a specific $\alpha_2$-adrenoceptor antagonist, atipamezole, on $\alpha_1$- and $\alpha_2$-adrenoceptor subtype binding, brain neurochemistry and behaviour in comparison with yohimbine. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 570–582.

Harley, C.W., 1987. A role for norepinephrine in arousal, emotion and learning? Limbic modulation by norepinephrine and the Kety hypothesis. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 11, 419–458.

Huang, M., Messing, R.B., Sparber, S.B., 1987. Learning enhancement and behavioral arousal induced by yohimbine. Life Sci. 41, 1083–1088.

Introini-Collison, I.B., McGaugh, J.L., 1989. Cocaine enhances memory in mice. Psychopharmacology 99, 537–541.

Introini-Collison, I.B., Saghafi, D., Novack, G.D., McGaugh, J.L., 1992. Memory-enhancing effects of post-training dipivefrin and epinephrine: involvement of peripheral and central adrenergic receptors. Brain Res. 572, 81–86.

Introini-Collison, I.B., Dalmaz, C., McGaugh, J.L., 1996. Amygdala, $\beta$-noradrenergic influences on memory storage involve cholinergic activation. Neurobiol. Learn. Mem. 65, 57–64.

Jacobs, B.L., Abercrombie, E.D., Fornal, C.A., Levine, E.S., Morilak, D.A., Stafford, I.L., 1991. Single-unit and physiological analyses of brain norepinephrine function in behaving animals. In: Barnes, C.D. (Ed.), Neurobiology of the Locus Coeruleus, Vol. 88. Elsevier, Amsterdam, pp. 159–165.

Jäkälä, P., Sirviö, J., Riekkinen, P.J., Haapalinna, A., Riekkinen, P., 1992. Effects of atipamezole, an $\alpha_2$-adrenoceptor antagonist, on the performance of rats in a five-choice serial reaction time task. Pharmacol. Biochem. Behav. 42, 903–907.

Jordan, S., Jackson, H.C., Nutt, D.J., Handley, S.L., 1995. Discriminative stimulus properties of ethoxy idazoxan. J. Psychopharmacol. 9, 223–228.

Lal, H., Shearman, G., Bennet, D., Hovart, A., 1983. Yohimbine: a beta carboline with behavioral and neurochemical properties common to anxiogenic drugs. Soc. Neurosci. Abstr. 9, 437.

Liljequist, R., Haapalinna, A., Åhlander, M., Ying, H.L., Männistö, P.T., 1997. Catechol-*O*-methyltransferase inhibitor tolcapone has minor influence on performance in experimental memory models in rats. Behav. Brain Res. 82, 195–202.

Llado, J., Esteban, S., Garcia-Sevilla, J., 1996. The $\alpha_2$-adrenoceptor antagonist idazoxan is an agonist at 5-HT$_{1A}$ autoreceptors modulating serotonin synthesis in the rat brain in vivo. Neurosci. Lett. 218, 111–114.

Luine, V., Villegas, M., Martinez, C., McEwen, B.S., 1994. Repeated stress causes reversible impairments of spatial memory performance. Brain Res. 639, 167–170.

McGaugh, J.L., 1989. Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. Annu. Rev. Neurosci. 12, 255–287.

McGaugh, J.L., Introini-Collison, I.B., Nagahara, A.H., Cahill, H., Broini, J.D., Castellano, C., 1990. Involvement of the amygdaloid complex in neuromodulatory influences on memory storage. Neurosci. Biobehav. Rev. 14, 425–431.

Meana, J.J., Callado, L.F., Pazos, A., Grijalba, B., Garciá-Sevilla, J.A., 1996. The subtype-selective $\alpha_2$-adrenoceptor antagonists BRL 44408 and ARC 239 also recognize 5-HT$_{1A}$ receptors in the rat brain. Eur. J. Pharmacol. 312, 358–388.

Mendelson, S.D., Quartermain, D., Francisco, T., Shemer, A., 1993. 5-HT$_{1A}$ receptor agonists induce anterograde amnesia in mice through a postsynaptic mechanism. Eur. J. Pharmacol. 236, 177–178.

Miralles, A., Olmos, G., Sastre, M., Barturen, F., Martin, I., Garcia-Sevilla, J.A., 1993. Discrimination and pharmacological characterization of I$_2$-imidazoline sites with [$^3$H]idazoxan and alpha-2 adrenoceptors with [$^3$H]RX821002 (2-methoxy idazoxan) in the human and rat brains. J. Pharmacol. Exp. Ther. 264, 1187–1197.

Pettibone, D.J., Pfleuger, A.B., Totaro, J.A., 1985. Comparison of the effects of recently developed $\alpha$2-adrenergic antagonists with yohimbine and rauwolscine on monoamine synthesis in rat brain. Biochem. Pharmacol. 34, 1093–1097.

Riekkinen, P.J., Sirviö, J., Riekkinen, M., Lammintausta, R., Riekkinen, P., 1992. Atipamezole, an $\alpha_2$-antagonist, stabilizes age-related high-voltage spindle and passive avoidance defects. Pharmacol. Biochem. Behav. 41, 611–614.

Robbins, T.W., Everitt, B.J., Cole, B.J., Archer, T., Mohammed, A., 1985. Functional hypotheses of the coeruleocortical noradrenergic projection: a review of recent experimentation and theory. Physiol. Psychol. 13, 127–150.

Sanger, D.J., 1986. Response decrement patterns after neuroleptic and non-neuroleptic drugs. Psychopharmacology 89, 98–104.

Sanger, D.J., Schoemaker, H., 1992. Discriminative stimulus properties of 8-OH-DPAT: relationship to affinity for 5-$HT_{1A}$ receptors. Psychopharmacology 108, 85–92.

Sara, S.J., Devauges, V., 1988. Priming stimulation of locus coeruleus facilitates memory retrieval in the rat. Brain Res. 438, 299–303.

Sara, S.J., Devauges, V., 1989. Idazoxan, an $\alpha$-2 antagonist, facilitates memory retrieval in the rat. Behav. Neural Biol. 51, 401–411.

Sara, S.J., Vankov, A., Hervé, A., 1994. Locus coeruleus-evoked responses in behaving rats: a clue to the role of noradrenaline in memory. Brain Res. Bull. 35, 457–465.

Savontaus, S., Raasmaja, A., Rouru, J., Koulu, M., Pesonen, U., Virtanen, R., Savola, J.-M., Huupponen, R., 1997. Anti-obesity effect of MPV-1743 A III, a novel imidazoline derivative, in genetic obesity. Eur. J. Pharmacol. 328, 207–215.

Scheinin, H., MacDonald, E., Scheinin, M., 1988. Behavioural and neurochemical effects of atipamezole, a novel $\alpha$2-adrenoceptor antagonist. Eur. J. Pharmacol. 157, 243–244.

Selden, N.R.W., Robbins, T.W.B., Everitt, J., 1990. Enhanced behavioral conditioning to context and impaired behavioral and neuroendocrine responses to conditioned stimuli following ceruleocortical noradrenergic lesion: support for an attentional hypothesis of central noradrenergic function. J. Neurosci. 10, 531–539.

Simson, P.E., Weiss, J.M., 1988. Responsiveness of locus ceruleus neurons to excitatory stimulation is uniquely regulated by alpha2 receptors. Psychopharmacol. Bull. 24, 349–354.

Sirviö, J., Riekkinen Jr., P., MacDonald, E., Airaksinen, M., Lammintausta, R., Riekkinen, P.J., 1992. The effects of alpha-2 adrenoceptor antagonist, atipamezole, on spatial learning in scopolamine-treated and aged rats. J. Neural Transm. 4, 99–106.

Sirviö, J., Jäkälä, P., Mazurkiewicz, M., Haapalinna, A., Riekkinen Jr., P., Riekkinen, P.J., 1993. Dose- and parameter-dependent effects of atipamezole, an $\alpha_2$-antagonist, on the performance of rats in a five-choice serial reaction time task. Pharmacol. Biochem. Behav. 45, 123–129.

Sjöholm, B., Voutilainen, R., Luoma, K., Savola, J.-M., Scheinin, M., 1992. Characterization of [$^3$H]atipamezole as a radioligand for $\alpha_2$-adrenoceptors. Eur. J. Pharmacol. 215, 109–117.

Sternberg, D.B., Isaacs, K.R., Gold, P.E., McGaugh, J.L., 1985a. Epinephrine facilitation of appetitive learning: attenuation with adrenergic receptor antagonists. Behav. Neural Biol. 44, 447–453.

Sternberg, D.B., Martinez, J., Joe, L., Gold, P.E., McGaugh, J.L., 1985b. Age-related memory deficits in rats and mice: enhancement with peripheral injections of epinephrine. Behav. Neural Biol. 44, 213–220.

Strupp, B.J., Bunsey, M., Levitsky, D., Kesler, M., 1991. Time-dependent effects of post-trial amphetamine treatment in rats: evidence for enhanced storage of representational memory. Behav. Neural Biol. 56, 62–76.

Valjakka, A., Riekkinen Jr., P., Sirviö, J., Nieminen, S., Airaksinen, M., Miettinen, R., Riekkinen, P., 1990. The effects of dorsal noradrenergic bundle lesions on spatial learning, locomotor activity, and reaction to novelty. Behav. Neural Biol. 54, 323–329.

Van Oene, J.C., de Vries, J.B., Horn, A.S., 1984. The effectiveness of yohimbine in blocking central dopamine autoreceptors in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. 327, 301–304.

Virtanen, R., Savola, J.-M., Saano, V., 1989. Highly selective and specific antagonism of central and peripheral $\alpha$2-adrenoceptors by atipamezole. Arch. Int. Pharmacodyn. Ther. 297, 190–204.

Vogel, W.H., Harris, N., 1991. Learning and memory of a water T-Maze by rats selectively bred for low or high plasma catecholamine stress responses. Behav. Neural Biol. 56, 113–117.

Weiss, J.M., Goodman, P.A., Losito, B.G., Corrigan, S., Charry, J.M., Bailey, W.H., 1981. Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. Brain Res. Rev. 3, 167–205.

Winter, J.C., Rabin, R.A., 1992. Yohimbine as a serotonergic agent: evidence from receptor binding and drug discrimination. J. Pharmacol. Exp. Ther. 263, 682–689.

Yavich, L., Sirviö, J., Haapalinna, A., Riekkinen, P.S., 1994. Some unusual effects of $\alpha_2$-adrenergic drugs on cortical high voltage spindles in rats. Eur. Neuropsychopharmacol. 4, 535–538.

Ylinen, A., Pitkänen, M., Pussinen, R., Haapalinna, A., Koivisto, E., Lappalainen, R., Sirviö, J., Riekkinen, P.S., 1996. A selective antagonist of alpha-2 adrenoceptors, facilitates the excitability of the dentate gyrus and improves the retention of the radial arm maze. Soc. Neurosci. Abstr. 22, 680.