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Tetrahydroaminoacridine and D-cycloserine fail to alleviate the water maze spatial navigation defect induced by hippocampal inactivation

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Abstract

The present study examined the efficacy of single and combined treatment with an anticholinesterase, tetrahydroaminoacridine (i.p.), and a glycine-B site partial agonist, D-cycloserine (i.p.; a positive allosteric modulator of NMDA receptors), in alleviating the deficit in water maze spatial navigation induced by electrolytic lesion of the medial septum or lidocaine infusion into the dorsal hippocampi. In medial septum-lesioned rats, a combination of tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1} facilitated acquisition of the water maze test more effectively than either of the drugs alone. Single or combined treatment with tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1} had no effect on the water maze deficit induced by hippocampal lidocaine infusion. These results suggest that combined treatment with tetrahydroaminoacridine and D-cycloserine can effectively stimulate water maze spatial navigation, and that functioning of the hippocampus is a prerequisite for this effect.

Keywords: Tetrahydroaminoacridine; D-Cycloserine; Water maze; Passive avoidance; Alzheimer disease

1. Introduction

Several studies support the theory that a loss of cholinergic cells in the nucleus basalis and medial septal area contributes to the development of clinical dementia in Alzheimer disease (Bowen et al., 1983; Reinikainen et al., 1988). This hypothesis is supported by results showing that tetrahydroaminoacridine, an anticholinesterase, that inhibits the breakdown of acetylcholine, can alleviate the symptoms of clinical dementia in Alzheimer's disease (Sahakian et al., 1993; Knapp et al., 1994; Solomon et al., 1996). However, tetrahydroaminoacridine may have only limited ability to stimulate memory performance if there is severe atrophy of the hippocampus (Riekkinen et al., 1995). Indeed, in clinical trials the therapeutic effects of anticholinesterase compounds have proven to be modest (Knapp et al., 1994). Therefore, the development of more effective strategies for the treatment of the cognitive defects associated with Alzheimer's disease is urgently needed.

The degeneration of glutamate-containing pyramidal projection neurons in areas important for memory function, such as the hippocampus and the surrounding medial temporal lobe cortical structures, is also observed in Alzheimer's disease (Francis et al., 1994; Braak and Braak, 1996). Indeed, a combined dysfunction of cholinergic and glutamatergic mechanisms in areas important for memory function may contribute to the memory loss observed in Alzheimer's disease.

NMDA receptors and cholinergic mechanisms may synergistically modulate neuronal functioning and memory processing in the hippocampus and cortex (Hirotsu et al., 1989; Collingridge and Singer, 1990; Ohno et al., 1992; Bliss and Collingridge, 1993; Ohno et al., 1994). Electrophysiological studies with a model of synaptic plasticity, long term potentiation, indicate that the induction, but not the expression of long term potentiation, is stimulated by activation of muscarinic cholinergic and NMDA receptors (Markham and Segal, 1990). Furthermore, acetylcholine, acting via muscarinic acetylcholine M1 and/or M3 receptors, may produce a long-lasting enhancement of glutamate-mediated membrane depolarization in auditory cortex neurons (Cox et al., 1994). Behavioral studies have also revealed that cholinergic and NMDA synapses may act synergistically to mediate performance in tests to assess learning and memory (Aigner, 1995). For example, Sirviö et al. (1992) investigated the anti-amnesic effect of D-cycloserine, which is thought to enhance the physiological

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functioning of NMDA receptors, in a pharmacological model of cholinergic decline. They found that intraperitoneal (i.p.) administration of D-cycloserine at a low dose (1 mg kg^{-1}) alleviated the scopolamine-induced deficits in spatial reference memory in the water maze. However, the possibility that combined treatment with a cholinesterase inhibitor and D-cycloserine may more effectively enhance memory has not yet been studied.

We designed the present study to investigate the hypothesis that combined treatment with D-cycloserine and tetrahydroaminoacridine (Riekkinen et al., 1990) may more effectively attenuate the decline in water maze performance observed in medial septum-lesioned rats. Since the severity of hippocampal atrophy may limit the ability of tetrahydroaminoacridine to stimulate memory performance, we investigated also the effect of tetrahydroaminoacridine and D-cycloserine treatment on the water maze performance of rats whose dorsal hippocampus had been infused with lidocaine. This method can identify treatments which require adequate functioning of the hippocampus for a beneficial effect on water maze navigation accuracy.

2. Materials and methods

2.1. Animals

Young (3.5–4 months old; $n = 12$ per group; 120 total) male Han–Wistar rats were used in the present study. The rats were housed in a controlled environment with food and water available ad libitum (temperature 22 ± 2°C, lights on: 0700–1900 h, humidity 60%). We had the permission of the municipal government of Kuopio to perform these studies.

2.2. Surgery, biochemistry and histology

The rats were deeply anesthetized with Chlornembutal®. The skull was flat during surgery. Medial septum (A: 0.0 mm, M: 0.0 mm, D: −7.0 mm; relative to bregma) lesions were made by the passage of anodal DC current (2 mA, 5 s) through stainless steel electrodes (0.4 mm diameter, 0.7 mm at the uninsulated tip) (Riekkinen et al., 1990). Controls were treated identically, but no current was applied (sham-lesioned). Rats recovered for 1 week before the start of behavioral studies.

Separate groups of rats were used for lidocaine microinfusion studies. The stainless steel cannulas (23 gauge) were bilaterally implanted in the dorsal hippocampus. The coordinates were: M: ±2.2 mm, A: −3.4 mm posterior to the bregma, D: −3.2 mm ventral, measured from the surface of the skull at the bregma. The cannulas were fixed to the skull with acrylic dental cement. Dummy cannulas were screwed in to keep the guide cannulas covered and patent. A recovery period of 1 week was allowed before the behavioral studies were started. Before the actual drug infusions (infusion cannula: 30 gauge), the dummy cannulas were removed. Lidocaine (2% in 0.1 M phosphate-buffered saline, pH 7.35) was infused first into the right hippocampus (2 min infusion time, 1 min diffusion time) and then into the left hippocampus (2 min infusion time, 1 min diffusion time). Half of the rats received the infusions in a reversed order. A interval of 5 min was allowed after the infusions were completed before behavioral training.

The medial septum-lesioned and control rats were decapitated 3 days after the end of behavioral testing. The brains of the rats were removed and dissected on ice. Hippocampi were removed bilaterally for biochemical analysis and stored at −75°C until assayed. A modified method of Fonnum (1975) was used to measure choline acetyltransferase activity in samples from all the medial septum-lesioned rats used in the study to examine the action of single and combined tetrahydroaminoacridine and D-cycloserine (Riekkinen et al., 1990). A brain sample containing the lesioned area from other rats of these groups was collected for histology.

All the rats that had hippocampal cannulas were decapitated 1 day after the end of the behavioral experiments and processed for histology as described below.

The brain samples containing the lidocaine infusion sites were placed into 4% formalin and 24 h later immersed in 30% sucrose. Serial sections were cut at 60 μm and every fourth section was mounted on a glass slide and stained with Cresyl violet for subsequent verification of cannula placement. The location of the infusion cannulas and medial septum lesions was histologically studied in all sections from all of the rats.

2.3. Drugs

The dose of tetrahydroaminoacridine (3 mg kg^{-1}) used was based on previous findings that 3 mg kg^{-1} tetrahydroaminoacridine attenuates medial septal lesion-induced water maze and passive avoidance deficits (Riekkinen et al., 1990, 1991), whereas slightly higher doses (> 5 mg kg^{-1}) are detrimental to the performance of these tasks. The D-cycloserine dose of 10 mg kg^{-1} was selected on the basis of our unpublished dose–response study, which showed that acute treatment with D-cycloserine at this dose facilitates the water maze behavior of medial septum-lesioned rats. Tetrahydroaminoacridine and D-cycloserine were dissolved in 0.9% NaCl (2 ml kg^{-1}) and injected i.p. 45 min before behavioral testing. Injections of NaCl (0.9%; 2 ml kg^{-1}) were used for control purposes. The drug treatments are described in detail in the results section.

In the experiment with medial septum- and sham-lesioned rats, five groups of 12 rats were used. One group of sham-lesioned rats was treated with vehicle. Four groups of medial septum-lesioned rats were treated with vehicle, tetrahydroaminoacridine 3 mg kg^{-1}, D-cycloserine 10 mg kg^{-1} or tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1}.

In the experiment with hippocampal lidocaine- and hippocampal vehicle-infused rats, five groups of 12 rats

were used. One group of vehicle-infused rats was treated with vehicle. Four groups of hippocampal lidocaine-infused rats were used and they were treated with vehicle, tetrahydroaminoacridine 3 mg kg^{-1}, D-cycloserine 10 mg kg^{-1} or tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1}.

2.4. Water maze

The swimming patterns of the rats as they searched the hidden platform were monitored with a computerized video tracking system (Riekkinen et al., 1990). The computer calculated the daily mean swimming speed (m s^{-1}), distance swam (cm) to the hidden platform and the crossing over the previous location of the escape platform. The daily escape distance swam was stored for statistical analysis of the effects of drug treatment on water maze acquisition. The starting locations (labeled North, South, East, and West) were located arbitrarily on the pool rim. The pool was divided into four quadrants (Southwest, Southeast, Northwest, and Northeast) and three annuli of equal surface area. Rats were placed in the water, with their nose pointing toward the wall, at one of the starting points. If the platform was located at the southwest quadrant, the first daily trial was started always from the north or east. The starting points of second and third trials were selected in a semi-random manner. Those rats that did not find the platform during the maximum duration of a trial (70 s) were placed on the platform by the experimenter. The rats were allowed to stay on the platform for 5 s. An intertrial interval of 30 s was used between trials.

The sham-lesioned and medial septum-lesioned rats were trained to find a hidden platform located in the middle annulus of the southwest quadrant on 5 consecutive days (three 70-s trials per day). On the 6th day, the platform was removed and spatial bias was assessed (crossings over the previous platform location) during a single 70-s trial.

Those rats that received a daily infusion of vehicle or lidocaine into the dorsal hippocampus before training trials were trained on 4 days only. The spatial bias was assessed immediately after the last training trial on the fourth day during a single 70-s trial.

2.5. Statistics

A one-way-analysis of variance (ANOVA) followed by Bonferroni post-hoc multiple group comparison was used to analyze data.

3. Results

3.1. Medial septum-lesioned rats

3.1.1. Escape performance

Medial septum-lesioned rats had a longer escape distance during the platform training days and a lower spatial bias during the 6th testing day than the sham-lesioned rats (Overall: $F(4,54) > 12.1$, $P < 0.01$; Bonferroni test: $P < 0.05$; for both) (Fig. 1). In medial septum-lesioned rats a single dose of either tetrahydroaminoacridine 3 mg kg^{-1} or D-cycloserine 10 mg kg^{-1} decreased the escape distance (Fig. 1) and significantly improved the spatial bias measure (Bonferroni test: $P < 0.05$, for both comparisons) (Table 1). Further, a combination of tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1} significantly decreased the escape distance more effectively than either tetrahydroaminoacridine 3 mg kg^{-1} or D-cycloserine 10 mg kg^{-1} alone (Bonferroni test: $P < 0.05$, for both) (Fig. 1). However, combined treatment with tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1} had only a non-significant tendency to improve the spatial bias measure as compared to that for either drug alone (Bonferroni test: $P > 0.05$; for both) (Table 1).

3.1.2. Swimming speed

Medial septum-lesioned rats swam faster than the sham-lesioned rats during the platform training period (Overall: $F(4,54) = 10.0$, $P < 0.01$; Bonferroni test: $P < 0.05$). Tetrahydroaminoacridine 3 mg kg^{-1} and combined tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1}-treated medial septum-lesioned rats swam as fast as the controls (Bonferroni test: $P < 0.05$; for both). D-cycloserine 10 mg kg^{-1}-treated medial septum-lesioned rats swam faster than the sham-lesioned rats (Bonferroni

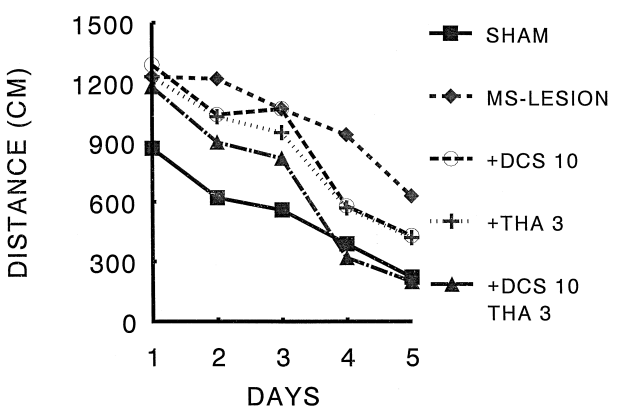

Fig. 1. Daily treatment (i.p.) with D-cycloserine and tetrahydroaminoacridine before the training trials during the 5-day platform training period facilitated the water maze spatial escape performance of medial septum-lesioned rats and a combined treatment with effective doses stimulated water maze navigation performance more effectively than either drug alone. During the training trials the escape platform was located in a fixed place. The effects of single or combined D-cycloserine 10 mg kg^{-1} and tetrahydroaminoacridine 3 mg kg^{-1} were evaluated. Escape distance to the hidden platform was used as an index of spatial learning during the 5 consecutive training days. *Y*-axis: escape distance to the hidden platform in centimeters. The values represent daily group means. *X*-axis: training days 1–5. Abbreviations: DCS, D-cycloserine; THA, tetrahydroaminoacridine.

Table 1
The effects of single or combined tetrahydroaminoacridine and D-cycloserine treatment administered before daily water maze trials on the deficit in spatial bias performance (a decreased number of platform crossings) induced by medial septum lesion (MS-l)

Groups	Sham-l	MS-l	THA 3	DCS 10	THA3 + DCS 10
crossings	4.3 ± 0.9	1.6 ± 0.5[a]	2.6 ± 0.7[a,b]	3.5 ± 0.7[b]	4.1 ± 0.9[b]

Groups	Vehicle	Lidocaine	THA3	DCS10	DCS10 + THA3
crossings	3.9 ± 2.1	0.4 ± 0.5[c]	0.3 ± 0.4[c]	0.3 ± 0.2[c]	0.4 ± 0.2[c]

[a] $P < 0.05$ vs. sham-lesioned.
[b] $P < 0.05$ vs. MS-lesioned vehicle treated (Duncan's post hoc test).
[c] $P < 0.05$ vs. vehicle infused (Duncan's post hoc test).
All the groups were trained for 5 days to find the hidden platform and on the 6th day a single spatial bias test was conducted. The platform was withdrawn during the spatial bias test and the rats were allowed to swim freely for 70 s.
The lack of an effect of single or combined tetrahydroaminoacridine and D-cycloserine treatment in rats subjected to dorsal intrahippocampal lidocaine infusions administered before water maze training (4 days of platform training) on spatial bias is shown. The spatial bias was measured immediately after the last platform trial, as described above.
The number of crossings over the previous platform location were calculated. The group means ± S.D. are shown. Doses are expressed mg kg^{-1}.
DCS, D-cycloserine
THA, tetrahydroaminoacridine.

test: $P < 0.05$), but as fast as the vehicle-treated medial septum-lesioned rats (Bonferroni test: $P < 0.05$) (sham-lesioned: 21.2 ± 1.2; medial septum-lesioned + vehicle: 28.3 ± 2.1; + tetrahydroaminoacridine 3 mg kg^{-1}: 22.3 ± 2.1; + D-cycloserine 10 mg kg^{-1}: 29.4 ± 2.5; + tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1}: 22.9 ± 2.5; group mean ± S.D., cm s^{-1}).

3.2. Hippocampal lidocaine-infused rats

3.2.1. Escape performance

We observed that daily administration of lidocaine into the dorsal hippocampus just prior to the behavioral training session clearly increased the escape distance and decreased spatial bias measured immediately after the last training trial on the 4th day of platform training (Overall: $F(4,54) = 18.1$, $P < 0.001$; Bonferroni test: $P < 0.05$; for both) (Fig. 2). Comparison of lidocaine-infused rats that were injected with vehicle, tetrahydroaminoacridine 3 mg kg^{-1}, D-cycloserine 10 mg kg^{-1} or the combination of tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1} revealed no drug treatment-induced improvement in escape distance or spatial bias measures (Bonferroni test: $P > 0.05$).

3.2.2. Swimming speed

Lidocaine infusion increased the swimming speed of rats compared with that of the vehicle infused rats in both experiments (Overall: $F(4,54) = 9.4$, $P < 0.01$). Tetrahydroaminoacridine 3 mg kg^{-1} and the combination of tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1} decreased the swimming speed of lidocaine-infused rats (Bonferroni test: $P < 0.05$); no difference was found between these two groups (Bonferroni test: $P > 0.05$). In contrast, D-cycloserine 10 mg kg^{-1} alone had no effect on the lidocaine-induced increase in swimming speed (Bonferroni test: $P > 0.05$) (vehicle-infused: 22.2 ± 1.3; lidocaine-infused + vehicle: 27.2 ± 2.2; + tetrahydroaminoacridine 3 mg kg^{-1}: 21.9 ± 2.4; + D-cycloserine 10 mg kg^{-1}: 30.0 ± 3.5; + tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1}: 23.9 ± 3.6; group mean ± S.D., cm s^{-1}).

3.3. Biochemistry and histology

Choline acetyltransferase activity (nmol mg $protein^{-1}$ min^{-1}) in the hippocampus of medial septum-lesioned rats was decreased significantly (sham-lesioned: 1.21 ± 0.16 vs. medial septum-lesioned: + vehicle: 0.54 ± 0.13; + tetrahydroaminoacridine 3 mg kg^{-1}: 0.57 ± 0.15; + D-cycloserine 10 mg kg^{-1}: 0.54 ± 0.11; + tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1}: 0.54 ± 0.13; mean ± S.D.; Bonferroni test: $P < 0.05$ vs. sham-lesioned). The choline acetyltransferase activity in the various medial septum-lesioned groups did not differ (Bonferroni test: $P > 0.05$).

The medial septum lesions destroyed tissue in the medial septum and vertical diagonal band nucleus. Histological analysis of Cresyl violet-stained sections revealed that the infusion cannulas had been accurately placed in the dorsal hippocampus.

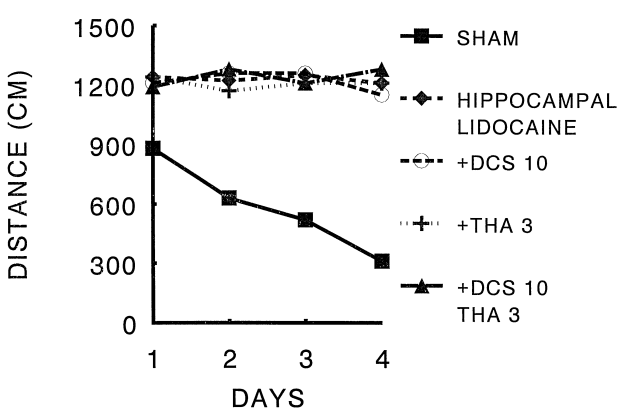

Fig. 2. Infusion of lidocaine into the dorsal hippocampus before the training trials disrupted water maze (4 training days, fixed platform location) spatial navigation in rats. Treatment with single or combined tetrahydroaminoacridine 3 mg kg^{-1} and D-cycloserine 10 mg kg^{-1} administered before the daily water maze training sessions had no effect on water maze spatial navigation. *Y*-axis: escape distance to the hidden platform in centimeters. The values represent daily group means. *X*-axis: training days 1–5. Abbreviations: DCS, D-cycloserine; THA, tetrahydroaminoacridine.

4. Discussion

The present study indicates that combined treatment with tetrahydroaminoacridine and D-cycloserine facilitates the water maze performance of medial septum-lesioned rats more effectively than either drug on its own. In contrast, neither drug alone or in combination, had an effect on the spatial navigation defect induced by hippocampal infusion of lidocaine. These results indicate that one needs a functioning hippocampus if one wishes to preserve the water maze performance-improving effects of cholinesterase inhibitors and NMDA modulators.

In our study we observed that destruction of the medial septal area by electrolysis produced a qualitatively similar effect on water maze performance as infusion of lidocaine into the dorsal hippocampus. However, a comparison between the experiments revealed that hippocampal inactivation by lidocaine infusion produced a more pronounced defect than medial septum lesioning, as the escape distances of lidocaine-treated rats were considerably greater than those of the medial septum-lesioned rats. Therefore, it is possible that the performance failure in the water maze test caused by medial septum lesion is to some extent caused by an impaired function of the septohippocampal projections or transection of some of the hippocampal outputs to subcortical structures. The site of action of systemically injected drugs to improve water maze navigation is difficult to pinpoint. However, our present study demonstrated that functioning of the hippocampus is essential for the stimulatory effect of D-cycloserine and tetrahydroaminoacridine on spatial navigation behavior. Therefore, it is possible that D-cycloserine and tetrahydroaminoacridine caused normalization of hippocampal functioning that is necessary for spatial information processing and learning in rats. At the present moment we are carrying out studies with hippocampal microinfusions to test this hypothesis.

It is relevant to compare the time course of action of drugs that block muscarinic acetylcholine or NMDA receptors to the time course of action of tetrahydroaminoacridine and D-cycloserine. Delivery of cholinergic or NMDA receptor antagonists before acquisition sessions impairs performance in several tests used to assess learning and memory. However, delivery of cholinergic or NMDA receptor antagonists immediately after training trials or only before performance is measured in pretrained animals has relatively modest, if any, effect on performance (Aigner, 1995). A similar time course of action has been described for NMDA antagonists. A non-competitive NMDA antagonist, MK-801, had no effect on water maze reference memory performance when injected after training trials but markedly disrupted water maze acquisition when administered before training (Heale and Harley, 1990). Furthermore, a non-competitive NMDA antagonist, disrupted acquisition but had no effect on retrieval of previously acquired spatial information during a spatial bias test in the water maze (Morris et al., 1986). These results suggest that the effects of tetrahydroaminoacridine and D-cycloserine on water maze escape behavior in medial septum-lesioned rats may be mediated by an increase in cholinergic and NMDA function, both of which improve functioning of the brain mechanisms necessary for acquisition of the water maze navigation task. Furthermore, our data may also indicate that combined activation of cholinergic and NMDA receptor function improves spatial navigation behavior more effectively than modulation of either of the systems alone.

We observed that D-cycloserine alleviated deterioration of spatial navigation accuracy induced by the medial septum-lesion induced, since it decreased escape distance and increased spatial bias. Furthermore, D-cycloserine had no effect on the locomotor hyperactivity produced by the medial septum-lesion, suggesting that the treatment alleviated only the dysfunction of spatial processing in medial septum-lesioned rats. In fact, we confirmed our previous finding that tetrahydroaminoacridine normalizes the swimming speed in medial septum-lesioned rats. However, tetrahydroaminoacridine 3 mg kg^{-1} decreased the swimming speed of medial septum-lesioned and hippocampal lidocaine-infused rats, suggesting that tetrahydroaminoacridine acts independently of the septohippocampal system to decrease the swimming speed. Furthermore, this also indicates that the decrease in swimming speed in medial septum-lesioned rats induced by tetrahydroaminoacridine is not causally related to improved spatial navigation in the water maze and is not mediated by the same cholinergic synapses.

The present results suggest that drugs that positively modulate NMDA receptors may have positive therapeutic effects and that combination therapy with a cholinesterase inhibitor and a NMDA modulator could stimulate cognitive functions more effectively in Alzheimer's disease than either agent alone. However, D-cycloserine is an experimental molecule that is only suitable for investigating the therapeutic actions of compounds that stimulate NMDA receptors, because its efficacy in the treatment of Alzheimer's disease was rather poor. However, our data also confirm previous clinical evidence that severe dysfunction of the hippocampus may limit the efficacy of drugs, such as tetrahydroaminoacridine, to restore memory in Alzheimer's disease patients.

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