

# Cholinergic manipulations and passive avoidance in the rat: effects on acquisition and recall

**W. Jeffrey Wilson and Jennifer A. Cook**

Department of Psychological Sciences, Indiana University - Purdue University Fort Wayne, 2101 Coliseum Blvd. East, Fort Wayne, IN 46805, USA

**Abstract.** A review of the literature concerning cholinergic manipulations and passive avoidance reveals that state-dependency is usually not controlled adequately, nor is acquisition of the passive avoidance task ascertained before retention is tested. These problems make interpretation of results difficult. We report three experiments on 129 rats, controlling both of these factors, in which scopolamine and pilocarpine impaired both acquisition and retention of a passive avoidance response. Lesions of the nucleus basalis magnocellularis had no effect on this task. The results suggest that an optimal level of central cholinergic activity exists for learning and memory, and that deviations from this optimal level impair acquisition and retention.

## INTRODUCTION

The role of central cholinergic systems in learning and memory processes has come under increasing investigation since the late 1970's and early 1980's when a number of investigators demonstrated degeneration of acetylcholine-producing tissue in the brains of patients with Alzheimer's disease (Davies and Maloney 1976, Perry et al. 1977, Davies 1979, Whitehouse et al. 1981, 1982, Coyle et al. 1983). Manipulations of central cholinergic activity in animal subjects, either *via* pharmacological manipulations or lesions of the nucleus basalis magnocellularis (nbM), have been carried out in an effort to model Alzheimer's disease, with varying degrees of success. In this paper we review some of the work that has been done involving the effects of manipulations of central cholinergic function on passive avoidance performance, an often used measure of memory. We then present data from three experiments that examine the specificity of the effects of cholinergic manipulations on either acquisition or recall.

Passive avoidance is the behavioral procedure of choice in many studies of learning and memory, probably because it requires little special training of the subjects, and because results are available quickly. Unfortunately, comparisons across studies are made difficult by the fact that very different procedures are employed in studies of passive avoidance in different laboratories. Different tasks are employed (e.g., step-through, step-down, etc.), and different shock parameters are used (varying intensities and numbers of escapable or inescapable shocks). Perhaps more serious, however, are two specific problems to be addressed in this paper: in studies involving pharmacological or surgical manipulations, state-dependency is usually not taken into account, nor is the subjects' acquisition of the task assessed before recall is tested.

Drug-state-dependency can only be controlled by the administration of the drug of interest before both the training and testing phases of the passive avoidance procedure. Any deficits in passive avoidance performance during the test, when a drug has

been administered solely in training or prior to the test, might result from state-dependent learning: the subject's memory of the training remains intact and functional, but the subject fails to recognize the situation as the one encountered during training, as the internal discriminative stimuli differ. Groups of subjects can be examined that have received the drug during only one of the sessions, but a group must be included that receives drug in both sessions to control for this effect.

A second problem in the interpretation of drug effects on passive avoidance arises because little concern seems to be given to whether the drug has its effects on acquisition or retention/recall of the task. One can be sure that the drug manipulation is affecting memory only if the task to be remembered has in fact been learned. Passive avoidance studies in which a single training trial is given provide no such assurance. In these studies, a drug given prior to training might inhibit the acquisition of the task, or the memory trace, or both. In a study adequately controlling state-dependency, that is, a study involving the administration of drug on both the training and testing sessions, the locus of the drug-induced deficit in performance in the test session cannot be determined if no assurance exists that learning in fact occurred during acquisition. To ensure that learning has occurred, the subject should be trained to some criterion during acquisition, allowing effects of the drug on acquisition of the task to be assessed. Memory can then be measured independently in the test session. Lacking a criterion for acquisition, learning and memory in this procedure are confounded.

In the experiments described herein we used a design that controls for both of these major problems. Rats were trained to criterion on Day 1, so that learning could be assessed, and so that any deficits in the test session could be attributed more correctly to memory impairment. We presume that rats that are all trained to the same criterion should have learned to the same extent, or at least to a more equal extent than rats that receive the same number of learning trials regardless of their performance. To control for state-dependency, the drug of interest

was administered to four groups of rats, during training alone, test alone, both, or neither. Control injections of saline were given if drug was not. Such a procedure allows us to examine the extent to which state-dependency might influence the rats' performance independent of any specific drug effects on learning or memory.

## EXPERIMENT I

### Introduction

Considerable interest has been expressed in the amnesic effects of scopolamine, to the extent that the effects of this muscarinic antagonist are sometimes used in animals as a model of Alzheimer's disease. Many studies of scopolamine's effect on learning and memory have employed the passive avoidance procedure. In a review of studies of passive avoidance that employed pharmacological manipulations, Bammer (1982) summarizes in tabular form 19 articles on scopolamine's effects on the performance of mice (14 articles) or rats (5 articles) in this task, and we have found 46 articles (29 using rats, 16 mice, and 1 both rats and mice) published since his review (see Table I). Scopolamine usually, but not always, proved detrimental to the performance of the passive avoidance task. Unfortunately, drug-state-dependency was rarely controlled properly; instead scopolamine was usually given before either the training or test session alone. Proper controls were used in only 7 of the studies reviewed by Bammer (1982); of the 13 experiments described in those 7 studies, scopolamine had no effect in 7, it produced a deficit in 5, and it enhanced performance in 1. Of the additional articles that we found, few addressed state-dependent learning. Elrod and Buccafusco (1988) explicitly controlled for state-dependency by administering scopolamine prior to both training and test sessions in one of the four experiments that they report. They found no evidence of state-dependent effects of scopolamine. Nisshimura et al. (1990) addressed state-dependent effects of morphine on memory, and demonstrated that changes in the internal state pro-

TABLE I

Studies examining scopolamine's effect on passive avoidance, published since Bammer's (1982) review. Complete references appear in the reference section

Alfano and Petit 1985	Matsouka et al. 1992
Altman et al. 1987	Mondadori et al. 1992
Bianchi and Panerai 1993	Murphy and Boast 1985
Bignami et al. 1992	Myslivecek 1984
Chopin and Briley 1992	Nabeshima et al. 1991
Cruz-Morales and Prado-Alcala 1992	Nagatani et al. 1991
Cruz-Morales et al. 1992	Nisshimura et al. 1990
Dawson et al. 1991	Okuyama et al. 1993
Decker et al. 1990	Piercey et al. 1987
DeNoble et al. 1986	Porsolt et al. 1987
Drago et al. 1990	Quirarte et al. 1993
Drago et al. 1993	Riekkinen et al. 1990
Duran et al. 1990	Rush 1986
Elrod and Buccafusco 1988	Rush and Streit 1992
Galliani et al. 1987	Schettini et al. 1992
Laborit and Zerbib 1987a, 1987b, 1989a, 1989b	Spignoli and Pepeu 1987
Lo Conte et al. 1982	Worms et al. 1989
Lopez et al. 1991	Yamamoto and Shimizu 1987
Lorenzini et al. 1992	Yamamoto et al. 1990
Loullis et al. 1983	Yamamoto et al. 1993
Mashkovsky et al. 1991	Zanotti et al. 1986
	Zerbib and Laborit 1990

duced by morphine can overcome scopolamine-induced memory deficits. However, they failed to address the implications of this finding for the many studies concerning scopolamine's amnesic effects. Lorenzini et al. (1992) used daily one-trial passive avoidance training sessions, with drug injections prior to each session. While this procedure adequately controls for state-dependency, it leaves open the question of whether scopolamine is affecting acquisition or recall of memory. In other studies (e.g., LoConte et al. 1982, Spignoli and Pepeu 1987), training occurred 30 min prior to testing, and scopolamine was administered prior to training. It is likely that drug effects may have persisted, but the injection experience prior to the test session was absent. In these studies, scopolamine interfered with successful performance of the passive avoidance

task. Loullis et al. (1983) administered scopolamine *via* implanted minipumps for a 15 day period, then trained and tested their rats after removal of the pumps. It is unlikely that state-dependence could have played a role in their results, and interestingly they found that scopolamine pretreatment improved performance in the passive avoidance task. It is clear that a study that adequately controls for state-dependency is needed.

With regard to the issue of the assessment of learning during the training session, only two of the papers reviewed by Bammer (1982) involved training to criterion. Wiener and Messer (1973) allowed their rats to escape the shock, and trained them until they stayed on the safe side of the apparatus for 2 min after their last escape. They found that intrahippocampal administration of scopolamine after the training session had no effect on performance in the test session. Feigley (1974) used a discrete trials procedure, and trained his rats until they remained on the safe side of the apparatus on two successive trials for 60 s following the beginning of a trial. He noted that acquisition of the passive avoidance task by young rats was disrupted by low doses of scopolamine, and that much higher doses were required to disrupt acquisition in adult rats. Feigley tested only trials to acquisition, he did not measure retention. Of the studies reported since Bammer's review, few involved a criterion, either explicit or implicit. Altman et al. (1987), in a step-through passive avoidance task in mice, allowed their subjects to escape the shock, and stopped the training session when the mice had either received 5 shocks or remained on the safe side for 60 s after receiving at least two shocks. Thus some of their subjects were trained to criterion, while some were not. Scopolamine had no effect on retention of passive avoidance in that experiment. Riekkinen et al. (1990) trained rats until they remained on the safe side of the apparatus for 60 s; high doses of scopolamine interfered with acquisition, but had no effect on the subsequent retention of the task. Murphy and Boast (1985) used a one-trial step-through task with their rats, and excluded any rats that reentered within 60 s the compartment in which they had been

shocked. Scopolamine produced a deficit in passive avoidance performance in these rats, but was administered only on the training session, so state-dependency could be involved. Myslivecek (1984) gave neonatal rats scopolamine during training in a procedure in which the "pup is incited by a gentle air stream to move toward an electrified grid" (p. 207) until it fails to move onto the grid within 60 s on two successive trials. Performance in the test session was worse in pups that had received scopolamine in training, but again a state-dependent effect cannot be ruled out. Elrod and Buccafusco (1988) examined the effects of scopolamine on acquisition in one of their experiments, but did not train the rats to criterion in the experiments involving 24 h retention intervals.

In this experiment, we examined the effects of scopolamine on both the acquisition and subsequent performance of a passive avoidance task. State-dependency was controlled, and subjects were trained to criterion so that scopolamine's effects on memory *per se* could be determined.

## Method

### SUBJECTS

Thirty-two female Sprague-Dawley rats obtained from Harlan Sprague-Dawley (Indianapolis, IN) served as subjects. Rats were housed individually with *ad lib.* access to food and water throughout the experiment, and were maintained on a reversed 12 h light-dark cycle. Experimentation was conducted on each rat during the dark phase at approximately the same time each day, no earlier than 1 h after lights off, and no later than 1 h before lights on. Rats were approximately 120 days old at the start of the experiment.

### DRUGS

Scopolamine hydrobromide (Sigma) was dissolved in isotonic saline, and was prepared fresh on a daily basis. Isotonic saline served as a control injection.

## APPARATUS

A rectangular Plexiglas chamber consisting of two compartments of equal size (22 x 23 x 21.5 cm) separated by a common wall served as the passive avoidance apparatus. The floor of the chamber was composed of 5 mm stainless steel rods (spaced 15 mm center to center) that could be electrified by a locally constructed high voltage DC source through a neon shock scrambler. The clear plexiglas walls and ceiling of one compartment were covered with black paper; the other compartment was illuminated by the house light of the ventilated, sound attenuating enclosure in which the chamber was located. A 9 x 9 cm doorway was located in the center of the common wall, at floor level. A pivoting, counter-weighted door covering this opening could be opened by the operation of a solenoid. The chamber pivoted on the center rod of the floor, activating a magnetic reed switch when the rat crossed from one compartment into the other. Experimental control and data collection were achieved *via* an IBM-PC compatible computer, a locally developed computer interface, and custom software.

## PROCEDURE

A step-through passive avoidance procedure was employed, in which the rats were trained to criterion on Day 1 and tested for retention of the task 24 h later. On Day 1 the rat was placed in the light compartment, and 60 s later the door into the dark compartment opened. Five seconds after the rat entered the dark compartment (with entry being defined as closure of the magnetic reed switch) a 1.0 mA shock was applied to the floor of the chamber. This shock remained on until the rat returned to the light compartment. This procedure was repeated every time the rat entered the dark side, until the rat attained the criterion of remaining on the light side for 60 s after the offset of the previous shock, a procedure similar to that used by Riekkinen et al. (1990). At this point the session was terminated. The latency to step through, number of shocks re-

ceived, and latency to escape each shock were recorded by the computer.

On Day 2 retention of the passive avoidance task was tested. The rat was placed on the light side, and 60 s later the door was opened. The test session continued until the rat crossed to the dark side, or until 600 s had elapsed. The latency to cross was recorded for each rat; rats that did not cross in the time allotted received a latency score of 600 s.

A 2x2 (drug/saline on Day 1 x drug/saline on Day 2) factorial design was employed, in which rats received either scopolamine (1 mg/kg, i.p., in a solution of 1 mg/ml isotonic saline) or an equal volume of vehicle on Day 1, during training, or on Day 2, during the test. The injection was given 20 min prior to the start of the behavioral session on each day. This design allowed independent assessment of the effects of drug on acquisition and recall, and allowed drug-state dependency to be assessed.

Separate analyses of variance were used to compare the step-through latencies, escape latencies, and trials to criterion of the two super groups (scopolamine *vs.* saline) for Day 1, and the step-through latencies of the four groups (scopolamine/scopolamine, scopolamine/saline, saline/scopolamine, and saline/saline) for Day 2.

## Results

In acquisition, rats that received scopolamine required on average more than twice as many trials ( $5.44 \pm 1.52$  *vs.*  $2.31 \pm 0.41$ , mean  $\pm$  standard error of the mean,  $F(1,30)=4.00$ ,  $P<0.06$ ) to reach the criterion of remaining on the safe side for 60 s. During the Test session, a main effect of drug treatment during test was apparent, with the rats that received scopolamine crossing to the dark side faster than the rats receiving saline ( $F(1,28)=4.70$ ,  $P=0.05$ ), regardless of the drug that they had received during training ( $F(1,28)=0.21$ , NS) (see Fig. 1). There was no interaction between training condition and test condition ( $F(1,28)=0.75$ , NS). Of the rats receiving saline in the test Session, 15 out of 16 did not cross within 600 s, while 11 of the 16 scopolamine rats failed to do so.

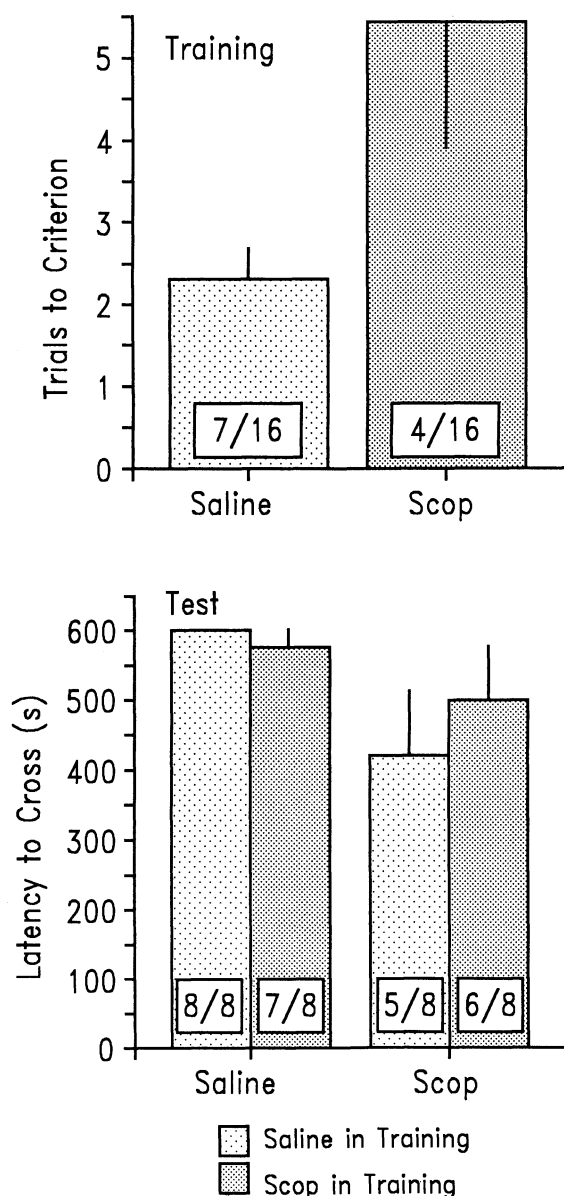


Fig. 1. Effects of scopolamine on acquisition (top) and recall (bottom) of the passive avoidance task in Experiment I. The top figure shows the mean number of trials required by the rats to reach criterion. The boxed ratios indicate the proportion of rats that reached criterion in a single trial (e.g., 7 of 16 rats receiving saline). The bottom figure presents the mean latency to enter the dark chamber in the test session. The boxed ratios indicate the proportion of rats that performed perfectly, remaining on the safe side for 600 s (e.g., 7 of 8 rats in the scop(olamine)/saline condition).

Rats from both the saline and scopolamine conditions escaped the first shock that they received in

training with equal latencies (saline:  $3.89 \pm 0.95$  s, scopolamine:  $3.85 \pm 1.09$  s,  $F(1,30)=0.001$ , NS). The rats receiving scopolamine tended to take longer to cross to the dark side for the first time during training (saline:  $16.87 \pm 3.62$  s, scopolamine:  $28.30 \pm 8.46$  s), but this difference was not significant ( $F(1,30)=1.54$ ,  $P=0.223$ ).

## Discussion

Scopolamine disrupted both the acquisition and the subsequent performance of the passive avoidance task, although its effect on performance was more powerful. That these effects are not due to motor effects of the drug treatment is made clear by the comparable escape latencies of the two groups, and by the increased latency to cross during training in the scopolamine rats. If anything, the drug tended to decrease locomotor activity, which would argue against a motor explanation for both the increased number of trials required to reach criterion, and the lower latency to cross during the test session. The lack of an interaction between drug condition during training and during test indicates the absence of state dependency (see also Elrod and Buccafusco 1988); the results can rightfully be ascribed to effects of the drug on acquisition and retention or recall of the passive avoidance task.

Because scopolamine impairs the acquisition of the passive avoidance task, it is very important that we trained our rats to criterion before testing the drug's effect on retention/recall. We can be confident that scopolamine in fact impairs retention or recall because we know that our rats had learned the task. In the absence of training to criterion, the locus of the effect of the drug during the test session could not have been determined. The two groups would almost certainly have differed in the test, but not because of the presence of the drug during that session. Instead, they would have differed because the comparison would have been made between rats that had learned and rats that had not. It is clear that training to criterion is essential if one wishes to study drugs that might impair retention or recall of a task.

## EXPERIMENT II

### Introduction

Experiment One demonstrated that the muscarinic antagonist scopolamine disrupts both the acquisition and subsequent performance of a passive avoidance task in rats. In Experiment Two we extended our analysis of the role of cholinergic mechanisms in passive avoidance by examining rats that had received lesions of the nbM, and thus presumably suffered a depletion in central acetylcholine. Some of these rats received the muscarinic agonist pilocarpine as we expected that the lesion would produce a deficit in performance, and we wanted to examine the ability of this pharmacological manipulation to overcome the lesion's effect.

### Method

#### *SUBJECTS AND SURGERY*

Seventy-three female Sprague Dawley rats from the same supplier as in Experiment One served as subjects. Rats were housed as described above. All rats underwent a surgical procedure prior to this experiment. Anesthesia was achieved by an injection of sodium pentobarbitol (42 mg/kg i.p., Butler). Bilateral electrolytic lesions of the nbM (1.3 mm posterior to Bregma,  $\pm 2.3$  mm from the midline, and 7.6 mm ventral to skull surface, from the atlas of Paxinos and Watson 1986) were produced in 39 rats by passing 1.5 mA DC current for 15 s through an anodal stainless steel insect pin electrode insulated except for 0.5 mm at the tip. An anal cathode was used. Sham surgeries were conducted in 34 rats using an identical procedure, except that no current was passed. The scalp incision was closed with wound clips, and the rats were allowed at least one week to recover prior to the behavioral testing. (We have noted previously that nbM lesions produce deficits in feeding, so each rat was given a chocolate chip cookie in milk on the day after surgery, and as necessary to maintain food intake *after that*.) Eight nbM rats and 3 sham operated

rats died either during surgery or prior to behavioral testing.

#### *DRUGS*

Pilocarpine nitrate (Sigma) and scopolamine methyl bromide (Sigma) were dissolved in isotonic saline. Drug solutions were prepared on a daily basis.

#### *APPARATUS*

The apparatus used in Experiment One was employed.

#### *PROCEDURE*

The behavioral training and testing were identical to the procedures employed in Experiment One, except that the number of fecal boli in the chamber at the end of the Test session was recorded for each rat.

A 2x2x2 (nbM/sham x Day 1 drug/saline x Day 2 drug/saline) factorial design was employed. The drug condition involved the peripheral muscarinic antagonist methyl scopolamine (1 mg/kg i.p., in a 1 mg/ml solution of isotonic saline) administered 20 min prior to the behavioral session, and pilocarpine (3 mg/kg, in a 3 mg/ml solution of isotonic saline) administered 15 min prior to the session. The saline condition involved two injections of isotonic saline (1 ml/kg, i.p.) administered 20 and 15 min prior to the session. As before, this allowed the independent assessment of the effects of pilocarpine on acquisition and recall of the passive avoidance task, and controlled for drug-state dependency.

Separate analyses of variance were used to compare step-through latencies, escape latencies, and trials to criterion of the lesion and drug conditions on Day 1, and to compare the step-through latencies and fecal boli counts of the lesion and drug conditions on Day 2.

### Results

Histological examination of the lesion site revealed that bilateral lesions of the nbM were not

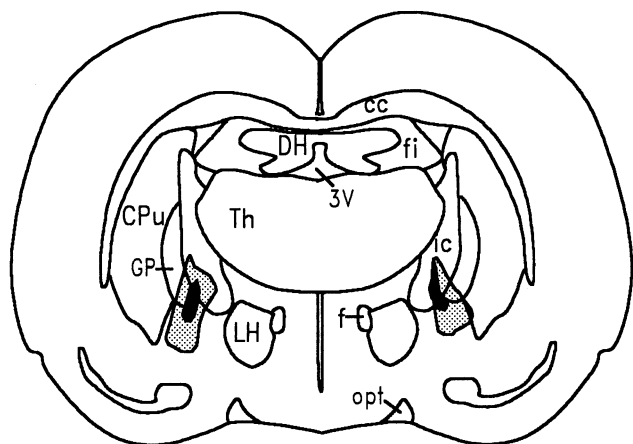


Fig. 2. Maximum and minimum extent of the nbM lesions. (Abbreviations: CPu, caudate-putamen; DH, dorsal hippocampus; GP, globus pallidus; LH, lateral hypothalamus; cc, corpus callosum; f, fornix; fi, fimbria; ic, internal capsule; opt, optic tract; after Paxinos and Watson 1986).

achieved in six rats; data from these subjects were excluded from further analysis. The extent of the largest and smallest successful lesions is illustrated in Fig. 2.

In acquisition, rats receiving pilocarpine required slightly more trials to reach criterion than did rats getting control injections of saline ( $F(1,52)=3.507$ ,  $P<0.07$ ). (See Figure 3). The nbM lesion had no effect on acquisition ( $F(1,52)=0.217$ , NS), and there was no Lesion  $\times$  Drug interaction ( $F(1,52)=0.693$ , NS). In the test session pilocarpine reduced the latency to enter the dark side ( $F(1,48)=5.404$ ,  $P<0.05$ ). The nbM lesion had no effect on this measure ( $F(1,48)=0.082$ , NS), nor did the drug condition in training ( $F(1,48)=0.143$ , NS). A significant interaction was present between drug condition in training and drug condition in testing ( $F(1,48)=4.954$ ,  $P<0.05$ ). Rats that had received control injections of saline on both days performed perfectly; those that had received pilocarpine on either day were impaired in the test. No other interactions were significant (Lesion  $\times$  Drug in training:  $F(1,48)=0.467$ ; Lesion  $\times$  Drug  $F(1,48)=0.023$ ; Lesion  $\times$  Drug  $\times$  Test  $F(1,48)=0.831$ ).

An examination of the non-parametric measure of the number of rats failing to cross within 600 s

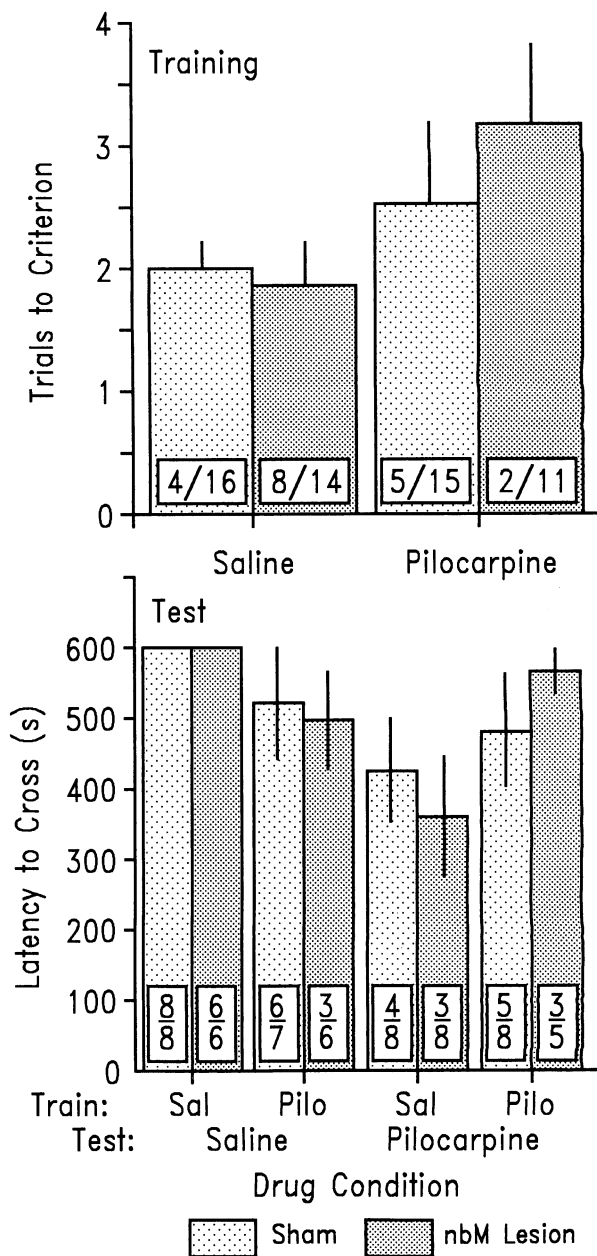


Fig. 3. Effects of pilocarpine and nbM lesions on the passive avoidance task of Experiment II. The mean number of trials to criterion is presented in the top figure. The boxed ratios indicate the proportion of rats that reached criterion in a single trial. The mean latency to enter the dark chamber appears in the lower figure. The boxed ratios present the proportion of rats that performed perfectly, remaining on the safe side for 600 s. Abbreviations: Sal, saline; Pilo, pilocarpine.

during the test reveals the same sort of drug effect as is shown by the examination of latency data. All rats receiving saline during both training and test



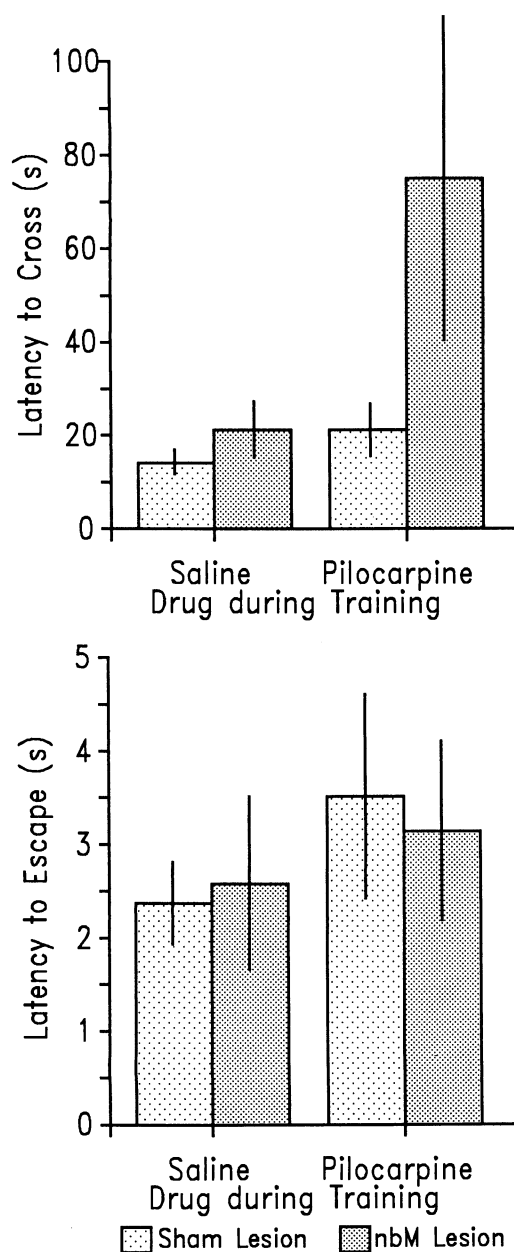


Fig. 4. Motor/motivational effects of pilocarpine and the nbM lesions. The upper figure presents the mean latency to enter the dark side for the first time during training. The mean latency to escape the first shock is presented in the lower figure.

failed to cross, while many rats that received pilocarpine in one or both sessions did cross.

During training all groups were equally fast to escape the shock, with neither the lesion ( $F(1,52)=0.004$ ) or the pilocarpine ( $F(1,52)=0.981$ ) having an effect (See Fig. 4). There was no interac-

tion ( $F(1,52)=0.109$ ). However, the nbM lesion significantly increased the latency to cross to the dark side in training ( $F(1,52)=4.048$ ,  $P<0.05$ ), and the pilocarpine tended to do the same ( $F(1,52)=3.816$ ,  $P<0.06$ ). The Lesion  $\times$  Drug interaction approached significance ( $F(1,52)=2.679$ ,  $P=0.108$ ), suggesting that the pilocarpine had a greater effect in the lesioned than in the sham-operated animals.

Pilocarpine and methyl scopolamine administered prior to the test session ( $F(1,48)=47.440$ ,  $P<0.0005$ ), or on the training day ( $F(1,48)=16.115$ ,  $P<0.0005$ ) almost eliminated defecation during the test session. The nbM lesion itself had no effect on defecation during the test ( $F(1,48)=0.060$ , NS), but interacted with the effect of the drug treatment during training such that lesioned rats given pilocarpine and methyl scopolamine in training were less likely than sham-lesioned controls to defecate in the test session ( $F(1,48)=4.890$ ,  $P<0.05$ ). Training and test session drug treatments also interacted, such that rats that had received saline on both days defecated more than those that had received pilocarpine and methyl scopolamine on either or both days ( $F(1,48)=14.758$ ,  $P<0.0005$ ).

## Discussion

Two important results were found in Experiment Two. First, pilocarpine disrupted the acquisition and performance of the task in both the nbM and sham lesioned rats. The impaired performance of rats that received pilocarpine during only the training or test session suggests that state-dependent learning might have played a role, but the impaired performance of the rats that received pilocarpine on both days indicates a specific effect of the drug treatment on memory. Second, the nbM lesion did not disrupt either the acquisition or later performance of the passive avoidance task.

That lesions of the nbM had no apparent effect on acquisition or recall of the passive avoidance task is hard to reconcile with the drug effects. Others have reported impaired passive avoidance performance following nbM lesions in rats (e.g., LoConte et al. 1982, Hepler et al. 1985, Murphy and

Boast 1985). The lack of lesion effect on learning and memory in our study might reflect our use of electrolytic lesions rather than the more commonly employed excitotoxic lesions. Lacking specific assay data concerning levels of acetylcholine remaining in the cortex following the lesion, it is possible that the lesion produced little or no depletion of cortical acetylcholine. We have presented the results in the interest of completeness, realizing that the absence of an effect is difficult to interpret.

In addition to the effects on learning and memory, the most dramatic effect of the drug treatment was the increase in the latency to cross to the dark side for the first time during the training session, most notable in the nbM lesioned rats. This effect was negligible in the sham-lesioned controls, and probably does not represent an impairment of motor abilities, as the nbM lesioned rats given the drugs were no slower to escape shock than the other groups. We believe this effect might provide behavioural evidence suggesting supersensitive cholinergic receptors following the nbM lesion, although why this should manifest itself as a decrease in exploratory behaviour is unclear. This result also suggests that the lesion did affect central cholinergic systems, making more difficult an understanding of the lack of effect of the lesion on our learning and memory measures.

## EXPERIMENT III

### Introduction

The impairment of learning and memory caused by the administration of pilocarpine in Experiment Two was somewhat surprising, as we expected this cholinergic agonist to have an effect opposite that of scopolamine. To ensure that this effect was not due simply to a debilitatingly large dose of pilocarpine, we ran an additional experiment in which several doses of pilocarpine were administered, and their effects on acquisition and recall were assessed. Because Experiment Two demonstrated the possibility of state-dependency, and in order to reduce the number of subjects necessary, rats in this experi-

ment received the same drug treatment on both days of the experiment. Groups of rats receiving drug on Day 1 and saline on Day 2, or *vice versa*, were not included.

### Method

#### SUBJECTS

Twenty-four female Sprague-Dawley rats from the same supplier as in Experiment One served as subjects. The rats were housed as described above.

#### DRUGS

Pilocarpine nitrate (Sigma) and scopolamine methyl bromide (Sigma) were dissolved in isotonic saline. Drug solutions were prepared on a daily basis.

#### APPARATUS

The apparatus used in Experiment One was employed.

#### PROCEDURE

Rats were randomly assigned to four groups of six subjects each. Passive avoidance training and testing proceeded as described above. Rats in each group received either 0, 0.33, 1.00, or 3.00 mg/kg pilocarpine, preceded by either saline (for the 0 mg/kg group) or 1.0 mg/kg methyl scopolamine, prior to both sessions.

### Results

A dose-dependent increase in the number of trials required to reach criterion was apparent during training; there was no evidence of a facilitative effect of the drug. Pilocarpine produced a dose-dependent increase in the number of rats that crossed during the test session, with 5 of 6 subjects performing perfectly at the 0 mg/kg dose and only 1 of 6 doing so at the 3.00 mg/kg dose (trend analysis for

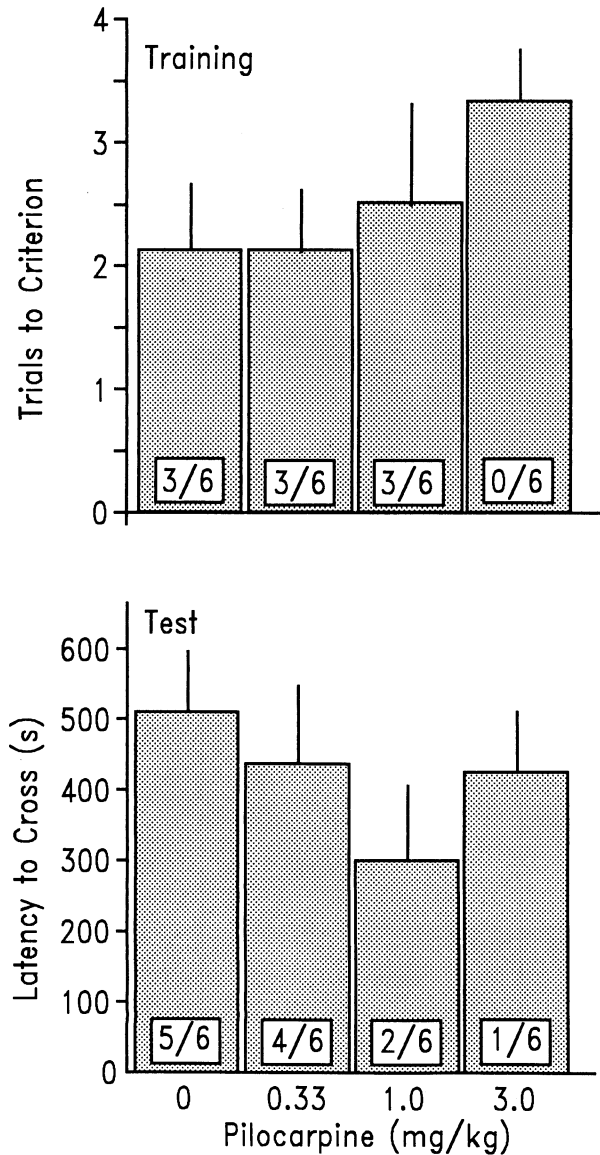


Fig. 5. Dose-dependent nature of the pilocarpine effect. As the dose of pilocarpine increases, the rats require more trials to reach criterion (upper figure). At the highest dose, none of the rats attained criterion in a single trial. During the test session (lower figure), increasing the pilocarpine dose resulted in fewer rats remaining on the safe side for all 600 s (boxed ratios). An interaction between motor and mnemonic effects resulted in the biphasic effect on latency to cross during the test session.

proportions,  $P < 0.05$ ). The latency scores during the test session did not vary in a monotonic fashion. Instead, the mean latency to cross decreased up through 1.00 mg/kg, and then increased at the 3.00 mg/kg dose (See Fig. 5). This probably reflects the

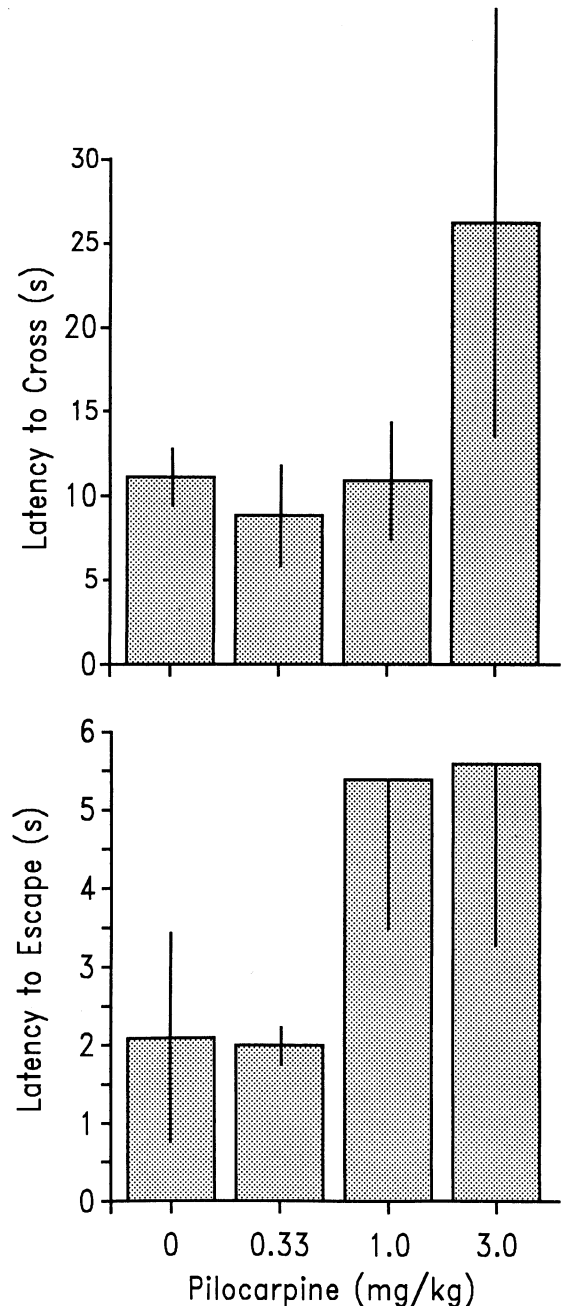


Fig. 6. Dose-dependent motor/motivational effects of pilocarpine. The mean latency to enter the dark side for the first time in the training session is presented in the upper figure. Mean latencies to escape the initial shock are presented in the lower figure.

motor-depressive effects of this drug: latencies to cross and latencies to escape during training both were increased by the higher doses of pilocarpine (See Fig. 6). As in the previous experiment, pilocar-

pine plus methyl scopolamine caused a dose-dependent reduction in defecation during both training and test sessions.

## Discussion

This experiment demonstrated that the impairment of learning and memory produced by pilocarpine in Experiment Two was not simply a reflection of a debilitatingly large dose of the drug. Dose-dependent increases in the number of trials to criterion, and the probability of crossing during test, indicate that the activation of central muscarinic receptors can interfere with learning and memory, as can their blockade by scopolamine.

## GENERAL DISCUSSION

When state-dependency is adequately controlled, and acquisition is assured by training the subjects to criterion, manipulations of the central cholinergic system impair both learning and retention/recall of a passive avoidance task. The muscarinic antagonist scopolamine interfered with both the acquisition of the passive avoidance task, and with subsequent performance of the task. This result is consistent with the generally held view of the effects of anticholinergics in learning and memory, and furthermore clarifies the locus of that effect by indicating that both acquisition and recall are affected. It also exonerates those investigators who have not controlled for state-dependent effects of scopolamine in tests of its effects on memory: as demonstrated in Experiment I, and in agreement with the results of Elrod and Buccafusco (1988), state-dependent effects do not seem to be a problem with this drug, at least in a passive avoidance setting.

Roitblat et al. (1989) and Buhot et al. (1989) have suggested that scopolamine has a major effect on the encoding of information, a conclusion that is supported by our results. Buhot et al. (1989) argued that scopolamine does not affect retrieval. Beatty and Bierley (1986), adequately controlling for state-dependent learning, demonstrated that both encoding (acquisition) and retrieval of information

about a radial arm maze are affected. Our data support this position. It is of course possible that task differences (open-field, radial arm maze, passive avoidance) interact with the drug's effects in such a way that cross-task comparisons are made difficult. Perhaps a within-subjects experiment examining scopolamine's effects on the acquisition and retrieval of several different behaviors would be of value.

The muscarinic agonist pilocarpine also impairs passive avoidance performance. By training the rats to criterion in Experiments II and III we were able to demonstrate that both acquisition and subsequent recall of this task were affected. In addition, we demonstrated that state-dependent learning can occur, and should be taken into account by investigators who administer pilocarpine.

The exact site within the brain at which these cholinergic manipulations disrupt passive avoidance is of course not addressed by these experiments. The lack of effect of our nbM lesions on passive avoidance seems to rule out this area, but others have reported deficits in passive avoidance following this lesion. Another possibility is that the septohippocampal pathway is more critically involved in this particular behavior. Direct application of scopolamine or pilocarpine into the hippocampus or neocortex might provide insight into this issue.

The results of these experiments implicate central cholinergic systems in two aspects of memory: its acquisition and retrieval. Interestingly, both blocking central muscarinic receptors with scopolamine, and activating those receptors with pilocarpine, impaired the passive avoidance performance, suggesting that a certain level of activity in cholinergic systems is optimal for learning, and that any deviation from this optimal level is detrimental.

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## REFERENCES

- Alfano D. P., Petit T. L. (1985) Postnatal lead exposure and the cholinergic system. *Physiol. Behav.* 34: 449-455.
- Altman H.J., Stone W.S., Ogren S.O. (1987) Evidence for a possible functional interaction between serotonergic and cholinergic mechanisms in memory retrieval. *Behav. Neural Biol.* 48: 49-62.
- Bammer G. (1982) Pharmacological manipulations of neurotransmitter involvement in passive avoidance responding: A review and some new results. *Neurosci. Biobehav. Rev.* 6: 247-296.
- Beatty W.W., Bierley R.A. (1986) Scopolamine impairs encoding and retrieval of spatial working memory in rats. *Physiol. Psychol.* 14: 82-86.
- Bianchi M., Panerai A.E. (1993) Reversal of scopolamine-induced amnesia by the GABA-sub(B) receptor antagonist CGP 35348 in the mouse. *Cog. Brain Res.* 1: 135-136.
- Bignami G., Allewa E., Chiarotti F., Laviola G. (1992) Selective changes in mouse behavioral development after prenatal benzodiazepine exposure: A progress report. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 16: 587-604.
- Buhot M.-C., Soffié M., Poucet B. (1989) Scopolamine affects the cognitive processes involved in selective object exploration more than locomotor activity. *Psychobiology* 17: 409-417.
- Chopin P., Briley M. (1992) Effects of four non-cholinergic cognitive enhancers in comparison with tacrine and galanthamine on scopolamine-induced amnesia in rats. *Psychopharmacology* 106: 26-30.
- Coyle J.T., Price D.L., Delong M.R. (1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* 219: 1184-1190.
- Cruz-Morales S.E., Duran-Arevalo M., Diaz del Guante M.A., Quirarte G., Prado-Alcalá R.A. (1992) A threshold for the protective effects of over-reinforced passive avoidance against scopolamine-induced amnesia. *Behav. Neural Biol.* 57: 256-259.
- Cruz-Morales S.E., Prado-Alcalá R.A. (1992) Participa el sistema colinérgico en la memoria de un aprendizaje mediado por niveles altos de reforzamiento negativo? *Rev. Mex. Psicol.* 9: 71-75.
- Davies P. (1979) Neurotransmitter-related enzymes in senile dementia of the Alzheimer's type. *Brain Res.* 171: 319-327.
- Davies P., Maloney A.J.F. (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* 2: 1403.
- Dawson G.R., Bentley G., Draper F., Rycroft W., Iversen S.D., Pagella P.G. (1991) The behavioral effects of heptyl physostigmine, a new cholinesterase inhibitor, in tests of long-term and working memory in rodents. *Pharmacol. Biochem. Behav.* 39: 865-871.
- Decker M.W., Gill T.M., McGaugh J.L. (1990) Concurrent muscarinic and  $\beta$ -adrenergic blockade in rats impairs place-learning in a water maze and retention of inhibitory avoidance. *Brain Res.* 513: 81-85.
- DeNoble V. J., Repetti S. J., Gelpke L. W., Wood L. M., Keim K. L. (1986) Vinpocetine: Nootropic effects on scopolamine-induced and hypoxia-induced retrieval deficits of a step-through passive avoidance response in rats. *Pharmacol. Biochem. Behav.* 24: 1123-1128.
- Drago F., Grassi M., Valerio C., Spadaro F., Dagata V., Lauria N. (1990) Effects of vinburnine on experimental models of learning and memory impairments. *Pharmacol. Biochem. Behav.* 37: 53-57.
- Drago F., Mauceri F., Nardo L., Valerio C., Genazzani A.A., Grassi M. (1993) Effects of cytidine-diphosphocholine on acetylcholine-mediated behaviors in the rat. *Brain Res. Bull.* 31: 485-489.
- Duran-Arevalo M., Cruz-Morales S.E., Prado-Alcalá R. A. (1990) Is acetylcholine involved in memory consolidation of over-reinforced learning? *Brain Res. Bull.* 24: 725-727.
- Elrod K., Buccafusco J.J. (1988) An evaluation of the mechanism of scopolamine-induced impairment in two passive avoidance protocols. *Pharmacol. Biochem. Behav.* 29: 15-21.
- Feigley D.A. (1974) Effects of scopolamine on activity and passive avoidance learning in rats of different ages. *J. Comp. Physiol. Psychol.* 87: 26-36.
- Galliani G., Cesana R., Barzaghi F. (1987) Reversal of scopolamine-induced amnesia in the mouse by arecoline. *Med. Sci. Res.* 15: 313-314.
- Hepler D.J., Wenk G.L., Cribbs B.L., Olton D.S., Coyle J.T. (1985) Memory impairments following basal forebrain lesions. *Brain Res.* 346: 8-14.
- Laborit H., Zerbib R. (1987a) Phorbol esters antagonize scopolamine-induced amnesia of a passive avoidance. *Res. Commun. Psychol. Psychiatr. Behav.* 12: 105-117.
- Laborit H., Zerbib R. (1987b) Role of various second messengers in the memorization of a passive or active avoidance. *Res. Commun. Psychol. Psychiatr. Behav.* 12: 193-204.
- Laborit H., Zerbib R. (1989a) Action du PMA (phorbol-myristate-acétate), de la scopolamine, du propranolol, et de l'oxotrémorine sur la mémorisation d'un test d'évitement passif ou actif. *L'Encéphale* 15: 22-35.
- Laborit H., Zerbib R. (1989b) Different environmental situations modify scopolamine-induced amnesia in mice assessed with a passive avoidance test. *Res. Commun. Psychol. Psychiatr. Behav.* 14: 143-156.
- Lo Conte G., Bartolini L., Casamenti F., Marconcini-Pepeu I., Pepeu G. (1982) Lesions of cholinergic forebrain nuclei: Changes in avoidance behavior and scopolamine actions. *Pharmacol. Biochem. Behav.* 17: 933-937.
- Lopez C.M., Govoni S., Battaini F., Bergamaschi S., Longoni A., Giaroni C., Trabucchi M. (1991) Effect of a new cognitive enhancer, alpha-glycerylphosphorylcholine, on scopolamine-induced amnesia and brain acetylcholine. *Pharmacol. Biochem. Behav.* 39: 835-840.

- Lorenzini C.A., Baldi E., Bucherelli C., Tassoni G. (1992) Mipratripe cancels scopolamine effects on the rat's acquisition of passive avoidance responses in two multitrial paradigms. *Pharmacol. Biochem. Behav.* 41: 715-718.
- Loullis C.C., Dean R.L., Lippa A.S., Meyerson L.R., Beer B., Bartus T. (1983) Chronic administration of cholinergic agents: Effects on behavior and calmodulin. *Pharmacol. Biochem. Behav.* 18: 601-604.
- Mashkovsky M.D., Andreyeva N.I., Parshin V.A., Golovina S.M. (1991) Antiamnesic properties of some antidepressants. *Farmakol. Toksikol.* 54: 4-5.
- Matsuoka N., Maeda N., Yamazaki M., Ohkubo Y., Yamaguchi I. (1992) Effect of FR121196, a novel cognitive enhancer, on the memory impairment of rats in passive avoidance and radial arm maze tasks. *J. Pharmacol. Exp. Ther.* 263: 436-444.
- Mondadori C., Gentsch C., Hengerer B., Ducret T., Borkowski J., Racine A., Lederer R., Häusler A. (1992) Pretreatment with aldosterone or corticosterone blocks the memory-enhancing effects of nimodipine, captopril, CGP 37 849, and strychnine in mice. *Psychopharmacology* 109: 383-389.
- Murphy D.E., Boast C.A. (1985) Searching for models of Alzheimer's disease: A comparison of four amnesic treatments in two behavioral tasks. *Ann. N. Y. Acad. Sci.* 444: 450-452.
- Murray C.L., Fibiger H.C. (1985) Learning and memory deficits after lesions of the nucleus basalis magnocellularis: Reversal by physostigmine. *Neuroscience* 19: 1025-1032.
- Mysliviček J. (1984) Some neurotransmitter mechanisms underlying early inhibitory conditioning. *Act. Nerv. Super.* 26: 206-207.
- Nabeshima T., Tohyama K., Murase K., Ishihara S., Kameyama T., Yamasaki T., Hatanaka S., Kojima H., Sakurai T., Takasu Y. (1991) Effects of DM-9384, a cyclic derivative of GABA, on amnesia and decreases in GABA-sub(A) and muscarinic receptors induced by cycloheximide. *J. Pharmacol. Exp. Ther.* 257: 271-275.
- Nagatani T., Yamamoto T., Takao K., Hashimoto S., Kasahara K., Sugihara T., Ueki S. (1991) Pharmacological profile of a potential anxiolytic: AP159, a new benzothieno-pyridine derivative. *Psychopharmacology* 104: 432-438.
- Nisshimura M., Shiigi Y., Kaneto H. (1990) State dependent and/or direct memory retrieval by morphine in mice. *Psychopharmacology* 100: 27-30.
- Okuyama S., Imagawa Y., Ogawa S., Araki H., Otomo S., Sakagawa T., Yamada S., Shima K. (1993) Effect of VA-045 on a closed head injury model in rats. *Life Sci.* 53: PL273-278.
- Paxinos G., Watson C. (1986) *The rat brain in stereotaxic coordinates*. Academic Press, Sydney.
- Perry E.K., Gibson P.H., Blessed G., Perry R.H., Tomlinson B.E. (1977) Neurotransmitter enzyme abnormalities in senile dementia. *J. Neurol. Sci.* 34: 247-265.
- Piercey M.F., Vogelsang G.D., Franklin S.R., Tang A.H. (1987) Reversal of scopolamine-induced amnesia and alterations in energy metabolism by the nootropic piracetam: Implications regarding identification of brain structures involved in consolidation of memory traces. *Brain Res.* 424: 1-9.
- Porsolt R. D., Lenégre A., Avril I., Stéru L., Doumont G. (1987) The effects of exifone, a new agent for senile memory disorder, on two models of memory in the mouse. *Pharmacol. Biochem. Behav.* 27: 253-256.
- Quirarte G.L., Cruz-Morales S.E., Díaz del Guante M.A., García M., Prado-Alcalá R.A. (1993) Protective effect of under-reinforcement of passive avoidance against scopolamine-induced amnesia. *Brain Res. Bull.* 32: 521-524.
- Reikkinen P., Sirvio J., Aaltonen M., Riekkinen P. (1990) Effects of concurrent manipulations of nicotinic and muscarinic receptors on spatial and passive avoidance learning. *Pharmacol. Biochem. Behav.* 37: 405-410.
- Roitblat H.L., Harley H.E., Helweg D.A. (1989) The effects of scopolamine on proactive interference and spatial delayed matching-to-sample performance in rats. *Psychobiology* 17: 402-408.
- Rush D. K. (1986) Reversal of scopolamine-induced amnesia of passive avoidance by pre- and post-training naloxone. *Psychopharmacology* 89: 296-300.
- Rush D.K., Streit K. (1992) Memory modulation with peripherally acting cholinergic drugs. *Psychopharmacology* 106: 375-382.
- Schettini G., Ventra C., Florio T., Grimaldi M., Meucci O., Scorziello A., Postiglione A., Marino A. (1992) Molecular mechanisms mediating the effects of L- $\alpha$ -glycerylphosphorylcholine, a new cognition-enhancing drug, on behavioral and biochemical parameters in young and aged rats. *Pharmacol. Biochem. Behav.* 43: 139-151.
- Spignoli G., Pepeu G. (1987) Interactions between oxiracetam, aniracetam, and scopolamine on behavior and brain acetylcholine. *Pharmacol. Biochem. Behav.* 27: 491-495.
- Whitehouse P.J., Price D.L., Clark A.W., Coyle J.T., DeLong M.R. (1981) Alzheimer's disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* 10: 122-126.
- Whitehouse P.J., Price D.L., Struble R.G., Clark A.W., Coyle J.T., DeLong M.R. (1982) Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science* 215: 1237-1239.
- Wiener N.I., Messer J. (1973) Scopolamine-induced impairment of long-term retention in rats. *Behav. Biol.* 9: 227-234.
- Worms P., Gueudet C., Péro A., Soubrié P. (1989) Systemic injection of pirenzepine induces a deficit in passive avoidance learning in rats. *Psychopharmacology* 98: 286-288.
- Yamamoto M., Ooyama M., Ozawa Y., Okada M., Tada S., Yamaguchi T., Endo H. (1993) Effects of indoloxazine hydrochloride, a cerebral activator, on passive avoidance

- learning impaired by disruption of cholinergic transmission in rats. *Neuropharmacology* 32: 695-701.
- Yamamoto M., Shimizu M. (1987) Cerebral activating properties of indeloxazine hydrochloride. *Neuropharmacology* 26: 761-770.
- Yamamoto M., Shimizu M., Yatsugi S. (1990) Effects of a new cerebral activator, indeloxazine hydrochloride on electroencephalogram and disturbed learning behavior in rodents. *Res. Commun. Psychol. Psychiatr. Behav.* 15: 1-15.
- Zanotti A., Valzelli L., Toffano G. (1986) Reversal of scopolamine-induced amnesia by phosphatidylserine in rats. *Psychopharmacology* 90: 274-275.
- Zerbib R., Laborit H. (1990) Chronic stress and memory: Implication of the central cholinergic system. *Pharmacol. Biochem. Behav.* 36: 897-900.

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