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31 December 1979; revised 27 March 1980

Age-Related Changes in Passive Avoidance Retention: Modulation with Dietary Choline

Abstract. *Two studies were performed to evaluate the effects of dietary choline manipulation on behavior in mice. Retired breeder mice (8.5 months old) were placed on purified diets that were either deficient in free choline or choline-enriched. After 4.5 months, the mice were trained in a single-trial, passive avoidance task and tested for retention either 24 hours or 5 days later. Their performance was compared with that of mice, of various ages, that were maintained on a control diet. The two salient findings were (i) a dramatic decrease in retention of the task in senescent mice (23 months and older) and (ii) marked behavioral differences between choline-deficient and choline-enriched mice (13 months old). In fact, the choline-enriched mice performed as well as 3-month-old mice, whereas the choline-deficient mice performed as poorly as the senescent mice. In a replication, three groups of retired breeder mice were placed on the same choline-deficient diet; control and enriched groups were given choline through their drinking water. Again, retention of learning was superior in the choline-enriched mice and inferior in the choline-deficient mice. These studies demonstrate that dietary manipulation of choline can significantly alter behavior in ways that are qualitatively and quantitatively similar to those occurring across the life-span of the mouse. Thus certain behavioral changes that occur with age might be modulated through appropriate precursor control.*

Evidence has accumulated that cholinergic dysfunctions may play a particularly important role in the memory impairments that occur with old age. This evidence, involving psychopharmacological (*1–4*), biochemical (*5*), and electrophysiological (*6*) studies of humans and infrahumans, has stimulated interest in the possibility that these age-related impairments might be reduced by pharmacological manipulation of the cholinergic system. Despite the compelling empirical support for this approach, attempts to pharmacologically modify the age-related impairments have thus far proven therapeutically disappointing (*7*).

It has been suggested that dietary manipulation of precursors to the cholinergic system may provide an alternative method of enhancing presynaptic cholinergic activity (*8*). Numerous studies have demonstrated that systemic or dietary manipulation of choline, the precursor for synthesis of acetylcholine, increases central cholinergic activity. (Manipulation of lecithin, the normal dietary source of choline, has the same effect.) In addition to the somewhat controversial findings that acetylcholine levels are altered by precursor manipulation (*8, 9*), significant changes in the activity of the synthesizing enzyme choline acetyltransferase (*10*) and in the amount of nicotinic receptors (*11*) and transsynaptic, cholinergically stimulated dopamine activity (*12*) have also been reported.

These biochemical studies, when considered with the cholinergic model for memory deterioration in old age, logically suggest that geriatric cognition might be improved by providing abundant amounts of choline or lecithin. However, clinical studies have failed to demonstrate reliable or therapeutically relevant effects in the cognitively impaired elderly (*13*). In fact, there is no empirical evidence that, by altering precursor availability, we can induce in the aged brain any of the changes that reportedly have been induced in the young healthy brain; or that any consistent behavioral changes occur at all. Although it is still too early to determine whether methods utilizing precursor control of the cholinergic system will be developed to reverse age-related memory impairments, other heuristically interesting possibilities should also be examined. For example, if age-related changes in the cholinergic system are at least partially responsible for the memory impairments, and if dietary manipulation of choline significantly affects cholinergic function, then it might be possible to modulate the rate at which the memory impairments occur with age by varying the availability of dietary choline.

We tested C57B1/6j mice of various age groups for memory of a one-trial, passive avoidance task either 24 hours or 5 days after training (Fig. 1). The results (Fig. 2) demonstrate that aged mice suffer impairments in learning and memory that appear similar to those found in aged rats, monkeys, and humans (*1, 3, 6*). After this "life-span" test, two different groups of retired breeder mice (8.5 months old) were given free access to purified diets that were either choline-deficient or choline-enriched (*14*). Because the life-span tests revealed that reliable passive avoidance deficits are not present at this age, it seems reasonable to assume that the major neurochemical

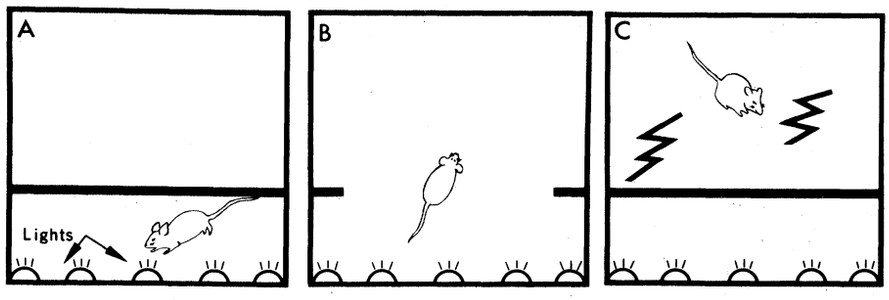

Fig. 1. Schematic representation of apparatus and procedure used to assess retention of passive avoidance across the life-spans of the mice and between choline groups. For training, each mouse was placed in the front chamber of a two-chambered apparatus (A). After a brief orientation period, a partition was raised, allowing the mouse to freely explore the apparatus, during which time it soon entered the second, darker chamber (B). Once the mouse was inside the second chamber, the partition was quickly lowered and a 0.3-mA shock was applied to the floor grids for 3 seconds (C). After this single training trial, the mouse was returned to its home cage to await testing for retention 24 hours or 5 days later. Testing was accomplished in exactly the same manner as the training, except the latency for entering the rear chamber was now measured. Pilot data demonstrated that if mice were not shocked on the training trial, latencies for entering the rear chamber were very low. However, if the mice were shocked on the training trial, latencies were consistently higher, but decreased with time. Thus higher latencies on the test day presumably reflect greater retention of the passive avoidance training trial.

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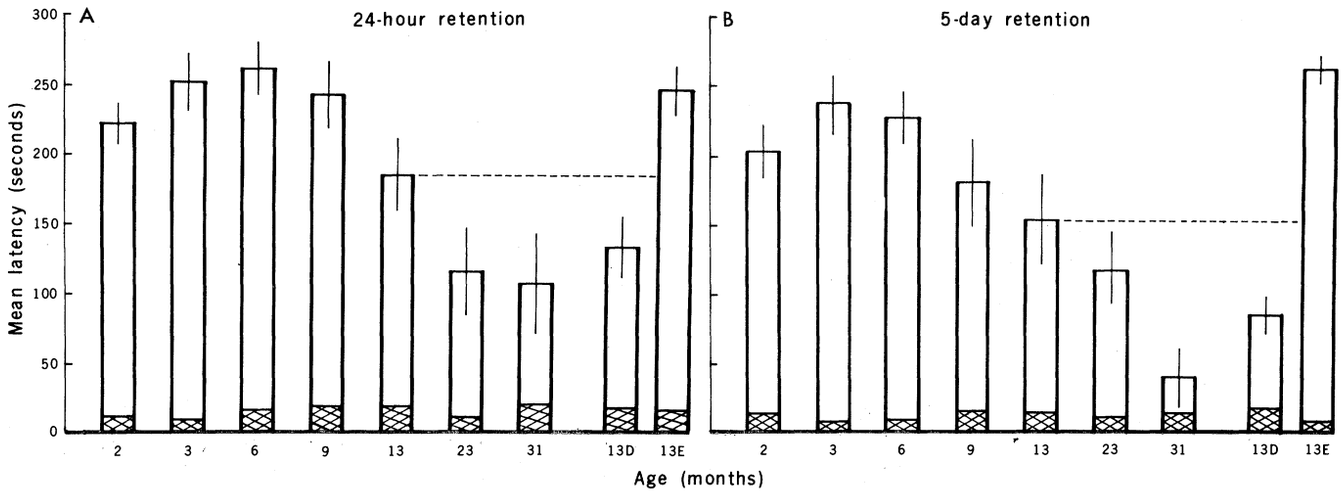

Fig. 2. Retention of the single-trial, passive avoidance task across the life-spans of C57B1/6j mice. Retention is expressed as latency to reenter the rear chamber (A) 24 hours or (B) 5 days after the aversive experience. A significant decrease in retention occurred across the life-spans of mice tested either 24 hours [F (6, 108) = 6.65, $P < .001$] or 5 days [F (6, 113) = 7.38, $P < .001$] after training. Because no consistent age-related differences in latency were observed on the training day (crosshatched bars) [F (6, 108) = 1.44, $P > .10$ and F (6, 113) = 0.97, $P > .10$ for the 24-hour and 5-day groups, respectively], it is reasonable to assume that this behavioral deficit is not due to differences in activity or responsiveness, but probably reflects impairments in retention of the training trial. In addition to this task, over a dozen other behavioral evaluations were made across the life-spans of the mice. The retention deficits in the senescent mice reflect the most severe behavioral deficits observed (*23*). The two right-hand bars depict the reliable difference in retention by 13-month-old mice, maintained on diets either deficient (*D*) or enriched (*E*) in choline for 4.5 months, when tested 24 hours [t (36) = 3.87, $P < .001$] or 5 days [t (38) = 10.72, $P < .001$) after training. Again, there were no reliable differences between training trial latencies ($t < 1$ in both cases). Note that the 13-month-old mice maintained on the choline-deficient diet performed as poorly as the senescent (23 months old) mice ($t < 1$ in both retention conditions), whereas the 13-month-old mice maintained on the choline-enriched diet performed as well as the young (6 months old) mice ($t < 1$ in both retention cases). The vertical lines within each bar represent the standard error of the mean for each group.

alterations responsible for the deficits were not yet severe in these mice.

When the animals became 13 months old (after 4.5 months on the diet), they were trained and tested on the same passive avoidance task used to assess the life-span changes. Half the mice were tested for retention 24 hours after training; the other half, 5 days after training. In both retention conditions, dramatic differences between diet groups occurred (Fig. 2).

These findings were replicated in a second study in which three groups of retired breeder mice were placed on the same purified, choline-deficient diet. The control and enriched groups were supplemented with choline chloride through their drinking water (*15*). This permitted the caloric intake to be more easily controlled and choline intake to be more carefully monitored. Also, the smaller amount of choline for the enriched group allowed us to assess the effects of more normal choline levels.

Clear differences in 24-hour retention were observed by the third month of choline manipulation and persisted into the fourth (and final) month (*16*). The choline-deficient group exhibited a 60 percent decrease in latency (compared to the control group), whereas the choline-enriched group exhibited a 25 percent increase. The more modest effects of choline enrichment in this replication presumably reflect the more moderate amount of supplemental choline (2.7 times the normal amount versus 15 times the normal amount in the first study).

To our knowledge, the results of these studies provide the first clear evidence that manipulation of cholinergic precursor availability can significantly alter behavior. Further, these behavioral changes are qualitatively and quantitatively similar to those occurring naturally across the life-span of the mouse. As such, they offer the heuristically interesting possibility that certain age-related changes in behavior can be modulated by long-term control of precursor availability. An important question concerns how long into the life-span increased choline will continue to retard the onset of age-related memory losses. Similarly, it remains to be seen whether long-term choline administration might reverse existing cognitive impairments in aged subjects. It is conceivable that the cholinergic dysfunction that presumably contributes to the age-related cognitive impairments also renders the aged brain relatively incapable of responding to additional precursor stimulation in the manner of the younger brain (*17*). If so, it would be best to intervene before the behavioral impairments and neurochemical dysfunctions fully develop.

An issue of crucial importance concerns the neurochemical changes that may be induced by choline manipulations. The literature suggests that alterations in central cholinergic function occur during such manipulations and that these alterations play a significant role in the behavioral impairments. However, this hypothesis needs to be confirmed. The relevant neurochemical changes may not be limited to alterations in the synthesis and release of acetylcholine. For example, it was recently reported that choline also interacts postsynaptically, binding to muscarinic receptor sites and thereby possibly inducing weak but direct cholinomimetic effects (*18*). It is also possible that the neurochemical effects of long-term choline manipulation may not even be limited to the cholinergic system. The conversion of choline to acetylcholine is believed to be a relatively minor pathway in the metabolism of choline (*19*), and other important roles of choline, including phospholipid synthesis, have been identified (*20*). Phospholipids are very important in the microenvironment of β-adrenergic (and presumably other) receptor-enzyme systems (*21*). Increasing the synthesis of phosphatidylcholine increases the number of β-adrenergic receptor sites and the degree of membrane fluidity, thereby increasing adenylate cyclase coupling. Therefore, it is possible that altering the availability of choline (which serves as a precursor for phosphatidylcholine) (*20*) may not only affect the turnover of acetylcholine, but also may affect more general neurotransmit-

ter mechanisms involved with receptor recognition sites and cyclic nucleotide coupling (*22*).

Clearly, more work is needed to identify which factors contribute to the behavioral effects induced by choline alterations, and how these factors are related to similar changes occurring naturally with age. By examining these relations, it might be possible to gain some insight into the etiology of the neurobehavioral impairments that occur with old age, and how they might be alleviated.

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14. Purified diet 785 (Bio-Serv) was used for the deficient group. Independent blind bioassays (Raltech) revealed less than 1 mg of choline per gram of chow in the deficient diet and 12 to 15 mg of choline per gram of chow in the enriched diet. Control chow (Purina) was estimated to contain approximately 1.6 mg of choline per gram.
15. Drinking water for the control groups was supplemented with choline chloride (1.5 mg/ml), making their daily consumption of choline normal. The choline-enriched group was given 4.0 mg of choline per milliliter of water, or 2.67 times the normal amount. Mice in all three groups consumed approximately 5 ml of water per day. Chow consumption averaged approximately 3 g per mouse per day. The mean weight of the mice during the last month of the study was 40 g.
16. An analysis of variance across the three choline conditions yielded F (2, 24) = 57.1, $P < .0001$; individual t-tests demonstrated significant effects of both choline-deficient and choline-enriched conditions [for choline controls (mean latency, 218.4 seconds) versus choline-deficient mice (mean latency, 65.7 seconds), t (12) = 6.70, $P < .001$; for choline controls versus choline-enriched mice (mean latency, 277.7 seconds), t (12) = 2.60, $P < .02$].
17. It has already been documented that the activity of choline acetyltransferase and the number of muscarinic receptor binding sites decrease significantly in the aged brain (*5*). Either of these factors could contribute to a dampened response to precursor availability. Still other possibilities involve unknown dysfunctions in postsynaptic, cyclic nucleotide coupling mechanisms (*6*) or in presynaptic choline uptake mechanisms.
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22. Probable interactions with other neurotransmitter systems, especially dopamine, also cannot be ruled out as a major contributing source [W. K. Creese and S. H. Snyder, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, R. F. Killan, Eds. (Raven, New York, 1978), p. 377].
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28 December 1979; revised 10 March 1980

Tritiated Imipramine Binding Sites Are Decreased in Platelets of Untreated Depressed Patients

Abstract. *The high-affinity binding of tritiated imipramine to platelet membranes was compared in samples from 16 untreated depressed women and 21 age-matched controls of the same sex. The maximal binding in the depressed group was significantly lower than that of the controls, although the affinity constants were similar. These results suggest that binding of tritiated imipramine in human platelets may represent a biochemical index of depression, possibly reflecting similar changes in the brain.*

Until recently, tricyclic antidepressant drugs were thought to act by inhibiting neuronal uptake of monoamines (*1*). This monoamine hypothesis was challenged by the advent of "atypical" antidepressant drugs, such as iprindole, that do not inhibit neuronal monoamine uptake (*2*). Furthermore, the demonstration that tricyclic antidepressant drugs can have direct postsynaptic effects on β-adrenoceptor-linked adenyl cyclase (*3*) suggested that their actions are more complex than previously supposed (*4*).

The high-affinity binding site for [^{3}H]imipramine possesses many of the properties expected for the site of action of tricyclic antidepressant drugs (*5*), thus providing a possible direct approach to the study of the mode of action of these drugs. Recent work has shown that high-affinity binding sites for [^{3}H]imipramine exist in human platelets and that their properties are apparently identical to those of high-affinity binding sites for [^{3}H]imipramine in rat brain (*6*). These findings make it possible to test the working hypothesis that the high-affinity binding site for [^{3}H]imipramine may be involved in the pathogenesis of depression. An essential requirement for such a study is the availability of a homogeneous population of clearly diagnosed depressed patients who have had no antidepressant treatment.

Previously we had shown that maximal binding of [^{3}H]imipramine in human platelets decreases with age in a control population, whereas the values of affinity constants do not change with age (*7*). In this study, we compared a group of female volunteers (20 to 65 years old) with 16 untreated depressed female patients of the same age range and demonstrated that the maximal binding of [^{3}H]imipramine is significantly decreased in the depressed patients, while the affinity constant is unaltered.

Platelet membranes were prepared as already described (*6*). Twenty milliliters of blood was withdrawn by antecubital venipuncture and collected into plastic tubes containing sodium citrate (final concentration, 0.38 percent). Platelets were obtained from platelet-rich plasma by centrifugation at 16,000*g* for 10 minutes. The platelets were washed twice with buffer (5 m*M* tris-HCl, 20 m*M* EDTA, 150 m*M* NaCl, *p*H 7.5), and membranes were prepared by hypotonic lysis (5 m*M* tris-HCl, 5 m*M* EDTA, *p*H 7.5), homogenization (Polytron), and centrifugation at 39,000*g* for 10 minutes.

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