MK801 retards acquisition of a classically conditioned response without affecting conditioning-related alterations in perforant path-granule cell synaptic transmission

GILBERT B. ROBINSON

University of New Brunswick, Fredericton, New Brunswick, Canada

The contributions of *N*-methyl-D-aspartate (NMDA) receptor activity to classical conditioning of the rabbit nictitating membrane (NM) response and conditioning-related alterations of the hippocampal perforant path-granule cell response were examined. Subcutaneous injections of the non-competitive NMDA antagonist MK801 (0.00, 0.05, or 0.10 mg/kg) were administered 75 min prior to each daily training session in a standard delay paradigm. MK801 administration resulted in a significant dose-dependent retardation in acquisition of the classically conditioned NM response. There were no consistent dose-dependent effects of MK801 on NM topography or on conditioned or unconditioned stimulus sensitivity. MK801 administration did not disrupt conditioning-related potentiation of perforant path-granule cell responses. The within-trial decrease in spike amplitude, relative to the between-trial response, was also not affected by MK801 administration. These results suggest that NMDA-mediated activity contributes to classical conditioning of the rabbit NM response and raise the possibility that conditioning-related alterations of perforant path-granule cell synaptic transmission are not NMDA dependent.

There has been much interest in and debate about the relationship between long-term potentiation (LTP), a use-dependent, long-lasting increase in the strength of neural connections, and behavioral plasticity. One prominent issue is whether or not LTP and behavioral plasticity share common mechanisms. The induction of LTP at both hippocampal Schaffer collateral-CA1 pyramidal cell synapses (Collingridge, Herron, & Lester, 1988; Harris, Ganong, & Cotman, 1984) and perforant path-dentate gyrus granule cell synapses (Abraham & Mason, 1988; Gilbert & Mack, 1990; Robinson & Reed, 1992) is dependent upon *N*-methyl-D-aspartate (NMDA) receptor-mediated activity. Similarly, several lines of investigation have raised the possibility that NMDA-mediated synaptic activity is involved in the acquisition of learned behaviors that are dependent upon the hippocampus (Heale & Harley, 1990; Morris, 1989; Shapiro & Caramanos, 1990; Shapiro & O'Connor, 1992). Although the existence of NMDA-dependent hippocampal LTP is accepted, the role of NMDA receptors in behavioral plasticity is still controversial. Potential differences in the dose-response curve for

NMDA antagonism of LTP and learning, and potential sensorimotor side effects of NMDA antagonists, have contributed to the controversy (Hargreaves & Cain, 1992; Keith & Rudy, 1990).

There is no *a priori* basis, however, to assume that the dose-response curve for NMDA antagonism of LTP and behavioral plasticity should be similar (Gallagher, 1990). It is not likely that the physiological pattern of activity that occurs during learning mimics the intense, synchronous activity pattern evoked to induce LTP. Therefore, it may not be appropriate to reject reports of learning impairments following the administration of NMDA antagonists at doses that may not be capable of blocking LTP. Examination of the effects of an NMDA antagonist on learning and on proposed learning-related forms of hippocampal synaptic plasticity may provide a more valid comparison. Such a comparison is the purpose of the present study.

Substantial potentiation of neural responses occurs during classical conditioning of the rabbit nictitating membrane (NM) response. There is, for example, a within-trial increase in action potential discharge frequency of both hippocampal pyramidal cells (Berger, Alger, & Thompson, 1976; Berger, Rinaldi, Weisz, & Thompson, 1983) and cells of the deep cerebellar nuclei (McCormick & Thompson, 1984)—brain regions implicated in acquisition of the NM response (Berger et al., 1983; McCormick et al., 1981). In addition, significant potentiation of the hippocampal dentate granule cell response to input from the entorhinal cortical perforant path fibers occurs

This research was supported by grants from the Medical Research Council of Canada and from the Natural Sciences and Engineering Research Council of Canada. The expert assistance of Evelyn Stillwell is gratefully acknowledged. I also would like to thank R. L. Port for his constructive comments on earlier drafts of the manuscript. Correspondence should be addressed to G. B. Robinson, Department of Psychology, University of New Brunswick, Fredericton, NB, E3B 6E4 Canada (e-mail: robinson@unb.ca).

during NM conditioning (Robinson, 1992; Weisz, Clark, & Thompson, 1984). The conditioning-related potentiation effects are similar, in many respects, to electrically induced LTP (Berger & Scialabasi, 1988).

In the following study, the effect of the noncompetitive NMDA receptor/channel antagonist (+)-5-methyl-10, 11-dihydro-5H-dibenzo(a,d) cyclohepten-5,10-imine hydrogen maleate (MK801; Kemp, Foster, & Wong, 1987) on acquisition of the classically conditioned NM response and on conditioning-related alterations of perforant path-granule cell synaptic transmission were examined within the same group of animals. The characteristics of the NM response and the animals' sensitivity to the conditioning stimuli were also analyzed, to determine possible sensorimotor side effects of MK801 administration. Dosages of 0.05- and 0.10-mg/kg MK801 were chosen for this study. In chronic preparations, these dosages disrupt LTP (Gilbert & Mack, 1990; Robinson & Reed, 1992) and appear to retard learning in animals while leaving the necessary sensorimotor capacities intact (Heale & Harley, 1990; Shapiro & Caramanos, 1990). Preliminary results have appeared elsewhere (Robinson, 1991).

METHOD

Subjects

The subjects were 34 male New Zealand white rabbits (*Oryctolagus cuniculus*) weighing 2.0–2.5 kg. The rabbits were housed individually and maintained on a 12:12-h light:dark cycle with ad lib access to food and water. All behavioral testing took place during the first half of the light cycle.

Surgical Procedures

The surgical procedures were similar to those described in detail elsewhere (Robinson, 1992; Robinson & Reed, 1992). Briefly, the rabbits were deeply anesthetized with halothane and were implanted with a bipolar stimulation and a monopolar recording electrode in the perforant path and hilus of the dentate gyrus, respectively, of the left hemisphere. The electrode leads were inserted into a head-cap; the assembly was then anchored to the skull with dental acrylic. After surgery, the rabbits were administered penicillin (300,000 units, i.m.), placed under a heat lamp, and closely monitored. At least one day prior to the start of training, the rabbits were again anesthetized with halothane and a small nylon loop was sutured in the left NM.

Conditioning Procedure

Following a 2- to 3-week recovery period, the rabbits were adapted to restraint in a Plexiglas box located within a sound-attenuating chamber. On the 2nd day of adaptation, 216 baseline granule cell responses to activation of the perforant path were recorded. The activation frequency was identical to that used during subsequent conditioning (see below). The stimulation intensity was sufficient to evoke a population spike amplitude of 3–4 mV.

Conditioning began on the following day, with 24 of the rabbits randomly assigned to one of three groups. Subcutaneous injections of saline (0.0 mg/kg MK801; $n = 7$), 0.05-mg/kg MK801 ($n = 10$), or 0.10-mg/kg MK801 ($n = 7$) were administered 75 min prior to each conditioning session. A tone (1 kHz, 82 dB, 400 msec) served as the conditioned stimulus (CS), and a corneal airpuff (5 psi, 100-msec duration) served as the unconditioned stimulus (US). The interstimulus interval was 300 msec, with the tone and airpuff co-

terminating. Training consisted of 12 blocks of trials per day. Each block consisted of eight paired CS-US trials, followed by one CS-alone trial. The intertrial interval averaged 40 sec (range, 30–50 sec).

Hippocampal dentate granule cell responses were evoked by perforant path stimulation applied midway between each trial and 220 msec after tone onset within each trial. Stimulation intensity was held constant (chosen during the adaptation session) throughout training. The responses were sampled at 10 kHz and saved to disk for later analysis. Figure 1A illustrates the general experimental design for stimulus presentation both between trials and within a trial.

NM extension of at least 0.5 mm during the CS-US interval was considered a conditioned response (CR). The number of trials to eight CRs in nine consecutive trials was recorded for each animal (Criterion 1). Training continued until a CR rate of at least 80% during an entire session (Criterion 2) was obtained, or for a maximum of 10 sessions. NM responses were saved to disk for later analysis.

A fourth group of rabbits ($n = 6$) received 10 sessions in which an equal number of specifically unpaired CS and US presentations occurred in a pseudorandom sequence. The intertrial interval ranged between 15 and 25 sec (mean, 20 sec). The protocol for perforant path-granule cell stimulation and recording was identical to that utilized in animals that received paired presentations of the CS and US.

A fifth group of rabbits ($n = 4$) were used only for classical conditioning, because the electrode placements were not suitable for evoking classic perforant path-granule cell responses. The conditioning procedure was identical to that described above. Initial training occurred following the administration of saline and continued until a CR rate of greater than 80% occurred over four consecutive sessions. The rabbits then received three additional training sessions, each of which began 75 min following the administration of 0.10-mg/kg MK801.

CS and US Sensitivity Tests

The animals served as their own controls for the CS and US sensitivity tests, with the order of testing in the drugged and nondrugged states being counterbalanced. MK801 or saline was administered 75 min prior to the start of each test. As a result of computer failures, data for both CS and US sensitivity were not available from all the animals. The effect of 0.05- and 0.10-mg/kg MK801 on CS sensitivity was available from 10 and 5 rabbits, respectively. The effect of 0.05- and 0.10-mg/kg MK801 on US sensitivity was available from 5 and 8 rabbits, respectively.

Four different tone intensities (82, 74, 57, and 45 dB) were used for the CS sensitivity tests. Each intensity was presented for 3 blocks of 9 trials. All trials were reinforced. The average intertrial interval was 40 sec. A given intensity was presented throughout a block. The sequence of presentation across blocks was 82, 82, 74, 57, 45, 45, 57, 74, 82, 74, 57, and 45 dB. To monitor the effect of tone intensity on granule cell responsivity to perforant path input, test pulses also were applied to the perforant path 220 msec after tone onset within each trial and midway between each trial (i.e., during the intertrial interval).

For US sensitivity tests, rabbits received four blocks of eight trials at each of three intensities (1, 3, and 5 psi, 100-msec duration). The order of presentation was 5, 3, 1, 1, 3, 5, 5, 3, 1, 1, 3, and 5 psi, with a given intensity being presented throughout a block. The average intertrial interval was 40 sec.

Data Analysis

Four characteristics of the NM response were analyzed: (1) the peak amplitude; (2) the area under the NM response; (3) the onset latency, representing the time from CS onset to response criterion (0.5 mm of NM movement); and (4) the peak latency, represent-

A

B

C

Figure 1. (A) Examples of nictitating membrane (NM) conditioned responses (CRs) and perforant path-granule cell evoked responses, to illustrate the experimental strategy. A perforant path-granule cell response was evoked midway between conditioning trials and 220 msec after tone onset within each trial. The arrows represent the onset of the conditioned stimulus (CS) and unconditioned stimulus (US). (B) Example of NM CR. Four measures of NM topography were obtained: peak amplitude, area (shaded area), latency to onset, and latency to peak amplitude. On CS-US trials, peak amplitude and area were separated into a CR (prior to US onset) component and unconditioned response (following US onset) component. (C) Example of perforant path-granule cell field potential. Changes in both the slope of the excitatory postsynaptic potential (measured between two fixed points on the initial positivity) and the amplitude of the population spike (height of a line from the peak of the negativity to a tangent joining spike onset and offset) were monitored throughout all experiments.

ing the time from CS onset to peak amplitude (see Figure 1B). Group differences in NM topography were analyzed using a one-way analysis of variance (ANOVA).

CS and US sensitivity were analyzed in a within-subjects repeated measures ANOVA (.05- or .10-mg/kg MK801 vs. saline $\times$ CS or US intensity).

The perforant path-granule cell response was reduced to two parameters of interest (see Figure 1C). Conditioning-related changes in the first, the population excitatory postsynaptic potential (EPSP), were monitored by measuring the slope during the initial 1 msec of positivity. Changes in the amplitude of the population spike, defined as the height of a line drawn from the peak of the negativity to a tangent joining spike onset and offset, were used to monitor conditioning-related changes in cell discharge.

Figure 2. Effect of MK801 on acquisition of the nictitating membrane (NM) conditioned response (CR). Two learning criteria were analyzed: the number of trials to eight CRs within nine trials (panel A), and a CR rate of at least 80% for an entire session (panel B). MK801 administration resulted in a dose-dependent increase in trials to criterion. Illustrated are the mean and SEM (T bars).

Figure 3. Effect of MK801 on the mean ($\pm$ SEM) percent conditioned responses (CRs) per session for the saline, 0.05-, and 0.10-mg/kg groups. The animals in these three groups received at least 4, 5, and 7 days of conditioning, respectively.

RESULTS

CR Acquisition

MK801 administered prior to each training session resulted in a significant dose-dependent increase in trials to criterion on both the first [$F(2,21) = 10.49$, $p < .0007$] and second criterion [$F(2,21) = 16.22$, $p < .0001$]. Follow-up analysis (Tukey's, $p < .05$) indicated that the three groups differed significantly from each other on both criteria (see Figure 2). One animal in the 0.05-mg/kg MK801 group failed to reach either criterion by the 10th day of conditioning, and a 2nd animal in that group failed to meet the second criterion. In contrast, 3 rabbits from the 0.10-mg/kg MK801 group did not meet either criterion, and 2 others in that group failed to meet the second criterion.

The animals in the saline, 0.05-, and 0.10-mg/kg MK801 groups received at least four, five, and seven training sessions, respectively. The average percent CRs of each group, for each session, are illustrated in Figure 3. For all groups, there was an increase in CR rate across training. A repeated measures ANOVA revealed significant effects of group [$F(2,21) = 27.66$, $p < .001$] and session [$F(3,63) = 41.61$, $p < .001$], and a significant group $\times$ session interaction [$F(6,63) = 9.52$, $p < .001$] over the first four sessions.

At the conclusion of training, the means ($\pm$ SEM) of the percent CRs for the saline, 0.05-, and 0.10-mg/kg

Figure 4. Illustrated are the mean ($\pm$ SEM) percent conditioned responses (CRs) elicited by 4 rabbits over the initial 10 training sessions that occurred following MK801 administration and during 2 subsequent drug-free training sessions. MK801 appeared to block acquisition of the conditioned stimulus–unconditioned stimulus association; the CR acquisition curve in subsequent drug-free sessions was similar to that for animals initially trained following saline administration.

groups were 92.1 ± 2.1 , 74.9 ± 9.6 , and 49.4 ± 14.1 , respectively. A one-way ANOVA revealed a significant group effect [$F(2,21) = 3.54$, $p < 0.05$], with follow-up analysis (Tukey's, $p < .05$) indicating that the saline group differed significantly from the 0.10-mg/kg group but not from the 0.05-mg/kg group. The 0.05- and 0.10-mg/kg groups also did not differ significantly.

The 4 animals that failed to meet the first criterion were subsequently trained following saline administration. These tests occurred 48 h following the last conditioning session under the drug. Figure 4 illustrates the percent CRs exhibited by these 4 animals during the 10 sessions in which testing took place following MK801 administration and the 2 days of testing following saline administration (only 2 days are illustrated, because 1 rabbit met criterion on the 2nd day). The percent CRs during the initial 10 sessions were never greater than $6.5\% \pm 4.2\%$ ($M \pm SEM$). When rabbits were subsequently trained following saline administration, the percent CRs during the first and second session were $18.5\% \pm 11.2\%$ and $49.3\% \pm 18.1\%$, respectively. These response rates are comparable to those exhibited by the 7 saline pretreated rabbits ($10.3\% \pm 5.3\%$ and $62.7\% \pm 9.3\%$, during Training Sessions 1 and 2, respectively; see Figure 3).

The effect of MK801 on the performance of previously acquired CRs was examined in a separate group of 4 animals. Figure 5 illustrates the percent CRs exhibited by this fifth group of animals during the last three sessions that occurred following saline administration and the first three

sessions following the administration of 0.10-mg/kg MK801. MK801 administration did not disrupt the rate of responding to the previously acquired CS–US association.

Conditioning-Related Alterations in Synaptic Transmission

Conditioning-related alterations in granule cell responsiveness to perforant path input are illustrated in Figure 6. A repeated measures ANOVA (group $\times$ between trials vs. within trial $\times$ day) indicated that a significant increase in spike amplitude occurred between the 1st day of training and criterion [$F(1,26) = 12.00$, $p < .002$] and a significant suppression of spike amplitudes evoked within trials [$F(1,26) = 69.72$, $p < .001$]. The group effect and the interaction were not significant. Similar analysis of the EPSP indicated that potentiation of EPSP slope from the 1st training day to criterion was significant [$F(1,26) = 4.86$, $p < .037$]. The effects of group and stimulus condition and the interaction were not significant.

NM Topography

The characteristics of the unconditioned response (UR) on the first day of conditioning are illustrated in Figure 7A. The mean ($\pm$ SEM) number of URs (NM movement greater than 0.5 mm) on the 1st day of conditioning for the saline, 0.05-, and 0.10-mg/kg groups was 80.0 ± 6.6 , 76.0 ± 5.8 , and 69.6 ± 7.0 , respectively. The differences were not significant ($p = .56$). MK801 ad-

Figure 5. Effect of MK801 administration (0.10 mg/kg) on previously acquired conditioned responses (CRs). Data are from 4 rabbits initially trained following saline administration. Subsequent training sessions, following MK801 administration, had no significant effect on the previously acquired CR.

Figure 6. Effect of MK801 on perforant path-granule cell responses obtained between (solid lines) conditioning trials and within (dashed lines) each conditioning trial. Illustrated are the percent change, relative to the pretraining baseline response, in spike amplitude (upper panels) and excitatory postsynaptic potential (EPSP) slope (lower panels) on the 1st day of conditioning and on the day Criterion 2 was met. Data from the final conditioning session were used for rabbits that failed to meet Criterion 2.

ministration also did not significantly affect UR amplitude, area, onset latency, or peak latency.

The characteristics of the CR, prior to US onset, on the day rabbits met Criterion 2 (at least 80% CRs for an entire session) are illustrated in Figure 7B. If criterion was not met, data from the 10th conditioning session were utilized. There were no significant group differences in CR amplitude, area, onset latency, or peak latency. Analysis of the peak amplitude and area, following US onset, also failed to indicate a significant effect of group (Figure 7C, right-hand panels).

The preceding analyses of CR topography were contaminated by subsequent UR occurrences on CS-US trials.

Therefore, CR topography on CS-alone test trials also was examined (Figure 7D). The mean ($\pm$ SEM) number of CRs on CS-alone test trials was 11.0 ± 0.2 , 9.0 ± 1.2 , and 6.4 ± 1.6 , for the saline, 0.05-, and 0.10-mg/kg groups, respectively. The group differences were not quite significant ($p = .063$). Analysis of CR amplitude, area, onset latency, and peak latency also revealed no significant effect of group.

CS Sensitivity

CS sensitivity was significantly reduced by the administration of 0.05-mg/kg MK801 (Figure 8, upper left panel) [$F(1,9) = 19.43$, $p < .002$] but not by the 0.10-mg/kg

Figure 7. Effect of MK801 on nictitating membrane (NM) topography. MK801 did not significantly alter (A) unconditioned response (UR) topography on the 1st day of conditioning or (B) conditioned response (CR) topography the day rabbits attained a CR rate of at least 80% for an entire session (the last conditioning session was used for rabbits that did not reach criterion). The topography of the CR on the day rabbits reached Criterion 2 was also separated into a CR (prior to unconditioned stimulus, US, onset) and a UR component (post-US onset). MK801 did not significantly affect the amplitude or area of either the CR (C, left-hand panels) or UR (C, right-hand panels) component of the response. MK801 also did not significantly affect CR topography on CS-alone test trials on the day Criterion 2 was met (D). Illustrated are the mean and SEM (T bars) for each response measure.

Figure 8. Effect of MK801 on conditioned stimulus (CS) sensitivity (upper panels). The percent conditioned responses (CRs) decreased significantly with 0.05-mg/kg but not with 0.10-mg/kg MK801. The decrease with the former dose occurred only in response to the two lower CS intensities (* $p < .05$). Thus, the effect of MK801 on CS sensitivity was variable and did not appear to be dose dependent. The lower panels illustrate that there was no significant effect of drug dosage or CS intensity on the perforant path-granule cell spike amplitude evoked within (dashed lines) or between (solid lines) trials.

dose (Figure 8, upper right panel) [$F(1,4) = 5.56$, $p = .078$]. Follow-up analysis of the 0.05-mg/kg group revealed a significant decrease in the percent CRs at 45 and 57 dB, but not at the two higher CS intensities. As expected, for both the 0.05- and the 0.10-mg/kg groups, there was a significant decrease in the percent CRs as CS intensity decreased [$F(3,27) = 34.79$, $p < .001$, and $F(3,12) = 31.62$, $p < .001$, respectively].

Population spikes evoked during occurrence of the CS were significantly (at least, $p < .001$) smaller than those evoked between trials. MK801 did not significantly affect the amplitude of population spikes evoked within or between CS presentations (see Figure 8, lower panels).

US Sensitivity

Neither the percent URs (Figure 9, upper left panel) nor UR amplitude (Figure 9, lower left panel) were dependent on whether testing occurred following saline or 0.05-mg/kg MK801. The percent URs also did not differ significantly depending on whether animals were tested following saline administration or 0.10-mg/kg MK801 (Figure 9, upper right panel). The effect of 0.10-mg/kg MK801 on UR amplitude was not quite significant (Figure 9, lower right panel) ($p = .058$).

As expected, the percent URs decreased significantly as US intensity decreased, for both the 0.05-mg/kg [$F(2,8) = 10.01$, $p < .007$] and the 0.10-mg/kg [$F(2,14) = 65.63$,

Figure 9. Effect of MK801 on unconditioned stimulus sensitivity. Illustrated are the percent unconditioned responses (URs) at each intensity for animals tested following both saline and 0.05-mg/kg MK801 (upper left panel) and both saline and 0.10-mg/kg MK801 (upper right panel). MK801 did not significantly affect the percent URs or UR amplitude (lower panels).

$p < .001$] comparisons. There was also a significant decrease in UR amplitude with decreasing US intensities for both the 0.05-mg/kg [$F(2,8) = 13.05, p < .003$] and the 0.10-mg/kg [$F(2,14) = 18.24, p < .001$] groups.

DISCUSSION

This study represented a within-subjects comparison of the effects of the NMDA antagonist MK801 on acquisition of the classically conditioned rabbit NM response and on conditioning-related alterations in granule cell responsiveness to input from the perforant path. The principal finding was that MK801 administration had significant dose-dependent effects on acquisition rate, without significantly affecting sensorimotor behavior or conditioning-related changes in perforant path-granule cell synaptic transmission.

Effect of MK801 on Behavioral Conditioning

MK801 retarded acquisition of the NM CR in a dose-dependent manner. Whether MK801 retarded acquisition rate by blocking learning is more difficult to answer. On the average, CR rates increased across training in MK801 pretreated rabbits. As noted previously, however, 4 animals did not exhibit an increase in CR rate across training sessions. This finding suggests that MK801 had blocked learning, an interpretation consistent with the effect of other noncompetitive NMDA antagonists on classical conditioning of the eyeblink response (Thompson & Disterhoft, 1991). Nevertheless, the increased CR rate exhibited by some animals requires the cautious interpretation that MK801 retards the rate of acquisition of the classically conditioned NM response.

When the 4 animals that showed no evidence of CR acquisition were subsequently trained following saline ad-

ministration, the percent CRs during the first two conditioning sessions were comparable to that observed in rabbits initially trained following saline administration. Since these animals did not respond at or near criterion during the first drug-free session, it would appear that MK801 had affected learning rather than behavioral performance. Furthermore, if the effect of MK801 on CR acquisition was the result of a performance deficit, MK801 should also disrupt performance of previously acquired CRs. This possibility, examined in 4 separate animals, was found not to be the case. Previously acquired CR rates were maintained when these rabbits were subsequently tested following the administration of 0.10-mg/kg MK801. Therefore, MK801 administration does not disrupt the performance of a previously acquired CR.

A number of additional results are also consistent with the interpretation that MK801 affects learning rather than performance. First, various measures of NM topography were not significantly affected by MK801 administration. Second, MK801 administration did not result in consistent dose-dependent effects on either CS or US sensitivity. However, both CS and US sensitivity were somewhat reduced following MK801 administration. In a subsequent study of the effects of MK801 on latent inhibition learning (Robinson, Port, & Stillwell, 1993), administration of 0.10-mg/kg MK801, but not 0.05-mg/kg MK801, was found to significantly reduce the percent CRs and UR amplitude. Despite the reduced CS sensitivity, latent inhibition learning did occur. In the present study, CS sensitivity was significantly reduced only by the 0.05-mg/kg dose of MK801 but only at intensities lower than that used in conditioning. Thus, in agreement with previous studies (Shapiro & Caramanos, 1990; Thompson & Disterhoft, 1991), the learning deficit does not appear to be a secondary consequence of a sensorimotor impairment.

Whether the learning deficits were the result of disrupting NMDA-mediated activity in the hippocampal formation cannot be determined with systemic injections. The hippocampal formation does exhibit learning-dependent plasticity (Berger et al., 1983), and its role in certain aspects of classical conditioning is well established (Akase, Alkon, & Disterhoft, 1989; Port, Mikhail, & Patterson, 1985; Schmajuk & DiCarlo, 1991; Solomon, Solomon, Vander Schaaf, & Perry, 1983). Hippocampal involvement in NM conditioning with a standard short-delay paradigm, for example, is limited to early in the learning process. As the response becomes consolidated, the necessity for hippocampal involvement decreases (Akase et al., 1989; see also Sears & Steinmetz, 1990). NMDA antagonists may interfere with establishment of a memory trace of the CS-US association, an effect similar to the disruptive effects of posttrial hippocampal stimulation (Salafia, 1987). In the unanesthetized rabbit, MK801 reduces both the magnitude and duration of LTP at perforant path-granule cell synapses (Robinson & Reed, 1992). If LTP is a neural substrate of associative learning, these findings raise the possibility that MK801 disrupts learning either by disrupting consolidation or by making the CS-US association less durable.

Conditioning-Related Alterations in Synaptic Transmission

MK801 did not significantly affect conditioning-related alterations of perforant path-granule cell synaptic transmission. Conditioning-related potentiation effects were found to occur in rabbits administered MK801. These conditioning-related potentiation effects, however, are short-lasting; that is, they are observable only while the animals are actively engaged in the behavioral task. Input/output responses obtained between NM conditioning sessions do not show this potentiation effect (Robinson, 1992). MK801 also does not affect electrically induced short-term potentiation. Thus, the inability of MK801 to block conditioning-related potentiation is not unexpected, if such changes reflect short-term neuronal plasticity. However, it is difficult to conclusively rule out an NMDA-mediated contribution to the conditioning-related potentiation effects, because the dosages of MK801 used in this study reduce but do not abolish LTP of perforant path-granule cell synaptic transmission (Robinson & Reed, 1992). High doses of MK801 (1.0–4.0 mg/kg) are also known to block paired-pulse facilitation of the population spike in urethane-anesthetized rats (Joy & Albertson, 1993).

The failure to find a significant group $\times$ time or group $\times$ condition (between vs. within trial) interaction in the analysis of potentiation of the population spike was unexpected. As illustrated in Figure 6, the rabbits that received paired training exhibited an average potentiation of spike amplitude that was greater than that observed in the rabbits given explicitly unpaired training. The differential spike amplitude observed between and within trials was also, on the average, much greater in animals given paired training as opposed to unpaired training. In the original report of conditioning-related potentiation effects (Weisz et al., 1984), the data were analyzed by training block. Significant differences were not found for all blocks. In the present study, the data were collapsed across all blocks. Whether this difference would account for the lack of a significant interaction is not known. It may also be that the test pulse intensity used in the present study was too high. Weisz et al. (1984) used an intensity sufficient to elicit population spikes that were approximately 30% of the maximum amplitude and found potentiation of approximately 40%–200% over training. Stimulation intensity in the present study was sufficient to evoke population spikes of 3–4 mV. These responses are approximately 60%–70% of the maximum amplitude (Robinson, 1992; Robinson & Reed, 1992). Thus, the small percent change in spike amplitude observed across training may have been the result of the large initial spike amplitude.

In addition to classical conditioning, hippocampal synaptic potentiation or LTP-like phenomena appear to occur during both operant conditioning (Skelton, Scarth, Wilkie, Miller, & Phillips, 1987) and spatial cognition (Sharp, McNaughton, & Barnes, 1985). These or other learning-related potentiation effects (e.g., potentiation of multiple-unit activity or evoked responses within the cerebellar interpositus nucleus or hippocampal CA1 pyrami-

dal cells) may be NMDA dependent. LTP also occurs both in the CA1 pyramidal cell layer and in the interpositus nucleus of the cerebellum (Racine, Wilson, Gingell, & Sunderland, 1986). LTP in the former structure is NMDA dependent (Collingridge et al., 1988; Harris et al., 1984). Whether LTP in cerebellar nuclei also is NMDA dependent is not known. It is possible that MK801 blocked conditioning-related potentiation effects within CA1 pyramidal cells or within cerebellar structures and thereby disrupted acquisition of the learned behavioral response. The effect of MK801 on learning-related potentiation of CA1 pyramidal cell activity and cerebellar multiple-unit activity, as well as on electrically induced LTP within the interpositus nucleus will have to be determined to address this possibility. In addition, it would have to be demonstrated that any apparent learning-related potentiation effects were not the result of changes in motor activity or behavioral state (Hargreaves, Cain, & Vanderwolf, 1990).

MK801 administration also did not disrupt the differential responsiveness of granule cells to perforant path input that is exhibited between and within trials. The presentation of sensory stimuli is known to inhibit granule cell activity (Vinogradova, 1975), unless paired with presentation of a US (Weisz, Clark, Yang, Thompson, & Solomon, 1982). As noted by Weisz et al. (1984), increased single-cell activity may decrease the amplitude of evoked responses by reducing the synchrony of cell discharge, because more cells would be refractory at the time of stimulation. Whatever the mechanism, the differential responsiveness is not affected by MK801.

Both the conditioning-related potentiation and the differential responsiveness of granule cells observed during conditioning may result from a change in the level of some tonic modulatory input (Weisz et al., 1984). Both the noradrenergic input from the locus coeruleus (Loy, Koziell, Lindsey, & Moore, 1981) and the cholinergic input from the medial septal nucleus (Chandler & Crutcher, 1983) modulate granule cell responsivity to input from the perforant path (Fantie & Goddard, 1982; Robinson, 1986; Robinson & Racine, 1985). Furthermore, septal inputs to the dentate gyrus participate in acquisition of the NM CR (Berry, 1982) and interact cooperatively with input from the entorhinal cortex in the induction of LTP (Robinson, 1986).

In summary, conditioning-related potentiation effects occur following the administration of dosages of MK801 that retard the rate of associative learning. The conditioning-related potentiation effect examined in the present study, however, is short-lasting and does not persist outside of the conditioning session (Robinson, 1992). Similarly, MK801 reduces or abolishes LTP but not short-term potentiation effects evoked by tetanization of the perforant path. It is still possible that MK801 administration may disrupt learning by interfering with the establishment of a longer lasting neural trace in regions outside of the hippocampal dentate gyrus.

REFERENCES

- ABRAHAM, W. C., & MASON, S. E. (1988). Effects of the NMDA receptor/channel antagonists CPP and MK801 on hippocampal field potentials and long-term potentiation in anesthetized rats. *Brain Research*, **462**, 40-46.
- AKASE, E., ALKON, D. L., & DISTERHOFT, J. F. (1989). Hippocampal lesions impair memory of short-delay conditioned eye blink in rabbits. *Behavioral Neuroscience*, **103**, 935-943.
- BERGER, T. W., ALGER, B., & THOMPSON, R. F. (1976). Neuronal substrate of classical conditioning in the hippocampus. *Science*, **192**, 483-485.
- BERGER, T. W., RINALDI, P. C., WEISZ, D. J., & THOMPSON, R. F. (1983). Single-unit analysis of different hippocampal cell types during classical conditioning of rabbit nictitating membrane response. *Journal of Neurophysiology*, **50**, 1197-1219.
- BERGER, T. W., & SCLABASSI, R. J. (1988). Long-term potentiation and its relation to hippocampal pyramidal cell activity and behavioral learning during classical conditioning. In P. W. Landfield & S. A. Deadwyler (Eds.), *Long-term potentiation: From biophysics to behavior* (pp. 467-497). New York: Alan R. Liss.
- BERRY, S. D. (1982). Septo-hippocampal activity and learning rate. In C. D. Woody (Ed.), *Conditioning: Representation of involved neural function* (pp. 417-431). New York: Plenum.
- CHANDLER, J. P., & CRUTCHER, K. A. (1983). The septohippocampal projection in the rat: An electron microscopic horseradish peroxidase study. *Neuroscience*, **10**, 685-696.
- COLLINGRIDGE, G. L., HERRON, C. E., & LESTER, R. A. J. (1988). Frequency-dependent N-methyl-D-aspartate receptor-mediated synaptic transmission in rat hippocampus. *Journal of Physiology*, **399**, 301-312.
- FANTIE, B. D., & GODDARD, G. V. (1982). Septal modulation of the population spike in the fascia dentata produced by perforant path stimulation in the rat. *Brain Research*, **252**, 227-237.
- GALLAGHER, M. (1990). Robinson et al. (1989) deserves another look. *Psychobiology*, **18**, 258-260.
- GILBERT, M. E., & MACK, C. M. (1990). The NMDA antagonist, MK-801, suppresses long-term potentiation, kindling, and kindling-induced potentiation in the perforant path of the unanesthetized rat. *Brain Research*, **519**, 89-96.
- HARGREAVES, E. L., & CAIN, D. P. (1992). Hyperactivity, hyperreactivity, and sensorimotor deficits induced by low doses of the N-methyl-D-aspartate non-competitive channel blocker MK801. *Behavioural Brain Research*, **47**, 23-33.
- HARGREAVES, E. L., CAIN, D. P., & VANDERWOLF, C. H. (1990). Learning and behavioral-long-term potentiation: Importance of controlling for motor activity. *Journal of Neuroscience*, **10**, 1472-1478.
- HARRIS, E. W., GANONG, A. H., & COTMAN, C. W. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Research*, **323**, 132-137.
- HEALE, V., & HARLEY, C. (1990). MK-801 and AP5 impair acquisition, but not retention, of the Morris milk maze. *Pharmacology, Biochemistry & Behavior*, **36**, 145-149.
- JOY, R. M., & ALBERTSON, T. E. (1993). NMDA receptors have a dominant role in population spike-paired pulse facilitation in the dentate gyrus of urethane-anesthetized rats. *Brain Research*, **604**, 273-282.
- KEITH, J. R., & RUDY, J. W. (1990). Why NMDA-receptor-dependent long-term potentiation may not be a mechanism of learning and memory: Reappraisal of the NMDA-receptor blockade strategy. *Psychobiology*, **18**, 251-257.
- KEMP, J. A., FOSTER, A. C., & WONG, E. H. F. (1987). Noncompetitive antagonists of excitatory amino acid receptors. *Trends in Neuroscience*, **10**, 294-298.
- LOY, R., KOZIELL, D. A., LINDSEY, J. D., & MOORE, R. Y. (1981). Noradrenergic innervation of the adult rat hippocampal formation. *Journal of Comparative Neurology*, **189**, 699-710.
- MCCORMICK, D. A., LAVOND, D. G., CLARK, G. A., KETTNER, R. E., RISING, C. E., & THOMPSON, R. F. (1981). The engram found? Role

- of the cerebellum in classical conditioning of nictitating membrane and eyelid responses. *Bulletin of the Psychonomic Society*, **18**, 103-105.
- MCCORMICK, D. A., & THOMPSON, R. F. (1984). Neuronal responses of the rabbit cerebellum during acquisition and performance of a classically conditioned nictitating membrane/eyelid response. *Journal of Neuroscience*, **4**, 2811-2822.
- MORRIS, R. G. M. (1989). Synaptic plasticity and learning: Selective impairment of learning in rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. *Journal of Neuroscience*, **9**, 3040-3057.
- PORT, R. L., MIKHAIL, A. A., & PATTERSON, M. M. (1985). Differential effects of hippocampectomy on classically conditioned rabbit nictitating membrane response related to interstimulus interval. *Behavioral Neuroscience*, **99**, 200-208.
- RACINE, R. J., WILSON, D. A., GINGELL, R., & SUNDERLAND, D. (1986). Long-term potentiation in the interpositus and vestibular nuclei in the rat. *Experimental Brain Research*, **63**, 158-162.
- ROBINSON, G. B. (1986). Enhanced long-term potentiation induced in rat dentate gyrus by coactivation of septal and entorhinal inputs: Temporal constraints. *Brain Research*, **379**, 56-62.
- ROBINSON, G. B. (1991). Contribution of NMDA-mediated neuronal activity to classical conditioning of the rabbit nictitating membrane and conditioning-related potentiation of perforant path-granule cell responses. *Society for Neuroscience Abstracts*, **17**, 323.
- ROBINSON, G. B. (1992). Reversal learning of the rabbit nictitating membrane response following kindling-induced potentiation within the hippocampal dentate gyrus. *Behavioural Brain Research*, **50**, 185-192.
- ROBINSON, G. B., PORT, R. L., & STILLWELL, E. J. (1993). Latent inhibition of the classically conditioned rabbit nictitating membrane response is unaffected by the NMDA antagonist MK801. *Psychobiology*, **21**, 120-124.
- ROBINSON, G. B., & RACINE, R. J. (1985). Long-term potentiation in the dentate gyrus: Effects of noradrenaline depletion in the awake rat. *Brain Research*, **325**, 71-78.
- ROBINSON, G. B., & REED, G. D. (1992). Effect of MK801 on the induction and subsequent decay of long-term potentiation in the unanesthetized rabbit hippocampal dentate gyrus. *Brain Research*, **569**, 78-85.
- SALAFIA, W. R. (1987). Pavlovian conditioning, information processing, and the hippocampus. In I. Gormezano, W. F. Prokasy, & R. F. Thompson (Eds.), *Classical conditioning* (3rd ed., pp. 197-216). Hillsdale, NJ: Erlbaum.
- SCHMAJUK, N. A., & DiCARLO, J. J. (1991). A neural network approach to hippocampal function in classical conditioning. *Behavioral Neuroscience*, **105**, 82-110.
- SEARS, L. L., & STEINMETZ, J. E. (1990). Acquisition of classically conditioned-related activity in the hippocampus is affected by lesions of the cerebellar interpositus nucleus. *Behavioral Neuroscience*, **104**, 681-692.
- SHAPIRO, M. L., & CARAMANOS, Z. (1990). NMDA antagonist MK-801 impairs acquisition but not performance of spatial working and reference memory. *Psychobiology*, **18**, 231-243.
- SHAPIRO, M. L., & O'CONNOR, C. (1992). N-Methyl-D-aspartate receptor antagonist MK-801 and spatial memory representation: Working memory is impaired in an unfamiliar environment but not in a familiar environment. *Behavioral Neuroscience*, **106**, 604-612.
- SHARP, P. E., MCNAUGHTON, B. L., & BARNES, C. A. (1985). Enhancement of hippocampal field potentials in rats exposed to a novel, complex environment. *Brain Research*, **339**, 361-365.
- SKELTON, R. W., SCARTH, A. S., WILKIE, D. M., MILLER, J. J., & PHILLIPS, A. G. (1987). Long-term increases in dentate granule cell responsivity accompany operant conditioning. *Journal of Neuroscience*, **7**, 3081-3087.
- SOLOMON, P. R., SOLOMON, S. D., VANDER SCHAAF, E., & PERRY, H. E. (1983). Altered activity in hippocampus is more detrimental to classical conditioning than removing the structure. *Science*, **220**, 329-331.
- THOMPSON, L. T., & DISTERHOFT, J. F. (1991). The NMDA receptor/channel complex and associative learning: Opposite effects of PCP and D-cycloserine. *Society for Neuroscience Abstracts*, **17**, 486.
- VINOGRADOVA, O. S. (1975). Functional organization of the limbic system in the process of registration of information: Facts and hypotheses. In R. L. Isaacson & K. H. Pribram (Eds.), *The hippocampus* (Vol. 2, pp. 3-70). New York: Plenum.
- WEISZ, D. J., CLARK, G. A., & THOMPSON, R. F. (1984). Increased responsivity of dentate granule cells during nictitating membrane response conditioning in rabbit. *Behavioural Brain Research*, **12**, 145-154.
- WEISZ, D. J., CLARK, G. A., YANG, B., THOMPSON, R. F., & SOLOMON, P. R. (1982). Activity of dentate gyrus during NM conditioning in rabbit. In C. D. Woody (Ed.), *Conditioning: Representation of involved neural function* (pp. 131-145). New York: Plenum.

(Manuscript received October 22, 1992;
revision accepted for publication July 21, 1993.)