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Serial Injections of MK 801 (Dizocilpine) in Neonatal Rats Reduce Behavioral Deficits Associated with X-Ray-Induced Hippocampal Granule Cell Hypoplasia

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MICKLEY, G. A., J. L. FERGUSON AND T. J. NEMETH. *Serial injections of MK 801 (Dizocilpine) in neonatal rats reduce behavioral deficits associated with X-ray-induced hippocampal granule cell hypoplasia.* PHARMACOL BIOCHEM BEHAV **43**(3) 785–793, 1992.—MK 801 (NMDA antagonist) has been shown to protect newborns from hypoxia-induced brain damage. Here, we determined if (+)-5-methyl-10,11-dihydroxy-5*H*-dibenzo(*a*,*d*)cyclohepten-5,10-imine (MK 801) could attenuate behavioral deficits associated with early radiation-induced hypoplasia of fascia dentata granule cells. We pretreated neonatal rats (n = 20) with MK 801 (0, 0.1, or 0.2 mg/kg, IP) before each of eight fractionated, head-only doses of X-rays (13 Gy total) administered during the first 16 days postpartum. Other rats (n = 18) received the same drug treatments but were sham irradiated. At age 16 months, water-escape latencies to a submerged platform were measured in a water maze.

Irradiated rats with hippocampal damage exhibited impaired learning (longer latencies to find the platform) than did sham-irradiated subjects. Moderate doses of MK 801 (0.1 mg/kg) facilitated the learning of the water maze by irradiated subjects but did not enhance the number of their fascia dentata granule cells. Higher doses (0.2 mg/kg) of MK 801 provided no behavioral benefits. In fact, this dose significantly impaired the learning of the water maze by sham-irradiated rats and potentiated the granule cell hypoplasia observed in irradiated subjects. Thus, early MK 801 treatment produces dose-dependent behavioral protection for rats with radiation-induced hippocampal damage. Future studies may reveal the neurophysiological and neuroanatomic substrates of this behavioral recovery.

Water maze Hippocampus Fascia dentata MK 801 Dizocilpine Behavior Brain damage Rats X-rays Ionizing radiation

X-IRRADIATION of partially shielded neonatal rat brain can cause selective hypoplasia of fascia dentata granule cells. Bayer and Peters (8) and others (43–45) utilized this technique to study the behavioral consequences of hippocampal damage. Lesions of hippocampal granule cells, whether through neonatal irradiation (68) or by colchicine treatment (66), effectively remove a major input to the hippocampus (through the perforant path from entorhinal cortex) and produce behavioral consequences similar to total hippocampalectomy (68). For example, Bayer et al. (7) described locomotor hyperactivity, reduced spontaneous alternation in a T maze, and retarded acquisition of a passive avoidance task in rats with early radiation-induced hippocampal damage. More recently, we (45) replicated and extended this work by revealing that rats with hypoplasia of the fascia dentata granule cells exhibit perseverative spontaneous turning, with few reversals, in a bowl-shaped apparatus. These data illustrate behavioral effects of early radiation exposure and suggest a role for hippocampal granule cells in working memory (54), response inhibition (2,17,33), and spatial mapping (51).

The primary mechanism producing radiation-induced hypoplasia of fascia dentata granule cells is presumed to be alteration of genetic material (27). However, recent data are consistent with the hypothesis that excitatory amino acid (EAA) neurotransmitters (e.g., glutamate) acting through NMDA receptors may also play a role in the production of hippocampal damage and/or behavioral alterations following X-irradiation. First, glutamate acts as a neurotransmitter at perforant path/granule cell synapses located at the site of radiation-induced hypoplasia (9). Second, behavioral effects of radiation-induced hippocampal damage are similar to those observed following NMDA-induced excitotoxic damage to

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hippocampal neurons (60,61) or acute antagonism of brain NMDA receptors (42,59,69,74). Third, because glia play an important role in glutamate metabolism (64) radiation-induced destruction of glia (65,67) may have the effect of potentiating EAA neurotoxicity. Fourth, ionizing radiation exposure alters glutamate dehydrogenase and produces time-dependent changes in neuronal glutamate levels (1,32,62). These alterations in EAA may be biologically important in neonates because glutaminergic systems are in flux, and therefore quite plastic, during the early postnatal period (73). For example, Erdo and Wolff (19) showed a dramatic rise in the levels of glutamate during postnatal weeks 2 and 3. Likewise, in the human hippocampus there is also a transient increase of NMDA binding sites during development (58). In fact, neonatal rat brain is significantly more sensitive to glutamate and NMDA excitotoxicity than is the adult brain (28,41). Thus, although the evidence is circumstantial, early X-ray-induced hippocampal granule cell hypoplasia, and accompanying behavioral deficits, may be mediated, in part, by alterations in EAAs acting through NMDA receptors. We tested this hypothesis further by administering an NMDA antagonist [(+)-5-methyl-10,11-dihydroxy-5*h*-dibenzo(*a*,*d*)cyclohepten-5, 10-imine (MK 801)] before each X-ray exposure and then recording behavioral and neuroanatomic changes in irradiated and sham-irradiated rats.

MK 801 is a phencyclidine (PCP)-like compound that displays even greater potency than PCP in binding to the PCP receptor and antagonizing the excitatory and toxic actions of NMDA (29). MK 801 can protect neurons against traumatic injury, ischemic, anoxic, or epilepsy-related brain damage (all of which are postulated to be NMDA receptor-mediated processes) (14,22,31,40,49,52,56). Here we administer MK 801 concurrently with partial-head exposures to X-rays to determine if this procedure might alter a) the radiation-induced hypoplasia of fascia dentata granule cells and/or b) the behavioral deficits associated with this neuropathology.

METHOD

Subjects

Pregnant rats [Crl: CD(SD)BR] obtained from Charles River Laboratories (Kingston, NY) and screened for evidence of disease were housed in a facility accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Temperature and relative humidity in the animal rooms were held at 19–21°C and 50 ± 10%, respectively, with ≥10 air changes/h. Full-spectrum lighting was cycled at 12-h intervals (lights on at 0600 h) with no twilight. Rats used in these experiments came from a total of 13 different litters. On the day of birth (day 1), litters were culled and up to eight males/litter were reared together. Based upon a random selection process, from 1 to 6 of these rats in each litter were actually used in the experiments reported here. All rats were weaned at the same time (on day 26 postpartum) and then individually housed in microisolator, polycarbonate cages on hardwood chip contact bedding. Rats were given ad lib Wayne Rodent Blox and acidified water (HCl, pH 2.5 to prevent the spread of *Pseudomonas*). Palatability studies indicate that animals do not prefer tapwater to acidified water and that there are no deleterious effects of this water treatment over the lifetime of the subject [for review, see (70)].

Irradiation

Subjects from each litter were randomly assigned to either the X-irradiated or the sham-irradiated (control) group. Irradiated rats received collimated X-rays (Phillips Industrial 300 kvp X-ray machine, Phillips, Inc., Mahwah, NJ; configured with 1.5 mm of copper filtration, with a half-value layer of 2.5 mm copper) delivered dorsally, in the coronal plane, through a narrow slot in a loose-fitting whole-body lead shield. X-rays were confined to that area of the head previously determined to contain the hippocampus. Determinations of the location of the hippocampal formation relative to external landmarks (e.g., snout, eyes, ears) were made during preliminary dissections of other neonatal rats. These external landmarks were subsequently used to set the position of the slot in the lead shield during our irradiation procedure. The measurements and anatomic landmarks we used for shield placement corresponded closely to those previously reported by Bayer and Peters (8). The slot in the shield was the opening between two movable lead strips (22.8 × 6.8 × 2 cm) suspended just above the heads of rats in the radiation exposure array. The opening extended laterally beyond the full width of each rat head and varied between 5–10 mm in the anterior/posterior plane to accommodate the growth of the head/brain over the course of the radiation treatment [see (8) for a complete explanation of this procedure]. Irradiated rats were exposed to 2.0 gray (Gy; 1 Gy = 100 rad) on postnatal days 1 and 2 (day of birth = postnatal day 1) and to 1.5 Gy on postnatal days 5, 7, 9, 12, 14, and 16 for a total partial-head-only dose of 13 Gy. Doses were determined by using Exradin 0.05 cc tissue-equivalent ion chambers with calibration traceable to the National Institute of Standards and Technology. X-rays were delivered at a rate of 0.49 Gy/min (total irradiation time = 3.0–4.0 min) at a depth of 2 mm in tissue. Sham-irradiated control rats were restrained for the same time period as irradiated rats but were not exposed to X-rays.

The entire anterior/posterior extent of the hippocampal formation was irradiated, as were brain areas dorsal and ventral to this structure [see (55) for a listing of these other brain areas]. Brain structures anterior and posterior to the slot in the lead were shielded. At the time of our postnatal radiation exposures, the rat brain contained three remaining populations of dividing (and therefore radiosensitive) cells: neuronal precursors of granule cells in the hippocampus, cerebellum, and olfactory bulbs (6,7). Two of these major neuronal precursor populations (in the cerebellum and olfactory bulbs) were covered by the radioopaque shielding. Unshielded were the mitotic (radiosensitive) granule cells of the dentate gyrus and the mature neurons in other brain structures residing in the same coronal plane as the hippocampus. This procedure produces selective hypoplasia of granule cells in the dentate gyrus (8,25) while sparing the radioresistant (13,26) mature neurons of other brain structures. The technique has been validated through a variety of neuroanatomic methods (6,25,75).

Drug Injections

Rats were randomly assigned to a drug treatment group. Within 5 min of the start of each irradiation or sham-irradiation procedure, rats were injected (IP) with either 0 (saline control), 0.1, or 0.2 mg/kg MK 801 in a volume of 0.03 ml. Therefore, each rat received eight injections during the first 16 days postpartum. Mortality resulting from the MK 801 treatments was not significantly different from that observed following saline control injections. See Table 1 for information about experimental group size.

Apparatus and Procedures

Following irradiation, rats were allowed to mature for approximately 17 months (see Table 1) before behavioral testing

TABLE 1

NUMBER, AGE, AND WEIGHT OF RATS AT THE TIME OF WATER MAZE TESTING

Radiation Treatment and Drug Administration	*n*	Age*	Weight†
Irradiated (saline)	8	507 ± 8	865 ± 51
Irradiated (0.1 mg/kg MK 801)	7	508 ± 7	877 ± 52
Irradiated (0.2 mg/kg MK 801)	5	512 ± 8	772 ± 38
Sham irradiated (saline)	7	502 ± 7	992 ± 70
Sham irradiated (0.1 mg/kg MK 801)	5	501 ± 5	959 ± 36
Sham irradiated (0.2 mg/kg MK 801)	6	508 ± 8	915 ± 74

*Ages are the mean days ± SEM for each group.
†Weights are mean g ± SEM for each group.

began. At this time, we recorded performance on a water maze (see below). We tested 17-month-old rats in the hopes of revealing hippocampally mediated deficits in spatial memory (20,57). Aged rats are more susceptible than young rats to drug-induced deficits in water maze performance (38). Thus, we expected radiation-induced deficits in performance of this spatial water maze task to be more prominent in old subjects. In a different time frame, other spontaneous behavior [i.e., spontaneous locomotion and rotation; see (45) for a description of these procedures] were also recorded. However, data from these other tests are not presented in this article.

Water maze. Water mazes have been used extensively to measure behavioral dysfunctions associated with hippocampal damage and NMDA receptor manipulation (42,47,59,61). In the current study, we measured water-escape latencies to a submerged platform located in a Morris-type water maze (47). The water maze was an oval stock-watering tank (manufactured by Rubbermaid, Inc., Frederick, MD) 140 × 90 × 60 cm deep. The tank was filled to 50 cm with clear water (27°C). The escape platform was a clear plastic disk (12.5 cm diameter × 1.2 cm) mounted on a clear plastic stand and submerged 2 cm below the surface of the water in the center of one of the tank's quadrants. Rats never showed evidence of detecting the platform unless they touched it. The tank rested on the floor of a 4.6 × 6.1-m room with overhead fluorescent lights and surrounded by a rich array of laboratory furnishings.

All trials lasted 30 s with 30 s between trials. Initial water acclimation consisted of two trials, the first with no platform. On the second trial, the rat was held facing the submerged platform (5 cm away) and released to climb up on it three times. Three to 4 weeks after this initial acclimation, rats were tested in the water maze. The test session consisted of eight trials. For each trial, the rat was placed in the water immediately facing the tank's wall at the middle of quadrant one or, on alternate trials, of quadrant 3. For the first and eighth trials, no platform was in the tank. For the second to the seventh trials, the platform was submerged in quadrant 4. The rat was given 30 s to locate and climb up on the platform. The rat remained on the platform for 2 s. If the rat did not find the platform in 30 s, it was guided to the goal. After the rat resided for 20 s on the platform, the subject was placed in a dry plastic holding box and given the next trial in 10 s (30 s total between trials). We recorded time spent in each quadrant, order of quadrants crossed, and latency to the platform.

Rats are natural swimmers and swim effectively when placed into the water for the first time. In these tests, no rat was in the water for more than 4 min and none shivered when removed. At the end of the session, subjects were thoroughly dried with towels and a hair dryer.

Body weights. Rats were weighed on 14 days during the irradiation period (postnatal days 1, 2, and 5–16) and on 9 days after irradiation—but before weaning (days 17–24 and 26), as well as 2 days after weaning (days 27 and 28). Weights were also recorded at times associated with behavioral testing.

Histology

After behavioral testing, rats were anesthetized and perfused with heparinized saline followed by 10% buffered formalin. Brains were embedded in paraffin, serially sectioned (6 μm) (in the sagittal plane), and then stained with cresyl violet and luxol fast blue (37). All brains received a preliminary review to confirm radiation-induced damage to the dentate gyrus [for full documentation of this effect, see (44)]. In addition, some of the brains were analyzed in more detail (see Table 2). A single section (approximately 1.9 mm lateral to the midline) (55) was used for this analysis to (a) estimate the degree of fascia dentata injury and (b) survey the other brain areas (olfactory bulb and cerebellum) that, although shielded from irradiation, are known to contain granule cells mitotic at the time of radiation treatment. We counted the total number of granule cells that could be visualized in the single section of the dentate gyrus used in this analysis. Cell counts were accomplished under 250× total magnification by an observer blind to the experimental results. Nuclear cell counts were used to avoid the error caused by double or triple nucleoli. The size, cytoplasmic staining, and nuclear structure of granule cells usually makes them distinguishable from glial cells (4,63). However, the possibility cannot be ruled out that some of the astroglial cells may have also been counted. The impact of this possible error is reduced by the fact that the number of glial cells in the fascia dentata is extremely low (36). In addition, after neonatal irradiations similar to those described here Bayer and Altman (5) reported that the granule cell population remains significantly reduced into adulthood while the glia show an initial reduction in number followed by a complete regeneration to normal levels within 60–90 days (6). Thus, our cell counts in the fascia dentata of the 1-year-old adult rat would presumably not reflect a radiation-induced alteration in glial population. To confirm that the shielding of other brain areas was sufficient, we also counted granule cells in a 0.04 mm^2 area in the cerebellum and olfactory bulb. Further, we evaluated the sparing of other more mature, and therefore less radiosensitive, hippocampal structures by counting the thickness of the CA1 pyramidal cell layer that was dorsal to the dentate and directly in the path of the X radiation.

Statistical Analyses

Unless otherwise stated, data were analyzed within the framework of a two-way analysis of variance [ANOVA: radia-

TABLE 2

HISTOLOGICAL DATA DERIVED FROM ANALYSIS OF SAGITTAL SECTIONS OF RAT BRAIN

Radiation Treatment and Drug Administration	*n*	Anatomic Areas*			
		OB†	CB‡	DG§	CA1§
Irradiated (saline)	5	460.5	660.4	225.6	2.5
		(4.6)	(78.7)	(32.2)	(0.5)
Irradiated (0.1 mg/kg MK 801)	5	499.2	755.0	227.0	2.7
		(32.4)	(70.0)	(22.9)	(0.4)
Irradiated (0.2 mg/kg MK 801)	4	353.5	659.8	165.3#	2.7
		(96.7)	(105.0)	(25.7)	(0.5)
MEAN irradiated		442.5	694.0	219.6**	2.66
SEM irradiated		(34.2)	(45.9)	(17.8)	(0.23)
Sham irradiated (saline)	3	475.3	457.5	2153.0	3.0
		(29.2)	(159.5)	(143.0)	(0.3)
Sham irradiated (0.1 mg/kg MK 801)	4	474.0	725.0	2248.5	3.2
		(12.7)	(53.9)	(106.3)	(0.1)
Sham irradiated (0.2 mg/kg MK 801)	4	425.3	620.3	1873.8	3.7
		(48.4)	(54.2)	(291.4)	(0.8)
MEAN sham irradiated		459.8	618.3	2086.2	3.33
SEM sham irradiated		(17.05)	(38.8)	(119.7)	(0.27)

*OB, olfactory bulb; CB, cerebellum; DG, dentate gyrus of hippocampus; CA1, CA1 of hippocampus.

†Mean cell counts in 0.04 mm^2 area. Number in parentheses is the SEM.

‡Mean cell counts in total fascia dentata. Number in parentheses is the SEM.

§Mean pyramidal cell layer thickness (numbers of cells). Number in parentheses is the SEM.

#Significantly different from irradiated, saline-treated rats ($p < 0.05$, see text).

**DG cell counts in irradiated rats are significantly lower than those in sham-irradiated subjects ($p < 0.001$).

tion treatment (radiation/sham irradiation) × drug treatment (saline/0.1 mg/kg/0.2 mg/kg); $\alpha = 0.05$] (72). Either Newman-Keuls posthoc tests or *t*-tests [with the Bonferroni correction so as to reduce the probability of Type I errors, (46)] were used to specify the individual group differences. We also used *t*-tests to evaluate single a priori hypotheses (34).

RESULTS

MK 801, at a repeated dose of 0.1 mg/kg, produced an improvement in water maze acquisition. It did not, however, reverse the radiation-induced hypoplasia of fascia dentata granule cells.

Water Maze

During Trials 2–4 of the water maze task (early learning), there was not a significant difference between the treatment groups in their latency to arrive at the hidden platform (see Fig. 1). Thus, neither radiation-induced hippocampal damage nor early treatments with MK 801 altered the initial latencies to find the platform. However, during Trials 5–7 (late learning) several group differences appeared. During these trials, rats that had received X-ray treatments and saline control injections exhibited significantly longer latencies to arrive at the safe platform than did sham-irradiated rats with similar control injections, $t(13) = -3.67$, $p < 0.5$. Our low dose of MK 801 (0.1 mg/kg) protected irradiated rats from this behavioral deficit. Animals that received this drug treatment found the safe platform with latencies that were a) significantly less than those exhibited by saline-treated irradiated rats but b) not significantly different from the latencies of sham-irradiated animals that received either saline or 0.1 mg/kg MK 801 [drug effect, $F(2, 32) = 7.508$, $p < 0.5$; drug × radiation treatments interaction, $F(2, 32) = 3.635$, $p < 0.05$; and Newman-Keuls tests, $p < 0.05$]. Conversely, the higher dose of MK 801 (0.2 mg/kg) provided no such behavioral benefit. Both irradiated and sham-irradiated rats that received this higher dose of MK 801 exhibited latencies comparable to those recorded from irradiated rats that received saline control injections as neonates. In fact, during training Trials 5–7 sham-irradiated rats that received serial doses of 0.2 mg/kg MK 801 took significantly longer to find the platform than did sham-irradiated rats that received control saline injections (Newman-Keuls, $p < 0.05$).

During Trials 1 and 8 (when no platform was present in the water maze), animals in all treatment groups spent similar amounts of time swimming in the quadrant of the maze where the safe platform was placed during trials 2–7. The total number of maze quadrants crossed during Trials 1 and 8 was also similar for our treatment groups. These data suggest that, independent of radiation or drug treatment history, animals showed similar characteristics of spatial exploration and swimming activity in the absence of a goal. We also determined the spontaneous locomotor activity (in a 1-h session) for some rats in this study ($n = 34$) [see (44) for details on this procedure]. The results from this measure were then correlated with each subject's latency to reach the hidden platform on Trials 5–7 of maze acquisition. This analysis revealed

a direct correlation between latency to find the hidden platform and locomotor activity, $r(32) = 0.38$, $p < 0.05$. This correlation suggests that hypoactivity may not be an important factor in the delayed acquisition of our water maze task.

Weight Data

Body weight at the time of maze testing did not correlate significantly with water maze learning [latency to arrive at the hidden platform, Trials 5-7) $r(36) = -0.18$]. However, as expected rat weight increased over time and this change was eventually modulated by radiation treatment history. Two repeated-measures ANOVAs [radiation treatment (radiated/sham irradiated) × drug dose (saline/0.1/0.2 mg/kg MK 801) × day] were calculated to determine weight changes during the irradiation procedure (postnatal days 1, 2, and 5–16) as well as following radiation treatments (days 17–24, 27, and 28). During postnatal days 1–16, there was a significant time effect, $F(13, 416) = 594.5$, $p < 0.5$ (suggesting growth), but neither radiation exposure nor drug treatment effects were statistically significant. Following the end of radiation treatments, body weight continued to increase over time, $F(10, 320) = 2130.24$, $p < 0.05$, and irradiated rats could eventually be differentiated from sham-irradiated subjects. A significant interaction between radiation treatment and time, $F(10, 320) = 17.27$, $p < 0.05$, was further explored by conducting two-way ANOVAs between treatment groups on particular days. When significant differences were found, t-tests [employing the Bonferroni correction, see (46)] were used to make paired comparisons. Within our measurement schedule, the first statistically significant radiation effect was observed on postnatal day 26, $F(1, 32) = 4.4$, $p < 0.05$, and persisted through the remainder of these early body weight assessments [day 27, $F(1, 32) = 4.7$, $p < 0.05$; day 28, $F(1, 32) = 6.07$, $p < 0.05$]. Irradiated rats that received low (0.1 mg/kg) doses of MK 801 or control injections weighed significantly less than similarly dosed sham-irradiated subjects on each of these days, $t(9–13) = 1.94–2.24$, $p < 0.05$. However, a comparison between sham-irradiated rats that received 0.2 mg/kg MK 801 and the corresponding group of irradiated rats revealed no significant differences in body weight on postnatal days 24 and 26–28 ($p > 0.05$). This finding may be placed in perspective by the fact that both irradiated and sham-irradiated rats that received 0.2 mg/kg MK 801 had weights that approximated those of irradiated rats in other drug treatment conditions (i.e., received saline or 0.1 mg/kg MK 801) and were lighter than sham-irradiated rats in these groups. For example, on postnatal day 28 combined groups that received the high dose of MK 801 had significantly lower body weights than did rats that were either saline treated or 0.1 mg/kg treated and sham irradiated, $t(21) = -2.49$, $p < 0.05$.

Adult weight changes paralleled, in some ways, those observed in young rats. A comparison of rat body weights at the time of the maze test (see Table 1) revealed that radiation-induced weight reductions observed early in this experiment were also present 19 months later [two-way ANOVA, radiation effect, $F(1, 32) = 6.284$, $p < 0.05$. Further, early serial doses of MK 801 produced a consistent, but small (not statistically significant), reduction in body weight (compared to saline-injected controls) in our mature animals.

Histology

Exposure of a portion of the neonatal cerebral hemispheres to early, fractionated doses of ionizing radiation produced a selective reduction of granule cells of the hippocampal dentate

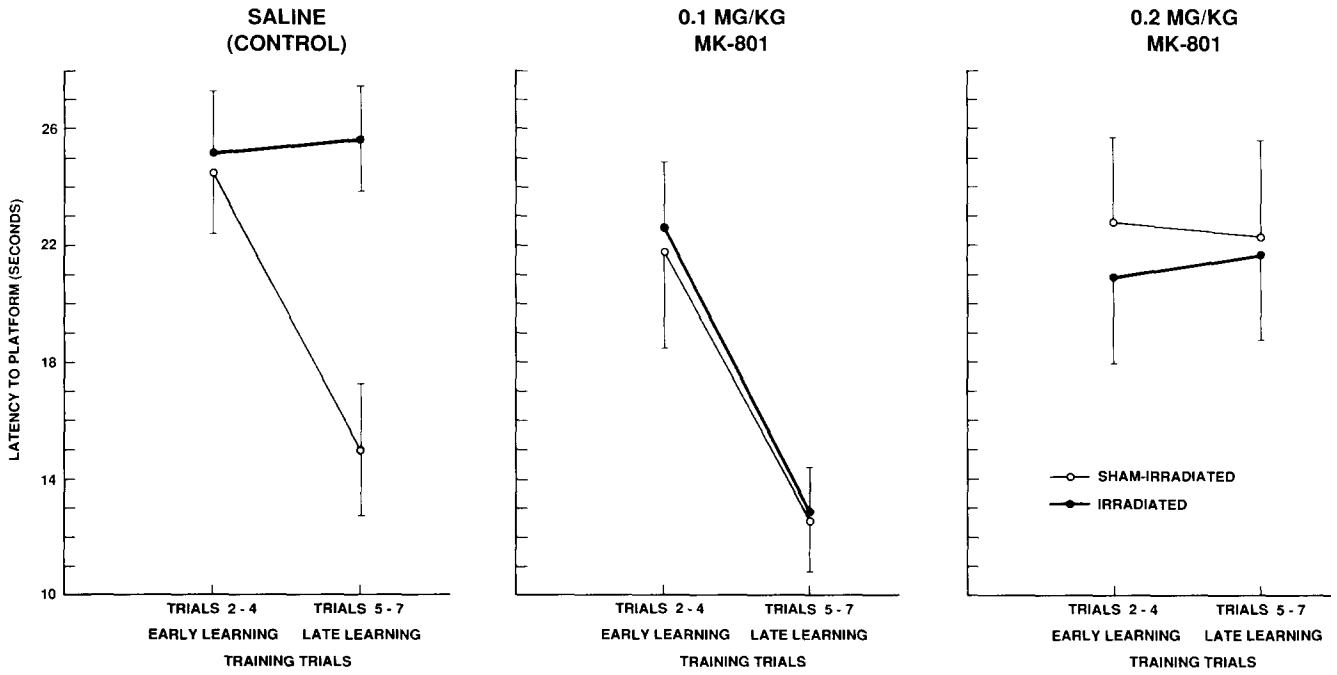

FIG. 1. Adult rat performance on a water maze task expressed as latencies (in seconds) to find a hidden platform. Data reflect mean latencies across three training trials (Trials 2–4 reflect early training; Trials 5–7 reflect late training). Radiation-induced hippocampal damage causes longer latencies to find a hidden platform late in maze learning. Repeated pretreatment of neonates with 0.1 mg/kg MK 801 (NMDA antagonist) eliminates this effect of radiation exposure. However, higher doses of MK 801 (0.2 mg/kg) retard maze learning in sham-irradiated controls. Variance indicators are SEM.

gyrus while sparing other brain areas (see Table 2). A two-way ANOVA (radiation treatment × drug treatment) revealed that exposure of the neonatal rat hippocampus to ionizing radiation produced a significant, $F(1, 24) = 308.17$, $p < 0.05$, depletion of dentate granule cells. Granule cell counts in the irradiated fascia dentata (mean = 219.6 ± 17.8) numbered approximately 11% of those observed in the hippocampus of sham-irradiated subjects (mean = 2,086.2 ± 119.7). Granule cell densities in the olfactory bulb and cerebellum were similar for subjects in both the irradiated and sham-irradiated groups. Likewise, the thickness of the pyramidal cell layer of CA1 was not altered by the radiation treatments. Using irradiation procedures similar to ours, Bayer and Peters (8) previously determined that X-irradiation destroys approximately 85% of the granule cells in the dentate gyrus of the hippocampus. As in our procedure, this technique spares a) hippocampal pyramidal cells and adjacent brain nuclei (e.g., caudate) in the path of the X-rays and b) shielded neurons anterior and posterior to the hippocampus (7,45).

A two-way ANOVA (radiation treatment × drug treatment) revealed that MK 801 treatments changed neither the CA1 pyramidal cell layer thickness nor the granule cell counts in the olfactory bulb or cerebellum. Likewise, serial injections of 0.1 mg/kg did not alter the number of fascia dentata granule cells in most treatment groups. Still, dentate granule cells were fewer in both irradiated and sham-irradiated rats that received 0.2 mg/kg MK 801. This reduction was statistically significant only in the case of irradiated rats treated with the higher dose of the drug [a priori, $t(7) = 2.11$, $p < 0.05$].

We calculated correlations between the anatomic data of saline-treated rats (irradiated and sham irradiated) and the results of their behavioral tests. This analysis revealed a significant relationship between the latency to find the hidden platform late in training (mean of Trials 5-7) and the number of cells in the fascia dentata, $r(6) = -0.69$, $p < 0.05$ (30). These data suggest that low granule cell numbers in the dentate gyrus predict long latencies (i.e., poor performance) late in water maze learning. There was not a corresponding statistically significant relationship between fascia dentata granule cell counts and early maze performance, $r(6) = 0.29$, $p > 0.05$. Likewise, none of the other anatomic parameters measured (e.g., cells in olfactory bulb, cerebellum, or CA1 cell layer thickness) correlated significantly with early or late maze performance.

DISCUSSION

Rats with radiation-induced hypoplasia of fascia dentata granule cells exhibited a learning deficit in the water maze. However, when irradiated neonatal rats were treated with our low dose of MK 801 (0.1 mg/kg) and then tested as adults their maze performance was not significantly different from sham-irradiated controls. These drug-induced behavioral benefits were observed despite the fact that radiogenic hippocampal damage in these animals was not significantly different from that of saline-treated irradiated rats. A higher dose of MK 801 (0.2 mg/kg) did not produce these behavioral benefits and, in fact, inhibited maze learning in control animals and potentiated hippocampal damage in irradiated subjects.

Our use of MK 801 in an attempt to antagonize radiation-induced hypoplasia of fascia dentata granule cells was not effective. Still, we observed significant *behavioral* benefits months after administration of the NMDA antagonist. This raises questions about the underlying physiological mechanisms of MK 801-induced antagonism of radiogenic learning deficits. While the current data do not speak to these points directly, our experimental paradigm has similarities to other experiments where MK 801 has been shown to modulate compensatory neural responses following sensory deprivation of the neonatal visual system. For example, blockade of NMDA receptors prevents the disconnection of deprived visual pathways and, when visual stimulation is reinstated, also prevents recovery of initially deprived afferents (16,23,35). Chronic MK 801 is effective in stimulating neuronal growth—especially in infant brain (22). Thus, NMDA receptor mechanisms are involved in the mediation of various forms of neuronal plasticity (10) including neuronal differentiation during organogenesis and the experience-dependent pruning of synaptic connections during early postnatal development (16). Other relevant studies have shown how, following transient cerebral ischemia, receptor binding on dentate granule cells can change dramatically in the absence of any cell loss (71). Perhaps MK 801's antagonism of NMDA activity modulates compensatory neuronal interconnections or receptor populations in such a way to allow maze learning in the presence of few fascia dentata granule cells (3,39).

Low doses (0.1 mg/kg) of MK 801 improved the water maze performance of irradiated rats. However, our higher dose (0.2 mg/kg) did not have this effect on irradiated rats and, in fact, inhibited maze learning in sham-irradiated control animals. Recent data suggest that NMDA receptors control neuronal plasticity in a way that is quite sensitive to the level of agonist present. For example, a high dose of NMDA induces retraction of retinal ganglion cell (RGC) neurites while low doses induce elongation of RGCs [(50; but also see (15)]. This dose-dependent modulation of neuronal growth could be an important tool of the developing nervous system.

It is difficult to fully explain the mechanisms by which our higher dose of MK 801 (0.2 mg/kg) lowered fascia dentata granule cell counts. We might point out, however, that under certain circumstances MK 801 has been shown to produce transient pathomorphological reactions in selected brain regions of the adult rat (53). Further, Duncan et al. (18) recently reported that a single 1-mg/kg (IP) dose of MK 801 causes inhibition of DNA synthesis in the neonatal brain. This inhibition may interfere with neurogenesis in the hippocampus. Thus, if NMDA antagonism is sufficiently strong functionally beneficial modulations of plastic neuronal interconnections (described above) may be replaced by significant changes in neurogenesis, gliogenesis, and the production of pathomorphological reactions.

Our observation that selective doses of MK 801 block radiation-induced learning deficits usually associated with fascia dentata granule cell hypoplasia is consistent with the hypothesis that NMDA receptors are involved in the brain's response to ionizing radiation. However, it may also be the case that some nonspecific physiological change produced by MK 801 could be playing a role in the behavioral radioprotection we report here. In particular, the hypothermia that accompanies MK 801 administration (11) has been shown to mediate some of the drug's antiischemic activity. This finding is not universal, however (24). MK 801-induced hypothermia may also play a role in its radioprotective actions since low temperature has been shown to reduce radiation-induced cell killing (21).

The changes in body weight reported here are of interest because, in many cases, they paralleled behavioral phenomenon [see also (15)]. For example, both reductions in weight and deficits in maze learning were observed in rats with fascia dentata granule cell hypoplasia; 0.1-mg/kg doses of MK 801 tended to normalize both body weights and maze acquisition

of irradiated animals; and higher drug doses (0.2 mg/kg) tended to produce weight reductions and selective impairments on water maze performance. These data might lead to the hypothesis that reduced body weights cause a lethargy or some other generalized debilitation that secondarily produces the deficits in maze learning that we report here. However, several findings do not support this notion. First, radiation-induced hippocampal damage such as that described here causes locomotor hyperactivity (44,45) and hyperresponsiveness to startle stimuli (43) rather than lethargy. Similarly, rats in all treatment groups in this study crossed a similar number of maze quadrants—thus suggesting similar swimming capabilities. Further, body weight did not correlate well with performance on the maze task. These data suggest that reduced body weight is neither a good indicator of lethargy nor an optimal predictor of water maze performance.

The long-term behavioral effects of repeated MK 801 administration in neonates may be compared and contrasted with those described following acute dosing of the NMDA antagonist in adult rats. A variety of studies indicate that MK 801 can produce acute a) sensorimotor disturbances (74), b) dose-dependent malaise (48), and c) impairment in the acquisition of hippocampal-dependent spatial learning tasks (12, 42,59) in adult rats. On the other hand, serial doses (0.2 mg/kg) of MK 801 in young rats (postnatal days 9–15) did not alter subsequent (day 36) motor activity, startle responsiveness, or water maze performance (42). These data are consistent with our findings in that we observed no long-term effects of serial neonatal doses of 0.1 mg/kg MK 801 on the water maze acquisition of our sham-irradiated control animals. However, neonatal doses of 0.2 mg/kg MK 801 produced marked deficits in maze learning that could be observed in adulthood. Because we did not test our animals as neonates/young adults, and McLamb et al. (42) tested only young animals, we do not know the extent to which the phenomenon we observed is age dependent. Our earlier and more frequent dosing of MK 801 may also be factors in producing the late behavioral deficits we observed in mature rats. The importance of appropriate dose selection in both the acute and repeated administration of MK 801 is illustrated by the fact that a 0.2-mg/kg (IP) dose of MK 801 can produce an acute "gross intoxication" (74) while 0.1 mg/kg evoked no such effect.

Our results reflect long-term and/or long-latency behavioral changes following early, repeated exposure to an NMDA antagonist. They also suggest a role for NMDA receptors in behavioral dysfunctions associated with X-ray-induced hypoplasia of hippocampal granule cells. Future studies will explore neuroanatomic and neuropharmacological correlates of the behavioral phenomena reported here.

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