JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1999, 50, 1, 139—151

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A PARTIAL AGONIST AT STRYCHNINE-INSENSITIVE GLYCINE SITES FACILITATES SPATIAL LEARNING IN AGED RATS

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1-Aminocyclopropanecarboxylic acid (ACPC) is a high affinity ligand at strychnine-insensitive glycine sites of the N-methyl-D-aspartate (NMDA) channels and exhibits partial agonist properties in both biochemical and electrophysiological measures. While ACPC was reported active in animal models used to evaluate potential antidepressants and anxiolytics, its effects on learning and memory are unknown. In the present study we investigated the effects of ACPC on spatial learning in the Morris water maze. On a schedule of 12 learning trials, one trial per day, mature male Wistar rats (3 months of age) rapidly acquired the task. Electroconvulsive shocks applied after each of the learning trials markedly inhibited the consolidation of spatial memory. Administration of either a muscarinic agonist, arecoline (1 mg/kg) or ACPC (250 or 400 mg/kg) 20 min before each of the learning trials did not affect the acquisition of spatial learning. Aged (16 months old) male Wistar rats demonstrated difficulties in the acquisition of spatial learning task. In these subjects, ACPC administered 20 min before each of the learning trials at a dose of 400, but not 250 mg/kg, facilitated the acquisition of spatial memory as indicated on trials 3—5. ACPC did not affect the strength of spatial memory as assessed at the end of conditioning, by measuring swimming behavior of rats in the pool with platform removed. It is suggested that ACPC may alleviate learning deficits observed in the elderly.

Key words: *glycine/NMDA sites, 1-aminocyclopropanecarboxylic acid, ACPC, spatial learning, arecoline, electroconvulsive shock, rats.*

INTRODUCTION

N-methyl-D-aspartate (NMDA) receptor antagonists have been proposed as potential therapeutics for a number of disorders including stroke, depression, anxiety and drug addiction (for reviews see ref. 1—3). However, the majority of these compounds produce a variety of undesirable side-effects, including psychotomimetic-like effects, ataxia, neurotoxicity and impairment of learning and memory. To date, the only NMDA receptor antagonists that have been successfully used in clinical settings are low affinity, voltage-dependent

channel blockers including dextromethorphan, memantine or amantadine (1). Potentially more attractive for drug development (and less explored) are antagonists and partial agonists acting at the strychnine-insensitive glycine recognition site at NMDA receptors (1, 4, 5). Unlike the channel blockers (6) or competitive NMDA antagonists (7), these compounds do not produce neurodegenerative changes in the cingulate/retrosplenial cortex (8, 9) or psychotomimetic-like effects (10, 11).

Converging lines of evidence indicate that activation of NMDA receptors is necessary for brain plasticity and learning. Perhaps the first observation on the attenuation of learning and memory by NMDA receptor antagonists concerned the effects of phencyclidine on the repeated acquisition procedures in nonhuman primates (12) and on delayed matching to sample paradigm in pigeons (13). The same, inhibitory effects were subsequently reported in maze procedures and other tasks in rodents (14—17). Some studies indicate that partial and full antagonists of the glycine/NMDA sites may also attenuate learning and memory processes. For example, in rats responding with lever pressing for food delivery, 3-amino-1-hydroxy-2-pyrrolidinone [R(+)HA-966] impaired accuracy of the task performance (18). After intracerebroventricular (i.c.v.) administration, the glycine/NMDA antagonist, 7-chloro-kynurenic acid (7-Cl-KYN) attenuated passive avoidance learning (19, 20). Spatial learning was also impaired by intra-hippocampal administration of 7-Cl-KYN in the three-panel runway (21) or after i.c.v. administration in the Morris water maze (22). However, other studies demonstrate that impairment of learning by other and more specific glycine/NMDA antagonists is minor or undetectable, as is in the case of ACEA 1021 (5-nitro-6,7-dichloro-1,4-dihydroquioxaline-2,3 dione) in the 8-arm maze (23), MDL 104,653 in the repeated acquisition of behavioral chains schedule (17), as well as kynurenic acid and 7-Cl-KYN in the passive avoidance test (24).

Facilitation of glutamatergic transmission by increasing glycinergic tone may produce the reverse, beneficial effect on learning and memory processes. However, in the majority of reports, facilitation of learning was observed in animals whose learning capabilities were reduced by brain lesions or administration of amnestic agents. A high dose of glycine (750 mg/kg) itself reversed the learning deficit produced by enthorinal cortex lesion in a brightness discrimination task (25). Several investigators reported facilitation of learning by a partial glycine/NMDA agonist, D-cycloserine. Such effects were found in the passive avoidance task (26) and the radial maze (27) when learning capabilities were impaired by scopolamine and hippocampal lesion, respectively. Scopolamine-induced learning deficit was reversed by D-cycloserine in the Morris water maze (28—31). Typically, in these experiments, D-cycloserine produced no effects on spatial learning by itself.

The objective of the present experiments was to determine whether a partial agonist at glycine/NMDA sites, 1-aminocyclopropanecarboxylic acid (ACPC)

(32,33) could influence the acquisition of spatial learning in the Morris water maze. It was hypothesized that since ACPC behaves as a partial agonist in neurochemical and electrophysiological measures (e.g. 32, 34, 35), it will facilitate spatial learning. In addition, electroconvulsive shock (ECS) and arecoline's effects on spatial learning were investigated as a negative and positive control, respectively.

MATERIALS AND METHODS

Subjects

Male Wistar rats (~3 months of age, „mature" (300 g) and 16 months of age, „aged" (450 g)) were housed under standard laboratory conditions for at least 2 weeks before experiments started. Animals were kept in plastic cages, four rats per cage (58 × 37 × 19 cm) in the animal room with a controlled light-dark cycle (lights on 7:00; off: 19:00). Water and commercial food were available ad libitum.

Drugs

ACPC was kindly donated by Dr. M-L Maccecchini, Symphony Pharmaceuticals, VA, USA. Arecoline HCl was purchased from the commercial supplier (Sigma). Both drugs were dissolved in physiological saline; the volume of injections was 1 ml/kg (arecoline) or 4 ml/kg (ACPC).

Water Maze training

Rats were trained to find a metal platform, which was submerged 1 cm below the water surface in the swimming pool (50 cm high, 180 cm in diameter) as described previously (36,37). The platform was positioned half way between the wall and the center of the circular pool and remained in this position throughout the twelve training days. There was 1 swimming trial on each training day. Each trial started from one of the four compass points around the pool perimeter, with the sequence e.g., N, E, S, W, etc. Rats were gently placed into the water, facing the wall and the latency to find the platform was measured for each rat. Subjects were kept on the platform for 30 sec, after which the next trial started. If the rat failed to find platform, it was directed to the platform by experimenter. After completion of the swimming trial, rats were transferred to the "drying" cage and later, to their home cages. The experimental room contained numerous visual cues and had dispersed illumination, which allowed videotaping.

Experimental schedule

Prior to examining the effects of ACPC, the sensitivity of experimental setup to detect the memory-attenuating and -facilitating treatments was investigated in 4 groups of mature rats. The first 2 groups were trained as described and received either sham-electroconvulsive shock (ECS) (N=20) or ECS (150 mA, 0.5 sec) (N=8), immediately after each training session. The next 2 groups were treated ~ 20 min before each of the training sessions with placebo (saline) (N = 12) or 1 mg/kg of arecoline (N = 12). In a separate experiment, 3 groups of mature rats were treated with 0, 250 or 400 mg/kg of ACPC (N = 12, 15 and 12, respectively). The last experiment was

carried in a manner identical to the previous one with the exception that the aged rats were used. The number of rats for ACPC at doses 0, 250 or 400 mg/kg were 22, 11 and 10, respectively.

For each group of rats, on the 13th day of training, a „transfer test" was carried out. The transfer test consisted of 1 min. videotaped observation of swimming behavior in the pool with the platform removed. The analysis of swimming paths was carried out off-line using the EYE (J. Długopolski, Kraków, Poland) and Track-Analyzer (38) software. Before the transfer test, rats received no injections.

Statistics

The latencies to find the platform were analyzed using repeated-measures MANOVA and post hoc Newman-Keuls test (Statistica 5.0). Where appropriate, the Student's *t*-test was used to compare the training quadrant preference, otherwise one-way ANOVA was used.

RESULTS

All placebo-treated rats trained in the Morris water maze demonstrated rapid acquisition of spatial memory. As expected (36), ECS impaired spatial learning in mature rats. This effect was statistically significant when all 12 trials were subjected to the MANOVA: $F(1, 26) = 26.13$, $P < 0.001$ (*Fig. 1*).

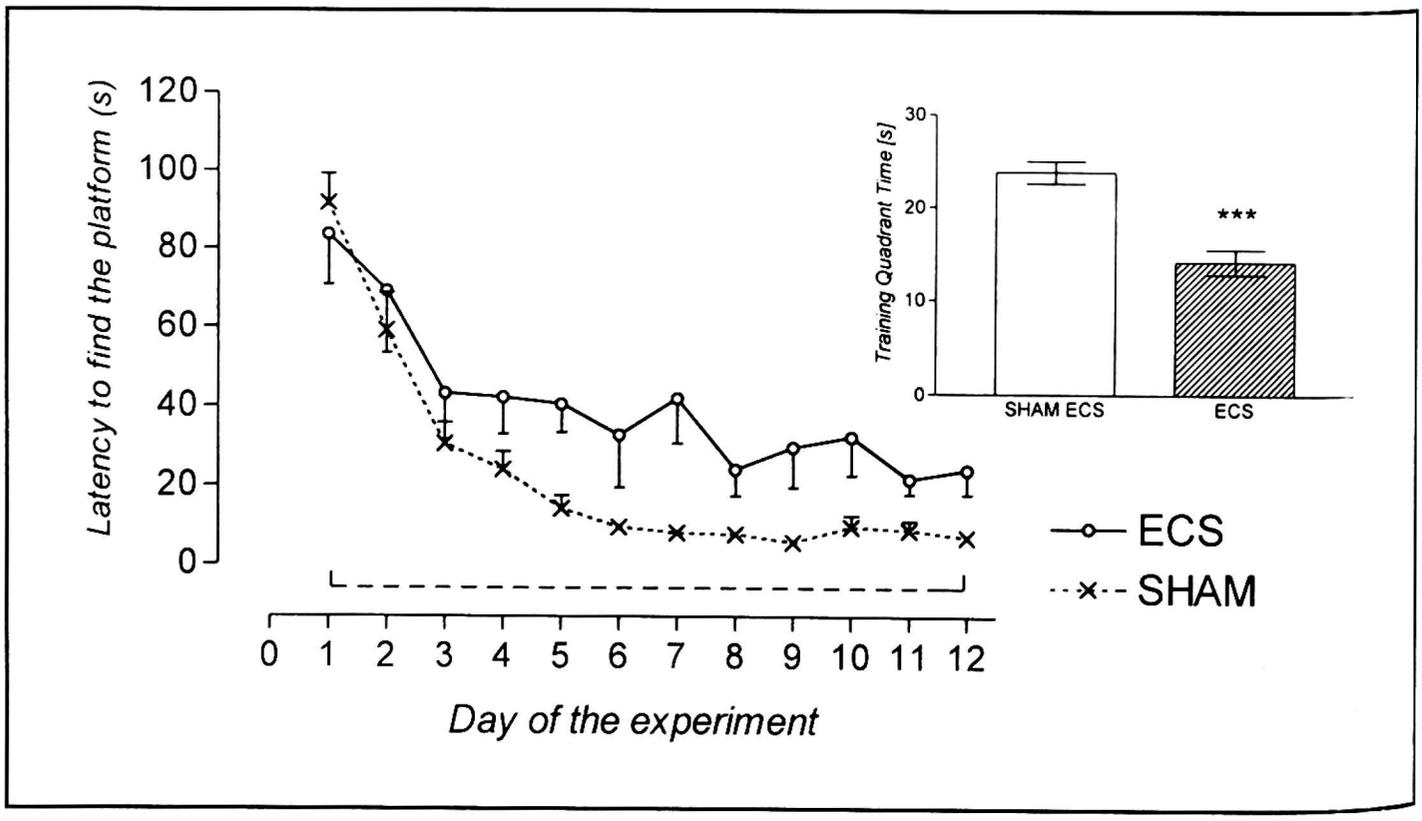

Fig. 1. Effects of Electroconvulsive Shock on the Acquisition of Spatial Learning in 3 Months Old (Mature) Rats. Presented are mean + or — S.E.M. escape latencies of control rats and rats that received electroconvulsive shocks (ECS) after each of the 12 swimming trials. The dashed line indicates that ECS-treated rats had significantly (MANOVA) longer latencies to find platform throughout the whole 12 swimming trials. Inset presents mean ± S.E.M. time spent by rats in the training quadrant during the transfer test (day 13 of experiment). Asterisks indicate statistically significant difference (Student's *t*-test).

In addition, ECS treatment resulted in the less preference for the training quadrant during the transfer test (*Fig. 1*, inset) $t = 4.52$, df $= 26$, $P < 0.001$. Treatment with arecoline prior to each of the swimming trials did not result in an alteration of the latencies to find the platform in mature rats: MANOVA ($F(1, 22) < 1$, *Fig. 2*. The inset of *Fig. 2*. shows that that there were no differences in the preference for the training quadrant on the transfer test.

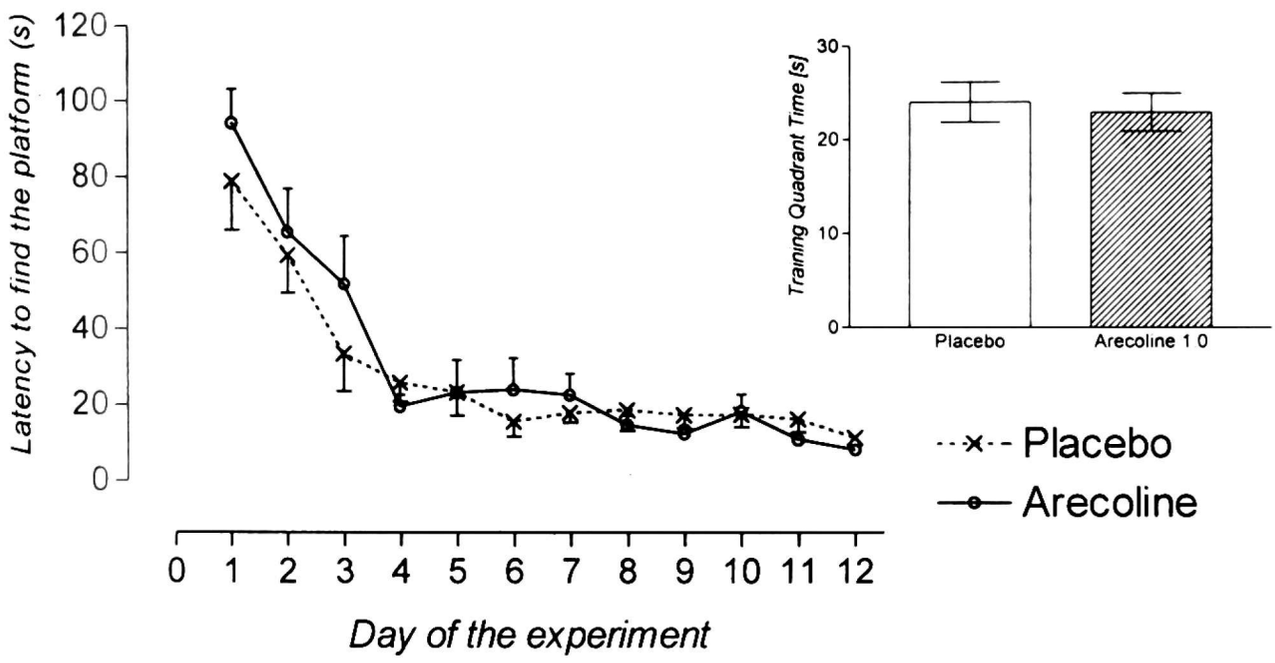

Fig. 2. Effects of 1 mg/kg of Arecoline on the Acquisition of Spatial Learning in 3 Months Old (Mature) Rats. Presented are mean + or — S.E.M. escape latencies of control rats and rats treated with arecoline 20 min before each of the 12 swimming trials. Inset presents mean ± S.E.M. time spent by rats in the training quadrant during the transfer test.

Mature rats did not respond to the treatment with ACPC with any alteration of swimming latencies (MANOVA: $F(2, 36) = 1.43$, $P > 0.05$, *Fig. 3*). The inset of *Fig. 3.* shows that there were no differences in the preference for the training quadrant on the transfer test.

When the ability to locate the platform position was compared between placebo-treated mature and aged rats, the latter group demonstrated markedly longer swimming latencies. This was observed for the first 8 days of training (MANOVA: $F(1, 32) = 6.29$, $P < 0.025$, *Fig. 4*). There were no differences between mature and aged rats in the preference for the training quadrant during the transfer test (*Fig. 4*, inset). The slower learning of aged rats compared to mature rats was not due to the deficits in sight, because a separate experiment, in which the speed of learning was compared when the platform was made visible did not demonstrate any differences (data not shown). In addition, there were no differences in the swim speed among groups tested (data not shown).

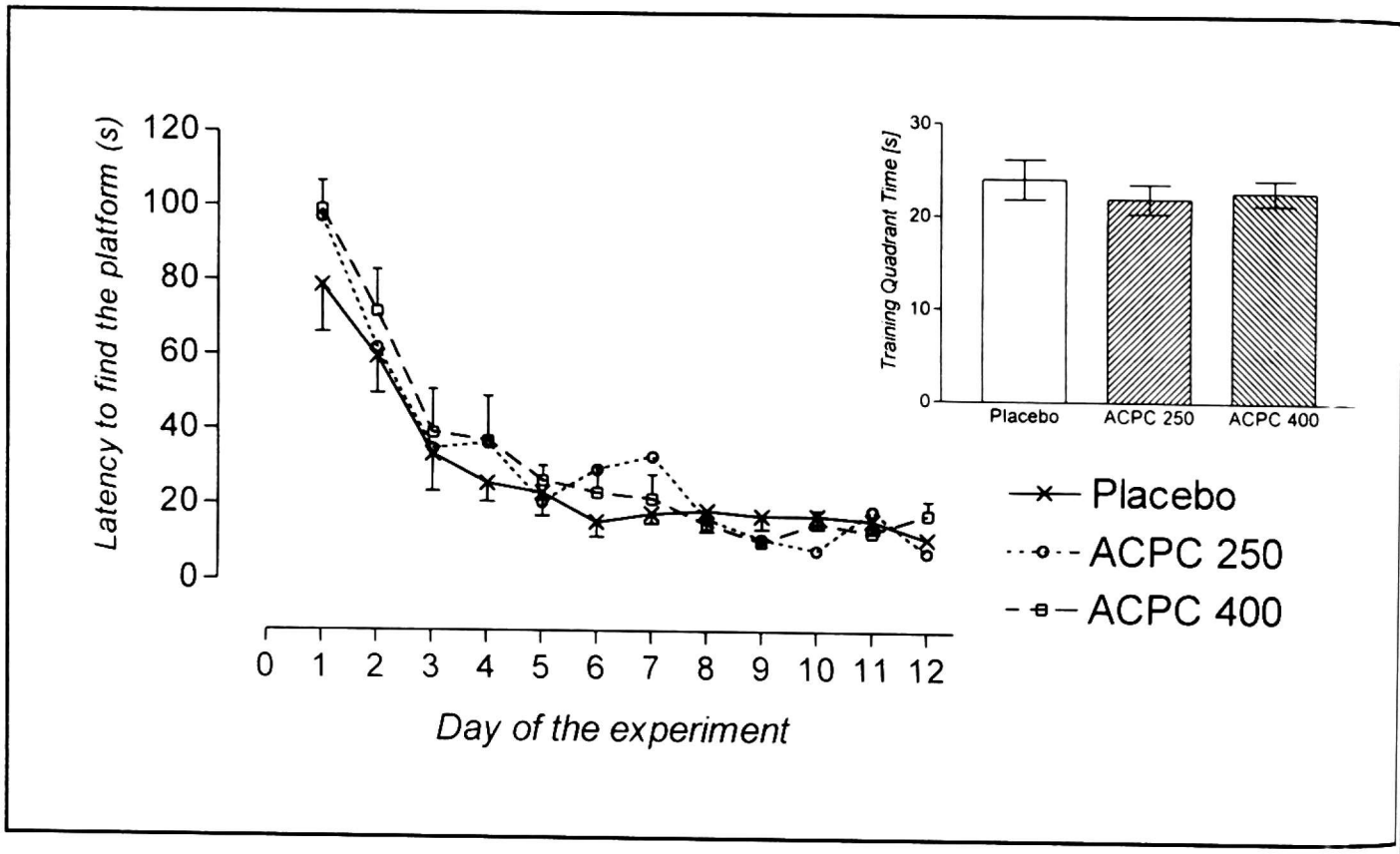

Fig. 3. Effects of 250 and 400 mg/kg of ACPC on the Acquisition of Spatial Learning in 3 Months Old (Mature) Rats. Presented are mean + or — S.E.M. escape latencies of control rats and rats treated with 250 and 400 mg/kg of ACPC 20 min before each of the 12 swimming trials. For the rats treated with 250 mg/kg of ACPC, the S.E.M. is not shown for clarity. Inset presents mean ± S.E.M. time spent in the training quadrant by rats during the transfer test.

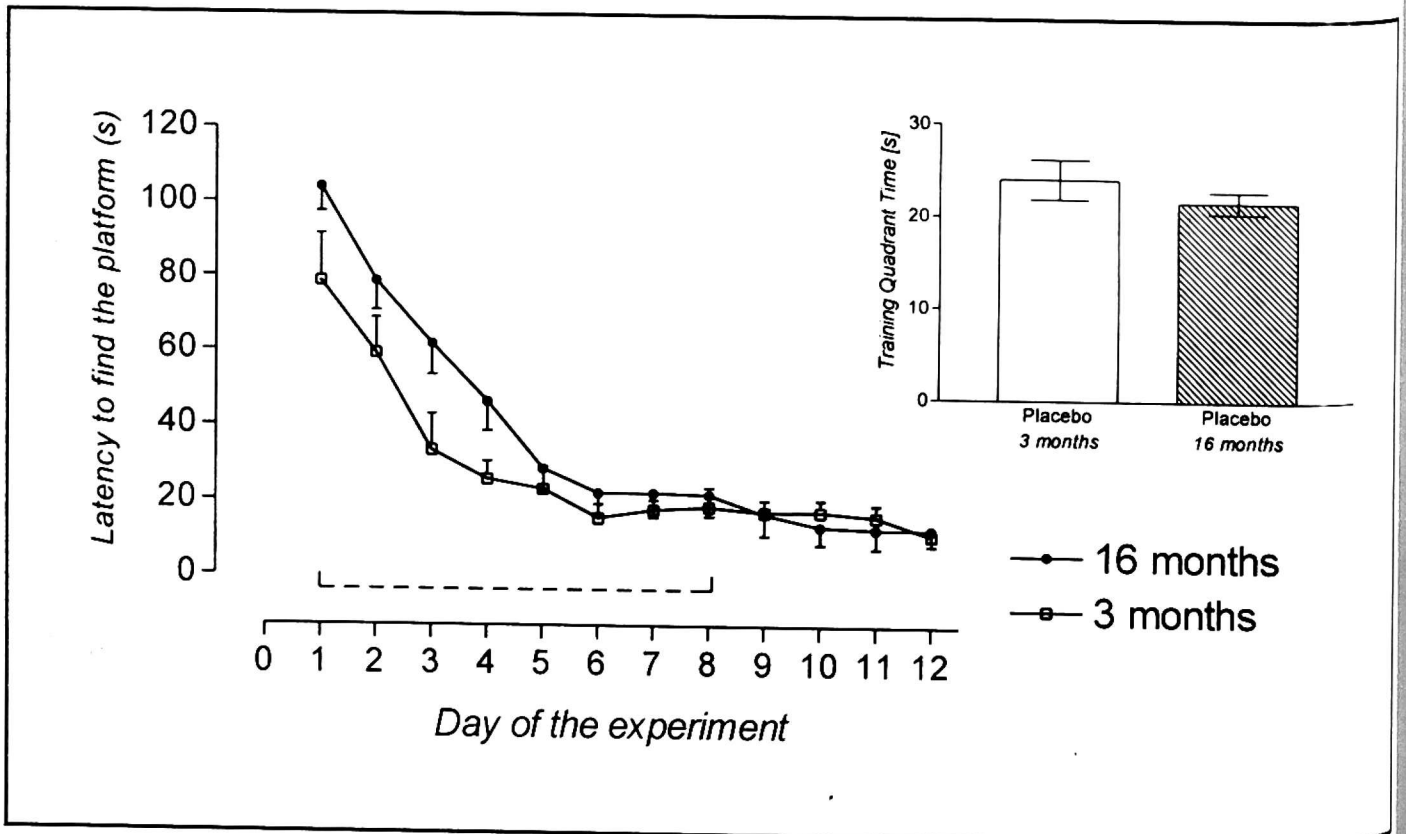

Fig. 4. The Effects of Aging on the Acquisition of Spatial Learning in Morris Water Maze. Presented are mean + or — S.E.M. escape latencies of rats being 3- and 16-months of age. The dashed line indicate that 16 months old rats were finding the platform slower than their 3-months old controls during the first 8 trials (MANOVA). Inset presents mean ± S.E.M. time spent by rats in the training quadrant during the transfer test.

Latencies to find the platform were compared for aged rats treated with ACPC. The MANOVA revealed differences among groups when latencies of days 3, 4 and 5 were compared: $F(2, 40) = 3.73$, $P < 0.05$ (*Fig. 5*). Post-hoc Newman Keuls test showed that aged rats treated with 400 mg/kg of ACPC before each of the learning trials were finding platform faster compared to placebo-treated controls, $P < 0.05$. There were no differences in the swim speed during the acquisition trials (data not shown) or training quadrant preferences during the transfer test (*Fig. 5*, inset).

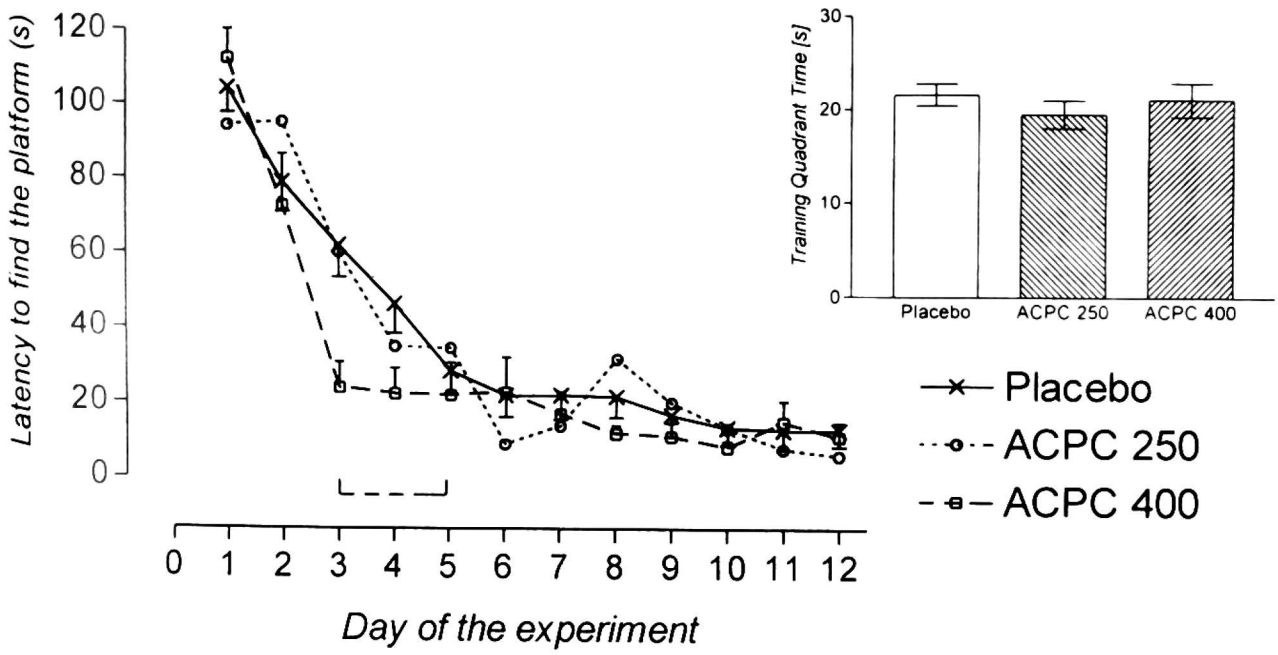

Fig. 5. Effects of 250 and 400 mg/kg of ACPC on the Acquisition of Spatial Learning in 16 Months Old (Aged) Rats. Presented are mean + or — S.E.M. escape latencies of control rats and rats treated with 250 and 400 mg/kg of ACPC 20 min before each of the 12 swimming trials. For the rats treated with 250 mg/kg of ACPC, the S.E.M. is not shown for clarity. The dashed line indicate that rats treated with 400 mg/kg of ACPC were finding the platform faster than placebo-treated controls on trials 3, 4 and 5 (MANOVA). Inset presents mean ± S.E.M. time spent by rats in the training quadrant during the transfer test.

DISCUSSION

The present results may be summarized as follows: ACPC facilitated spatial learning only in aged rats and only at a dose of 400 mg/kg. These effects were detectable only on days 3, 4 and 5 of the training session lasting for 12 days. Electroconvulsive shock attenuated consolidation of spatial memory in mature rats. Neither arecoline nor ACPC affected acquisition of spatial learning in mature subjects.

Investigators of memory facilitating drugs lack both a reliable model of human dementia and a standard, recognizable memory-facilitating reference

compound. The lack of these two basic tools in the learning and memory research (as opposed to other areas of psychopharmacology) is a serious obstacle in drug development. This prompts for a development of a variety of animal models of dementia, of which none to date seems to be fully satisfactory. The 3-months old (mature) rats used in the present study appeared insensitive to the treatment with ACPC and arecoline, a muscarinic agonist reported to facilitate learning. For arecoline, such effects were found in the active avoidance learning in mice (39) and social memory in rats (40). In these experiments arecoline facilitated the long-term and short-term memories, respectively, but only when given immediately *after* the learning trials. Although arecoline was demonstrated to facilitate spatial learning, data from this laboratory demonstrate that such effects were noted when it was administered just before the retention trial i.e., affecting the retrieval of spatial information (41). Since the search of literature data fails to provide evidence that arecoline facilitates the acquisition of spatial memory in the intact, relatively young animals, we attempted to use it as a positive control for ACPC, based on findings mentioned earlier (39—41).

The failure of ACPC and arecoline to affect acquisition of spatial learning in 3-months old rats is perhaps due to the fact that the Morris water maze test (42) is not very sensitive to investigate the effects of cognitive enhancers. In fact, data showing facilitation of learning in this test in the intact, relatively young rats are sparse at best. In a comprehensive review on the effects of different drugs on spatial learning, Brandeis and collaborators (43) reported that among 38 experiments, spatial learning was facilitated in only 2 instances (physostigmine and α-MSH). In addition, all examples of the facilitatory effects of D-cycloserine on learning processes were reported in subjects whose learning capabilities were somewhat reduced.

Aged rats showed impairment in spatial learning in the Morris water maze, which agrees with earlier reports (44). However, in these subjects, the facilitatory effect of ACPC on spatial learning was relatively modest and short lasting. One possibility to explain this finding may be that in aged subjects, beyond day 6 of training, the performance reached an optimum and that there was no room for further improvement („floor effect"). Indeed, a direct comparison of learning curves between 3- and 16-months old rats showed statistically significant longer swimming latencies of the latter group only when the latencies of days 1—8 were subjected to MANOVA. This indicates that beyond that day, aged rats were finding the platform as efficiently as 3 months old rats. Consistently, 3- and 16-months old rats did not differ in swimming preferences for the training quadrant as shown on the „transfer test" (*Fig. 4*, inset). Miyagawa and collaborators (45) demonstrated recently that aging does not affect spatial learning in a uniform way and that aged rats can be subdivided into the groups of memory-intact and memory-impaired individ-

uals. If such pre-selection would be done in the present study, perhaps the differences between mature and aged rats, as well as between placebo treated and ACPC treated aged rats, would be more remarkable. However, such pre-selection would preclude the possibility of studying the effects of ACPC on the acquisition of spatial information in experimentally naive subjects.

An alternative hypothesis explaining a relatively short-lived effect of ACPC on spatial learning may be considered. As noted by Lopes and colleagues (46), the pharmacological effects of chronically administered ACPC are either maintained (e.g., anxiolytic actions (47)), persists for some time after the washout period (e.g. neuroprotective actions (48, 49)), or are lost following chronic treatment (e.g., forced swim test (48) and *in vitro* neuroprotective actions (50)). Interestingly, the favorable cognitive effects of another glycine/NMDA partial agonist, D-cycloserine also seem to dissipate with the time this compound is administered. For example, Quartermain *et al.*, (51) found that D-cycloserine facilitated consolidation and retrieval of learning in the thirst-motivated linear maze in mice. However, the beneficial effects of D-cycloserine were not observed in mice pretreated with D-cycloserine twice daily for 15 days, suggesting a desensitization to its effects.

Regarding the specificity of facilitation of spatial learning by ACPC, two issues should be brought about. First, it is unlikely that ACPC improved the sight of aged rats, because in our hands, the intact, 16-months old rats did not differ from the mature rats in spatial learning, when the platform was made visible. Next, ACPC might increase the locomotor activity. This explanation is also unlikely, because ACPC did not influence swim speed in the present experiment, and appear not markedly affect locomotor activity in Trullas *et al.*, (52) and Lopes *et al.*, (46) studies.

The pharmacological profile of ACPC include anxiolytic (47), neuroprotective (48—50) and antidepressant effects (48) and is typical for compounds that inhibit the function of NMDA receptors (for reviews see (1—3)). Although ACPC is regarded as a partial agonist of the glycine/NMDA receptors, it has a rather high efficacy, and its memory-facilitating effects may be due to its glycine-mimetic properties. Its positive effects on learning in the aged but not mature rats may be due to the fact that aged rats demonstrate severe decline of the [^{3}H]-glycine binding sites in the telencephalic regions including the hippocampus and cerebral cortex (53, 54), suggesting that glycine may more potently enhance NMDA receptor function in aged animals (55).

Studies with antagonists and partial agonists of the glycine/NMDA receptors indicate that these compounds may have more favorable therapeutic and side-effect profile than other types of NMDA receptor antagonists, as they appear to produce no neurotoxicity in the cingulate/retrosplenial cortex (8, 9), and no psychotomimetic effects at anticonvulsant doses (10, 11). Unlike some noncompetitive NMDA receptor antagonists, the glycine/NMDA site antagon-

ists produce neither phencyclidine-like interoceptive stimulus (56, 57) nor phencyclidine-like motor stimulatory effects in rodents (11, 58).

Since our data demonstrate that ACPC not only fails to impair the acquisition of spatial learning but rather facilitates it in the aged rats, ACPC appears to be an attractive candidate for drug development for the other clinically useful antidepressant, anxiolytic and anti-addictive uses. Further studies are, however, required to elucidate the possible mechanism of these cognitive effects.

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Received: November 10, 1998
Accepted: January 1999

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