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Parallel Recovery of MK-801–Induced Spatial Learning Impairment and Neuronal Injury in Male Mice

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BROSNAN-WATTERS, G., D. F. WOZNIAK, A. NARDI AND J. W. OLNEY. *Parallel recovery of MK-801–induced spatial learning impairment and neuronal injury in male mice.* PHARMACOL BIOCHEM BEHAV. **62**(1) 111–122, 1999.—The relationship between spatial learning impairment and reversible neuronal injury in the posterior cingulate/retrosplenial (PC/RS) cortex induced by MK-801 in male mice was studied using a four-corner holeboard task. Mice were dosed with 1 mg/kg MK-801 and tested on acquisition of a new "baited" hole at 5 or 12 h posttreatment. Acquisition in drugged mice was impaired at 5 h, but not at 12 h posttreatment. Their retention performances were unaffected 24 h after either the 5 or 12 h posttreatment acquisition sessions. MK-801 (1 mg/kg) was found to induce locomotor hyperactivity and some sensorimotor impairment at 5 h posttreatment, which could have contributed to the acquisition deficit. However, nonassociative effects of the drug were not prominent because this same dose did not impair holeboard performance at 5 h posttreatment when the task was well learned. Histologic experiments showed that many injured neurons (containing cytoplasmic vacuoles) were present in the PC/RS cortex at 5 h posttreatment but the reaction was essentially reversed at 12 h posttreatment. The results suggest that the acquisition impairment and neuronal injury induced by MK-801 evolve and recover in parallel according to a similar time schedule. © 1998 Elsevier Science Inc.

MK-801 Dizocilpine Spatial learning and memory Mice Sensorimotor Neuronal injury NMDA receptor antagonist

THE *N*-methyl-D-aspartate (NMDA) subtype of glutamate (Glu) receptor is thought to play an important role in learning and memory. This is based on evidence that it mediates the induction of long-term potentiation (LTP), a putative electrophysiological correlate of memory, and that systemic administration of NMDA antagonist drugs interferes with various kinds of learning [e.g., (17)]. For example, it is well established that low doses of NMDA antagonists disrupt spatial learning in rats (2,5,6,7,9–12,14,18,25–27,32,33,35) and mice (4,13,24,31). In mice, a low dose of MK-801 (0.05 mg/kg, SC) impairs acquisition on the rotating holeboard task (4). Specifically, MK-801–treated mice take longer to learn the location of a new "baited" hole, which is different from the location learned during a pretreatment session when no drugs are administered. This impairment appears to be a true disturbance in learning/memory processes rather than the result of nonassociative effects induced by the drug because this dose of MK-801 does not produce hyperactivity or sensorimotor disturbances that are likely to disrupt performance of the holeboard task (4). In addition, it has been shown in mice that a much higher dose (e.g., MK-801, 10 mg/kg IP) disrupts the same type of memory parameter (acquisition of a new baited hole) on the holeboard task, but the impairment is chronic and perhaps permanent with residual impairment for up to 5–6 months posttreatment (34). Both the transient and permanent memory disrupting effects in mice pertain to acquisition of new information, while retention of learned information appears to be unaffected by these compounds (4,34). Although it is not known what region(s) of the brain mediates the memory functions disrupted by NMDA antagonist drugs, the hippocampus is assumed to be involved, on the basis of extensive evidence suggesting the importance of this brain region for spatial memory functions.

Systemic treatment with an NMDA antagonist in rodents can cause either reversible injury or irreversible degeneration

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of cerebrocortical neurons (8,22,34). The neurons predominantly affected are pyramidal neurons in layers III and IV of the posterior cingulate/retrosplenial (PC/RS) cortex. Doses in the range of 0.5 to 1 mg/kg cause reversible injury consisting of vacuole formation from endoplasmic reticulum and mitochondria. In rats, the reaction reaches peak severity at approximately 4 to 6 h posttreatment, then recedes in the ensuing 6–10 h, and becomes undetectable by 24 h. Whether this injury is similarly reversible in the mouse has, to our knowledge, not yet been reported. However, permanent irreversible degeneration of PC/RS cortical neurons has been shown to occur over a 3–10-day period following a high dose of an NMDA antagonist (e.g., MK-801 at 5 to 10 mg/kg, IP) in both rats (7,8) and mice (34).

The relationship between the acquisition-impairing effects of NMDA antagonists and their ability to injure or kill cerebrocortical neurons is unclear. The brain region most frequently implicated in spatial learning is the hippocampus. However, studies conducted in rats have shown that ablative lesions of the PC/RS cortex impair spatial learning in the Morris maze (29,30), and excitotoxic lesions in mice have been reported to impair performance on spatial discrimination reversal learning in a T-maze (16). Although PC/RS cortical neurons are selectively injured by doses of NMDA antagonists that impair spatial learning acquisitions, hippocampal neurons do not sustain detectable injury from such treatment. Thus, on the basis of the hypersensitivity of PC/RS cortical neurons to the toxic effects of NMDA antagonists, it is logical to propose that a selective functional disturbance in PC/RS cortical neurons may play some role in the acquisition-impairing effects of these compounds. This proposal is consistent with the observation that the acquisition-impairing effect is converted from a transient to a permanent effect when the dose of NMDA antagonist is raised from a noninjurious dose to a dose that induces permanent neuronal loss in the PC/RS cortices. Here we have explored the relationship further by treating mice with a dose of MK-801 that is known to induce transient reversible neuronal injury in the PC/RS cortical area and by determining whether this results in an acquisition disturbance that is similarly transient and reversible. To our knowledge, this relationship has not been investigated in any animal species.

EXPERIMENT 1: EFFECTS OF 1 mg/kg MK-801 ON SPATIAL LEARNING EVALUATED 5 HOURS POSTTREATMENT

The decision to conduct holeboard testing at 5 h posttreatment was based on pilot work that suggested that 5 h after being treated with 1 mg/kg MK-801, mice were able to move around their cages without showing signs of gross sensorimotor disturbances or alterations in locomotor activity. In addition, pilot histologic work indicated that there was substantial neuronal injury in the PC/RS cortical area at this posttreatment time.

METHOD

Subjects

Twenty-seven outbred male albino mice (Harlan ICR) that were between 2–3 months old at the beginning of habituation served as subjects in Experiment 1. Mice were housed in large polypropelene cages, with a maximum of 10 mice to a cage in a temperature- and humidity-controlled animal quarters. They were maintained on continuous access to food and water until the beginning of the experiment, and on a 12L:12D cycle.

Apparatus

The holeboard has been described previously (3,4,34). Briefly, it consists of a square floor that contains 16 holes arranged in a 4×4 matrix enclosed by Plexiglas sides. Although there are two different versions of the task reflecting different stimulus configurations (four-corner and row), only the four-corner configuration was used in the present experiments. In the four-corner version of the task, an opaque Plexiglas insert is placed on the floor of the apparatus covering all but the four corner holes. There is a Froot Loop (Kellogg's) in every exposed hole, which is made inaccessible by being placed under a screen at the bottom of the hole. The screen allows the odor of the food to emanate from the hole, but does not allow access to the food. When an individual hole is baited, a piece of Froot Loop is placed on top of the screen, making the food accessible. The entire apparatus rests on a turntable so that it can be rotated easily. A start tube is placed in the center of the apparatus, and its removal is used to initiate a trial.

Procedure

Food restriction. Mice were weighed during handling and habituation to establish baseline body weights before being exposed to food restriction procedures, which resulted in slowly lowering their body weights to approximately 85–90% of their free-feeding weights. The mice were weighed daily during the course of the experiment.

Habituation. Mice were handled extensively and allowed to explore the holeboard apparatus for 2 min per day for 2 weeks. During this time, all the holes were covered. During the next 3 days, with the holes still covered, pieces of Froot Loop were made available to the mice by scattering them about the enclosure. During the next phase, mice were food restricted as previously described, and for 4 days were placed in the apparatus with all four holes open, and with a piece of Froot Loop in every hole. They were allowed 3 min to find and eat the reinforcement from the four holes.

Pretreatment acquisition and retention. After handling and habituation, the mice were trained on the four-corner version of the holeboard task where each mouse was required to learn the spatial location of the one hole out of four that was baited (contained a Froot Loop piece) on every trail. A trial consisted of releasing a mouse from the start tube and allowing it to poke its head into the holes until it retrieved the reinforcer or until 3 min elapsed. If a mouse poked its head into the baited hole and retrieved the reinforcer on its first poke for a given trial, a correct trial was recorded. If a mouse poked its head up to eye level (operational definition of a hole poke) into a nonbaited hole on its first hole poke, this was scored as an incorrect trial, although the mouse was allowed to continue to poke until it retrieved the reward. To prevent mice from using odor or other proximal cues to locate the correct hole, the apparatus was washed with a scented detergent and rotated 90 degrees (on a pseudorandom basis) between each trial. Thus, although the actual baited hole was different from the one used on the immediately preceding trial, it was always located in the same position relative to distal cues in the room. The total number of pokes it took the mouse to retrieve the reward, as well as the latency to find the reward, were recorded. A massed trials protocol was used where training continued for up to 1.5 h or until the mouse reached a criterion of eight correct trials out of nine consecutive trials. Only mice that were able to reach criterion within the 1.5-h time limit were included in the study. Twenty-four hours after acquisition training, the mice were given a retention test using the

same criterion to determine if they would perform as if they remembered the spatial location of the baited hole learned on the previous day.

Drug treatment and posttreatment acquisition and retention. Two weeks after pretreatment retention, the mice were divided into two groups matched on the basis of their pretreatment acquisition performance (trials to criterion). Half were injected (SC) with 1 mg/kg MK-801, and the other group was injected with vehicle. The MK-801 was dissolved in distilled water and adjusted to a neutral pH (7.4 ± 0.1) with NaOH. Five hours after drug or vehicle injection, the mice were run individually as in the pretreatment phase, although testing order was derived by using pairs of mice, one from each treatment group with members of pairs and order of pairs being randomly determined. The protocol for the posttreatment acquisition and retention was identical to the pretreatment acquisition and retention, except that mice were trained to a new hole. Thus, the design of the present experiment is similar to a reversal learning paradigm in that a previously learned baited hole location is no longer correct and a new baited hole location must be learned. Perseverative responding to the hole that was baited during pretreatment was also evaluated during posttreatment. A perseveration score was calculated for each mouse by determining the number of hole pokes that were made to the hole that was baited during pretreatment (but no longer baited) out of the total number of hole pokes emitted during posttreatment, multiplied times 100. The mice were tested by an experimenter who was "blind" with regard to the treatment status of the animals. The experiment was run in two replicates with 13 mice in the first replicate (MK-801, $n = 6$; vehicle controls, $n = 7$) and 14 in the second (MK-801, $n = 7$; vehicle controls, $n = 7$).

Statistical Analyses

An ANOVA model containing two between-subjects variables (group and replicate) and two within-subjects variables (pre- vs. posttreatment tests; acquisition vs. retention) was used to analyze acquisition scores (trials to criterion). Planned comparisons were conducted to evaluate performance between groups following drug/vehicle treatment and others were conducted within each group to determine potential differences between pre- and posttreatment performances. Similar ANOVA models were used to evaluate performance when error scores were used as the dependent variable. A *t*-test was used to evaluate group differences in perseveration scores. Two types of error scores were used: wrong trials to criterion and total number of incorrect nose pokes to criterion. A wrong trial was defined as a trial on which the first nose poke was incorrect. Because a mouse was allowed to make nose pokes until it made one into the correct hole, it could make several incorrect pokes within a single trial. The incorrect nose poke score was a summation of the incorrect pokes within and across all trials. Computer-generated *p*-values equal to 0.000 are expressed as $p < 0.0005$.

RESULTS

The acquisition and retention data from Experiment 1 are presented in Figs. 1A and 2A. The results of the ANOVA indicated that the effects of group, replicate, and group by replicate interaction were nonsignificant. However, the within-subjects effect of acquisition vs. retention was significant, $F(1, 23) = 181.53$, $p < 0.0005$, as was the group by acquisition vs. retention interaction, $F(1, 23) = 5.72$, $p = 0.025$. Also, the within-subjects effect of pre- vs. posttreatment was significant, $F(1, 23) = 6.18$, $p = 0.021$, as was the group by pre- vs. posttreatment interaction, $F(1, 23) = 5.72$, $p = 0.014$. All other interactions were nonsignificant. Univariate *F*-tests were conducted for each of the pre- and posttreatment acquisition and retention tests to better understand the nature of the significant group by acquisition vs. retention and group by pre- vs. posttreatment interactions. The only instance where there were significant differences between groups in terms of trials to criterion was during the posttreatment acquisition test where the MK-801–treated mice required significantly more trials to criterion than vehicle controls, $F(1, 25) = 10.26$, $p = 0.004$. Because the effect of replicate was not significant, nor was any interaction involving replicate significant, the data were combined from the two replications when evaluating other effects.

The nature of the between-subjects acquisition effect during posttreatment was clarified by the planned within-subjects comparisons for each group. Specifically, the vehicle controls showed a significant improvement in acquisition performance from pretreatment to posttreatment sessions. $F(1,12) = 8.55$, $p = 0.01$, whereas the MK-801–treated group showed no such improvement. In contrast to the acquisition data, the two groups of mice performed similarly during retention for both pre- and posttreatment testing. Partitioning the significant acquisition vs. retention effect through subsequent comparisons helped to clarify the nature of this effect. Specifically, the vehicle controls showed a significant improvement in performance (took fewer trials to reach criterion) during retention compared to acquisition for both the pretreatment, $F(1, 13) = 58.39$, $p < 0.0005$, and posttreatment $F(1,13) = 51.23$, $p < 0.0005$, suggesting that learning took place during acquisition training under both testing conditions. The same was true for the MK-801–treated mice during pretreatment, $F(1, 12) = 29.05$, $p < 0.0005$, and during posttreatment testing, $F(1, 12) = 32.62$, $p < 0.0005$. The mean (±SD) perseveration scores for the MK-801–treated and vehicle controls were 16.79 ± 2.36 and 17.23 ± 4.40, respectively. A *t*-test on these data indicated that the groups did not differ significantly.

The results of a comparison involving errors to criterion during acquisition (data not shown) indicate that the MK-801–treated mice committed more errors in reaching criterion than did the vehicle controls, $F(1, 25) = 11.22$, $p = 0.003$, thus confirming the trials to criterion data. A similar comparison of wrong trials to criterion also confirmed the trials to criterion data, in that the vehicle control mice also had significantly fewer wrong trials to criterion, $F(1, 25) = 9.11$, $p = 0.006$. A comparison of the vehicle control group's pretreatment vs. posttreatment errors to criterion also confirmed the trials to criterion comparisons, $F(1, 12) = 12.91$, $p = 0.004$, as did wrong trials to criterion comparisons, $F(1, 12) = 8.98$, $p = 0.01$. Similar comparisons for the MK-801–treated animals also confirmed the trials to criterion findings, in that the treated animal's performance did not significantly improve between pretreatment and posttreatment using either of the errors-to-criterion measures.

EXPERIMENT 2: EFFECTS OF 1 mg/kg MK-801 ON SPATIAL LEARNING EVALUATED AT 12 HOURS POSTTREATMENT

Twelve hours was the next posttreatment time used for conducting holeboard testing following MK-801 (1 mg/kg) administration because pilot histological work suggested that vacuolar injury to PC/RS cortical neurons was essentially reversed by this posttreatment time.

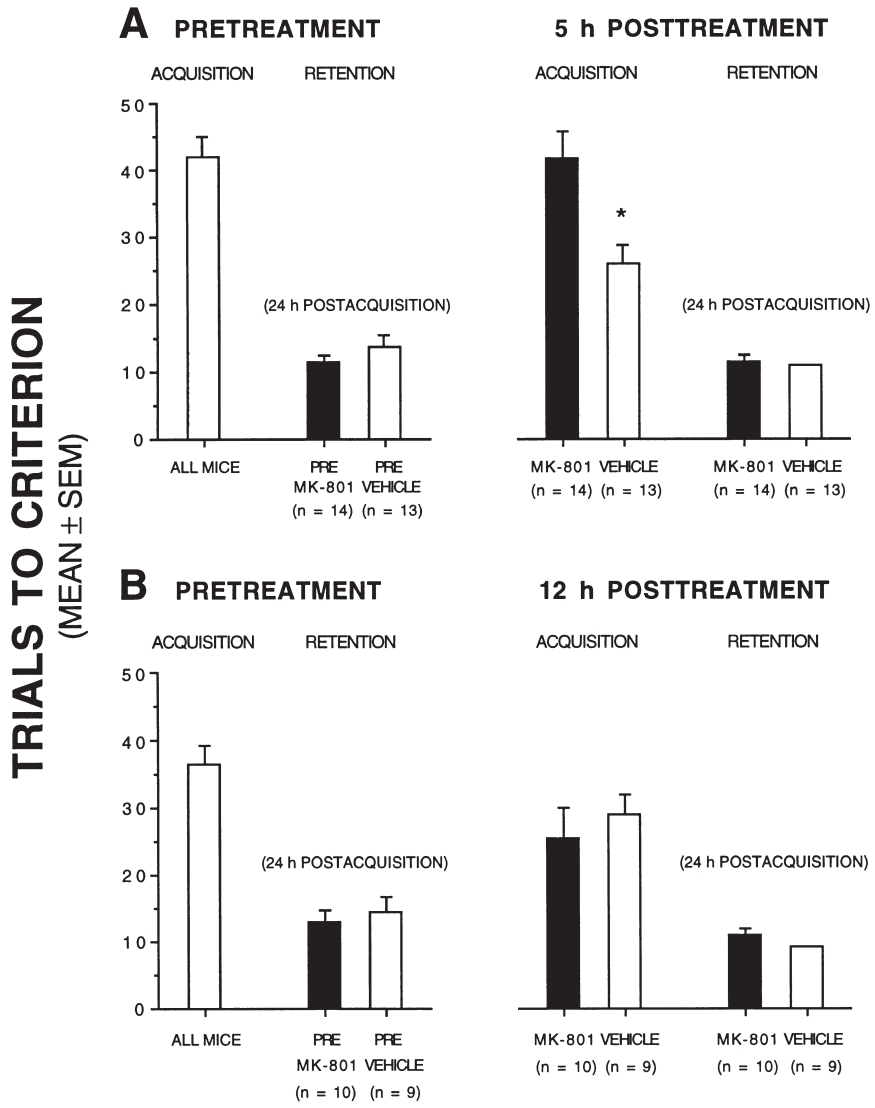

Fig. 1. Trials to criterion during acquisition and retention under pretreatment and posttreatment conditions and as a function of posttreatment time interval. (A) This represents data from Experiment 1 in which mice were trained on the four-corner holeboard task and then were split into two groups that were matched according to trials to criterion during pretreatment acquisition when no treatments were administered. The groups also performed similarly when given a retention test 24 h after acquisition. Beginning approximately 2 weeks later, groups of mice were injected SC with either MK-801 (1 mg/kg) or vehicle and were tested 5 h later on the holeboard again to evaluate their ability to acquire the location of a new "baited" hole, which was different from the location of the baited hole used during pretreatment. The vehicle control mice took significantly (*) fewer trials to reach criterion than the MK-801–treated mice ($p = 0.004$). In addition, the vehicle control mice required significantly fewer trials to reach criterion during acquisition following vehicle/drug treatment compared to levels observed during pretreatment acquisition ($p = 0.01$), while the MK-801–treated mice showed no such improvement. Retention performances were not different between the groups when mice were tested 24 h posttreatment. (B) This represents data from Experiment 2. In this experiment, mice were also trained on the four-corner holeboard task, and two groups of mice were formed by matching them in terms of holeboard acquisition scores (trials to criterion) on a pretreatment task when no treatments were administered. The groups also did not differ in retention performance when tested 24 h later. Approximately 2 weeks later the two groups of mice were administered either vehicle or 1 mg/kg MK-801, and then were tested on acquiring the location of a new baited hole at 12 h posttreatment. The groups did not differ in terms of acquisition performance, nor did they differ on a retention test group given 24 h later.

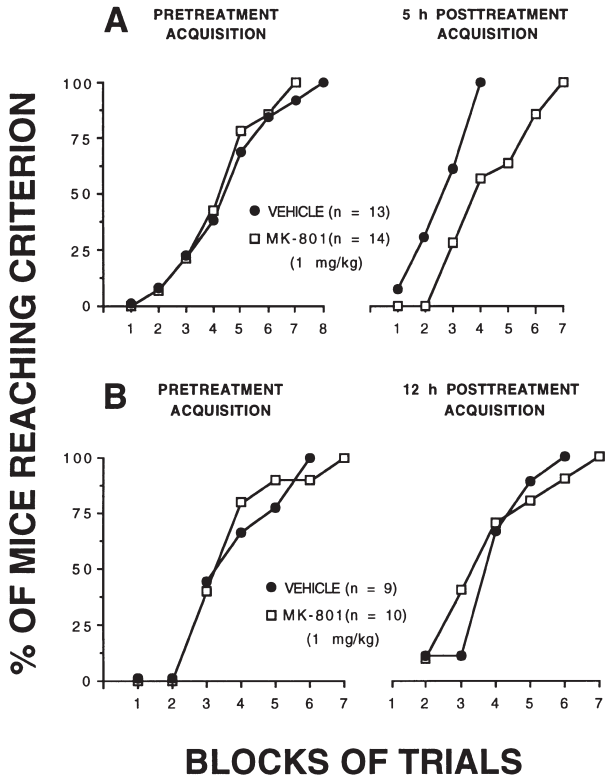

Fig. 2. Cumulative percent of mice reaching criterion during acquisition as a function of blocks of trials. Acquisition performance on the four-corner holeboard over time as it pertains to the 5-h posttreatment test (Experiment 1) is represented in A. The data show that the mice were well matched according to pretreatment performance when no treatments were administered. Beginning approximately 2 weeks later when groups of mice were dosed with either vehicle or 1 mg/kg MK-801 and were tested 5 h posttreatment, the MK-801–treated mice reached criterion more slowly than the vehicle controls. (B) This shows holeboard acquisition performance pertaining to the 12 h posttreatment test (Experiment 2). Again, groups were well matched according to pretreatment acquisition scores when no treatments were administered. However, no differences in acquisition performance were observed when groups of mice were treated with either vehicle or 1 mg/kg MK-801 and then tested 12 h posttreatment.

METHOD

Subjects

Nineteen naive male mice of the same age and strain as were used in the previous experiments served as subjects. They were maintained under the same conditions as the animals in the previous experiments.

Apparatus

The apparatus was the same as that used in Experiment 1.

Procedure

Food restriction, habituation, and the acquisition/retention procedures were similar to those used in Experiment 1. The main difference was that posttreatment testing took place 12 h after injection (SC) with 1 mg/kg of MK-801 ($n = 10$) or vehicle ($n = 9$). The experimenter was "blind" to the treatment condition of the mice, and retention testing was conducted 24 h after acquisition.

Statistical Analysis

An ANOVA containing one between-subjects variable, groups (control vs. MK-801) was conducted as a planned comparison to evaluate differences in trials to criterion during acquisition performance following drug/vehicle treatment. A repeated-measures ANOVA was used to determine if acquisition performance (trials to criterion) was significantly different during posttreatment compared to pretreatment levels, and a repeated-measures ANOVA was also used to evaluate performance during acquisition vs. retention.

RESULTS

The data from the acquisition and retention tests in Experiment 2 are shown in Figs. 1B and 2B. Because the groups were matched post hoc on trials to criterion during pretreatment acquisition, differences in acquisition performance during pretreatment were not an issue. From observing the posttreatment acquisition data, it appeared that vehicle and drug-treated groups performed similarly during this test. The ANOVA confirmed that there was no significant effect of 1 mg/kg MK-801 at 12 h posttreatment, $F(1, 17) = 0.37$, $p = 0.55$. In fact, a repeated-measures ANOVA revealed that, although the means of the two groups were similar, the acquisition performance of the drug-treated mice was significantly better during posttreatment, $F(1, 9) = 6.86$, $p = 0.03$, compared to pretreatment levels, although the control group did not change significantly, $F(1, 8) = 3.11$, $p = 0.12$. With regard to the retention data, the vehicle controls showed significant improvement in performance during retention compared to acquisition for both pretreatment, $F(1, 8) = 52.61$, $p = 0.0001$, and posttreatment sessions, $F(1, 8) = 34.67$, $p < 0.0005$, suggesting that learning took place during training under both testing conditions. The same was true for the MK-801–treated mice during pretreatment, $F(1, 9) = 29.44$, $p = 0.0004$, and during posttreatment testing, $F(1, 9) = 10.67$, $p = 0.01$. There were no differences in retention performance between the two groups under either testing condition.

Using errors to criterion and wrong trials to criterion (data not shown) as dependent variables to evaluate acquisition yielded some results that were similar to the findings from the analyses using the trials to criterion data. Specifically, ANOVAs using errors and wrong trials to criterion yielded no significant effect of drug treatment, $F(1, 17) = 0.10$, $p = 0.76$, $F(1, 17) = 0.12$, $p = 0.74$, respectively. However, when errors and wrong trials to criterion were used as dependent variables, the control group performed significantly better during posttreatment acquisition compared to pretreatment levels, $F(1, 8) = 6.45$, $p = 0.04$, $F(1, 8) = 6.13$, $p = 0.04$, respectively, as did the drug-treated group, $F(1, 9) = 12.43$, $p = 0.006$, $F(1, 9) = 10.88$, $p = 0.004$, respectively. This is in contrast to the findings from the analysis of the trials-to-criterion data where the control group did not show significant improvement.

To summarize, at 12 h posttreatment, holeboard acquisition performance of MK-801–treated mice was not significantly impaired compared to that of controls.

EXPERIMENT 3: EFFECTS OF 1 mg/kg MK-801 ON ACTIVITY AND SENSORIMOTOR PERFORMANCE AT 5 HOURS POSTTREATMENT

Because a significant impairment in holeboard acquisition was found at 5 h following administration of 1 mg/kg MK-801

(Experiment 1), it became important to document whether sensorimotor or activity disturbances existed at this posttreatment time in order to evaluate whether it was likely that certain nonassociative effects contributed to the holeboard acquisition deficit.

METHOD

Subjects

Three to 4-month-old male Harlan ICR mice were used for the 1-h activity test and sensorimotor battery. They were maintained on continuous food and water and were housed as described above. They were handled in a manner similar to the mice used in Experiment 1.

Activity and Sensorimotor Battery

One-hour activity test. Locomotor activity was evaluated over a 1-h period, 5 h after mice received SC injections of either 1 mg/kg MK-801 ($n = 12$) or vehicle ($n = 12$), according to previously published procedures (4,34,35). Briefly, this involved placing a mouse in one of two translucent polystyrene (rat) cages, which contained woodchip bedding and recording the number of beam breaks registered by three pairs of photoelectric cells placed across the width of the cages. Two chambers were used, with one mouse from each treatment group per chamber, and mice were run in pairs to control for time of testing. The order of testing of individual animals was pseudorandomly determined, in that a mouse from one condition was randomly chosen, and then one mouse from the other condition was chosen to be run at the same time. The activity chambers were counterbalanced across treatments such that half of the MK-801–treated mice were tested in one chamber and half in the other and the same was true for the vehicle controls. Testing took place between 0700 and 1600 h in a quiet room continuously illuminated by fluorescent lights.

Sensorimotor battery. Mice ($n = 11$) were first evaluated on the sensorimotor battery during a habitation trial with no treatment, then injected with saline, and 5 h later were evaluated again. The next day, mice were injected with 1 mg/kg MK-801, and at 5 h posttreatment were evaluated again on the battery of four sensorimotor tests. These tests have been shown to be sensitive (especially ledge and platform tests) for detecting dose-related impairments in sensorimotor performance following systemic administration of MK-801 (3,31,32). Within a given session, all of the mice were evaluated on one test before proceeding onto the next test until all four tests in the battery were completed. The testing order of individual subjects was pseudorandomly determined. The individual tests are briefly described below. For additional details concerning the apparatuses and procedures, see Brosnan-Watters et al. (3).

Walking initiation. Each mouse was placed in the middle of a square outlined by white cloth tape (21 × 21 cm) on a smooth black surface of a large table top. The time (in seconds) it took each mouse to leave the square (place all four paws outside of the tape) was recorded. The maximum time allowed was 60 s.

Ledge. Each mouse was timed for how long it could maintain its balance on a narrow (0.75 cm thick) Plexiglas ledge without falling (60-s maximum). A score of 60 s was also assigned if the mouse traversed the entire length (51 cm) of the Plexiglas ledge and returned to the starting place in under 60 s without falling.

Inclined screen. Each mouse was placed on an elevated wire mesh grid inclined to 60 degrees. It was placed in the middle of the screen with its head oriented downward toward the floor and timed (in seconds) for how long it remained on the screen without falling. The maximum length of a trial was 60 s. A mouse also received a score of 60 s if it turned 180 degrees and climbed to the top of the apparatus and rested its forepaws on top without falling.

Platform. Each mouse was timed (in seconds) for how long it remained on a small (1.0-cm thick; 3.0-cm diameter) elevated (30 cm) circular platform. A maximum score of 60 s was assigned if the mouse remained on the platform for the maximum amount of time or if it could, without falling, climb down the very thin pole (1 cm diameter) that supported the platform.

Padding was placed under each apparatus that was evaluated above the floor (inclined screen, platform and ledge) to prevent pain or injury resulting from falling.

Statistical Analysis

The activity data (total number of beam breaks over a 1-h period) were analyzed using an ANOVA containing one between-subjects variable, group (saline vs. 1 mg/kg MK-801), to evaluate the effects of this dose of MK-801 at 5 h posttreatment. A repeated-measures ANOVA containing one within-subjects variable, trials (saline vs. 1 mg/kg MK-801), was used when appropriate for each sensorimotor test to evaluate the effect of 1 mg/kg of MK-801 at 5 h posttreatment compared to saline. Data from the habituation trials were not used in the statistical analyses.

RESULTS

One-Hour Activity Test

The 1-h activity data displayed in Table 1 show that the MK-801–treated mice had higher activity counts than did the controls, and the ANOVA confirmed that this difference was statistically significant $F(1, 22) = 7.31$, $p = 0.01$.

TABLE 1

1-HOUR LOCOMOTOR ACTIVITY AND SENSORIMOTOR BATTERY

Tests	Saline Mean* (±SEM)	MK-801 Mean* (±SEM)
Activity	657.8 (187.85)	1214.9† (85.5)
Ledge	60.0 (0.00)	36.9† (7.33)
Platform	51.8 (5.61)	27.3† (7.87)
Inclined screen	60.0 (0.00)	60.0 (0.00)
Walking initiation	6.4 (1.03)	5.5 (2.70)

*For activity, values represent the mean number of photocell beam breaks over a 1-h period. For the sensorimotor tests, values represent mean time (s) spent on ledge, platform, or inclined screen or within the square for the walking initiation test.
†$p = 0.01$.

Sensorimotor Battery

Walking initiation. Data from the walking initiation test appear in Table 1. The ANOVA indicated that performance (time to leave the square) was not different following saline injections compared to that observed after administration of 1 mg/kg MK-801 when evaluated at 5 h posttreatment, $F(1, 10) = 0.12$, $p = 0.74$.

Ledge. As can be seen in Table 1, mice performed better (stayed on the ledge longer) under the saline treatment than after MK-801, and the ANOVA confirmed that these differences represented a significant effect of drug treatment, $F(1, 10) = 9.86$, $p = 0.01$.

Inclined screen. The data from this test (Table 1) indicate that it is a very easy test for the mice, and that this dose of MK-801 has no effect on the mice at 5 h posttreatment. Because all the scores were 60 s, no analysis was performed on these data.

Platform. The data from this test (Table 1) indicated that the performance (ability to remain on the platform) of the mice was impaired 5 h after MK-801 administration compared to performance observed after saline injections. These differences represented a significant effect of drug treatment, $F(1, 10) = 9.20$, $p = 0.01$.

In summary, these results indicated that mice were significantly more active at 5 h posttreatment, and that the MK-801 treatment impaired performance on the ledge and platform portions of the sensorimotor battery.

EXPERIMENT 4: EFFECTS OF 1 mg/kg MK-801 ON A WELL-LEARNED TASK AT 5 HOURS POSTTREATMENT

Although 1 mg/kg MK-801 induced behavioral changes such as heightened locomotor activity or certain sensorimotor impairments, it was not clear that these effects would alter holeboard performance. To help clarify whether the sensorimotor and/or activity disturbances induced by 1 mg/kg MK-801 would affect holeboard performance, mice were trained for 4 days on the holeboard and then on day 5 they received 1 mg/kg MK-801 and were tested on the holeboard at 5 h posttreatment. Two groups of mice, which differed in terms of previous experience with the holeboard procedure, were tested using this protocol (see Subjects below). If MK-801 impaired holeboard performance when the task was well learned, it would provide evidence that nonassociative factors may have affected acquisition in Experiment 1.

METHOD

Subjects

Three- to 4-month-old Harlan ICR mice that were housed in the same manner as previously described in Experiment 1 served as subjects in this experiment. One group consisted of naive mice ($n = 5$), and the other was composed of mice that had served as vehicle controls in the 12-h posttreatment experiment (Experiment 2) and, thus, had previous experience with the holeboard procedure ($n = 8$).

Apparatus

The four-corner version of the holeboard apparatus previously described in Experiment 1 was used.

Procedure

Habituation and food restriction. The habituation and food restriction procedures were similar to that described for Experiment 1.

Acquisition and postdrug testing. Naive mice were trained to criterion (same as in Experiment 1) to go to one hole in the four-corner configuration of the holeboard over 4 consecutive days. In the case of the nonnaive mice, this was a hole to which they had not been previously trained. On day 5, they were challenged with a 1-mg/kg dose (SC) of MK-801, and were tested 5 h later to determine if the drug affected their performance.

Statistical Analyses

An ANOVA containing one between-subjects variable, group (naive vs. experienced), and one within-subjects variable, training days (day 1 to day 5), was used to evaluate performance over the 5 days of testing, and p-values were adjusted using the Greenhouse-Geisser statistic because there were more than two levels of the within-subjects variable. Planned comparisons were conducted on the data from day 1 vs. day 2 and day 4 vs day 5.

RESULTS

The data, seen in Fig. 3, suggest that there was no effect of treatment on holeboard performance in either group when the task was well learned. The results of the overall ANOVA indicated that the effect of group was nonsignificant, while the effect of training days and the group by training days interaction were significant, $F(4, 44) = 18.78$, $p < 0.0005$; $F(4, 44) = 4.71$, $p = 0.03$, respectively. Subsequent univariate F-tests indicated that the differences between groups on day 1, $F(1, 11) = 4.83$, $p = 0.05$, likely accounted for the significant group by training days interaction because no significant differences were observed between groups during any of the other training days. Within the naive group there was a significant effect of training, $F(4, 16) = 8.14$, $p = 0.03$. At least part of this effect was due to mice showing a significant improvement in performance from day 1 to day 2, $F(1, 4) = 9.52$, $p = 0.04$, as would be expected from the results of Experiment 1. However, there was no difference in performance between day 4 and after the drug challenge on day 5, $F(1, 4) = 0.085$, $p = 0.79$, indicating that this dose of MK-801 had no effect on holeboard performance when tested at 5 h posttreatment and when the task was well learned. Similar results were found for the experienced group. Specifically, there was a significant effect of training, $F(4, 28) = 8.82$, $p = 0.002$, a significant improvement in performance from day 1 to day 2, $F(1, 7) = 10.89$, $p = 0.01$, but there was no difference in performance levels between day 4 and after the drug challenge on day 5.

EXPERIMENT 5: HISTOLOGIC EVALUATION OF THE EFFECTS OF 1 mg/kg MK-801 AT 5 AND 12 HOURS POSTTREATMENT

METHOD

Subjects

Male mice that were of the same age and strain as those used in the previous experiments served as subjects. They were maintained on continuous access to food and water and were housed as has been described for the previous experiments.

Procedure

There were three treatment groups of mice: saline controls ($n = 3$); mice that received 1 mg/kg MK-801 (SC) and were perfused at 5 h posttreatment ($n = 6$); and mice that received

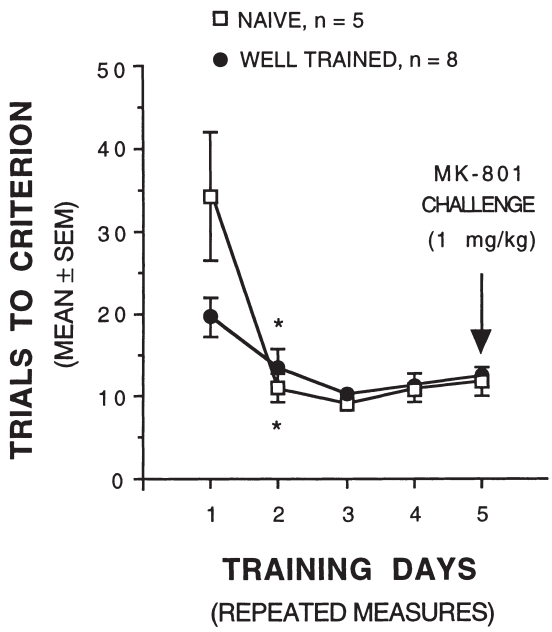

Fig. 3. Effects of MK-801 (1 mg/kg) on performance of a well-learned task (Experiment 4). Two groups of mice were trained on 4 successive days and then were challenged with MK-801 on day 5. One group was composed of naive mice ($n = 5$) and the other contained mice that had served as vehicle controls for the 12-h posttreatment experiment and, thus, were well trained ($n = 8$). The two groups performed similarly except on day 1 when the naive mice required more trials to reach criterion, $F(1, 11) = 4.83$, $p = 0.05$. Within-subjects planned comparisons concerning performances on day 4 vs. those on day 5 for each group were nonsignificant, indicating that MK-801 did not impair performance. Similar planned comparisons between performances on day 1 vs. day 2 indicated a significant reduction on day 2 concerning the number of trials needed to reach criterion for both the naive, $F(1, 4) = 9.52$, $p = 0.04$, and well-trained mice, $F(1, 7) = 10.89$, $p = 0.01$. These findings are similar to the acquisition vs. retention findings in Experiments 1 and 2. See text for greater details on the statistical analyses.

1 mg/kg MK-801 (SC) and were perfused at 12 h posttreatment ($n = 6$). At the appropriate times posttreatment, mice were deeply anesthetized with 14% chloral hydrate before undergoing intracardiac perfusion with a solution of 2.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M sodium phosphate buffer. Brains were cut in 1.0-mm transverse slabs and further fixed in osmium tetroxide, dehydrated in alcohol, cleared in toluene, and embedded in araldite. They were sectioned at approximately 1 μm on an ultramicrotome and stained with methylene blue/azure II, and were evaluated using light microscopy.

To quantify the extent of neuronal injury at 5 vs. 12 h posttreatment with MK-801 (1 mg/kg), numbers of vacuolated PC/RS cortical neurons were counted in coronal sections from three different rostrocaudal levels of the PC/RS cortex according to the atlas of Slotnick and Leonard (28): 2.1; 2.5; and 2.9 mm posterior (−) to bregma. All other brain regions on these sections were also inspected for the presence of vacuolated neurons. In addition, during the process of cutting through the transverse slabs to arrive at the desired rostrocaudal levels, sections were regularly saved (at approximately every 20 μm) and examined for the presence of vacuolated neurons in all brain regions. In this manner, much of the brain was examined, between 0.5 mm anterior to bregma to 3.4 mm posterior to bregma, for the presence of vacuolated neurons. The slides were evaluated by an experimenter who was "blind" to the treatment status of individual animals from which the brain sections were sampled. Vacuolated neurons were identified visually using a light microscope, and cell plots were constructed using software (mdplot, Minnesota Datametrics), which integrated information from a digitizer attached to a microscope stage and recorded the location of the vacuolated cells. Cell plots were constructed from individual mice that were representative of the two drug-treatment groups.

Statistical Analyses

ANOVAs containing one between-subjects variable (treatment groups) and one within-subjects variable (rostrocaudal level) were used to analyze the vacuolated neuron data. Additional between-groups comparisons were conducted at each rostrocaudal level.

RESULTS

Injured pyramidal neurons containing cytoplasmic vacuoles were observed in layers III and IV of the PC/RS cortex (Fig. 4A), but not in any other examined brain region. Vacuolated PC/RS cortical neurons were observed in six out of six mice treated with MK-801 and perfused 5 h posttreatment, and in three out of six drug-treated mice that were perfused at 12 h posttreatment. No vacuolated neurons were observed in the saline controls (Fig. 4B). Cell plots from representative mice in the two drug-treated groups show the distribution of vacuolated neurons as a function of rostrocaudal level within the PC/RS cortical area (Fig. 5). The cell plots and graph of the mean (+SEM) vacuolated neuron counts for the drug-treated mice (Fig. 6) indicate that much larger numbers of vacuolated PC/RS cortical neurons were present at each rostrocaudal level in mice killed at 5 h posttreatment compared to that observed in mice killed at 12 h posttreatment. Actually, there were very few vacuolated neurons present in the brains of mice sacrificed at 12 h posttreatment. In fact, no vacuolated neurons were observed in two mice from the 12-h posttreatment group, while only one vacuolated neuron was observed in another mouse from this group. This is in contrast to the vacuolated neuron counts observed in the mice killed at 5 h posttreatment which typically ranged from 25 to 60 per section.

The ANOVA on the vacuolated neuron data confirmed that a significantly larger number of vacuolated PC/RS cortical neurons were observed in mice that were treated with 1 mg/kg MK-801 and sacrificed 5 h posttreatment compared to that observed in mice treated with the same dose of MK-801 and sacrificed 12 h posttreatment, $F(1, 10) = 14.20$, $p = 0.004$. The ANOVA also indicated a nonsignificant effect of rostrocaudal level and a nonsignificant group by rostrocaudal level interaction. Additional between-group comparisons confirmed that the differences between the two groups were significant at all three rostrocaudal levels; for −2.1 mm from bregma, $F(1, 10) = 10.66$, $p = 0.008$; for −2.5 mm from bregma, $F(1, 10) = 17.77$, $p = 0.002$; and for −2.9 mm from bregma, $F(1, 10) = 10.93$, $p = 0.008$. In summary, many more PC/RS cortical neurons were observed to be injured at each of the three rostrocaudal levels sampled in mice killed at 5 h posttreatment compared to the numbers observed in mice killed at 12 h posttreatment.

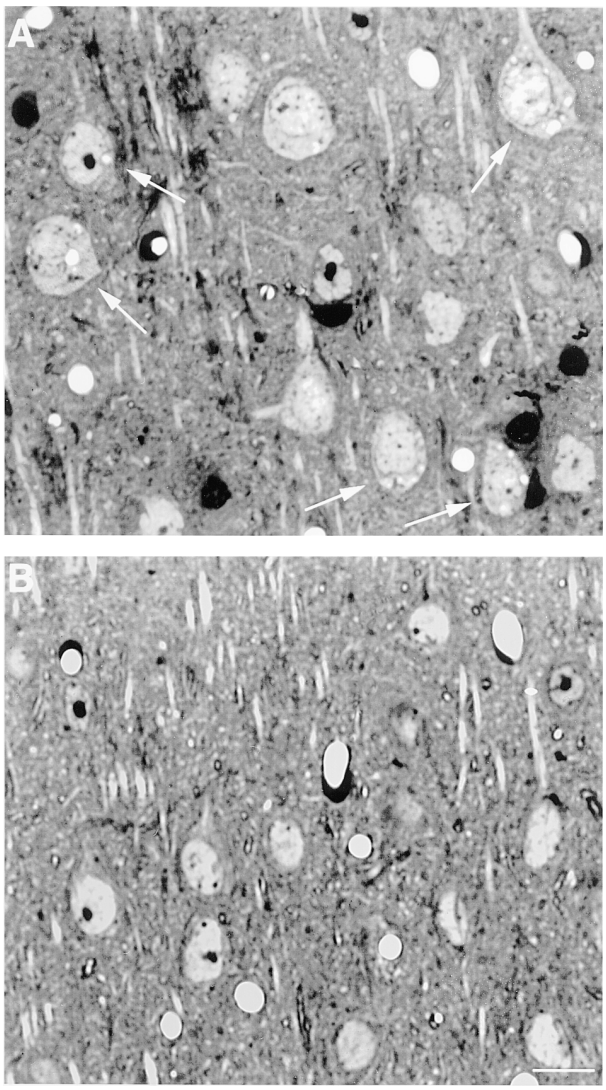

Fig. 4. The acute vacuole reaction induced by MK-801 (1 mg/kg) in the mouse PC/RS cortex. (A) This is a light photomicrograph showing pyramidal neurons in layer III of the PC/RS cortex of a representative mouse injected SC with 1 mg/kg MK-801 and then killed 5 h posttreatment. Brains were embedded in plastic and sections were cut at 1 μm and stained with methylene blue/azure II. Note the conspicuous vacuoles in the cytoplasm of many of these pyramidal neurons (arrows). (B) This shows the PC/RS cortex from a saline control animal. Note that the PC/RS cortical neurons in the saline control mouse do not contain any of the cytoplasmic vacuoles found in the MK-801–treated animal shown in A. Scale bar equals 20 μm.

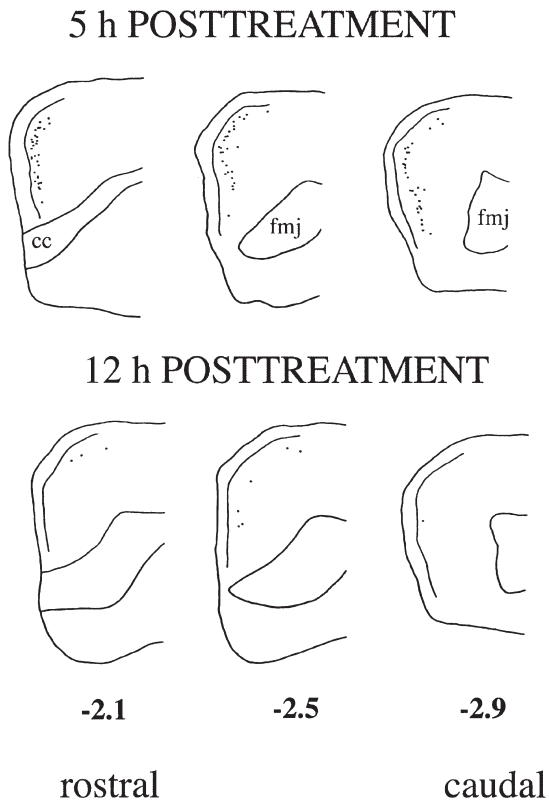

Fig. 5. Schematic representation of the distribution of PC/RS cortical neurons containing cytoplasmic vacuoles at three different rostrocaudal levels following administration of 1 mg/kg MK-801 and after being killed at either 5 h posttreatment (top row) or 12 h posttreatment (bottom row). Each dot represents one vacuolated neuron. Coronal sections were taken from three different rostrocaudal levels of the PC/RS cortical area [2.1, 2.5, and 2.9 mm posterior (−) to bregma] according to the atlas of Slotnick and Leonard (28). The curved line just deep to the medial cortical surface represents layer 2 granule cells. Note that many more PC/RS cortical neurons were observed to be vacuolated at each of the three rostrocaudal levels in the mouse killed at 5 h posttreatment compared to the one killed at 12 h posttreatment. Abbreviations: cc—corpus callosum; fmj—forceps major of the corpus callosum.

GENERAL DISCUSSION

In the present study, we show, in male mice, that a dose of MK-801 that induces transient neuronal injury in the PC/RS cortical area that is histologically demonstrable at 5 h, but is virtually gone by 12 h posttreatment, transiently impairs a type of spatial learning acquisition on the same time schedule. Our finding that substantial numbers of vacuolated neurons were present at three different rostrocaudal levels of the PC/RS cortical area at 5 h posttreatment while very few were present at 12 h posttreatment suggests that MK-801-induced neuronal vacuolization is a reversible phenomenon in mice as it is in rats. Our behavioral findings following MK-801 treatment were parallel to our histologic results in that acquiring a new baited hole location was clearly impaired at 5 h posttreatment but not at 12 h posttreatment. The significant acquisition impairment shown by the MK-801–treated mice at 5 h posttreatment was evident for trials to criterion as well as for errors and wrong trials to criterion. In addition, the vehicle controls showed significant improvement in their acquisition performance relative to their pretreatment scores, while the MK-801–treated mice did not improve. In contrast, at 12 h posttreatment, there was no evidence of any impairment in holeboard acquisition in the MK-801–treated mice.

It should be emphasized that the acquisition impairment induced by MK-801 at 5 h posttreatment refers to the acquisition of a "new" baited hole, which was tested approximately 2 weeks after the original baited hole location was acquired during pretreatment training when no drugs were adminis-

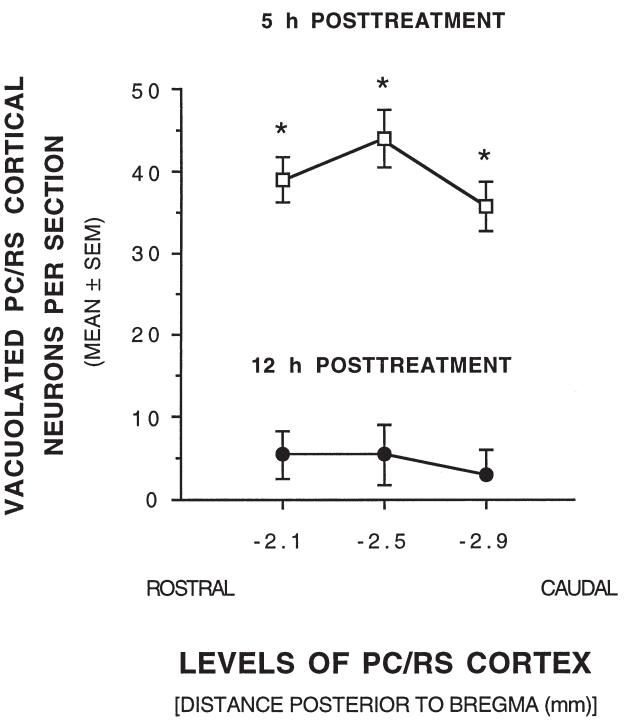

Fig. 6. Numbers of vacuolated PC/RS cortical neurons as a function of the posttreatment interval and rostrocaudal level of brain section. Mice were dosed with 1 mg/kg MK-801 and were killed 5 or 12 h later and their brains were processed as described in Fig. 4. Note that there are significantly (*) greater numbers of vacuolated neurons at each rostrocaudal level in the mice from the 5-h posttreatment group compared to that observed in the 12-h posttreatment group. See text for details of the statistical analyses and *p*-values.

tered. In this regard, acquisition of a new baited hole location is similar to reversal learning where the mice must disregard certain cues pertinent to the original baited hole location and learn other information relevant to the new baited hole location. The lack of group differences in perseveration scores in the 5 h posttreatment experiment indicates that MK-801 did not impair the new baited hole acquisition by increasing perseveration to the initial baited hole. It is also important to note that although acquisition of a new baited hole location was not impaired at 12 h after MK-801 (1 mg/kg) administration, it is not clear whether the drug would affect acquisition of the original baited hole location at 12 h posttreatment if no pretreatment training session were given. In the present study, we focused on evaluating the effect of MK-801 on acquiring a new baited hole location because we have found in previous work that matching groups according to pretreatment acquisition scores and using a pre- vs. posttreatment design provides the sensitivity necessary for evaluating subtle drug effects.

In the present study and in preliminary histological work, we have surveyed a large portion of the mouse brain and have not observed vacuolated neurons in any area besides the PC/RS cortical area following a 1 mg/kg dose of MK-801. The present results, coupled with those in a previous work (34) in which we reported that a dose of MK-801 that kills PC/RS cortical neurons in male mice, also induces a permanent impairment in spatial learning acquisition, suggest a strong association between the acquisition-impairing action of MK-801 and the injurious action of MK-801 on PC/RS cortical neurons. This association and evidence from ablation studies in the rat (29,30), suggesting that PC/RS cortical neurons may mediate spatial learning functions, implicate the toxic effects of MK-801 on these neurons as a candidate mechanism to explain the acquisition-impairing effects of MK-801. Recent studies in the rat have suggested that excitotoxic PC/RS cortical lesions that spare the fibers in the cingulum bundle may not have a significant effect on spatial learning/memory (1,19,20). However, the generality of these findings in the rat may be questioned because certain standard spatial learning tests like the Morris water navigation task or the radial arm maze have not yet been used to evaluate the effects of excitotoxic PC/RS cortical lesions. More important for the present study is work conducted in the mouse that suggests that excitotoxic PC/RS cortical lesions do impair spatial learning, but they do so in a way that is qualitatively different from that which occurs following radiofrequency lesions that do not spare the cingulum bundle (15,16). The results of the present study are consistent with those from the study involving excitotoxic PC/RS cortical lesions in mice suggesting a role for PC/RS cortical neurons in spatial learning/memory in this species.

Although there is an association between the acquisition-impairing effects of MK-801 and the toxic action of MK-801 on PC/RS cortical neurons, it is not clear whether the drug affects learning/memory processes directly or influences performance through other nonassociative factors. The results from Experiment 3 suggest that MK-801 does induce certain other behavioral changes that may not be directly related to learning/memory processes. Specifically, heightened locomotor activity was found following MK-801 (1 mg/kg) administration at 5 h posttreatment and performance on two out of the four sensorimotor tests (ledge and platform tests) were found to be impaired following this dose at this posttreatment time. By themselves, these results suggest that holeboard performance could have been affected by these nonassociative factors. However, our finding in Experiment 4 that the same dose of MK-801 (1 mg/kg) did not affect performance after the holeboard task was well learned, suggests that MK-801 may induce certain behavioral changes not related to learning/memory, but that these changes may not be disruptive enough to alter holeboard performance. However, because there are design differences between Experiments 1 and 4, particularly with regard to recency of training in the holeboard, it is possible that subtle nonassociative factors could have affected acquisition in Experiment 1 but not affected performance in Experiment 4.

There are several other issues, however, that remain unresolved with regard to the role of the PC/RS cortical area in spatial learning and memory. For example, in our study involving chronic acquisition deficits following high dose MK-801 (10 mg/kg) treatment (34), we pointed out that because the damage was relatively small and apparently focally restricted to a single forebrain region it remained to be demonstrated whether the long-term acquisition impairment could be attributed to such brain damage. Also, in experiments involving a very low dose of an NMDA antagonist (e.g., 0.05 mg/kg MK-801), there are not observable pathomorphological changes in PC/RS cortical neurons at the light microscopic level. It is possible, however, that a low dose of MK-801 would disrupt the activities of PC/RS cortical neurons for a very brief period of time, during which spatial learning acquisition would be disrupted. Furthermore, it might be argued

that the transient acquisition-impairing action of MK-801 at low doses that are not toxic to PC/RS cortical neurons could be explained as well or better by blockade of LTP in the hippocampus. However, it is important to note that the learning/memory impairment induced by nontoxic doses of MK-801 is the same type of acquisition deficit that is altered transiently by a reversible PC/RS toxic dose or is altered permanently by an irreversible PC/RS toxic dose. It would be difficult to explain this feature of the observed phenomenon on the basis of transient interference in synaptic communications between neurons in the hippocampus that do not show signs of either reversible or irreversible injury from the MK-801 treatment. Another problematic issue pertains to the fact that the generality of results across species concerning the neurotoxic effects of NMDA antagonists on spatial learning is not presently known. Other than our own work in mice, we know of no other published studies in which other animal species have been used to investigate the effects of histologically verified neurotoxic doses of NMDA antagonists on spatial learning. Finally, it should be acknowledged that additional work is required to determine whether the acquisition-impairing effect of MK-801 found in the present study reflects a specific deficit in spatial learning or whether it is part of a more general cognitive disturbance.

The memory-impairing effects of systemically administered NMDA antagonists are usually attributed to a disruption of neurotransmission at NMDA receptors in hippocampal circuits (17,25,26), although there is no definitive evidence to substantiate this interpretation. Data from our studies involving the neurotoxicity of NMDA antagonists in rats suggest that a circuit mediates the neurotoxic reaction in the PC/RS cortex, which involves not only PC/RS cortical neurons but neurons also in the thalamus, basal forebrain, and brain stem (21,23). In addition, data from studies involving intracranial injections of NMDA antagonists and other agents in rats suggest that several transmitter pathways may need to be activated simultaneously for the neurotoxic reaction to occur (21). Specifically, our findings suggest that blockade of NMDA receptors in the mammalian brain induces a network disturbance in which Glu ceases driving certain gamma-aminobutyric acid (GABA) neurons, and these GABA neurons cease inhibiting certain cholinergic and Glu excitatory neurons that convergently innervate specific pyramidal neurons in the PC/RS cortices. These excitatory neurons, when released from inhibition, hyperactivate the PC/RS cortical neurons, resulting in either reversible injury or death of the PC/RS cortical neurons. Thus, it may be an oversimplification to conceive of PC/RS cortical neurons as the sole source of the association between the neurotoxic reaction and the spatial learning acquisition impairment induced by NMDA antagonists because several brain regions and transmitter systems may be involved in the neurotoxic reaction. Thus, for the moment, it seems reasonable to suggest that drug effects on PC/RS cortical neurons may play a role in NMDA antagonist-induced spatial learning impairments. This tenet does not assume that the PC/RS cortical area plays a primary role in these deficits, nor does it rule out the possibility that drug effects in other areas (e.g., CA1 hippocampal neurons) may play a role in mediating these effects. Nevertheless, it is important to recognize that injury of PC/RS cortical neurons may play a role in the spatial learning impairments induced by NMDA receptor antagonists in mice.

In summary, the present study is the first, to our knowledge, to investigate the association between the degree of injury induced in PC/RS cortical neurons by MK-801 and the spatial learning impairments induced by the drug while providing some control for evaluating the role of nonassociative factors that may confound interpretation of learning/memory effects. Our data suggest that a dose of MK-801 that induces neuronal injury in PC/RS cortical neurons and impairs spatial learning acquisition does so on an acute basis in that the neuronal injury and spatial learning impairment both recover quickly in a parallel fashion.

ACKNOWLEDGEMENTS

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