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Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation *in vivo*

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ABSTRACT **An experimental drug, 1-(1,3-benzodioxol-5-ylcarbonyl)piperidine, that facilitates glutamatergic transmission in brain after systemic administration was tested for its effects on the induction of long-term potentiation in the hippocampus of rats. Intraperitoneal injections of the drug markedly increased the degree and duration of long-term potentiation; similar results were obtained with an analogue of 1-(1,3-benzodioxol-5-ylcarbonyl)piperidine that was also found to improve retention of memory in a radial maze task and in an odor-matching problem. These results define tools for enhancing long-term potentiation *in vivo* and confirm an important prediction from the hypothesis that long-term potentiation is a substrate of memory.**

Long-term potentiation (LTP) is a stable increase in synaptic strength known to occur at a variety of sites in the forebrain after brief periods of high-frequency afferent stimulation (1–3). The characteristics of LTP, including rapid development (4), persistence (5, 6), and synapse specificity (7, 8), are suggestive of a memory-encoding process; moreover, patterns of physiological activity that have a strong relationship with LTP induction (9–11) have been recorded from neurons during learning (12). The hypothesis, arising from these observations, that LTP is a substrate of memory predicts that manipulations that block the potentiation effect will cause anterograde amnesia and that manipulations that promote it will result in memory enhancement. While there have been a number of tests of the first prediction (13–16), the absence of drugs that facilitate LTP *in vivo* have precluded tests of the second.

Previous work (17) has shown that 1-(1,3-benzodioxol-5-ylcarbonyl)piperidine (BDP) increases the amplitude and duration of field excitatory postsynaptic potentials (EPSPs) recorded *in vitro* in slices of hippocampus while having little effect on the slope of responses. Studies with outside-out patches indicate that BDP modulates α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-receptor-gated currents (18). After intraperitoneal (i.p.) injections and for 2–3 h, the drug influences monosynaptic responses in the dentate gyrus *in vivo* in a manner similar to that observed in slices (17). Since compounds that facilitate AMPA-receptor-mediated responses promote the induction of LTP in hippocampal slices (19), BDP is a candidate for an enhancer of the potentiation effect *in vivo*. The experiments described in the present paper indicate that i.p. injections of BDP or a related compound have a marked influence on LTP in freely moving rats and, as expected from the LTP–memory hypothesis, improve retention of spatial and olfactory information.

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MATERIALS AND METHODS

To assess the time course of biodistribution of BDP (Cortex Pharmaceuticals, Irvine, CA), the drug was radiolabeled with carbon-11 as described (20) and injected into rats under conditions that simulated the dosage and mode of administration to be used (see below) in testing its effects on LTP. Biodistribution and pharmacokinetics were studied with positron emission tomography (PET) by using procedures for small animals that are described elsewhere (21). Radiolabeled BDP was diluted with nonlabeled drug and injected i.p. at a dose of 100 mg/kg. Images of the brain were collected at four times after injection.

To determine potential facilitatory effects of BDP on magnitude and duration of LTP *in vivo*, adult male Long Evans rats were prepared for chronic recording. The animals were deeply anesthetized, then a recording electrode was lowered into the stratum radiatum of field CA1, and stimulating electrodes were positioned into field CA3 both ipsilateral and contralateral to the recording electrode to activate the Schaffer collateral and commissural projections to the recording site. Physiological recording was used to adjust the position of the electrodes to maximize the amplitude of the field EPSP elicited by single stimulation pulses. After these steps, the leads of the electrodes were connected to a headstage permanently affixed to the skull of the rat. Ten days later, the rats were acclimated to a chronic recording cage and to the attachment of a recording lead to the headstage. Recordings were collected for several days before the start of experimental sessions to ensure stability of the stimulating-recording arrangements (for a more complete description, see ref. 22).

LTP was induced using a stimulation paradigm involving pairs of short (30 msec) high-frequency (100 Hz) bursts of pulses delivered to the Schaffer collateral/commissural projections to hippocampal region CA1. A train of 10 such bursts produces a robust and extremely stable LTP effect in rats with chronically implanted electrodes, when the bursts are separated by 200 msec (i.e., "θ burst stimulation" or TBS) (22). Pilot studies for the present project indicated that five pairs of θ bursts delivered at the frequency of one pair every 30 sec induce a small and transient potentiation effect. Accordingly, this stimulation pattern was used to test whether i.p. injections of BDP would facilitate the induction of LTP. Nine animals were used in the experiments and each received TBS both in presence and absence of the drug to allow for within-subject (and within pathway) comparisons of

Abbreviations: BDP, 1-(1,3-benzodioxol-5-ylcarbonyl)piperidine; BDP-5, 1-(1,3-benzodioxol-5-ylcarbonyl)-1,2,3,6-tetrahydropyridine; LTP, long-term potentiation; EPSP, excitatory postsynaptic potential; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; PET, positron emission tomography; TBS, θ burst stimulation.

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the amount and duration of LTP produced. In all cases, baseline recordings (1 stimulation pulse per 20 sec) were collected for at least 60 min before attempts were made to induce LTP. Vehicle (cyclodextrin) or drug (120 mg/kg) was injected 45 min before TBS, and recording was continued for 180 min thereafter. Twenty-four and 48 h later, the rats were returned to the recording apparatus and field EPSPs were recorded for 60 min. The sequence of drug and vehicle administration was counterbalanced across animals, with a minimal period of 5 days between successive episodes of LTP induction; stimulation current was kept constant for a given subject throughout the experiment.

The behavioral equipment and protocols used to test learning and memory retention are described below; separate groups of adult male rats were used in the two behavioral studies.

RESULTS

Fig. 1*A* illustrates results from the PET study at four times after i.p. injection of BDP; as shown, the drug rapidly migrated from the injection site and crossed the blood–brain barrier. Time–activity curves for brain, heart, and liver (Fig. 1*B*) show that the drug reached a maximum in the brain in about 4 min and maintained a concentration of about 0.35 mM throughout the experiment. Thus, the time course for the biodistribution of BDP as measured by PET is consistent with the effects observed in earlier *in vivo* recording experiments (17); moreover, the effective concentration produced in the brain by this dosage would be expected to enhance AMPA currents (18).

Temporally spaced pairs of θ bursts produced a small and transient LTP under control conditions and this effect was markedly enhanced by systemic administration (prior to θ bursts) of BDP. Fig. 2 illustrates the results for one rat after vehicle or drug injection. As shown, the drug caused an increase in amplitude and decay time without affecting the slope of the baseline field EPSP and enhanced the effects of the paired θ bursts on potentiation of synaptic responses in field CA1; i.e., the increase in slope and amplitude produced by TBS was greater in magnitude and more persistent after drug than vehicle injection. Fig. 2*D* summarizes the data for the group of nine rats, and as shown, injections of the drug

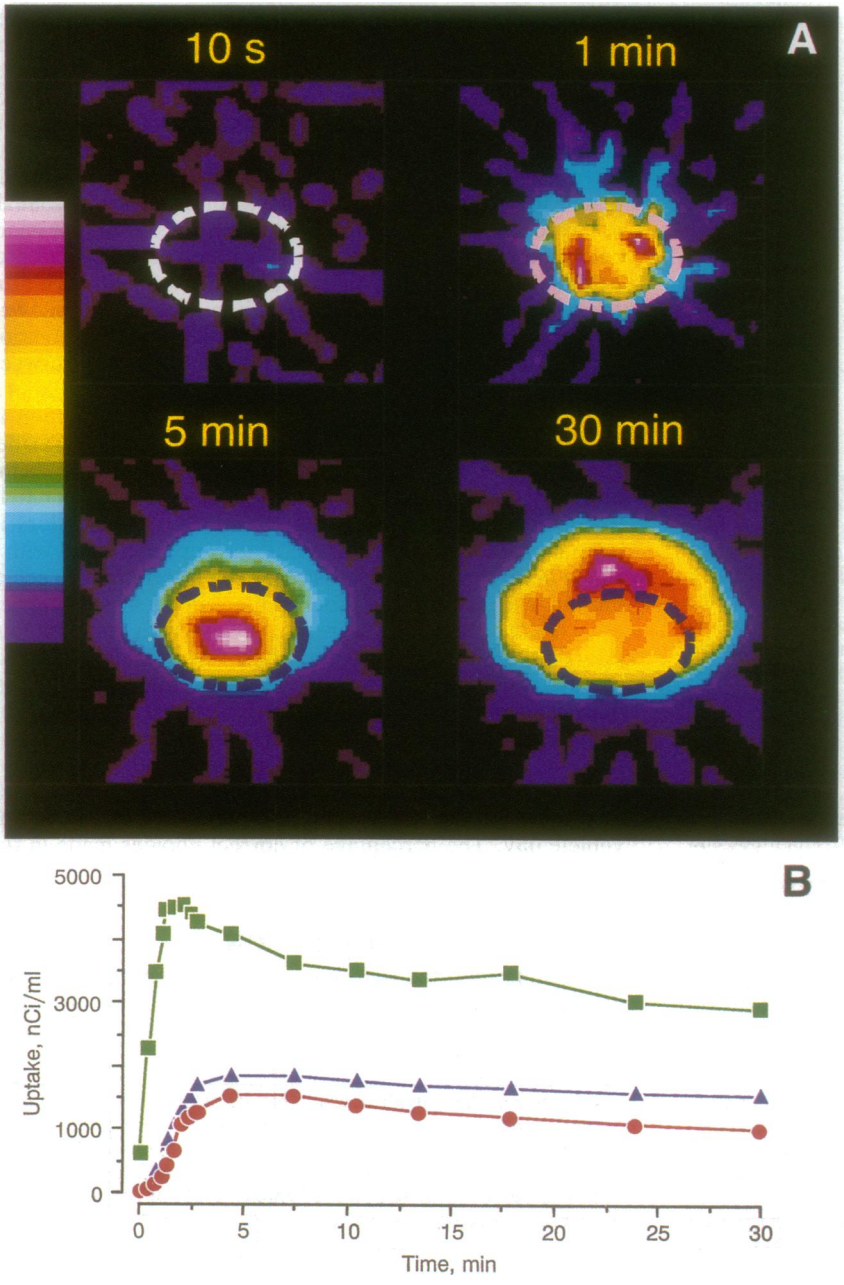

FIG. 1. Biodistribution and kinetics of the experimental drug BDP at various times after i.p. injection in rats. (*A*) PET images at four times after injection. For purposes of comparison, the brain has been outlined with a dashed line. The images were generated by a coronal slice with the ventral side toward the top of the figure. Spatial resolution by PET using ^{11}C is about 6 mm. Note that much of the early uptake occurs into the brain, while little drug is found in surrounding tissue within the first minutes after injection. At 30 min, equilibration has occurred between brain and surrounding areas. In a separate experiment, the brain was removed 55 min after injection and the radioactivity was determined in hippocampus, caudate, cerebellum, and cerebral cortex and found to vary by only 10% across the four regions. (*B*) Time–activity curves for the regional distribution of 250 μCi of [^{11}C]BDP (1 Ci = 37 GBq) coinjected i.p. with 20 mg of nonlabeled BDP in a 200-g rat (squares, liver; triangles, heart; circles, brain). In a separate experiment, activity was determined in plasma to be essentially constant from 5 to 52 min after injection and in the brain to decrease less than 50% in the same period.

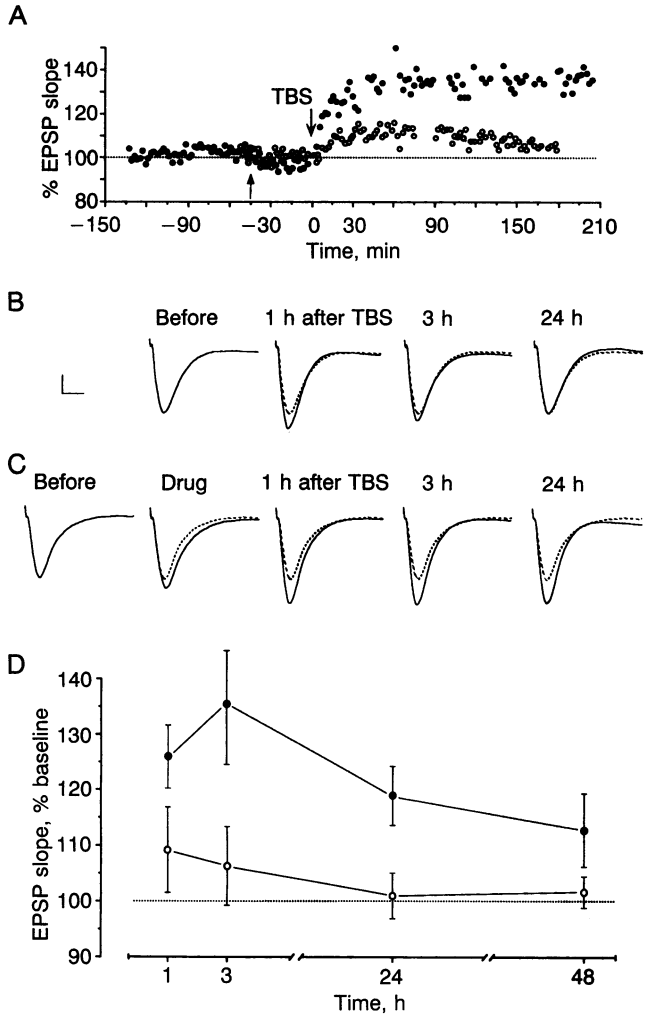

FIG. 2. Effect of a drug (BDP) that facilitates glutamatergic transmission on the induction of LTP in hippocampus of freely moving animals. (*A*) Slope of dendritic-field EPSPs (average of four responses per data point) recorded on separate days in stratum radiatum of field CA1 before and after induction of LTP in a rat with chronically implanted stimulating and recording electrodes in the Schaffer collateral/commissural system. Open circles, experiment in which TBS was delivered after systemic administration of vehicle solution (arrow pointing up, time point of injection); solid circles, TBS was delivered after injection of the drug. Note that the drug did not increase the slope of the field EPSP but markedly enhanced the potentiation of the slope produced by TBS. The illustrated effects are typical of those obtained in a group of nine rats. (*B* and *C*) Averaged field EPSPs ($n = 4$) from the experiment shown in *A*, where LTP was induced after injection of vehicle solution (*B*) or drug (*C*). Note that the drug has no effect on the slope but increases both amplitude and decay time of baseline EPSPs (see second response in *C*). (Calibration bars: 2.5 mV/5 msec.) (*D*) Amount and duration of LTP obtained in a group of nine rats injected with vehicle (open circles) or drug (solid circles) before delivering TBS. LTP is expressed as an increase in the slope of the field EPSP (mean ± SEM); each time point contains the average of 16 consecutive responses per animal. The difference in LTP within animals (intrasubject comparisons) between drug and vehicle condition was statistically significant at 3 h ($T = 4.19$; $P < 0.002$) and 24 h ($T = 2.89$; $P < 0.01$) and marginally significant at 1 h and 48 h ($T = 1.74$; $P < 0.06$; paired one-tailed t test). Responses to single pulses delivered to a second stimulating electrode positioned in the contralateral field CA3 were recorded throughout the experiments in seven of nine rats to provide an index of the stability of the recording arrangements (data not shown). In one rat, the control response increased steadily after TBS given in presence of the drug; this did not occur for the potentiated response. Dropping the animal in question did not affect the magnitude or statistical significance of the group data comparing LTP in presence vs. absence of the drug. The group means of the slope of responses to the second electrode (i.e., control responses) were 107 ± 3% at 1 h, 109 ± 5% at 3 h, 99 ± 10% at 24 h, and 102 ± 23% at 48 h in the drug condition and 102 ± 4% at 1 h, 104 ± 3% at 3 h, 101 ± 5% at 24 h, and 101 ± 7% at 48 h in the vehicle condition, as predicted by the synapse specificity of the LTP effect. Drug effects on amplitude and decay time of control responses were of the size routinely observed for baseline EPSPs and lasted for 2–3 h.

prior to TBS greatly facilitated the subsequent induction of LTP. Note also that the differences in degree of potentiation were still evident 24 and 48 h later, sampling times that occurred well after the physiological effects of the drug on amplitude and decay time had dissipated. A second injection of the drug in two rats enhanced LTP (relative to potentiation in the same pathway under control conditions) to a degree equivalent to that obtained after the first injection. Finally, a structural analogue [1-(1,3-benzodioxol-5-ylcarbonyl)-1,2,3,6-tetrahydropyridine, BDP-5; Cortex Pharmaceuticals] of BDP that also enhances field EPSPs *in vitro* and *in vivo* (Fig. 3) was tested in three rats by using the same protocol as described for BDP and was found to facilitate LTP induction in field CA1 [percent LTP at 3 h was 129 ± 9 for drug injections and 105 ± 4 for vehicle injections; mean ± SEM ($T = 3.94$; $P < 0.03$); percent LTP at 24 h was 114 ± 6 for drug injections and 98 ± 1 for vehicle injections ($T = 3.18$; $P < 0.04$); see Fig. 3*E*].

Behavioral studies were carried out with i.p. injections of BDP-5 at dosages found to facilitate LTP *in vivo*. Rats were first given extensive training in an eight-arm radial maze in which single rewards (chocolate chips) were located at the terminus of each arm (for a description of a similar study, see ref. 17). The animals were allowed access to four arms and then tested later with free access to all arms. At shorter intertrial intervals, experienced rats made very few (zero or one) reentry errors before obtaining the four remaining rewards but, with 8-h delays, typically committed one to three such errors. Accordingly, 12 rats were repeatedly tested in the maze with 8-h delays; on 6 days the animals received a vehicle injection 30 min before the first session, and on another 3 days, the animals received an injection of BDP-5 (120 mg/kg at a concentration of 40 mg/ml). The drug trials were conducted once per week, preceded and followed by a vehicle test day; the volume of vehicle and drug solution injected was kept constant within a rat and varied between rats according to weight (average, 1.0 ± 0.25 ml). Fig. 4*A* summarizes the results; as shown, a sizeable reduction in reentry errors occurred on the drug injection days. A further test of the effects of the drug on memory was conducted using olfactory cues ejected from random positions in an open field (for a description on preparation and delivery of odors in learning paradigms, see ref. 14). Rats were presented with a single odor in a morning session and then required to select that odor from a group of four in the second session carried out 6 h later. Daily tests were run, always with the same four odors but with the one used as a test odor varying from day to day; water rewards were given for responses to the test odor in the morning session and for selecting it from the group of four in the second session. Sixteen rats were used and received an injection of vehicle 30 min before the test session on 6 days and the drug (120 mg/kg) on another 3 days, each drug injection being preceded and followed by at least one vehicle day. The percentage of correct choices made in the second session was significantly greater on the drug vs. vehicle injection days (Fig. 4*B*).

DISCUSSION

The results reported here indicate that drugs that facilitate central glutamatergic transmission via modulation of AMPA receptors promote the induction of LTP *in vivo*. This prob-

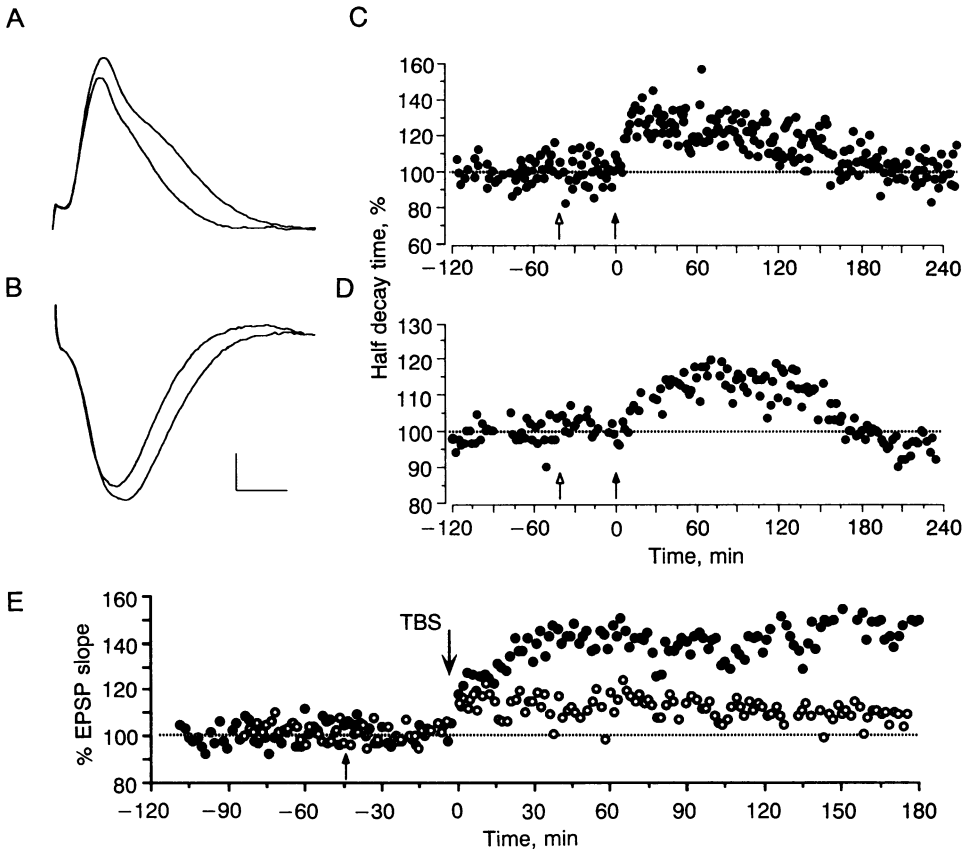

FIG. 3. Changes in synaptic transmission in the hippocampus of freely moving animals after systemic injection of BDP-5, an analogue of BDP. (*A* and *B*) Averaged field EPSPs ($n = 4$) recorded in the hilus of the dentate gyrus in response to stimulation of the perforant path (*A*) or recorded in stratum radiatum of field CA1 to stimulation of the Schaffer collaterals (*B*) in a freely moving rat prior to i.p. injections of the drug (lower amplitude trace) and at the peak of the drug effect (120 mg/kg; superimposed trace). The effects shown closely resemble those obtained in hippocampal slices with BDP-5 and related compounds. (Calibration bars: 1 mV/5 msec.) (*C*) Time course of the drug effect on half decay time of the responses shown in *A*. Open arrow, vehicle injection; solid arrow, drug injection. The maximum averaged effects (five animals) caused by the drug (percent change from baseline at 60 min after injection) for four synaptic parameters of responses elicited by perforant path stimulation were as follows: amplitude, +10 ± 2; slope, +3 ± 4; area, +25 ± 4; half-width, +20 ± 2. (*D*) Same as in *C* but for the response shown in *B*. Maximum averaged effects (eight animals) were as follows: amplitude, +3 ± 2; slope, −10 ± 4; area, +13 ± 3; half-width, +12 ± 2. (*E*) Slope of dendritic field EPSPs (average, $n = 4$) recorded on separate days in stratum radiatum of field CA1 before and after induction of LTP in a rat with chronically implanted stimulating and recording electrodes in the Schaffer collateral/commissural system. Open circles, experiment in which TBS was delivered after systemic administration of vehicle solution (arrow pointing up, time point of injection); solid circles, TBS was delivered after injection of the drug. The illustrated effects are typical of those obtained in a group of three rats.

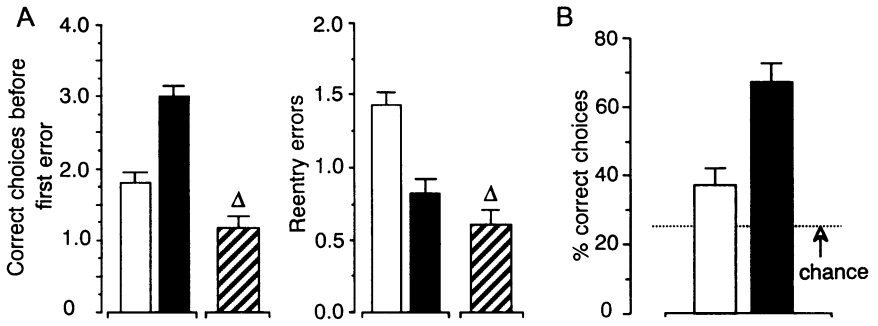

FIG. 4. Effects of BDP-5, a drug that facilitates induction of LTP *in vivo*, on retention in two behavioral paradigms. (*A*) (*Left*) The number of correct choices in an eight-arm radial maze made before reentering any of four arms that had already been visited in a previous (8 h earlier) session. Shown are averages (+SE) for a group of 12 rats for days on which vehicle injections (open bar) or drug injections (solid bar) were given 30 min prior to the first of the two sessions. The hatched bar indicates the mean of the within-rat differences for drug vs. vehicle days; as shown, the animals made more correct choices before committing an error on drug-injection days ($T = 5.15$; $P < 0.0002$, paired t test). (*Right*) The number of reentries (errors) into arms already visited made by the rats before collecting all four available rewards during the second of two sessions in an eight-arm radial maze (8 h between sessions). Open and solid bars are as in *Left*. The hatched bar summarizes the average within-rat improvement (i.e., reduction in the number of reentries) on drug- vs. vehicle-injection days ($T = 5.31$; $P < 0.0001$; paired t test). (*B*) The percentage of trials in which rats selected the test odor from a group of four simultaneously present odors on days in which the animals had been injected with vehicle (open bar) or drug (solid bar) prior to a first session in which they were presented with the test odor alone. The interval between the first session (test odor alone) and second session (four odors) was 6 h. An average score for six vehicle-injection and three drug-injection days was calculated for each rat; the summarized results are means ± SEM for 16 animals ($T = 4.03$; $P < 0.0006$; paired t test).

ably reflects greater depolarization of postsynaptic cells during TBS of their afferents, an effect that should result in an enhanced response by the voltage-sensitive *N*-methyl-D-aspartate receptors (19). The experiments used a stimulation paradigm that induces a weak form of LTP; whether the drugs would have any effects on the robust and extremely stable potentiation obtained with trains of bursts (22) is an open question.

If the weak form of LTP occurs during behavior and contributes to the encoding of one or more types of transient memory, then it is reasonable to expect that the drugs administered prior to learning would enhance such variants of memory. In accord with this, recent work indicates that BDP improves retention in radial mazes; specifically, rats injected with the drug prior to a first session in the maze made fewer reentries into arms visited during that session when tested 8 h later (17, 23). This observation was confirmed and extended in the present study using the analogue compound BDP-5: the drug, at dosages that promote LTP in a manner indistinguishable from that for BDP, produced a significant improvement in the retention of both spatial and olfactory cues, as predicted from the hypothesis that LTP is the substrate of memory.

It is also possible, however, that the behavioral effects of the compounds are to some measure due to facilitation of glutamatergic transmission rather than to the enhanced LTP that results from facilitation. The effects of the drugs were considerably more pronounced on LTP than on field EPSPs and potentiation has a more obvious relationship to the lasting consequences of learning episodes than does the duration of monosynaptic responses. The development of other pharmacological tools for facilitating the induction of LTP would provide the means for more firmly testing the linkage between potentiation and memory. But as they stand, the findings reported here confirm an important prediction of the LTP–memory hypothesis and also suggest an approach for the development of memory-enhancing pharmaceuticals.

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