Pharmacology Biochemistry & Behavior, Vol. 30, pp. 519–523. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

BRIEF COMMUNICATION

The Effect of Pirenzepine on Spatial Learning in the Morris Water Maze

A. J. HUNTER[1] AND F. F. ROBERTS

Department of Neuropharmacology, Glaxo Group Research Ltd. Ware, Herts, England SG12 ODJ

Received 23 June 1987

HUNTER, A. J. AND F. F. ROBERTS. *The effect of pirenzepine on spatial learning in the Morris Water Maze.* PHARMACOL BIOCHEM BEHAV 30(2) 519–523, 1988.—The effects of the selective M_1-muscarinic antagonist, pirenzepine, were studied on the Morris Water Maze, a test of spatial learning in the rat. Pirenzepine (0, 10 or 30 μg) was administered into lateral ventricle during acquisition of this task. Although 30 μg of pirenzepine impaired acquisition of the spatial aspects of the task, treated animals still appeared to be able to acquire a taxon strategy. A low dose of pirenzepine (10 μg) produced a slight deficit but this was only visible in a "spatial probe" trial. Although these results are consistent with the belief that muscarinic M_1-receptors are involved in spatial learning, it cannot be excluded that the effects recorded were mediated by muscarinic M_2-receptors, due to the low selectivity of pirenzepine.

Spatial learning Morris Water Maze Pirenzepine

CENTRALLY acting muscarinic antagonists such as scopolamine and atropine have been reported to impair memory in a wide range of spatial tasks in both rodents and primates, e.g., the radial arm maze [22], spontaneous alternation [20] and spatial matching to sample tasks [18].

The Morris Water Maze is a novel test of spatial learning in which the rat has to learn to locate a hidden platform (island) placed in a pool of opaque water [14]. The island lies about one centimetre below the surface of the water and therefore the rat has to learn to locate it by means of distal cues external to the maze. Performance on this task has been shown to be susceptible to manipulations of central cholinergic systems in rats, being impaired by both anticholinergic drugs [9, 23, 25] and lesions of the nucleus basalis [25]. Damage to the hippocampus or neocortex, areas of the brain which receive extensive cholinergic innervation (from the medial septal area and nucleus basalis respectively), also impair performance on this task [11, 15, 19], and improvements in performance have been reported in hippocampally lesioned animals given cholinergic rich septal grafts [7]. Given the current interest in the possibility that selective muscarinic agonists (or antagonists) may be efficacious in the treatment of Alzheimer's disease [17], it seemed worthwhile to investigate the effects of the M_1-selective antagonist, pirenzepine, on the task.

Radioligand binding studies with pirenzepine have shown that the distribution of muscarinic receptor subclasses in the brain is not uniform. Sites with a high affinity for pirenzepine predominate in the hippocampus and cortex [8]. However, although cholinergic input to the hippocampus and cortex is severely affected in Alzheimer's disease [3], the high-affinity pirenzepine binding sites, which are presumably postsynaptic [2], are unaltered. These areas of the brain have also been shown to play a key role in regulating memory processes in both animals and man (see [18]). Pirenzepine has been shown to impair passive avoidance learning in the mouse when given by intracerebroventricular injection [5], although an improvement with a low dose of pirenzepine has also been observed [12]. Intrahippocampal injection of pirenzepine has also been shown to impair performance on a rewarded non-matching to sample T-maze task [13]. It was therefore of interest to examine the effects of pirenzepine on the performance of a reference memory task, the Morris Water Maze.

Animals

Thirty-four experimentally naive male Lister Hooded rats were used for this study with 11 or 12 animals in each group. They weighed 300–400 g and were obtained from Olac

[1]Requests for reprints should be addressed to A. J. Hunter at her present address: Astra Neuroscience Research Unit, 1 Wakefield Street, London, WC1N 1PJ.

(Bicester, England). They were housed in groups of three with food and water available ad lib and were maintained on a 12 hour light (6 a.m.–6 p.m.) on/off cycle. All behavioural testing was carried out during the light phase of their cycle.

Two weeks before the experiment each rat was anaesthetised with 5% chloral hydrate and a cannula implanted in the right lateral ventricle following the method of Caulfield *et al.* [5]. At the end of the experiment the rats were given an injection of dye via the cannula and the cannula position verified on autopsy.

Apparatus

The water maze consisted of a square tank made of perspex (122×122 cm), filled to a depth of 24 cm with water, which was made opaque by the addition of latex compound E308 (Williams Division, Morton-Thiokol, Hounslow, UK). The temperature of the water was maintained at approximately 29°C. This temperature is higher than that used in previous studies [14,24] but was chosen for our studies as many of the drugs tested in our laboratory cause a lowering of body temperature (although pirenzepine, of course, does not). As hypothermia has been shown to impair spatial learning [16] a temperature of 29°C was used to avoid any cooling effects due to the water temperature. The island was 11 cm in diameter and was positioned approximately 1 cm below the surface of the water. The island could be placed in one of 4 possible symmetrical positions in the tank. Each trial was recorded by means of a video recorder and the path taken by the rat analysed using a video-digitising system (HVS, Kingston, Surrey, UK) linked to an Apple IIE computer. The path was then stored on disc for further analysis.

Procedure

The data to be reported here are pooled from two experiments. Drugs were administered intracerebroventricularly in a volume of 3 μl, 10 minutes before testing on three successive days, with each rat receiving the same treatment on each day. Testing consisted of 6 successive training trials on each day with an additional trial on day 3 (trial 19) when the island was removed from the tank (spatial probe trial). For a given rat the island remained in the same position over all 18 trials, although the position of the island varied between rats in a counter balanced fashion.

On each trial the rat was allowed a maximum of 100 seconds to find the hidden island. Rats which found the island in less than 100 seconds were allowed to remain on it for 10 seconds and then removed. Rats which failed to find the island were placed on it for 10 seconds at the end of each trial. To prevent the use of a simple taxis strategy, the rat was started from a different quadrant of the pool on adjacent trials.

Rats were then tested on a fourth day with a visible island, painted black, whose surface was 1 cm above the surface of water. Drugs or vehicle solution were administered as on the previous days. The island was placed in the quadrant of the pool opposite to the one which had contained the island to which the rats had been trained to on the previous 3 days of testing. Rats were given 6 trials (trials 20–25) to this visible island to check for any drug effects on vision, motor coordination or motivation to escape from the water.

Data Analysis

The following variables were analysed: latency to find island, path length, speed, time in the island quadrant and annulus crossings, each island position corresponding to an

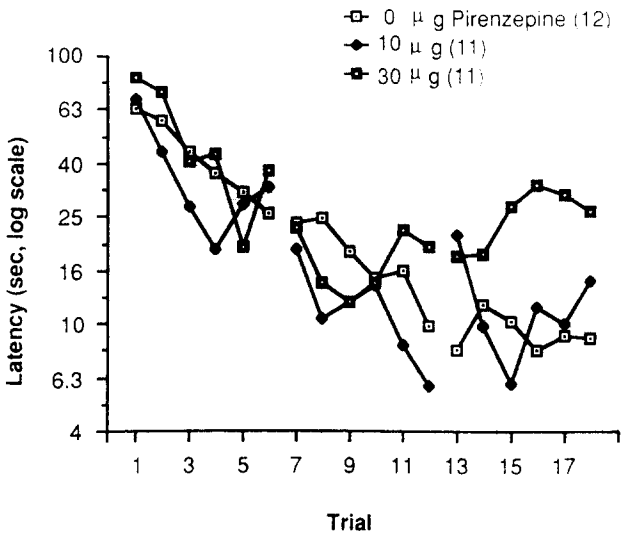

FIG. 1. The effect of ICV pirenzepine on acquisition. Geometric mean latencies to find the hidden island are shown on a log scale over the first 18 training trials on days 1–3. 0 μg (n=12), 10 μg (n=11) or 30 μg (n=11) pirenzepine was administered ICV 10 minutes before the first trial on each day. (N)=number of animals in group.

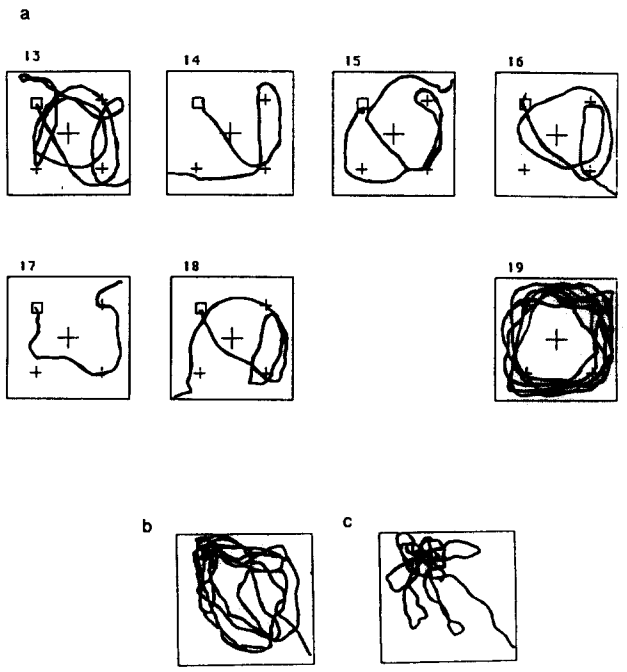

FIG. 2. (a) Path plots of a rat treated with 30 μg pirenzepine ICV. These plots were obtained when an image analyser (HVS Ltd., Kingston, Surrey) linked to an Apple IIE was installed. The experiment was subsequent to that described in this paper but used a similar protocol. The straight lines indicate when the rat swam under water. The small square indicates the position of the island. The numbers identify which trial each path plot relates to. There was no island in trial 19. (b) Path plot of a rat treated with 10 μg of pirenzepine in trial 19. (c) Path plot of a control rat in trial 19.

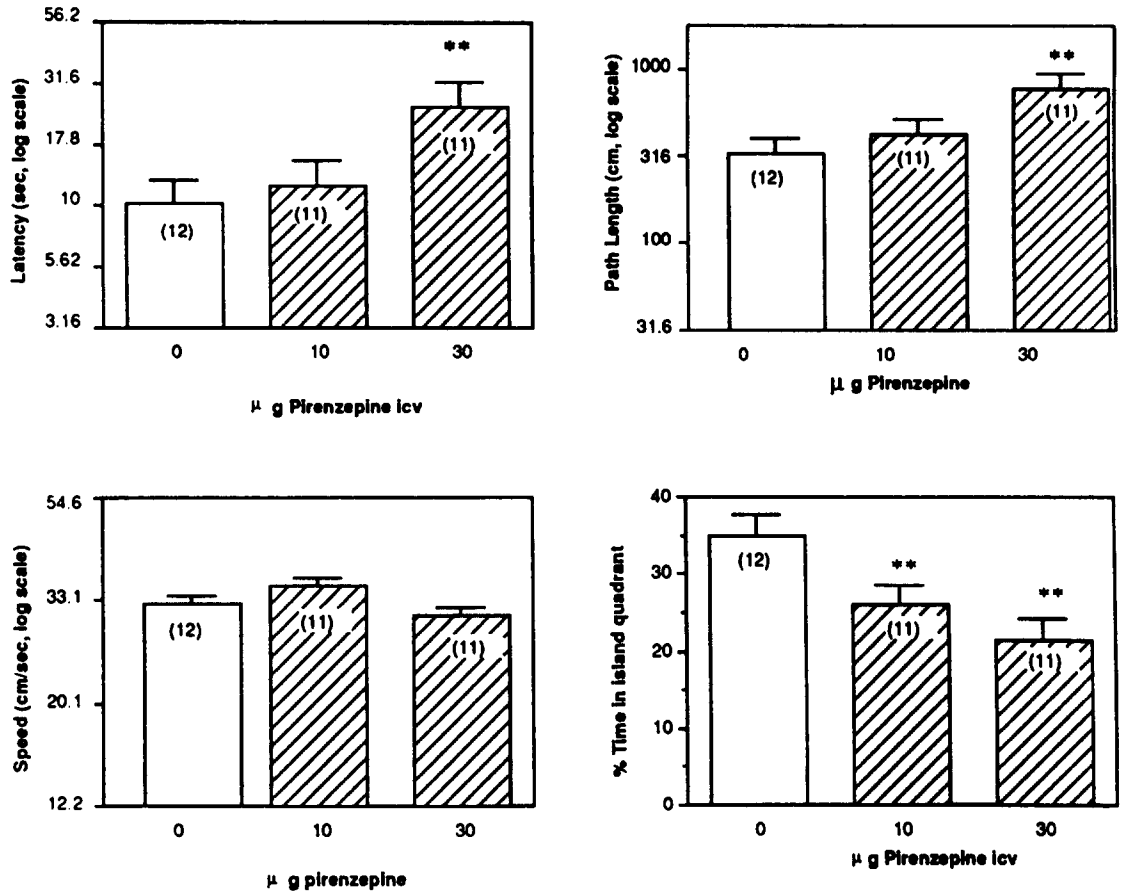

FIG. 3. The effect of ICV pirenzepine on latency, path length, speed and percentage time in the island quadrant in trials 13–18. Geometric mean values for latency, speed and path length are plotted on a logarithmic scale. The numbers of rats in each group are shown in brackets. The F values for the treatment effects (with 2,31 degrees of freedom) in the analysis of variance were: latency 10.2**, path length 11.5**, speed 2.97, % time 6.53* (*$p<0.05$, **$p<0.01$.) Values of t (with 31 degrees of freedom) were estimated from the residual mean square in the analysis of variance, the data obtained with the pirenzepine-treated groups being compared with the data from the control group: latency $t=0.76$ (10 μg pirenzepine), 4.27** (30 μg pirenzepine); path length: $t=1.38$, 4.68**, the error bars shown in the figure were estimated from the residual mean square of the analysis of variance. Speed: $t=1.65$, -0.78, % time: $t=-2.31$**, -3.55**.

annulus. Only the trials from the third day of training (trials 13–18) were considered in the analysis as the high number of rats with maximum latencies on days 1 and 2 invalidated analysis of the data by parametric statistics. Even over training trials 13–18 the distributions of latency and path length data tended to show positive skewness and the variances increased with the mean. A logarithmic transformation was therefore used. The percentage times in the island quadrant and percentage annulus crossings were not transformed prior to analysis.

Data from each rat over trials 13–18 were summarised by a mean value and these were subjected to a one-way analysis of variance. In addition the data from pirenzepine-treated rats were compared with that of the control by a t-value calculated from residual variance of the analysis of variance.

For the spatial probe trial, data for the time in the 'island' quadrant and the percentage annulus crossings was subjected to a one-way analysis of variance, as described above.

Drugs

Pirenzepine HCl was dissolved in a modified Krebs solution for administration via the intracerebroventricular (ICV) cannula. Control animals received the modified Krebs solution alone. The modified Krebs solution had the following composition: 120 mM NaCl, 4.7 mM KCl, 1.3 mM $MgSO_4$ 7 H_2O, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose, 2.5 mM $CaCl_2$. Pirenzepine HCl was kindly supplied by Dr. R. Hammer, Boehringer Ingerheim Zentrale GmbH.

RESULTS

Although data from trials 1–12 were not analysed statistically it can be seen from the latencies shown in Fig. 1 that both controls and the treated rats improved their performance over time and by trial 13 the control and pirenzepine-treated animals appear to have reached a constant level of performance. By watching the swimming path of the rats treated with the highest dose of pirenzepine it could be seen that they appear to locate the island by swimming at a fixed distance and in a fixed direction around the sides of the pool (Fig. 2a). Such behaviour is not apparent in naive animals or control animals that have learned the task (Fig. 2c).

The data from trials 13–18 are summarised in Fig. 3 together with the statistical analysis. It can be seen that 30 μg pirenzepine produces a significant increase in path length

and decrease in percentage time in the island quadrant compared with the control group. There was no significant difference in speed across treatments.

The data obtained for the 19th 'no-island' trial are shown in Fig. 4 together with the statistical analysis. Pirenzepine- (10 μg and 30 μg) treated rats crossed all 4 possible island positions with equal frequency, whereas control rats did not. Consistent with this the treated rats spent less time in the 'island' quadrant than control rats. This cannot be attributed to a visual impairment because no significant differences were seen between control and treated animals with any of the measures with a visible island.

DISCUSSION

In these experiments pirenzepine-treated rats improved their performance over time, showing that they had acquired some knowledge of the task. The change in swimming pattern seen in pirenzepine-treated rats suggests that they were using a taxon, or procedural strategy rather than a truely spatial one. A similar pattern of impairment is seen with scopolamine and atropine-treated rats [9, 23, 24].

The pattern of impairment produced by pirenzepine on the water maze is similar to that procedure by septal or retrohippocampal lesions [19]. In the Morris Water Maze, rats with septal lesions are initially unimpaired compared to controls. As training progresses, the unlesioned rats learn the spatial aspects of the task and those with septal lesions become impaired relative to the unlesioned (Hunter and Roberts, unpublished observation). In contrast, rats with lesions of the nucleus basalis are markedly impaired on early trials compared to controls but with training these animals eventually reach the same level of performance as the controls (Hunter, unpublished observations; [25]). This recovery has been shown, in rats given bilateral ibotenic acid lesions of the nucleus basalis, to be dependent upon extensive post-lesion training [1]. This dichotomy between the effects of septal and nucleus basalis lesions is interesting as it could suggest that different types of learning may be involved and that these may be mediated by different brain areas. The fact that pirenzepine mimics the effects of septal lesions more closely than those seen with nucleus basalis lesions could therefore reflect differential access of the drug to the cerebral cortex as opposed to the hippocampus, the hippocampus receiving a cholinergic innervation from the septum and the cortex receiving a cholinergic innervation from the nucleus basalis. Quaternary compounds such as pirenzepine administered via lateral ventricles could have ready access to the surrounding hippocampus, but might penetrate less readily to the cortex.

Differential penentration could, however, confound any interpretation of the receptor subtype mediating the effects of pirenzepine. Although pirenzepine is designated as M_1-selective, this selectivity is not great; the relative affinity at M_1- and M_2-receptor subtypes is approximately ten-fold [8]. Thus, pirenzepine could be mediating its effects on spatial learning by acting on M_2-receptors. On the other hand pirenzepine was more potent on passive avoidance in mice than the receptor subtype unselective quaternary compound N-methyl scopolamine [3]. The doses of pirenzepine that affected passive avoidance were also less than those reversing oxotremorine induced salivation or tremor.

In conclusion, the M_1-subtype selective antagonist, pirenzepine, appears to have similar actions on the Morris Water Maze to those of unselective muscarinic antagonists. However, while data are consistent with the involvement of M_1-receptors the low selectivity of pirenzepine cannot exclude an action at M_2-receptors. Identification of the muscarinic receptor subtype involved would be greatly facilitated if a more selective and brain penetrating antagonist was available.

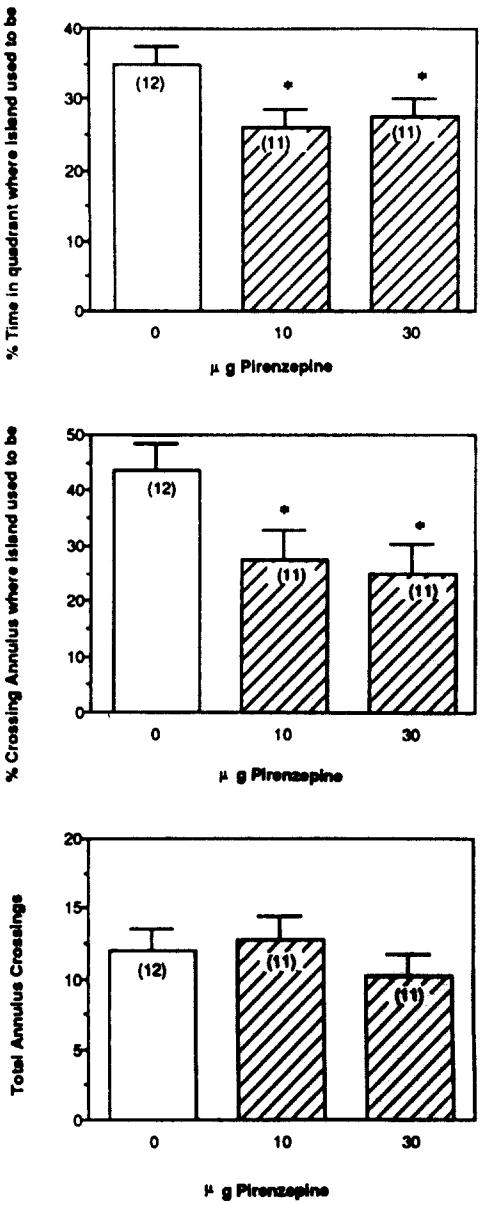

FIG. 4. The effect of pirenzepine on the behaviour of rats in trial 19, the extinction trial. The number of rats in each group are shown in brackets. The F values (with 2,31 degrees of freedom) for treatment effects in the analysis of variance were: % time 3.8*, % annulus crossings 4.4*, total annulus crossings*. (*$p<0.05$, **$p<0.01$.) Values of t (with 31 degrees of freedom) were estimated from the residual error in the analysis of variance, the data obtained from the pirenzepine groups being compared with the data from the control group. % time: 2.57* (10 μg pirenzepine), 2.13* (30 μg pirenzepine), % annulus crossings $t=2.19$*, 2.53*, total annulus crossings $t=0.37, 0.83$. The error bars shown in the figure were estimated from the residual mean square of the analysis of variance.

ACKNOWLEDGEMENT

We would like to thank John Forster for statistical advice.

REFERENCES

1. Bartus, R. T., M. J. Pontecorvo, C. Flicker, R. L. Dean and J. C. Figueiredo. Behavioural recovery following bilateral lesions of the nucleus basalis does not occur spontaneously. *Pharmacol Biochem Behav* **24**: 1287–1292, 1986.
2. Birdsall, N. J. M., E. C. Hulme and J. M. Stockton. Muscarinic receptor heterogeneity. *Trends Pharmacol Sci* **5**: 4–8, Suppl, 1984.
3. Bowen, D. M., S. J. Allen and J. S. Benton. Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. *J Neurochem* **41**: 261–272, 1983.
4. Caulfield, M. P. C., G. A. Higgins and D. W. Straughan. Central administration of the muscarinic receptor subtype-selective antagonist pirenzepine selectivity impairs passive avoidance learning in the mouse. *J Pharm Pharmacol* **35**: 131–132, 1983.
5. Caulfield, M. P. C., K. F. Clover, D. A. Powers and T. Savage. A rapid and convenient method for the implantation of cerebroventricular cannulae in rats. *J Pharmacol Methods* **9**: 231–236, 1983.
6. Dunnett, S. B. Comparative effects of cholinergic drugs and lesions of the nucleus basalis of fimbria-fornix on delayed matching in rats. *Psychopharmacology (Berlin)* **87**: 357–363, 1985.
7. Dunnett, S. B., F. H. Gage, A. Bjorklund, Low W. C. Steneviv and S. D. Iversen. Hypocampal deafferentation: transplant-derived re-inervation and functional recovery. *Scand J Physiol* Suppl 1, 104–111, 1982.
8. Hammer, R., C. P. Berrie, N. J. M. Birdsall, A. S. V. Burgen and E. C. Hulme. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* **283**: 90–92, 1980.
9. Hunter, A. J., F. F. Roberts and C. A. Tutty. Scopolamine impairs performance on the Morris water maze in both naive and trained rats. *Br J Pharmacol* **87**: 41P, 1986.
10. Hunter, A. J. and F. F. Roberts. The effect of septal lesions on the performance of trained rats on the Morris water maze. In preparation.
11. Kolb, B., R. J. Sutherland and I. Q. Whishaw. A comparison of the contributions of frontal and parietal association cortex to septal localization in rats. *Behav Neurosci* **97**: 13–27, 1983.
12. Longoni, A., D. Braida, R. Biagetti, M. Sala and E. Gori. Effects of intracerebroventricular administration of pirenzepine on memory processing in rats. In: *Neuroendocrine System and Aging*, edited by P. Vezzadini, A. Facchini and G. Labo. Rijswijk, The Netherlands: Eurage, 1986, pp. 293–298.
13. Messer, W. S., G. J. Thomas and W. Hoss. Selectivity of pirenzepine in the central nervous system. II. Differential effects of pirenzepine of a representational memory task. *Brain Res* **407**: 37–45, 1987.
14. Morris, R. G. M. Spatial localization does not require the presence of local cues. *Learn Motiv* **12**: 239–249, 1981.
15. Morris, R. G. M., P. Garrud, J. N. P. Rawlins and J. O'Keefe. Place navigation impaired in rats with hippocampal lesions. *Nature* **297**: 681–693, 1982.
16. Panakhova, E., O. Buresova and J. Bures. The effect of hypothermia on the rats spatial memory in the water tank task. *Behav Neural Biol* **42**: 191–196, 1984.
17. Perry, E. K. The cholinergic hypothesis—ten years on. *Med Bull* **42**: 63–69, 1986.
18. Ridley, R. M., N. G. Barratt and H. F. Baker. Cholinergic learning deficits in the marmoset produced by scopolamine and ICV-hemicholinium. *Psychopharmacology (Berlin)* **83**: 340–345, 1984.
19. Schenk, F. and R. G. M. Morris. Dissociation between components of spatial memory in rats after recovery from the effects of retro-hippocampal lesions. *Exp Brain Res* **58**: 11–28, 1985.
20. Squire, L. R. Effects of pre-trial and post-trial administration of cholinergic and anticholinergic drugs on spontaneous alteration. *J Comp Physiol Psychol* **69**: 69–75, 1986.
21. Squire, L. R. and N. Butters (Eds.). *Neuropsychology of Memory*. New York: Guilford Press, 1984.
22. Stevens, R. Scopolamine impairs spatial maze performance in rats. *Physiol Behav* **27**: 385–386, 1981.
23. Sutherland, R. J., I. Q. Wishaw and J. C. Regehr. Cholinergic receptor blockade impairs spatial localization by distal cues in the rat. *J Comp Physiol Psychol* **96**: 563–573, 1982.
24. Whishaw, I. Q. Cholinergic receptor blockade in the rat impairs local but not taxon strategies for place navigation in a swimming pool. *Behav Neurosci* **99**: 979–1005, 1985.
25. Whishaw, I. Q., W. T. O'Connor and S. B. Dunnett. Disruption of central cholinergic systems in the rat by basal forebrain lesions atropine: Effects of feeding, sensormotor behaviour, locomotor activity and spatial navigation. *Behav Brain Res* **17**: 103–115, 1985.