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Vitamin E Deprivation in Rats: Some Behavioral and Histochemical Observations

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SARTER, M. AND A. VAN DER LINDE. *Vitamin E deprivation in rats: Some behavioral and histochemical observations.* NEUROBIOL AGING 8(4) 297–307, 1987.—Rats deprived of vitamin E from age of 4 weeks were tested in four independent behavioral experiments and compared with a group fed a control diet. During a 14-minute session in a hole-board, no differences in the level and the course of habituation of parameters of activity and exploration were found. A second group of animals was trained in an automatically controlled six-arm radial tunnel maze. Although no differences were found in various activity measurements, the deprived animals showed a slightly impaired spatial concept formation during 8 acquisition sessions. Testing their relearning ability of the same maze 18 days later, the vitamin E deprived animals showed a significant impairment. In a third experiment, animals were trained 16 days in the same maze configuration and at day 17 they were exposed to the mirror image of the radial maze. Both groups mastered this reversal with an increased level of activity but without differences in patrolling efficiency. In a fourth behavioral experiment, the effects of scopolamine on deprived animals were examined. Compared to the controls, the vitamin E deprived animals were relatively insensitive to the effects of scopolamine. Autofluorescent neuronal lipofuscin accumulation was found especially in the hippocampus (CA3) of vitamin E deprived animals. Based on these results, the usefulness of vitamin E deprivation as an animal model for accelerated normal aging is discussed.

Vitamin E Hole-board Radial arm tunnel maze Lipofuscin Learning Memory Aging Rat

VITAMIN E is recognized as a biological antioxidant which may protect unsaturated fatty acids against oxidation [5,17] and may act more generally as a free radical scavenger stopping membrane-damaging chain reactions [38]. Dietary vitamin E deprivation is a procedure used frequently to induce neuronal lipofuscin accumulation [9, 20, 22, 23, 34]. This symptom seems to appear before peripheral effects of the vitamin deprivation develop and seems to be irreversible, in contrast to the lipofuscin accumulation caused by other treatments (protein-calorie malnutrition, hyperbaric oxygen exposure) [9, 18, 37]. Some studies indicate species differences. For example, after vitamin E deprivation neuronal changes in rat brain appear earlier than in monkeys, and in the latter lengthier deprivation is required to induce deposition of lipid products within neurons [9]. Furthermore, the cell alterations remain longer at a reversible level in primates [9,34].

Lipofuscin accumulation in the brain is a prominent and consistent manifestation of aging [30]. In normal aging, there seems to be a linear relationship between the accumulation of lipofuscin in neurons and age. In brains of demented people, however, the lipofuscin accumulation increases dramatically and is quite unrelated to age [8]. It is worth noting that the question whether lipofuscin (for its structure see [1,36]) has cytotoxic effects or is a harmless by-product of normal cellular aging is still under discussion [7].

In order to study systematically the behavior of rats deprived of vitamin E from the age of 4 weeks, four independent behavioral experiments were made using a hole-board and the tunnel maze, both equipped with automated data recording equipment. The hexagonal tunnel maze, which was originally designed by Bättig and co-workers (e.g., [3,24]) was selected to permit testing of animals without reinforcement procedures, to permit changing the configuration of the maze, and especially because it allows conclusions on spatial cognitive concept formation by the animals since extra-maze-cues are not available. In an additional experiment, the effects of vitamin E deprivation in adult rats on lipofuscin accumulation were examined by the use of fluorescence microscopy.

GENERAL METHOD

Animals

A total of 60 male Wistar rats (Department Tierzucht und -haltung, Schering, Berlin) were used and kept in a

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TABLE 1

SOME COMPONENTS OF THE VITAMIN E-FREE AND THE CONTROL DIET (ACCORDING TO THE PRODUCER'S ANALYSES)

Component	Vitamin E-Free (mg/kg)	Control (mg/kg)
crude protein	172 717,6	172 650,0
fat	50 563,5	53 530,5
fibre	30 930,6	30 890,6
ash	55 135,9	54 922,3
water	89 173,8	99 783,6
disaccharides	96 905,4	110 364,7
polysaccharides	467 440,1	455 800,0
lysine	15 322,0	15 322,0
methionine	8 635,0	8 635,0
Vitamin E	0,5	163,9
choline chloride	1 000,0	1 011,5
inositol	100,0	111,0
caprylic acid	1 550,0	2 500,0
lauric acid	22 250,0	2 500,0
myristic acid	9 500,0	2 500,0
palmitic acid	5 500,0	2 700,0
stearic acid	3 700,0	1 250,0
linoleic acid	2 700,0	35 050,0
arachinic acid	100,0	2 500,0

Note: For a series of other components similar values for both diets are given by the supplier. The fatty acids listed were a part of the fat. The fat was given in a triglyceride form.

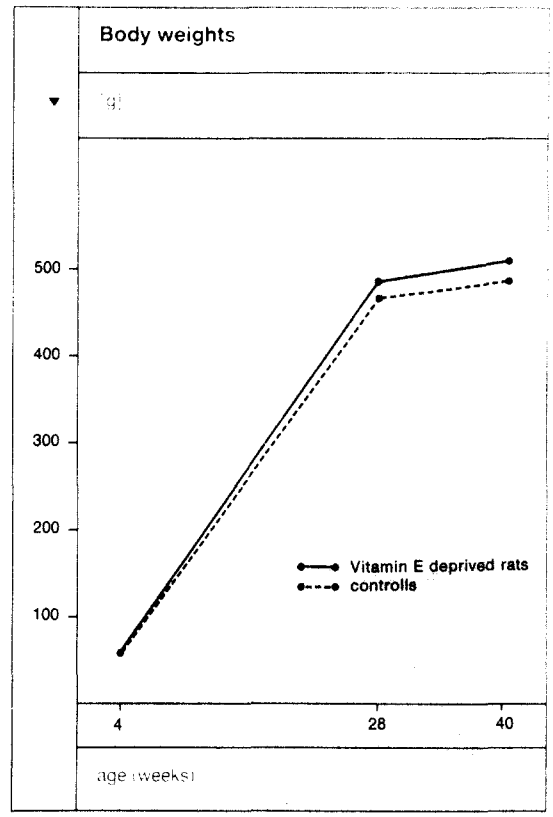


FIG. 1. Body weights (means) of the animals on both diets at different ages. The value at the age of 4 weeks was recorded just before switching from the standard laboratory food to the diets.

temperature-controlled room (22±1°C) with a 12 hr light/dark cycle. Young rats (1 month) were distributed randomly to the vitamin E-free diet or control diet, respectively. Both groups received food and water ad lib (5 animals per cage). At the beginning of the different experiments, the animals had eaten the diets for between 9 and 12 months (specified below for each experiment). None of the animals was used for more than one experiment.

Diets

Both the vitamin E-deficient diet and the control diet were obtained from ALTROMIN (Lage, F.R.G.). Table 1 indicates some components (mg/kg) of both diets. Supplier's data on the vitamin E content of the diets were confirmed using an HPLC method, developed by Dr. H. H. Schneider and D. Seel (Department of Neuropsychopharmacology, Schering AG). Although the content of certain unsaturated acids also varies between the two diets (e.g., especially lineoleic acid) due to the use of different base materials, neither diet was deficient in these constituents and the results obtained are not likely due to these particular variations.

Briefly, the diets were ground up with ascorbic acid. For internal standardization, α-tocopherol was added to aliquots of the chow. Following several steps of extraction with ethyl acetate and evaporation, the heptan-phase was measured by the HPLC method on a silica gel column (250×4 mm). The effluent was monitored at 293/370 nm with a Kratos FS 970 fluorescence detector. The results precisely confirmed the suppliers data on the vitamin E content of the diets.

EXPERIMENT 1

The hole-board represents an open field of increased environmental complexity. The behavioral parameters extracted from the photo-beam controlled recordings have been interpreted in terms of activity and explorative behavior, the latter being indicated by head-dipping behavior [11]. During a test session of about 10 minutes, habituation of the activity and exploration-related parameters has been demonstrated especially in psychopharmacological studies [12,15].

METHOD

Ten animals from each diet group were used. At the day of testing, the animals had been fed the diets 32 weeks from age 4 weeks.

Apparatus

The hole-board apparatus consisted of a square open field (65×65 cm) with 16 holes (3 cm diameter) arranged in four parallel rows of four holes each. The field was surrounded by vertical Plexiglas walls 30 cm high. Automation of the hole-board was provided by photo-beam devices which also monitor the holes. Using an automatic counter, data were collected and printed out each second minute. The information permitted evaluation of total horizontal activity and number of head dips.

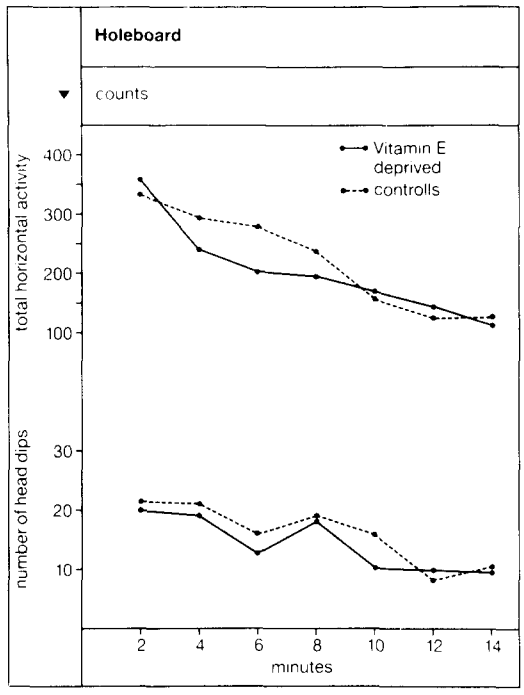


FIG. 2. Total horizontal activity and number of head dips (means) as counted from 14 minute sessions in the hole-board.

Procedure and Statistics

Testing was performed between 2 p.m. and 6 p.m. In random order, individual animals from both groups were put into the center of the hole-board and counting was started. Following the end of testing, the hole-board was cleaned and the next trial was started. The total time tested was 14 minutes per animal. Each of the two (dependent) variables "total horizontal activity" and "number of head dips" was separately analyzed using a repeated measurement model with one between factor (treatment on the two levels of "diet" and "no diet") and one within factor (time with 7 levels dividing the 14 minutes session into periods of 2 minutes intervals). Tests concerning the within factor or interactions with the within factor were carried out using adjusted degrees of freedom according to Greenhouse-Geisser corrections. These corrections ensure that the nominal error rate of the F-test is kept even in case the sphericity assumption about the residuals' covariance matrix underlying the model is violated [2].

RESULTS

Figure 1 shows the development of the body weights of the animals of both groups throughout the time of feeding the diets. There were clearly no differences between both groups.

Figure 2 summarizes descriptively the results of the hole-board test.

Statistical analysis did not reveal significant effects of the dietary treatment on total horizontal activity, $F(1,18)=0.34$, $p>0.56$, nor on the number of head dips, $F(1,18)=0.46$, $p>0.50$. Although the effect of the intra-session-habituation was significant for all parameters, there was no significant interaction between this variable and the dietary treatment.

DISCUSSION

The rats fed a vitamin E-free diet and those fed control diet did not differ with respect to their locomotor activity and exploratory behavior as indicated by the hole-board test. This result corresponds closely to that of Lal *et al.* [14] who tested animals which were fed a vitamin E deficient diet for as long as 14 months.

EXPERIMENT 2

The tunnel maze system was originally developed by Bättig and co-workers. It consists of walls and a ceiling which form a unit which can be lifted. The maze configuration can be changed by interchangeable barriers (see Fig. 3).

It has been shown frequently (for review see [3]) that animals reach the highest level of locomotor activity during the first two days of testing and that they develop quite rapidly a spatial concept formation of the actual maze configuration even in the absence of extra-maze-cues. As no reinforcement is necessary to encourage the animals to explore the maze, the tunnel system appears to represent an appropriate environment for rats. The spatial concept formation may reflect a species-specific cognitive ability, namely to explore systematically a defined environment with a quite consistent frequency. The tunnel maze system has been shown to measure sensitively the effects of various psychopharmacological treatment, brain lesions or of sex and strain on different parameters of locomotion, exploration, habituation and cognitive concept formation [3,24]. In our experiment, vitamin E-deficient and control animals were trained for 8 days in a 6-arm radial configuration and, to test their long-term memory capacity, retested following an 18-day break.

METHOD

Subjects

Ten animals from each diet group were used. At the first day of testing, the animals had been fed the diets for 45 weeks from week 5. At the beginning of testing they weighed 475±53 g (vitamin E-deficient) and 481±43 g (controls).

Apparatus

The tunnel maze is illustrated in Fig. 3. Forty-two infrared photocell unites are distributed throughout the tunnels and interfaced to an IBM XT computer system equipped with the PCI-20000 system (Burr-Brown Corp.). The diagonal diameter of the maze is 1.4 m, the tunnels are 8 cm wide and 15 cm high. The ceiling and side walls were fitted together to form a unit which can be lifted to remove the animal and to clean the floor. The whole maze was constructed of black Plexiglas. Software for data recording and preliminary data analysis was written by Dr. R. Oettinger (ETH Zürich). The maze configuration used in this experiment is shown in Fig. 4.

Procedure

The testing was performed between 12 and 5 p.m. in a dark room. The animals of both groups were tested in random order each day. They were dark adapted in the test room for half an hour before testing. For removal of animals and cleaning the maze a dim light was turned on and off again as soon as the next trial was started. The animals were handled for 20 min daily during the week preceding testing.

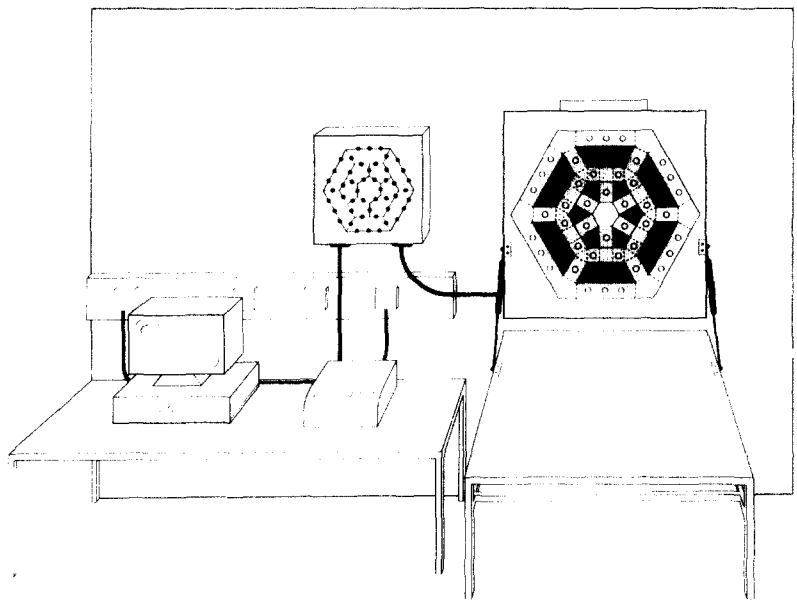


FIG. 3. Schematic illustration of the tunnel maze system. The ceiling and the walls are lifted up here. The position of the photocells are schematically indicated by circles, the sites of possible barriers by broken lines. On the left, a display showing the actually activated photocell, the switchboard and the PC are illustrated.

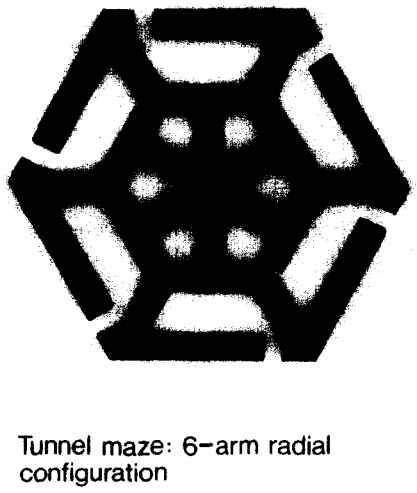


FIG. 4. Maze configuration used in experiment 2.

Each animal was put into the maze by a ceiling door at the center, the door was closed, and data recording was started. Each trial was terminated automatically after 600 seconds, then the animals were removed and the maze base cleaned. Defecation and urination usually ceased on the second day of testing. Animals were trained for 8 days, followed by an 18 days break and then 2 days of relearning completed the test.

Data Analysis

From each session, a number of parameters were calculated: total number of photocells interrupted (total activity), total number of different photocells interrupted (maximal 42 which is the total number of photocells on the maze); for

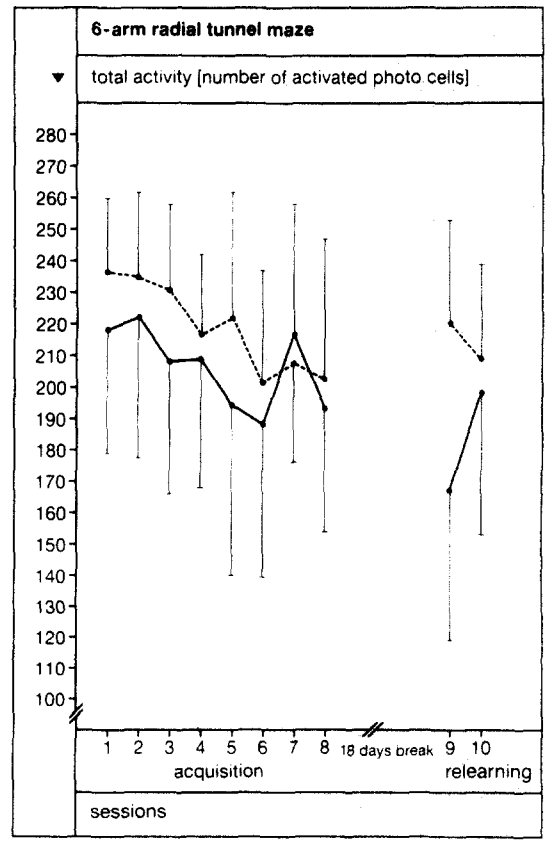


FIG. 5. Total activity (means, SD) of both groups of animals during acquisition and relearning of the maze configuration shown in Fig. 4. Broken lines: control animals; unbroken lines: vitamin E deprived.

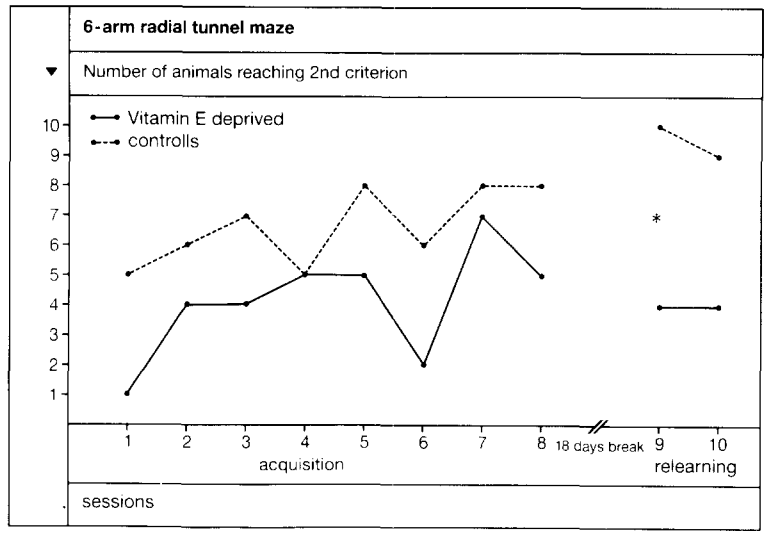


FIG. 6. Number of animals which reached a second exploratory round (2nd criterion).

eleven different behavioral decisions, the frequency and the average transition times were given; for the first 6 arm entries and for the first and second complete round of exploration (all the six different arms), the number of activated photocells, the time needed, the number of repetitions, the position of the first repetition and the number of entries into the short blind arms were recorded. Finally the total number of activating of photocells situated at the entries of the arms (seen from center view), the middle and the end of it, and those in the short blind arms were noted. Thus, the number of incomplete arm entries could be calculated (number of "enter"-photocells minus number of "end"-photocells).

Statistical analysis compared between both groups the level (median of values from sessions of the acquisition period) and slope (median of differences between values of consecutive times during acquisition) and the slope of the transition (from the last acquisition session to the first relearning session) of some of these behavioral variables. The comparisons were carried out using the Kruskal-Wallis test ($\alpha=0.05$, family error rate giving a significance level of $\alpha'=0.016$ for each univariate test). Of the behavioral parameters available from this method, only total activity and total explored area are reported here. The interdependence of these variables was not taken into account. The number of animals which reached the second exploratory round (2nd criterion) is given for each session and for the session before and after the break. These numbers were compared using Fisher's exact probability test ($\alpha=0.025$ for each comparison). Finally, rank correlations have been computed between total activity and explored area for the three periods of the experiment.

RESULTS

During acquistion, no difference between the groups in level or time course of locomotor activity was found. The transition from acquisition to relearning differed with respect to the slope since the controls showed a higher activity level during the first relearning session compared to the last acquisition session than the deprived animals (see Fig. 5). The difference, however, did not reach significance ($H_1=4.69$; $p=0.03>0.016$).

Deprived animals showed a non-significantly reduced level of exploration of the whole area compared to the controls ($H_1=4.28$; $p=0.03>0.016$). However, the vitamin E deprived animals explored a smaller area during the first relearning session compared to the last acquisition session and, therefore, the slopes of the transition lines for both groups are significantly different ($H_1=6.21$; $p=0.012<0.016$).

Although locomotor activity of both groups did not differ during acqusition, the number of animals of the deprived group which reached a second exploratory round (that is, the six different maze arms were completely explored for the second time) was smaller, though not significantly, than that of the control group at the last acquisition session (see Fig. 6; $n_1=n_2=10$; $x_1=8$; $x_2=5$; $p>0.025$). Following the 18-day break, all animals of the control groups reached this criterion, but only 4 from the deprived group ($n_1=n_2=10$; $x_1=10$; $x_2=4$; $p<0.025$).

The Spearman rank correlation coefficients between total activity and total explored area were $r_s^1=0.5195$ for the level and $r_s^2=0.035$ for the slope of the acquisiton period, and $r_s^3=0.579$ for the slope of the transition to the relearning period.

DISCUSSION

The lower locomotor activity and, significantly, the smaller area explored by deprived animals on the first day of relearning seems very difficult to interpret especially with respect to the rapid recovery to the control level at the 2nd day of relearning. It could be speculated that the degree of environmental novelty might have been higher following the 18 days break for the deprived animals than for the controls. However, the difference between both groups on the very first day of the experiment was smaller than that following the break.

It is of interest that, during relearning, the deprived animals recovered rapidly in terms of locomotor activity and explored area, but not in terms of exploratory efficiency (see

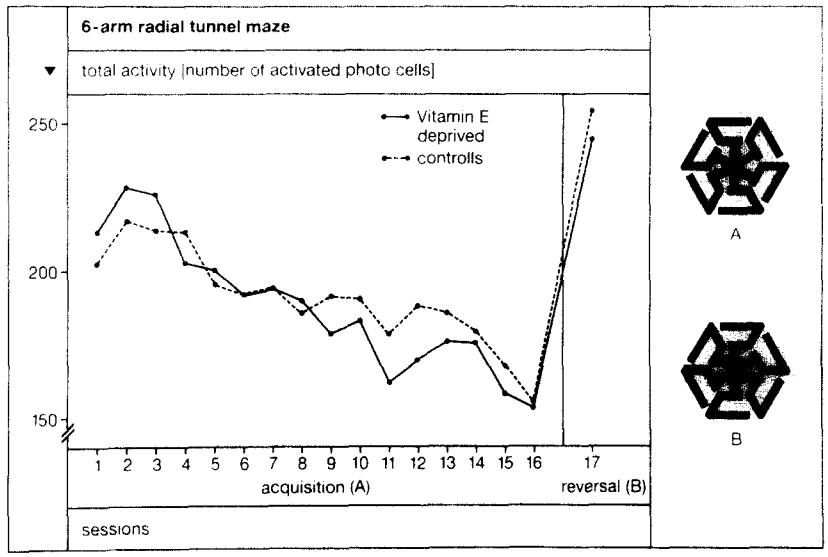


FIG. 7. Total activity (means) of both groups of animals during acquisition and reversal of a 6-arm radial tunnel maze. At the right, the configuration given during acquisition (A) and the reversed on (B) are schematically illustrated.

Fig. 6). This observation indicates that the measures of locomotor activity, explored area, and behavioral efficiency are to a degree independent. The result of the rank correlations suggests that locomotor and exploratory activity habituate independently during acquisition. In terms of the level and transition slope, measures of activity are correlated only moderately.

Therefore, it may be possible to speculate about a cognitive disturbance arising from the vitamin E deprivation which is inferred from the number of animals of both groups reaching the performance criterion during relearning (see above). This disturbance may best be referred to as a deficit in reference memory. The following experiment was planned in order to test working memory by testing the animals in a reversed spatial configuration of the 6-arm radial tunnel maze.

EXPERIMENT 3

Vitamin E deprived rats have been reported to be impaired in learning a delayed alternation task [14]. This result clearly suggests a deficit in working memory abilities.

Reversal learning is thought to test predominantly working memory, since it requires the extinction of a previously reinforced response and, simultaneously, the acquisition of the alternative one. In maze learning tasks, the reversal implicates a modification of the cognitive concept about the environment. If vitamin E deprived animals in fact are impaired in such a task, this finding would contradict the idea of an animal model of accelerated aging, since aged animals do not seem to be impaired in various reversal tasks [28,33].

Procedure

The procedure in this experiment was in principle the same as in the former one. Ten animals of each group were tested for 16 consecutive sessions (600 seconds each) in the 6-arm radial maze. At day 17, the spatial configuration was reversed as illustrated in Fig. 7 (right part). The entrance into an arm in configuration A becomes a blind arm in B and vice versa. As in the former experiment, levels and slopes of the acquisition period and the transition from the last day of acquisition to the reversal session were computed.

RESULTS

Figure 7 shows that locomotor activity of both groups habituated during acquisition. The reversed configuration induced an increase in activity in both groups, and activity indeed reached a higher level than at the beginning of the experiment. In none of the behavioral parameters were differences between the groups found, including the number of entries into blind arms. Similarly, the number of animals reaching the 2nd exploratory round was similar in both groups and increased as well during the transition from acquisition to reversal.

DISCUSSION

The long acquisition period (16 sessions) yielded a relatively high degree of habituation. The reversal induced a shift in activity and performance in both groups. Thus, vitamin E deprivation did not affect performance in the spatial reversal of such a maze. This result suggests that there were no signs of increased behavioral stereotypy or inflexibility of the spatial abilities of vitamin E deprived rats.

EXPERIMENT 4

In experiment 4, possible interactions between the administration of scopolamine and vitamin E deprivation have been examined. Such an experiment may be of interest for several reasons.

Neuronal lipofuscin accumulation as a result of either aging or vitamin E deprivation has been documented to be most dense in hippocampal neurons [6]. Although vitamin E deprivation had no significant effects on activities of ChAT and AChE (choline acetyltransferase, acetylcholinesterase) [23], some lowering of activity was observed in hippocampus. Accordingly, as both hippocampal injury and scopolamine-treatment are well known to reduce the ro-

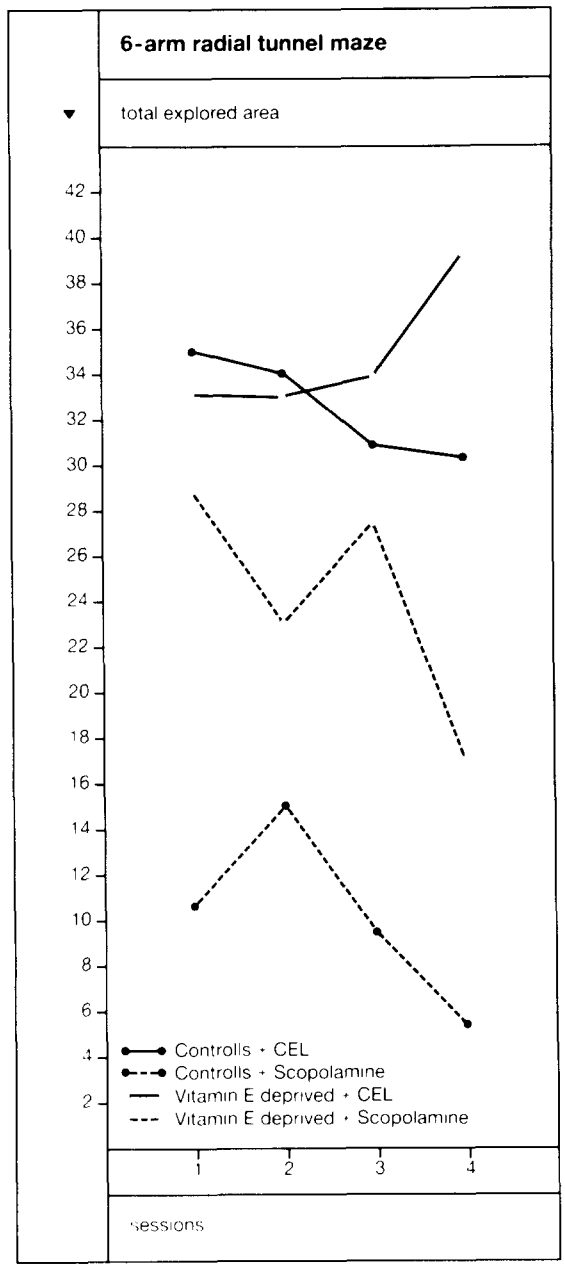


FIG. 8. Total explored area (number of *different* photocells activated maximally 42; means) of the four groups during 4 sessions of acquisition of a 6-arm radial maze.

dent's ability to learn spatial tasks [4,31], if lipofuscin accumulation has cytotoxic effects, then synergistic effects of vitamin E deprivation and scopolamine might be expected.

METHOD

From each group, 14 animals were used. At the first day of testing, the animals had been fed the diets for 40 weeks from week 5. In each group the animals were randomly distributed to the vehicle or scopolamine treatment.

Two days before the experiment started, all animals were injected with the vehicle (Cremofor EL, BASF Ludwigshafen; 10% v/v in physiological saline; 1 ml/kg, IP) in order to habituate them to the procedure. On test days, the animals were injected 20 minutes before the test with either vehicle or scopolamine (scopolamine hydrobromide, Sigma; 0.5 mg/kg/ml, IP; dissolved in Cremofor EL).

The 6-arm radial tunnel maze was used as described above. The animals explored the maze on four successive days and for 480 seconds each day.

Statistical analysis was performed by computing the Kruskal-Wallis analysis of variance test results based again on the level and slope of the curves of the four groups over these 4 sessions ($\alpha=0.05$, family error rate).

TABLE 2

NEURONAL LIPOFUSCIN CONTENT IN CRUDELY DEFINED BRAIN AREAS

Area	Controls n=5	Vitamin E Deprived n=5
cerebral cortex	+	++
n. accumbens	0	+
striatum	0	+
hippocampus	+	+++
thalamus	0	+
cerebellum	0	++

0: no lipofuscin present.
+: small amount of lipofuscin granules in few neurons.
++: large amount of lipofuscin granules in few neurons or a small amount of lipofuscin granules in many neurons.
+++: large amount of lipofuscin granules in many neurons.

RESULTS

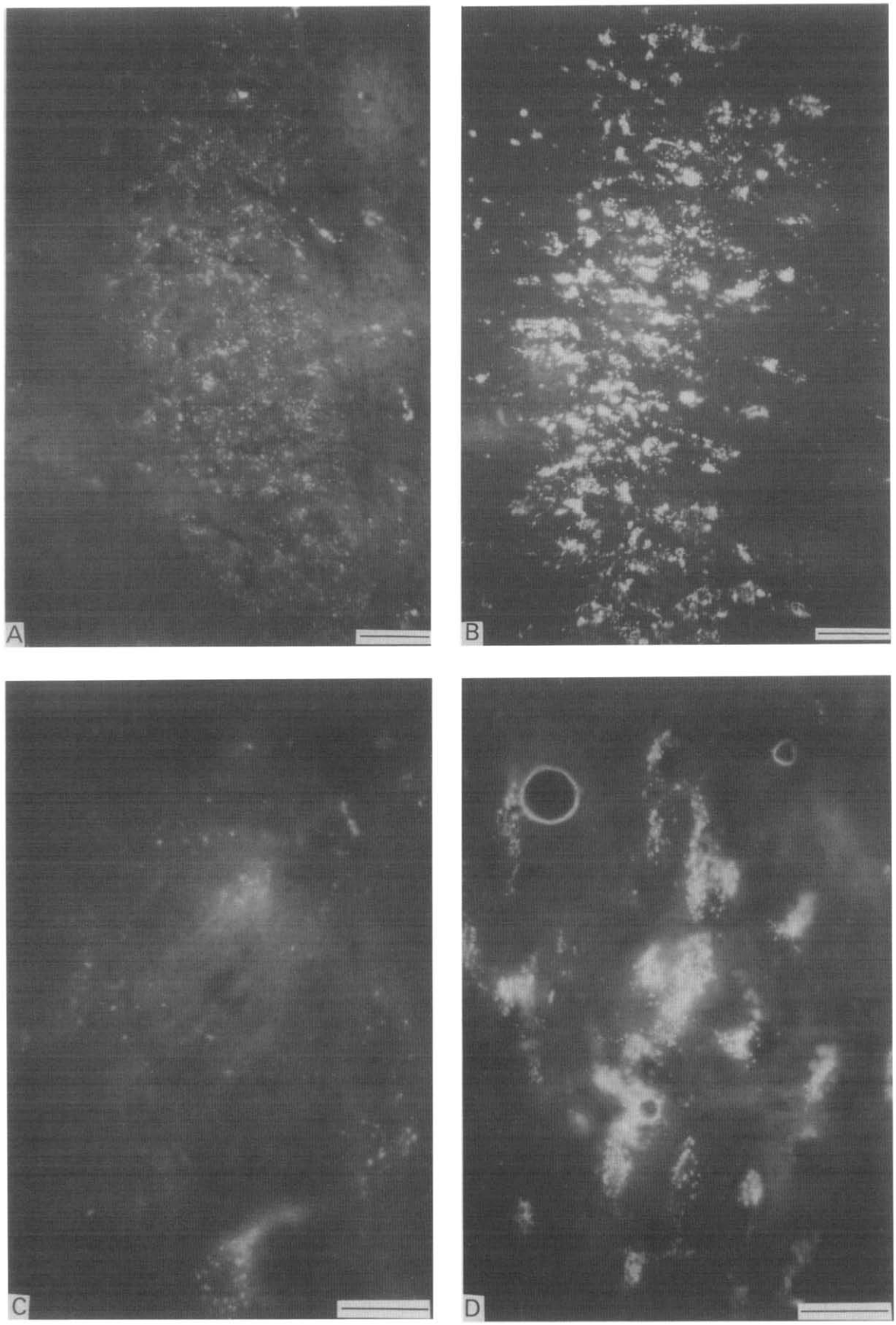
Scopolamine administration inhibited locomotor activity in control animals as well as in vitamin E deprived animals ($H_3=19.03$; $p=0.0003<0.025$). The effects of scopolamine on locomotor activity in vitamin E deprived animals were weaker than those in the control group. The slopes of the activity curves over the 4 sessions did not differ between the 4 groups ($p=0.75$).

The same result is shown for the total explored area (Fig. 8). Control, scopolamine-treated animals activated about 1/4 of total maze photocells per session while the vitamin E deprived animals activated more than half ($H_3=16.59$; $p=0.0009<0.025$).

DISCUSSION

This experiment demonstrates that scopolamine reduces significantly locomotor and exploratory activity of animals in the tunnel maze. The effects of scopolamine in the vitamin E deprived animals were clearly smaller than in the controls and thus do not support the idea of a higher susceptibility to scopolamine as result of the vitamin E deprivation.

This unexpected result may be best interpreted in terms of kinetic changes as a result of the vitamin E deficiency. However, little is known of the pharmacokinetics of scopolamine or of the effects of vitamin E deprivation on drug metabolism. Effects of vitamin E deprivation have been found on the metabolism of nitrofurantoin, phenolsulfonphtalein or barbital [19,32]. It could be speculated that the effects of vitamin E on the function of the smooth fraction of the hepatic endoplasmic reticulum [16] might alter the me-


A
B
C
D

tabolism of scopolamine, possibly even the rate of its metabolism to scopoline and scopine [29].

On the other hand, a direct, receptor-mediated effect cannot be conclusively excluded. Although it has been shown by Noda *et al.* [23] that Vitamin E deficiency alters ChAT activities in various brain areas only insignificantly, any effect of the diet on the pharmacological potency of scopolamine should be verified in future experiments.

EXPERIMENT 5

In this experiment, 5 animals of each group were examined by the use of fluorescence microscopy in order to describe crudely the degree of the neuronal lipofuscin accumulation in the brain of the vitamin E deprived rats. Lipofuscin emits a bright yellow-green to orange fluorescence and, therefore, can be easily demonstrated when excited with ultraviolet light.

METHOD

From each group, 5 animals were used. They had been fed the diets for 46 weeks (age: 50 weeks). They were sacrificed, their brains were removed and immediately frozen in isopentane chilled by liquid nitrogen. Coronal sections (40 μm) were cut by a cryostat (Leitz) and examined microscopically (Leitz Ploemopak; Filter block D, i.e., excitation wavelength: 340–410 nm; emission filter: LP 460). Photomicrographs were prepared with Ektachrome 400. Lipofuscin accumulation in different brain areas was estimated semiquantitatively as indicated in Table 2 and in a blind way by two persons.

RESULTS

Table 2 summarizes the results. In all areas examined, there was a higher lipofuscin content in the vitamin E deprived animals than in the controls. The highest content of autofluorescent lipofuscin granules was found in hippocampal areas. Figure 9 illustrates the striking differences between controls and vitamin E deprived animals in this brain region. In comparison to the hippocampus, neither cortical nor cerebellar areas showed such a high density of granules within such a high number of neurons (see Table 2).

DISCUSSION

Hippocampal accumulation of lipofuscin has been found previously, either as a result of aging (e.g., [10]) or vitamin E deprivation (e.g., [14, 20, 23]). Although in this and other studies considerable variability between individual animals in cytoplasmic density of neuronal lipofuscin is observed, the differences between vitamin E deprived and control animals observed here were dramatic.

GENERAL DISCUSSION

The results of the different experiments suggest that:
(a) vitamin E deprivation did not alter locomotor and exploratory activity measured by the use of the hole-board;
(b) the vitamin E deprived animals were significantly impaired in relearning of a 6-arm radial tunnel maze following an 18-day break of training;
(c) the vitamin E deprived animals mastered the reversed configuration of the tunnel maze as well as the controls;
(d) the vitamin E deprived animals showed weaker effects when treated with scopolamine than the controls and,
(e) the highest density of lipofuscin accumulation as a result of vitamin E deprivation was found in hippocampal areas.

The following discussion will be guided by 2 general questions:
(1) Does vitamin E deprivation induce defined behavioral alterations and does the lipofuscin accumulation represent its biological basis?
(2) Does vitamin E deprivation represent a useful animal model for accelerated aging?

Behavioral Alterations as a Result of Vitamin E Deprivation

The behavioral alterations found in our experiments are very subtle, especially when it is considered that the dependency of the behavioral variables was not taken into account in statistical analysis. However, the behavioral impairment of the vitamin E deprived animals during the relearning of the 6-arm radial maze (experiment 2) did not seem to be related to non-cognitive behavioral components (locomotor activity).

To our knowledge, only Lal and co-workers have studied the cognitive effects of vitamin E deprivation [14]. As in our experiments, they did not find effects on exploratory or locomotor activity. However, their deprived rats needed more trials to acquire a conditioned avoidance response than the age-matched controls and they were impaired in a delayed alternation task under different delay intervals. Part of the results of Lal's experiments refer to working memory, whereas the one effect seen in our experiments has to be discussed rather in terms of reference memory. The most striking effect of the vitamin E-deficient diet in Lal's experiments, however, was the impaired retention of a one-trial experience, also a task dependent on reference memory.

Does the Accumulation of Lipofuscin Relate to the Cause of the Behavioral Alterations?

Like numerous previous studies (for review, see [27]) we have shown that vitamin E deprivation induces a remarkable accumulation of lipofuscin which, in our experiment, was especially high in the hippocampus. Therefore, it does not seem a matter of debate that the lipofuscin accumulation represent a major pathological consequence of the vitamin E deprivation in the CNS. There are some studies which reported a variety of further neuropathological signs as, for instance, loss of sensory axons in the posterior columns, sensory roots and peripheral nerves [22]. In rats, however, behavioral signs of sensory axonpathy were never observed, neither in our nor in Lal's experiments.

On the other hand, the impaired relearning performance of our vitamin E deprived animals corresponds to the im-

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FIG. 9. Lipofuscin accumulation in the CA3 field of the hippocampus in control (A,C) and vitamin E-deficient animals (B,D). Scales in A and B: 100 μm, in C and D: 50 μm. Leitz Ploemopak, Filter D, Ektachrome 400.

paired spatial-reference memory in hippocampal lesioned rats [21]. In order to support such an idea, however, it seems necessary to detect clear-cut signs of neuronal alterations of pyramidal hippocampal neurons which are filled with lipofuscin granules. Moreover, lipofuscin accumulation in the course of normal aging may have more defined functional consequences than when induced by vitamin E deprivation: the studies of Brizzee and Ordy [6,7] suggest at least a correlation between cognitive abilities, neuronal loss and lipofuscin accumulation in the hippocampus CA1 zone and in area 17. However, it is important to note that the tissue distribution of lipofuscin may not be the same in aged and vitamin E deprived animals.

Dietary Vitamin E Deprived Animals as a Model for Accelerated Aging

Lipofuscin accumulation represents one of the most prominent alterations of the aging brain, in animals as well as in man. From this point of view, vitamin E deprivation clearly induces a characteristic sign of neuronal aging in young adult animals. The purpose of animal models of aging and age-related cognitive disturbances implies, however, that behavioral impairments of the animals can be measured reliably and related to the experimentally induced neuronal changes (for review, see [26,27]). These requirements do not seem to be satisfied at present by the properties of the vitamin E deprivation model. However, it is still too early to decide finally about the model; by the use of more sensitive behavioral models or completely different ones (in terms of their psychological validity) and by measuring various biochemical events [10,23], the animal model might be developed and it could mirror human aging at both a neuropathological and psychological level. Since lipofuscin accumulation in demented people was found to be even higher than in aged ones and not any more related to age [8], a cytotoxic function of lipofuscin or, at least, its involvement in cytotoxic processes seems a more appropriate hypothesis than the assumption of a harmless cellular by-product. Thus, further studies should be concentrated on studying more intensely the behavioral effect of vitamin E deprivation and should focus on changes of neuronal functions (on a biochemical or electrophysiological level) as a result of lipofuscin accumulation.

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REFERENCES

1. Arnhold, J. Die Entstehung von Lipofuscin. *Ztr Altersforschung* **34:** 179–182, 1979.
2. Barcikowski, R. S. and R. R. Rowbey. Decisions in single group repeated measures analysis: statistical tests and three computer packages. *Am Stat* **38:** 148–150, 1984.
3. Bättig, K. Spontaneous tunnel maze locomotion in rats. In: *Application of Behavioral Pharmacology in Toxicology*, edited by G. Zbinden, V. Cuomo, G. Racagni and B. Weiss. New York: Raven Press, 1983, pp. 15–26.
4. Becker, J. T., J. A. Walker and D. S. Olton. Neuroanatomical bases of spatial memory. *Brain Res* **200:** 307–320, 1980.
5. Bieri, J. G. and P. M. Farrell. Vitamin E. *Vitam Horm* **34:** 31–75, 1976.
6. Brizzee, K. R. and J. M. Ordy. Age pigments, cell loss and hippocampal function. *Mech Ageing Dev* **9:** 143–162, 1979.
7. Brizzee, K. R., T. Samorajski, D. L. Brizzee, J. M. Ordy, W. Dunlap and R. Smitz. Age pigments and cell loss in the mammalian nervous system: Functional implications. *Aging* **21:** 211–229, 1983.
8. Dowsen, J. H. Neuronal lipofuscin accumulation in ageing and Alzheimer dementia: A pathogenic mechanism? *Br J Psychiatry* **140:** 142–148, 1982.
9. Einarson, L. and I. R. Telford. Effect of vitamin E-deficiency on the central nervous system in various laboratory animals. *Biol Skr Dan Vrid Selsk* **11:** 1–81, 1960.
10. Ferrendelly, J. A., W. G. Sedgwick and V. Suntzeff. Regional energy metabolism and lipofuscin accumulation in mouse brain during aging. *J Neuropathol Exp Neurol* **30:** 638–649, 1971.
11. File, S. E. and A. G. Wardill. Validity of head dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia* **44:** 53–59, 1975.
12. File, S. E., S. Pellow and L. Wilks. The sedative effects of DL 281,872, like those of chlordiazepoxide, are reversed by benzodiazepine antagonists. *Psychopharmacology (Berlin)* **85:** 295–300, 1985.
13. File, S. E. and A. G. Wardill. The reliability of the hole-board appartus. *Psychopharmacologia* **44:** 47–51, 1975.
14. Lal, H., S. Pogacar, P. R. Daly and S. K. Puri. Behavioral and neuropathological manifestations of nutritionally induced central nervous system "aging" in the rat. *Prog Brain Res* **40:** 129–140, 1973.
15. Ljungberg, T. and U. Ungerstedt. Automatic registration of behavior related to dopamine and noradrenaline transmission. *Eur J Pharmacol* **36:** 181–188, 1976.
16. Lucero, S., F. A. de la Iglesia, G. Lumb and G. Fener. Nutritional influences on drug metabolism. *Rev Can Biol* **32:** 69–75, 1973.
17. Lucy, J. A. Functional and structural aspects of biological membranes: A suggested structural role for vitamin E in the control of membrane permeability and stability. *Ann NY Acad Sci* **203:** 4–11, 1972.
18. Manocha, S. L. and S. P. Sharma. Reversibility of lipofuscin accumulation caused by protein malnutrition in the motor cortex of squirrel monkeys. *Saimiri scireus*. *Acta Histochem* **58:** 219–231, 1977.
19. Meshali, M. M. and C. H. Nightingale. Effect of alpha tocopherol (vitamin E) deficiency on intestinal transport of passively absorbed drugs. *J Pharmacol Sci* **65:** 344–348, 1976.
20. Miyagishi, T., N. Takahata and R. Iizuda. Electron microscopic studies on the lipo-pigments in the cerebral cortex nerve cells of senile and vitamin E-deficient rats. *Acta Neuropathol* **9:** 7–17, 1967.
21. Morris, R. G. M. An attempt to dissociate "spatial-mapping" and "working-memory" theories of hippocampal function. In: *Neurobiology of the Hippocampus*, edited by W. Seifert. London: Academic Press, 1983, pp. 405–432.
22. Nelson, J. S., C. D. Fitch, V. W. Fischer, G. O. Broun and A. C. Chov. Progressive neuropathologic lesion in vitamin E-deficient rhesus monkeys. *J Neuropathol Exp Neurol* **40:** 166–186, 1981.
23. Noda, Y., P. L. McGeer and E. G. McGeer. Lipid peroxides in brain during aging and vitamin E-deficiency: Possible relation to changes in neurotransmitter indices. *Neurobiol Aging* **3:** 173–178, 1982.

24. Oettinger, R., J. R. Martin, E. Rosenberg and K. Bättig. Effects of tunnel maze complexity on caffeinic hyperactivity in the rat. *Pharmacol Biochem Behav* **23:** 85–90, 1985.
25. Reichel, W., J. Hollander, J. H. Clark and B. L. Strehler. Lipofuscin pigment accumulation as a function of age and distribution in rodent brain. *J Gerontol* **23:** 71–78, 1968.
26. Sarter, M. Measurement of cognitive abilities in senescent animals. *Int J Neurosci* **32:** 765–774, 1987.
27. Sarter, M. Animal models of brain aging and related diseases. *Comp Gerontol* **1:** 4–15, 1987.
28. Sarter, M. and H. J. Markowitsch. Reduced resistance to progressive extinction in senescent in rats: A neuroanatomical and behavioral study. *Neurobiol Aging* **4:** 203–215, 1983.
29. Scheurlen, M., H. Bittiger and B. Ammann. Simple radioligand binding assay for the determination of urinary scopolamine. *J Pharmacol Sci* **73:** 561–563, 1984.
30. Schlote, W. and J. W. Boellaard. Role of lipopigment during aging of nerve and glia cells in the human central nervous system. *Aging* **21:** 27–74, 1983.
31. Spencer, D. G. and H. Lal. Effects of anticholinergic drugs on learning and memory. *Drug Dev Res* **3:** 489–502, 1983.
32. Statham, C. N., R. F. Minchin, H. A. Sasame, S. H. Kim and M. R. Boyd. Effects of vitamin E on the distribution and metabolism of nitrofurantoin in rats. *Drug Metab Dispos* **13:** 532–534, 1985.
33. Stephens, D. N., R. Weidmann, D. Quartermain and M. Sarter. Reversal learning in senescent rats. *Behav Brain Res* **17:** 193–202, 1985.
34. Sulkin, N. M. and P. Srivanij. The experimental production of senile pigments in the nerve cells of young rats. *J Gerontol* **15:** 2–9, 1960.
35. Tappel, A. L. Will antioxidant nutrients slow aging process? *Geriatrics* **23:** 97–105, 1968.
36. Taubold, R. D., A. N. Siakatos and E. G. Perkins. Studies on chemical nature of lipofuscin (age pigment isolated from normal human brain). *Lipids* **10:** 383–390, 1975.
37. Zuckermann, B. M. and M. A. Geist. Effects of nutrition and chemical agents on lipofuscin formation. In: *Age Pigments*, edited by R. S. Sohal. Amsterdam: Elsevier, 1981, pp. 283–302.