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Enhanced Acquisition of Reversal Training in a Spatial Learning Task in Rats Treated With Chronic Nimodipine

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McMONAGLE-STRUCKO, K. AND R. J. FANELLI. *Enhanced acquisition of reversal training in a spatial learning task in rats treated with chronic nimodipine.* PHARMACOL BIOCHEM BEHAV **44**(4) 827-835, 1993.—Nimodipine levels were measured in blood and brain of rats implanted SC with sustained-release pellets of nimodipine (0, 10, 20, or 40 mg). Dose-dependent levels of nimodipine were detected in both plasma and brain. These results indicated the possible usefulness of these pellets in behavioral studies where long-term treatment is required. Therefore, the effects of chronic nimodipine, using 40-mg pellets, were examined on the performance of young, middle-aged, and aged rats in the Morris water maze. Following implantation of either nimodipine or placebo pellets, rats were trained for 6 days (three sessions/day) followed by 6 days of reversal training. During both initial and reversal training, every sixth trial was a probe trial. During initial training, there were clear age-related acquisition deficits in place training, with no effects of chronic nimodipine. Nimodipine did, however, enhance performance of rats during the first and second probe trials of reversal training. Time spent in the training quadrant by nimodipine-treated rats was approximately 30% longer on the first reversal probe and 35% longer on the second reversal probe than time spent in this quadrant by placebo-treated animals. These results indicate that chronic nimodipine enhances the performance of normal animals in reversal training on a spatial learning task.

Nimodipine Sustained-release pellets Chronic treatment Spatial learning Morris water maze Calcium channel antagonist

EVIDENCE is rapidly accumulating that calcium channel antagonists may have beneficial effects in various disorders of the CNS, including ischemia, degenerative disorders, brain damage, and age-associated memory impairment (21,22). Due to the high doses required to produce effects in the CNS by many calcium channel antagonists, their usefulness in the treatment of these disorders has been limited (23). Nimodipine, a 1,4-dihydropyridine with calcium channel antagonist activity (6,9,14,15), was found to have cerebrovasodilatory and neuronal effects at doses low enough not to interfere with peripheral circulation (5,7,8). Given this finding, nimodipine has been investigated in animal models as a potential therapeutic for the treatment of numerous disorders of the CNS. In animal studies of cognitive performance, IV nimodipine has been shown to accelerate the acquisition of associative learning in aging rabbits using trace eye-blink conditioning (2), while repeated oral administration of nimodipine facilitates the recovery of learned behavior in rats with an excised visual neocortex when trained on a brightness discrimination task in a Y-maze (11).

The use of nimodipine to treat CNS disorders will likely require chronic treatment. The methods used for long-term administration of this agent in animal models have included repeated injection, catheterization, intubation, or placing the drug in food or water. Difficulties do, however, arise from these methods of treatment, including the stress of repeated injections, invasive surgical techniques, and the inability to accurately regulate the dose and time course of drug levels when administered ad lib. Sustained-release pellets of nimodipine have been suggested as a useful route of administration for chronic treatment because the stress associated with drug administration is less likely to interfere with behavioral or physiological procedures. Therefore, the first goal of this investigation was to evaluate the usefulness of sustained-release, SC pellets as a method of chronic treatment with nimodipine.

Spatial memory testing is particularly sensitive to changes in cognitive ability in rodents. A recent study has shown that implantation of nimodipine pellets improves performance of young rats in a radial arm maze (13). Performance in the Morris water maze, a spatial information memory task, demonstrates sensitivity to age-related cognitive deficits (18). Therefore, the second goal of this investigation was to evalu-

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ate the effects of nimodipine pellets on the performance of young, middle-aged and aged rats in the Morris water maze to provide additional information on the behavioral effects of this agent.

EXPERIMENT 1

METHODS

Sustained-release, SC pellets of nimodipine were produced by Innovative Research of America (Toledo, OH). In a preliminary study, 0-, 10-, 20-, and 40-mg sustained-release nimodipine pellets were surgically implanted in young adult, male Long-Evans rats (approximately 300 g) (n = 4 per group). All animals were obtained from Charles River Laboratories. They were housed singly, with food and water available ad lib. The animal facility was maintained on a 12 L: 12 D cycle with lights on at 7:00 a.m. Rats were anesthetized with 40 mg/kg pentobarbital (IP), after which a patch of fur was shaved from the nape of the neck using small-animal clippers, and a small incision (1–1.5 cm) was made. Using a slightly curved microdissecting forceps, a blunt dissection of fascia down the side of the neck was carried out, creating a pocket into which a pellet was then placed. The incision was closed using 3-0 silk suture material (Ethicon, Inc., Somerville, NJ). Rats were returned to their home cages and monitored for reaction to the surgical procedure. Blood samples were taken by tail bleeds at various time points (6 and 24 h and 4, 7, 10, and 14 days) after implantation. The whole blood was placed in microcentrifuge tubes and centrifugation was carried out at 12,320 × g for 15 min at 4°C. Plasma was removed and frozen for later analysis. At the end of 3 weeks (21 days following implant), rats were decapitated and blood and brain samples were collected. Blood was treated as above, while the cortex, hippocampus, and brain stem were dissected from each brain and frozen for later analysis.

In a second study, independent groups of rats (n = 4 per group) were implanted with 40-mg nimodipine pellets using the same surgical technique described above. The rats were sacrificed by decapitation at 1, 7, 14, 28, 35, 42, 49, and 56 days following pellet implantation. Blood and brain samples (cortex, hippocampus, and brain stem) were collected and treated as above. The plasma and brain sections were frozen for later analysis.

A modification of the capillary gas chromatographic

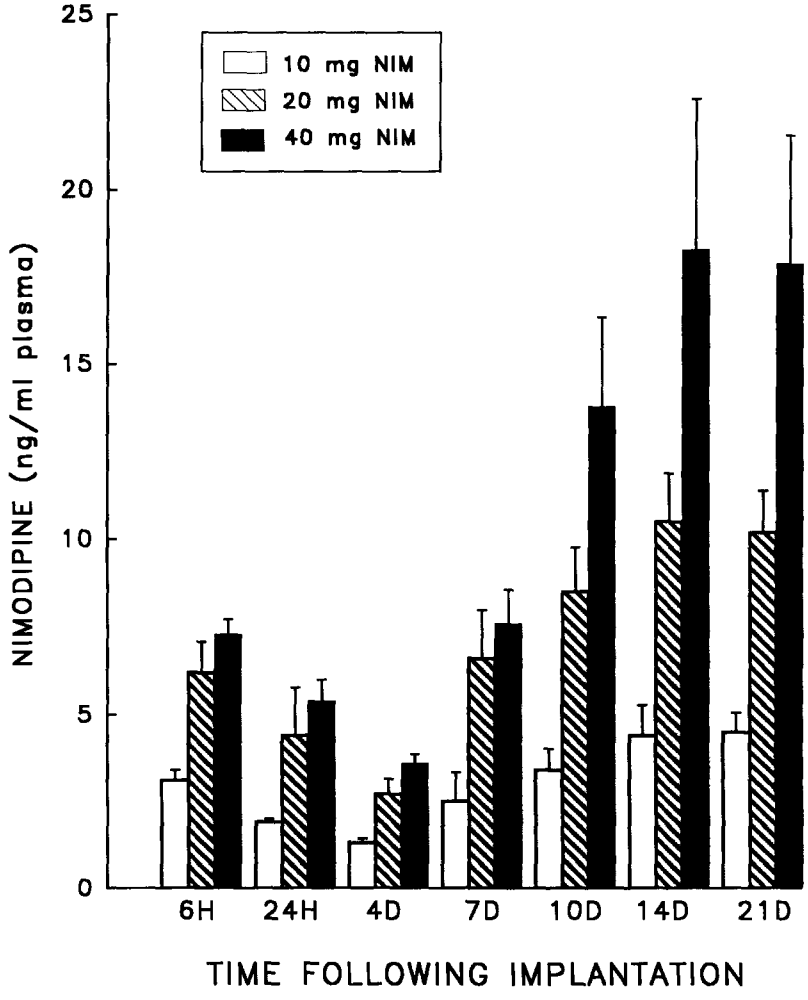

FIG. 1. Nimodipine levels (ng/ml, mean ± SEM, n = 4) in plasma at selected times (H = hours, D = days) following implantation of 10-, 20-, or 40-mg nimodipine (NIM) pellets in young adult rats.

method previously described by Krol et al. (10) was used to analyze the samples for the presence of nimodipine.

RESULTS AND DISCUSSION

In this initial study, there was clear evidence of a dose-dependent relationship in the plasma levels of nimodipine from days 10 to 21 (Fig. 1). Nimodipine levels in plasma reached peak values 10 days following implantation for all three doses and remained near maximum through the final time point examined (21 days). The higher levels of nimodipine measured during the first 24 h, relative to the value at 4 days, may be due to a prolonged effect of anesthesia upon drug metabolism or uptake. Brain levels of nimodipine displayed a dose-dependent relationship in the three regions analyzed on day 21 (Fig. 2). The analysis of brain regions (cortex, hippocampus, and brain stem) on day 21 produced similar nimodipine levels across the different brain regions.

The nimodipine levels in plasma of rats implanted with 40-mg sustained-release nimodipine pellets reached peak by day 14 (13.7 ± 1.41 ng/ml) (Fig. 3). Substantial drug levels were maintained through day 35 (14.7 ± 1.40 ng/ml), following which the levels of nimodipine in plasma began to decline. As can be seen in Table 1, in contrast to plasma, substantial levels of nimodipine were measured as early as day 1 in the different brain regions analyzed, although there appears to have been a more rapid uptake of nimodipine into the cortex and brain stem when compared to the hippocampus.

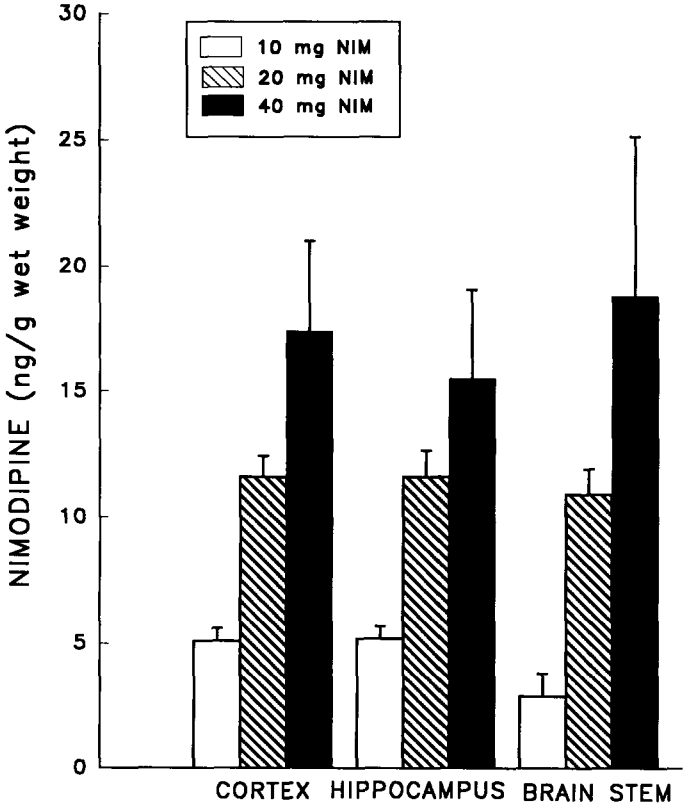

FIG. 2. Nimodipine levels (ng/g wet weight, mean ± SEM, $n = 4$) in selected brain regions 21 days following implantation of 10-, 20-, or 40-mg nimodipine (NIM) pellets in young adult rats.

EXPERIMENT 2

METHODS

Male Long-Evans rats of three ages—young (4 months old), middle-aged (11–13 months old), and aged (20–28 months old)—were used. Animals were obtained as 4-month-old retired breeders from Charles River Laboratories and maintained following the housing, food, water, and lighting procedures described in Experiment 1. Prior to experimentation, all rats were visually inspected and only rats free of obvious health defects were used. Rats of each age were randomly assigned to receive either a 40-mg nimodipine or a placebo pellet. Implantation of the sustained-release pellets was performed using the surgical procedures described above. After insertion of the pellet was completed, rats were returned to their home cages and handled daily (2 min/day) for 1 week prior to behavioral training.

Behavioral training was carried out in a galvanized steel tank measuring 58 cm high with a diameter of 1.47 m. White polyvinyl chloride (PVC) collars were secured to the floor of the tank in the center of each quadrant. An escape platform was constructed of white PVC piping, and was 34.5 cm high, 11.5 cm in diameter, and covered on top with white wire mesh. The escape platform fit securely on the collar but could be moved by the experimenter. Prior to daily testing, the maze was filled with water to a depth 1 cm above the escape platform (35.5 cm) and maintained at 26 ± 2°C. The water was made opaque by adding 0.9 kg powdered milk. Numerous stationary visual cues were present in the testing room, including wall hangings, video equipment, and the experimenter. Data were recorded using the Multiple Zone Distance Traveled program of the Videomex-V Motion analysis system (Columbus Instruments International Corp., Columbus, OH).

On day 7 following pellet implantation, each animal was habituated to the maze for 90 s with the escape platform absent. Given the levels of nimodipine found in Experiment 1, behavioral training began 10 days following surgery. Animals were trained three trials per day using a 60-s intertrial interval (ITI). For each animal, the position of the goal platform was assigned randomly to one of the four possible locations and remained in the same position for the first 6 days of training. Rats were placed into the maze from one of the four equally spaced positions around the perimeter of the tank. The starting locations varied with the stipulation that each location be used once in a block of four trials. A time limit of 120 s was placed on each training trial. If the platform was not located, the animal was placed onto the platform, where it remained for 60 s. The latency to escape was recorded. Every sixth trial was a probe trial, during which the platform was withdrawn and animals were placed in the maze for 30 s. The amount of time the animal spent in each quadrant was assessed. Following initial training (6 days) to find the submerged platform in one spatial location, the platform was moved to the quadrant opposite its original position. An additional 6 days of training with the platform in its new reversed location were carried out as described above.

At the completion of the reversed training trials (6 days), rats were decapitated and blood samples were collected and analyzed for the presence of nimodipine as in Experiment 1.

RESULTS AND DISCUSSION

Consistent with Experiment 1, the sustained-release pellets resulted in moderately high nimodipine plasma levels in the treated animals, measured at the end of training (28 days) (Table 2). There were no group differences on this measure.

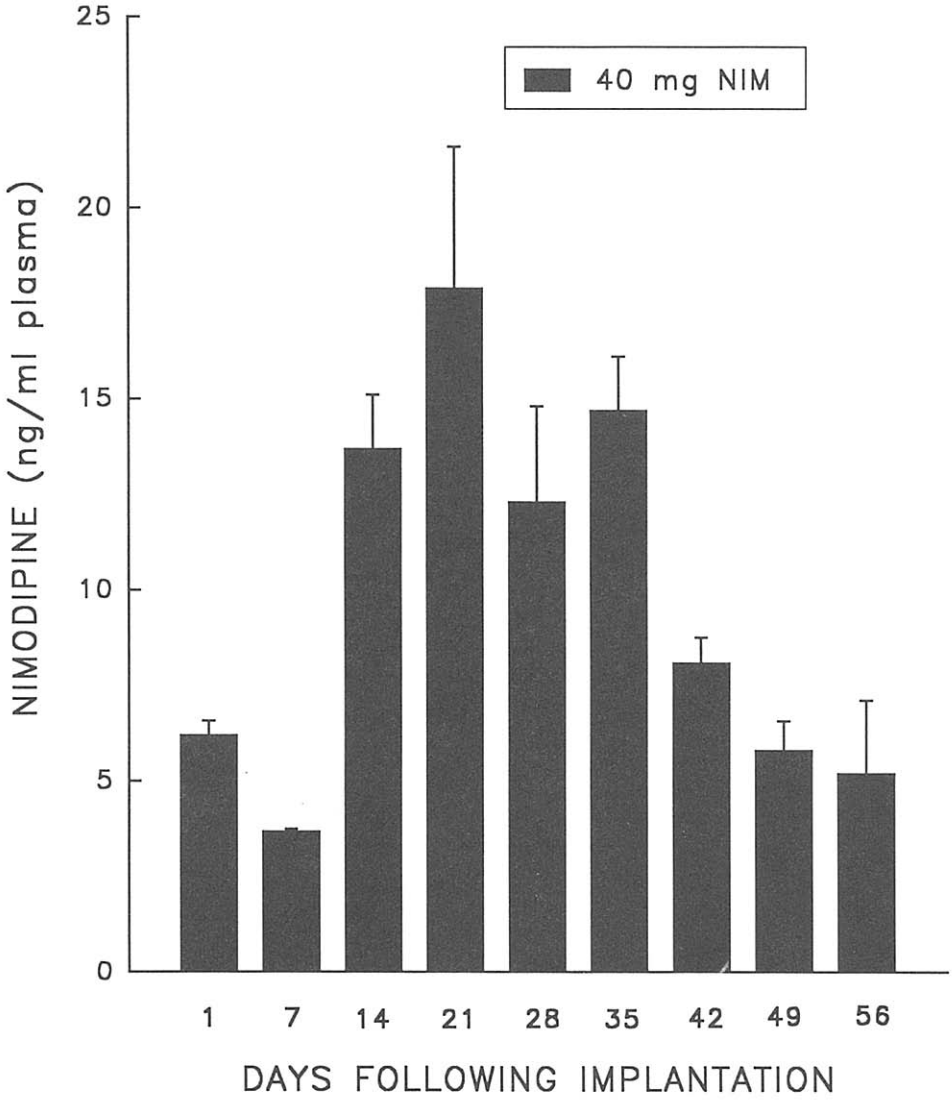

FIG. 3. Nimodipine levels (ng/ml, mean ± SEM, n = 4-8) in plasma at selected times following implantation of a 40-mg nimodipine (NIM) pellet in young adult rats.

TABLE 1

NIMODIPINE LEVELS IN BRAIN REGIONS AT SELECTED TIMES FOLLOWING IMPLANTATION OF A 40-mg PELLET

Day Following Implantation	Brain Region		
	Cortex	Hippocampus	Brain Stem
1	15.9 ± 2.55	9.1 ± 0.23	19.3 ± 5.27
7	10.6 ± 1.16	7.4 ± 0.71	14.0 ± 2.37
14	24.4 ± 6.36	14.3 ± 2.46	22.5 ± 1.74
21	17.4 ± 3.66	15.5 ± 3.56	18.8 ± 6.30
28	11.5 ± 2.04	16.4 ± 3.30	18.9 ± 3.13
35	17.2 ± 2.95	11.7 ± 0.75	25.6 ± 4.02
42	9.5 ± 0.91	9.2 ± 1.23	12.1 ± 1.05

Values (in ng/g wet weight) represent mean ± SEM; n = 4 for each group.

TABLE 2

NIMODIPINE LEVELS IN PLASMA USING A 40-mg PELLET MEASURED AT THE END OF TRAINING IN THE DIFFERENT AGE GROUPS

Group	N	Plasma Nimodipine (ng/ml)
Young	17	14.5 ± 1.6
Middle-aged	13	11.0 ± 1.8
Aged	16	14.0 ± 2.0

Values represent mean ± SEM.

The mean value of plasma nimodipine across all age groups was approximately 13 ng/ml.

During initial training, as expected, all animals reduced their escape latencies over the course of the 15 training trials, as revealed by a two-way analysis of variance (ANOVA) with repeated measures [trial effect, $F(14, 1{,}162) = 37.39$, $p < 0.001$] (Fig. 4). In addition, there were clear age-related deficits in acquisition [age effect, $F(2, 83) = 12.40$, $p < 0.001$]. The rate of acquisition did not differ between these age groups (age × trial interaction, not significant). There was no effect of nimodipine treatment on escape latency during initial training. In addition, the measures obtained during the three probe trials (with the goal removed), administered during initial training, indicated no significant drug treatment effects (Fig. 5).

Performance during the reversal procedure was superior to that of initial training during the early trials, as indicated by the reduced escape latencies (Fig. 6). The asymptotic performance on the reversal was approximately the same as during initial acquisition. In general, rats improved their performance over the course of the 15 reversal trials [trial effect, $F(14, 1{,}190) = 14.78$, $p < 0.001$]. As with initial training, there was no effect of nimodipine treatment on escape latency during the reversal training.

Analysis of the probe trials during the reversal training did reveal a significant enhancement of performance by nimodipine (Fig. 7). Nimodipine-treated rats spent significantly more time in the new goal quadrant during the probe trial following the first five training trials of the reversal [drug treatment effect, $F(1, 84) = 10.71$, $p < 0.01$]. Time spent in the goal quadrant by nimodipine-treated rats was approximately 30% longer than that of placebo-treated rats on the first probe trial. A significant effect of nimodipine was also observed during the second probe trial of the reversal [drug treatment effect, $F(1, 84) = 16.79$, $p < 0.001$]. On the second probe trial, nimodipine-treated animals spent about 35% more time

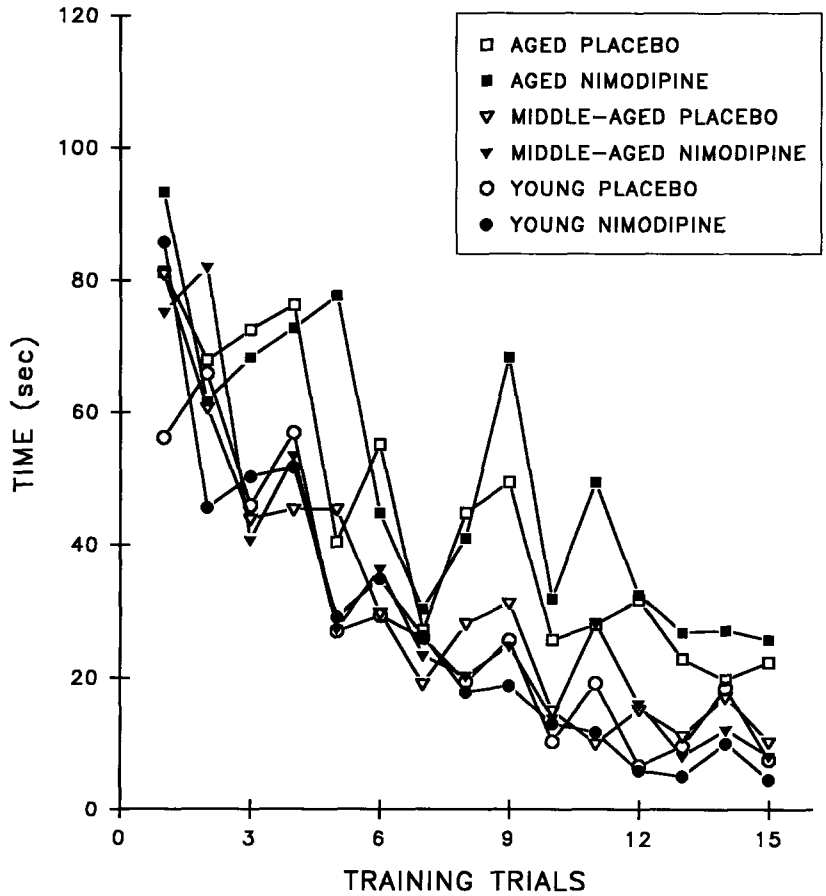

FIG. 4. Mean escape latencies across initial training trials 1–15 for young, middle-aged, and aged rats treated with either placebo or nimodipine (40 mg) pellets (n = 13–17/group). Probe trials were given between trials 5 and 6, 10 and 11, and after trial 15.

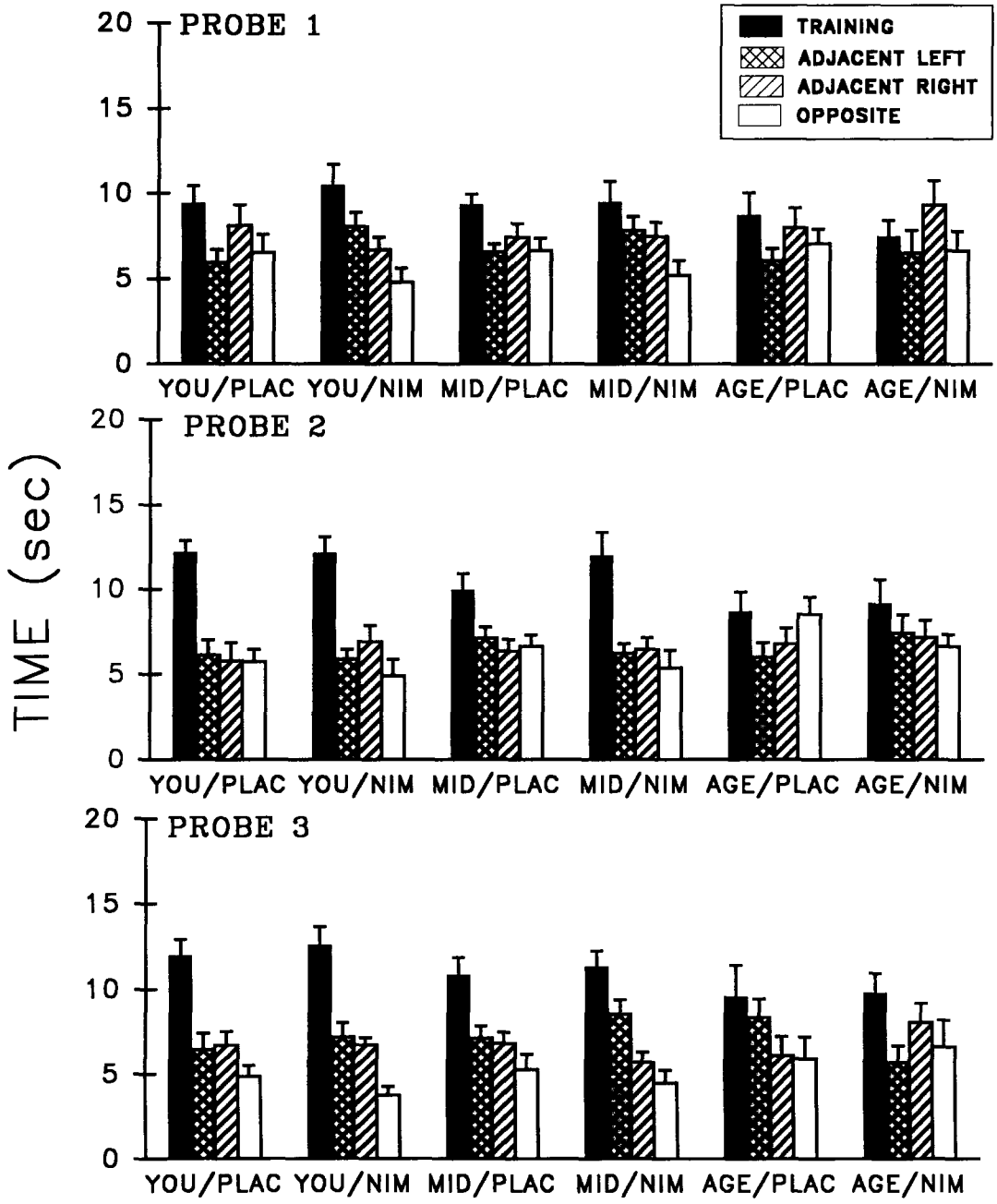

FIG. 5. Mean number of seconds (±SEM, n = 13-17), of a maximum 30 s, spent by young (YOU), middle-aged (MID), and aged (AGE) rats treated with either placebo (PLAC) or nimodipine (40 mg) (NIM) pellets during the three probe trials of initial training. Adjacent left and right bars depict times spent in quadrants to the left and right of the training quadrant, respectively, and the opposite bar depicts time in the quadrant directly opposite the training quadrant.

in the goal quadrant than did placebo-treated rats. By the final probe trial of the reversal, all groups spent approximately the same time in the training quadrant and there was no longer a significant effect of nimodipine.

GENERAL DISCUSSION

The present experiments indicate that chronic nimodipine treatment with SC sustained-release pellets improves performance of reversal training on a spatial learning task in rats. Animals from all three age groups treated with nimodipine spent more time in the training quadrant during the first two probe trials during reversal training. Because the nimodipine effect was only seen on probe trials of reversal training, and nimodipine did not alter performance during initial training nor during training on the reversal, it appears that nimodipine did not alter reference memory required for learning of this task in normal animals (1). In these animals, nimodipine ap-

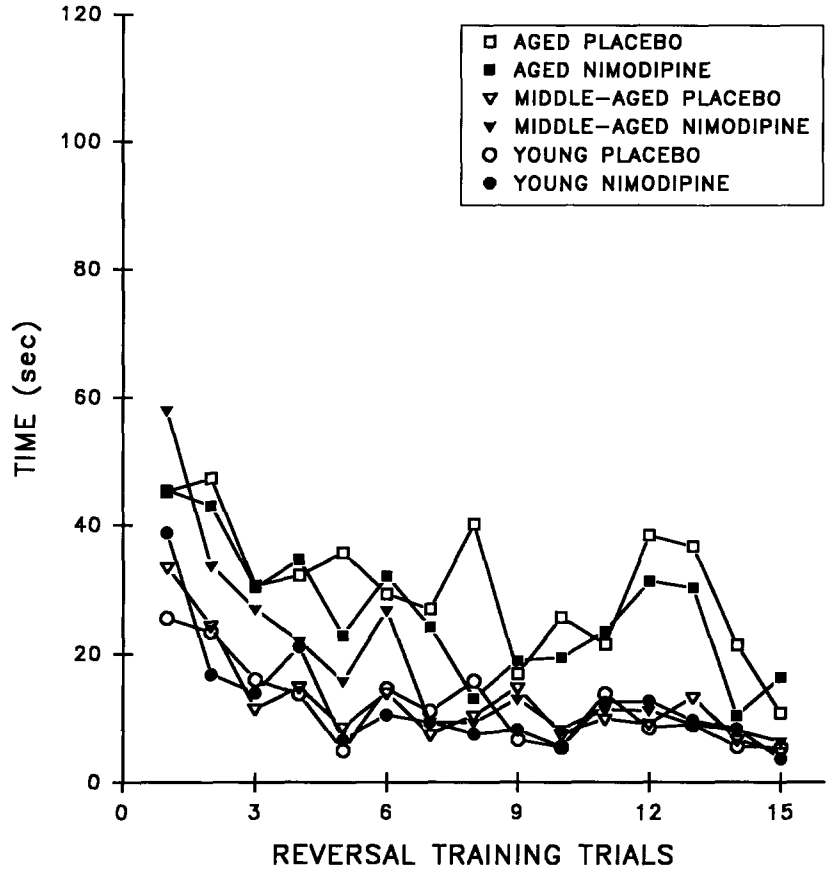

FIG. 6. Mean escape latencies across reversal training trials 1-15 for young, middle-aged, and aged rats treated with either placebo or nimodipine (40 mg) pellets (n = 13-17/group). Probe trials were given between trials 5 and 6, 10 and 11, and after trial 15.

pears to improve memory required for a difficult discrimination rather than produce a general enhancement of cognitive performance.

The significant effect of nimodipine on this aspect of performance was not dependent upon age because animals of the three age groups tested did not exhibit a differential beneficial response to drug treatment. In addition, nimodipine did not reduce the age difference in performance seen during initial or reversal training. Therefore, these results do not directly address the therapeutic potential of this agent for the treatment of age-related cognitive deficits. There is the possibility, however, that with a larger group of aged animals, separated into those with impaired or unimpaired performance (4), an effect of nimodipine might be demonstrated in a subset of aged animals.

The present findings agree with previous studies that demonstrated beneficial effects of nimodipine in normal young animals (5,13). Numerous other investigations have shown beneficial effects using nimodipine in both lesion-induced models of cognitive deficits (3,11,16) as well as in aged animals (2,12,19,20). The relationship between these two groups of studies, as well as the neural substrates and mechanisms contributing to the beneficial effects of nimodipine, are far from clear and require further investigation.

In a recent publication, McCarthy and TanPiengco demonstrated, using patch clamp methodology, that nimodipine blocks L-type calcium channel current with high affinity in neurons (14). Additional studies have described other neuronal effects of nimodipine, including the enhancement of synaptic transmission measured with intracellular recordings in the hippocampal slice (17). Whether any of these specific neuronal effects are responsible for the cognitive-enhancing effect of nimodipine in normal animals, or whether a more general pharmacological effect is responsible, such as the vasodilation of cerebral arterioles, is unclear at present. Further, it is likely that the neuronal mechanisms responsible for the beneficial effects of nimodipine upon cognition may be considerably different in the intact and in the damaged or degenerating nervous system. Given the ubiquitous and critical role of calcium in the CNS, there is also the possibility for multiple mechanisms of action of nimodipine under both normal and pathologic conditions.

SC implantation of sustained-release pellets of nimodipine resulted in therapeutically relevant plasma and brain levels of drug by days 10-14 following implantation that were maintained through at least day 35. There was some indication that significant nimodipine levels were evident more rapidly in brain than in plasma. From these data, it appears that these pellets may provide a useful route of administering nimodipine when chronic treatment is desired. However, to ensure

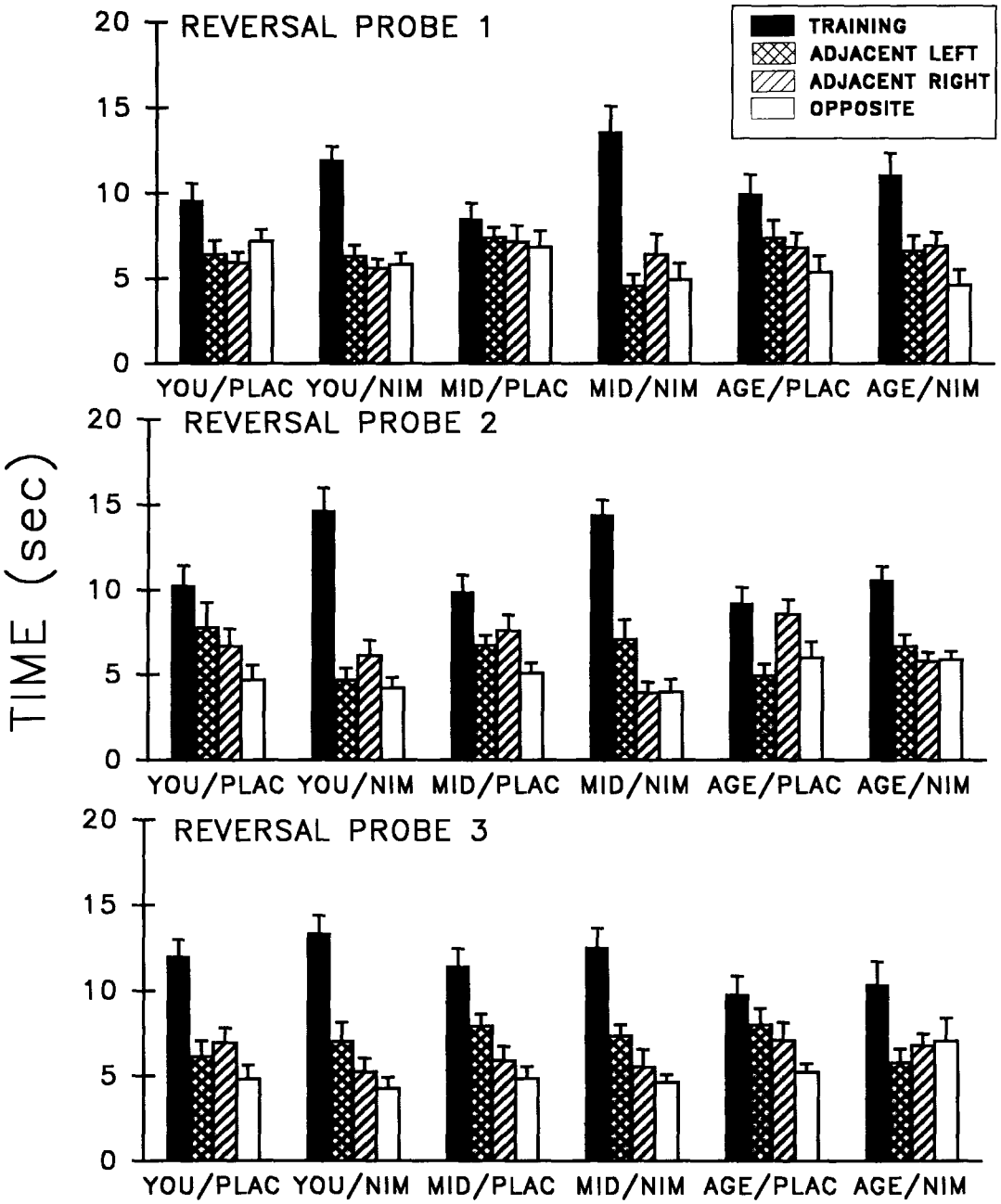

FIG. 7. Mean number of seconds (±SEM, n = 13-17), of a maximum 30 s, spent by young (YOU), middle-aged (MID), and aged (AGE) rats treated with either placebo (PLAC) or nimodipine (40 mg) (NIM) pellets during the three probe trials of reversal training. The quadrants are designated as in Fig. 5.

that the desired dose of drug has been delivered it is critical that blood plasma levels are monitored for the presence of nimodipine. In subsequent studies, we found significant variations in the nimodipine levels with different batches of pellets. The form of drug used in producing these pellets is also important. For example, when micronized nimodipine was used in the pellet formulation, the release of drug was prevented.

In summary, the present experiments provide additional evidence for the beneficial effect of nimodipine upon cognitive performance. Chronic treatment with nimodipine resulted in enhanced memory of reversal training, as demonstrated during probe trials. The enhanced performance was found in all age groups tested, indicating that this enhancement by nimodipine is present in normal animals and not restricted to pathologic conditions.

ACKNOWLEDGEMENTS

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