

RAPID COMMUNICATION

Nimodipine Improves Spatial Working Memory and Elevates Hippocampal Acetylcholine in Young Rats

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LEVY, A., R. M. KONG, M. J. STILLMAN, B. SHUKITT-HALE, T. KADAR, T. M. RAUCH AND H. R. LIEBERMAN. *Nimodipine improves spatial working memory and elevates hippocampal acetylcholine in young rats.* PHARMACOL BIOCHEM BEHAV 39(3) 781–786, 1991.—The calcium channel blocker nimodipine has been reported to improve cognitive performance in aged and brain-damaged animals. In the present study, the effects of nimodipine and placebo on spatial working memory and hippocampal acetylcholine were studied in young Fischer-344 rats. Nimodipine or placebo was administered via subcutaneously implanted, sustained-release pellets. Each active pellet contained 20 mg of nimodipine and released the drug over approximately 21 days. Two days after the drug or placebo pellets were implanted, training in the 8-arm radial maze started and continued for 12 days. Rats were required to learn a win-shift strategy. Nimodipine-treated animals learned the maze more rapidly than a placebo-treated group as indicated by the number of correct choices out of the first eight arms visited ($p < 0.001$). Treated rats also made twice as many choices per unit time during the first week of training ($p = 0.005$). To assess hippocampal acetylcholine release, in vivo microdialysis was performed while animals were awake and unrestrained, 19–21 days after pellet implantation. A probe with a 3 mm semipermeable tip was placed in the hippocampus (CA1 and dentate gyrus), and individual μl dialysate samples were collected at 2 $\mu\text{l}/\text{min}$ and immediately analyzed by high performance liquid chromatography with electrochemical detection. Significantly higher extracellular ACh levels were found in nimodipine-treated rats (71.4 ± 3.6 nM; $n = 4$) compared to controls (52.5 ± 2.5 nM; $n = 5$) ($p = 0.003$) and in another group of rats of the same age that received identical drug treatment. It appears that sustained nimodipine treatment improves working memory and increases extracellular acetylcholine release in the hippocampus of young rats.

Nimodipine Spatial memory Learning Hippocampus Acetylcholine Microdialysis
Calcium channels

NIMODIPINE is a potent calcium (CA) channel antagonist with a higher affinity for the central nervous system than other drugs of this class (9). When tested in a variety of behavioral paradigms with old or impaired animals, it appears to have beneficial effects on memory and other cognitive functions. In rats, nimodipine improves performance in both a passive avoidance task and the 8-arm radial maze following memory disruption induced by exposure to hypoxia (7,22). It has also been reported to facilitate associative learning in aged rabbits when administered either via acute intravenous infusion (5) or following 28 days of inclusion in the diet (23). A beneficial effect on the performance of old rats in a water maze was observed following

one week of oral nimodipine treatment (20). In aging primates performing a nonmatching-to-sample task, nimodipine treatment improved recent memory function (19). Nimodipine has been reported to facilitate recovery of memory retrieval ability following large neocortical lesions (11), and to improve performance on a DRL (differential reinforcement of low rates of responding) schedule of reinforcement following hippocampal lesions (6). When tested in elderly patients with chronic cerebrovascular disorders, nimodipine appears to enhance learning and memory (3). It has also been reported to improve certain aspects of memory in patients with Alzheimer's disease (25), and to selectively dilate cerebral blood vessels (9). The central effects of nimo-

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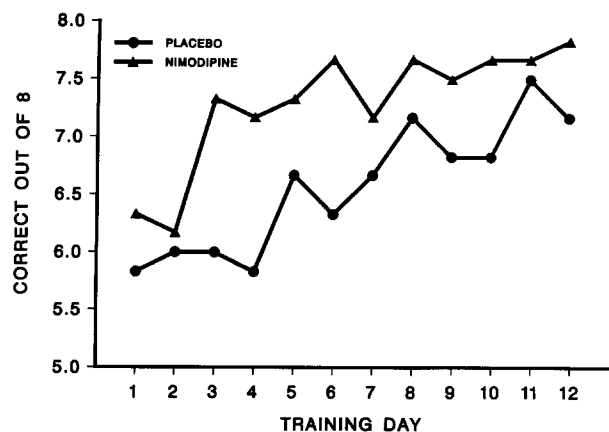


FIG. 1. Mean number of correct choices out of the first eight in the radial arm maze over days of testing for nimodipine- and placebo-treated rats.

dipine have been summarized and discussed in an extensive review (21).

The underlying mechanism(s) responsible for the effects of nimodipine on memory are unresolved. Although it is a potent CA channel blocker under certain conditions, its central effects could also be attributable to other mechanisms of action (14).

This study was designed to determine whether sustained-release nimodipine treatment would alter acquisition using a version of the 8-arm radial maze known to require spatial working memory (15,16). Since spatial working memory is considered to be mediated by cholinergic hippocampal neurons (2,17), *in vivo* changes in extracellular acetylcholine (ACh) concentration were assessed during nimodipine treatment in several rats used for behavioral testing, and in another group of young rats which received exactly the same drug treatment. Unlike most previous studies which focused on the beneficial effects of nimodipine in old, brain-lesioned, or ischemic laboratory animals, this study reports improved cognitive performance in young, healthy animals.

METHOD

Animals

Male Fischer-344 rats, 5 months old, and weighing approximately 330 g at the beginning of this study, were used as subjects. They were obtained from Charles River Labs, Kingston, NY. The rats were individually housed in hanging wire mesh cages and maintained on a 12 h light/dark cycle (lights on at 0600 h).

Drug Treatment

Twelve rats were anesthetized with phenobarbital (55 mg/kg, IP) and a single sustained-release pellet containing either nimodipine (20 mg total, released over 21 days) or placebo (Innovative Research of America, Toledo, OH) was implanted subcutaneously in the nape of the neck. Six animals selected at random received nimodipine pellets, and six received placebo. All of these animals were used for behavioral testing and most for *in vivo* microdialysis. Another group of ten Fisher-344 rats of the same age were also implanted with placebo ($N=5$) and

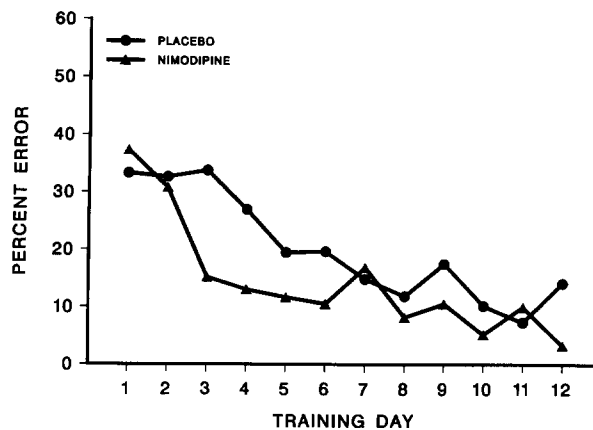


FIG. 2. Mean percent errors in daily radial arm maze sessions for nimodipine- and placebo-treated rats.

nimodipine ($N=5$) pellets, but were only used for microdialysis.

Behavioral Testing

The 8-arm radial maze consisted of eight equally spaced arms (70 cm long, 12 cm wide, 20 cm high) radiating from an octagonal central arena 36 cm in diameter. A food cup, baited with one 45-mg food pellet (Bioserve, Inc., Frenchtown, NJ), was placed at the end of each arm of the maze. Following one week of exposure to the laboratory environment, including daily handling, rats were food-restricted for one week (12–13 g of rat chow per day) and lost 10–15% of total body weight as compared to a control group fed *ad lib*. On the next two days, rats were fed only baiting pellets to familiarize them with the reinforcer used in the maze. On these days, they were placed in the maze for five minutes, and a few additional food pellets were randomly scattered on its floor. On the following day, one pellet was placed in each food cup, and rats were permitted to search for the food for five minutes. Four days later, nimodipine or placebo pellets were implanted. Two days after surgery, behavioral testing began and continued for 12 consecutive days, between 0800 and 1200 h each day.

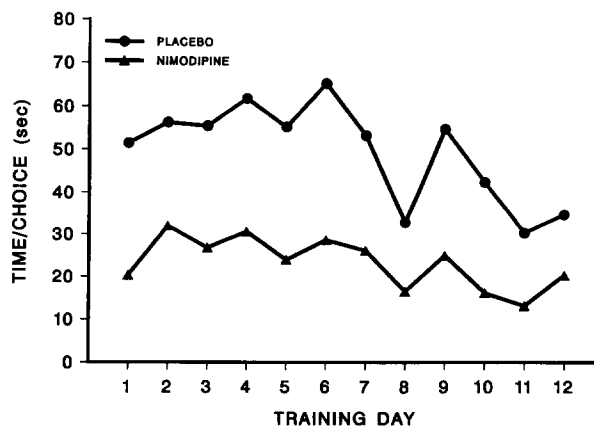


FIG. 3. Mean time per choice in the 8-arm radial maze over days of testing for nimodipine- and placebo-treated rats.

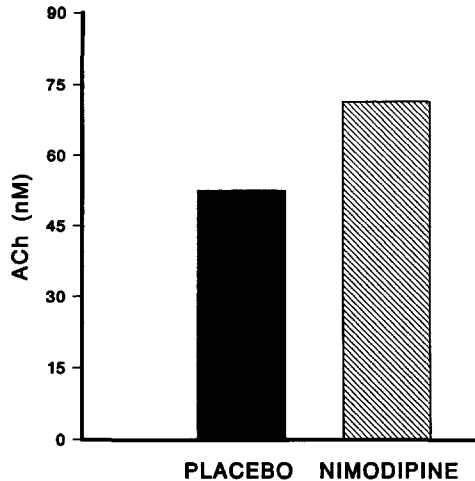


FIG. 4. Mean extracellular hippocampal acetylcholine levels in nimodipine- and placebo-treated rats.

Each training session started when the rat was placed in the center of the maze inside a transparent acrylic barrier that prevented access to the arms of the maze. After 20 s, the barrier was removed, and the rat was permitted free access to all the baited arms. The rat was required to learn a win-shift foraging strategy; visited arms were not rebaited. Reentries into previously visited arms were recorded as errors. The number of errors, as well as the time to complete a trial, were monitored. The number of correct entries in the first eight choices was also recorded. Each session was terminated when all eight arms had been visited, when 16 choices had been made, or at the end of 10 min, whichever came first.

Three parameters were recorded: number of correct choices out of the first eight; percent errors per day; and total time for completion of the session divided by the total number of choices (referred to as "time per choice").

Plasma Nimodipine Assays

On the 17th day after pellet implantation, approximately 0.5 ml of blood was withdrawn from the tail vein of each animal and held on ice. Each sample was centrifuged to separate out serum and stored at -20°C . Nimodipine levels were assayed using capillary gas chromatography (10).

Microdialysis

After behavioral testing was completed, *in vivo* microdialysis was performed to assess extracellular hippocampal ACh levels in five animals from each group. On the 17th or 18th day following pellet implantation, rats were anesthetized with a mixture of urethane (500 mg/kg) and alpha-chloralose (50 mg/kg) IP. A guide cannula (Carnegie Medicin, Stockholm, Sweden) was then implanted in the cranium of each rat using a small animal stereotaxic device (David Kopf, Tujunga, CA). Following a 48–72 h recovery period, a dialysis probe with a 3 mm semipermeable membrane at its tip was inserted through the guide cannula to monitor ACh. During all microdialysis sessions, the rats were free to move in a transparent acrylic bowl with sloping sides (height=35 cm; diameter at the top=39 cm). A liquid swivel mounted on a counterbalanced arm was attached to the rim of the bowl (CMA/120; Carnegie Medicin, Stockholm,

Sweden). All microdialysis measurements were conducted between 0800 and 1600 h. When placed in the bowl, the rats were usually inactive, except for a brief initial period of exploration. The permeable region of the probe was located in the CA1 and dentate gyrus (DG) regions of the hippocampus. The stereotaxic coordinates for the 3-mm permeable region of the probe were: AP = -3.8 ; L = $+1.6$ with respect to bregma; and V = 1.7 – 4.7 with respect to dura (18). Using a CMA/100 microinjection pump (Carnegie Medicin), the probe was perfused at a rate of $2\ \mu\text{l}/\text{min}$ with Ringers solution containing $10\ \mu\text{M}$ of neostigmine to inhibit acetylcholinesterase (AChE) activity. Individual samples were collected every 15 minutes. The first two samples were discarded, and the next four individually assayed and the results averaged. Samples were immediately analyzed using high performance liquid chromatography with electrochemical detection (HPLC-EC). The HPLC was fitted with an MF-8910 ACh assay kit (Bioanalytical Systems, West Lafayette, IN), which contains an analytical column and an enzyme column (AChE plus choline oxidase). Percent ACh recovery for every probe was estimated *in vitro*. Mean recovery was 19.6 ± 0.7 percent (mean \pm SEM). However, all data are reported without correction for recovery, since *in vitro* recovery values appear to provide unreliable estimates of *in vivo* recovery (1,8).

Histology

To verify the placement of the microdialysis probe, animals were euthanized and their brains removed and fixed in 10% neutral buffered formalin and processed routinely for paraffin embedding. Six micron coronal sections at the level of the hippocampus were cut serially and stained with hematoxylin and eosin (H&E).

Statistical Analysis

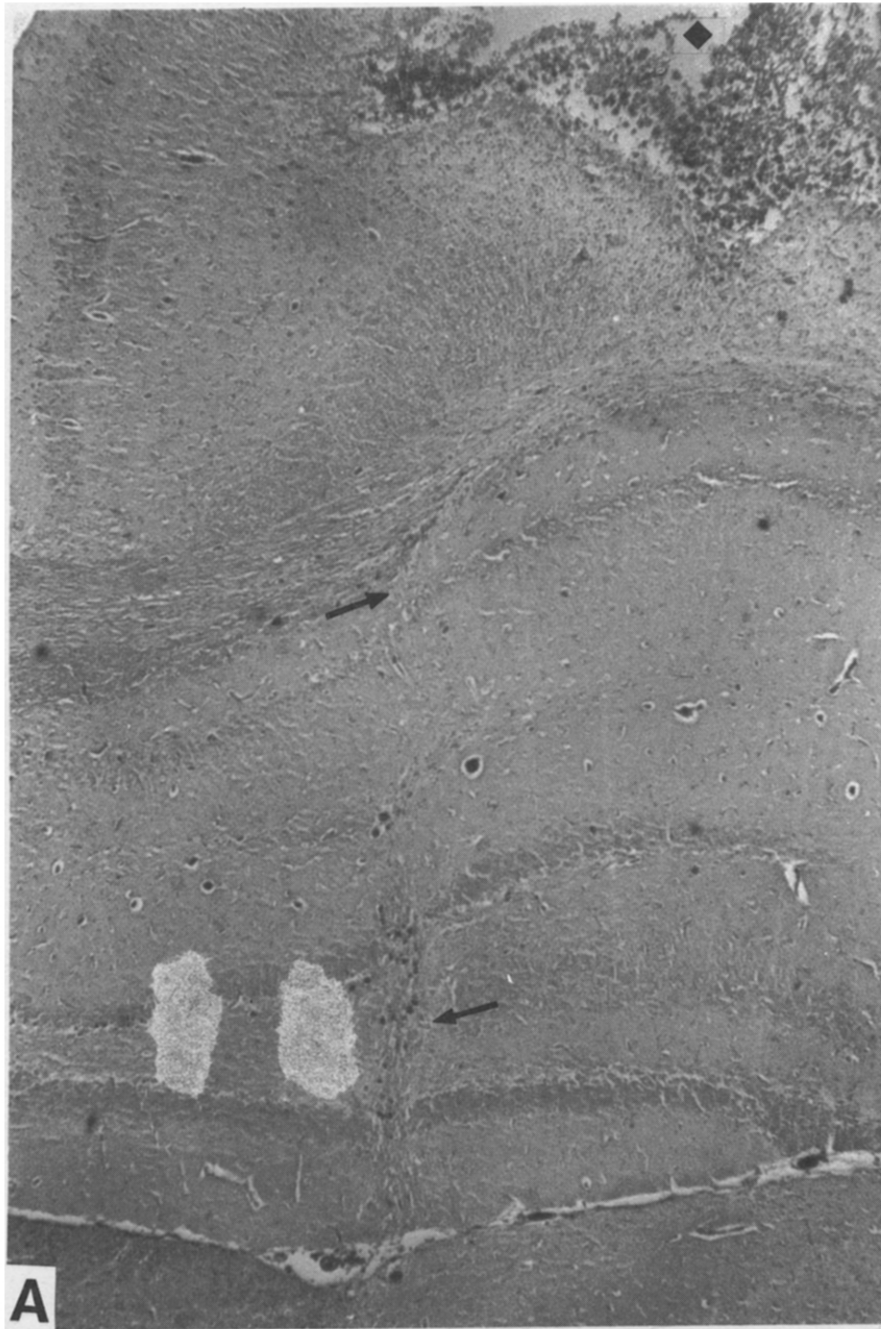
Analysis of variance (ANOVA) was employed to evaluate behavioral and microdialysis results. For the behavioral part of the study, two-way ANOVAs, with one between-subject variable, drug, and one within-subject variable, test day, were employed. Microdialysis derived differences in ACh levels across groups were tested using one-way between-subject ANOVAs. Differences of $p < 0.05$ were considered to be statistically significant.

RESULTS

Behavior

Figure 1 illustrates the pattern of learning, as assessed by the number of correct responses out of the first eight choices, in each experimental group. On days one and two of testing, there was little difference between groups; however, by day three, performance of the nimodipine-treated animals was substantially better than that of controls. By day eight, nimodipine-treated animals achieved mean levels of performance that were near perfect (7.7 correct out of eight) while control animals failed to achieve this level of performance even after 12 days of testing. ANOVA showed a significant effect of drug ($F = 25.51$, $p = 0.0005$) and day ($F = 5.96$, $p < 0.0001$) but no significant drug-by-day interaction.

A similar pattern was observed for the percent error parameter (Fig. 2) with nimodipine-treated animals appearing to perform more accurately starting on day three and achieving near perfect performance by day eight. However, this difference was not statistically significant, although there was a clear improve-



ABOVE AND FACING PAGE

FIG. 5. (A) Section through the hippocampal CA1 and DG regions showing microdialysis probe track marked with arrows. Cannula site, marked by a diamond, is visible in the upper layers of the cortex. H&E staining. Original magnification $\times 73$. (B) Higher magnification of the hippocampal CA1 sub-field illustrating the microdialysis probe track; note normal appearance of nearby neurons. H&E staining. Original magnification $\times 292$.

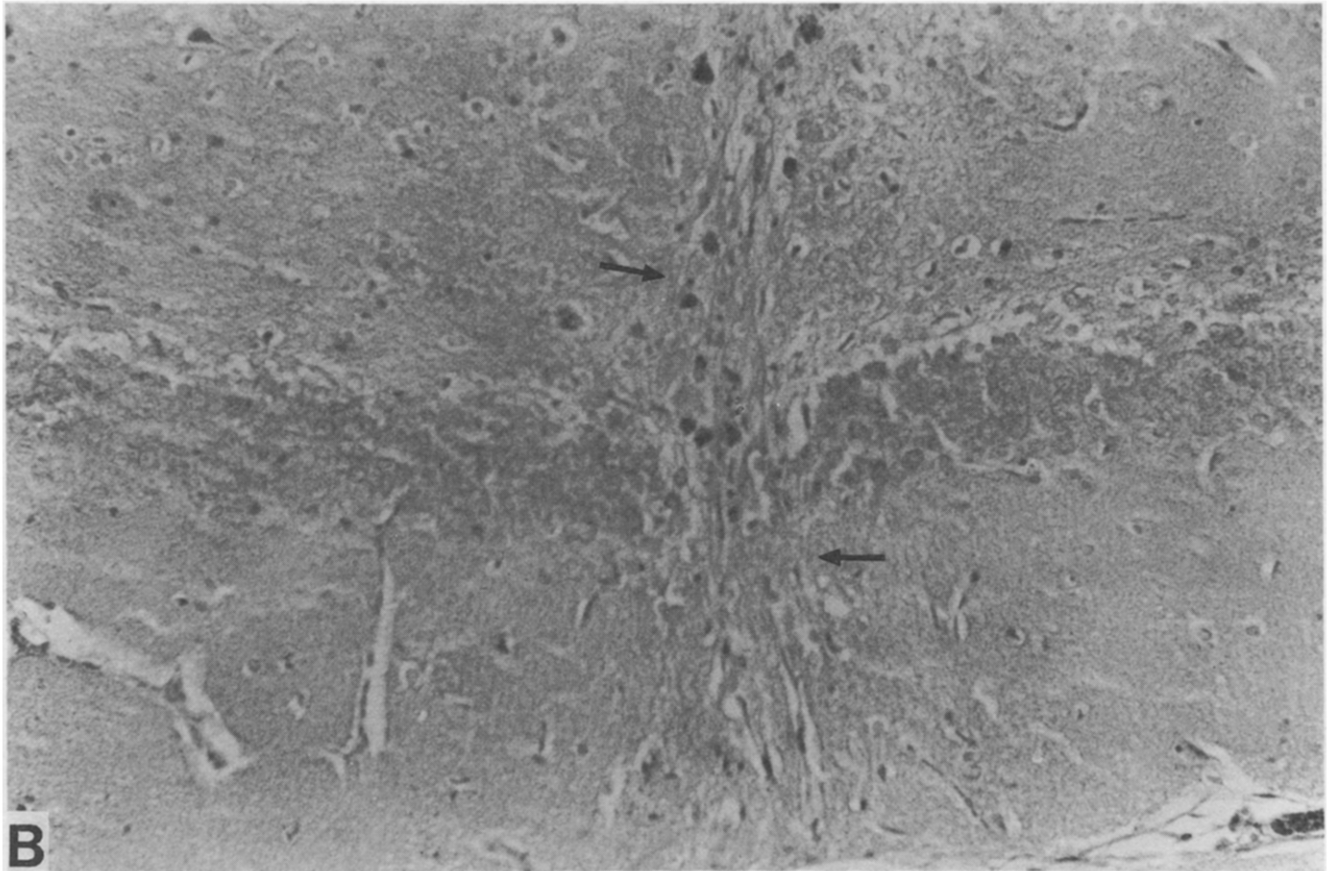
ment over days ($F=6.22$, $p<0.0001$). There was no drug-by-day interaction.

The third parameter assessed, time per choice, is presented in Fig. 3. Differences between placebo and nimodipine-treated rats were present throughout testing, with nimodipine-treated rats performing the task more rapidly. ANOVA detected a drug effect ($F=12.90$, $p=0.005$) as well as a day effect ($F=3.74$,

$p=0.0001$), but not a drug-by-day interaction.

Plasma Nimodipine Levels

On the 17th day after pellet implantation, plasma nimodipine concentration was 10.2 ± 0.4 ng/ml (mean \pm SEM) in the nimodipine-treated animals and undetectable in the plasma of placebo-treated rats.



Microdialysis

On drug days 17 and 18, cannulae were implanted in five animals that were randomly selected from each group tested in the 8-arm radial maze. One nimodipine-treated rat did not survive surgery. Hippocampal ACh levels were assessed on drug days 19–21, and found to be significantly elevated by nimodipine treatment (71.4 ± 3.6 nM; mean \pm SEM), as compared to placebo treatment (52.5 ± 2.5 nM) ($F = 19.83$, $p = 0.003$) (Fig. 4).

Hippocampal ACh levels were also assessed in the ten rats not tested in the 8-arm radial maze. Five animals from this group who received nimodipine and five with placebo pellets had cannulae implanted on days 17–18 and were tested on days 19–21 (one nimodipine-treated animal did not survive surgery). In this group of animals, probes with 2 mm membranes were used, and four individual samples of 40 μ l each were collected at a rate of 2 μ l/min starting 30 min after insertion of the probe. Samples were analyzed as before and averaged. Nimodipine-treated rats had significantly elevated hippocampal ACh levels (90.1 ± 14.9 nM) compared to placebo-treated animals (53.3 ± 4.38 nM) ($F = 7.13$, $p = 0.032$). The ACh levels of the second two groups of microdialysis animals could not be formally compared to ACh levels in the radial arm tested animals, since different-sized probes were employed. However, the magnitude and direction of the effects observed were very similar across groups.

Histology

Placement of the microdialysis probe was verified in the H&E stained serial sections. In all brains examined, the track of

the probe was observed to be within the CA1-DG regions of the hippocampus. In all animals, no detectable damage was observed in cells adjacent to the probe track. Representative sections from two animals are shown in Fig. 5.

DISCUSSION

This study indicates that sustained-nimodipine treatment improves certain aspects of cognitive performance in young rats learning the 8-arm radial maze. Statistically significant effects between drug groups were observed in two of the three parameters assessed. Animals in the nimodipine-treated group made more correct entries in their first eight choices and performed the task more rapidly, as indicated by the time-per-choice parameter. Therefore, it appears that nimodipine improves both accuracy (correct entries) and speed (time per choice) in normal, healthy rats when they acquire a task requiring spatial working memory.

In addition to improving spatial working memory function, nimodipine treatment also affected extracellular hippocampal ACh. Large, statistically significant increases in ACh were observed not only among the animals tested in the radial maze, but also in a second group of rats. These findings are consistent with electrophysiologic measurements reporting increased hippocampal cell firing in aged rabbits (24), as well as in young and old rats (13) following nimodipine treatment.

The mechanism whereby sustained nimodipine treatment caused an elevation in hippocampal ACh levels is uncertain. The effects of nimodipine on the hippocampal cholinergic system may be related to its effects on calcium channels, or via some other mechanism of action. Studies of calcium uptake into rat whole

brain synaptosomes suggest that nimodipine may have a minimal effect on central calcium channels at concentrations in the range probably present in the brains of our animals (14), although dose-effect relationships *in vitro* may be considerably different from those observed *in vivo*. Furthermore, other calcium channel blockers such as nifedipine and flunarizine do not increase hippocampal firing (24), suggesting the possibility of a unique mechanism of action for nimodipine.

Nimodipine has been shown to increase release of dopamine in electrically stimulated brain slices taken from the striatum and cerebral cortex of rats (14). In the same study, nimodipine was found to inhibit the release of ACh from these same brain regions. Since data from electrically stimulated brain slices and *in vivo* microdialysis are not directly comparable, it cannot be concluded that nimodipine has different effects on different brain regions; however, this hypothesis cannot be excluded.

Evidence from pharmacologic, electrophysiologic, and behavioral studies unequivocally demonstrates that cholinergic hippocampal neurons are critical for the performance of tasks with significant spatial working memory components (2, 4, 17). This

study supports that body of literature by demonstrating both an increase in the release of hippocampal ACh, and an improvement in spatial working memory, as consequences of sustained treatment with nimodipine. Future studies should define the mechanisms of action of nimodipine and its relationships to spatial working memory.

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