



Uncompetitive NMDA Receptor Antagonists Attenuate NMDA-induced Impairment of Passive Avoidance Learning and LTP

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Summary—In general, *N*-methyl-D-aspartate (NMDA) receptor antagonists inhibit learning and long term potentiation (LTP). However, it has been suggested that direct tonic, i.e. non-temporal, activation of NMDA receptors, in contrast to learning, may lead to an increase in synaptic “noise” and, in turn, to a loss of association detection. In the present study, a two-choice passive avoidance task and LTP *in vitro* (CA1 hippocampal region) were used to address this issue. Dark avoidance learning was impaired by systemic NMDA administration (starting at 25 mg/kg) that was not related to either toxic effects or state-dependent learning. NMDA-induced amnesia was antagonized by ((+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate (MK-801) and 1-amino-3,5-dimethyladamantane (memantine), starting at low doses of 0.05 and 2.5 mg/kg, respectively, in a bell-shaped dose–response relationship. A competitive NMDA receptor antagonist CGP-39551 failed to reverse NMDA-induced amnesia. In hippocampal slices, NMDA (10 μ M) depressed (S)- α -amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid (AMPA) receptor-mediated field potentials in CA1 and also caused a moderate reduction of LTP induction/expression. It was this latter effect that was antagonized by memantine (1 μ M). Thus, under conditions of tonic activation of NMDA receptors, uncompetitive NMDA receptor antagonists can paradoxically reverse deficits in learning and synaptic plasticity. © 1997 Elsevier Science Ltd.

Keywords—MK-801, memantine, CGP-39551, passive avoidance learning, LTP, hippocampal slice.

The crucial role of the glutamatergic system in learning processes has been indicated by numerous studies (Morris *et al.*, 1986; for reviews see Collingridge and Singer, 1990; Danysz *et al.*, 1995b). In this respect, the predominant role of glutamate receptors of the *N*-methyl-D-aspartate (NMDA) type seems proven. The voltage- and use-dependent blockade of NMDA receptors-coupled channels by Mg²⁺ make them uniquely suitable for mediation of activity-dependent, long lasting neuronal modifications, in line with Hebbian theory (Nowak *et al.*, 1984; Cotman and Monaghan, 1988; Collingridge and Singer, 1990). The basic principle is the transformation of quantitative associatively interacting inputs into a qualitative synaptic modification as soon as a certain threshold is achieved. A great deal of evidence for the role of NMDA receptors in learning derives from

pharmacological studies where antagonists of NMDA receptors have been shown to disrupt acquisition of various learning task (Morris *et al.*, 1986; Advokat and Pellegrin, 1992; Danysz *et al.*, 1995b). The precise mechanism of NMDA receptor antagonist action has not found consensus, i.e. it could reflect effects on acquisition, storage of association or alternatively on memory supporting processes (arousal, sensory input etc.). Moreover, some apparent inconsistencies are evident. For example, Mondadori *et al.* (1989), reported an improvement of learning after NMDA receptor antagonists in passive avoidance and social recognition tasks (Lederer *et al.*, 1993). This effect was only apparent when the performance of control animals was very low. At present, such a positive action of NMDA receptor antagonists on learning remains highly controversial and the possible mechanism elusive.

Since most behavioural studies have shown only negative effects of NMDA receptor antagonists on learning, it has been suggested, and indeed reported, that memory enhancement can be obtained by administration of NMDA receptor agonists or partial agonists, assuming

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that low doses are used that produce neither convulsions nor excitotoxic damage (Flood *et al.*, 1990; Parada-Turska and Turski, 1990; Sahai, 1990; Chessell *et al.*, 1991; Myhrer and Paulsen, 1992; Mirchandani *et al.*, 1994). In spite of such positive findings, other authors failed to see a positive effect of NMDA agonists on learning (Ungerer *et al.*, 1988; Holmes *et al.*, 1993;) and, in two studies, even impairment was observed (Jones *et al.*, 1989; Cohn and Cory-Slechta, 1994). This is, in our opinion, not particularly surprising in the view of the above highlighted principle of the role of NMDA receptors in synaptic plasticity; simply continuous, non-contingent stimulation should rather lead to a persistent decrease in the signal to noise ratio and/or sensitivity of secondary processes downstream to NMDA receptor activation and, in turn, to deterioration of the detection capacity of the system. The same argumentation was used to explain the negative effect of either NMDA itself or Mg^{2+} removal on the induction of LTP in hippocampal slices (Coan *et al.*, 1989; Izumi *et al.*, 1992).

If indeed amnesia produced by NMDA receptor agonists is evoked by non-physiological, low level, continuous activation of NMDA receptors, it should be attenuated by voltage-dependent uncompetitive NMDA receptor antagonists such as (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate ((+)-MK-801) and 1-amino-3,5-dimethyl-adamantane (memantine), (Chen *et al.*, 1992; Parsons *et al.*, 1993; Frankiewicz *et al.*, 1996). The present study was devoted to address this issue using the passive avoidance test in rats and long term potentiation (LTP) in hippocampal slices *in vitro*.

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats weighing 200–220 g or 160–180 g at the beginning of behavioural or long-term potentiation experiments, respectively, were housed in polyethylene cages (4–5 per cage) with free access to food and water. Conditions in the animal room were kept constant at 20°C, 50% relative humidity and a 12/12 hr dark/light cycle with light on at 06.00 hr. Experiments were performed strictly according to the animal rights commission allowance # F 77–35 (Hessen).

Passive avoidance apparatus

The two choice dark avoidance apparatus consisted of three identical sized compartments (25 × 22 × 20 cm; w × d × h) connected so that access from the start compartment to both choice compartments could be controlled by a vertically sliding door (6 × 9 cm; h × w), (Misztal and Danysz, 1995). Each compartment was equipped with a source of light and a grid floor connected to a shock generator (Geltz Labortechnik, Germany).

Behavioural procedure

The behavioural procedure was based on a previous

study (Misztal and Danysz, 1995). On day 1, each animal received 5 min of handling while on the next day rats were handled again for 5 min and then habituated to the apparatus (5 min) by placing them in the start box. During habituation only one (light) choice compartment was available. To avoid directional preference, for 50% of rats from each group the left compartment was lit (and therefore available) and for 50%, the right one. On day 3 (training), rats usually received i.p. injection of the tested agents and after 30 min (if not stated otherwise) were placed in the starting compartment with free access to both choice compartments (the light compartment was the same as on day 2). Time to enter the light and/or dark compartment was recorded. Immediately after the rat entered the dark compartment, the door was closed, two electroshocks (1 mA, 1 sec) were delivered (3 sec apart) and the rat was returned to its home cage until the retention test (see below). The shock level was selected on the basis of preliminary titration experiments. One of the criteria was avoidance of the dark compartment upon presentation to the apparatus during the retention test, without pronounced general behavioural inhibition expressed as failure to enter the light compartment. On day 4 (retention) the rat was placed in the starting compartment with both choice compartments open and the time to enter the light and dark compartment was recorded. The cut off time was 300 sec. During retention tests, no shocks were delivered. For statistical analysis, the following parameters were used: (1) during training: latency to leave the start box (as a measure of general inhibition/activation) and to enter the dark box; (2) during retention: latency to leave the start box (as a measure of non-specific inhibition), latency to enter the dark box (as a measure of dark avoidance).

Long-term potentiation

Chloroform-anaesthetized rats were decapitated and the brains were removed rapidly and immediately cooled on ice (2–4°C). Transverse hippocampal slices (400 µm thick) were cut (FTB Vibracut) and stored in artificial cerebro-spinal fluid (aCSF) containing in (mM): NaCl 124, KCl 3, NaHCO₃ 26, NaH₂PO₄ 1.2, CaCl₂ 2, MgSO₄ 1 and D-Glucose 10, bubbled with 95%O₂/5%CO₂ at room temperature for at least 2 hr. Drugs were added to this aCSF and pH was adjusted to 7.35 after equilibrium with 95%O₂/5%CO₂. In experiments with memantine, slices were stored in aCSF additionally containing 1 µM of this antagonist for at least 4 hr, which allows it to achieve equilibrium blockade (Frankiewicz *et al.*, 1996). This concentration was chosen as it is clinically relevant (see Discussion).

The CA3 region was dissected and discarded. Slices were then placed on a nylon mesh in an interface chamber (BSC-HT, Medical Systems) and perfused at a rate of 0.8 ml/min⁻¹ with aCSF at 33°C in an oxygen-enriched (95% O₂/5% CO₂) humidified atmosphere. After at least 30 min of incubation in the recording chamber, a glass recording electrode (2–3 MΩ, filled with aCSF) was

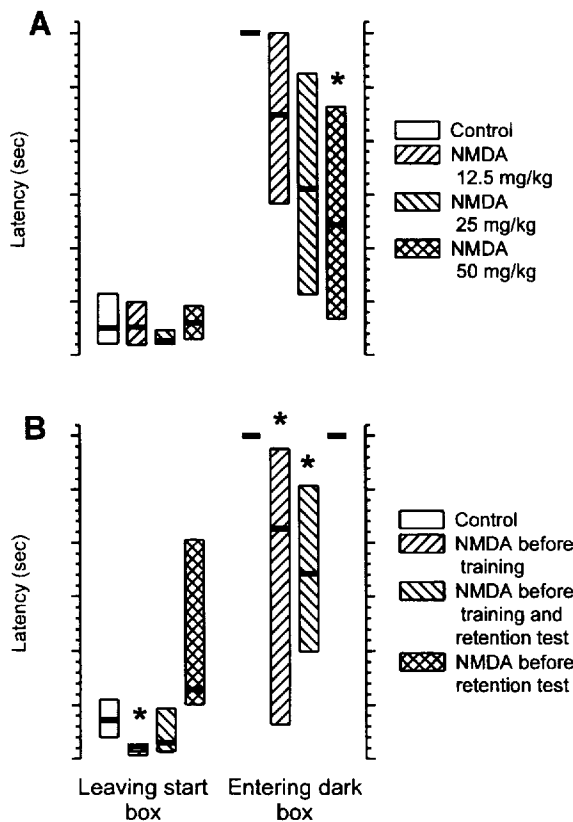


Fig. 1. Effect of NMDA on passive avoidance learning. (A) Different doses of NMDA were administered 30 min. before training. $N = 7-9$. Kruskal-Wallis test values (H) were 1.6 and 11.7 for latency to leave the start box and to enter the dark box, respectively. (B) To test the state-dependency of the learning impairing effect, NMDA (25 mg/kg) was administered 30 min. before training, or 30 min. before retention testing or both. $N = 7-8$. Kruskal-Wallis test values (H) were 15.0 and 12.1 for latency to leave the start box and to enter the dark box, respectively. Results are expressed as medians and 1st and 3rd quartiles as a measure of variation. * $p < 0.05$ as compared to control group (Mann-Whitney test).

positioned in the dendritic layer of area CA1 to record extracellular field excitatory post-synaptic potentials (fEPSP). A concentric, bipolar tungsten stimulating electrode (inner $\varnothing 50 \mu\text{M}$, outer $\varnothing 125 \mu\text{M}$, 100 k Ω , Science products, Germany) was placed 500–700 μm away from the recording electrode but at the same dendritic level to activate the Schaffer collateral commissural fibres. Extracellular recordings were made in response to constant voltage (20–25V, i.e. $\approx 200-250 \mu\text{A}$, square pulse for 20 μsec) single shock stimulation once every 15 sec. (DS2 isolated stimulator, Digitimer). This short stimulus duration was used to minimize stimulus artefacts and allowed stable recordings for at least 4 hr. Stimulus intensities were adjusted to evoke fEPSPs of half maximal amplitude. Responses were recorded with an Axoclamp 2a (Axon Instruments, CA, U.S.A.), further amplified and AC filtered at 0.3 Hz with an Axon AL-2130, and digitized using TIDA for

Windows (HEKA), before being stored on an IBM PC for off-line analysis. The bath was earthed via a silver chloride bar connected to the axoclamp headstage.

Field potentials were sensitive to blockade by GYKI 52466, indicating their mediation via AMPA receptors (data not shown). The slope of the rising phase of the fEPSP (mV/ms^{-1}) was measured between 20–80% of the peak amplitude and was assessed semi-automatically by AUTESP for IBM (Garching Instruments, Munich). A single tetanic stimulation (100 Hz for 1 sec, at unchanged intensity and pulse width) was used to evoke LTP.

Chemicals

The following agents were used in the course of the present study; 1-amino-3,5-dimethyladamantane hydrochloride (memantine; Merz and Co., Germany), (+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5, 10-imine maleate (MK-801; RBI, U.S.A.), CGP-39551

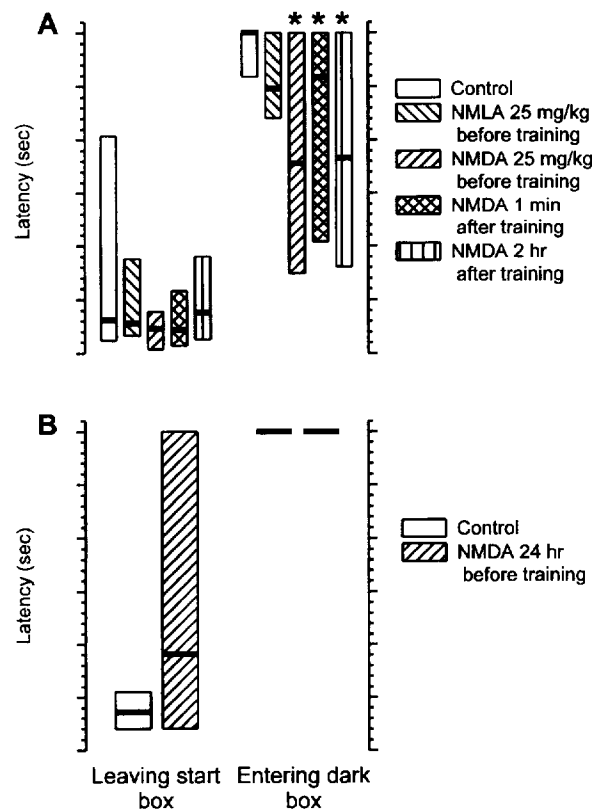


Fig. 2. Effects of NMDA on passive avoidance learning. (A) Time dependency of the learning impairing effect of NMDA. NMDA was administered before the training or 1 min or 2 hr afterwards. An additional group of animals was injected with NMLA (25 mg/kg) before the training. $N = 26, 9, 9, 26$ and 25, respectively. Kruskal-Wallis test values (H) were 4.2 and 13.2 for latency to leave the start box and to enter the dark box, respectively. (B) To test the possible involvement of toxic actions in the learning impairing effect of NMDA, NMDA (25 mg/kg) was administered 24 hr. before training. $N = 8$. Results are expressed as medians and 1st and 3rd quartiles as a measure of variation. * $p < 0.05$ as compared to control group (Mann-Whitney test).

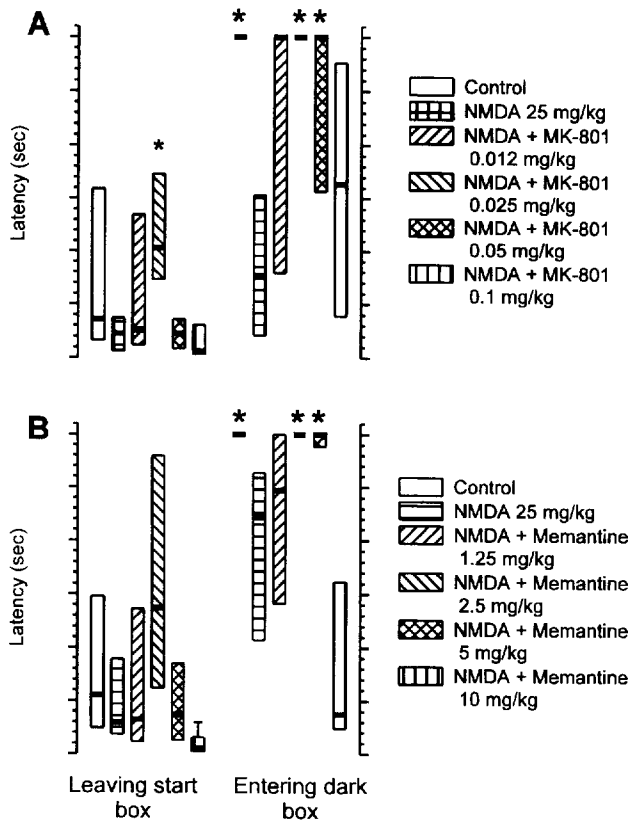


Fig. 3. Effect of uncompetitive NMDA receptor antagonists on NMDA-induced passive avoidance impairment. (A) NMDA and MK-801 were administered 30 min before training. $N = 16, 14, 8, 6, 8$ and 8 , respectively. Kruskal–Wallis test values (H) were $12, 7$ and $29, 0$ for latency to leave the start box and to enter the dark box, respectively. (B) NMDA and memantine were administered 30 min before training. $N = 16, 16, 8, 8, 8$ and 8 , respectively. Kruskal–Wallis test values (H) were $19, 6$ and $35, 3$ for latency to leave the start box and to enter the dark box, respectively. Results are expressed as medians and 1st and 3rd quartiles as a measure of variation. * $p < 0.05$ as compared to NMDA group (Mann–Whitney test).

(DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid ethyl ester; Ciba Geigy, Switzerland), CGP-37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentanoic acid; Ciba Geigy) *N*-methyl-L-aspartate (NMLA; Sigma, St Louis, U.S.A.) and *N*-methyl-D-aspartate (NMDA; Sigma). All substances were dissolved in saline and pH was adjusted to 7.4 ± 0.4 .

Statistical analysis

Statistical analysis was performed with SigmaStat statistical software (Jandel Scientific, U.S.A.) with use of non-parametric ANOVA (Kruskal–Wallis test) which, if significant, was followed by the Mann–Whitney test for pairwise comparison. A difference at $p < 0.05$ was regarded as significant. Results are expressed as medians and percentiles as a measure of variation. In the case of LTP, fEPSP slopes were normalized with respect to the

30 min control period prior to tetanic stimulation or NMDA application and expressed as means \pm SEM, and were analyzed by two-way ANOVA (treatment \times time).

RESULTS

None of the treatments used affected significantly the behavioural parameters measured during the training, i.e. latency to leave the start box and latency to enter the dark box (not shown). NMDA injected systemically (i.p.) 30 min before the passive avoidance training produced dose-dependent amnesia, as evidenced by a reduced latency to enter the dark (shock associated) compartment during the retention test performed 24 hr later ($p < 0.05$, Fig. 1A). On the basis of this experiment, the dose of 25 mg/kg was selected for further studies. The amnesic effect was also seen if additional injection of NMDA was given before the retention test, indicating the impairment

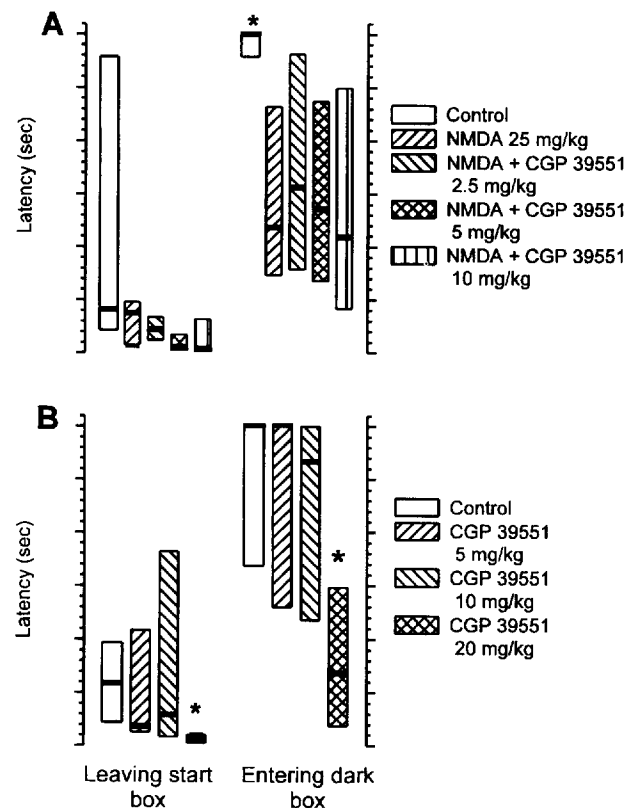


Fig. 4. (A) Effect of CGP-39551 on NMDA-induced passive avoidance impairment. NMDA and CGP-39551 were administered 30 min and 2 hr before training, respectively. $N = 8$. Kruskal–Wallis test values (H) were $9, 7$ and $10, 3$ for latency to leave the start box and to enter the dark box, respectively. (B) Effect of CGP-39551 on passive avoidance learning. CGP-39551 was administered 2 hr before training. $N = 8$. Kruskal–Wallis test values (H) were $12, 5$ and $8, 9$ for latency to leave the start box and to enter the dark box, respectively. Results are expressed as medians and 1st and 3rd quartiles as a measure of variation. * $p < 0.05$ as compared to NMDA group (Mann–Whitney test).

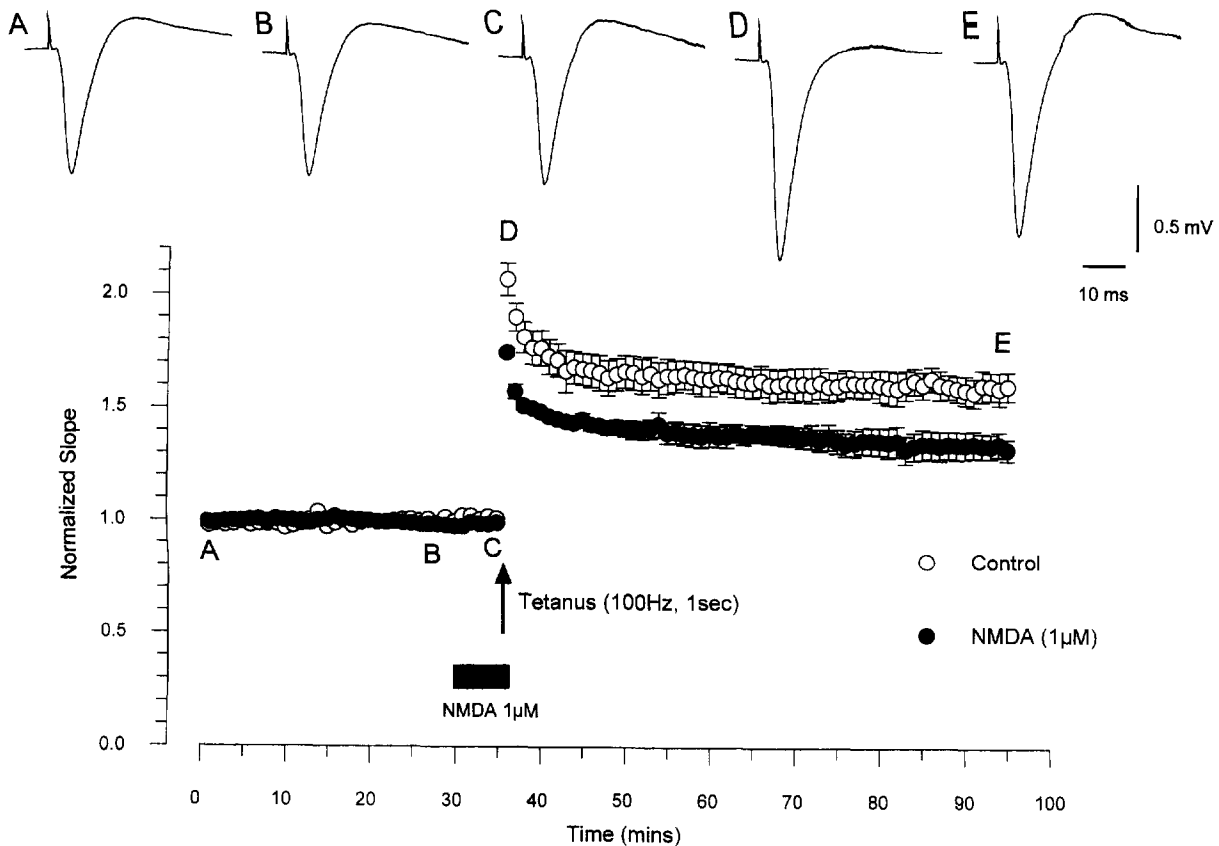


Fig. 5. Effect of short incubations with low concentrations of NMDA on LTP induction/expression in hippocampal slices. Traces were averaged in groups of four consecutive responses ($4 \times 15 \text{ sec} = 1 \text{ min}$) and were then normalized with respect to the grouped average slope of responses during the 30 min prior to NMDA application and have been plotted as means \pm SE against time (control $N = 6$, NMDA $N = 4$). The raw data presented illustrate a representative example of the level of LTP seen after tetanic stimulation in the presence of NMDA ($1 \mu\text{M}$). Each superimposed trace is the average of four consecutive responses. The relation of these responses to the time course of the recording session are given by (A) through (E) on the lower graph. High frequency stimulation (100 Hz, 1 sec) was given at the time indicated by the arrow. NMDA ($1 \mu\text{M}$) was present as indicated by the bar.

was not related to state-dependent learning ($p < 0.05$, Fig. 1B). In contrast, no deteriorating effect on retention was detected when NMDA was injected 30 min before the retention trial alone (Fig. 1B). In this experiment also, the latency to leave the start box was decreased in one group ($p < 0.05$, Fig. 1B). Pre-training injection of the less active isomer, NMLA, did not affect passive avoidance performance (Fig. 2A). NMDA also impaired passive avoidance learning when it was injected shortly, or 2 hr after the training ($p < 0.05$, Fig. 2A). However, NMDA failed to change either passive avoidance training performance or retention latency if injected 24 hr before the training (Fig. 2B).

An uncompetitive NMDA receptor antagonist, MK-801 dose-dependently attenuated the deficit induced by NMDA in a bell-shaped pattern dose-response relationship, i.e. was effective at doses of 0.025 and 0.05, but not higher or lower ($p < 0.05$, Fig. 3A). The doses of MK-801 (and all other antagonists used) were based on our previous study in an identical experimental design. Therefore, MK-801 produces significant impairment at

a dose of 0.2 mg/kg while at 0.1 mg/kg, a strong but non-significant trend is seen towards a shortened latency to enter the dark box (Miszta and Danysz, 1995). A similar characteristic was seen with memantine which was an effective antagonist of NMDA-induced amnesia at doses of 2.5 and 5 mg/kg, but neither at 1.25/nor 10 mg/kg ($p < 0.05$, Fig. 3B). In our previous study, 10 mg/kg was the lowest memantine dose producing learning deficits in this task. In contrast, a competitive antagonist of NMDA receptors CGP-39551 at doses of 2.5 to 10 mg/kg failed to attenuate the effect of NMDA (Fig. 4A). CGP-39551 produced amnesia on its own at a dose of 20 mg/kg ($p < 0.05$, Fig. 4B).

Long-term potentiation

In control experiments, tetanic stimulation (100 Hz) induced stable potentiation of fEPSP slopes to $162 \pm 6\%$ ($n = 6$) of the basal level 50–60 min after tetanic stimulation (Fig. 5). In contrast to previous reports (Izumi *et al.*, 1992), short 5 min incubations with NMDA ($1 \mu\text{M}$) were unable to completely block the induction of

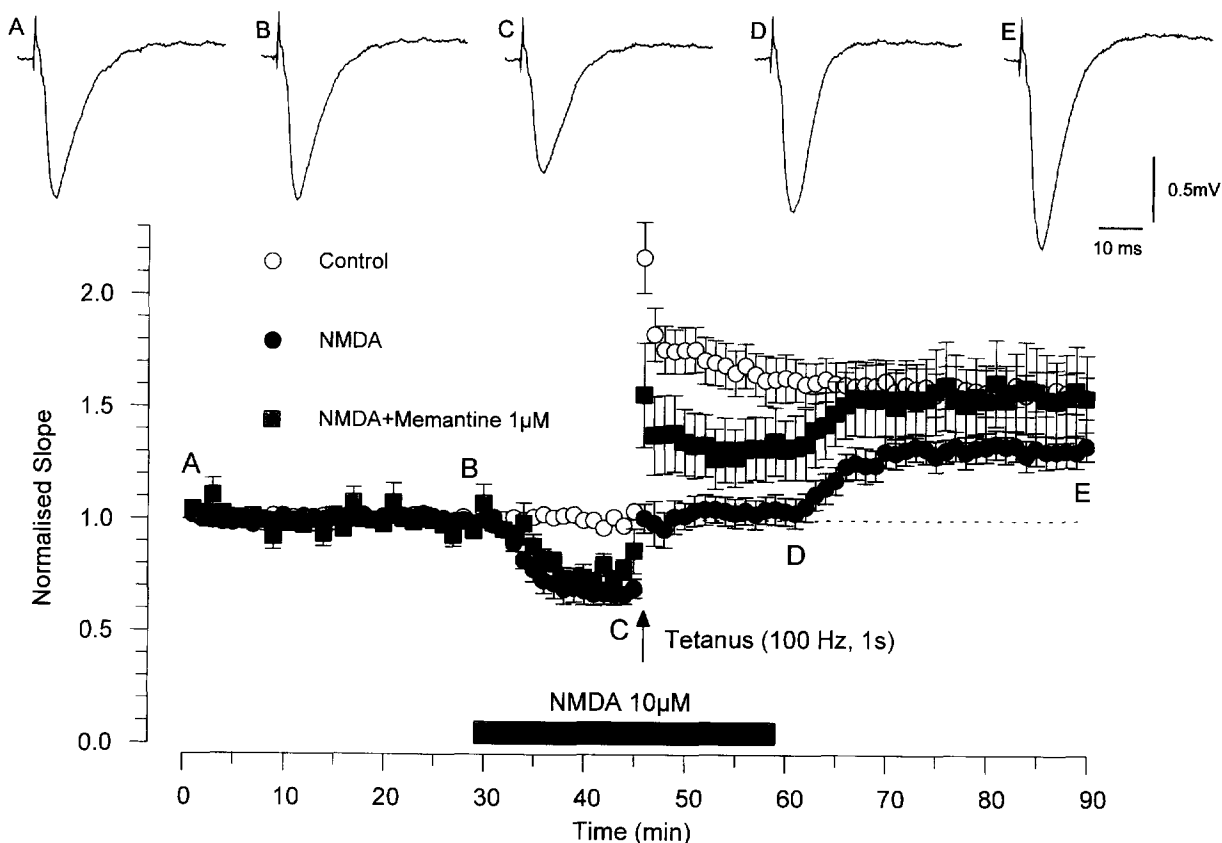


Fig. 6. Effect of NMDA and memantine on LTP induction/expression in hippocampal slices. Analysis and presentation as in Fig. 5. The raw data presented illustrate a representative example of the level of LTP seen in the presence of NMDA ($10 \mu\text{M}$) alone. The number of slices tested at each concentration were as follows: control $N=8$, NMDA $N=8$ and NMDA+memantine $N=8$. NMDA ($10 \mu\text{M}$) was present as indicated by the bar. Memantine ($1 \mu\text{M}$) was continuously present for at least 4 hr before induction of LTP. ANOVA over the whole recording period revealed a highly significant effect of time (89, 2070 $F=248$), treatment (2, 2157 $F=212$) and a time/treatment interaction (178, 1890 $F=2.43$): all $p < 0.0001$.

LTP ($138 \pm 7\%$, $n=4$). Similar short incubations with NMDA $10 \mu\text{M}$ were somewhat more effective ($124 \pm 10\%$, $n=6$), but a depression of baseline responses also became apparent (data not shown).

As such, further experiments were performed with longer 15 min pre-incubations of NMDA to allow this effect on baseline responses to reach equilibrium. In control experiments, tetanic stimulation (100 Hz) induced stable potentiation of fEPSP slopes to $157 \pm 7\%$ ($n=8$) of the basal level 35–45 min after tetanic stimulation (Fig. 6). NMDA ($10 \mu\text{M}$) caused a decrease of the control response to $69 \pm 5\%$ ($n=8$) without causing any apparent tonic depolarization or spreading depression. Although tetanic stimulation did produce an increase in fEPSP slope, this potentiation was only able to return the depressed response to control levels in the continued presence of NMDA ($103 \pm 5\%$, Fig. 6). After NMDA removal from the bath solution, the response recovered to $131 \pm 6\%$ of the control level.

The continuous presence of memantine ($1 \mu\text{M}$) only slightly reduced the depression of the control fEPSP by NMDA ($77 \pm 3\%$, $n=8$) but substantially preserved the

induction of LTP ($129 \pm 10\%$, $p < 0.05$) which then reached control levels of potentiation after removal of NMDA ($152 \pm 15\%$). No attempt was made to increase stimulus intensity to match responses to control levels, as such manipulations would be unlikely to reflect learning processes *in vivo* and a further, undefinable readjustment would also have been necessary following NMDA removal. However, the levels of LTP relative to the depressed response in the continuous presence of NMDA (i.e. control 0–5 min prior to, and potentiated 10–15 min after tetanic stimulation) were as follows: Control: $168 \pm 8\%$; NMDA $151 \pm 3\%$; NMDA + Memantine $169 \pm 7\%$.

Further experiments were performed to test whether the effects of NMDA on baseline responses are truly mediated by NMDA receptors. NMDA ($30 \mu\text{M}$) caused an even more pronounced reduction of baseline fEPSPs without depolarizing the slice, and this effect was antagonized completely by the competitive NMDA receptor antagonist CGP 37849 ($10 \mu\text{M}$, Fig. 7).

A final series of experiments was performed to assess whether longer applications of NMDA really cause stable

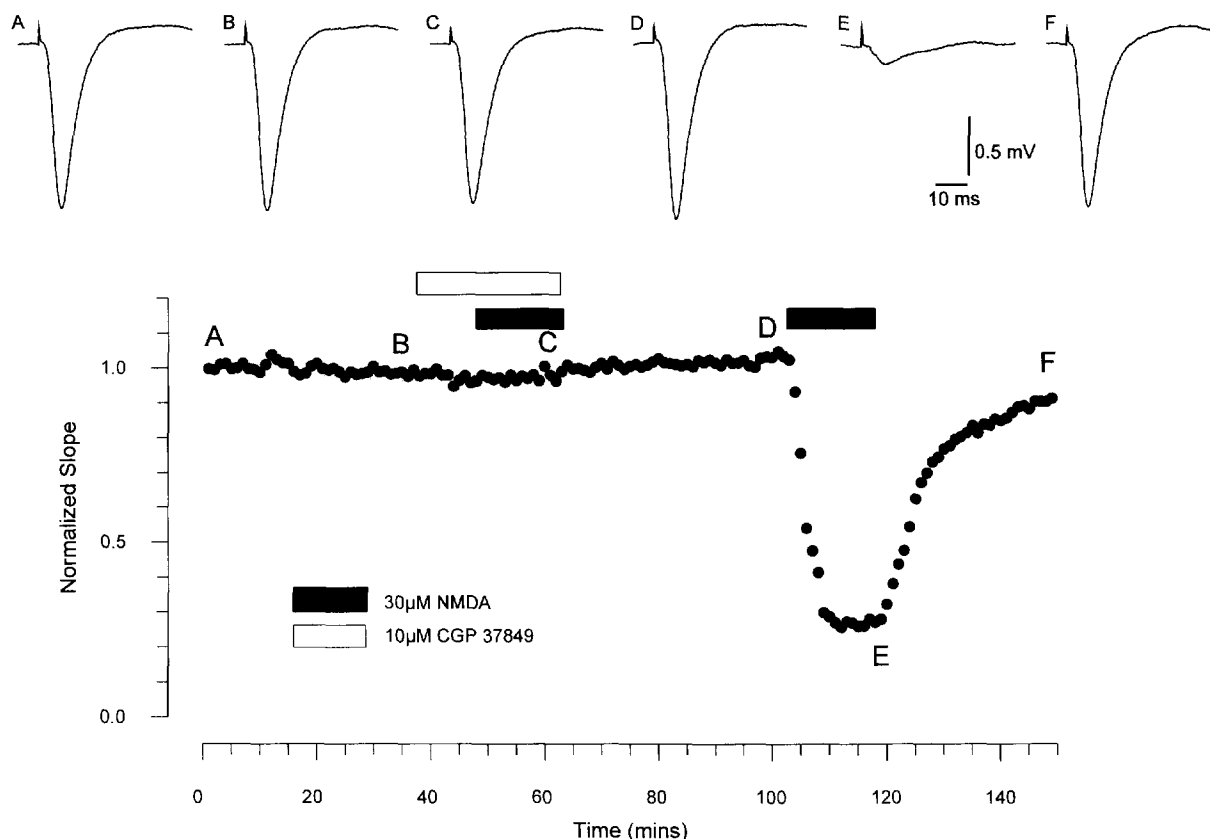


Fig. 7. Depression of AMPA receptor-mediated fEPSPs by high concentrations of NMDA in hippocampal slices. Analysis and presentation as in Fig. 5. The raw data presented illustrate a representative example of fEPSPs at the time points indicated on the lower graph, which shows the average of two experiments. NMDA (30 μ M) and CGP 37849 (10 μ M) were present as indicated by the filled and open bars, respectively.

depression of fEPSPs and if this effect is reversible. NMDA (10 μ M) for 1 hr caused a stable decrease of the control response by 10.0%, which reached equilibrium within 5–10 min without causing any apparent tonic depolarization or spreading depression (assessed without AC filtering, $N=4$, Fig. 8). This effect was fully reversible indicating no NMDA-induced LTP or LTD. Possible, depotentiating effects of NMDA were not assessed.

DISCUSSION

In the present study we report that NMDA, in an apparently specific manner, impairs passive avoidance learning if given before training (or shortly afterwards) but not if injected immediately before the retention test. This NMDA effect is not state-dependent, as demonstrated by the experiment where NMDA was ineffective when given before the retention test alone but was effective when given before training alone or both before training and retention. The NMDA-induced impairment also probably does not involve toxic effects since in rats injected with NMDA 24 hr before the training no deficit was seen. If the effects of NMDA were due to toxicity, then such effects would be expected to be greater after

24 hr than after 30 min (Bandopadhyay and Debelleroche, 1995). Moreover, the doses used in the present study were relatively modest, as compared to convulsive effects of NMDA in rats and mice which appear at 100–200 mg/kg (Leander *et al.*, 1988; Ormandy and Jope, 1991; Bisaga *et al.*, 1993). Whilst it cannot be excluded that NMDA produces this impairment of learning in an unspecific manner, the lack of NMDA effect when given before the retention test, the absence of any apparent behavioural inhibition and the inactivity of NMLA makes this seem unlikely.

One critical issue for the present study is the assumption that NMDA affects respective receptors in the CNS directly. This could be brought into doubt considering its highly hydrophilic structure. Nevertheless, there are several indications that relatively low doses of NMDA do indeed cross the intact blood–brain barrier. It has been reported that after systemic administration NMDA (10–60 mg/kg) produces CNS related effects such as dipsogenic effects in pigeons (Baron and Woods, 1993), a clear drug discrimination cue that is antagonized by competitive NMDA receptor antagonists (Bennett *et al.*, 1988; Willetts and Balster, 1989; Grech *et al.*, 1993, 1995), induction of γ -motoneuronal discharges in cats

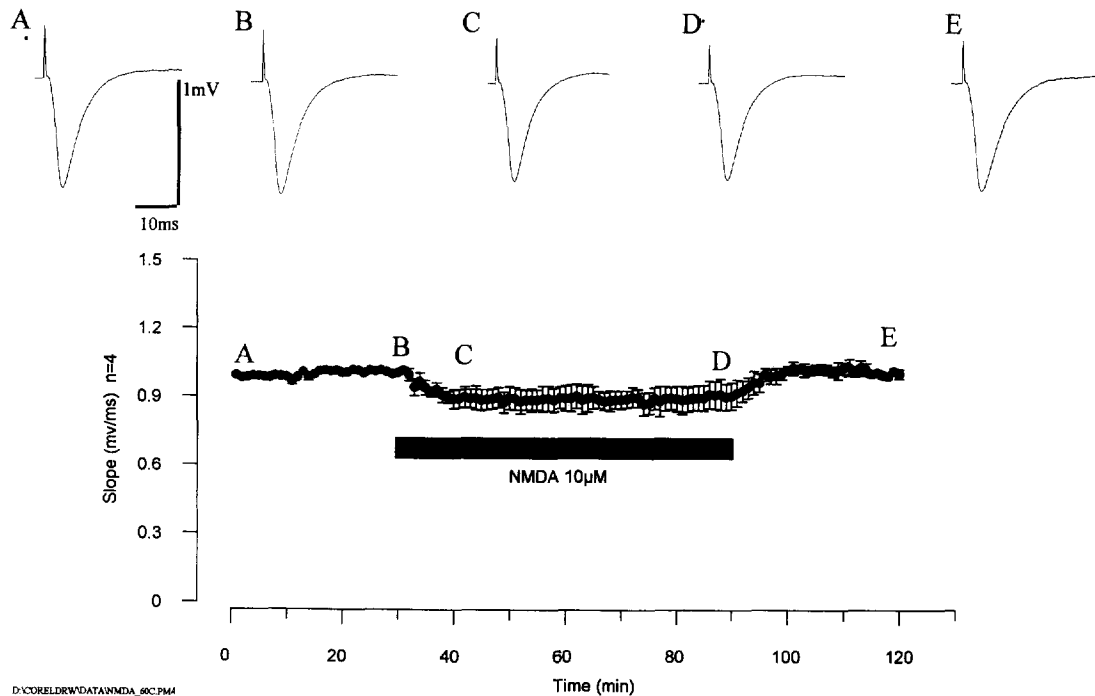


Fig. 8. Long lasting, reversible depression of fEPSPs by NMDA in hippocampal slices. The raw data presented on the top illustrate a representative example of fEPSPs at the time points indicated on the lower graph, which shows the average of four experiments (mean \pm SE). NMDA ($10 \mu\text{M}$) was present for 1 hr as indicated by the bar.

(Polc, 1987) and attenuation of MK-801 induced locomotion in mice (Irifune *et al.*, 1995).

The learning impairing effect of NMDA described here is in line with previous findings obtained by Jones *et al.* (1989) in a passive avoidance test in mice and later by Cohn and Cory-Shlechta (1994) in the repetitive learning lever pressing procedure in rats. At first sight, this may seem to contrast with the accepted role of NMDA receptors in learning processes and LTP induction. However, there are several mechanisms by which NMDA could produce detrimental effects on learning.

1. After NMDA administration, a direct, tonic (i.e. non-temporal), activation of NMDA receptors might occur. This, in contrast to learning, may lead to an increase in synaptic "noise" and in turn to a loss of association-detection sensitivity.
2. Tonic NMDA receptor activation may cause long term changes in the sensitivity of secondary processes by, for example, the generation of nitric oxide (Izumi *et al.*, 1992; Kato and Zorumski, 1993) or accommodation of Ca^{2+} -dependent mechanisms (Danysz *et al.*, 1995a)
3. NMDA could preferentially increase the activity of inhibitory GABAergic pathways.

Although the effects of NMDA given prior to behavioural training might be related to any one or several of these mechanisms, the impairing effect of post-training administration of NMDA would seem to be best explained by depotentiation.

In support of the possibility that NMDA might produce selective impairment of memory, we also observed a modest effect of NMDA in reducing the induction and/or expression of LTP in hippocampal slices. Unfortunately, a decrease in pre-LTP values of the fEPSP was also seen at higher concentrations, making interpretation of the concentration-dependence of this effect more difficult. Since after NMDA removal the fEPSPs partially reached potentiated levels, it is not clear whether the observed effect represents inhibition of induction *per se* or the expression of enhanced EPSPs or both. On the basis of present results, both effects seem likely.

Previously Izumi *et al.* (1992) showed that prior exposure of hippocampal slices for 5 min to very low concentrations of NMDA ($0.2\text{--}2 \mu\text{M}$) completely prevents the induction of LTP in the CA1 region. This effect was shown to be clearly dependent on NMDA receptor stimulation, subsequent Ca^{2+} influx and nitric oxide production (Izumi *et al.*, 1992; Kato and Zorumski, 1993). Post-tetanic exposure to NMDA also had an inhibitory effect (*ibid.*). We were unable to see such pronounced effects of low concentrations of NMDA in the present experiments. The reasons for this discrepancy remain elusive but could, for example, be due to the use of higher stimulus intensities and the recruitment of NMDA receptors in baseline responses in the study of Izumi *et al.* (1992), the authors set stimulus intensities to evoke population spikes of 50% maximum amplitude. Another major difference is that most of the recordings of Izumi *et al.* (1992) were made with the CA3 region intact,

which might be expected to increase the level of spontaneous activity in the slice.

Removal of Mg^{2+} has also been reported to prevent the induction of LTP in hippocampal slices by allowing tonic activation of NMDA receptors by endogenous glutamate (Coan *et al.*, 1989). The mechanism of this effect is also not clear, but in this context it is note worthy that others reported that low frequency NMDA receptor-mediated synaptic activity can prevent or even reverse LTP (Huang *et al.*, 1992; Malenka, 1994). One study even indicates that exposure of slices to high concentrations of glutamate may erase previously induced LTP without signs of toxicity (Harrison and Alger, 1993).

The receptor specificity of the NMDA-induced amnesia or LTP inhibition was demonstrated by the antagonism by an uncompetitive NMDA receptor-antagonist, memantine (Kornhuber *et al.*, 1989; Chen *et al.*, 1992; Parsons *et al.*, 1993). Interestingly, the decrease of fEPSP amplitude induced by NMDA was not attenuated by this low concentration of memantine but was antagonized completely by a high concentration of CGP 37849. Lower concentrations of CGP 37849 ($3 \mu M$) were less effective in blocking the depression of fEPSPs by NMDA but were still able to completely block the induction of LTP ($IC_{50} = 0.37 \pm 0.04 \mu M$, unpublished data), indicative of different sensitivities of these two processes to competitive NMDA receptor antagonists. Similar experiments were not performed with memantine due to problems of its acute access to and removal from the slice (Frankiewicz *et al.*, 1996). Previously Coan *et al.* (1989) found that in hippocampal slices, (CA1) removal of Mg^{2+} produces blockade of LTP which can then be restored by a low concentration ($20 \mu M$) of the competitive NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (APV).

In analogy to data obtained with LTP, in the passive avoidance test NMDA-induced amnesia was antagonized by low doses of memantine (2.5 and 5 mg/kg) and of MK-801, another uncompetitive NMDA receptor antagonist (0.025 and 0.05 mg/kg). At higher doses, 0.1 and 10 mg/kg for MK-801 and memantine, respectively, no positive effect was seen. This is not surprising considering that these doses are only 50% lower than doses producing clear-cut passive avoidance learning impairment on their own (Misztal and Danysz, 1995). Thus, NMDA receptor antagonists at low doses can paradoxically enhance learning (attenuate learning deficit) and this effect may possibly be related to a restoration of signal to noise ratio following tonic activation of NMDA receptors. However, in the present study the competitive NMDA receptor antagonist CGP-39551 failed to restore normal learning after the NMDA challenge. A basic difference exists between uncompetitive and competitive NMDA receptor antagonist, since only the former class produces voltage-dependent block. Therefore, it can be assumed that memantine and MK-801 inhibit NMDA receptors and prevent their tonic low level activation by NMDA. In contrast, synaptic activity results in pro-

nounced transient glutamate release and in turn, stronger depolarization (through AMPA receptors) leading finally to removal of uncompetitive antagonist block during rapid repetitive stimulation. This mode of action resembles, to a certain extent, the effects of the endogenous NMDA receptor antagonist Mg^{2+} (Parsons *et al.*, 1993). Since competitive antagonists completely lack this "switch effect", the "improving" and "impairing" doses may overlap.

Memantine has been shown to have cognition enhancing effects in moderately demented patients (Ditzler, 1991; Pantev *et al.*, 1993), which may also seem to be surprising in the view of the accepted role of NMDA receptors in learning (Collingridge and Singer, 1990; Danysz *et al.*, 1995b). The present data raise the possibility that such cognitive effects of memantine could result from the restoration of signal-to-noise ratio. The doses producing antagonism of NMDA-induced amnesia (2.5 and 5.0 mg/kg) would be expected to produce serum levels of approximately $0.5\text{--}1.0 \mu M$, which is within the therapeutic range (Danysz *et al.*, 1994; Quack *et al.*, 1995). On the other hand, an amnesic effect is seen at a dose of 20 mg/kg (Misztal and Danysz, 1995) leading to much higher serum levels, $5.4 \mu M$ (Danysz *et al.*, 1995b), and *in vitro* inhibition of LTP is seen with an $IC_{50} = 11.6 \mu M$ (Frankiewicz *et al.*, 1996). It should be stressed at this point that there are reasons to believe that the brain interstitial concentration of memantine is slightly lower than serum levels (approximately 40%), and much lower than the total levels in brain homogenates (20–30 times, Danysz *et al.*, 1994; Quack *et al.*, 1995).

In conclusion, the present study indicates that the continuous, non temporal activation of NMDA receptors leads to both passive avoidance learning impairment and more moderate inhibition of LTP *in vitro*. This effect is attenuated by the uncompetitive NMDA receptor antagonist memantine in the case of LTP and by both MK-801 and memantine in the behavioural paradigm.

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