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ORIGINAL INVESTIGATION

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HFS-induced long-term potentiation and LFS-induced depotentiation in area CA1 of the hippocampus are not good models for learning

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Abstract Spatial learning in rats has been shown to be dependent on the intact hippocampus and lesioning this region impairs learning performance. Long-term potentiation (LTP) and depotentiation (DP) of synaptic transmission have been suggested to model memory formation at the neuronal level. Recently it was shown that LTP in the dentate gyrus or area CA3 of the hippocampus is not essential for the ability to learn a spatial water maze task. Here we show that the metabotropic glutamate receptor agonist (1S,3S)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3S-ACPD), which acts predominantly at presynaptic sites, only marginally impaired spatial learning in a water maze or radial arm maze (three out of eight arms baited) when injected ICV (5 µl of a 20 mM solution). There also were small impairments in non-spatial and visual discrimination tasks, indicating that the small learning impairments were due to nonselective effects of the drug. The same dose depressed field EPSPs and completely blocked LTP induced by high-frequency stimulation (HFS, 200 Hz) in the CA1 region of the rat hippocampus in vivo. A lower (5 µl of a 10 mM solution) dose did not depress baseline but still blocked LTP. Injecting the same dose after induction of LTP blocked DP induced by low-frequency stimulation (LFS, 10 Hz). These results indicate that neither HFS-induced LTP nor LFS-induced DP in area CA1 are good models for the induction of synaptic changes that might underlie spatial learning in the rat.

Key words Depotentiation · Hippocampus · Learning · Long-term potentiation · Memory · mGluR · Radial arm maze · Synaptic plasticity · Water maze

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Introduction

Long-term potentiation (LTP) of neuronal synaptic responses has been discussed for 30 years as a possible model for synaptic changes which occur during learning (Lømo 1966; Bliss and Lømo 1973; McNaughton et al. 1986; Morris 1989; Bliss and Collingridge 1993). Since then, studies have been undertaken to investigate the correlation between synaptic changes that occur during learning and those that occur during LTP in vivo or in vitro, e.g. by blocking LTP induction with specific drugs and comparing the effect of these drugs on learning. While early results showed a close correlation (Morris 1989; Morris et al. 1986; Richter-Levin et al. 1994; Riedel et al. 1994), more recent evidence has been published which contradicts the postulated relationship, showing that doses of NMDA receptor antagonists that block LTP in the dentate gyrus do not automatically block spatial learning in rats (Bannerman et al. 1995; Saucier and Cain 1995). Furthermore, studies of mutant mice that lacked the glycoprotein Thy-1 showed that loss of LTP in the dentate gyrus (Nosten-Bertrand et al. 1996) did not prevent learning of spatial tasks, nor did loss of LTP at mossy fiber terminals in area CA3 in mice that did not express functional protein kinase A impair spatial learning (Huang et al. 1995). This led to the proposal that area CA1 in the hippocampus is the most important region for spatial learning, and that LTP-like processes in this area alone could lay down the memory trace required for learning (Huang et al. 1995). We therefore investigated whether LTP, or depotentiation (DP), which is another mechanism of synaptic plasticity, which could play a role in learning and memory formation (O'Dell and Kandel 1994), in area CA1 of the hippocampus, could be responsible for the preserved learning ability of animals. Area CA1 is known to be of importance for memory formation in humans (Zola-Morgan et al. 1986). Furthermore, it has been observed that area CA1 can perform pattern

completion of spatially coded neuronal firing during suppression of CA3 activity. This suggests that impairments of other hippocampal regions can be compensated for by area CA1 (Mizumori et al. 1989).

We used the metabotropic glutamate receptor (mGluR) group I and II agonist 1S,3S-ACPD which acts presynaptically to reduce excitatory postsynaptic potentials (EPSPs) in vitro (Jane et al. 1994; Vignes et al. 1995) and in vivo (Salt and Eaton 1995), though it also acts at a postsynaptic site to some degree (Davies et al. 1995). To ensure that the drug spread throughout the hippocampus, recordings of EPSPs were conducted in the dorsal and ventral area CA1, ipsilateral and contralateral to the unilateral injection site. To assess learning abilities of rats, we used spatial and nonspatial tests in the water maze and the radial arm maze. It has been shown before that solving spatial tasks in the radial arm maze (Jarrard 1986) or in the water maze is dependent on the hippocampal formation (Morris et al. 1982).

Materials and methods

Behavioural studies

Animals

Male Wistar rats weighing between 280 and 320 g were divided into two groups ($n = 7–8$ each). Animals received food ad lib for the water maze trials. The groups were then tested in the radial arm maze and received a 12 g per day diet which kept their weight at approximately 85% of their free feeding weight before and during eight-arm maze training days. Animals were held in opaque plastic cages, one animal per cage in a room with an 8 a.m. to 8 p.m. light/darkness cycle at 23°C.

All experiments and surgical procedures were conducted according to the regulations of the Irish Health Department and the European Community Directive 86/609/EC.

Surgery

Rats were anesthetized with 0.25–0.3 ml sodium pentobarbitone solution (60 mg/ml) (Rhône Mérieux, Ireland). Atropine sulphate (0.1 ml of a 10 μg/ml solution) was injected IM. Animals were oxygenated throughout surgery. Temperature was measured and kept to 35–37C° with a heating blanket. The local anesthetic xylocaine with adrenaline was injected in small doses into the scalp and the skin was cut open. Four 0.5 mm holes were drilled and three stainless steel screws were inserted into three of the holes. The animal was put into a stereotaxic frame and a cannula was inserted just above the right ventricle (coordinates 1.5 mm lateral, 3.5 mm posterior, 3 mm downwards from bregma). The cannula was made out of a 0.7 mm diameter syringe needle, length 12 mm, with smooth edges. A stylus made of stainless steel wire was inserted into the hole (Bilaney, Germany). The opening was sealed with super glue (Loctite, USA) and the final position fixed with acrylic dental cement (Assoc. Dental Works, UK). After surgery, 0.05 ml of buprenophine (Temgesic; Merk Sharpe Dome, UK) 0.3 mg/ml was given IP.

At the end of experiments, animals were killed with an overdose of pentobarbitone and ink was injected into the cannula. The location of cannula and ink was assessed, and animals with cannulae in wrong locations or with excessive damage/blood clots were discarded from data analysis.

Drugs

All drugs used in this study were obtained form Tocris Cookson (UK) and dissolved in saline (NaCl solution 0.9%). The pH was measured and adapted to 7.4 with NaOH when necessary. Rats were injected unilaterally ICV with 5 μl of a 20 mM drug solution or with saline 30 min before training.

Water maze

The pool for the maze was made of fiberglass and painted white. Diameter was 120 cm, height 48 cm, depth of water 34 cm (26 cm with the platform exposed in the visual platform task). The platform was made of transparent perspex, had an 8×8 cm surface and was 3 cm below the water surface in the water maze training and 4 cm above water level in the visual platform task. Animals were not able to see the platform when submerged due to surface glare/reflection. The water was kept at 23 ± 2°C. The pool was situated in a room with visual cues such as a door, a curtain, a black cover over a window, and a water heater on the wall. The animals' movements were recorded with a video camera attached to the ceiling. Data were analyzed using a tracking program written by James Mahon at TCD. The program measures the latency of animals to reach the platform and distance swum. During the transfer task, percentage of distance swum in four quadrants in which the pool was divided by the tracking program, and the number of crossings of a 20 cm diameter area where the platform was during the training period (annulus crossings) were calculated. Animals' heads were marked with a black water resistant felt tip marker to enable the program to identify the white rats in the water.

Training protocol

Acquisition phase of spatial task: days 1–4. On all days, rats were injected ICV with 5 μl of a 20 mM drug or vehicle 30 min before their first trial. Animals were put in the water from one of four different starting points which alternated clockwise. Each animal had six trials per day. The intertrial delay was about 5 min. Cut-off time for a trial was 180 s if the animal did not find the platform. In that case the animal was moved manually onto the platform and left there for 10 s.

Time needed to find the platform was measured (latency), and the distance swum in the maze. The swim speed was computed from these data.

Quadrant analysis: transfer test. On day 4, animals were given six training trials. After that, the platform was removed and the animal was given 60 s in the pool. The percentage of distance swum in the four quadrants, and annulus crossings made were measured. The annulus was defined as the perimeter of the area where the platform was located previously. It covered a circle 20 cm in diameter.

Nonspatial visible platform task. Rats were injected with drug or vehicle 30 min before the task and given three trials in the pool with the platform 4 cm exposed above water level to test visual discrimination of animals. The platform was moved randomly between trials in order to reduce the use of a spatial strategy by the animals. Time and distance swum was recorded and swim speed was computed.

Radial arm maze

The maze was made of 15 mm thick white plastic coated chip board and consisted of eight arms, each 19 cm wide, 60 cm long, and

30 cm high, and a central octagonal platform which was 46 cm in diameter. A white plastic weighing boat 5 cm wide was attached at the end of each arm. A 45 mg food pellet was put in this if the arm was baited. The animals were not able to see the food pellet from the centre platform. The animals were observed with a video camera from another room. The monitor was marked to allocate each arm a number from 1 to 8.

Training protocol

Pretraining sessions. Animals were made familiar with the maze on 2 days. All arms were baited with a 45 mg food pellet. No drugs were given.

Acquisition phase: days 1–4. Three arms were baited (arms 8,4,3). On all days, rats were injected ICV with 5 µl of a 20 mM drug solution or vehicle 30 min before their first trial. Each trial consisted of putting the animals into the centre of the platform. The latency for retrieving all three pellets was measured, and visits of the different arms was recorded. Cut-off time for the trial was 5 min, in which the animal had to find the pellets. Each animal was given six trials per day. The intertrial delay was about 6 min.

Data were analyzed for reference memory errors (RF), defined as entries into arms that were never baited, and for working memory errors (WM), defined as entries into arms that had been visited by the rat within the same trial previously.

Transfer task. In the transfer test, no arms were baited, and each animal had 1 min per trial to explore the maze. Six trials were given. Total number of arms visited as well as number of visits of arms that were baited previously was recorded.

Marked arm visual discrimination task

In a visual cue task, one arm had not been baited previously was baited and marked with a checkerboard pattern. Rats were given six trials to learn the task. Latency to retrieve the pellet, total number of arms visited as well as number of visits to arms that were baited previously were recorded.

Statistics

Results were analyzed using two-way repeated measure ANOVA (SYSTAT computer program). In order to ensure that the data for the different groups were normally and equally distributed, they were subjected to a logarithmic transformation.

The open field results and annulus crossing measures in the transfer test were analyzed using an unpaired, two-tailed Student's *t*-test. Transformation of data to ensure that the different groups were normally and equally distributed was not necessary.

The data from the quadrant analysis of the transfer test were subject to angular transformation. Since data expressed in percentages might not be equally distributed due to ceiling and bottom limit effects, data were transformed from "X" to "arcsin[square root (X)]" (see McNaughton 1993, for details). Using a computer program (INSTAT), one-way ANOVA with a post-hoc Tukey Kramer multiple comparison test was used to assess differences. Values presented are the mean ± SEM.

In vivo electrophysiology

Male Wistar rats weighing 200–300 g were used. The rats were anaesthetised with urethane (ethyl carbamate, 1.5 g/kg, IP) for the duration of all experiments. About 10% of the animals were additionally given a halothane/oxygen (1% halothane in pure oxygen; flow rate, 1 l/s) gas mixture during the implantation procedure to ensure deep anaesthesia throughout surgery. Recovery from halothane was assessed by measuring EEG activity, and usually took less than 30 min.

The animal was put into a stereotaxic frame and a cannula was inserted into the right lateral ventricle (coordinates 3.5 mm downward into the ventricle, 1.5 mm posterior to bregma, and 0.5 mm lateral to the midline). The cannula was made out of a 0.7 mm diameter syringe needle, length 12 mm. The final position was fixed with acrylic dental cement. Recordings of field EPSPs were made from the CA1 stratum radiatum of the right hippocampal hemisphere in response to stimulation of the Schaffer collateral/commissural pathway. Electrode implantation sites which were identified using stereotaxic coordinates relative to bregma and lambda, with the recording site located 3 mm posterior and 2 mm right of the midline, and the stimulating electrode 4 mm posterior to bregma and 3 mm right of the midline. Bipolar stimulating and monopolar recording electrodes consisted of two pieces of twisted tungsten wire attached to a connecting socket insulated along its length with a Teflon coat except at the tips. The electrodes were slowly lowered through the cortex and the upper layers of the hippocampus into the CA1 region until the appearance of a negative deflecting field EPSP. The electrodes were then fixed in place with cyanoacrylate glue and acrylic dental cement for the stimulation and recording of evoked field EPSPs. Stainless steel screws fixed to the skull served as ground (anterior 7 mm, lateral 5 mm) and reference (posterior 8 mm, lateral 1 mm) electrodes.

In the contralateral recordings of the dorsal and ventral hippocampus the measurements were taken from the left hippocampus which was contralateral to the cannula position. Coordinates for measurements in the dorsal hippocampus were identical to the coordinates of the ipsilateral hemisphere except that the left of the midline rather than right. Coordinates for ventral hippocampus measurements were: 5.8 mm posterior of bregma, 5.5 mm to the left for the electrodes; depth was 7 mm for stimulation and 7.8 mm for recording electrode. Analysis of electrode tracks was made post mortem to ensure correct locations.

In all experiments control EPSPs were evoked at a frequency of 0.033 Hz, and an input-output curve (i/o, stimulus intensity versus EPSP amplitude) plotted for each experiment at this test frequency. For the control EPSPs, the stimulation voltage intensity was adjusted to give an EPSP amplitude of 50% of maximum i/o amplitude. LTP was induced using three sets of trains of stimuli, each set consisted of ten trains of 20 stimuli, inter stimulus interval 5 ms (200 Hz), inter-train interval 2 s and inter-set interval of 5 min. The low frequency stimulation used to induce DP consisted of 900 stimuli at 10 Hz, which in previous studies in this laboratory was found to evoke maximal DP (Doyle et al. 1996). The stimulation intensity was increased to give an EPSP of 75% maximum amplitude during the stimulation used to induce LTP.

All recording and stimulating was performed using an on-line computerised oscilloscope/stimulator and data analysis interface system (MacLab/2e). Unless otherwise stated, all data are expressed as mean ± SEM% baseline EPSP amplitude. Results were analyzed by Student's *t*-test or Welch *t*-test which does not assume equal standard deviations between data sets.

Results

Water maze

Animals injected with 5 µl of a 20 mM 1S,3S-ACPD solution 30 min before training, a dose that produced no changes in spontaneous locomotor activity in an open field test (data not shown), learned the location

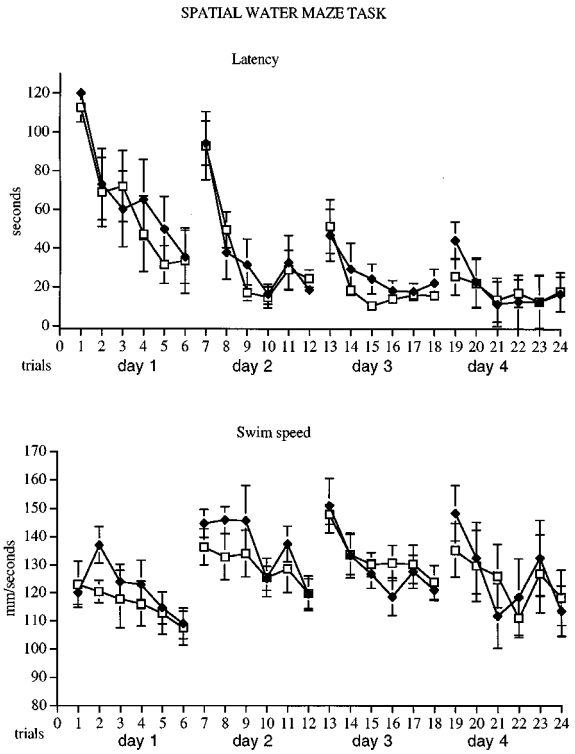


Fig. 1 Effect of 1S,3S-ACPD on learning a spatial task in a water maze. *Top*: time needed by animals to find a hidden platform in the water maze after an injection of 5 µl of a 20 mM 1S,3S-ACPD solution 30 min pre-training ICV. Drug group $n = 8$, saline group $n = 6$. No difference in performance was found between groups (two-way ANOVA). *Bottom:* swim speed of animals when searching for the platform. No difference between groups was found in a two-way ANOVA. υ 1S,3S-ACPD; o saline

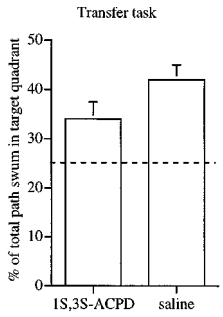


Fig. 2 Transfer task. Percentage of path length swum by rats in the quadrant which previously contained the platform. Rats were injected with 5 µl of a 20 mM 1S,3S-ACPD solution 30 min before testing. One-way ANOVA showed an overall difference over all quadrants ($P < 0.0001$). There was a difference between control group and drug group ($P < 0.01$). However, the drug group performed better than chance ($P < 0.01$; *broken line* marks chance performance)

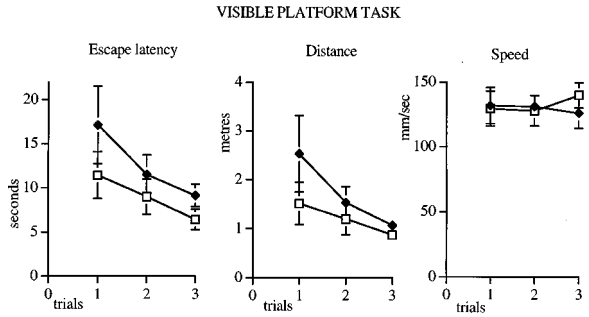


Fig. 3 Nonspatial visible platform task. The drug group needed more time to find the visible platform after injection with 5 µl of a 20 mM 1S,3S-ACPD solution 30 min before training ICV ($P < 0.05$, $n = 8$ per group). No difference was seen between groups in distance swum or in swim speed. υ 1S,3S-ACPD; o saline

of the hidden platform in the water maze as quickly as control animals. The swim speed was not affected by the drug either (Fig. 1).

In the transfer task, however, a small deficit was visible in the drug group. One-way ANOVA showed significant difference over all quadrants ($F = 7.4$, df 55, $P < 0.0001$). A post-hoc Student-Newman-Keuls multiple comparison test showed a higher percentage of the total distance swum in the target quadrant where the platform had been located previously in the control group compared to the drug group (q-value 4.0, $P < 0.01$) (Fig. 2). However, the number of annulus crossings by animals was not significantly different between groups (Saline 5.86 ± 1.03; 1S,3S-ACPD 4.62 ± 0.8). The drug group performed better than chance level as shown in a t-test ($t = 5.4$, df 5, $P < 0.01$), taking 25% of total swimming distance per quadrant as chance performance.

Latency in the nonspatial task with a visible platform was also slightly affected by the drug. In a two-way ANOVA a difference between trials ($F_{2,42} = 3.5$, $P < 0.05$) as well as between groups ($F_{1,14} = 5.1$, $P < 0.05$) was found. No difference was seen between groups in distance swum or in swim speed (Fig. 3).

Radial arm maze

The results of the radial arm maze experiments were consistent with those of the water maze tasks. Time needed to visit all three baited arms in a spatial task was not impaired by the drug (two-way ANOVA). A small increase in working errors was visible. Two-way ANOVA detected a difference between groups ($F_{1,15} = 29.9$, $P < 0.001$) and trials ($F_{23,336} = 5.6$, $P < 0.001$). No difference in numbers of reference memory errors committed was found (Fig. 4).

Furthermore, the drug group performed slightly worse in a transfer task with no arms baited. After injection of 1S,3S-ACPD there was no difference from controls in the total number of arms visited during a 1-min test trial. However, the number of visits of arms that were baited previously was higher in the control group as detected by two-way ANOVA. There was a difference between groups ($F_{1,15} = 9.2$, $P < 0.01$) and trials ($F_{5,72} = 3.6$, $P < 0.01$) (Fig. 5).

The drug group performed as well as the control group in a visual discrimination task in which one arm was baited and marked. Two-way ANOVA showed a

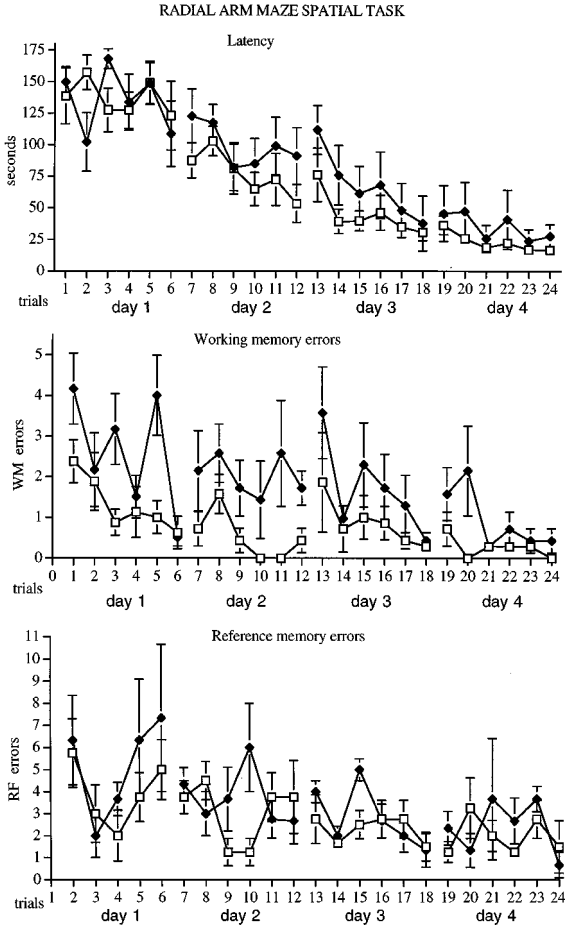


Fig. 4 Effect of 1S,3S-ACPD on learning a spatial task in a radial eight-arm maze. Drug group $n = 8$, saline group $n = 7$. *Top*: time required by animals to find three baited arms after an injection with 5 µl of a 20 mM 1S,3S-ACPD solution 30 min before training ICV. Two-way ANOVA detected no difference between groups. *Middle*: number of working memory errors committed. Two-way ANOVA detected a difference between groups ($P < 0.001$). *Bottom*: number of reference memory errors committed. No difference was seen between groups. υ 1S,3S-ACPD; o saline

difference between groups in time required to enter the marked arm between groups ($F_{1,15} = 7.2$, $P < 0.05$). There was no difference between groups in the number of arms visited or in visits to previously baited arms (Fig. 6).

In vivo electrophysiology

All groups contained six animals except when stated otherwise. HFS induced LTP in the control group (to 178 ± 16% 30 min after HFS; $t = 21.5$, *df* 10, $P < 0.001$ compared to baseline, alternate Welch *t*-test). LFS given 30 min later depotentiated LTP down to 120 ± 24% of baseline ($t = 17$, *df* 10, $P < 0.001$). A second HFS induced LTP again (175 ± 32% of baseline 30 min after HFS; $t = 19.6$, *df* 10, $P < 0.001$, Fig. 7a,b). Injecting 5 µl of a 20 mM 1S,3S-ACPD solution reduced the EPSP slope compared to baseline ($t = 21.5$, *df* 10, $P < 0.001$). HFS did not change the slope of EPSPs compared to baseline, nor did low frequency stimulation (LFS), as measured by *t*-test. A second HFS 90 min after the drug injection also failed to induce LTP. Comparison between drug and control group after HFS showed a significant difference ($t = 68.1$, *df* 10, $P < 0.0001$, Fig. 7a,b).

Injecting 5 µl of a 10 mM solution did not reduce the EPSP slope 30 min after injection compared to baseline. Neither HFS, LFS, nor the second HFS induced changes in the EPSP slope. Comparison between drug and control group after HFS showed a significant difference ($t = 47.4$, *df* 10, $P < 0.0001$). Injecting 5 µl of a 5 mM solution did not reduce the EPSP slope compared to baseline nor did it block LTP or DP. In these animals HFS produced LTP (174 ± 32 of baseline 30 min after HFS; $t = 24.5$, *df* 10, $P < 0.001$), LFS produced DP ($t = 18.1$, *df* 10, $P < 0.001$) compared to the slope of the EPSP after potentiation. Comparison between drug and control

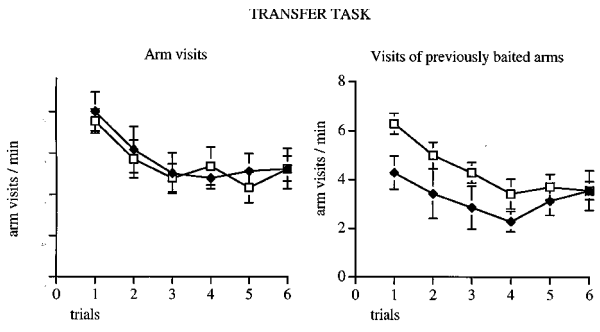


Fig. 5 Transfer task. After injection of 5 µl of a 20 mM 1S,3S-ACPD solution 30 min before training ICV animals showed no difference in the total number of arms visited during a 1-min test trials compared to controls. However, the number of visits to arms that had been baited previously was higher in the control group as detected by two-way ANOVA ($P < 0.01$). υ 1S,3S-ACPD; o saline

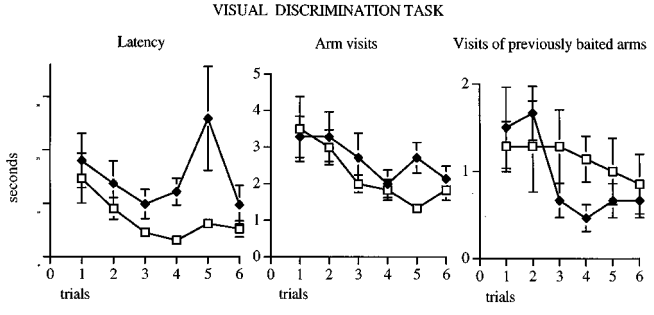


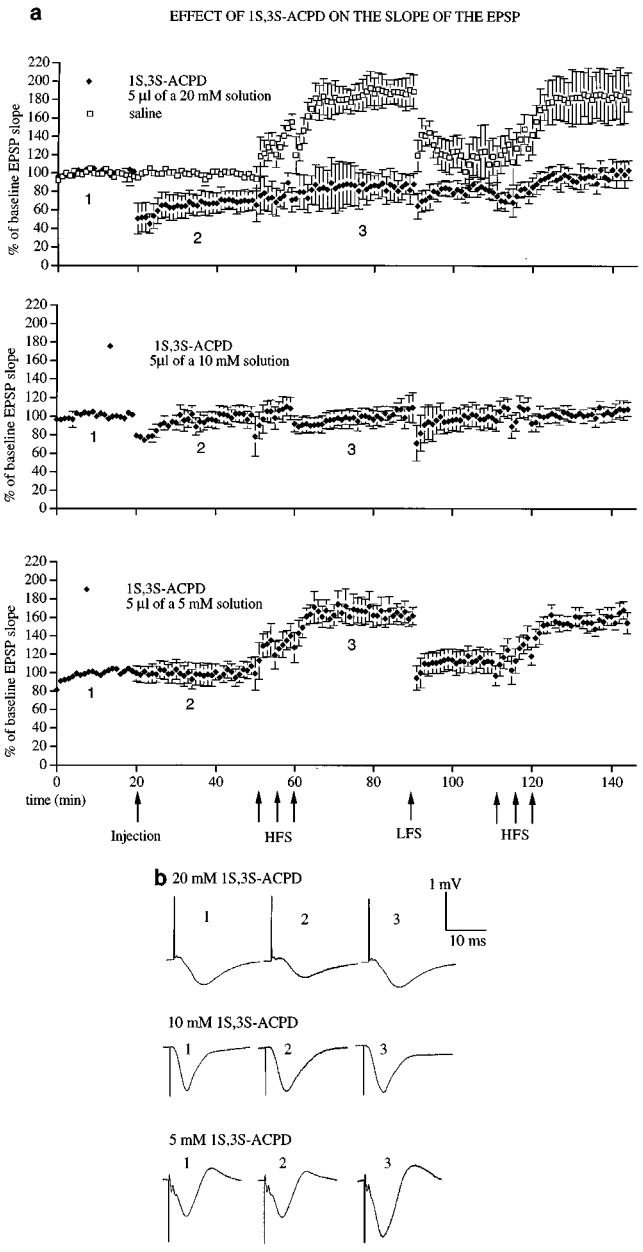
Fig. 6 Visual discrimination task. Time required by rats to visit a marked and baited arm. The drug group did not perform as well as the control group after injection of 5 µl of a 20 mM 1S,3S-ACPD solution 30 min before training ICV. Two-way ANOVA showed a difference between groups ($F = 3.0$, *df* 1/14, $P < 0.05$) but not between trials. There was no difference between groups in number of arms visited or in the number of visits of previously baited arms. υ 1S,3S-ACPD; o saline

group after HFS did not show a significant difference (Fig. 7a,b).

Injecting 5 µl of a 20 mM 1S,3S-ACPD solution after LTP induction prevented induction of DP by LFS. LFS induced DP in the control group (same control data as in Fig. 7a) but not in the drug group. Comparison between drug and control group after LFS showed a significant difference ($t = 47.9$, df 10, $P < 0.0001$; Fig. 8).

HFS was applied 4 h after drug injection when the drug would be expected to be washed out to test that the block of LTP was due to drug treatment and not to the inability of a chosen site to express LTP. After injection of 5 µl of a 10 mM 1S,3S-ACPD solution, no LTP or DP was observed after stimulation with HFS, LFS, and subsequent HFS as shown before in Fig. 7a. After applying HFS again 4 h after drug injection, LTP was induced (Fig. 9).

To ensure that the drug blocks LTP throughout the hippocampus, the effect of 1S,3S-ACPD on the slope of the EPSP of the dorsal and ventral CA1 area contralateral to the cannula side was measured. An aliquot of 5 µl of a 20 mM solution reduced the EPSP slope compared to baseline ($t = 4.1$, df 6, $P < 0.005$; $n = 4$). The result was practically identical to the measurement in the ipsilateral hippocampus (Fig. 7a), except that onset of baseline depression was about 2 min later. Neither HFS nor LFS changed the EPSP slope, nor did the second HFS induce LTP as measured by two-way ANOVA. In the control group, HFS induced LTP of the EPSP slope ($t = 5.9$, df 6, $P < 0.001$; $n = 4$). LFS induced DP of the potentiated EPSP slope ($t = 2.9$, df 6, $P < 0.01$). The second HFS again induced LTP compared to baseline after LFS ($t = 3.4$, df 6, $P < 0.01$).



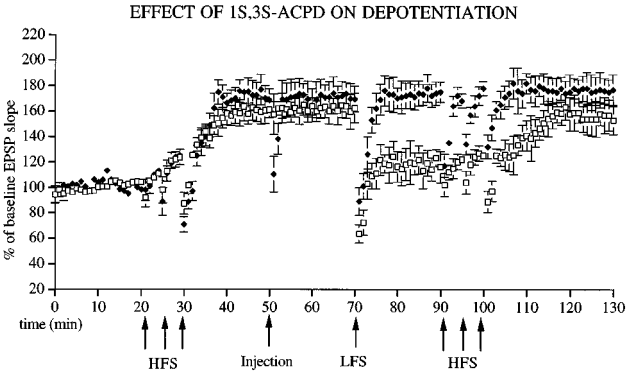


Fig. 8 Injecting 5 µl of a 10 mM 1S,3S-ACPD solution after LTP induction prevented DP. LFS induced DP in the control group ($P < 0.001$) but not in the drug group ($n = 6$ per group). Comparison between drug and control group after LFS showed a significant difference ($P < 0.0001$). υ 1S,3S-ACPD; o saline

Fig. 7 a The effect of 1S,3S-ACPD on the slope of field EPSPs of area CA1 of the rat hippocampus as recorded in vivo. The baseline was recorded 20 min before drug injection. All groups consisted of six animals. *Top*: in the control group, HFS induced LTP of the EPSP slope ($P < 0.0001$). LFS induced DP of the potentiated EPSP slope ($P < 0.0001$). The second HFS again induced LTP compared to baseline after LFS ($P < 0.0001$). An aliquot of 5 µl of a 20 mM solution reduced the EPSP slope compared to baseline ($P < 0.0001$). Neither high frequency stimulation (HFS) nor low frequency stimulation (LFS) changed the EPSP slope, nor did the second HFS induce LTP. Comparison between drug and control group after HFS showed a significant difference ($P < 0.0001$). *Middle*: injecting 5 µl of a 10 mM 1S,3S-ACPD solution did not affect the EPSP slope 30 min after injection compared to baseline. Neither HFS, LFS, nor the second HFS induced changes in the EPSP slope. Comparison between drug and control group after HFS showed a significant difference ($P < 0.0001$). *Bottom:* 5 µl of a 5 mM solution did not affect the EPSP slope compared to baseline. HFS produced LTP ($P < 0.0001$) compared to baseline, and LFS produced DP ($P < 0.0001$) compared to the potentiated level. Comparison between drug and control group after HFS did not show a significant difference. **b** Typical traces of field EPSPs during recording of *1* baseline, *2* 15 min after drug injection, *3* after HFS

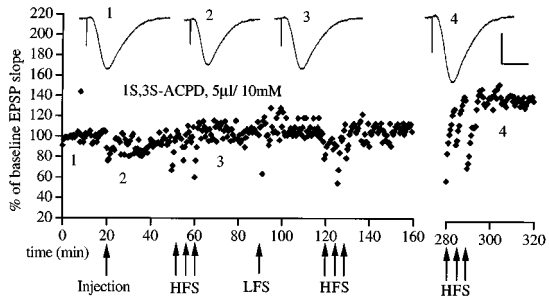


Fig. 9 Recovery from the effect of 1S,3S-ACPD on LTP induction (single animal test). After injection of 5 µl of a 10 mM 1S,3S-ACPD solution, no change in the slope of the EPSP was observed after stimulation with HFS, LFS, and subsequent HFS. When applying HFS again 4 h after injection of 1S,3S-ACPD, LTP was observed. Traces show typical EPSPs of *1*) baseline, *2*) after drug injection, *3*) after first HFS *4*) after HFS 4 h after drug injection. Traces show typical EPSPs of *1*) baseline, *2*) after drug injection, *3*) after HFS. Calibration bars 10 ms (x-axis), 1 mV (y-axis)

The effect of 1S,3S-ACPD on the slope of the EPSP of the ventral CA1 area contralateral to the cannulation site was tested in a single animal. Injection of 5 µl of a 20 mM solution reduced the EPSP slope compared to baseline. The onset of baseline depression was delayed by 5 min. Neither HFS nor LFS changed the EPSP slope. However, 4 h after drug injection HFS induced LTP. HFS induced LTP of the EPSP slope in the control rat (Fig. 10).

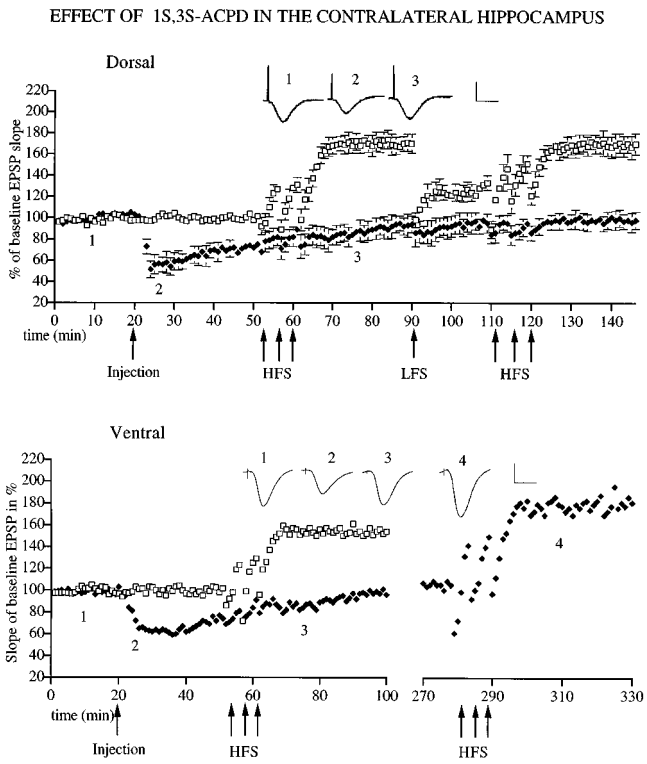


Fig. 10 *Top*: effect of 1S,3S-ACPD on the slope of the EPSP of the dorsal area CA1 contralateral to the cannula side ($n = 4$ per group). An aliquot of 5 µl of a 20 mM solution reduced the EPSP slope compared to baseline ($P < 0.001$). Neither HFS nor LFS changed the EPSP slope, nor did the second HFS induce LTP. In the control group, HFS induced LTP of the EPSP slope ($P < 0.001$). LFS induced DP of the potentiated EPSP slope ($P < 0.001$). The second HFS again induced LTP compared to baseline after LFS ($P < 0.001$). Traces show typical EPSPs of 1) baseline, 2) after drug injection, 3) after HFS. Calibration bars 10 ms (x-axis), 1 mV (y-axis). *Bottom*: effect of 1S,3S-ACPD on the slope of the EPSP of the ventral CA1 area contralateral to the cannulation site, single animal study. An aliquot of 5 µl of a 20 mM solution reduced the EPSP slope compared to baseline. Onset of baseline decrease was delayed by about 5 min. Neither HFS nor LFS changed the EPSP slope after drug injection. However, 4 h after drug injection HFS induced LTP. In the control group, HFS induced LTP of the EPSP slope. Traces show typical EPSPs of *1*) baseline, *2*) 15 min after drug injection, *3*) after HFS *4*) after second HFS 4 h after drug injection. υ 1S,3S-ACPD; o saline

Discussion

1S,3S-ACPD injected ICV at the dose used did not prevent rats from learning the spatial tasks in the radial arm maze or the water maze. However, though having performed better than chance, the drug group did perform worse than the control group in the transfer task in the water maze. In the radial arm maze the animals did learn the spatial and non-spatial tasks but made more errors in the acquisition phase. The animals injected with 1S,3S-ACPD also showed deficits in non-spatial tasks in both the water maze and radial maze.

These results suggest that the drug slightly impaired performance in both spatial and non-spatial tasks. Since previous experiments showed that the hippocampus is involved mainly in a spatial task and not in a visual discrimination task version of the water maze (Morris 1989; Bannerman et al. 1995) it seems likely that the behavioural deficits produced by this dose of 1S,3S-ACPD was due to some generalised effect on synaptic transmission throughout the brain in a non-selective way. The impairments observed, however, are negligible when compared to those found in rats learning a water maze after hippocampal lesions. Such animals needed considerably longer to find a platform than control rats and never performed better than chance in the transfer tasks (Morris et al. 1982; Bannerman et al. 1995). Furthermore, the impairments found in the radial arm maze task are very small compared with those induced by NMDA receptor antagonists (Butelman 1990; Ward et al. 1990) or those seen in hippocampectomised rats (Olton et al. 1979).

The electrophysiological studies showed that the drug dose used in the behavioural experiments had powerful effects on baseline EPSPs in CA1. Induction of LTP by HFS was blocked despite the powerful stimulation protocol which produced large LTP in controls. The induction of depotentiation by low-frequency stimulation was also blocked. Since a strong baseline reduction can prevent LTP induction, the drug dose was reduced. This eliminated the effect on baseline at the time when HFS was applied, yet LTP induction was

still blocked. The result suggests that the mechanism which reduces baseline EPSPs may be independent of the LTP blocking mechanism. However, paired-pulse facilitation was affected by this dose of 1S-3S-ACPD, indicating that some presynaptic effect was still evident. Reducing the drug concentration further abolished any effect on baseline or LTP induction, and paired-pulse facilitation was no longer affected (manuscript in preparation).

The block of LTP by 1S,3S-ACPD disappeared after 4 h, showing that the lack of LTP at the site chosen was not due to the inability of the site to produce LTP. Measurements in the dorsal and ventral CA1 area of the hippocampus contralateral to the cannula site gave similar results to those found in the ipsilateral hippocampus, showing that the drug had spread throughout the hippocampus during the period before HFS stimulation or training.

These results therefore indicate that HFS-induced LTP or LFS-induced DP in area CA1 do not provide a valid model for learning and memory formation. Other research in this laboratory showed that LTP induction of the population spike or of the slope of the EPSP in the dentate gyrus was also blocked after injection of 5 µl of a 20 mM 1S,3S-ACPD solution (manuscript in preparation). The results confirm earlier studies which showed that AMPA receptor antagonists can block LTP induction (Krug et al. 1982) but do not necessarily block memory formation (Burchuladze and Rose 1992).

One would expect that the complete obliteration of HFS-induced LTP by a drug should strongly impair if not block learning, especially as the area CA1 is known to be of great importance for memory formation (Zola-Morgan et al. 1986). However, the results presented here do not rule out that LTP or DP could have been obtained after drug injection by using different stimulation protocols, or that HFS-induced LTP or DP is a valid model for memory formation in other areas of the brain. Clearly, the voltages involved when applying HFS or LFS are much larger than what one would observe in an intact brain during learning. It is theoretically possible that the subtle synaptic changes that might go on during learning are of a different nature to those occurring during LTP/DP induction and that these natural changes are not blocked by the drugs employed. In support of Bliss and Lynch (1988) and of Barnes (1995), who sounded notes of caution against automatically accepting every form of LTP as a valid model for learning, and that the conditions under which LTP is obtained (slice type, buffer contents, temperature, etc.) are often very far removed from the in vivo situation, we suggest that researchers use protocols of inducing LTP or DP that are closer to neuronal activities occurring naturally in the living brain, and to focus more on research on LTP or DP in vivo. It is furthermore of interest to evaluate the difference in LTP/LTD expression between anaesthetised and freely moving rats. One could envisage that drug effects observed in freely moving rats when using electrophysiological methods reflect changes in learning mechanisms better. Generally speaking, it is of great importance when studying mechanisms of LTP to investigate if the artificial synaptic changes obtained after stimulation correlate with synaptic changes observed in learning tasks.

In conclusion, HFS-induced LTP in all major areas of the hippocampal formation, the dentate (Bannerman et al. 1995; Saucier and Cain 1995; Nosten-Bertrand et al. 1996), CA3 (Huang et al. 1995), or CA1, or DP in area CA1 (present study), cannot be considered to represent the induction mechanism of synaptic changes which might occur during learning.

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