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Nimodipine enhances spontaneous activity of hippocampal pyramidal neurons in aging rabbits at a dose that facilitates associative learning

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The functional activity of hippocampal neurons is strongly correlated with behavioral performance in a vertebrate model learning system, rabbit eyeblink conditioning. Using this system, we have previously shown that (a) complete removal of the hippocampus blocks acquisition of the conditioned response; (b) a calcium-dependent postsynaptic afterhyperpolarization is reduced in pyramidal cells recorded intracellularly in hippocampal slices taken from conditioned rabbits; and (c) nimodipine, a 1,4-dihydropyridine calcium-channel antagonist, facilitates acquisition of the conditioned response in aging rabbits. Although calcium-channel antagonists directly block neuronal calcium currents *in vitro*, they also alter cerebral blood flow *in vivo*. Thus, the effects of nimodipine on hippocampal neuronal activity in awake animals were examined, with controls for cerebrovascular changes. A total of 457 pyramidal cells and 160 theta cells were studied. During infusion of nimodipine, pyramidal cell firing activity was enhanced and theta interneuron activity was suppressed at all doses tested in aging animals. This effect was rapidly reversed when infusion of the drug ceased. The greatest enhancement of neuronal firing was seen at the most behaviorally effective dose of nimodipine. The enhancement of pyramidal cell firing was age-dependent, with greater increases in firing activity seen in aging than in young animals, but with a similar dose-dependent pattern of effects in the two age groups. Two other calcium-channel antagonists, nifedipine and flunarizine, did not significantly alter spontaneous firing rates of hippocampal neurons. A calcium-channel agonist, BAY-K-8644, produced less easily interpretable results. BAY-K-8644 enhanced interneuron activity at one dose, but enhanced pyramidal cell activity at a dose one log unit higher. The calcium-channel agonist's enhancement of pyramidal cell activity at the highest dose was sustained up to 1 h after drug infusion. Nimodipine's enhancement of the activity of hippocampal pyramidal cells is consistent with the hypothesis that these neurons, which play a necessary role in some forms of learning, may mediate the calcium-channel antagonist's behavioral effects.

INTRODUCTION

Aging is a complex and poorly understood process, apparently regulated by multiple mechanisms, only one of which is the simple passage of time. A great deal of emphasis has been focused lately on neuronal deficits associated with aging. Alzheimer's disease^{6,57,58,82} and Parkinsonism^{16,52} are two devastating pathological syndromes in humans closely linked to the aging process, producing severe physical and behavioral deficits. Although these disorders deserve increased clinical and research attention, the majority of the aging population exhibits little or no major neurological deficits, and certainly none of this magnitude²⁶. It has been proposed, however, that age-associated memory impairment (AAMI^{14,15,83}) may be quite common, and may impact a large proportion of the normal aging population. Dysfunctions of the hippocampus are implicated in the learning deficits associated with Alzheimer's disease⁸¹, and could be involved in other age-associated behavioral

deficits. Considerable anatomical^{18,28,48}, neurochemical^{2,24,25,40} and physiological^{4,5,39} data have been accumulated showing age-associated alterations in the mammalian hippocampus. The hippocampus is one potential central site of action of pharmacological agents that may ameliorate or eliminate the neuronal impairments associated with aging. Our convergent interests in hippocampal mechanisms underlying the learning process and age-associated deficits in these processes have led us to investigate impairments in learning associated with normal aging, using an animal model²⁹ of human learning¹¹, trace eyeblink conditioning in awake rabbits⁶⁹.

The deficits in learning exhibited by aging rabbits in the trace eyeblink conditioning task^{19,30,68,71,75} are functionally similar to those seen in young animals performing the same task after complete hippocampal removal^{55,70}. Both intact aging and hippocampectomized young subjects typically fail to learn the task successfully. Nimodipine, a 1,4-dihydropyridine L-type calcium-channel antagonist, facilitates associative learning of the

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trace-conditioned eyeblink response in aging rabbits when given peripherally^{19,75}. Nimodipine also reverses aging-associated deficits in two other learning tasks that show a similar dependence on intact hippocampal function: delayed non-matching-to-sample in aging primates⁶⁰ and spatial water-maze learning in rats^{61,65}. Strong evidence links the activity of hippocampal pyramidal neurons to acquisition of conditioned responses⁸, and indicates a deterioration of the functional activity of these cells with aging³. The physiology of hippocampal interneurons has rarely been studied in the context of aging or of calcium-channel blockade, and only infrequently in the context of learning. New recording⁷⁷ and unit isolation techniques⁵¹ now allow the activity of theta interneurons and of closely associated pyramidal cells to be studied concurrently. The present experiments were designed to examine the effects of nimodipine and related compounds on hippocampal neuronal activity, with simultaneous assessment of their effects on the activity of both pyramidal and theta cells.

Our goal was to determine if nimodipine affected the spontaneous firing rates of hippocampal neurons in our awake restrained rabbit preparation, in order to begin assessing potential neural mechanisms underlying nimodipine's behavioral facilitation in aging animals. Since nimodipine both facilitates learning¹⁹ and increases cerebral blood flow⁶⁴ in aging rabbits, it is possible that its facilitation of learning occurs as a consequence of improved brain perfusion. However, nimodipine blocks L-type calcium currents *in vitro* when the drug has direct access to hippocampal pyramidal cells⁴⁴, and recent studies indicate that nimodipine, unlike many calcium-channel blockers, readily crosses the blood-brain barrier⁸⁰. Thus, nimodipine's facilitation of learning could be due to direct actions at neuronal dihydropyridine receptors. To test these alternate hypotheses, the effects of calcium-channel blockade on hippocampal pyramidal cell activity were tested *in vivo* under the same conditions in which behavioral facilitation occurs. The effects of nimodipine were compared to those produced by other drugs which interact with calcium channels. The dihydropyridine nifedipine and the diphenylalkylamine flunarizine, 2 calcium-channel antagonists that relax vascular smooth muscle and alter cerebral blood flow^{7,63,80} but do not cross the blood-brain barrier as readily as nimodipine, and the dihydropyridine calcium-channel agonist BAY-K-8644, which constricts rather than relaxes the cerebrovasculature⁵⁹, were tested. Hippocampal cellular responses to these agents were compared across a range of doses in aging rabbits, and aging effects on the response to nimodipine were compared between young and aging animals.

MATERIALS AND METHODS

Animals

Aging (mean 47.3 ± 4.6 mo) or young adult (mean 4.1 ± 0.4 mo) female albino rabbits were individually housed, with free access to food and water, on a 12-h/12-h light/dark cycle, under conditions that replicated those used in earlier assays of nimodipine's effects on learned behaviors¹⁹. Rabbits were chronically implanted with moveable bundles of microwire electrodes and indwelling jugular catheters under sterile surgical conditions as described below, and hippocampal single-unit activity was monitored while series of doses of different calcium channel agents were infused systemically. Changes in the rate of spontaneous activity of pyramidal cells and of interneurons, readily identified via extracellular recordings from the CA1 region of dorsal hippocampus, were quantified as a function of age, drug, and dose.

Chronic catheterization

Animals were anesthetized with xylazine and ketamine-HCl. Catheters with non-exteriorized access ports (Access Technologies, Model GPV) were surgically implanted in the right jugular vein. Incisions were infiltrated with lidocaine HCl. An incision was made in the dorsal surface of the neck, and small pockets were formed

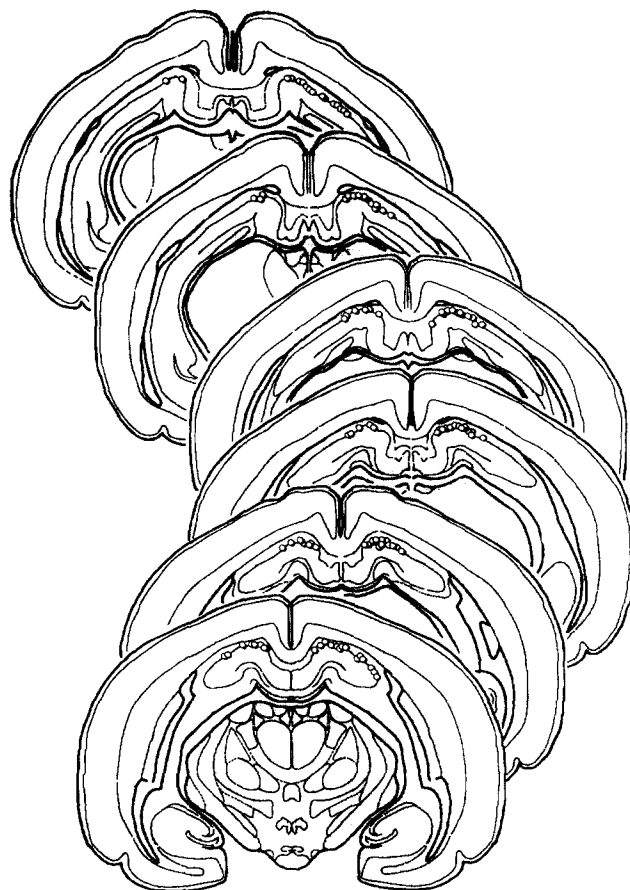


Fig. 1. Identification of electrode sites from which single- or multiple-unit activity was recorded in the CA1 region of the dorsal hippocampus of 9 aging and 3 young adult albino rabbits (*Oryctolagus cuniculus*). A total of 111 different recording sites (●) were used in the present study, an average of 9 sites/animal, with many sites used over multiple sessions as long as stable discriminable single-unit activity was present. Recording sites were identified in Nissl-stained sections via small marking lesions made after experimentation was terminated.

under the skin caudal to this. The jugular was then exposed via the neck. The access port (filled with heparin sodium, 1 kU/ml) was slipped into the dorsal pocket and sutured into place, with the catheter run under the skin, through a second pocket holding a loop of excess catheter, to the jugular. The jugular vein was ligated rostral to the point of entry and transected. The catheter was implanted into the jugular, ligated in place, and the incisions were closed. This procedure was previously used in assessing nimodipine's behavioral effects *in vivo*¹⁹. The chronic catheter was flushed daily with heparin sodium to maintain an open lumen.

Chronic recording instrumentation

Extracellular recording assemblies were implanted during the same surgical session, using techniques adapted from those used for chronic recording in freely-behaving rats^{76,77}. The recording assembly consisted of a microdrive containing a 'floating' bundle of ten 32- μm nichrome microwire electrodes within a stainless steel guide cannula³⁶, and allowed advancement of the electrodes in increments of approximately 20 μm in the awake animal. In most cases, twisted pairs of microwire stereotrodes were used (instead of untwisted single wires) in the electrode assembly to enhance common-mode rejection for single-unit isolation⁵¹. The guide cannula also served as a cortical indifferent electrode. The tips of the microwire

electrodes, cut diagonally at 30°, were implanted in the anesthetized rabbits over the dorsal hippocampus under stereotaxic guidance, and the microdrive assembly was cemented in place with dental acrylic. A restrainer consisting of closely opposed nylon screws with an acrylic base was affixed to the frontal plates of the skull to aid in nontraumatic immobilization of the subjects during recording. The craniotomy was filled with sterile petroleum jelly (refreshed as needed) to allow for electrode advancement and to cover the wound. Animals received Buprenex postoperatively to minimize discomfort and facilitate recovery. The rabbits used recovered rapidly from these procedures, exhibiting normal behavior within 2–3 days.

Neuronal unit recording

After 100 h or more of recovery from surgery, awake rabbits were restrained using snug bags with front and rear drawstrings, placed in a padded plexiglass stock, and habituated to a sound attenuated IAC double-walled Faraday chamber for at least 1 h. The bundles of microwire electrodes were then lowered into the pyramidal cell layer of CA1 of the dorsal hippocampal formation until one or more complex-spike cells were isolated extracellularly. Unit activity was recorded against an adjacent microwire and/or a cortical indifferent electrode using Grass P5 AC preamplifiers (gain $\times 10$ K; bandpass

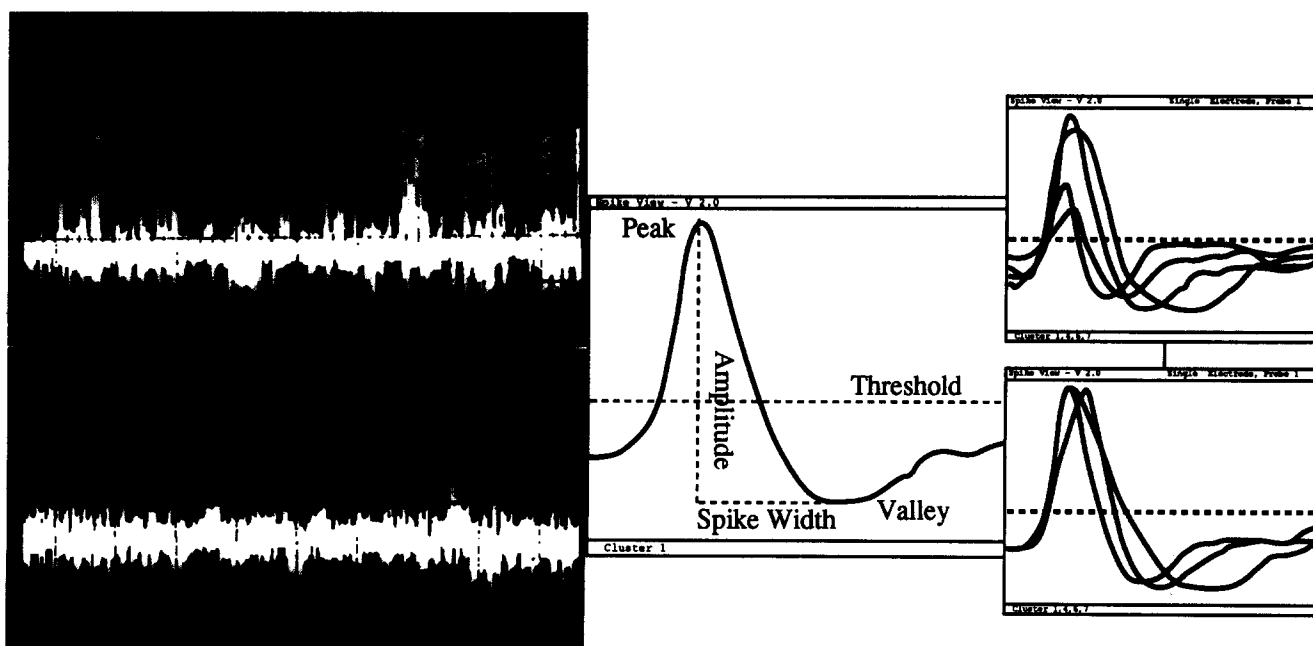


Fig. 2. Single-unit discrimination using the spike separation algorithms of a BrainWave™ workstation. The top trace, left, shows 1 s of a typical multi-unit signal recorded midway through stratum pyramidale of field CA1 in the dorsal hippocampus using a single microwire in a chronic driveable microelectrode³⁶, with clear theta frequency (4–8 Hz) modulation of the firing of many cells. The 2 smallest and the 2 highest amplitude units separated from this signal are shown in the inset at top right; a threshold of 170 μV above ground potential was used to select these and 4 other intermediate amplitude units (not shown, for clarity) for waveform parameter cluster cutting on the workstation. The 2 smallest units separated, with spike widths of 200 μs and of 160 μs , respectively, had spontaneous firing rates of 6.2 ± 0.4 Hz and of 11.6 ± 0.3 Hz, and were classified as theta interneurons, based on Ranck's⁵⁶ criteria. The two largest units, with spike widths of 540 μs and of 430 μs , respectively, had firing rates of 1.2 ± 0.1 Hz and 0.8 ± 0.2 Hz, and were classed as pyramidal cells. The latter 2 units occasionally exhibited complex-spike activity when observed on a storage oscilloscope at a higher sweep speed. The lower trace at left shows 5 s of a classic 'single-unit' signal recorded in the same rabbit, from a microwire in the same bundle located approximately 75 μm dorsal and 200 μm medial to the first recording site, on the dorsal edge of the pyramidal cell layer. Simple window discrimination of this 'unitary' signal (possessing fairly uniform peak time, valley time, and spike width parameters, however, revealed 3 units in this signal (inset, lower right), with firing rates of 1.4 ± 0.2 Hz, 0.5 ± 0.1 Hz, and 0.3 ± 0.1 Hz, respectively. The reliability of this separation and of these spontaneous firing rates were confirmed across 18 consecutive 5-min sampling intervals (a total of 90 min of continuous recording), an indication both of the stability of the recording preparation and of the power of the software spike separation algorithms. Many recordings exhibited not only such short-term stability, but also exhibited apparent long-term stability similar to that reported in other chronic preparations⁷⁷, with similar waveform parameters observed from the same electrodes over multiple days. For the present analysis, each waveform isolated on a given day was treated as a single-unit for that day's observation. No attempts were made to verify that cells were 'held' across multiple days.

0.3–3.0 kHz), and displayed on a storage oscilloscope. Single-unit activity was discriminated using a BrainWave™ workstation (see below) and stored online. In the event that waveform analyses indicated that a recording site was not stable compared to previous recordings, the electrode was advanced following a daily recording session to isolate new unit activity, if stable unit activity was not present on other electrodes within the same driveable bundle. At the completion of all experiments, rabbits were deeply anesthetized with intravenous barbiturate, all recording sites were marked via small lesions (10 μ A, 30 s) made through the recording electrodes, and the subjects were perfused transcardially with physiological saline followed by 10% formalin. Histological placement of the recording sites in fixed Nissl-stained tissue sections was compared to the characteristic firing patterns of the neurons recorded; for all cases of data reported here, electrode placement was within *stratum pyramidale* of the dorsal CA1 subfield. Figure 1 shows the recording sites used in the current study within the dorsal CA1 pyramidal cell layer in the aging rabbit.

Single-unit discrimination

A BrainWave™ workstation (BrainWave Systems, Boulder, CO) was used to record and analyze electrophysiological data on-line. This system, running on an Intel-80386 based microcomputer, performed software discrimination of unit waveforms using threshold, amplitude, time, and template matching parameters. The software algorithms used by the BrainWave™ workstation allowing simultaneous recordings to be made from multiple single neurons in real-time are detailed elsewhere⁵¹. Chronic unit activity was also collected and stored on a VCR with a digitizing adapter for later reanalysis on the workstation, to ensure that artifactual changes in the data induced by minor changes in waveform amplitude consequent to global changes in cerebral blood flow were eliminated. In all cases within single recording sessions, spike widths of single-units included for analysis varied by less than 1%, while spike amplitudes varied by less than 5% (e.g. between baseline, drug infusion periods, and post-infusion periods). Figure 2 illustrates some principles of the use of the BrainWave™ workstation for hippocampal single-unit discrimination.

Up to 4 channels of hippocampal extracellular single- or multiple-unit activity were analyzed, allowing simultaneous discrimination of the activity of an average of 15 single-units. The characteristic waveforms and firing frequencies of pyramidal cells and of 'theta' interneurons allowed quantitative separation of these 2 closely associated neuronal populations for study. Complex-spike units were differentiated from the other major behavioral class of hippocampal units, theta cells, based upon Ranck's criteria^{27,56}: complex-spike or pyramidal cells exhibit a low spontaneous rate of activity and a constant broad waveform (>500 μ s duration, measured between amplitude peak and trough) during awake quiet immobility; while theta interneurons have a higher tonic rate of activity, a narrower waveform (<400 μ s duration), and reliable phase-locking to the hippocampal EEG theta rhythm typified by robust autocorrelations of spike activity within the theta bandwidth (140–250 ms interspike intervals)⁵¹. Complex-spike activity was also considered, but not used as a rigid criterion for discriminating cell types, since the proportion of complex-spike bursts to single spike firings varied considerably between pyramidal neurons, and even for the same neuron within sessions. Data collection from a given recording site was terminated if classical 'single-unit' isolation from the site could not be maintained (i.e. peak-to-peak spike amplitudes greater than 4 times the peak-to-peak noise level on the channel were required), and data from such sessions were not included for analysis.

Drug infusion and data collection

Varying concentrations of nimodipine (Miles Pharmaceuticals), nifedipine (Sigma), flunarizine (Sigma), or BAY-K-8644 (Miles) were dissolved in 300 mol. wt. polyethylene glycol vehicle in light-resistant containers to prevent photo-sensitive reactions from occurring. All drugs were delivered via the chronic i.v. catheters to

awake restrained animals, in order to replicate the methodology of our earlier study of nimodipine's effects on associative learning¹⁹. Each series of 1 session of vehicle alone and 4 sessions of different doses of drug was delivered over a period of 5 successive days, followed by at least 2 days of rest between series (rabbits averaged 3 series of drugs each). Each animal received a pseudorandom choice of a different drug for each series of testing, yielding a series of cellular response measures to a range of doses for several drugs from each animal.

A vehicle control session was followed at 24-h intervals by a pseudorandom choice of 0.01, 0.1, 1.0, or 10 μ g/kg/min of drug infusion, as follows. Unit activity was continuously recorded during sessions, with single-neuron firing frequency averaged over 5-min samples to reduce spontaneous fluctuations in firing rate. Two baseline periods were followed by continuous equivolume i.v. infusion of drugs for 65 min, with data collected after 20, 40, and 60 min of infusion, with a final sample of activity assessed 20 min post-infusion. All recording and data analyses were performed blind.

Statistical analyses

Spontaneous single-unit firing rates were calculated across 5-min samples. Mean rates for the baseline, drug-treatment, and post-treatment periods were calculated. Initial statistical tests compared the effects on mean firing rates of the 3 different calcium-channel antagonists used, with appropriate adjustments made for unequal-sized groups. Two-factor ANOVAs (SuperANOVA, Abacus Concepts, Berkeley, CA) were used to compare mean firing rates of complex-spike or of theta cells in aging animals during baseline, drug-treatment, or post-treatment periods. Significant main effects of drug or of dose were evaluated using Scheffé post-tests, with a significance level of $P < 0.05$. Two-factor ANOVAs were also used to assess age-dependent effects of nimodipine infusion at different doses, with significant main effects of age or dose evaluated using Scheffé post-tests. Paired *t*-tests (two-tailed) were then used to test whether unit activity during infusion of specific doses of specific drugs (identified by Scheffé tests as significantly different from unit activity during infusion of other specific drug dosages) was significantly altered from baseline activity. Additional two-factor ANOVAs were used to compare the effects of nimodipine with those of BAY-K-8644, a calcium-channel agonist. Significant main effects of drug or of dose were evaluated using Scheffé post-tests. The results of all statistical analyses are presented below. Drug-treatment and post-treatment rates were also converted to percent differences from the mean baseline firing rate for simplicity of graphic presentation.

RESULTS

Cellular responses to infusion of 3 different calcium-channel antagonists or of 1 calcium-channel agonist were examined using 393 pyramidal neurons and 137 theta interneurons recorded from the dorsal hippocampi of 9 awake intact aging rabbits. Since nimodipine was the only calcium-channel antagonist that produced a significant effect on extracellularly recorded hippocampal single-unit activity in aging animals, and since only nimodipine has been extensively tested for behavioral effects in this preparation, only nimodipine's effects were compared across age groups. Thus, we examined the responses to nimodipine treatment of an additional 64 hippocampal pyramidal neurons and 23 theta cells in 2 young animals. Table I shows the number of cells tested in each condition.

TABLE I

Number of hippocampal single-units sampled in aging or young rabbits during infusion of calcium channel agents at each of 4 doses

Drug	Age group	Pyramidal cells					Theta cells				
		Dose ($\mu\text{g}/\text{kg}/\text{min}$)				All doses	Dose ($\mu\text{g}/\text{kg}/\text{min}$)				All doses
		0.01	0.1	1.0	10		0.01	0.1	1.0	10	
Nimodipine	Aging	27	25	31	29	112	10	6	13	9	38
	Young	17	16	18	13	64	8	4	6	5	23
Nifedipine	Aging	23	21	27	25	96	7	7	7	6	27
Flunarizine	Aging	28	27	22	21	98	9	9	9	9	36
BAY-K-8644	Aging	23	19	27	18	87	8	9	8	11	36
Total aging		101	92	107	93	393	34	31	37	35	137
Total young		17	16	18	13	64	8	4	6	5	23

In comparisons between calcium-channel antagonists, no significant interactions between drug and dosage were found in the two-factor ANOVAs during baseline, drug-treatment, or post-treatment periods for pyramidal cells or for theta cells. Further, no significant main effects of dosage independent of drug were observed. Significant main effects, however, for the calcium-channel antagonist infused were found for both classes of hippocampal neurons. Table II lists the compute F values, degrees of freedom, and associated probabilities for these tests. As indicated by Scheffé post-tests, nimodipine was the only calcium-channel antagonist which produced unit firing activity significantly different from that seen with either of the other drugs tested. Scheffé F -tests indicated that during the drug infusion period, unit activity from pyramidal and from theta neurons recorded from animals receiving nimodipine was significantly different from that of pyramidal and theta neurons recorded from animals receiving nifedipine ($F = 162.76$, $P < 0.004$, and $F = 979.95$, $P < 0.01$, respectively) or flunarizine ($F =$

102.27 , $P < 0.04$, and $F = 962.44$, $P < 0.02$, respectively). The rate of unit firing for either the pyramidal or theta cell types did not differ significantly among the different calcium-antagonist treatment groups during the baseline ($P > 0.36$ and $P > 0.59$, respectively) or during the post-infusion intervals ($P > 0.24$ and $P > 0.37$, respectively), indicating that the pools of neurons assessed in each group had similar firing properties in the absence of drug treatment.

Dose-dependent effects of nimodipine on hippocampal pyramidal neurons

Nimodipine altered the spontaneous firing rates of hippocampal neurons in awake aging rabbits. Nimodipine increased the firing of pyramidal cells but decreased the firing of closely associated theta interneurons at all doses tested, with the greatest magnitude effects seen at behaviorally effective doses (see Fig. 3 and Table II). Scheffé post-tests indicated significant differences in the magnitude of the drug-induced increases between all

TABLE II

The results of two-factor analyses of variance testing significance of interactions and main effects of calcium antagonist (Drug) used or of dose given (Dose) at all intervals tested for hippocampal theta and pyramidal neurons

The only significant effects are by drug, during drug infusion intervals. The resulting F scores (F), degrees of freedom (df), and associated probabilities (P) are listed.

Cell type	Testing interval	Drug \times dose			Drug			Dose		
		F	df	P	F	df	P	F	df	P
Theta cells										
	Baseline	0.61	6,105	>0.72	2.54	2,105	>0.08	0.15	3,105	>0.93
	Drug infusion	0.59	6,105	>0.81	3.51	2,105	<0.03	0.33	3,105	>0.81
	Post infusion	0.45	6,105	>0.84	3.02	2,105	>0.05	0.25	3,105	>0.86
Pyramidal cells										
	Baseline	1.68	6,294	>0.13	0.88	2,294	>0.42	0.86	3,294	>0.46
	Drug infusion	1.84	6,294	>0.12	7.89	2,294	<0.0005	1.84	3,294	>0.14
	Post infusion	1.75	6,294	>0.11	1.42	2,294	>0.24	0.45	3,294	>0.72

possible comparisons of doses except between the doses of 0.1 and 10.0 $\mu\text{g}/\text{kg}/\text{min}$ of nimodipine ($P > 0.62$). Infusion of the lowest dose of nimodipine, 0.01 $\mu\text{g}/\text{kg}/\text{min}$, increased pyramidal cell activity compared to baseline spontaneous activity ($t_{26} = 2.62$, $P < 0.02$). Significant increases in pyramidal cell activity relative to baseline were also seen during infusions of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ of nimodipine ($t_{24} = 7.89$, $P < 0.0001$) and of 10.0 $\mu\text{g}/\text{kg}/\text{min}$ of nimodipine ($t_{28} = 6.37$, $P < 0.0001$). The largest magnitude effect was seen during infusion of 1.0 $\mu\text{g}/\text{kg}/\text{min}$ of nimodipine, when spontaneous firing activity was enhanced over 90% compared to baseline spontaneous activity ($t_{30} = 6.81$, $P < 0.0001$). Behavioral dose-response curves in aging rabbits similarly show that doses of 1.0–2.0 $\mu\text{g}/\text{kg}/\text{min}$ of nimodipine produce maximal facilitation of learning in aging animals, while doses one log unit higher or lower are much less efficacious (Deyo, Straube and Disterhoft, unpublished observations). The enhancement of spontaneous pyramidal cell firing activity by nimodipine was rapidly reversed when drug infusion was stopped. At all doses of nimodipine tested, firing rates of pyramidal or theta neurons were not significantly different from baseline activity within 20 min post-infusion ($t_{455} = 0.81$, $P > 0.5$ and $t_{158} = 0.73$, $P > 0.5$). The spontaneous activity of theta interneurons in the dorsal hippocampus was significantly depressed only during nimodipine infusion ($F_{3,105} = 3.51$, $P < 0.03$), and post-tests indicated the effect was significant in comparison to the lack of effect of the other calcium-channel antagonists nifedipine ($F = 962.44$, $P < 0.02$) and flunarizine ($F = 979.95$, $P < 0.01$). There was no significant difference in the effects on interneuron activity between any of the doses of nimodipine tested, however ($F_{3,37} = 0.24$, $P > 0.87$). The suppression of theta interneuron firing rates during nimodipine infusion was significantly different from the firing rates observed

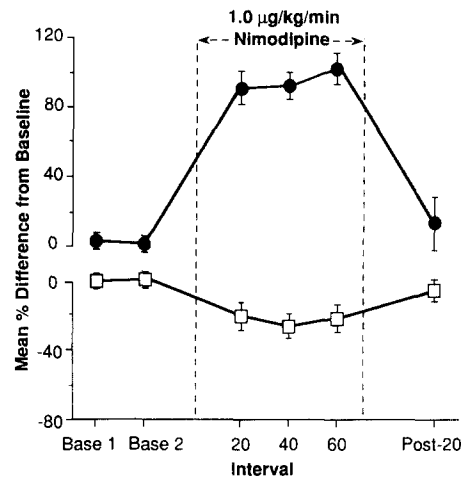


Fig. 3. Time-course of the dose-dependent effects of intravenous infusion of the 1,4-dihydropyridine nimodipine on spontaneous single-unit activity recorded from the dorsal hippocampus of aging rabbits. The mean spontaneous firing activity of hippocampal pyramidal neurons (●) and of hippocampal theta interneurons (□) are shown at each of the 5-min intervals measured: during 2 baseline periods; 20, 40, and 60 min after the start of drug infusion; and 20 min after drug infusion was terminated. The behaviorally effective dose of 1.0 $\mu\text{g}/\text{kg}/\text{min}$ of nimodipine produced maximal enhancement of pyramidal cell firing and moderate suppression of theta cell firing. As can be seen, the response to intravenous nimodipine in vivo had a fairly rapid onset (typically less than 8 min) and was markedly stable throughout drug administration at all doses tested. All doses of nimodipine produced a statistically significant enhancement of pyramidal neuron firing, which returned to rates similar to those observed during the pre-drug baseline within 20 min post-infusion.

during baseline periods, as determined by paired t -tests (lowest $P < 0.03$).

Age-dependent effects of nimodipine on hippocampal pyramidal neurons

The baseline spontaneous firing rate of pyramidal

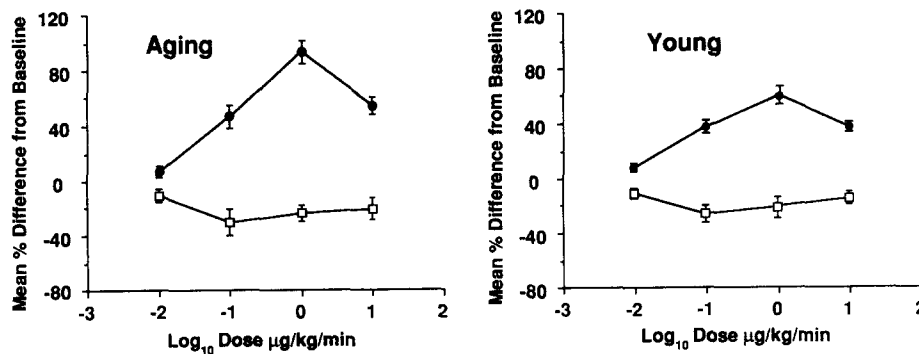


Fig. 4. Age-dependence of nimodipine's effects on hippocampal pyramidal neuron activity. The L-type calcium channel blocker enhanced the spontaneous firing rates of pyramidal neurons (●) in aging animals to a significantly greater degree than it did those in young animals. The enhancement of pyramidal cell firing rates seen in both groups was greatest at the behaviorally effective dose of 1.0 $\mu\text{g}/\text{kg}/\text{min}$ with a similar pattern of dose relationships and time-courses of the effects seen in both age groups. The effect of nimodipine on theta interneuron firing rates (□) was similar in both age groups at all doses tested.

neurons from aging subjects (mean 0.98 ± 0.05 Hz) was slightly lower than that for young subjects (mean 1.10 ± 0.19 Hz), but was not significantly different ($F_{1,456} = 0.65$, $P > 0.4$). Similarly, the baseline spontaneous firing rate of theta neurons from aging subjects (mean 7.16 ± 1.87 Hz) was slightly faster than that for young subjects (mean 6.65 ± 1.91 Hz), but was not significantly different ($F_{1,159} = 0.72$, $P > 0.5$).

Nimodipine infusion produced the greatest facilitation of spontaneous firing rate in pyramidal cells from aging subjects, the same subject group that shows facilitation of associative learning with nimodipine treatment. Pyramidal cell spontaneous firing rates were enhanced over 90% during infusion of $1.0 \mu\text{g/kg/min}$ of nimodipine in aging subjects, compared to the average 58% enhancement seen at the same dose in young subjects. Comparisons of firing rates of pyramidal cells in aging and young subjects revealed significant differences between age groups only during infusion of nimodipine ($F_{1,175} = 15.08$, $P < 0.001$). No differences in the effects of nimodipine on theta interneuron firing rates were found between age groups ($F_{1,60} = 1.08$, $P > 0.19$).

A pattern of dose-dependent facilitation of pyramidal cell firing similar to, but smaller than that observed in

aging rabbits was seen in the young subjects. When firing rates during drug infusion were compared to spontaneous baseline activity, the firing rates of pyramidal cells were significantly enhanced at doses of 0.01 ($t_9 = 3.63$, $P < 0.04$), 0.10 ($t_5 = 4.29$, $P < 0.03$), 1.0 ($t_{12} = 5.08$, $P < 0.01$), and 10.0 ($t_8 = 4.07$, $P < 0.03$) $\mu\text{g/kg/min}$ of nimodipine. As in aging subjects, these effects were rapidly reversed in young subjects by termination of nimodipine infusion, with firing returning to rates indistinguishable from baseline rates within 20 min post-infusion ($F_{3,61} = 0.94$, $P > 0.79$). Figure 4 illustrates the age-dependence of nimodipine's effects on pyramidal neuron activity.

Lack of effect of nifedipine, flunarizine, or vehicle on hippocampal neurons

As reported above, the dihydropyridine calcium-channel antagonist nifedipine and the diphenylalkylamine calcium-channel antagonist flunarizine, which affect vascular smooth muscle but do not cross the blood-brain barrier as readily as nimodipine, had no significant effects on hippocampal single-unit activity (see Fig. 5). None of the doses tested altered the spontaneous firing rates of the cells on average by more than 15%, and none

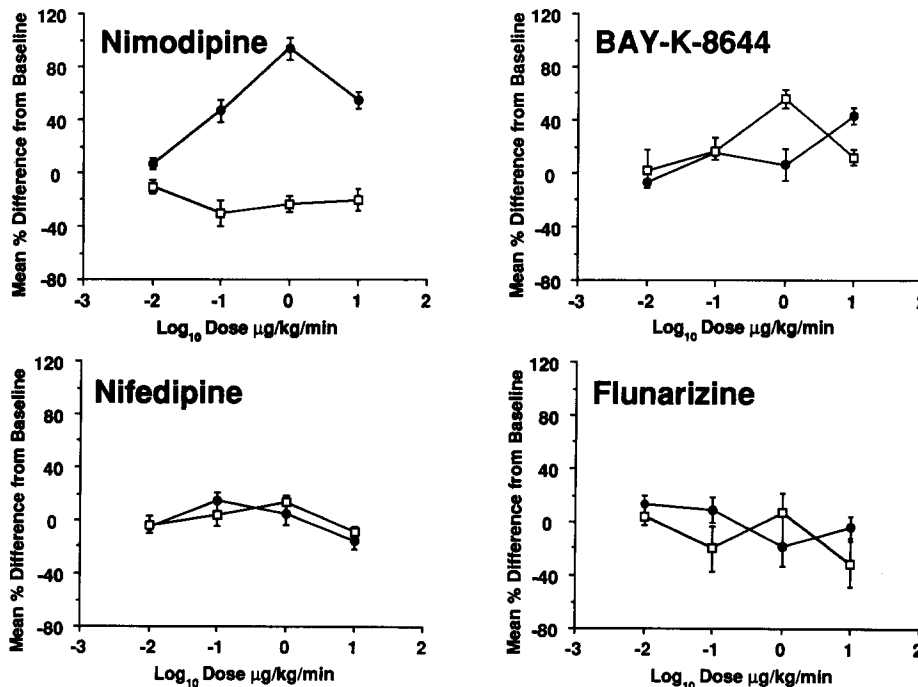


Fig. 5. Dose-response curves showing the effects of nimodipine and nifedipine (both dihydropyridine calcium channel antagonists) and of BAY-K-8644 (a dihydropyridine calcium channel agonist) and of the non-dihydropyridine flunarizine (a diphenalkylamine calcium channel antagonist) on the mean spontaneous firing rates of hippocampal neurons in aging rabbits. While nimodipine enhanced the firing rates of pyramidal cells (●) and slightly depressed the firing of theta cells (□), neither nifedipine nor flunarizine had significant effects on either cell type. BAY-K-8644 had generally opposite effects to those of nimodipine on theta cells, but only significantly enhanced the firing rates of pyramidal cells at the highest dose tested. The effects of BAY-K-8644 also did not terminate with the end of drug infusion, but continued up to 1 h after drug infusion was stopped.

produced a sustained change in firing rate throughout all periods of drug infusion as were seen with nimodipine treatment.

Effects of calcium agonist BAY-K-8644 different from calcium antagonist nimodipine

The calcium-channel agonist, BAY-K-8644, produced a different pattern of changes in neuronal activity, with no significant effects at lower doses, and a greater effect on interneurons than on pyramidal cells (see Fig. 5). Statistically, the pattern of firing activity of pyramidal cells showed a significant difference between treatment with the agonist or the antagonist only during drug infusion, with significant interactions for drug and dose ($F_{1,191} = 1.87, P < 0.005$). As seen in Fig. 5, low doses of BAY-K-8644 slightly suppressed or had no effect on pyramidal cell firing rates, while the highest dose enhanced firing rates. Nimodipine at all doses enhanced pyramidal cell firing rates. The interaction, attributable to BAY-K-8644's mixed suppressant and facilitatory effects across doses, may be due to the racemic nature of the drug (see Discussion, below). Due to the interaction, however, no definitive statement comparing calcium-channel agonist and antagonist effects can be made.

The largest change produced by BAY-K-8644 was seen at 1.0 $\mu\text{g}/\text{kg}/\text{min}$, with a significant enhancement of the firing rate of theta cells compared to baseline firing rates ($t_7 = 7.31, P < 0.01$). At a dose one log unit higher, however, no significant enhancement of theta interneuron activity occurred ($t_{10} = 0.81, P > 0.8$). Instead, 10.0 $\mu\text{g}/\text{kg}/\text{min}$ of BAY-K-8644 significantly enhanced pyramidal cell activity ($t_{28} = 4.69, P < 0.01$). The enhancement of pyramidal cells at the latter dose of BAY-K-8644 was long lasting, with data obtained 20 min post-infusion indistinguishable from that obtained during drug infusion ($t_{28} = 1.06, P > 0.5$). In most cases, enhancement of pyramidal cell activity persisted up to 1 h after drug infusion ended. No evidence of seizure activity was observed in the hippocampal EEG at any of the doses of BAY-K-8644 tested.

DISCUSSION

Nimodipine, a 1,4-dihydropyridine L-type calcium-channel antagonist, altered the spontaneous firing rates of hippocampal neurons in chronically instrumented awake rabbits in both a dose- and an age-dependent manner, while other calcium-channel antagonists had little or no effect. Nimodipine maximally increased the firing rate of hippocampal pyramidal cells at the same dose that previous work from our laboratory showed facilitated associative learning in aging rabbits¹⁹. The activity of closely associated theta interneurons was

decreased at the same dose and over the same time-course. These data support our working model that nimodipine enhances hippocampal function in aging animals. Changes in neuronal activity occur in the hippocampus during nimodipine treatment, and these changes are appropriate to those required in the context of behavioral learning. Nimodipine's alteration of neuronal activity, reaching a maximum of a 2-fold increase in the spontaneous firing of pyramidal neurons, is less than that observed during acquisition of a conditioned response by a young animal⁸, but is in the same direction and may be sufficient to allow further increases in activity in the context of learning. As seen, systemic administration of nimodipine is sufficient to produce neurophysiologically effective levels within the central nervous system, and to ameliorate age-associated behavioral deficits in rabbits^{19,20,75} and in rats^{61,65}.

Until now, no studies of single-unit activity in the aging rabbit hippocampus comparable to those available for the rat^{3-5,39} have been reported. This study is the first to assess the effects of calcium channel blockade on single-unit activity in the aging rabbit. The current study is also the first to demonstrate that nimodipine has direct effects on hippocampal neurons in intact animals, and that these effects are similar to those seen in hippocampal neurons as a consequence of learning. Several lines of evidence are discussed below in support of a working model of hippocampal function in learning, and the effects of nimodipine on this model.

Since nimodipine facilitates acquisition in aging rabbits but has no significant effect on acquisition of the conditioned response in an associative learning task in young rabbits¹⁹, we compared its effects on unit activity across age groups. As the behavioral data predict, nimodipine produced less enhancement of hippocampal pyramidal neuron activity in young animals than in old. The dose-response curves for pyramidal neurons in the two age groups were similar in direction, although the slopes of the curves were exaggerated in aging animals (see Fig. 4). Since nimodipine's effects on theta interneurons were not age-dependent, nimodipine's behavioral effects may be mediated by pyramidal neurons. This interpretation is consistent with earlier work showing that changes in the firing of pyramidal cells but not of theta interneurons are strongly correlated with behavioral conditioning⁸. A significant facilitation of learning by nimodipine in aging but not in young animals¹⁹ might be attributable to physiologically inadequate increases in pyramidal cell activity in young animals, or to ceiling effects in the behavioral measurements used. Using the non-specific calcium antagonist Mg^{2+} , different behavioral assays have revealed facilitation of learning in young as well as aging animals⁴⁵. Chronic feeding of Mg^{2+} to

rats also produced greater improvements in long-term frequency potentiation of aging hippocampal neurons (as compared to untreated aging controls) than in similarly treated young animals. Behavioral facilitation was seen in both age groups, although the greatest facilitation of maze learning was observed in aging rather than young animals. The high specificity of nimodipine for L-type calcium channels⁶⁴ and the more robust behavioral¹⁹ and neuronal age-dependent effects reported for this agent than for non-specific blockers suggest that L-type channels may be strongly affected by aging processes in the brain.

Calcium plays a complex role in the regulation of cellular processes in the hippocampus. Pyramidal neurons possess regenerative calcium spikes⁸⁴, and active regions of dendritic membrane in these neurons also possess calcium channels⁷⁹. A number of calcium-dependent potassium currents ($I_{K-Ca^{2+}}$) are involved in repolarization of hippocampal neurons after action potential generation^{1,31,34,41,42,47,66,74,85}. The hippocampus is particularly rich in binding sites both for dihydropyridine calcium-channel antagonists^{63,67} and for apamin and charybdotoxin, antagonists of 2 classes of calcium-dependent potassium channels^{53,73}. Several theories assert that calcium-dependent cellular processes are disturbed in cells from aging animals^{35,43,44}.

A working model of the neurobiological mechanism of nimodipine's behavioral actions is that peripherally administered nimodipine acts centrally. Its antagonism of neuronal L-type calcium conductance(s) would indirectly reduce the calcium-dependent slow-AHP in hippocampal pyramidal neurons of aging rabbits, similar to the AHP reductions observed in young hippocampal neurons after conditioning^{17,21,23}. Landfield and colleagues have shown that calcium currents are increased in hippocampal pyramidal neurons from aging rats, and that nimodipine reduces these currents more effectively in aging than young CA1 hippocampal neurons¹². Similarly, the slow AHP is increased in CA1 neurons from the aging rat hippocampus⁴⁶ compared to those seen in young hippocampal neurons. It has been suggested that the learning deficits that accompany normal aging may be due to difficulty in modulating calcium-dependent responses at the cellular level in the hippocampus^{35,43}. Nimodipine has been shown to reduce the AHP of CA1 pyramidal neurons⁵⁴, and to reduce calcium currents in these neurons both in *in vitro* slices¹² and in acutely dissociated cells⁹. Reduction of calcium currents and/or $I_{K-Ca^{2+}}$ and a consequent reduction in the calcium-dependent AHP of pyramidal cells would lead to reduced spike accommodation, and an increase in firing rate^{22,32}.

The data for pyramidal cells reported here are consistent with the mechanism of action outlined in the working

model described above. Further, a reduction in the firing rates of theta interneurons across the same range of doses of nimodipine is consistent with a central action of the drug, although the mode of action on these cells is unclear. Non-pyramidal interneurons in the CA1 region fire repetitive action potentials in response to depolarizing pulses and have a relatively brief AHP^{37,38,62}. The mechanism of nimodipine's reduction of interneuron firing rates reported here is not known, and the relatively small effects seen in the current data do not allow definitive conclusions to be drawn. The small magnitude of the decrease in theta cell activity may be related to the role of these cells in inhibitory feedback loops (at present poorly defined and understudied), and thus governed by mechanisms different from those acting on pyramidal cells. Until additional studies examining nimodipine's effects on non-pyramidal neuron membrane conductances are carried out, the mechanism of nimodipine's effects on these cells remains unknown. Ongoing biophysical studies in our laboratory are designed to address these and related issues⁵⁴.

The current findings do not rule out other potential central sites of action of nimodipine, including cerebellar regions which appear to have great importance in certain conditioning paradigms^{49,50,78}. Instead, they offer an additional piece of evidence indicating functional changes at the cellular level in the hippocampal region during nimodipine treatment which are consistent with earlier reports of changes at the cellular level during learning. The specific mechanism(s) of the changes, and the relation of changes in the hippocampus to changes in other regions both afferent and efferent to the hippocampus, are conjectural. Similarly, the current findings do not rule out potential non-specific neural mechanisms related to changes in arousal levels, although past behavioral work¹⁹ utilizing pseudoconditioned control animals indicates that behavioral responsivity is enhanced only to associative stimuli, rather than to randomly presented stimuli. The behavioral data thus suggest a much greater specificity of action of nimodipine upon the central nervous system than a change in general arousal levels.

As seen in Fig. 5, the effects of BAY-K-8644, frequently used as a calcium-channel agonist, did not inversely mirror those of nimodipine. In the case of pyramidal neuron activity, BAY-K-8644 slightly suppressed or had no effect on firing rates at lower doses, while the highest dose enhanced firing rates. Nimodipine, on the other hand, enhanced pyramidal cell firing rates at all doses tested. BAY-K-8644 is a racemic drug with mixed D- and L-isomers. It has been reported previously⁶⁴ that at higher doses, BAY-K-8644 may have both agonist and antagonist effects, which may account for the mixed

effects observed across doses in the present study. The effects of BAY-K-8644 on theta interneurons was the inverse of that seen with nimodipine, although only at a single dose. The persistent firing rate enhancement of pyramidal cells at the highest dose of BAY-K-8644 tested, lasting up to 1 h after drug infusion was terminated, cannot presently be explained. This persistence, and the mixed effects across cell types described above, indicate that further study of calcium-agonist effects on hippocampal neuronal activity in vivo is needed.

Finally, the possibility that some of the observed changes in neuronal activity are a consequence of improved perfusion of the highly vascularized hippocampus should be addressed. Many calcium antagonists have a direct dose-dependent effect on cerebral blood flow, which is the basis of many current clinical uses of these agents. Nimodipine is a potent cerebral vasodilator, and increases cerebral blood flow in a dose-dependent fashion⁶³. Dose-response curves of nimodipine's effects in unanesthetized rabbits indicate that doses of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ of intravenous nimodipine increase cerebral blood flow 2-fold, with an additional 40–50% increase found at a dose of 1.0 $\mu\text{g}/\text{kg}/\text{min}$ ³³. It is, however, reasonable to expect that blood-flow effects would be non-specific across cell types. The suppression of theta interneuron firing across the same range of doses that enhanced pyramidal neuron firing argues against a

non-specific effect of improved cerebral perfusion. Additionally, the data indicating a lack of effect of 2 other cerebrovasodilators, nifedipine and flunarizine, on the firing activity of either neuronal cell type argues strongly against non-specific cerebral blood flow-induced effects. A more parsimonious explanation is that peripherally administered nimodipine has a direct effect on neuronal membrane dihydropyridine receptors, as well as on receptors on vascular smooth muscle. Another recent study indicates that nimodipine crosses the blood-brain barrier more readily than other dihydropyridine calcium-channel antagonists⁸⁰.

The current findings are convergent with other lines of evidence supporting the hypothesis that disturbances in neuronal calcium regulation, including disturbances in the hippocampal region, underlie many of the deficits in learning and memory that are a result of the process of aging^{35,43}. The current study provides evidence that the 1,4-dihydropyridine calcium-channel blocker nimodipine can perhaps offset these age-associated disturbances, at least in the hippocampus, and restore neuronal activity to patterns approximating those required for normal behavioral function.

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