Psychopharmacology (1987) 93:470–476

**Psychopharmacology**


# Blockade of spatial learning by the $M_1$ muscarinic antagonist pirenzepine

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**Abstract.** Two experiments were conducted to determine the effects of the $M_1$ muscarinic receptor antagonist pirenzepine on place navigation in a water maze. In the first experiment rats were required to learn the location of a hidden platform following intracerebroventricular injections of equimolar doses of pirenzepine or scopolamine methylbromide. Both drugs dose-dependently impaired spatial learning according to both escape latency data and transfer test analysis. Pirenzepine was approximately 3 times less potent than scopolamine, a potency ratio which suggests $M_1$ receptor mediation of the impairment. In the second experiment pirenzepine ($1 \sim 92.3$ µg/rat ICV) was injected prior to training on a simultaneous place dicrimination task in the water maze. Impairments of choice accuracy were found with a dose of 20 µg/rat in the absence of any marked increases in either errors of omission or choice latency. These data suggest that $M_1$ receptor blockade impairs processes which are involved in spatial learning.

**Key words:** Place navigation – Scopolamine – Pirenzepine – Muscarinic $M_1$ and $M_2$ receptors – Rats

The purpose of these experiments was to examine the effects of the atypical muscarinic antagonist pirenzepine on place navigation and simultaneous place discrimination learning in a water maze. The role of central cholinergic neurons in learning has long been a focus for investigations (see Gold and Zornetzer 1983; Collerton 1986; Hagan and Morris 1987 for reviews) but interest was re-kindled by reports of a positive correlation between mental status and cholinergic markers in brains taken from patients with Alzheimer's disease (Perry et al. 1978). Many of the ensuing animal learning studies, for example those based on radial maze (Eckerman et al. 1980; Okaichi and Jarrard 1982; Watts et al. 1982) and place navigation tasks (Sutherland et al. 1982; Whishaw et al. 1985; Buresova et al. 1986; Hagan et al. 1986; Willner 1986), have essentially confirmed Macht's (1924) original observation that centrally acting muscarinic antagonists impair learning in rats. The conclusion that central cholinergic neurons play an important role in cognitive processes is further supported by experiments which demonstrate impaired learning following lesioning of forebrain cholinergic neurons (e.g. Hepler et al. 1985; Knowlton et al. 1985; Whishaw et al. 1985).

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To date, the experimental emphasis has been on defining which behavioural processes are sensitive to cholinergic intervention and relatively little attention has been paid to pharmacological aspects. However, this emphasis now requires revision as extensive evidence suggests that muscarinic binding sites in the brain and peripheral nervous system may exist in at least two subclasses, the so called $M_1$ and $M_2$ subtypes (see Birdsall and Hulme 1983; Eglen and Whiting 1985, 1986; Watson et al. 1985 for reviews).

The receptor heterogeneity concept is supported by ligand binding (Birdsall and Hulme 1976; Hammer et al. 1980, 1982), electrophysiological (Egan and North 1985, 1986; North et al. 1985; McCormick and Prince 1986) and biochemical (Meyer and Otero 1985) evidence. One of the most intriguing findings is that $M_1$ binding sites are largely restricted to forebrain structures such as cortex, hippocampus, nucleus accumbens and neostriatum in both rats (Wamsley et al. 1984; Spencer et al. 1985; Cortes and Palacios 1986) and humans (Cortes et al. 1986; Lin et al. 1986). Such a distribution suggests an important role for $M_1$ receptors in cognitive processes. However, conventional antagonists such as scopolamine and atropine, which have been widely used in behavioural studies, have high affinity for both binding sites, thus failing to discriminate between them.

Treating animals with the selective receptor antagonist pirenzepine, which has high affinity only for the $M_1$ subtype (Hammer et al. 1980, 1982), may reveal the behavioural functions of the $M_1$ receptors (Caulfield et al. 1983; Messer et al. 1985). In order to test the hypothesis that blockade of forebrain $M_1$ receptors impairs spatial learning the effects of pirenzepine on place navigation were investigated in a water maze. This task is known to be sensitive to both hippocampal (Morris et al. 1982, 1986) and cortical (Kolb et al. 1983) lesions and is impaired by pretraining injections of non-selective muscarinic antagonists such as scopolamine or atropine (Sutherland et al. 1982; Whishaw 1985; Whishaw et al. 1985; Buresova et al. 1986). Two experiments were conducted. The first was a dose-response study comparing pirenzepine with the non-selective antagonist scopolamine. In this study rats were trained to learn the spatial location of a submerged escape platform. In the second experiment the effects of various doses of pirenzepine were examined using simultaneous place discrimination, a task also known to be sensitive to both hippocampal lesions (Morris et al. 1986) and muscarinic antagonists (Hagan et al. 1986) but designed to provide separate measures of choice accuracy and latency. Pirenzepine is a highly polar

compound which crosses the blood-brain barrier very poorly. Intraventricular injections were therefore used throughout the series of experiments.

## Experiment 1

### *Methods*

*Subjects.* Naive adult male Hooded Lister rats (Harlan Olac Ltd., England) weighing approximately 200–300 g were housed singly in a temperature-controlled environment (22° C) with free access to food and water. Lights were on between 6 a.m. and 6 p.m. and all experiments were conducted between 8 a.m. and 4.30 p.m.

*Surgery.* Rats were anaesthetised with Nembutal® (60 mg/kg IP) and a stainless steel guide tube (0.64 mm external diameter) was implanted in the left lateral ventricle at the following co-ordinates relative to bregma (AP −0.8 mm, Lat: 1.5 mm, DV −3.0 mm) with bregma and lambda in the same horizontal plane and the tooth bar set at approximately 3.3 mm below the interaural line. Four dental screws were placed in the surrounding skull. The entire assembly was then covered in carboxylate cement and the wound dressed with antibiotic (Sterilon®). A stainless steel stylet, cut the same length as the guide tube, was inserted to keep the cannula patent. At least 1 week was allowed for recovery before the start of the experiments.

At the end of each experiment rats were deeply anaesthetised and the accuracy of cannula locations was checked by injecting methylene blue dye into the ventricle and visually inspecting its distribution following removal of the brains. Rats with misplaced cannulae were excluded from analysis.

*Behaviour.* The place navigation task has been described in detail previously (Morris et al. 1982, 1986). Briefly, rats were trained to find the spatial location of a platform (11 cm diameter) which was hidden in a circular black pool (2.1 m diameter) filled to a depth of 24.5 cm with water (24° C ± 1°). The platform was constructed of the same material as the pool, was covered with wire mesh and stood 1 cm below the water surface, making it almost invisible at water level. Four points on the pool rim (North, South, East, West) defined four 90° quadrants on the pool surface (NE, NW, SE, SW) and the platform occupied a position mid-way between the centre and rim of the pool along the 45° line. The platform remained in its allocated position throughout training but the starting position on each trial was randomly selected from N, S, E, or W. Conspicuous cues (racking, wall plates, door, ceiling frame, camera etc.) were provided around the pool. Behaviour was monitored via an overhead camera and the rat's, position was computed from the video image and stored, when required, on disc.

Each rat was given 120 s of adaptation to the pool prior to training. During training, a trial began when the rat, held facing the side wall, was immersed in the water. Latency to escape onto the hidden platform was recorded with a stopwatch. If a rat failed to locate the platform within 120 s it was placed on, or guided to it. The rat remained on the platform for 30 s, was removed and returned to a holding cage to await the next trial. Each rat received four trials on each of 4 treatment days, which were separated by at least 48 h. Rats were trained in squads of four or five with the platform in either one of two pool locations (NE or SW) and treatment conditions were randomly distributed throughout the training squads.

At the end of the last training trial a transfer test was conducted. This consisted of removing the platform from the pool and allowing the rats 60 s of free swimming. Two measures of spatial bias were calculated: i) the total number of occasions on which the rat crossed the exact location previously occupied by the platform (annulus crossings), ii) the total time spent searching within each of the four quadrants (quadrant swim time).

*Drug treatment.* Scopolamine methylbromide and pirenzepine dihydrochloride were dissolved in artificial cerebrospinal fluid and the pH adjusted to 7. Injections (5 µl in 30 s) were made using 25 µl syringes driven by a perfusion pump (CMA 100®) and attached to a syringe which protruded 1.5 mm below the guide tube. Scopolamine (28.9, 49.2, 83.6 µg/rat ICV) and pirenzepine (32.2, 54.8, 93.2 µg/rat ICV) doses were chosen to provide a comparison of the two antagonists at equimolar doses (72, 123, 209 nmoles).

*Statistics.* Escape latencies were analysed using Mann Whitney *U* tests. Data from the transfer tests were analysed using one-way analysis of variance followed by Dunnett's *t*-test (Winer 1971) when *F* ratios reached significance.

### *Results*

Placebo-treated rats rapidly learned the place navigation task and approached asymptotic escape latencies by day 4. Pretreatment with scopolamine caused highly significant dose-dependent increases in escape latency throughout training (see Fig. 1). Analysis of transfer test data revealed a significant drug effect on annulus crossings [$F=7.37$, $df=3,26$, $P<0.001$] and Dunnett's *t*-tests showed that all scopolamine-treated groups made significantly fewer annulus entries ($P<0.01$) than controls (see Fig. 2). Similarly, time spent swimming in the training quadrant was reduced by scopolamine treatment [$F=12.13$, $df=3,26$, $P<0.001$)]. This was confirmed after all doses ($P<0.005$) and in the case of the two highest doses swimming time in the training quadrant was reduced to chance levels (15 s).

Pirenzepine also caused a dose dependent increase in escape latencies (see Fig. 1). However, only the two higher doses (54.8, 93.2 µg/rat) caused impairments throughout training. The lowest dose significantly increased escape latencies on day 4 only. Analysis of transfer test data (see Fig. 2) revealed a highly significant effect of drug treatment on annulus entries [$F=5.4$, $df=3,28$, $P<0.01$]. In order to enable a direct comparison of potencies to be made, escape latencies on day 4 were plotted against drug dose expressed as nanomoles. These data are shown in Fig. 3.

Annulus entries were not significantly affected by the lowest dose of pirenzepine but were dose-dependently reduced by 54.8 µg/rat ($P<0.025$) and 93.2 µg/rat ($P<0.005$). Swimming time in the training quadrant was also reduced by pirenzepine [$F=8.07$, $df=3,28$, $P<0.001$]. The lowest dose of pirenzepine (32.2 µg/rat) did not affect time spent swimming in the training quadrant, but after both 54.8 µg ($P<0.025$) and 93.2 µg ($P<0.05$) time spent in the training quadrant was reduced. The total distance swam (control group mean = 19.07 m) was not significantly affected by either scopolamine [$F=1.59$, $df=3,26$] or pirenzepine [$F=1.44$, $df=3,28$]. No behavioural abnormalities were seen

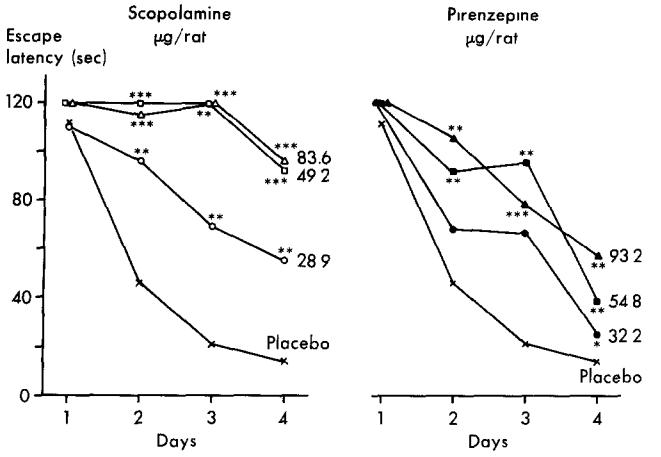


**Fig. 1.** The effects of ICV scopolamine and pirenzepine on escape latency in the place navigation experiment (Expt. 1). The four trial average was calculated for each rat and data shown are the group medians. Data from the placebo group are shown in both panels. * $P<0.05$; ** $P<0.01$; *** $P<0.001$

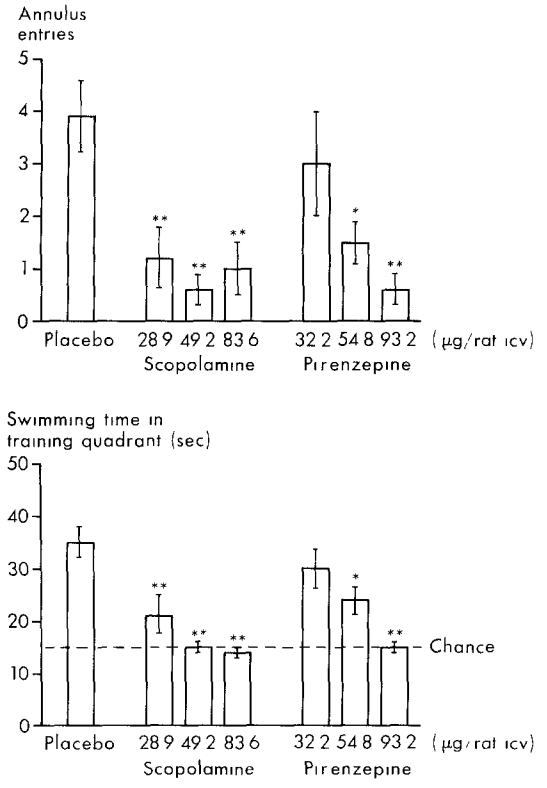


**Fig. 2.** The effects of scopolamine and pirenzepine on performance in a 60-s transfer test conducted after training on day 4. Data are means ± SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

after the two lowest doses of either scopolamine or pirenzepine. However, after the highest dose of scopolamine some rats had tremors and convulsions. These symptoms were never seen with pirenzepine but after the highest pirenzepine dose rats often swam in small tight circles.

*Discussion*

Place navigations learning was impaired by intracerebroventricular injections of scopolamine and pirenzepine in a

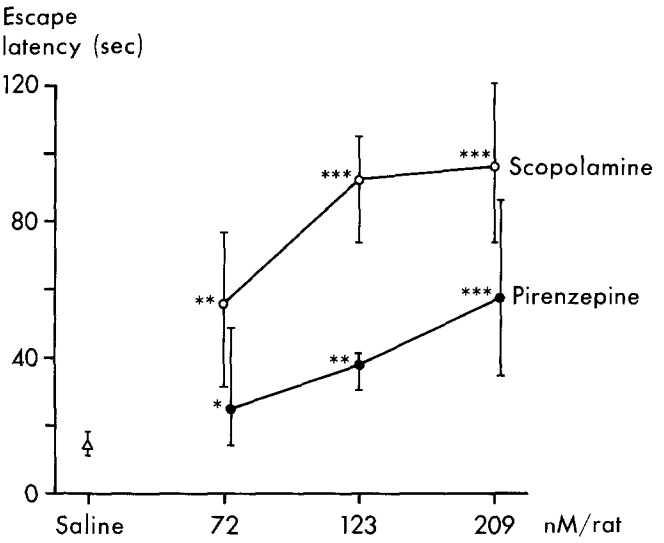


**Fig. 3.** Escape latencies on the last day of place navigation training plotted as a function of dose for scopolamine and pirenzepine. Doses of scopolamine (28.9, 49.2, 83.6 μg/rat) and pirenzepine (32.2, 54.8, 93.2 μg/rat) are expressed on a molar basis (72, 123, 209 nmoles/rat). Data are medians and *vertical bars* indicate the interquartile range. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

dose-dependent manner. This was evident in both the training phase, when escape latencies were increased, and during the transfer test when spatial bias in the search strategies of treated rats was greatly reduced. These data therefore support the findings of impaired place navigation reported in studies with systemic injections of non-selective antagonists (Sutherland et al. 1982; Hagan et al. 1986; Willner et al. 1986) and the pirenzepine data further suggest that blockade of $M_1$ receptors may be sufficient to impair spatial learning.

Estimates of pirenzepine's affinity for $M_1$ and $M_2$ binding sites in the rat brain (Tonnaer et al. 1987) show that scopolamine has a high, and approximately equal, affinity ($pK_i \sim 9.3$) for both receptor subtypes. The affinity of pirenzepine for $M_1$ forebrain sites ($pK_i$ 8.5) is about 6-fold lower than that of scopolamine. However, the affinity of pirenzepine for $M_2$ sites ($pK_i$ 6.6) is about 500-fold lower than that of scopolamine. This implies that, other factors being equal, pirenzepine induced blockade of $M_1$ receptors should occur at lower doses of the drug than $M_2$ blockade. Strictly quantitative comparisons are difficult to make in vivo where equilibrium conditions do not hold and the time of peak effect is unknown. Nevertheless, when effects on escape latency are compared on a molar basis (see Fig. 3) pirenzepine is approximately 3-fold weaker than scopolamine in impairing place navigation. This relatively small potency difference therefore supports the hypothesis that blockade of CNS $M_1$ receptor causes an impairment of spatial learning.

The experiment does not, however, address the question of which behavioural processes are impaired. The absence of obvious side effects with the lower doses of pirenzepine and failure to find any change in the total distance swum during the transfer test argues against any gross motor deficits interfering with performance. However, sensory or motivational effects of pirenzepine are more difficult to exclude on the basis of the available data. A second experiment was therefore carried out using simultaneous place discrimi-

nation to try to separate the effects of pirenzepine on performance and spatial learning processes. In this task, the rat is confronted on each trial with two superficially similar islands which are clearly visible above the water surface (Morris et al. 1986). However, only one island remained in a fixed position (the platform) and was sufficiently rigid to support the animal when it attempted to escape. The other island (the float), was randomly moved to different locations in the pool on every trial and was packed with expanded polystyrene so that it sank below the water when the rats attempted to escape. The procedure allowed a measure of choice accuracy as well as choice latency. Previous experiments have shown this version of the place navigation task to be sensitive both to systemic injections of atropine (Hagan et al. 1986) and to bilateral hippocampectomy (Morris et al. 1986).

## Experiment 2

### *Methods*

*Subjects and surgery.* Naive adult male Hooded Lister rats (Harlan Olac Ltd., England) were surgically prepared and housed as described in Experiment 1.

*Behaviour.* The experiment was conducted in the apparatus described in Experiment 1. The platform was placed at one of the quadrant locations (NE, SW) where it remained throughout an animal's training. The float was moved randomly throughout the remaining three quadrants from trial to trial and room cues were fully visible during training. Squads of four or five rats were trained using ten trials per day on 4 training days which were spaced 48–72 h apart. Drug treatment conditions were randomly distributed across training squads. Rats were immersed in the water from randomly selected starting points (N, S, E, W) and were allowed 60 s to identify and escape onto the platform. In case of failure to escape within 60 s (error of omission) the rat was guided to or placed on the platform where it remained for 30 s before being removed to a holding cage for the intertrial interval (2–3 min). On each trial the latency to choose and choice accuracy (platform always correct) were recorded. Choice was defined as snout or forepaw contact and did not include brushing with the side or incidental hindlimb contact.

*Drug treatment.* Pirenzepine was dissolved in artificial CSF and the pH adjusted to 7.0. Intracerebroventricular injections were made in 5 µl volumes injected over approximately 30 s using the methods described in Experiment 1. Pirenzepine was tested in doses of 1, 10, 20, 32.2, 54.8 and 93.2 µg/rat.

*Data analysis.* Three aspects of behaviour were recorded and analysed: choice latency, choice accuracy and errors of omission (failure to choose within 60 s). In order to assess the incidence of trials on which sensorimotor effects interfered with efficient task performance, errors of omission were analysed separately. Following their exclusion, the percentage of correct choices and a mean choice latency were calculated for each session. These data were compared using analysis of variance followed by Dunnett's *t*-test when significant *F* ratios were detected.

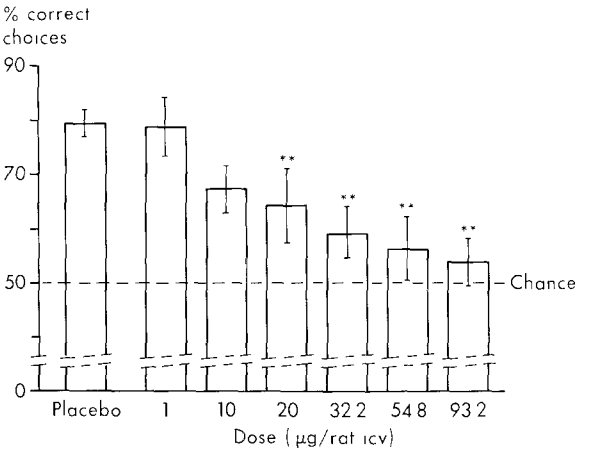


**Fig. 4.** The effects of pirenzepine on choice accuracy during day 4 of training in the two platform spatial discrimination task (Expt. 2). Data shown are mean ± SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

**Table 1.** The effects of pirenzepine on errors of omission and the maximum number of consecutive correct choices during 40 spatial discrimination trials

| Dose of pirenzepine (µg/rat ICV) | Total errors of omission | | Maximum consecutive correct choices |
|---|---|---|---|
| Placebo | 1.7±0.3[+] | (28) | 8.3±0.6 |
| 1 | 2.7±0.6 | ( 8) | 7.0±1.0 |
| 10 | 3.1±1.0 | ( 7) | 7.3±1.5 |
| 20 | 1.9±0.6 | ( 7) | 4.7±0.4*** |
| 32.2 | 4.1±0.8* | (10) | 4.7±0.4** |
| 54.8 | 7.2±1.2*** | (10) | 6.0±0.6* |
| 93.2 | 7.6±1.7*** | (10) | 4.7±0.3*** |

[+] Data are mean ± SEM
* $P<0.05$, *** $P<0.005$ compared to placebo
Numbers in parentheses are *N*'s per group

### *Results*

On day 1 placebo-treated rats performed at chance levels (50% choice accuracy) but acquired the discrimination task rapidly and were making approximately 80% correct choices by day 4. An overall two-way analysis of variance for unequal *N* was conducted with days as a repeated measure. This revealed a significant main effect of both treatments [$F=5.27$, $df=6,71$, $P<0.001$] and days [$F=14.8$, $df=3,213$, $P<0.001$] with a significant interaction between these two factors [$F=3.15$, $df=18,213$, $P<0.001$]. Evidence for pirenzepine induced impairment of choice accuracy emerged on day 3 [$F=3.2$, $df=6,71$, $P<0.001$] and on day 4 [$F=6.48$, $df=6,71$, $P<0.001$] when clear dose-dependent disruption was found across all groups. Choice accuracy on day 4 was impaired by pirenzepine in doses of 20 µg/rat and greater. These data are shown in Fig. 4. There were no significant effects of pirenzepine on choice accuracy on day 1 ($F<1$) or day 2 ($F=1.73$).

A separate analysis was conducted in which the longest run of consecutive correct choices was calculated from the 40 trials available for each rat. These data are summarised in Table 1. They provide an overall estimate of both choice accuracy and consistency and confirm the dose-dependent nature of pirenzepine's effects [$F=4.49$, $df=6,71$, $P<0.001$]. The maximum number of consecutive correct re-

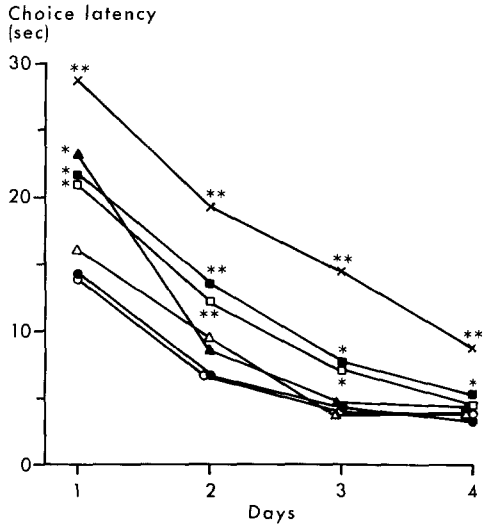


**Fig. 5.** The effects of pirenzepine on choice latency throughout training on the a two platform spatial discrimination task (Expt. 2). Data shown are mean±SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. × 93.2 μg, ■ 54.8 μg, □ 32.2 μg, ▲ 20.0 μg, △ 10.0 μg, ○ 1.0 μg, ● Placebo

sponses was significantly reduced by doses of 20 μg or more of pirenzepine.

Errors of omission were significantly higher in pirenzepine-treated rats [$F=8.63$, $df=6,71$, $P<0.001$] in the dose range 32~93.2 μg/rat (see Table 1). Over 75% of rats in each group (including placebo) made one or more errors of omission, usually during the 1st training day. The significant increase after the higher pirenzepine doses therefore largely represents an increased rate of omission on day 1, i.e. after initial exposure to the drug. No errors of omission were recorded on days 3 or 4, with the exception of two rats in the 93.2 μg group which each made one error.

Choice latency was calculated as the mean for each rat on each of the 4 training days and excluded data from trials on which errors of omission had been made. These data are summarized in Fig. 5. An overall two-way analysis of variance for unequal $N$ was conducted with days as a repeated measure. This revealed a highly significant effect of treatments [$F=24.4$, $df=6,71$, $P<0.001$] and days [$F=169.9$, $df=3,213$, $P<0.001$] with a significant interaction between these factors [$F=2.07$, $df=18,213$, $P<0.01$]. Choice latencies were increased on day 1 [$F=5.6$, $df=6,71$, $P<0.001$] in the dose range 20~93 μg (all $P$ values $<0.05$). On day 2 [$F=15.9$, $df=6,71$, $P<0.001$] and day 3 [$F=13.6$, $df=6,71$, $P<0.001$] increased latencies were found in the dose range 32~93 μg (all $P$ values $<0.05$). Finally, on day 4 [$F=10.1$, $df=6,71$, $P<0.001$] increased latencies were found only after 54 μg ($P<0.05$) and 93.2 μg ($P<0.005$).

## General discussion

Experiment 2 showed that pirenzepine dose-dependently impaired choice accuracy in a simultaneous spatial discrimination task when injected into the lateral ventricle prior to training. Choice accuracy on day 4 approached asymptote in placebo-treated controls but 20 μg/rat of pirenzepine impaired accuracy and at doses in excess of 32.2 μg/rat choice accuracy hardly rose above the 50% chance level. Similarly, the maximum number of consecutive correct choices was reduced by doses of 20 μg/rat and greater. Several aspect of the data argue that although pirenzepine causes sensorimotor or motivational impairments at high doses, these are insufficient to explain the more prominent effects on choice accuracy. First, choice accuracy impairments were found after 20 μg/rat with no increase in errors of omission and an increase in choice latency which was restricted to day 1. Furthermore, after a higher dose (32.2 μg/rat) errors of omission, although significantly higher than in controls, still only occurred on 10% of trials, compared to 4% in controls, and were entirely restricted to day 1. In addition, although choice latencies were increased by about 6 s after 32.2 μg/rat on days 1 and 2, by day 4 choice latency was not significantly higher than in controls despite the fact that choice accuracy at this point was at approximately chance levels. Only after the highest doses (54.8, 93.2 μg/rat) did the incidence of errors of omission (approx. 20%), combined with long choice latencies, plus observations of abnormal behaviour indicate that sensorimotor disruption may play a confounding role. Taken together, the data suggest that the transient disruptive effects of pirenzepine rapidly subside to reveal severe losses of choice accuracy which reflect disruption of spatial learning processes.

Although sensorimotor or motivational impairment can be excluded as satisfactory hypotheses, the present experiments do not shed any light on whether or not the deficit is selective for processes unique to spatial tasks or whether drug-induced impairments of attentional or perceptual mechanisms may explain the findings and predict more generalised learning impairments. Differentiating between these alternatives has been difficult using systemic injections of scopolamine or atropine and results have often been conflicting. For example, some authors argue in favour of task selective impairments on the basis of data which show that these antagonists block place navigation learning but do not impair learning of simple cue-guided navigation (Whishaw 1985; Willner et al. 1986). Others have argued that when differences in task difficulty (Okaichi and Jarrard; 1982) or discrimination (Hagan et al. 1986) are controlled, task-selective deficits are not found. Furthermore, claims that antagonist-induced impairments are selective for working memory tasks (Wirsching et al. 1984; Beatty and Bierley 1986; Beninger et al. 1986; Buresova et al. 1986) are not supported by the results of several studies showing that purely reference memory procedures are also impaired (Sutherland et al. 1982; Whishaw et al. 1985; Hagan et al. 1986) and that mixed working/reference memory tasks show no selective working memory impairment (Okaichi and Jarrard 1982). One hypothetical reason for the relatively non-specific behavioural effects of conventional anticholinergics is that, by virtue of their high affinity for all muscarinic receptors, widespread blockade of CNS receptors may inevitably result in a very wide range of behavioural effects. It will be interesting to see, in the future, if learning impairments induced by $M_1$ antagonists are more task selective than those of antagonists such as scopolamine or atropine, which show no receptor selectivity.

Impairment of spatial learning following ICV injections implicates a central site of action. However, studies of rabbit EEG (Whishaw et al. 1976) suggest that penetration of scopolamine into the adjacent tissue is poor, a problem which may be exacerbated with a polar structure such as pirenzepine. A rigorous test of this hypothesis will require

studies of the disposition of radiolabelled pirenzepine and scopolamine following ICV administration. Nevertheless, the doses required in our, and other experiments (Flood et al. 1981) do appear to be relatively high when it is considered that some forms of discrimination learning are impaired by peripherally administered doses of 62 μg/kg in rats (Warburton and Brown 1971) and delayed matching to sample is impaired by 20 μg/kg in primates (Pontecorvo and Evans 1985). Thus, ICV administration may not be the optimal route but in the absence of lipophilic $M_1$ antagonists peripheral administration is not possible.

The site of action for pirenzepine is a matter for speculation, but considering the relatively discrete distribution of $M_1$ receptor sites within the forebrain (Wamsley 1984; Spencer et al. 1985; Cortes and Palacios 1986; Cortes et al. 1986; Lin et al. 1986) it is feasible that both hippocampal and cortical sites may be involved in the mediation of spatial learning impairments. Indeed preliminary evidence from local injection experiments suggests hippocampal involvement (Messer et al. 1985). However, neither a striatal nor a nucleus accumbens contribution can be excluded on the basis of the present experiments. The distribution of $M_1$ sites in the forebrain, coupled with the observation that patients with senile dementia of the Alzheimer type (SDAT) have well preserved $M_1$ receptor populations (Mash et al. 1985), has encouraged the view that selective $M_1$ agonists may compensate for aspects of the cognitive deterioration characteristic of this disease (Mash et al. 1985; Cortes et al. 1986). Our data and other studies (Caulfield et al. 1983; Messer et al. 1985) support this hypothesis by showing that $M_1$ receptors play an important role in cognitive functions.

*Acknowledgement.* We wish to thank Dr. Karl Thomae GmbH for their generous gift of pirenzepine.

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Received April 9, 1987 / Final version June 24, 1987