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# Facilitation of glutamate receptors enhances memory

**(chronic recording/synaptic responses/spatial mazes/olfactory learning)**

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**ABSTRACT** **A benzamide drug that crosses the blood–brain barrier and facilitates DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated synaptic responses was tested for its effects on memory in three behavioral tasks. The compound reversibly increased the amplitude and prolonged the duration of field excitatory postsynaptic potentials in hippocampal slices and produced comparable effects in the dentate gyrus *in situ* after intraperitoneal injections. Rats injected with the drug 30 min prior to being given a suboptimal number of training trials in a two-odor discrimination task were more likely than controls to select the correct odor in a retention test carried out 96 hr later. Evidence for improved memory was also obtained in a water maze task in which rats were given only four trials to find a submerged platform in the presence of spatial cues; animals injected with the drug 30 min before the training session were significantly faster than vehicle-injected controls in returning to the platform location when tested 24 hr after training. Finally, the drug produced positive effects in a radial maze test of short-term memory. Well trained rats were allowed to retrieve rewards from four arms of an eight-arm maze and then tested for reentry errors 8 hr later. The number of such errors was substantially reduced on days in which the animals were injected with the drug before initial learning. These results indicate that a drug that facilitates glutamatergic transmission enhances the encoding of memory across tasks involving different sensory cues and performance requirements. This may reflect an action on the cellular mechanisms responsible for producing synaptic changes since facilitation of AMPA receptors promotes the induction of the long-term potentiation effect.**

Recent work indicates that facilitation of DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (glutamate) receptor-mediated transmission in slices of hippocampus reduces the amount of afferent stimulation needed to induce a maximal degree of long-term potentiation (LTP) without changing the LTP "ceiling" itself (1). Because there is evidence implicating LTP as a substrate for certain types of memory (2), it is possible that drugs that produce such effects in brain will reduce the amount of training needed for the formation of robust memory. The experiments reported here tested this idea by using a drug that crosses the blood–brain barrier and enhances synaptic responses in freely moving animals.

The carbonic anhydrase inhibitor cyclothiazide and the nootropic compound aniracetam enhance excitatory transmission *in vitro* by prolonging the open time of glutamate (AMPA) receptors (3–6). Cyclothiazide probably does not cross the blood–brain barrier (7) and aniracetam is rapidly metabolized in peripheral tissues to anisoyl γ-aminobutyric acid (GABA) (8), which we have found to have little effect on glutamatergic transmission. Accordingly, we synthesized a series of compounds that lack the labile imide function of aniracetam and tested their effects first with *in vitro* slices and then in brain after peripheral administration. Three separate structural modifications yielded a drug [1-(1,3-benzodioxol-5-ylcarbonyl)piperidine] with the requisite pharmacological characteristics. This compound was used in three behavioral paradigms and found to substantially improve memory encoding in each case. These results may be of significance for efforts to develop therapeutics directed at memory disorders.

## MATERIALS AND METHODS

Slices of hippocampus were prepared and maintained in an interface chamber using conventional methods. Field excitatory postsynaptic potentials (EPSPs) were recorded in the stratum radiatum of region CA1b and elicited by single stimulation pulses delivered once per 20 sec to a bipolar electrode positioned in the Schaffer commissural projections. Measures of the slope, amplitude, and duration of the responses were collected from digitized records.

Chronic stimulation electrodes were implanted in the perforant path and chronic recording electrodes were placed in the hilus of deeply anesthetized male rats (Long–Evans; 250 g). The final positions of the electrodes were determined by physiological criteria; i.e., the production of a large, short-latency field EPSP with minimal stimulation currents. The electrodes were then attached to a head connector, which was permanently affixed to the skull. One week later the rats were acclimated to a chronic recording cage and to the attachment of a recording lead to the head connector. Recordings were collected for 2–3 days before the start of experimental sessions to ensure the stability of the stimulation–recording arrangements (see ref. 9 for a more complete description). For drug testing, the rats were placed in the cage and 60–90 min of baseline recording was conducted; in most cases, the animals were then injected with vehicle (cyclodextrin) and then 30–45 min later with vehicle plus the experimental benzamide compound (120 mg/kg). Recordings were then continued for 2–3 hr. Seven rats were used, most of which were tested twice with the drug.

The behavioral equipment and paradigms used in the studies are described below; different groups of adult male rats were used in the separate experiments.

## RESULTS

Fig. 1 *B* and *D* illustrates results from slices for the benzamide; as shown, the compound produced an increase in the amplitude of the field EPSP and extended its duration and did so to a much greater extent than an equivalent concentration of aniracetam (Fig. 1 *A* and *C*). A number of benzamide compounds have been synthesized and shown to be more

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Abbreviations: AMPA, DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; LTP, long-term potentiation; EPSP, excitatory postsynaptic potential; GABA, γ-aminobutyric acid.

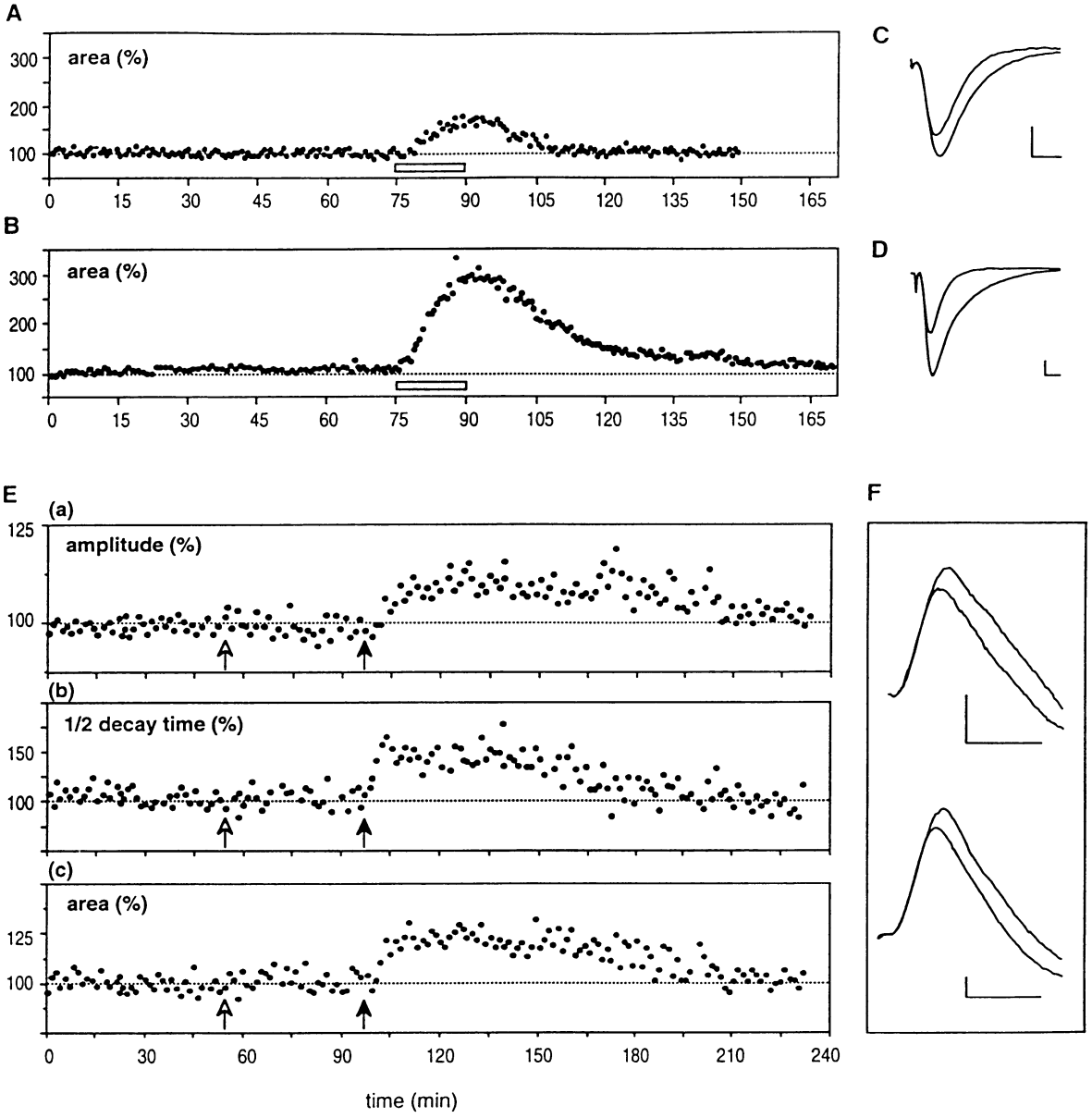


FIG. 1. Drug-induced facilitation of synaptic responses in hippocampus. (*A*) Areas of field EPSPs recorded in the CA1 region of a hippocampal slice before, during, and after a 15-min infusion (horizontal bar) of aniracetam at 1.5 mM. (*B*) A comparable experiment in which the same concentration of the experimental drug (see text) was used; the illustrated effects are typical of those obtained with the drugs. (*C*) Averaged field EPSPs from the experiment shown in *A* recorded before (upper trace) and at the end of (lower trace) infusion of aniracetam. [Bars = 0.5 mV (ordinate) and 5 msec (abscissa).] (*D*) Averaged field EPSPs from the experiment shown in *B* recorded before and after infusion of the experimental drug. Traces are shown on a longer time sweep than those in *C* so that the full effect of the drug can be illustrated. [Bars = 0.5 mV (ordinate) and 5 msec (abscissa).] (*E*) Evoked potentials recorded in the hilus of the dentate gyrus in response to stimulation of the perforant path in freely moving rats. Effects of an injection of carrier vehicle (25% cyclodextrin in saline; open arrow) or of the experimental compound (120 mg/kg) (solid arrow) are shown for three response parameters (amplitude, one-half decay time, and area). (*F*) Averaged evoked responses elicited by perforant path stimulation before (lower traces) and after (upper traces) drug injection. Results are illustrated for two freely moving rats with control (predrug) responses of very different amplitudes. [Bars = 1.25 mV (ordinate) and 5 msec (abscissa).]

potent than aniracetam but structure–activity relationships have not yet been established. Results from i.p. injections are summarized in Fig. 1 *E* and *F*. Peripherally administered drug enhanced the field EPSP evoked by perforant path stimulation in freely moving rats (Fig. 1*F*) much in the way that it did for responses elicited in slices. The effect had a rapid onset and persisted for considerable periods as shown in Fig. 1*E*; injections of the carrier vehicle had no effects on the evoked responses. Similar results were obtained from seven rats with chronically implanted electrodes (Table 1). These findings indicate that the compound crosses the blood–brain barrier and produces a reliable facilitation of central synapses. Rapid transport to brain after systemic injections has also been observed with positron-emission tomography (PET) scans using C-11-labeled analogue and variants of the benzamide have been found to produce physiological effects similar to those shown in Fig. 1 in anesthetized rats (G.R., J. Larson, P. Xiao, and G.L., unpublished data). Aniracetam at concentrations 4 times greater than those used in the experiments described above (i.e., at concentrations approaching the limit of solubility) had no detectable effect on the field EPSP in three rats with chronically implanted electrodes.

Behavioral studies were carried out with i.p. injections at dosages found to enhance glutamatergic transmission. The

Table 1. Percentage increases for three synaptic response parameters after i.p. injections of a drug (AMPA) that modulates glutamate receptors

| Parameter | % increase, mean ± SE |
|---|---|
| Amplitude | 19 ± 12* |
| Half-width | 12 ± 2** |
| Area | 32 ± 15** |

Evoked responses elicited by stimulation pulses (3 per min) delivered to the perforant path were recorded in the hilus of the dentate gyrus of freely moving rats. Average values for all responses collected for 30 min before and 30 min after injection of the drug (120 mg/kg) were compared in 13 experiments involving seven rats. Values shown are means (±SE) for the within-rat comparisons. *, $P < 0.01$; **, $P < 0.001$; paired $t$ tests.

drug reduced spontaneous activity (e.g., rearing) of rats in an open field but did not detectably change sensory-motor performance in response to visual or auditory cues (e.g., novel objects in an open field, clicks). Its effects on two-odor discrimination learning are summarized in Fig. 2*A*. This paradigm involved the simultaneous presentation of two odors from any of six positions in a semicircular open field; rats were given a water reward if they broke a photobeam immediately below the origin of the odor designated as correct. An incorrect selection caused a light to flash for 4 sec. Rats with extensive training become adept at learning new odor pairs and such animals were used in the drug experiments. Control (vehicle injections; $n = 27$) and experimental (drug injected; $n = 27$) rats were given four trials on a novel pair of odors; these discriminations were known from other studies (10) to be readily acquired by rats given 10 or more trials. Eight control and eight drug-treated rats were also given 15 trials on a difficult and slowly acquired discrimination; i.e., one in which normal rats improve slowly over blocks of 5 trials. Thus, 35 rats in each of two groups (vehicle or drug-injected 30 min prior to training) were given a suboptimal number of training episodes. Four days after training, the animals were given two unrewarded "probe" trials and the percentage of rats in each group selecting the correct odor was recorded. There were no evident differences between control and experimental rats during acquisition but the experimental rats performed well above chance levels on the probe trials 4 days after training while the controls did not (Fig. 2*A*). Omitting the data for the animals given the difficult discrimination did not affect the outcome of the analysis. It bears repeating that control rats given additional trials show excellent retention for these discriminations—thus, it appears that the drug reduced the amount of training needed for encoding of long-term memory.

A second test was carried out with the Morris water maze (2). In this paradigm, rats were first given 3 days of training (four trials per day with a 1-hr intertrial interval) in which they learned that a submerged platform was present in a large pool of opaque water surrounded by a black curtain. The position of the platform was varied across days. One week later, the rats were returned to the maze, injected with drug or vehicle, and given four training trials with the curtain removed and thus in the presence of a collection of large, extramaze (spatial) cues. Each of the four trials began from a different quadrant of the maze with the platform always in the same position. The time the rats took to return to the same location was then measured for a single test trial carried out 24 hr later. One vehicle and 1 experimental rat behaved aberrantly during the training session and were dropped from the study, leaving 16 animals in the control group and 17 in the experimental group. Fig. 2*B* summarizes the median scores for these two groups during the acquisition and probe trials. Performance for the individual animals in both groups improved over the course of the four training trials as evidenced by a decrease in the average latency for trials 3 and 4 combined versus that for trials 1 and 2 combined. The groups did not differ significantly on the first trial or on average latencies across trials. The scores for the probe trial were statistically different ($P = 0.015$; Mann–Whitney U test), with the drug-treated rats requiring less time to reach the platform location. The differences between the means of the groups did not reach statistical significance ($P = 0.07$; $t = 1.51$). Given that the frequency distributions for each group were skewed, the nonparametric statistic is the more appropriate test for differences.

The behavioral effects of the centrally active modulator of glutamate receptors were also examined in an eight-arm radial maze in which rats ($n = 15$) were tested repeatedly over a period of 3 months. Daily sessions involved two episodes, in the first of which four arms were blocked. The rats were allowed to enter the open arms to retrieve a food reward (chocolate chips) and then returned to their home cages. Six or 8 hr later they were returned to the maze, which now had all arms open but contained rewards only in the previously blocked arms (blocked arms were randomized across days). The number of incorrect entries (i.e., arms entered more than once) and the number of correct choices made before a reentry occurred were recorded. Previous studies indicate that rats show excellent retention on tests of this kind at 4–6 hr with an evident decay in memory thereafter (11, 12). This occurred in the present experiments as evidenced by an increase in reentry errors from 1.1 ± 0.6 to 1.6 ± 0.4 (mean ± SD) in tests carried out with 6- vs. 8-hr delays between sessions ($P < 0.01$; paired two-tailed $t$ test; six experiments at each delay). The experimental question investigated with the radial maze was whether the glutamate receptor modulator would prolong the duration of memory. Fifteen rats were given sham or saline injections (4 days), vehicle injections (6 days), or drug injections (6 days) on alternate days 30 min before the first trial and were then tested 8 hr later. Fig. 2*C* summarizes the results; the rats exhibited substantially better retention on drug-injection days than they did on control injection days ($P < 0.0001$; paired $t$ test). This was equally true for both reentry errors [$t(14) = 7.54$] and for number of correct choices [$t(14) = 5.55$] before an error. The effect held for 14 of the 15 rats—the lone exception exhibited excellent retention on control days and thus had little room for improvement. Since the rats had extensive experience with the maze before being injected with drug, it can be assumed that they were thoroughly familiar with the procedural aspects of the task. Moreover, there was no improvement over the several weeks of testing with control and drug injections, indicating that the experimental compound was not facilitating performance by promoting the encoding of "rules" pertinent to the task.

## DISCUSSION

These results indicate that facilitation of glutamatergic transmission causes a general improvement in memory encoding. The first two studies used a suboptimal number of training trials to test whether the experimental treatment would reduce the amount of training needed for stable memory. The two paradigms involved different rewards (food, escape), sensory cues (odors, spatial relationships), and locomotor behaviors (approach and nose poking, swimming). The third paradigm tested for effects on the duration of memory and involved still another set of behaviors. In all instances, the rats injected with drug prior to initial learning exhibited substantially better performance on a subsequent memory test. It is noteworthy that learning in the three behavioral tasks is dependent on the hippocampus (13–15) and is likely to involve cortical circuitries as well. AMPA receptors, the target of the experimental manipulation, are concentrated in these regions (16).

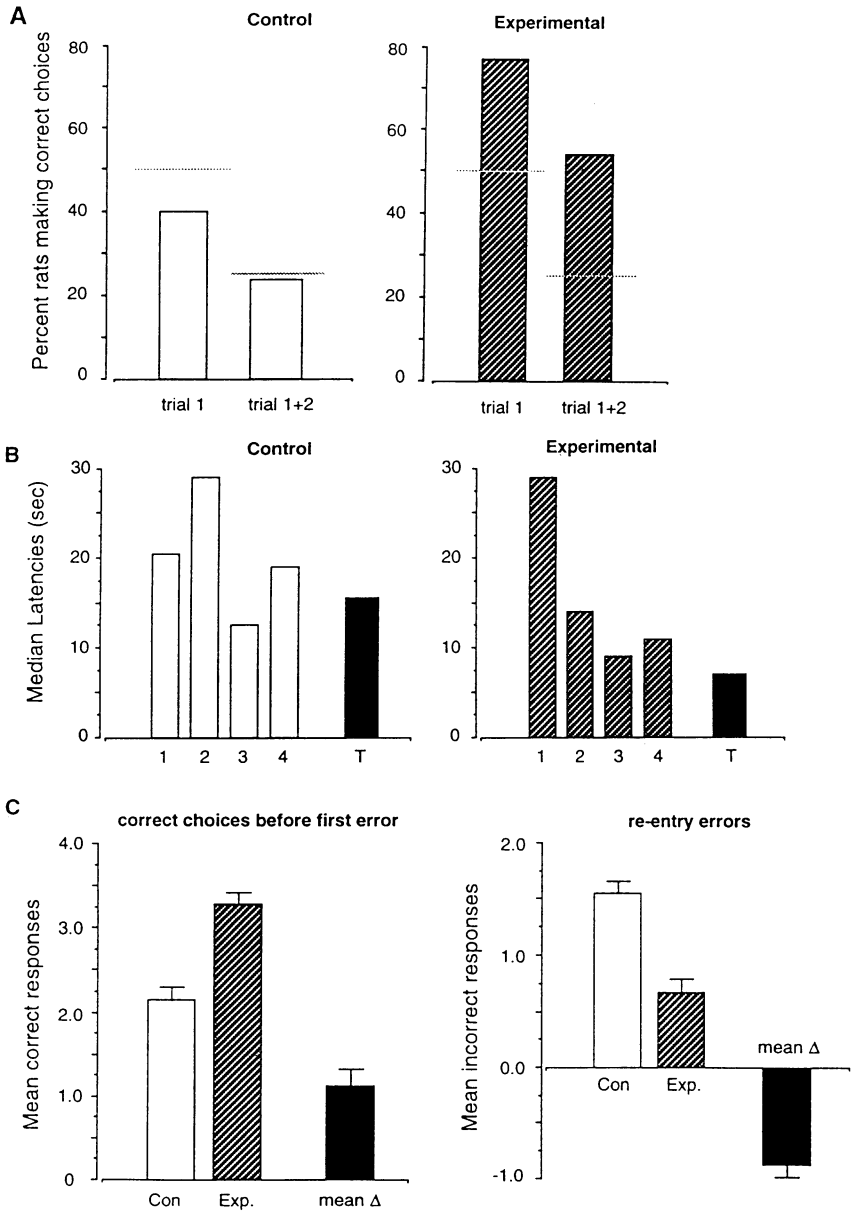


FIG. 2. Effects of a drug that facilitates glutamatergic transmission on learning in three behavioral paradigms. (*A*) Retention of a two-odor discrimination. Percentages of control ($n = 35$) and experimental ($n = 35$) rats selecting a correct odor on two nonrewarded trials given 96 hr after initial training; bars labeled trial 1+2 denote percentage of animals that chose the correct odor on both trials. Dotted lines indicate expected performance if the rats made random selections (i.e., 50% of the rats would select the correct odor on trial one and 25% would select it on trials one and two). The rats were given the centrally active drug (see Fig. 1) or vehicle 30 min before initial training. The experimental animals performed above chance levels on test trials 96 hr later ($P < 0.01$; $\chi^2$ goodness-of-fit test); performance of the control rats did not differ from that expected from chance responding. A description of the apparatus and paradigm can be found in ref. 10. (*B*) Retention in a Morris water maze. Control ($n = 16$) and experimental ($n = 17$) rats were given four trials (15-min intertrial interval) in a circular pool of water surrounded by large visual cues (extramaze spatial cues). Each trial began in a different quadrant of the maze. A submerged platform was located in the same position for each trial; height of bars denotes median latencies for the rats to find the platform. Twenty-four hours later the rats were given a single retention trial (T) with the platform in the same location as in the acquisition trials. The rats were injected with drug or vehicle 30 min before the four acquisition trials; differences between the groups on the retention trial were statistically significant ($P = 0.015$ Mann–Whitney U test). (*C*) Retention in an eight-arm radial maze. Rats were allowed free access to an eight-arm radial maze, each arm of which had a food reward at its terminus. Four arms were blocked in a first session. Eight hours later the animals ($n = 15$) were retested with all arms open; the number of reentries into already selected arms (reentry errors) and the number of correct choices made before a reentry error was committed were recorded. Open bars show scores (mean ± SE) for 10 days on which saline injections or vehicle injections were used; hatched bars are scores on 6 days on which the centrally active drug was injected 30 min before the first test session. Experiments were run over a period of 3 months; the first day of testing in any given block of sessions was not used for drug or control injections. Solid bars summarize means of the within-animal differences between control and experimental days ($P < 0.0001$; paired $t$ tests).

That facilitation of excitatory receptors enhances memory has an interesting counterpart in the effects caused by manipulations that facilitate inhibitory receptors. Benzodiazepines, which augment $GABA_A$ receptor-mediated hyperpolarizing currents, are known to produce anterograde amnesia in animals and humans (17, 18). Possibly then, the balance of fast excitatory transmission vs. the slower inhibitory responses affects the amount of training needed to form memory. This relationship could reflect an influence of excitation/inhibition on the rate at which experienced ani-

mals process information in the constrained environment of a learning task; i.e., in circumstances in which pertinent cues and pertinent responses are unambiguous and familiar. It is also possible that the balance of excitation vs. inhibition affects learning by an action on the machinery that encodes memory. Enhanced GABAergic transmission impedes the induction of LTP (19), while facilitation of excitatory receptors promotes the potentiation effect (1). This latter result presumably reflects the effects of the greater depolarization elicited by afferent bursts in the presence of the drug on the voltage-sensitive *N*-methyl-D-aspartate (NMDA) receptors; i.e., by enhancing the response of target cells to brief stimulation episodes, drugs that modulate the AMPA receptor increase the magnitude of the NMDA receptor-mediated currents that trigger LTP. Thus, if LTP is a substrate of memory, then enhancement of AMPA receptors would be predicted to improve learning, the result obtained in the present study. Since the stability as well as magnitude of LTP varies depending on the amount of afferent stimulation used for induction (20) and may differ across brain subsystems (9, 21), it is conceivable that the potentiation effect participates in both the decremental and stable forms of memory studied in the present experiments.

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