

THE CALCIUM HYPOTHESIS FOR ALZHEIMER'S DISEASE: INSIGHTS FROM ANIMAL AND HUMAN STUDIES

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SUMMARY

The calcium hypothesis of aging and neural degeneration is discussed in the context of its potential application to Alzheimer's disease. Calcium homeostasis in neurons and the effect of aging on calcium-related homeostatic mechanisms are extremely important potential contributors to alterations which may lead to neuronal deterioration and, ultimately, cell death. Eyeblink conditioning is a behavioral model which we have used to probe hippocampal changes in learning and aging. Hippocampal neurons demonstrate increased excitability after associative learning, as evidenced by reductions in the calcium-dependent afterhyperpolarization and spike frequency accommodation. Aging hippocampal neurons show reduced excitability by these indices, correlated with impaired eyblink conditioning ability in aging rabbits. The calcium channel antagonist nimodipine enhances excitability in aging hippocampal neurons and enhances behavioral learning rate in aging rabbits. A similar behavioral effect has been observed after nimodipine administration in humans. Eyblink conditioning and other hippocampally-dependent behaviors are being adapted for the mouse to enable further systematic examination of the calcium hypothesis of Alzheimer's disease in a powerful animal model which complements earlier work.

KEY WORDS: Trace eyblink conditioning, hippocampus, aging, Ca^{2+} antagonists, rabbit, mouse, human.
6 Figures, 48 references.

INTRODUCTION

Calcium is critically involved in cell function. In neurons, calcium regulates numerous processes, including but not limited to neurotransmitter release, development and maintenance of cytoarchitecture, gene transcription, and activation of enzyme systems (some of which themselves act to regulate intracellular Ca^{2+}). Calcium is required for a variety of "plastic" changes in the brain critical both to development and to learning and memory. Calcium is also likely to play a determining role in the cellular processes contributing to brain aging. These changes lead to a number of behavioral problems, from normal age-associated memory impairments to the severe dementia of Alzheimer's disease, and are of significant clinical importance.

The permissive role of intracellular free Ca^{2+} in a huge variety of cellular processes dictates a need for its precise regulation. Disruptions of this regulation, whether local or global, severe or minor, transient or acute, all lead to altered neuronal function. For example, recent studies from our laboratory and from others associate enhanced Ca^{2+} influx with aging-related changes at both the behavioral and the neurophysiological level. These findings support the calcium hypothesis (15,18), which posits that in the aging brain, increases in the average intracellular free Ca^{2+} concentration contribute to functional impairment, and eventually to cell death. The loss of neurons, which are post-mitotic in the adult animal, leads to behavioral deterioration and contributes to death of the entire organism. One promise of the calcium hypothesis is that the functional impairment of a patient at a particular stage of aging-related

disease may be relieved by reducing intracellular free Ca^{2+} in aging neurons. Since calcium dysregulation terminating in cell death is progressive, varying by degree between cell populations, the hypothesis also suggests that the rate of neuronal loss may be reduced by decreasing free Ca^{2+} . Our own work has focused on altering Ca^{2+} influx and measuring both the neuronal and the behavioral functional consequences.

This chapter focuses on data that illustrate the role of Ca^{2+} influx in cellular mechanisms contributing to learning and memory. We provide evidence linking altered Ca^{2+} influx with learning deficits and changes in the electrophysiological properties of hippocampal neurons critically involved in learning, using a rabbit model of normal aging. We detail effects of aging on calcium-dependent protein kinase C (PKC), an enzyme system that serves multiple functions, including an important role in regulation of intracellular Ca^{2+} . We discuss a new mouse model with great potentials for examining genetic determinants of age-associated changes in neuronal calcium regulation. We also present evidence that Ca^{2+} channel antagonists can ameliorate aging-related neurophysiological and behavioral changes, and discuss the relevance of these findings both to normal aging and to the more severe problems confronted in Alzheimer's disease.

DYSREGULATION OF INTRACELLULAR CALCIUM IN AGING

Numerous mechanisms maintain calcium homeostasis in neurons, which are generally summarized in Figure 1. Three major transmembrane sources of calcium are: 1) influx via voltage-gated calcium channels, of which there are at least four major classes, with numerous subclasses differentially expressed in different cell types; 2) influx through other ligand-gated receptors, such as the NMDA receptor/channel complex, with variant subtypes again differentially expressed in different populations of neurons; and 3) through activation of $\text{Na}^+/\text{Ca}^{2+}$ exchangers. Free intracellular Ca^{2+} also is released from intracellular stores (in organelles such as calciosomes, the endoplasmic reticulum, and the mitochondria) and is released from Ca^{2+} -binding proteins such as calmodulin, calbindin, and parvalbumin. Calcium is bound to and released from numerous enzymatic proteins. Immunohistochemical evidence from our laboratory indicates alterations in PKC activity in aging (28). Transmembrane calcium efflux occurs via ATP-driven pumps and $\text{Na}^+/\text{Ca}^{2+}$ exchangers. As stated, the calcium hypothesis posits that disruption of intracellular calcium regulation causes some of the neuronal deterioration in Alzheimer's as well as in normal aging (15,18). The root causes for these disruptions, involving both genetic and environmental factors, have not been definitively described, although the state of the art now allows us to begin probing the basic mechanisms. It is possible that excessive Ca^{2+} influx, sustained excess free Ca^{2+} levels, or impaired buffering of intracellular calcium are results, not causes, of the molecular and cellular mechanisms leading to the development of Alzheimer's disease and of the impairments seen even in non-demented aging individuals. If the calcium hypothesis is correct, the accelerated age-related deterioration seen in some individuals is calcium-linked, and should therefore be amenable to empirical assessment and treatment. It is critically important to test the calcium hypothesis empirically, both in animal models and in clinical settings, and to modify the hypothesis if necessary to fit the observed data.

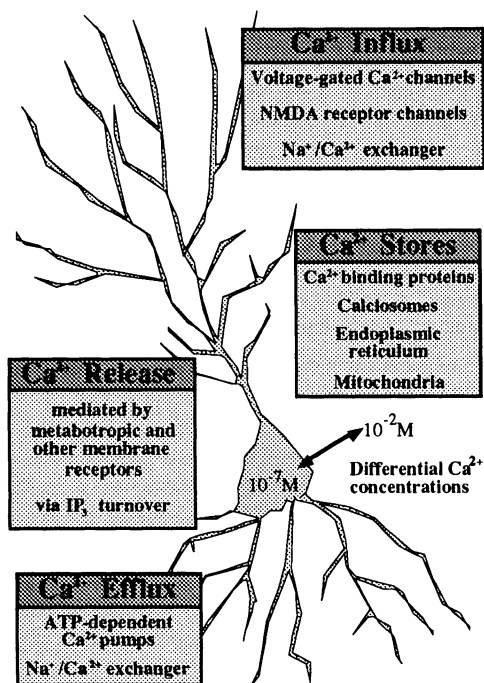


Figure 1. Calcium homeostasis in young neurons buffers calcium from a number of sources (both intra- and extracellular) to maintain intracellular free calcium concentrations in the nanomolar range. Calcium influx occurs via voltage-operated calcium channels (VOCCs, including the L-type channels blocked by nimodipine), via ligand-gated receptors, such as a type of excitatory amino acid receptors (N-methyl-D-aspartate receptors, NMDA-Rs), and via $\text{Na}^+/\text{Ca}^{2+}$ exchange proteins. Calcium is stored intracellularly in a variety of ways, including binding with calcium-binding proteins (CaBPs, including calmodulin, calbindin, and parvalbumin), and sequestration in cellular organelles including calciosomes, the endoplasmic reticulum, and mitochondria. A number of membrane receptors activate second messenger cascades that liberate intracellular stores of calcium, and calcium-dependent intracellular calcium-release has been described. Calcium efflux via energy-dependent calcium pumps and via $\text{Na}^+/\text{Ca}^{2+}$ exchange proteins also regulate intracellular free calcium concentrations. Disruptions of these processes can have profound consequences both to the neuron and to the organism to which it belongs.

Calcium is directly involved in deterioration and cell death in a variety of disease processes. An intensively studied example is glutamate neurotoxicity accompanying ischemic episodes (5,22). In ischemia, glutamate release is enhanced, resulting in sustained neuronal depolarization. Calcium influx is then enhanced both through NMDA-receptor gated channels and through voltage-gated Ca^{2+} channels. Enhanced calcium release from intracellular stores also occurs, as a result of metabotropic receptor activation. Free cytosolic Ca^{2+} levels would be raised as a result of all of these processes, creating acute cellular damage via various pathways, including reduced cellular energy metabolism (30) and activation of catabolic enzymes including a calcium-activated neutral protease (calpain) which can degrade neuronal structural proteins (20,32). Sustained elevation of intracellular Ca^{2+} leads to a number of destructive cascades: 1) the generation of phospholipase A_2 , and production of superoxide radicals, with subsequent cellular toxicity; 2) activation of phospholipase C, which activates protein kinase C, which phosphorylates and increases calcium channel activity, and IP_3 generation which releases additional Ca^{2+} from internal stores; 3) generation of calcium-calmodulin-dependent protein kinase II, which phosphorylates presynaptic synapsin I, leading to further glutamate release; 4) activation of endonucleases, which fragment DNA; and 5) production of nitric oxide synthase, which inhibits mitochondrial respiration, the citric acid cycle enzyme aconitase, and DNA synthesis (5,22). Thus, even small disruptions of calcium homeostasis have devastating consequences, while sustained disruptions can be fatal for neurons. A simplified summary of calcium's ubiquitous role in neuronal function, and potential sites for disruption of Ca^{2+} homeostasis, are shown below (Figure 2).

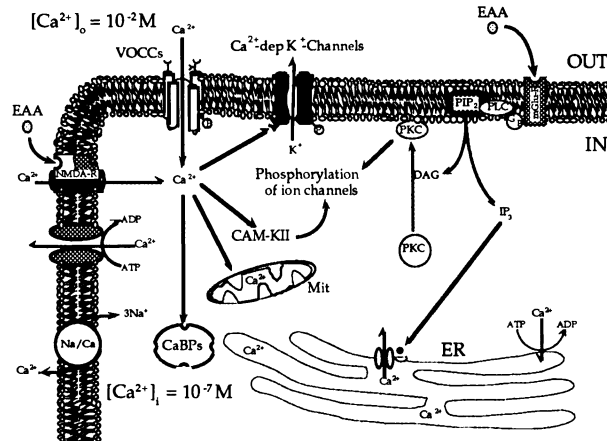


Figure 2. Increased intracellular free Ca²⁺ increases activity of the Ca²⁺-dependent enzyme PKC, increasing protein phosphorylation and amplifying initial increases in free Ca²⁺. Phosphorylation of CABPs may reduce their capacity for Ca²⁺ buffering. Increased free Ca²⁺ would increase activity of calcium-dependent enzymes, including calcineurin, calpains, phospholipase A₂, Ca²⁺-calmodulin-dependent protein kinase (CAM-KII), and phospholipase-C (PLC), altering protein and phospholipid metabolism. Ligand-gated receptors, including metabotropic receptors, liberate inositol triphosphate (IP₃) and diacylglycerol (DAG). Increased IP₃ production would increase Ca²⁺ release from intracellular stores. Depolarizing responses to excitatory amino acids would increase Na⁺ influx, altering activity of the Na⁺/Ca²⁺-exchange antiporter. Altered metabolic demands and an overload of intracellular free Ca²⁺ could overwhelm the calcium pump (Ca²⁺/Mg²⁺-ATPase).

CALCIUM HOMEOSTASIS AND OTHER NEGATIVE FEEDBACK LOOPS

When Ca²⁺ concentrations reach high levels, they stimulate calcium-activated regulatory processes in a negative feedback loop to restore calcium to resting levels. A good example of such a calcium-activated process is the post-burst afterhyperpolarization (AHP), a hyperpolarizing voltage shift from the resting membrane potential that occurs after a burst of action potentials (13,17). Briefly, burst depolarization activates voltage-dependent calcium channels. The calcium influx through these channels activates outward calcium-dependent potassium currents. When these outward currents hyperpolarize the neuronal membrane, voltage-sensitive calcium currents are inactivated. This feedback loop prevents uncontrolled firing activity under normal conditions. However, calcium-dependent systems can show saturation. Under pathological conditions, intracellular calcium concentrations could swamp homeostatic control mechanisms, leading to edema and cell death. As currently formulated, the calcium hypothesis does not presume that massive acute calcium overloads are the primary source of age-associated pathologies. Instead, both aging and Alzheimer's disease presumably represent cumulative effects of small disturbances in calcium homeostasis, with each disturbance resulting in progressive small but significant breakdowns of the regulatory process.

LEARNING IN AGING MAMMALS

The behavioral task which we use as a model system for analyses of the neurobiological changes which occur during the learning process in young and aging brain is eyeblink conditioning. Eyeblink conditioning is a

Pavlovian or associative learning task in which the human or animal is presented with a tone conditioned stimulus (CS) paired with an air puff unconditioned stimulus (US) to the corneal region. Eyeblink conditioning is a well defined task which has been extensively studied behaviorally and with various neurobiological approaches (11). It also has the advantage of being appropriate for use with a variety of species, including humans (Figure 3). We have done most of our experiments with rabbits but have recently been extending our studies to the human, in an attempt to determine the generalizability to humans of observations we and others have made in rabbits (3,4,9). We have also begun experiments with eyeblink conditioning in the mouse, in order to allow us to more easily do coordinated behavioral, physiological and anatomical experiments in an animal model which closely mimics Alzheimer's disease. This approach will be discussed in more detail below.

A major interest in our recent research program has been to determine the cellular mechanisms by which hippocampal neurons change during associative learning, and to characterize alterations in hippocampal neurons which may contribute to learning deficits in aging. We thus use a variant of eyeblink conditioning which probes hippocampal function, trace eyeblink conditioning, to study age-associated learning deficits of aging rabbits and aging humans. In this paradigm, the subject must form a short-term memory trace of a brief tone CS in order to appropriately time the occurrence of a conditioned eyeblink response (see Figure 3). We chose this paradigm because trace eyeblink conditioning is dependent upon an intact hippocampus for its successful acquisition (24,35). Trace conditioning appears to tap the critical involvement of the hippocampus in processing and storing temporal aspects of stimulus contingencies (33).

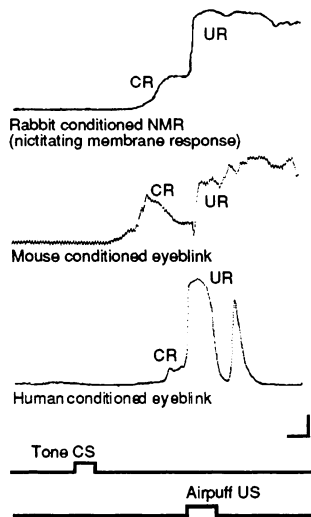


Figure 3. Trace eyeblink conditioning can be used to train subjects from a variety of species. As applied in our laboratory, a 100 ms duration pure tone CS is paired, after a 500 ms stimulus-free "trace interval", with a 150 ms duration airpuff US sufficient to elicit a reliable unconditioned eyeblink response (UR). After sufficient pairings, the CS elicits a conditioned eyeblink response (CR). Numerous control paradigms are available and routinely used, including pseudoconditioning, stimulus generalization, and extinction. Since stimulus and response relationships are precisely temporally defined, the task lends itself readily to a variety of neurobiological preparations.

Acquisition of eyeblink conditioning is impaired for both aging rabbits and for aging humans (4,38,45,46). Woodruff-Pak and Solomon and their colleagues have demonstrated that eyeblink conditioning is noticeably impaired in Alzheimer's patients as compared to age-matched controls (34,47). Notably, hippocampally-dependent learning tasks may be impaired in aging animals and humans because this structure is affected relatively early by the cellular changes associated with aging (1,38). We have recently

completed a systematic analysis of performance by rabbits in trace eyeblink conditioning across the age span (38). Rabbits begin to show major deficits in acquisition of this task as early as 24 months of age, with a plateau at around 30 to 36 months of age (the oldest population we tested). Other data indicates further

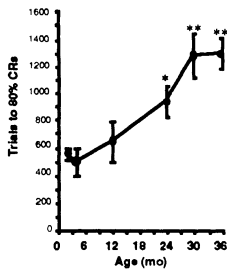


Figure 4. Associative learning of some tasks declines with increasing age. Trace eyeblink conditioning in rabbits is comparable in rabbits up to 12 mo of age, but declines by 24 mo of age. Further impairments are seen with increasing age. These deficits were not accompanied by losses in sensory acuity, nor in motor performance. Instead, they appeared to be specific associative deficits of aging.

declines with increased aging. Additionally, with increasing age an increasingly larger percentage of the rabbit population were severely impaired in learning the trace conditioning task (i.e. they exhibited much more severe learning deficits than their age-matched cohorts). This heterogeneity in performance by the aging rabbits is reminiscent of comparable findings in other species, including humans, where some aging individuals show learning rates which are comparable to those of young adults.

Ca²⁺-mediated Changes in Learning and Aging

Hippocampal pyramidal neurons recorded *in vivo* during and after eyeblink conditioning demonstrate dramatic increases in excitability. These changes are evidenced by dramatic changes in firing rate to conditioned stimuli as they gain behavioral significance (2,8,43). We have demonstrated that one cellular mechanism likely to underly this excitability increase is a reduction in the post-burst afterhyperpolarization (AHP), mediated by an outward Ca²⁺-mediated potassium current, and by an accompanying reduction in spike frequency accommodation (7,26,37). A reduction in the AHP current would make pyramidal neurons less hyperpolarized in the period immediately after a series of action potentials are fired, making it easier for successive action potentials to be initiated (13). The time course of the AHP and accommodation reductions are appropriate for a structure presumably involved in temporary storage of learned associations, as they are transferred from short- to long-term storage (26,37). Our immunohistochemical data shows alterations in PKC staining after learning that are consistent with reduced AHP and accommodation measures in hippocampal pyramidal neurons (40,41).

It is quite striking that both the post-burst AHP and accommodation are *increased* in hippocampal CA1 pyramidal neurons from aging rabbits (26; see Figure 5A). This increase was first observed in hippocampal pyramidal neurons from aging rats by Landfield, who suggested that an AHP increase would reduce neuronal excitability, impeding involvement of hippocampal neurons in the changes which accompany learning (19). A decreased ability of hippocampal neurons to mediate plastic changes could be involved in the impaired learning capacity seen in many aging mammals, as discussed above. Further study has shown that the Ca²⁺ action potential is actually increased in CA1 hippocampal neurons from aging rabbits (25; see Figure 5B). Note that the increase in the Ca²⁺ action potential is in the slow depolarizing plateau which follows the

initial peak of Ca^{2+} influx. This is temporally appropriate for mediating an increase in the slow Ca^{2+} -mediated post-burst AHP described above in aging neurons.

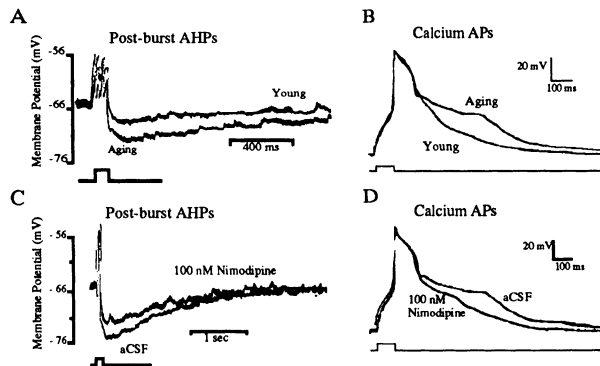


Figure 5. The slow post-burst AHPs of aging CA1 pyramidal neurons were significantly enhanced in comparison with those of young neurons at the same resting potentials, being both longer in duration and greater in amplitude (A). Interestingly, the slow plateau phase of the Ca^{2+} action potentials of hippocampal neurons was greatly enhanced in aging (B), indicating a significant increase in calcium influx during depolarizing events. Sustained increases in Ca^{2+} influx are a likely basis for the enhanced calcium-dependent AHP observed in aging hippocampal neurons. Although greatly enhanced in aging CA1 neurons, both post-burst AHPs (C) and Ca^{2+} action potentials (D) were significantly reduced by low concentrations (100 nM) of nimodipine. Concentrations in the micromolar range were required for effect in young CA1 neurons. Treatment with nimodipine restored Ca^{2+} influx to levels typical of young neurons. The Ca^{2+} -dependent AHP was also restored to levels typical of young pyramidal cells.

Effects of the Ca^{2+} Antagonist Nimodipine on Neurons and Learning in Aging

We tested the possibility that pharmacological blocking voltage-dependent Ca^{2+} influx into neurons might alter physiological function and learning rate in aging rabbits. Nimodipine, a lipophylic dihydropyridine which crosses the blood barrier quite effectively and which reduces calcium influx through L-type channels, was administered intravenously at $1 \mu\text{g}/\text{kg}/\text{min}$. Aging rabbits which received nimodipine learned the trace eyeblink conditioning task as fast as young rabbits, and considerably faster than aging control rabbits (6). A follow-up study demonstrated that the $1 \mu\text{g}/\text{kg}/\text{min}$ dose of nimodipine was most effective in increasing learning rate in aging rabbits (16).

An important issue, of course, is what effect nimodipine has on hippocampal neurons in aging rabbits. We have tested this in a variety of ways. First, we showed that intravenous nimodipine enhanced baseline firing rates in conscious, sitting rabbits in a dose- and age-dependent fashion (36). The dose which caused a maximal increase in CA1 pyramidal cell firing rate was $1 \mu\text{g}/\text{kg}/\text{min}$, the same dose which maximally increased learning rates. Nimodipine increased baseline firing rate considerably more in aging than in young rabbits. We next showed that nimodipine applied to hippocampal slices reduced the post-burst AHP in aging CA1 pyramidal neurons at concentrations as low as 100 nM (26; see Figure 5C). Concentrations as low as 10 nM reduced spike frequency accommodation in aging CA1 neurons. Finally, 100 nM nimodipine was shown to be effective in reducing the depolarizing plateau potential in aging hippocampal Ca^{2+} action potentials (25;

see Figure 5D). The calculated behaviorally effective extracellular nimodipine concentration has been estimated to be approximately 75 nM (26). The low concentrations of nimodipine which were effective in aging hippocampal slices had no comparable effect on the AHP, accommodation or calcium action potentials in neurons from young rabbits. Recall that nimodipine had no effect on learning rate in young rabbits.

Nimodipine Improves Eyeblink Conditioning in Impaired Aging Humans

Eyeblink conditioning can be used to compare across species, an important consideration for experiments focused on developing pharmacological strategies for improving learning in aging. We used this approach to see if the nimodipine effects seen in aging rabbits generalized to aging humans. We recently completed a double-blind placebo-controlled study evaluating the effects of nimodipine on eyeblink conditioning in aging (60 - 75 yr) and young (20 - 30 yr) human subjects (3). All subjects were screened physically and with a neuropsychological testing battery to insure that they were within normal ranges for their age groups. We found that aging, but not young, subjects who received nimodipine (60 mg tid for 3 mo) acquired the conditioned eyeblink response to a higher level than did their age-matched placebo controls. This effect was most marked after three months of treatment. It was also dramatically more apparent in the half of the subjects who performed at the lowest levels, in terms of percent conditioned responses, during baseline testing, i.e., the effect was most dramatic in those aging subjects who were initially most impaired.

We are not aware of another such clear demonstration of a pharmacological enhancement of learning in both humans and animals in which exactly the same behavioral test was used for the cross-species comparison. Our results clearly suggest that nimodipine has promise as a cognitive enhancer in aging subjects. In addition, they offer clear support for the calcium hypothesis of aging and dementia. It is not likely that nimodipine would be effective as a cognitive enhancer in Alzheimer's subjects with serious and advanced neuronal deterioration in the hippocampus. But because nimodipine is so safe and well tolerated, the possibility of using it prophylactically is appealing. Progression of degenerative disease could theoretically be slowed, if treatment with a calcium channel blocker was begun early enough. Such a scenario, of course, assumes that excess calcium influx contributes to the neuronal loss in a degenerative disease such as Alzheimer's. The rationale for such an assumption was discussed above.

Hippocampal-Dependent Learning in the Mouse

Given that hippocampal neurons are likely to be "multitasking", i.e., responsive to a variety of integrated/configural stimuli, multiple tasks or variations of tasks should be more likely to reveal the complexity of stimuli that activate hippocampal neurons. However, other tasks are more difficult to implement with rabbits due to their size and limited behavioral capabilities. An appealing approach is to develop eyeblink conditioning in a species where many tasks can be examined, and where existing databases from behavior, physiology and genetics can be utilized. The literature base for behavioral genetics of the mouse, advances in transgenics and gene knockout procedures, plus a short life span, make the mouse a powerful model to adapt for eyeblink conditioning, especially for studies regarding age-related learning

deficits and the possibility of pharmacological and physiological studies in transgenic mouse models of Alzheimer's disease. Therefore, we are developing eyeblink conditioning in the restrained mouse.

Several mice have gone through conditioning procedures. An example of the progression in *delay* conditioning is shown for a young mouse that exhibited 94% CRs (Figure 6A). In this figure an upward deflection is a blink. The duration and timing of the tone and airpuff are indicated at the bottom of the figure. Following this success several mice were trained in the trace paradigm described for our rabbit and human experiments. An example of four trials from a trace conditioned mouse is shown (Figure 6B). Data from a control mouse given explicitly unpaired stimuli (Figure 6C) are also shown. Notice that this mouse blinks to the airpuff alone trials, but not to the tone alone trials.

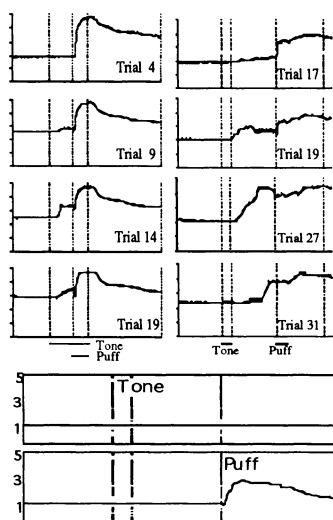


Figure 6. Examples of eyeblink responses elicited in mice during conditioning in the delay paradigm (top left), trace paradigm (top right), and during pseudo-conditioning (bottom). The tone CS and air puff onset times are indicated. Eyeblink responses were measured as differences in reflectance from the cornea with an infrared diode.

The results of our preliminary studies suggest young adult mice can be conditioned to blink in either the delay or trace paradigm while restrained, while middle aged mice may have difficulty learning and remembering, and that pseudoconditioning can be prevented. In the best case, the level of delay conditioning (94%) exceeded that of young freely moving F1 hybrid rats run by Weiss and Thompson (44). After eyeblink conditioning, the mice are run in a Morris water maze, a sensitive way to evaluate spatial learning (23). The combination of eyeblink conditioning with maze tasks will probe two important behavioral functions of the hippocampus, i.e. association of spatially and of temporally-related stimuli. Correlations of behaviors in different tasks address issues of whether or not different functions of the hippocampus are differentially impaired by age or neurodegenerative disease. Trace eyeblink conditioning clearly exploits the temporal demands placed upon memory formation, as do delayed matching to sample (DMS) and delayed nonmatching to sample (DNMS) tasks. Unpaired controls in eyeblink conditioning equate most aspects of the paradigm except for the contiguity of the stimuli to be associated. However, other aspects of the task may be affected by aging, and other neural systems are known to be involved. The cerebellum is particularly problematic, because it is essential for delay *or* trace eyeblink conditioning (39), and it is severely affected by aging (29).

Damage to the cerebellar system can be assessed by training the mice in the delay paradigm or by use of other more purely sensorimotor evaluations such as the rotorod.

The Morris water maze exploits spatial demands on memory formation. Allocentric memory is especially needed for the swimming maze, while egocentric memory can be measured by evaluating spontaneous alternation behavior in a Y maze. The effects of emotionality and other motivational behaviors can be tested by activity in an open field. An examination of this combination of behaviors is difficult in rabbits and humans, but is fairly easy to accomplish in mice.

The behavioral genetics of mice are also worth exploiting. There are many strains that can be evaluated, and the new transgenic and knockout technologies are suited for the mouse. While there is the problem of developmental defects as a result of adding or deleting a gene, significant progress has already been made using transgenic and knockout techniques and mutant mice. For example, Yamaguchi *et al.* (48) inserted the human amyloid precursor protein 695 into the mouse and found that these mice were retarded in learning the Morris water maze (however, amyloid deposits were not found). Games *et al.* (10) succeeded in producing a transgenic mouse with amyloid deposits, but have not yet tested the mice behaviorally. Silva *et al.* (31) knocked out the gene for calcium calmodulin-dependent kinase II and found that the mice were impaired in hippocampal LTP and in spatial learning. Grant *et al.* (12) found that the mutant mouse missing the *fyn* tyrosine kinase gene was impaired in LTP and spatial memory. Spontaneous mutations can also be bred to propagate a new line. Jamot *et al.* (14) found that a mutated strain of the C57BL/6J mouse exhibited deficits in the radial arm maze which correlated with the size of intra- and infrahippocampal mossy fiber projections. Finally, the mouse is a cost-effective species to maintain in large numbers over a long time span.

We are developing a behavioral battery with the mouse model to use in future systematic extensions of the work reported and ongoing with rabbits and humans. Some systematic experiments, especially those which involve prophylactic pharmacological interventions over the life span of the animal, should be facilitated with the mouse model. The ability to do eyeblink conditioning experiments with mice will also allow us to use the power of this behavioral preparation in combination with physiological, pharmacological and biophysical experiments to study new transgenic or knockout mouse models of Alzheimer's disease as they become available.

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