Calcium Blockers and Memory in the Aging Brain

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Associative learning is accompanied by a number of changes in the brain, many of which are mediated by calcium. Our experiments have used trace eyeblink conditioning in the rabbit as a model system to examine these learning-induced changes. The hippocampus, a temporal lobe structure known to be required for storage of new learned information both in humans and in our animal model, has been the major focus of our investigations. We have previously shown that pyramidal neurons recorded *in vitro* in hippocampal slices taken from trained animals show a conditioning-specific reduction in the amplitude and duration of their post-burst afterhyperpolarization (AHP). This AHP is mediated by an outward, calcium dependent potassium current, and serves to reduce neuronal firing rates. The training-induced reduction of the AHP, which is postsynaptic and localized to the hippocampus, is a likely cellular mechanism for conditioning specific increases in firing by hippocampal neurons *in vivo*. Hippocampal calcium regulation, we will argue, plays a critical role in learning and memory.

Aging animals and humans show well-documented learning deficits.[1-4] Interestingly, the calcium dependent AHP is increased in hippocampal neurons from aging animals.[5] Neuronal free-cystolic calcium levels are also increased.[6] More recently, it has been shown that calcium currents are also increased in hippocampal neurons from aging rats.[7] These and similar changes in hippocampal calcium regulation are hypothesized to contribute learning deficits associated with aging,[8] and lead us to hypothesize that calcium channel blockers may facilitate learning in aging animals either by directly reducing the AHP, or by offsetting disruptions in intracellular calcium buffering.

Aging rabbits are severely impaired in the eyeblink conditioning task. Our experiments have shown that intravenous administration of nimodipine, a dihydropyridine calcium channel blocker, causes aging rabbits to learn the eyeblink conditioning task as fast as young controls.[9] Learning by young rabbits was not enhanced by nimodipine. More recently, we have shown that orally administered nimodipine also causes an enhancement of learning in aging rabbits when compared to aging controls.[10] The enhancement by oral nimodipine, at the dose tested, was not as dramatic as that with intravenous administration. We have also found that oral nimodipine affects open field behaviors in aging rabbits, with treated aging animals behaving more like young controls than untreated aging control rabbits. These experiments suggest that this particular calcium channel blocker may facilitate learning in other contexts besides associative learning. To date, we have not tested the behavioral effects of other calcium channel blockers.

The possibility that neuronal calcium channel blockade may be therapeutically useful for dealing with age-related learning, cognitive, or general behavioral deficits will be discussed. This hypothesis is directly testable using eye-

blink conditioning, which shows parallel decreases in learning ability in aging humans and rabbits; with other, more "cognitive" learning tasks known to be especially sensitive to hippocampal function; and with tests sensitive to the altered interaction of aging animals with their environment, such as our open field analyses. The memory loss associated with "normal" aging (Age Associated Memory Impairments: AAMI) should be most amenable to interventions altering neuronal calcium channels. The learning and general cognitive impairments associated with Alzheimer's disease may also be reversible, especially in early stages of the disease process, via calcium channel blockade. In both of these clinical syndromes, impaired learning is associated with hippocampal dysfunction.

The Hippocampus and Trace Eyeblink Conditioning

New Zealand rabbits were conditioned using standard procedures.[11] The animals were restrained in padded Plexiglas stocks, and eyeblinks were measured with an infrared optical transducer and analyzed on-line. The conditioned stimulus (CS) was an 85 db, 6 kHz pure tone, presented within a sound attenuated chamber. The unconditioned stimulus (US) was a 2.5 psi airpuff to the eye, just sufficient to elicit a reliable eyeblink (the unconditioned response, UR). In this trace conditioning paradigm, the CS and US were separated by a trace or no-stimulus interval. The animal was required to maintain a memory or "stimulus trace" of the CS in order to correctly time the conditioned response (CR) and avoid the US. Conditioned responses were defined as eyeblinks which occurred during or after CS presentation but prior to US onset. Training trials were presented at an average of 1/min in blocks of 80 trials/d. Pseudoconditioned control rabbits were presented with an equal number of unpaired CS and US presentations.

Some of our recent experiments have evaluated the contribution of the hippocampus to the successful acquisition and extinction of this behavioral paradigm.[12] We have found that hippocampectomized rabbits acquire the paired stimulus short interval (300 msec trace) eyeblink paradigm at a normal rate, but have profound difficulty extinguishing their conditioned responses when tone CSs are later presented alone. On the other hand, hippocampectomized rabbits are totally unable to acquire a long interval (500 msec trace) eyeblink task, even after 25 successive days of training sessions (Figure 1). Our results thus provide us with evidence that the hippocampus is critically involved in at least one form of associative learning, trace eyeblink conditioning, and that disruption of hippocampal function results in severe learning deficits.

Almost all identified pyramidal neurons recorded in the CA1 and CA3 subfields of the hippocampus in trace-conditioned rabbits are functionally modulated during eyeblink conditioning. Berger and Thompson[15] have described a dramatic correlation between the firing of hippocampal pyramidal neurons and the conditioned behavioral response in an associative eyeblink paradigm, which they termed "neural modeling." We have recorded cells during the hippocampally dependent long-interval (500 msec trace) conditioning trials which show clear modelling of the behavioral response, similar to that described by Berger and colleagues, but with a novel additional sensory response to the tone CS (Figure 2). This non-habituating sensory response to the CS is almost certainly unique to trace conditioning, as it has not previously been reported. Other neurons show clear inhibitory responses during the CS-US interstimulus trace interval, which may be followed by enhanced responses to the US. This inhibition of firing during the trace interval may be a mechanism for control of the temporal characteristics of the conditioned response mediated by the hippocampus.

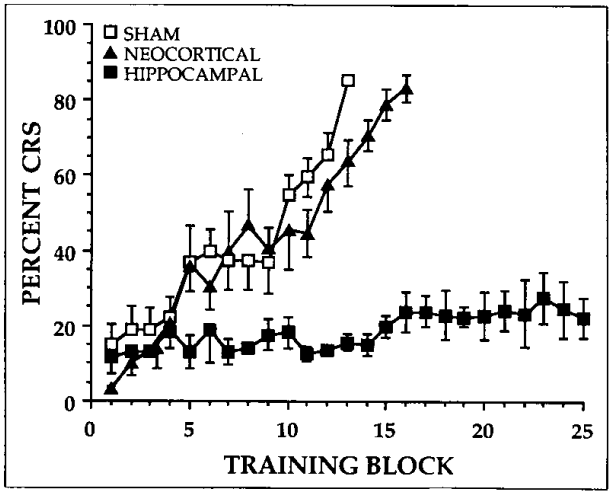

Figure 1. The effects of complete hippocampal lesions on 500 msec trace eyeblink conditioning in young rabbits. The learning curves within each group were "Vincentized" to normalize the number of sessions to criterion to that of the slowest learner, yielding an equal number of training blocks for each subject within each group. These curves permit visualization of various stages of acquisition by sham and neocortically lesioned control animals, and clearly illustrate the severe learning impairment of hippocampectomized rabbits. In control subjects, the first learning stage is seen as an increase in responses over the first two to five sessions. This initial stage is followed by a plateau stage (lasting two to three sessions) in which acquisition levels off. Control rabbits then increase their conditioned responses until behavioral criterion (80% CRs) is reached. Hippocampectomized rabbits show none of these stages of acquisition. The error bars indicate SEM (n = 6 subjects per group).

Calcium Dependent Afterhyperpolarization and Associative Learning

In several replications of our *in vitro* brain slice experiments, young adult animals were first trained to a behavioral criterion of 80% CRs, were pseudoconditioned, or served as naive controls. Hippocampal slices were taken 24 hours later, and neurons were impaled and studied intracellularly. A conditioning-specific reduction in the calcium-mediated AHP response was observed following a

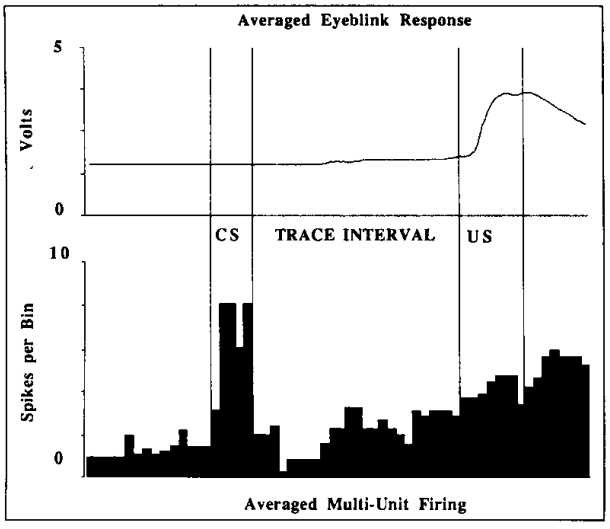

Figure 2. The firing activity of hippocampal pyramidal cells recorded during long-interval (500 ms) trace eyeblink conditioning. Averaged behavioral responses and the related post-stimulus time histograms for behavioral trials at criterion are shown. Typically, a non-habituating sensory response to the CS was followed by robust "neural modelling" of the CR and UR eyeblink. Although the neural modelling is similar to that previously reported during delay eyeblink conditioning,[15] the CS component of the response appears to be unique to the hippocampally dependent trace conditioning task.

burst of action potentials elicited by intracellular current pulses in CA1 pyramidal neurons.[16,17] The fact that the AHP was reduced in neurons in slices, separated from their normal afferent and efferent connections, strongly argues that the mechanisms mediating the AHP reductions are localized to the hippocampus. The AHP response elicited by current injection was demonstrable in slices in which synaptic transmission had been blocked by TTX and TEA, arguing that the mechanisms producing the AHP reductions are localized in postsynaptic cells.[17]

The AHP is generated by a calcium-dependent outward potassium current.[18-21] This potassium current, activated by the calcium influx during an action potential, hyperpolarizes the membrane potential relative to its resting level, and limits firing in hippocampal pyramidal cells during and after bursts of action potentials.[22,23] A reduction in the AHP after conditioning would make cells more excitable. We suggest that a reduction in the calcium-dependent AHP *in vivo* could be one mechanism underlying the increased firing in response to the tone CS which occurs in eyeblink conditioning.[24]

Nimodipine Facilitation of Eyeblink Conditioning in Aging Rabbits

Nimodipine is one of the family of dihydropyridine calcium channel antagonists[25-28] and is currently in use in humans to control some consequences of subarachnoid hemorrhage, a type of stroke. Our interest in this drug stemmed from preliminary observations suggesting that nimodipine enhanced learning in aging humans who had suffered strokes or other cerebrovascular disorders.[29] In addition, we were intrigued by the possibility that nimodipine might be effective in enhancing learning by a direct reduction of the calcium-dependent AHP described above. As discussed earlier, associative learning reduces the AHPs in hippocampal pyramidal neurons from young adult animals. Landfield and Pitler[5] have shown that hippocampal neurons from aged brains exhibit increased, rather than reduced, AHPs. This suggests that the learning deficits which accompany normal aging may be due to difficulty in modulating calcium-dependent responses at the cellular level. More recently, Landfield has shown that calcium currents are increased in pyramidal neurons from aging rats, and that nimodipine reduces these currents.[7] We wished to test the possibility that nimodipine would facilitate acquisition of learned eyeblink responses and, if so, if it acts in the brain by directly modulating the AHP of hippocampal neurons. These are the behavioral implications of Landfield's biophysical experiments regarding calcium-mediated events in the hippocampus.

Eyeblink conditioning in the rabbit is an ideal preparation for evaluating pharmacological agents to counteract aging-related learning deficits. The training parameters are similar in humans and rabbits, and behavioral acquisition of this task is dramatically impaired in both elderly humans and aging rabbits.[1,30,31] We tested the hypothesis that nimodipine facilitates learning in normal aging by studying the effects of nimodipine on the acquisition of the conditioned eyeblink response in young and aging rabbits.[9] Young (mean age, 3 months) and aging (mean age, 37 months) rabbits received vehicle only or 1.0μg/kg/min of nimodipine via a chronic indwelling intrajugular catheter, with the experimenter blind to individual drug conditions. Vehicle control animals and nimodipine-treated animals were evaluated on their abilities to acquire a criterion of four CRs in any block of five trials. Aging control rabbits learned significantly more slowly than did young controls. However, nimodipine markedly facilitated acquisition of the response by aging rabbits. Nimodipine-treated aging animals performed at levels near that of young controls (Table I). No significant drug effects were detected on measures of the CR amplitude, indicating that nimodipine increases the number but not the strength of responses. Unconditional response amplitude was also unchanged, indicating that nimodipine's effect on learning was not due to non-specific increases in responsiveness to the corneal air puff. To further differentiate direct effects on learning from nonassociative performance enhancements, additional nimodipine-treated rabbits (both young and aging) were pseudoconditioned. Trace-conditioned nimodipine-treated aging rabbits demonstrated significantly more CRs than pseudoconditioned animals, which gave no evidence of learning. Since nimodipine-treated aging animals increased their responses to the CS only when it was paired with the US, it appears that nimodipine specifically facilitated associative learning in this task.

We have also begun to explore the behavioral effects of oral nimodipine administration.[10] Female rabbits (mean age, 40 months) were fed either a diet of 860 ppm nimodipine in a standard rabbit chow (n = 7) or the rabbit chow

TABLE. SUMMARY OF THE BEHAVIORAL EFFECTS OF NIMODIPINE

	EYE-BLINK CONDITIONING*,† MEAN TRIALS TO CRITERION		OPEN FIELD BEHAVIORS		
	IV	ORAL	CROSSINGS	GROOMING (ORAL ONLY)	REARING
Young-Controls (3 month)	387.5 ± 95.5	440.0 ± 87.5	35.65 ± 6.2	4.15 ± 0.5	7.00 ± 1.5
Aging-Controls (38 ± 1.3 month)	842.5 ± 153.6‡	1306.0 ± 255.8§	61.27 ± 8.3§	0.60 ± 0.2§	0.60 ± 0.6§
Aging-Nimodipine* (39 ± 1.6 month)	175.8 ± 50.5‖	723.5 ± 179.6*	31.65 ± 7.3*	1.30 ± 0.5§	4.60 ± 1.9*

* Comparisons were made within drug administration method only.
† Animals were trained to a criterion of 4 CRS in any sequential block of 5 trials.
‡ Comparison with young-controls is statistically significant, $p < 0.05$.
§ Data presented are for the most effective dosages studied to date: 860 ppm oral dose and 1.0 μg/kg/min IV nimodipine.
‖ Comparisons with aging controls is statistically significant, $p < 0.05$.

alone (n = 7) for 28 days prior to conditioning and throughout training. An additional seven young female rabbits (mean age, 3 months) were maintained on the control diet. All experiments were carried out double-blind. Animals fed the nimodipine diet reached the behavioral criterion of four CRs out of five consecutive trials faster than the aging controls (Table I). As expected, young controls also reached criterion faster than aging controls. Although the nimodipine-treated aging animals appeared to learn slower than young-controls, this difference was not statistically significant. There are three important implications from these data: (1) We have again demonstrated the utility of the eye-blink conditioning task as an animal model of age-related learning deficits, since the task was particularly sensitive in detecting the learning deficits afflicting aging rabbits; (2) Oral nimodipine ameliorated these deficits; and (3) Although oral nimodipine at the dose tested improved learning by aging rabbits, oral administration (at least at this dose) does not appear to be as potent as the intravenous treatment used earlier (Table I). It should be noted that we have also found that intravenous nimodipine also facilitates aging rabbits acquiring the even more strict criterion of eight CRs out of any block of 10 trials.[9] We have found that only 33% of normal aging controls are capable of reaching an eight CRs out of 10 trials criterion within 2000 trials, while nearly all aging control subjects (over 90%) can reach the simpler four out of five criterion.

While running our first group of animals in the double-blind oral experiments, we noticed that some aging animals had very different activity patterns in their home cages than did others. Since aging individuals are also susceptible to a decline in general activity, in motor skills, and in affect, and given that nimodipine has been reported to improve motor skills and activity in aging rats and mice,[32-34] we felt that it was important to systematically test these observations. We selected tests of open field behaviors, which, although unreported in the rabbit, have been well studied in a number of species, including chickens, dogs, cats, and particularly in both young and aging rats and mice.[35]

Young rabbits tended to engage in less ambulation and more rearing and grooming than did aging control animals (Table I). When young rabbits did move around the open field, they tended to stay closer to the walls of the apparatus, avoiding the center of the open field. Aging rabbits did not seem to attend as strongly to the open field environment. They roamed the entire open field, showed no preferences for any particular section, and exhibited little or no grooming. As indicated in Table I, oral nimodipine-treated aging rabbits behaved more like young control rabbits, showing significantly less ambulation in the open field than did aging control rabbits. In addition, treated animals reared almost as frequently as did young controls, and both groups showed more rearing than aging controls. Finally, aging nimodipine-treated rabbits were intermediate between young and aging controls in frequency of grooming bouts, and both aging nimodipine-treated and young control rabbits showed significantly more grooming than aging controls.

Our measurements of open field behaviors extend our analysis of the cognitive effects of calcium channel blockers to more general areas than simple associative learning. Our results are consistent with other experiments on sensorimotor systems in the aging rat,[36] which show that a diet containing nimodipine reversed age-related deficits in walking pattern, enhanced ability to cross a broad bridge, enhanced conduction velocity in the sciatic and caudal sensory nerves, and increased fiber density observed histologically in the sciatic nerve. These data, evaluating behavioral, neurophysiological, and histological measures, suggest that chronic calcium channel blockade causes a general improvement in the "quality of life" of aging mammals. Our data in improved open field behaviors in the aging rabbit are consistent with these from the rat model of aging. Comparable neurophysiological and neuroanatomical measures would presumably demonstrate comparable alterations in the peripheral nervous system of the rabbit.

It is quite striking to compare these results in experimental animals with the synopsis of double-blind studies on a large human patient population (more than 1000 patients).[37] These studies showed a general improvement over three months in nimodipine-treated rather than control patients on the Sandoz Clinical Assessment Geriatric Scale. This scale is a general measure of impairment of cognitive, affective, social, and somatic functions. The improvement seen in these aged patients as a consequence

of nimodipine treatment suggests that the "improved quality of life" seen in aging rats and rabbits treated with nimodipine is also an accurate and appropriate description of the preclinical data for the aged human.

There are at least three lines of evidence from our experiments which point to the hippocampus as a likely area to focus our search for a mechanism by which nimodipine acts to ameliorate age-related learning deficits: (1) Lesions of the hippocampus result in learning deficits similar to, although more severe than, those seen in aging rabbits[13,14]; (2) The firing rates of hippocampal pyramidal cells mimic the conditioned eyeblink response in young rabbits[15,38]; (3) The calcium-dependent afterhyperpolarization (AHP) responses of hippocampal pyramidal cells are reduced in animals that have been trace conditioned. Since hippocampal neurons from aged animals exhibit larger AHPs than those from young animals, learning deficits in the aged may be attributable to a declining ability to regulate calcium-dependent AHPs. We are currently pursuing experiments exploring the effects of nimodipine on hippocampal neurons recorded *in vivo* in the aging rabbit. This is a first step both in describing the neural mechanisms underlying nimodipine's behavioral facilitation in aging, and in describing hippocampal neuronal activity in our model of the neurobiological effects of aging.

Action of Nimodipine on Hippocampal Neuron Firing in Aging Rabbits *in Vivo*

A chronic multiple-electrode assembly[39] previously used in rats[40] has been successfully adapted for use in the rabbit in conjunction with our Brain-Wave™ computerized extra-cellular recording system. This assembly allows the simultaneous isolation of a large number of single-units in the awake, behaving rabbit. We have simultaneously examined the activity of up to 12 single units (up to 8 per electrode), and thus studied interactions between pyramidal cells and closely associated interneurons[41,42] as well as between different pyramidal cells. As in the rat,[43,44] the stability of recording offered by the Kubie electrode allows the activity of individual cells to be followed over periods of time several orders of magnitude longer than was previously possible using other chronic or acute microelectrode techniques.

We are currently investigating the effects of calcium channel antagonists and agonists on hippocampal single-unit activity in aging rabbits. Preliminary data indicate that nimodipine increases the firing rate of hippocampal pyramidal cells at the same dose which facilitates behavior in aging rabbits.[45] The activity of closely associated interneurons is decreased at the same dose and over the same time course (Figure 3). Since the activity of hippocampal pyramidal cells in eyeblink conditioning models the behavioral response, it is possible that facilitation of pyramidal cell activity underlies the improvements observed following nimodipine treatment. The firing rate decrease by interneurons and coincident increase by pyramidal cells is consistent with nimodipine's facilitation of hippocampal function during learning. Other calcium channel antagonists, including flunarizine,[46] and the calcium channel agonist BAY-K-8644 are also being tested for their effects on hippocampal circuit single-unit activity. We have presently only studied the effects of calcium channel blockade in hippocampal neurons in aging rabbits. We also intend to investigate the effect of these agents on hippocampal neurons in young rabbits, to differentiate effects which are specific to the aging population. Until now, no studies of single-unit activity in the aging rabbit hippocampus comparable to those available for the rat[47,48] have been reported.

Are Studies in Aging Rabbits Applicable to Humans?

Our experimental program is designed to investigate the cellular mechanisms by which aging impairs learning in rabbits. We have investigated pharmacological intervention with calcium channel blockers, since a number of lines of evidence indicate alterations in calcium metabolism in the aging brain. Dysfunctions of the hippocampus, the temporal lobe structure which has been our focus, are implicated in the learning deficits associated with "normal" Age Associated Memory Impairments and with those seen in Alzheimer's disease.[49] We have shown that the trace eyeblink conditioning task is also hippocampally dependent.[13,14] We then hypothesized that nimodipine acts to enhance hippocampal function during the learning process. Since eyeblink conditioning was initially developed as an objective measure for the analysis of associative learning in humans,[50] and since there are clear parallels between age-related learning deficits in this task in the human and the rabbit, eyeblink conditioning provides an ideal paradigm to evaluate neuropharmacological agents useful in treating these deficits.

The anatomical and neurophysiological consequences of "normal aging" in the hippocampus have been well

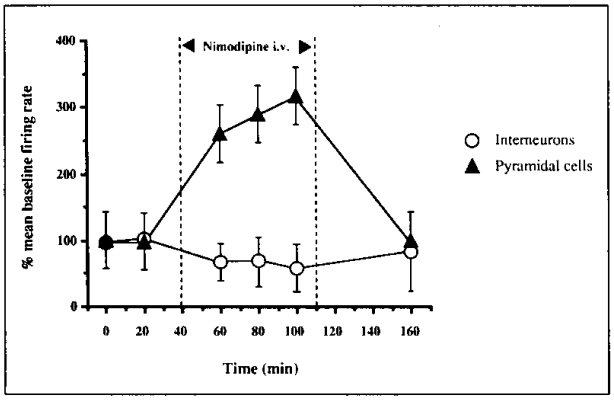

Figure 3. The effects of intravenous nimodipine (1 mg/kg/min) on the activity of hippocampal pyramidal cells and interneurons recorded in the awake aging rabbit. This behaviorally effective dose of nimodipine increased the mean firing frequency of pyramidal cells while decreasing that of hippocampal interneurons over the same time course. The effects on firing activity were reversible when the drug infusion ended. The error bars represent SEM.

described in humans and in experimental animals.[51-55] One interesting finding is that not all aging animals show learning and/or neurobiological deficits. Some subgroups seem to learn as well as young controls. The same is also true in human aging: some people seem not to experience learning and memory problems as they age. Many others, however, notice deficits, and have been described as suffering from AAMI.[56] They have not suffered clinical traumas, such as a stroke, but instead appear to suffer the behavioral side effects of lesser neuronal dysfunctions characterized in aging experimental mammals, many of which involve the hippocampus. This large population of aging people, we postulate, could be helped by therapeutic treatment with calcium channel blockers, because these people have relatively intact if impaired hippocampal circuitry.

One group of the aging human population with well-documented learning deficits are those afflicted with Alzheimer's disease. Compelling evidence suggests that the memory deficits associated with Alzheimer's disease are likely the result of the destruction of hippocampal input and output circuitry resulting from the formation of plaques and tangles in the entorhinal cortex.[49,57,58] We feel that it is not likely that calcium channel blockers such as nimodipine would have significant therapeutic benefits in a population with fully developed Alzheimer's disease. It would be difficult to understand how such a substance could reverse deficits essentially caused by anatomical lesions throughout brain structures required to lay down new memories. Again, however, such a hypothesis is testable in humans and in animals with various lesions to the hippocampal circuitry, ranging from the nearly complete lesions described earlier which abolish trace eyeblink conditioning,[13,14] to more limited lesions of selected portions of the hippocampal circuitry which have been proposed as animal models of Alzhiemer's pathology.[59-62] There is the possibility, of course, that calcium channel blockers could be effective in slowing the Alzheimer's disease process, by interacting with subcellular processes involving calcium early in the disease. The intriguing results of Finger and Dunnett which show that the nimodipine facilitates the survival of grafted brain tissue may be relevant to such speculation.[63]

We are also interested in studying other cognitive or learning related tasks which are disturbed by aging, to determine if these deficits might also be reversed by calcium channel blockers, such as nimodipine. Some ideal tasks to examine include delayed nonmatching-to-sample and serial list learning. Both of these human memory tests are known to involve hippocampal function[64] and have animal equivalents for use in laboratory testing.[64-66] Other tests of hippocampal function in aging could also be developed, and would likely have immediate clinical application.

Comments and Conclusions

Our laboratory has demonstrated that the dihydropyridine calcium antagonist nimodipine markedly facilitates associative learning in aging rabbits. Further, we have demonstrated in young adult rabbits that one change induced by classical conditioning is a reduction in the afterhyperpolarization (AHP) that follows a burst of action potentials in hippocampal CA1 neurons. This AHP is generated via a calcium-dependent potassium conductance. Nimodipine blocks neuronal calcium conductance, and may reduce AHPs in hippocampal neurons of aging rabbits, thereby inducing a biophysical change similar to one found in the young adult hippocampus following learning. This pharmacologically induced reduction of the AHP may in turn facilitate learning.

We should stress some qualifications that we are well aware of when evaluating and interpreting the data discussed. We have concentrated our discussion on the hippocampus, a brain region profoundly affected by aging. And, we have used trace eyeblink conditioning, a hippocampally dependent associative learning task, in our behavioral studies. But it is clear that the aging process affects all brain regions, even if the hippocampus appears to be especially vulnerable.[57] Given the methods of nimodipine administration in our behavioral and *in vivo* single neuron recording studies, the drug should reach the entire brain and could have its positive behavioral effects through brain regions other than the hippocampus. This issue is likely to remain open, although further accumulation of evidence linking hippocampal changes in calcium regulation with learning will continue to strengthen our hypothesis that the hippocampus is critically involved.

The second major qualification we should stress is that we have concentrated our discussion on nimodipine's blockade of neuronal calcium channels. Nimodipine and other dihydropyridines also block calcium channels in smooth muscle, thereby increasing cerebral blood flow.[67] It is possible that the behavioral effects we have noted in aging rabbits is being mediated by enhanced cerebral blood flow rather than by direct blockade of neuronal calcium channels. Nimodipine has been shown to cross the blood-brain barrier.[34] Further studies demonstrating specific and reversible binding of peripherally administered dihydropyridines to brain receptors *in vivo* are needed. Our ongoing studies on the effects of nimodipine (which crosses the blood-brain barrier) and of flunarizine (a calcium channel blocker that does not enter the brain) on hippocampal single-unit activity are also designed to address the issue of blood flow effects. We are also presently measuring brain and serum levels of nimodipine in our behavioral experiments.

At this time, it appears that pharmacological blockade of neuronal calcium channels can have positive effects, reversing some forms of aging-related learning deficits. We have demonstrated one specific example, using the rabbit eyeblink conditioning paradigm, in which both oral and intravenous administration of a dihydropyridine calcium channel antagonist (nimodipine) facilitates learning in aging animals.[9,10] We have presented evidence that indicates that the hippocampus is both profoundly affected by aging, and is critically involved in specific forms of learning, including trace eyeblink conditioning. We have seen that some physiological properties of hippocampal neurons are regulated by calcium-dependent mechanisms, that hippocampal pyramidal neurons model associative eyeblink responses, and that calcium channel blockers change the activity of these neurons *in vivo*. Further, we have shown that learning induces changes in a calcium dependent response to localized hippocampal neurons. Each line of evidence presented is convergent with the

hypothesis that disturbances in neuronal calcium regulation, perhaps centered in the hippocampal region, underlie many of the deficits in learning and memory which are a result of the process of aging. Our work on the rabbit model of associative learning should be relevant to scientists and clinicians concerned with human age-related learning deficits and indicates that nimodipine or other calcium channel blockers may have applications in the treatments of such disorders. □

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