Radboud Repository

Radboud University Nijmegen

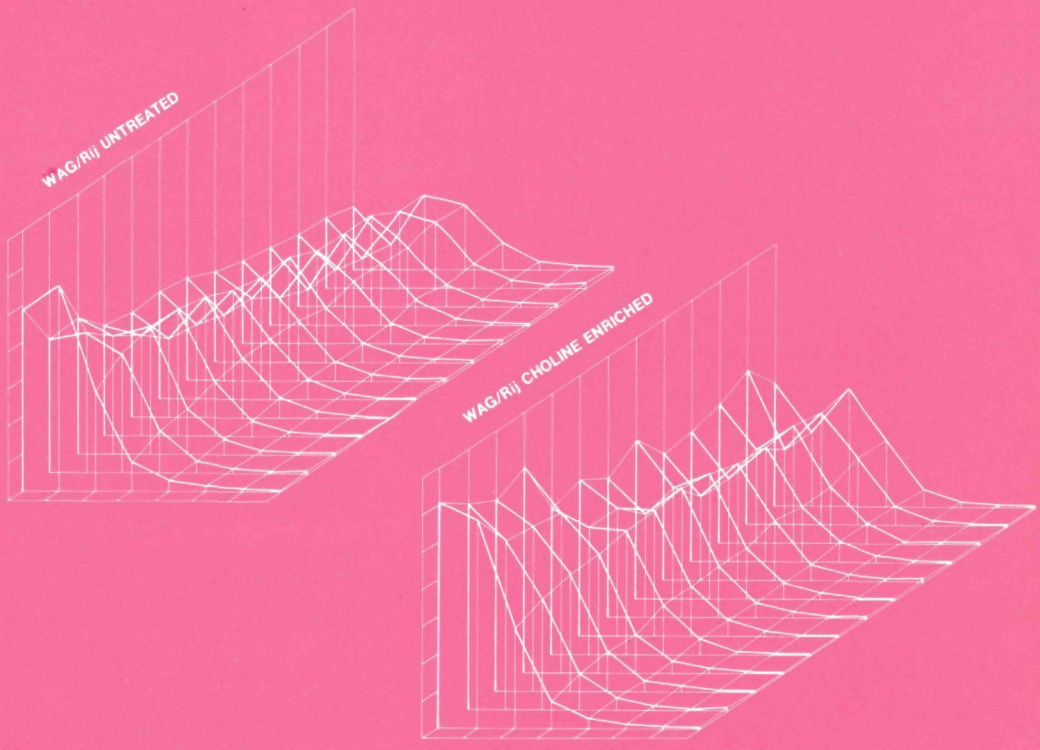
PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/113649

Please be advised that this information was generated on 2017-12-06 and may be subject to change.

BEHAVIORAL CONSEQUENCES OF CHRONIC DIETARY CHOLINE ENRICHMENT



F. Josef van der Staay

BEHAVIORAL CONSEQUENCES OF CHRONIC DIETARY CHOLINE ENRICHMENT

BEHAVIORAL CONSEQUENCES OF CHRONIC DIETARY CHOLINE ENRICHMENT

Een wetenschappelijke proeve op het gebied van de
Sociale Wetenschappen

Proefschrift
ter verkrijging van de graad van doctor aan
de Katholieke Universiteit te Nijmegen,
volgens besluit van het college van decanen in het
openbaar te verdedigen op
donderdag 25 mei 1989
des namiddags te 3.30 uur

door

Franz Josef van der Staay
geboren op 7 juni 1951 te Goch

Maastricht 1989

Promotores:
Prof. Dr. J.M.H. Vossen
Prof. Dr. J. Jolles (Rijksuniversiteit Limburg)

Co-referent:
Dr. W.G.M. Raaijmakers (Rijksuniversiteit Limburg)

This research was conducted at the Department of Comparative and Physiological Psychology while F.J. van der Staay was supported by a research grant from the Catholic University of Nijmegen (Universitaire Onderzoekpool, project nr. SW 3-81) awarded to dr. W.G.M. Raaijmakers, and dr. J.M.L. Kerbusch.

Financial support for the purchase of aged animals was given by a grant from 'Interdepartementaal Stimuleringsfonds Gerontologie',
SOOM project nr. 83-2-59.

ISBN 90-9002811-0

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

Man's increased lifespan is paralleled by an increase in age-related impairments and diseases (Brody, 1985) Dementias, progressive clinical states which are characterized by a deterioration of intellect, memory, judgment and abstract thinking (American Psychiatric Association DSM III-R, 1987), are among the most disabling impairments in elderly people For example, dementia of the Alzheimer type affects about 5 percent of the population over 60 years of age (Coyle, Price, & DeLong, 1983 Katzman 1986) Census projections indicate that a dramatic increase in the number of Alzheimer s patients can be expected in the future (Brody, 1985, Anderson, 1986) The prevalence of dementias is increasing, and 50 to 60 percent of the demented suffer from Alzheimer's disease (Katzman 1986)

Different therapeutic strategies have been proposed for the treatment of the age-related decline of cognitive functions which are observed in the aged, and more severely in demented people (reviewed by Craik, 1977, Winblad, Hardy, Bäckman, & Nilsson, 1985, Jolles, 1986a) Some of these therapies are already applied in the clinic while others, for example transplantation of embryonic neurons in the aged brain (Bjorklund, & Gage, 1986), are still at a preclinical stage Clinical therapies range from the prescription of drugs supposed to increase cerebral blood flow or to stimulate the brain metabolism (Swaab, & Fliers, 1986) to pharmacological manipulations of neuroendocrine (e g ACTH and vasopressin Jolles, 1986b) and neurotransmitter systems (Swaab, & Fliers, 1986)

In the context of aging and dementia, the central cholinergic neurotransmitter system has received special attention A pronounced decline of the central cholinergic activity has been observed in the brains of Alzheimer patients (Perry, 1980) The magnitude of the decrease in cholinergic function correlates with the severity of the cognitive impairments in these patients (Katzman, 1986) The presumed causal relationship between the decreased cholinergic activity and the cognitive dysfunctions has led Bartus and co-workers to advance their so-called 'cholinergic hypothesis of geriatric memory dysfunction' (Bartus, Dean, Beer, & Lippa, 1982)

This hypothesis implies that treatments should focus on increasing central cholinergic activity One such treatment strategy consists of supplementing the diet with the acetylcholine precursors choline or lecithin (Bartus, Dean, & Beer, 1984, Lieberman, & Abou-Nader, 1986) It has been reported that an increased availability of these precursors increases cholinergic activity (Haubrich, Wang Clody, & Wedeking, 1975) In turn, enhanced cholinergic activity should be accompanied by a reduction in the cognitive impairments (Bartus, Dean, & Beer, 1984) Clinical trials with this precursor therapy, however, have generally yielded disappointing results (Bartus, Dean, Pontecorvo, & Flicker, 1985)

This thesis describes a series of experiments in which the behavioral consequences of chronic dietary choline supplementation were assessed in rats We studied basal behaviors such as the speed of adaptation to a novel environment, emotional reactivity, and the circadian drinking pattern Learning and memory were assessed in inhibitory (passive) and active avoidance

tasks, in an incompletely acquired operant bar-press task, and in complex temporal and spatial discrimination tasks

Avoidance paradigms were used to investigate whether the positive results that were found earlier with mice (Bartus, Dean, Goas, & Lippa, 1980 Leathwood, Heck, & Mauron, 1982, Davis, & Trombetta, 1984, Mervis, Horrocks, Wallace, & Naber, 1984, Muma, & Rowell, 1984) could be reproduced with rats Other behavioral paradigms were included to gain more insight into the effects of chronic dietary choline enrichment than that provided by aversively motivated tasks Complex temporal (e g Meck, & Church, 1987a, 1987b) and spatial (e g Davis, Idowu, & Gibson, 1983) discrimination performance can be influenced by cholinergic drugs Therefore, discrimination tasks are considered suitable paradigms to assess the behavioral effects of chronic dietary choline enrichment Moreover, it has been found consistently that rats show an age-related decrease of their performance in complex spatial discrimination tasks

Two animal models were used an 'aging model', and a 'genetic model' The senescent rat possesses high face validity as a model for the aging human This 'aging model', however, may be of limited value (discussed in a separate paragraph, later in this Chapter) Therefore, the (young-) adult Brown Norway (BN) rat, which has lower cholinergic activity than other strains of rats and performs very poorly in shock-motivated tasks, was included as a potential genetic model for the age-related decline in learning and memory, and in central cholinergic activity The validity of this genetic model' was assessed

THE 'CHOLINERGIC HYPOTHESIS OF GERIATRIC MEMORY DYSFUNCTION'

According to Bartus and colleagues (Bartus, Dean, Beer, & Lippa, 1982), the 'cholinergic hypothesis of geriatric memory dysfunction' should meet three deductive requirements

1) Cholinergic markers in the brains of subjects suffering from age-related memory loss should indicate specific dysfunctions
2) The reduction of central cholinergic function in young subjects by pharmacological agents should produce cognitive impairments similar to those found in the elderly
3) The enhancement of central cholinergic activity should ameliorate the age-related cognitive impairments

As far as the first two requirements are concerned there is evidence in favor of the 'cholinergic hypothesis', although some reservations have been expressed, especially with respect to the second requirement

Ad 1) The aging brain, dementia, and cholinergic neurotransmission

The enzymes that synthesize neurotransmitters have been studied as potential indices of age-associated changes in neurotransmission In the central nervous system a reduction in the activity of cortical choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine (ACh), as well as a decrease in markers of other neurotransmitter systems have been found to accompany normal aging and dementias (McGeer, & McGeer, 1975, 1978, Gottfries, Adolfsson, Aquilonius, Carlsson, Eckernas, Nordberg, Oreland, Svennerholm, Wiberg, & Winblad, 1983, Winblad, Hardy, Bäckman, & Nilsson, 1985, Lieberman, & Abou-Nader, 1986, Farooqui, Liss, & Horrocks, 1988; Mann, 1988)

The most pronounced decline, however, is in the activity of ChAT (McGeer, & McGeer, 1978, Perry, 1980, Collerton, 1986) A severe functional deterioration of the central cholinergic

system (Coyle, Price, & deLong, 1983) is seen as one of the most important and consistent symptoms accompanying Alzheimer type dementia (Collerton, 1986)

Neuroanatomically, a profound degeneration of ACh-releasing cells in the nucleus basalis of Meynert (nbM), localized in the basal forebrain, has been found in Alzheimer patients (Coyle, Price, & DeLong, 1983, Davison, 1987) This nucleus provides the major cholinergic input to the neocortex Perry and co-workers (Perry, Candy, Perry, Irving, Blessed, Fairbairn, & Tomlinson, 1982) however, observed a very pronounced decrease in cortical ChAT activity and only a slight loss of neurons in brains from Alzheimer patients.

Cell loss in the nbM and a reduction of cholinergic activity in the cortex have also been observed in patients suffering from other types of dementias such as Picks's disease (Uhl, Hilt, Hedreen, Whitehouse, & Price, 1983, but not confirmed by Tagliavini, & Pilleri, 1983), Parkinsons s disease (Candy, Perry, Perry, Irving, Blessed, Fairbairn, & Tomlinson, 1983, Whitehouse, Hedreen, White, & Price, 1983, Tagliavini, Pilleri, Bouras, & Constantinidis, 1984), Creutzfeldt-Jakob disease (Arendt, Bigl, & Arendt, 1984), and Korsakoff's disease (Arendt, Bigl, Arendt, & Tennstedt, 1983) Taken together, these findings provide ample evidence that the degeneration and loss of cholinergic cells provide a morphological correlate of the reduced cortical cholinergic activity that might play a crucial role in several types of dementias The precise relationship between cell loss and reduced cortical ChAT activity, however, remains to be determined (Plotkin, & Jarvik, 1986)

A clear correlation between the severity of the cognitive impairments and the severity of the histological abnormalities (senile plaques and the reduction of cortical ChAT activity) has been found in patients with Alzheimer's disease (e g Perry, Tomlinson, Blessed, Bergman, Gibson, & Perry, 1978, Wilcock, Esiri, Bowen, & Smith, 1982, Davies, 1985, Katzman, 1986)

It should be stressed that the cholinergic hypothesis does not exclude the involvement of neurotransmitter systems other than the cholinergic system in age-associated cognitive impairments and dementias (Bartus, Dean, Pontecorvo, & Flicker, 1985) The literature on the relationship between the cholinergic system and geriatric memory function has been reviewed extensively during the last few years (e g Bartus, Dean, Beer, & Lippa, 1982, Coyle, Price, & DeLong, 1983; Bartus, Dean, & Beer, 1984, Bartus, Dean, Pontecorvo, & Flicker, 1985, Collerton, 1986, Kopelman, 1986, Plotkin, & Jarvik, 1986).

Ad 2) Scopolamine induced amnesia

Drachman (1977, Drachman, & Leavitt, 1974) demonstrated that treatment with scopolamine, a powerful and specific anticholinergic agent that blocks cholinergic receptors, disrupted the performance of female and male students on a battery of tests measuring memory and cognition The subjects showed impaired memory storage and impaired non-mnemonic cognitive functions Drachman stated that there was a marked similarity between the pattern of cognitive impairments induced in the young subjects by scopolamine and the impairments seen in aged subjects (Drachman, & Leavitt, 1974) Scopolamine-induced memory impairments have gained widespread acceptance as a model for dementias (e g Broks, Preston, Traub, Poppleton, Ward, & Stahl, 1988) Beatty, Butters, and Janowski (1986), however, doubt whether scopolamine-induced memory impairments in healthy young subjects adequately mimic the full pattern of impairments shown by patients with cortical or subcortical dementias They found that scopolamine did not induce generalized impairments in episodic and semantic memory tasks or increase the sensitivity to interference and the disposition for perseverative errors, which are characteristic of patients suffering from Alzheimer's disease

Similarly, objections to scopolamine-induced amnesia as a model for age-related cognitive impairments have been expressed by Flood and Cherkin (1986), who assessed the effects of scopolamine on the retention of mice in a series of experiments

Ad 3) Treatment of age-related memory dysfunction

The third point implies that treatments which improve central cholinergic neurotransmission should ameliorate age-related memory dysfunctions Research on the precursor therapy has yielded contradictory results, most of them disappointing, since beneficial effects have been attained only sporadically (extensively reviewed by Bartus, Dean & Beer 1984, Bartus, Dean, Pontecorvo, & Flicker, 1985, and summarized by Collerton, 1986, Perry, 1986)

STRATEGIES FOR THE MANIPULATION OF CENTRAL CHOLINERGIC (DYS)FUNCTIONS

Three possible strategies for the treatment of cholinergic dysfunction have been described (Drachman, Glosser, Fleming, & Longenecker, 1982, Coyle, Price & DeLong, 1983)

The first involves the administration of an anticholinesterase, for example physostigmine, which prolongs the 'survival of actylcholine in the synaptic cleft by inhibiting the activity of acetylcholinesterase (AChE) the enzyme that hydrolyzes ACh In two studies performed by Drachman (1977, Drachman, & Leavitt, 1974) with human volunteers attempts were made to reverse the scopolamine induced cognitive impairments by physostigmine, an inhibitor of AChE Only one of these attempts was successful (Drachman, 1977) Drachman is one of the first to attempt to reduce the cognitive impairments which were caused by decreased cholinergic activity, by means of pharmacological interventions

The second strategy consists of directly stimulating post-synaptic cholinergic receptors with an ACh agonist, for example arecoline (e g Flood, & Cherkin, 1988) However, the treatment with anticholinesterases or with direct receptor agonists have a number of disadvantages They might produce very distressing side-effects (Growdon, 1979), their action is relatively short, thus necessitating frequent dosing, and the therapeutic window is very narrow (Weinstock, Razin, Chorev, & Tashma, 1986)

The third therapeutic strategy is believed to be free from these disadvantages The approach consists of increasing the availability of the ACh precursor choline or lecithin by increasing the concentration of these substances in the diet The rationale behind this approach which has been termed precursor therapy' (Bartus, Dean, & Beer, 1984), is that the enhanced availability of ACh precursors leads to an increase in cholinergic activity, presumably via an increased synthesis of ACh (Growdon, 1979), and that the increased cholinergic activity in turn abolishes the cognitive impairments accompanying the inadequate cholinergic tone

POSSIBLE MODES OF ACTION OF THE SUPPLEMENTED DIETARY CHOLINE

Supplementation with exogenous choline and phosphatidylcholine increases the levels of choline in the brain (Wurtman, Hirsch, & Growdon, 1977, Flentge, & van den Berg, 1979, Jope, 1982) but it is not clear whether it also raises the brain ACh level (Leathwood, & Schlosser, 1986) Only a few studies have shown an increased choline level accompanied by an increased ACh level in the brain (e g Haubrich, Wang, Clody, & Wedeking, 1975, Cohen, & Wurtman, 1975, 1976, Hirsch, & Wurtman, 1978), whereas most studies have failed to find such an effect

(e.g. Flentge, & van den Berg, 1979; Brunello, Cheney, & Costa, 1982; Jope, 1982). It is likely that choline supplementation increases intra-neuronal ACh levels under conditions of increased neuronal demand (Schmidt, & Wecker, 1981; Jenden, Weiler, & Gundersen, 1982; Wecker, 1986).

Although it is assumed that precursor loading predominantly affects the synthesis of ACh, other modes of action have been proposed.

Choline as ACh agonist

Choline might act as a direct ACh agonist (Consolo, Ladinsky, & Gomeni, 1979; Krnjević, & Reinhardt, 1979). Krnjević and co-workers (Krnjević, Reinhardt, & Ropert, 1982) estimated that choline is about ten times weaker than ACh at the postsynaptic site. Instead of increasing the synthesis and release of ACh, choline might act directly at cholinergic receptors. This suggestion, however, has been criticized. There is evidence to suggest that choline is first incorporated into other compounds (lipid bound) (Schmidt, & Wecker, 1981; Tuček, 1985; Wecker, 1986). Cholinergic neuron firing might mobilize bound choline which is then utilized for the synthesis of ACh rather than that it acts directly.

Role of choline in membrane synthesis

The choline molecule can be incorporated into cell membrane phospholipids, predominantly phosphatidylcholine (Farooqui, Liss, & Horrocks, 1988). The fluidity of a cell membrane is determined in part by its content of phosphatidylcholine (Shinitzky, 1986).

If an organism is at rest, the pool of free choline may provide sufficient material for the synthesis of ACh. However, under conditions of enhanced cerebral activity, this pool may not be able to supply sufficient quantities of choline. In this case, choline may be liberated from membrane phospholipids. Blusztajn and colleagues (Blusztajn, Maire, Tacconi, & Wurtman, 1984) elaborated this possibility and advanced their 'autocannibalism hypothesis'. They proposed that, in Alzheimer's disease, the degradation of phosphatidylcholine in cholinergic cells is more rapid than its synthesis under conditions in which there is a short supply of free choline. This might alter the composition of the membranes (an altered balance between phosphatidylcholine and other lipids), which might ultimately affect the cell's ability to synthesize new membranes, and thus might impair cell membrane function permanently. Supplementation with exogenous choline might help to reinstate the balance between the membrane lipids and might lead to the synthesis of membranes (Farooqui, Liss, & Horrocks, 1988).

Choline and neuronal plasticity

Mervis and co-workers (Mervis, Parker, El-Yabroudi, Byler, Scherer, McLaughlin, Makely, Dvorak, & Wierdl, 1987, unpublished report) have provided experimental evidence from animals for the view that dietary choline supplementation affects neuronal plasticity. They fed 13-month-old C57Bl/6NNIA mice with chow supplemented with choline, phosphatidylcholine or a commercial soy lecithin for 11 months. Dendritic intersections of pyramidal cells in layer V of the fronto-parietal cortex were analyzed. There was a trend toward a reduction in dendritic branching in untreated 24-month-old mice. The three supplementations with choline increased dendritic branching and dendritic growth in the area most distal to the soma. The amount of dendritic material exceeded that found in 13-month-old animals. Comparable results had been obtained in an earlier study by Mervis and colleagues (Mervis, Horrocks, Demediuk, Wallace, Meyer, Beall, Caris, & Naber, 1985).

Further evidence for the effect of chronic dietary choline manipulations on synaptic plasticity has been found by Bertoni-Freddari and colleagues (Bertoni-Freddari Mervis Giuli & Pieri, 1985) in the cerebellar glomeruli of aging mice In a morphometric study they observed an age-related decrease in the total synaptic contact area and in the number of synaptic contacts per unit volume of tissue sample, and an increase in the average length of the synaptic junctions. Dietary choline enrichment, starting at the age of 10.5 months and lasting for 8 5 months prevented the development of age-associated changes Because only very few fibers in the cerebellar glomeruli are cholinergic, this finding suggests that chronic dietary choline supplementation also affects non-cholinergic structures

In conclusion choline supplementation may play a role in membrane maintenance membrane fluidity and neuronal plasticity apart from its role in cholinergic neurotransmission (as precursor of ACh or as direct cholinergic agonist) The effects of dietary choline supplementation may be due to one or any combination of these modes of action

Choline supplementation via choline chloride in the drinking water or via lecithin in the diet

Attempts to ameliorate age-related deficits of passive avoidance retention in mice and rats by chronic dietary choline (Bartus, Dean, Goas & Lippa, 1980, Davis, & Trombetta 1984, Mervis, Horrocks, Wallace & Naber, 1984, van der Staay, Raaijmakers, & Collijn, 1986) and lecithin enrichment (Muma, & Rowell, 1984) have been reported to be successful In Muma and Rowell s study, however, the step-through inhibitory avoidance of additional groups of aged mice which received a chronically choline- instead of lecithin-enriched diet did not improve

Although lecithin is a more efficient nutritional source of choline in the brain than supplemented choline is (Wurtman, Hirsch, & Growdon, 1977, Leathwood, & Schlosser, 1986) choline was supplemented as choline chloride instead of as lecithin in the experiments described in this thesis We did this for several reasons

Firstly, lecithin is of nutritional value as it consists of phosphatidylcholine (20% or more, depending on the degree of purity), fatty acids, and other substances (Wurtman, 1979) This makes it necessary to use purified diets in experimental and control conditions in order to balance the amount of fat and the calories consumed by the animals

Secondly, we wanted to start choline enrichment immediately after weaning in some experiments and Bell and co-workers (Bell, & Lundberg, 1985, Bell, & Slotkin, 1985) reported that commercial soy lecithin preparations retarded sensorimotor maturation when fed perinatally or during the early juvenile period (shortly after weaning) The lecithin-fed animals showed a retarded cellular development in the cerebral cortex, an effect that was still apparent in the adult animals (Bell, & Slotkin, 1985) Thus, a treatment with lecithin, if started early, might have detrimental effects As far as we know, such findings have not been reported for dietary choline treatment when given as choline chloride in the drinking water

Thirdly, Sanders and colleagues (Sanders, Ackroff, Collier, & Squibb, 1984) have pointed out that some purified diets, although balanced and composed to meet all nutritional needs, retard growth in rats. In order to circumvent the problems associated with lecithin enrichment and purified diets, choline was supplied via the drinking water in our study and the rats received standard rat chow This approach assured a) that all animals received a balanced diet which guaranteed normal growth (see also Appendix B of this thesis), b) that differences observed

between diets could not be attributed to undetected deficiencies induced by the synthetic diets themselves and c) that the only experimentally induced difference between untreated and choline-enriched animals was the amount of choline consumed

ANIMAL MODELS FOR THE STUDY OF CHOLINERGIC NEUROTRANSMISSION AND AGING

Animal models can be helpful when evaluating precursor therapy Small rodents especially possess a number of advantages they have a relatively short lifespan (two to three years) their environment can be strictly controlled, and they show age-related impairments of learning and memory (e g Elias, & Elias 1976) The short lifespan provides the opportunity to test the possible prophylactic or therapeutic effects of drug interventions on the aging process in a short period of time (Mervis 1981)

Various behavioral, anatomical and neuro-chemical characteristics can be mimicked by genetic variations Central cholinergic neurotransmission has been found to show clear genetic variability Overstreet and co-workers (Overstreet Russell Crocker & Schiller 1984) genetically selected a line of rats with increased sensitivity to the AChE inhibitor diisopropyl fluorophosphate Roderick (1960) showed that cortical cholinesterase activity responded to bidirectional selection in two genetically heterogeneous populations of different origin Genetic variability of AChE in the cortex was also found by Kerbusch and co-workers (Kerbusch, van der Staay, & Hendriks, 1981) in a Mendelian cross breeding study with rats, and by Kerbusch (1974) and Raaijmakers (1978) in diallel cross studies with mice

The survival characteristics of populations are also under genetic control Miyamoto and co-workers (Miyamoto, Kiyota Yamazaki, Nagaoka, Matsuo, Nagawa & Takeda 1986) maintain two sublines of mice one of which shows biological characteristics of accelerated aging (SAM-P senescent-accelerated prone mouse), whereas the other shows normal aging (SAM-R senescent-accelerated resistant mouse) The SAM-P mouse shows an earlier onset of the age-related deterioration in learning and memory that is correlated with the accelerated aging

These examples might suffice to demonstrate that parameters of the cholinergic system and survival characteristics respond to genetic selection Further evidence for genetic factors in aging is provided by the fact that inbred strains of mice (Russell, 1972) and rats (Burek, 1978, Gleiser, & Shain, 1986, Masoro, 1980) show considerable differences in the means and distributions of their lifespans

Appropriate lines of mice or rats can be selected from the enormous genetic pool provided by the various inbred strains of rats and mice (Altman, & Katz, 1979 Festing, 1980) According to Russell (1972) inbred strains possess a number of advantages that make them valuable for aging research Firstly, genetically different lines provide controlled differences for experimental designs Secondly, they increase the reproducibility of results as a consequence of the reproducibility of individuals within specific strains (and of F_1 hybrids from inbred strains) Thirdly, the use of inbred strains offers the advantage of predictability As the genotype is specified exactly, knowledge about an inbred strain accumulates with every experiment Results from different studies in which the same genotypes are used can be compared more readily Because longitudinal studies are quite time-consuming, even in rodents with their relatively short lifespan, the most frequently applied experimental design in aging research

consists of cross-sectional comparisons between age groups The use of a specific genotype eliminates the potential source of error variation due to genetic fluctuation

Animal models Limitations and new developments

A general limitation of animal models is that they lack any true analogy to the human disease state they are supposed to be a model for (Gamzu, 1985) As Mervis (1981) pointed out animal models suffer from the major limitation that they do not show the pathologies that characterize the age-related human neuropathologies of the Alzheimer type dementia Senile neuritic plaques and neurofibrillary tangles, both key morphological changes of this disease, seem to be absent in the aging rodent brain

Armstrong and colleagues (Armstrong, Hersh, & Gage, 1988), however recently observed morphological changes in the neocortex of aged Fischer 344 rats that were very similar to the cortical alterations found in the brains of Alzheimer patients cholinergic processes (swellings or grape- like clusterings) in ChAT-positive fibers were enlarged in the neocortex, predominantly in the cingulate cortex

Arendash and colleagues (Arendash, Millard, Dunn, & Meyer, 1987) reported that neuropathological and neurochemical changes were observed in the brains of rats that had received excitotoxic lesions of the nucleus basalis magnocellularis (the animal analogue of the human nucleus basalis of Meynert) 14 months earlier Besides neuronal atrophy and neuronal loss, neuritic plaque-like structures and neurofibrillary changes were observed which resembled the abnormalities found in the brains of Alzheimer patients

The findings of Armstrong and colleagues and of Arendash and co-workers still need to be confirmed If the observed changes can be reproduced then a useful animal model for Alzheimer s disease and for the relationship between cholinergic dysfunctions and cognition (remember that nBM lesions predominantly destroy cholinergic neurons) may become available

Neuronal cell loss in subcortical and cortical areas of senescent rodents has only been observed incidentally (e g Brizzee, & Ordy, 1979, Landfield, Rose, Sandles, Wohlstadter & Lynch, 1977, Landfield Braun, Pitler Lindsey, & Lynch 1981) Most studies, however, have not found any evidence for an age related drop in the number of neurons (e g Freund, 1980, Peters, Feldman & Vaughan, 1983) Hornberger and co-workers (Hornberger, Buell, Flood, McNeill, & Coleman, 1985) investigated the number and size of cholinergic neurons in the basal forebrains of 7-, 15-, and 53-month-old mice There was no age-related cell loss, but the cell size declined with age Thus, age-associated neuronal changes might be more subtle in rodents than in humans

A decrease in dendritic branching, a thinning of the apical dendrites, and a variable loss of spines have been observed in aged mice and rats (Mervis, 1981) Dendritic spines are postsynaptic receptor sites (the function of dendritic spines is reviewed by Coss, & Perkel, 1985) The loss of dendritic spines might be a factor causing age-related cognitive and memory impairments in the rodent However, it is not yet clear whether the loss of synapses in the senescent rodent is a generalized phenomenon in the central nervous system (Curcio & Hinds, 1983, Scheff, Anderson, & DeKosky, 1985) In senescent rats, cytomorphological changes and cell loss are often absent or they occur only moderately This contrasts with the evidence from behavioral studies in aged rodents in which age-related impairments in cognitive functions and memory are generally found

Impairments of learning and memory in the aging rodent

Rats and mice show age-related impairments of learning and memory in a great variety of tasks The precise nature of the cognitive deficits in aged rats is still poorly understood, but there are indications that acquisition is affected predominantly (Ingram, 1988) This impairment may be due to poor memory processing (Gallagher, & Pelleymounter 1988, Pontecorvo, Clissold, & Conti, 1988), but more research on the mechanisms underlying the deficiencies observed in aging animals is needed

The performance of rodents in complex spatial discrimination tasks has been found to be especially affected by aging (Gallagher, & Pelleymounter, 1988) In particular, place learning tasks such as the radial maze (e g Barnes Nadel, & Honig, 1980) the Morris water maze (e g Rapp, Rosenberg, & Gallagher, 1987), and the holeboard (e g van der Staay, Raaijmakers, Sakkee, & van Bezooijen, 1988) are sensitive to age-related impairments

Age-related differences become evident if the development of response strategies and the availability of local cues that mark the correct goal(s) are precluded (Gallagher & Pelleymounter, 1988) This is consistent with the observation gained from research on normal aging and dementia in humans, that the most pronounced effects of aging are found in non-guided free recall tasks (Winblad, Hardy, Backman, & Nilsson, 1985) An age related decrease has also been found in non-spatial tasks provided they are complex An example is the 14-unit Stone multiple T-maze in which the solution depends on 14 successive correct left/right discriminations (Ingram, 1985, 1988)

Equally well established are the learning and memory deficits of aged rodents in shock-motivated tasks (reviewed by Sprott, & Stavnes, 1975, McNamara, Benignus, Benignus, & Miller, 1977, Kubanis, & Zornetzer, 1981) such as inhibitory (passive) avoidance (e g Gold, McGaugh, Hankins, Rose, & Vasquez, 1981), shuttle-box avoidance (e g Rüthrich, Wetzel, & Matthies, 1982), and spatial reversal in a T-maze (Zornetzer, Thompson, & Rogers, 1982) The results gained from these tasks indicate that aged rats show impaired acquisition and deficits in short- and long term retention

Temporal discrimination tasks such as fixed interval (FI) and differential reinforcement of low-rate responding (DRL) can be considered as 'free recall' tasks in which behavior is not guided by external cues Studies using this type of tasks in aging research are rather scarce The available evidence, however, indicates that, in contrast with other tasks, there is no, or only a transient, impairment of the acquisition of a temporal discrimination (e g Hamm, Knisely, & Dixon, 1983, Lejeune, Jasselette, Nagy, & Peree, 1986) It has been reported that aged rats show an age-related memory impairment at the FI task (Campbell, & Haroutunian 1981)

The (young-) adult Brown Norway rat as a 'genetic model' of age-associated behavioral and neurochemical decline

We decided to use young-adult and adult rats of two inbred strains BN/BiRij and WAG/Rij[1] Animals from both strains are commercially available during their whole natural lifespan This makes it possible to use aging rats of both strains to extend knowledge gained from the genetic

[1] Van Luijtelaar and Coenen (1986) reported that WAG/Rij rats show a high incidence of electrocortical abnormalities If these findings would have been known earlier then rats from another strain would have been used in our experiments

model to the naturally aging animal Moreover, a vast amount of information about age-related pathologies and about the survival curves of both rat strains is available (Burek, 1978)

Rats of the BN strain have been described as poor learners (de Koning-Verest, Knook, & Wolthuis, 1980) with low central cholinergic activity (Gilad, & Gilad, 1981, Gilad & McCarty, 1981 Gilad Rabey, & Shenkman, 1983) Sherman and co-workers reported that a reduction of cholinergic activity accompanies aging in rats (Sherman Kuster Dean, Bartus, & Friedman, 1981) In the mouse, an age-related decrease in the activity of the central cholinergic system and of passive avoidance performance has also been found (Strong, Hicks, Hsu, Bartus, & Enna, 1980) Concerning these two characteristics (poor learning and low central cholinergic activity), young BN rats were assumed to provide an adequate genetic model for age-related behavioral and neurochemical changes Thus, it was assumed that in this model sub-optimal functioning of central cholinergic neurotransmission impaired cognitive functions in the (young-) adult BN rat

A crucial question concerning the validity of this model is whether the BN rats suffer from a general cognitive impairment or whether the impairments are task specific Only preliminary conclusions about cognitive impairments can be drawn if a single task is used, as performance on tasks depends on cognitive and non-cognitive functions (e g perception, motivation, motor coordination) The impairment may be caused by any non cognitive function (Olton, 1985) Thus cognitive impairments should be independent of the particular requirements of a task (Squire, & Zola-Morgan, 1985) and should be apparent in different procedures in order to exclude non-cognitive explanations and to support an interpretation in terms of underlying cognitive and mnemonic processes (Gamzu, 1985)

OBJECTIVES OF THE PRESENT STUDY

As stated in the 'cholinergic hypothesis of geriatric memory (Bartus, Dean, Beer, & Lippa, 1982), the age-related decrease in the cholinergic neurotransmission seems to play an important role in the occurrence of cognitive impairment in aging and dementia We wanted to study whether cognitive and non cognitive functions in an 'aging model and a genetic model' could be modulated by chronic dietary choline supplementation Thus, broadly formulated the objectives of the present study were

1) To assess the effects of dietary choline supplementation on behavior of the rat from adulthood to senescence A broad range of behavioral paradigms was used adaptation to a novel environment, emotional reactivity, basal diurnal drinking pattern, acquisition and retention of aversively and appetitively motivated tasks and temporal and spatial discrimination
2) To evaluate the (young-) adult Brown Norway rat as a genetic model of age-associated impairments
3) To extend the behavioral analyses in order to gain insight into the possible role of non-cognitive and non mnemonic factors in the different learning and memory tasks

In all experiments in which the effects of chronic dietary choline supplementation were studied in aging rats, the treatment was intended to be prophylactic We expected that the treatment would ameliorate the age-related impairments of the rats from both strains in these experiments The preventive approach of choline treatment was suggested first by Bartus and colleagues (Bartus, Dean, Goas, & Lippa, 1980) The effectiveness of the treatment in the

first place depends upon the anatomical and functional integrity of the cholinergic neurons if choline is assumed to act as precursor of ACh (Bartus, Dean, Beer, & Lippa, 1982; Drachman, Glosser, Fleming, & Longenecker, 1982).

As aging might be accompanied by cell shrinkage and cell loss, the aging brain may not be able to utilize the supplemented choline (Plotkin, & Jarvik, 1986). The younger brain, on the other hand, might be able to do so. Therefore, all treatments were started at ages that are not characterized by gross behavioral deficits. In some of the experiments, treatment started immediately after weaning and was continued throughout the life of the animal. In experiments in which the animals were tested at relatively young ages, the treatment was intended to be curative instead of preventive in the BN rats, as the low cholinergic activity and the poor performance in shock motivated tasks is already observed at a young age in this particular strain.

SHORT DESCRIPTION OF THE EXPERIMENTS

This section provides an overview of the experiments performed. The reasons for the selection of particular experimental paradigms are discussed in the introductions to the chapters.

In *Chapter 2* the retention performance of BN and WAG rats in an aversively motivated inhibitory (passive) avoidance task was assessed. In the first experiment we investigated whether BN rats perform as well as WAG rats, and whether female rats perform as well as male rats. The main objective of this experiment was to assess whether adult BN rats can be used as a 'genetic model' of age-related cognitive impairments. In the second and third experiment, the effects of chronic dietary choline supplementation on retention performance in the inhibitory avoidance task were studied. The treatment was started shortly after weaning in both experiments, and the animals were tested when they had reached the age of seven (second exp.) or 15.5 months (third exp.), respectively. In a fourth experiment aged rats were tested on this task at the age of about 27 months, after having been on the choline supplemented diet for 5.5 months.

In the first experiment of *Chapter 3* we questioned whether BN rats and female rats of both strains (BN and WAG) show a generalized retention deficit that is independent from the motivational (aversive vs. appetitive) system involved. The extent of the impairment on retention performance of BN rats was compared with that of WAG rats, and the performance of female rats was compared with that of male rats. Twenty-four hour retention of an appetitively motivated incompletely acquired bar-press response in a Skinnerbox was used as the behavioral paradigm. In a second experiment, the effects of chronic dietary choline enrichment on the performance of male BN and WAG rats in this task were studied.

In *Chapter 4* the effects of chronic dietary choline supplementation on timing behavior of BN and WAG rats were studied in a series of experiments. In the first two experiments, a DRL 8" (differential reinforcement of low rate responding) schedule was used; in the third experiment, the rats were trained on the more complex DRL 16" schedule.

In *Chapter 5* we assessed whether dietary choline enrichment affects spatial discrimination performance in a holeboard. In the first experiment, the rats were treated with choline from weaning and were tested at the age of 13 months. In the second experiment, the age-related decrease in the spatial working and reference memory of BN rats was assessed with 4-, 13-, 19-, 25-, and 30-month-old BN rats. Based on the results from this experiment, aging BN and

WAG rats were chronically treated with extra choline during the period in which clear age-associated impairments usually develop Testing in the holeboard task was performed at the age at which the BN rats showed impaired spatial discrimination performance in the previous experiment

In *Chapter 6* we studied whether chronic dietary choline enrichment affects basal behaviors In the first experiment, we assessed whether the treatment influenced the adaptation of BN and WAG rats to a novel environment, and the diurnal pattern of drinking In a second experiment, the effects of the choline supplementation on 'emotional reactivity', as measured in the open field test, were evaluated

In the first two experiments of *Chapter 7* the question of whether BN rats might provide a genetic model' for the age-related decrease in central cholinergic activity was addressed In addition, the effects of chronic dietary choline enrichment on the hippocampal and cortical activities of the enzyme choline acetyltransferase (which synthesizes the neurotransmitter acetylcholine) and of the sodium dependent high-affinity choline uptake system were assessed in the second experiment The third experiment was a preliminary study of age-related changes of these cholinergic markers in the BN strain

CHAPTER 2

EFFECTS OF CHRONIC DIETARY CHOLINE ENRICHMENT ON THE BEHAVIOR OF RATS IN AN INHIBITORY AVOIDANCE TASK

SUMMARY

We first investigated whether the performance of adult Brown Norway (BN) rats in an inhibitory avoidance task is worse than that of WAG rats and thus whether these animals could be used as a 'genetic model of age-associated decrease in learning and memory Secondly we questioned whether the behavior of BN and WAG rats in the inhibitory avoidance task, in particular their retention performance could be modified by chronic dietary choline enrichment We found (exp 2I and 2II) that BN rats performed very poorly when compared with WAG rats Female rats performed poorer than males (exp 2I) Chronic dietary choline enrichment had no effect on the retention performance in the inhibitory avoidance task of adult (exp 2II), middle-aged (exp 2III) and senescent rats (exp 2IV) The middle-aged and senescent rats of both strains showed a poor retention performance (exp 2III and 2IV) Comparisons across experiments suggest that WAG rats probably show an age-related decline in retention performance, while BN rats perform very poorly at all ages During the habituation phase of the inhibitory avoidance task choline-treated rats spent more time in the light compartment and entered that compartment more frequently than the untreated controls This finding suggests that chronic dietary choline enrichment might reduce emotional reactivity However, this effect varied across the strains and experiments and needs to be further investigated It remains to be seen whether the BN strain can be used as a genetic model of age-associated impairments in learning and memory as non mnemonic factors might be responsible for the poor retention of these animals in the inhibitory avoidance task

INTRODUCTION

The inhibitory (passive) avoidance task is widely used to assess the effects of all kinds of experimental manipulations on learning and memory (Gold, 1986) In this task, an animal is placed into the brightly illuminated compartment of a two-compartment box The other compartment is dark As mice and rats prefer the dark (or alternatively, avoid the light) (e g Aulich, Spielhofen, & Raaijmakers, 1974, Aulich, 1976), they will quickly step into the dark compartment where a noxious stimulus (usually an electric footshock) is delivered The animal is retested after a retention interval Normally, the animal now inhibits its preferred response i e the tendency to enter the dark compartment The step-through latency exceeds that measured before the noxious shock was given (Sprott, & Stavnes, 1975) Shorter step-through latencies are interpreted as being an index of impaired memory performance (Gold, 1986)

Age-related decreases of learning and memory in rodents are well-documented (reviewed by Sprott, & Stavnes, 1975, Elias, & Elias, 1976, Kubanis, & Zornetzer, 1981) An inhibitory avoidance task has been used in many of the studies on aging The step through latencies of

aged rodents in this task are shorter than those of younger animals (rats e g Gold & McGaugh, 1975 McNamara Benignus Benignus, & Miller, 1977, Lippa, Pelham, Beer, Critchett, Dean, & Bartus, 1980 Rigter, Martinez & Crabbe, 1980 Gold McGaugh Hankins, Rose & Vasquez, 1981, Bartus, Dean, Sherman Friedman & Beer, 1981 Martinez & Rigter, 1983, mice e g Bartus, Dean, Goas & Lippa, 1980, Dean, Scozzafava, Goas, Regan, Beer, & Bartus, 1981)

Ordy and co-workers (Ordy, Brizzee, Kaack, & Hansche, 1978) observed neuronal loss in the cortex of aged rats that occurred concomitantly with a decline in short term (2 and 6 hours) inhibitory avoidance retention Other studies have found an age-associated loss of pyramidal cells in the hippocampus (Landfield, Braun, Pitler, Lindsey, & Lynch, 1981), which was correlated with impaired memory performance (Landfield Rose, Sandles, Wohlstadter, & Lynch, 1977, Brizzee, & Ordy, 1979) At present, however there is not sufficient experimental evidence to conclude that the age-related decline in inhibitory avoidance performance is caused by neuropathological or cytomorphological changes

Schwegler and Lipp (Lipp, & Schwegler, 1982, Schwegler & Lipp, 1983) found that, over a wide range of mouse and rat genotypes, the structural variability of the hippocampal mossy fiber connections correlates with the animals' performance in a two-way active avoidance task The poorer the avoidance performance was, the more mossy fibers made synaptic contact with basal dendrites of the pyramidal cells in the regio inferior of the hippocampus These findings were corroborated by Dimitrieva and co-workers (Dimitrieva, Gozzo Dimitriev, & Ammasari-Teule 1984) Apart from showing that a direct correlation between neuroanatomical characteristics and avoidance learning might exist, the significance of these findings for the age-related deterioration in avoidance performance remains to be determined

A cholinergic involvement in the retention performance in inhibitory avoidance tasks has been suggested (e g Deutsch, & Rogers, 1979, Deutsch, 1983, excellently reviewed by Hagan, & Morris, 1988 an extensive review about the effects of cholinergic drugs and pharmacological agents that affect non-cholinergic neurotransmitter systems has been compiled by Bammer, 1982) For example, the muscarinic receptor antagonist scopolamine was found to decrease step-through latencies (Deutsch, 1983) Further evidence for a cholinergic involvement has been derived from the finding that neurotoxic lesions of the nuclei of the basal forebrain -which are the main origins of cortical cholinergic innervation have disruptive effects on the retention performance in inhibitory avoidance tasks (e g Friedman, Lerer, & Kuster, 1983)

Although the etiology of the age-associated decline in learning and memory performance in rodents has not yet been established, one particular hypothesis has received considerable attention during the last decade, namely the cholinergic hypothesis of geriatric memory' (Bartus, Dean, Beer, & Lippa, 1982, see also General Introduction' of this thesis) This hypothesis is mainly based on the observation that a severe functional deterioration of central cholinergic neurons is one of the characteristics of patients suffering from Alzheimer type dementia (Perry, 1980) In short, this hypothesis states that cholinergic dysfunction is the major cause of the memory impairment Thus, it has been attempted to pharmacologically modulate central cholinergic functions (Davidson, Haroutunian, Mohs, Davis, Horvath, & Davis, 1986) One approach consists of enhancing the availability of the acetylcholine precursor choline (or lecithin) The rationale behind this approach, which has been characterized as 'precursor therapy' (Bartus, Dean, & Beer, 1984), is that the enhanced availability of acetylcholine precursors raises cholinergic activity (e g Cohen, & Wurtman, 1975, Haubrich, Wang, Clody, & Wedeking, 1975, Wurtman, Hefti, & Melamed, 1981) and thus diminishes cognitive impairment

Research on the precursor therapy has yielded contradictory results, most of them disappointing (reviewed by Bartus, Dean, Pontecorvo & Flicker, 1985) Only a few studies have been successful in ameliorating the age-related deficits in the retention performance in inhibitory tasks in mice which were chronically treated with a choline-enriched diet (Bartus, Dean, Goas & Lippa, 1980, Davis & Trombetta, 1984, Mervis, Horrocks, Wallace, & Naber, 1984)

In the present study a series of four experiments were performed to investigate whether chronic dietary choline enrichment affects the behavior of rats in the inhibitory avoidance task In the first three experiments young-adult, adult, and middle-aged rats of two inbred strains were used the pigmented BN/BiRij and the albino WAG/Rij Rats of the BN strain are poor learners (de Koning-Verest Knook, & Wolthuis, 1980) with a low central cholinergic activity (Gilad & Gilad 1981, Gilad & McCarty, 1981, Gilad, Rabey, & Shenkman, 1983, see also Chapter 7) These characteristics (poor learning and low central cholinergic activity) have also been found in aging mice and rats (Strong, Hicks, Hsu, Bartus, & Enna, 1980, Sherman, Kuster, Dean, Bartus, & Friedman, 1981) Thus, we consider that the BN strain could be used as a genetic' model of age-related behavioral and neurochemical changes

Because the classification of BN rats as poor learners compared with WAG rats was based on results from a shock-motivated 'drink-test (Wolthuis, Knook & Nickolson, 1976 De Koning-Verest, Knook, & Wolthuis, 1980), the first experiment was designed to investigate whether this strain difference could also be demonstrated in a one-way inhibitory avoidance task The drink-test' experiments of Wolthuis and co-workers were done with female rats, while we intended to use male rats in our study Female rats are reported to perform less well than male rats in inhibitory avoidance tasks (e g van Oyen, van de Poll, & de Bruin, 1979, Drago, Bohus, Scapagnini, & de Wied 1980) Therefore, animals of both sexes were used in the first experiment If BN males showed less severe (or even absent) deficits in inhibitory avoidance tasks, then the genetic model would be invalid

In the second experiment we assessed the effects of chronic dietary choline enrichment on the behavior of adult BN and WAG rats in the inhibitory avoidance task In the third experiment the choline concentration was doubled and middle-aged rats of both strains were used In the fourth experiment a more direct model of aging was studied senescent BN and WAG rats were tested after several months of dietary choline supplementation

Apparatus and General Methods

Apparatus The inhibitory avoidance apparatus consisted of a light compartment and a dark compartment, each measuring 40 * 25 * 40 cm The light compartment was made of transparent perspex(R) and was not covered by a lid A bulb mounted above it provided an illumination of 1500 lux at its floor, which was made of grey PVC The dark compartment was made of opaque, black perspex and was covered by a black lid The floor consisted of a metal grid (diameter of stainless steel bars 3 3 mm, free space between bars 9 9 mm) connected to a shock scrambler The two compartments were separated by a guillotine door that could be raised 10 cm When the door was open, the illumination in the dark compartment was about 3 lux The equipment was placed in a room illuminated by two red fluorescent tubes

General Methods Two habituation sessions, one shock session, and a retention session were given separated by inter-session intervals of 24 hours In the habituation sessions the rat was allowed to explore the apparatus for 10 minutes The rat was placed in the light compartment. After an accomodation period of 15 s the guillotine door was opened so that all parts of the

apparatus could be visited freely In the shock session the guillotine door between the compartments was lowered as soon as the rat had entered the dark compartment with its four paws, and a scrambled footshock (0 5 s, 1 mA) was administered The rat was then immediately removed from the apparatus and put back into its home cage The procedure during the retention session was identical to that of the habituation sessions The position of the rat in the apparatus was registered continuously during all sessions with a keyboard connected to an AIM 65 microcomputer Three variables were selected for further analyses

1) the step-through latency, that is the latency of entering the dark compartment for the first time (in s),
2) the time spent in the light compartment (in s), and
3) the frequency of entering the light compartment

As suggested by Richardson and Riccio (1986), besides the step-through latency, the time spent in the the light compartment in the retention session might also be used as an index of retention In addition the time spent in the light compartment during habituation sessions might provide a measure of 'emotional reactivity Aulich (Aulich, Spielhofen, & Raaijmakers, 1974, Aulich, 1976) has shown that treatments which reduce emotional reactivity increase the time spent in the light compartment of a light dark preference box The frequency with which the rat entered the light compartment during the habituation sessions was taken as a measure of locomotor activity This measure might provide an additional index for 'emotional reactivity' during habituation sessions Aulich and Spielhofen (1977) reported that the number of compartment entries was higher in less 'reactive' rats

If the rat did not enter the dark compartment, the first two variables were ascribed a latency of 600 s As variables measured in units of time (latency of entering the dark compartment and time spent in the light compartment) tend to show heavily skewed distributions, they were transformed to the natural logarithm (ln (s)) before statistical evaluation (cf Kerbusch, van der Staay, & Hendriks, 1981, Bond, 1984)

EXPERIMENT 2I INHIBITORY AVOIDANCE STRAIN AND SEX DIFFERENCES

Aim of this experiment was to determine whether young-adult BN rats show a poorer performance in the inhibitory avoidance task than WAG rats Both male and female rats were tested in order to detect sex differences and possible strain by sex interactions

MATERIAL AND METHODS

Animals A total of forty rats of the BN/BiRij and the WAG/Rij strains (ten female and ten male rats per strain) were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands, at the age of 12 weeks (see Appendix C, first experimental protocol) When the animals were 16 weeks old they were housed individually in standard Makrolon™ cages (31 * 28 * 20 cm) The light regimen was reversed (light on between 20 00 and 8 00) when rats were 21 weeks old

Methods At the age of 23 weeks, all animals were tested in an open field (not reported) The animals were 24 weeks old when they were tested in the inhibitory avoidance task, as described in the section Apparatus and General Methods'

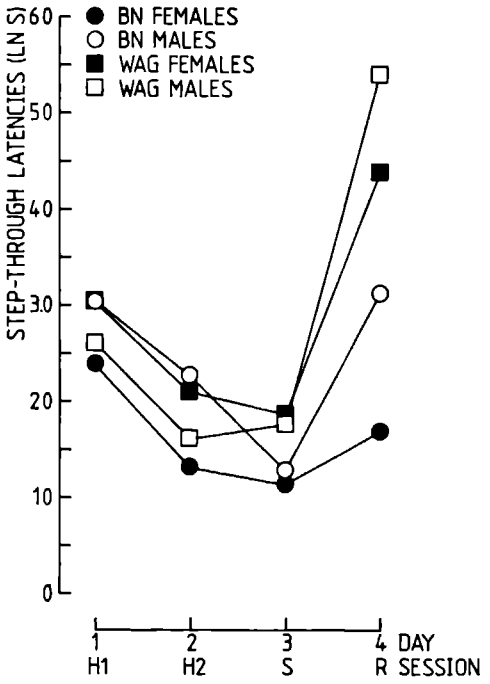


FIGURE 2.1: Step-through latencies (transformed to the natural logarithm: ln s) of BN and WAG female and male rats in a one-trial inhibitory avoidance task.
Abbreviations: H1, and H2: first and second habituation session, S: shock session, R: retention session.

RESULTS

Step-through latency, time spent in the light compartment, and frequency of entering the light compartment were analyzed by a two-factorial strain (BN vs. WAG) by sex (female vs. male) analysis of variance (ANOVA).

Step-through latency (see Fig 2.1): A strain by sex interaction was found for the step-through latencies of the first ($F_{1,36}$ = 9.17, $p < 0.01$) and second habituation session ($F_{1,36}$ = 9.93, $p < 0.01$). Post-hoc Duncan analyses revealed that the BN females had shorter step-through latencies than the BN males in both habituation sessions while those of the female and male WAG rats were not different. A sex difference was no longer apparent in the shock session. The step-through latencies of the two strains were different ($F_{1,36}$ = 7.94, $p < 0.01$), those of the BN rats being faster than those of the WAG rats (average: 3.5 s for BN, 6 s for WAG rats).

The BN rats showed a very poor retention when compared with the WAG rats ($F_{1,36}$ = 43.92, $p < 0.01$), and the female rats of both strains showed a poorer retention than the male rats ($F_{1,36}$ = 10.79, $p < 0.01$; on the average: 5.5 s for BN females, 23 s for BN males, 80.5 s for WAG females, and 223 s for WAG males). As the BN rats had shorter step-through latencies than the WAG rats in the shock session, different step-through latencies between the two strains in the retention session could merely have mirrored a response tendency that already existed in the shock session. To explore this possibility, an analysis of covariance was performed on the step-through latencies of the retention session, with the step-through

latencies of the shock session as covariate The retention differences between the strains ($F_{1,35}$ = 30 30 p < 0 01) and the sexes ($F_{1\,35}$ = 10 79 p < 0 01) were confirmed The above mentioned explanation thus could be ruled out

Time spent in the light compartment (see Fig 2 2, lower panel) The time spent in the light compartment during the habituation and retention sessions quite closely paralleled the findings for the step-through latencies A strain by sex interaction was found in both habituation sessions ($F_{1\,36}$ = 5 03 p < 0 05 and $F_{1\,36}$ = 6 53, p < 0 05 respectively) which probably reflects the fact that the difference between the sexes is not in the same direction in the two strains The precise nature of this interaction, however, could not be determined because a post-hoc Duncan analysis did not confirm differences between the strain by sex groups Strain differences were found in the retention session the WAG animals spent more time in the light compartment than BN rats ($F_{1\,36}$ = 4 68 p < 0 05)

Frequency of entering the light compartment (see Fig 2 2, upper panel) The frequency of entering the light compartment in the habituation sessions was not different for the strains and sexes However, BN rats entered the light compartment more frequently than the WAG rats in the retention session ($F_{1\,36}$ = 4 68, p < 0 05), and the female rats entered the light compartment more often than the male rats ($F_{1\,36}$ = 7 69, p < 0 01)

Discussion

Animals of both strains showed clearly different inhibitory avoidance retentions, as shown by both indices of retention, 'step-through latency' and time spent in the light compartment Both sexes of the BN strain showed a very poor retention Their step-through latencies in the retention test hardly differed from the latencies observed in the first habituation session This corroborates the findings of McCarty, Kirby, and Garn (1984) who found that BN rats

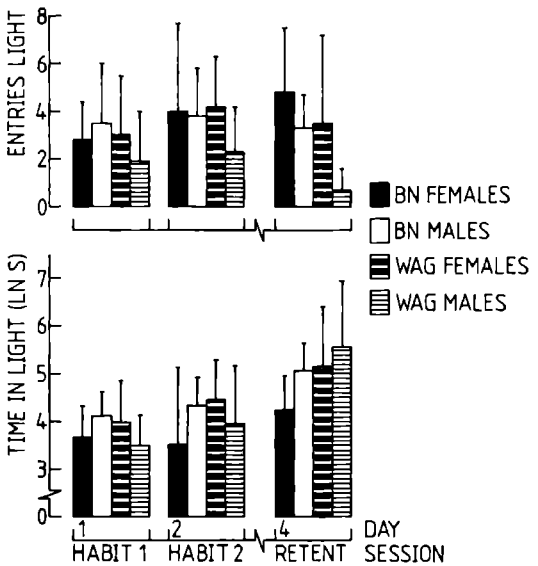


FIGURE 2 2 Frequency of entering the light compartment (upper panel), and time spent in the light compartment (lower panel) of BN and WAG female and male rats in a one-trial inhibitory avoidance task The vertical bars indicate standard deviations

l a poor retention performance in the inhibitory avoidance task when compai
ats.

strain difference in avoidance retention observed in the present experiment ca

and that female rats performed less well than males Thus, it is unlikely that differences in light aversion caused the sex differences found in our experiment Paré (1969) investigated the threshold for grid shocks in female and male rats He found that female rats had a lower threshold than male rats This makes it unlikely that female rats performed less well because the shock was less aversive

EXPERIMENT 2 II EFFECTS OF CHOLINE ON INHIBITORY AVOIDANCE OF YOUNG-ADULT RATS

The aim of this experiment was to evaluate the effects of chronic dietary choline enrich ment on inhibitory avoidance performance Male rats of both strains (BN and WAG) were used The previous experiment demonstrated that there was a clear difference in inhibitory avoidance behavior between the strains The BN rats showed a very poor retention performance and might therefore provide a genetic model of age-associated impairments of retention in an inhibitory avoidance task It is therefore of interest to study the effect of chronic dietary choline enrichment on the performance of the two strains in an inhibitory avoidance task We hypothesized that choline enrichment would ameliorate the poor retention in the BN strain because these animals show a poor performance in inhibitory avoidance tasks and have a lower central cholinergic activity Only male rats were used in this experiment

MATERIAL AND METHODS

Animals A total of forty male rats was used (see Appendix C, second experimental protocol) Ten litters with two littermates each of the BN/BiRij and the WAG/Rij strain were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands at the age of

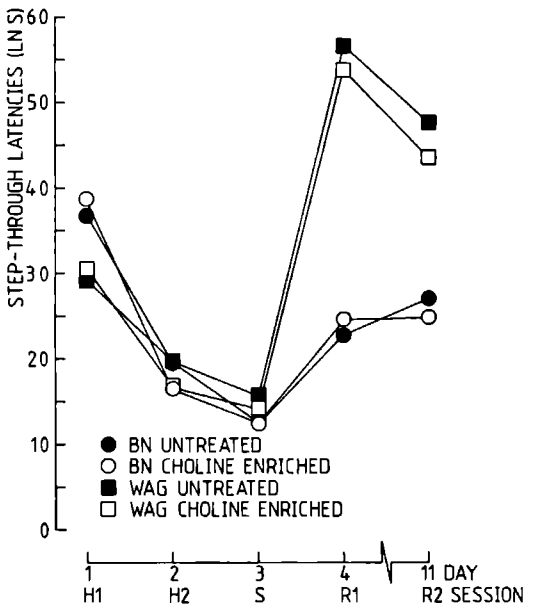


FIGURE 2 3 Step-through latencies (ln s) of untreated and chronically choline-enriched BN and WAG rats in a one-trial inhibitory avoidance task Abbreviations H1, and H2 first and second habituation session, S shock session, R1 and R2 first and second retention session

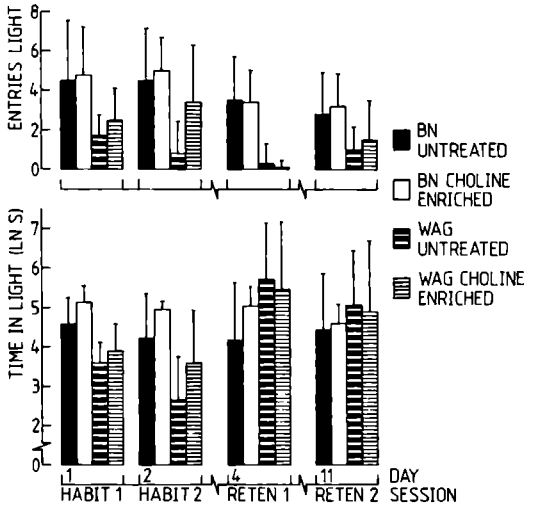


FIGURE 24 Frequency of entering the light compartment (upper panel) and time spent in the light compartment (lower panel) of untreated and chronically choline-enriched BN and WAG rats in a one-trial inhibitory avoidance task The vertical bars indicate standard deviations

three weeks They were housed in pairs in standard Makrolon™ cages and were habituated to a reversed day/night cycle (light being on from 20·00 to 8·00)

Methods At the age of five weeks one animal from each litter was randomly assigned to the choline enrichment condition The standard laboratory chow that was fed to all animals contained about 1700 mg choline per kilogram (Hope Farms) In addition 2·5 mg choline chloride ($C_5H_{14}ClNO$ Merck) per ml was added to the drinking water of the choline enrichment groups Based on the average amount of food and water consumed (see also Appendix B) it was estimated that the intake of choline from the drinking water ranged between two- and three times the amount taken in via the normal chow When the animals were 26 weeks old they were housed individually in standard Makrolon™ cages The animals were tested in the inhibitory avoidance task when they were 31 weeks old, as described in Apparatus and General Methods In addition to the 24 hour retention test, avoidance was also assessed a second time eight days after the administration of the shock

RESULTS

The same variables used in the previous experiment were analyzed by a two factorial strain (BN vs WAG) by treatment (untreated vs choline enriched) ANOVA[1]

Step through latency (see Fig 2·3) The WAG rats had shorter step-through latencies than the BN rats in the first habituation session ($F_{1,36} = 8·61$, $p < 0·01$) Neither strain nor treatment had a differential effect on the step-through latencies of the second habituation session and of the shock session

[1] A strain by treatment by litters within strains ANOVA would have been appropriate Because the special error terms for this analysis (MS litters, and MS litters by treatment) proved to be homogeneous, and because this was the case for all experiments in which litter designs were used all effects were tested against the pooled error terms (= MS Within of the two factorial design) This simplifies the description of results without loss of accuracy with respect to the evaluation of strain differences, treatment effects, and their interactions

The BN rats performed less well than the WAG rats in both retention sessions (24-hour retention session: $F_{1,36} = 38.54$, $p < 0.01$; on the average: 10 s for BN animals, 250 s for WAG animals; 8-day retention session: $F_{1,36} = 15.24$, $p < 0.01$; on the average: 14 s for BN rats, 63 s for WAG rats). Choline enrichment had no effect on retention performance ($F_{1,36} < 1.0$, ns).

Time spent in the light compartment (see Fig. 2.4, lower panel): During the first and second habituation session the BN rats spent more time in the light compartment than the WAG rats ($F_{1,36} = 38.98$, and $F_{1,36} = 19.86$, resp.; both p-values < 0.01). The choline-treated animals spent more time in the light compartment during the first ($F_{1,36} = 5.71$, $p < 0.05$) and second ($F_{1,36} = 6.46$; $p < 0.05$) habituation session. During the first retention session the WAG rats spent more time in the light compartment than the BN rats ($F_{1,36} = 5.41$; $p < 0.05$), but this difference was no longer apparent during the second retention session.

Frequency of entering the light compartment (see Fig. 2.4, upper panel): The BN rats entered the light compartment consistently more often than the WAG rats during habituation (first habituation session: $F_{1,36} = 13.71$, $p < 0.01$; second habituation session: $F_{1,36} = 13.56$, $p < 0.01$), and retention (24-h retention session: $F_{1,36} = 48.81$, $p < 0.01$; 8-day retention session: $F_{1,36} = 9.70$, $p < 0.01$). The choline-treated rats entered the light compartment more frequently than the untreated animals during the second habituation session ($F_{1,36} = 4.64$, $p < 0.05$).

Discussion

Chronic dietary choline enrichment had no effect on the retention performance in the inhibitory avoidance task, neither after 24 hours nor after 8 days. Memory-modulating effects of the choline treatment -like those observed in experiments with mice (Bartus, Dean, Goas , & Lippa, 1980; Davis, & Trombetta, 1984; Mervis, Horrocks, Wallace, & Naber, 1984)- were not found. The strain difference in retention was comparable to that found in experiment 2.1. The BN rats showed a poorer avoidance performance than WAG rats. This difference was still observed in a second retention test after eight days.

Choline enrichment reduced light aversion and heightened locomotor activity during the habituation sessions. The treated animals of both strains spent more time in the light compartment than untreated rats. Moreover, in the second habituation session, the frequency of entering the light compartment was higher in the choline-treated rats. The longer time spent in the light and the heightened frequency of entering the light compartment during habituation might be interpreted as a decreased 'emotional reactivity' (Aulich, Spielhofen, & Raaijmakers, 1974; Aulich, 1976; Aulich, & Spielhofen, 1977) after chronic dietary choline supplementation. Similar effects of choline enrichment were not found in the retention sessions. The BN rats entered the light compartment more frequently and spent more time in the light compartment than the WAG rats, and might thus be less 'emotionally reactive' than the WAG rats.

In summary, the retention performance in the inhibitory avoidance task was not influenced by chronic dietary choline enrichment, but the frequency of entry into the light compartment and the time spent in the light compartment during the habituation sessions were modulated by the treatment. These effects might be interpreted as showing that the treatment reduced 'emotional reactivity' of the animals. In addition, the BN rats showed fewer signs of 'emotional reactivity' than the WAG rats. The BN rats showed poorer retention than the WAG rats as indicated by the short step-through latencies, and by the fact that they spent less time in the light compartment during the first retention session.

EXPERIMENT 2 III EFFECTS OF CHOLINE ON INHIBITORY AVOIDANCE OF MIDDLE-AGED RATS

A potential cause for the lack of effects of the chronic dietary choline enrichment on inhibitory avoidance performance is the relatively modest concentration of choline used in the previous experiment Another factor that might have contributed to the lack of a treatment effect is the age of the animals when they were tested Since adult rats were used in experiment 2 II, we now used middle-aged rats Van der Staay et al (van der Staay Raaijmakers, & Collijn, 1986) found that dietary choline enrichment improved the retention performance of one- and two-year-old female rats of the inbred CPBB strain in an inhibitory avoidance task This result suggests that the treatment effect might not be restricted to aged animals but might already be found in rats that are older than one year The concentration of choline chloride was doubled to enhance the effectiveness of the choline treatment

MATERIAL AND METHODS

Animals Thirty-two male rats randomly selected from a group consisting of sixty animals, were used (see Appendix C, third experimental protocol) Ten litters with three littermates each of the BN/BiRij and the WAG/Rij strain were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands at the age of five weeks They were housed in pairs in standard Makrolon™ cages and habituated to a reversed day/night cycle (light being on from 20 00 to 8 00)

Methods At the age of seven weeks two subgroups of five litters each were formed from both strains by random assignment Two of the three littermates from the first subgroup, and one littermate per litter from the second subgroup were randomly assigned to the choline treatment condition The remaining animals were assigned to the untreated control condition Thus the choline-enriched group and the control group of both strains group consisted of 15 animals Choline enrichment consisted of adding 2.5 mg choline chloride/ml to the drinking water

When the animals were 28 weeks old they were housed individually in standard Makrolon™ cages The choline concentration was doubled[2] (5mg choline chloride/ml water) starting at the age of 33 weeks Based on the average amount of food and water consumed (see also Appendix B) it was estimated that the amount of choline taken in via the drinking water was minimally four and maximally six times that taken in via the standard laboratory rat chow (Hope Farms).

From the 15 animals per strain by treatment group, eight animals were randomly selected (compare Table 21) and tested in the inhibitory avoidance task in week 68, as described in 'Apparatus and General Methods' Before the assessment of performance in the inhibitory avoidance task, the animals had already been trained on a DRL 8"- and DRL 16"-task (see Chapter 4) and on a spatial discrimination task (see Chapter 5)

[2] At that time we obtained results from a DRL (differential reinforcement of low-rate responding) experiment in which rats had been put on a choline-enriched diet (2 5 mg choline chloride/ml tap water) We hypothesized that stronger enrichment effects than those found in that particular experiment might be obtained by supplying a higher concentration of choline

TABLE 21 Example of selection of animals for the inhibitory avoidance experiment within a strain Note that one or two rats per litter were selected randomly If two rats of the same litter participated in the study the restriction held that both littermates belonged to different treatment conditions Littermates are indicated by the letters C (choline-enriched) and U (untreated) Animals selected for the present study are printed bold

Subgroup 1				Subgroup 2					
Litter									
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
C C **U**	C **C** U	**C** C **U**	**C** C **U**	**C** C **U**	**C** U **U**	**C** U **U**	**C** U **U**	C **U** U	C **U** U

RESULTS

The same variables used in the previous experiments were analyzed by a two factorial strain (BN vs WAG) by treatment (untreated vs choline-enriched) ANOVA.

Step-through latency (see Fig 25) No strain differences or effects of choline enrichment on step-through latencies were found in any of the sessions (all $Fs_{1\,28}$ with associated probabilities > 010)

Time spent in the light compartment (see Fig 26, lower panel) The BN rats spent more time in the light compartment than the WAG rats during the first ($F_{1,28}$ = 3022 p < 001) and second habituation session ($F_{1\,28}$ = 3058 p < 001) and during the retention session ($F_{1\,28}$ = 481 p < 005) A strain by treatment interaction ($F_{1\,28}$ = 865 p < 001) was found for the first habituation session Post-hoc Duncan comparisons revealed that the choline enriched WAG rats spent more time in the light compartment than the untreated animals

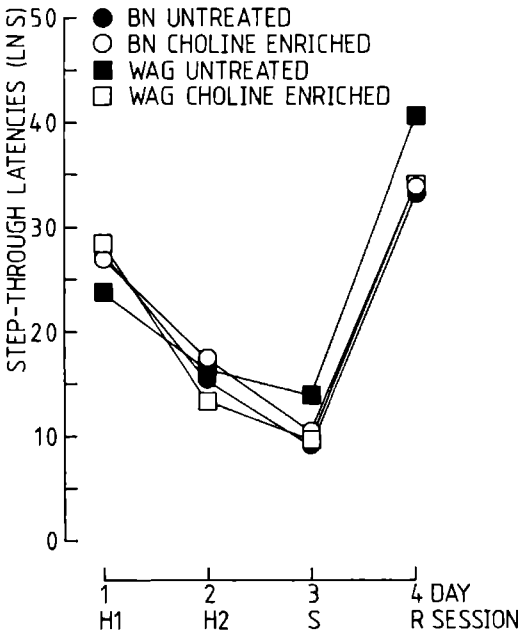


FIGURE 25 Step-through latencies (ln s) of untreated and chronically choline-enriched BN and WAG rats in a one-trial inhibitory avoidance task Abbreviations H1, and H2 first and second habituation session, S shock session, R. retention session

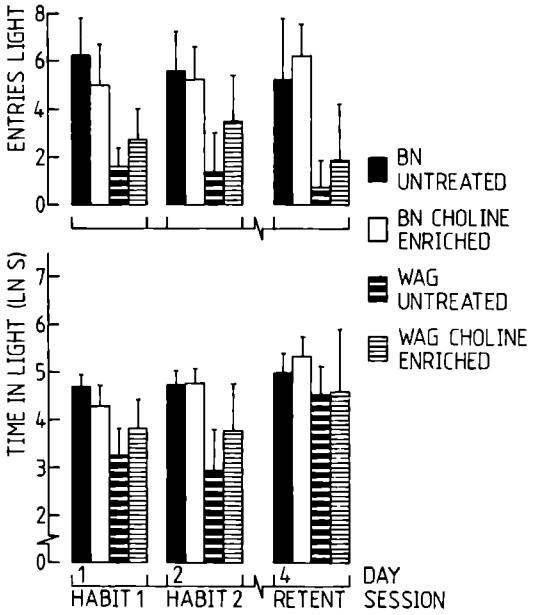


FIGURE 26 Frequency of entering the light compartment (upper panel), and time spent in the light compartment (lower panel) of untreated and chronically choline enriched BN and WAG rats in a one trial inhibitory avoidance task The vertical bars indicate standard deviations

Frequency of entering the light compartment (see Fig 26 upper panel) The BN rats entered the light compartment more often than the WAG rats during the first ($F_{1\,28}$ = 50 06 $p < 0\,01$) and second habituation session ($F_{1\,28}$ = 25 44, $p < 0\,01$) and during the retention session ($F_{1\,28}$ = 42 67, $p < 0\,01$) Strain by treatment interactions were found in the first ($F_{1\,28}$ = 5 97 $p < 0\,05$) and second habituation session ($F_{1\,28}$ = 4 42, $p < 0\,05$) Post-hoc Duncan comparisons confirmed that the treatment effect was restricted to the WAG strain The choline treated WAG rats entered the light compartment more often than the untreated animals

DISCUSSION

In the present experiment middle-aged (15 5-month-old) rats were used In the previous experiments (young-) adult (exp 2I 5 5-month-old, exp 2II 7-month-old) animals had been tested It appeared that the strain differences in avoidance retention observed in young adult and adult rats were no longer apparent mainly due to the decrease of retention performance in the middle-aged WAG rats Strain differences in step-through latencies during the habituation sessions were also no longer apparent in the middle aged animals

However, the differences between the strains for the frequency of entering the light compartment and the time spent in the light compartment, which had been observed in experiment 2 II were also observed with the middle-aged animals The BN rats were more active -as measured by the number of entries into the light compartment- and they avoided the light compartment less than the WAG rats in all sessions Although a longer time spent in the light compartment during the retention session might be taken as an index of better retention (Richardson, & Riccio, 1986), this interpretation contrasts with the observation that there were no differences in the retention performance, as indicated by the step-through latencies

The choline-treated WAG rats were more active (in both habituation sessions) and spent more time in the light (in the second habituation session) than their untreated controls These choline treatment effects were similar to those found in experiment 2II. However, these effects were found in both strains in that particular experiment, while they were restricted to the WAG strain in the present experiment Chronic dietary choline supplementation thus might have reduced the emotional reactivity of the WAG rats (Aulich, 1976)

EXPERIMENT 2IV EFFECTS OF CHOLINE ON INHIBITORY AVOIDANCE OF AGED RATS

The results obtained so far did not support the idea that choline treatment might be effective in (young-) adult or middle-aged rats Therefore a final experiment was performed with senescent rats of both strains that had received choline for 5 5 months prior to inhibitory avoidance testing The purpose of this experiment was to specifically assess whether chronic dietary choline supplementation might improve the retention of avoidance behavior of aged rats

A decreased inhibitory avoidance performance of rats aged 24 months or more has often been reported (e g Gold & McGaugh, 1975, McNamara, Benignus Benignus & Miller 1977, Lippa, Pelham, Beer, Critchett, Dean, & Bartus, 1980, Gold, McGaugh, Hankins, Rose, & Vasquez, 1981, Bartus, Dean, Sherman, Friedman & Beer, 1981, Martinez & Rigter, 1983) We hypothesized that chronic dietary choline enrichment would ameliorate the age-related deterioration in retention performance in both strains

MATERIAL AND METHODS

Animals Forty-eight male rats were used (see Appendix C, fifth experimental protocol) Twenty-four rats of the BN/BiRij and WAG/Rij strains were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands, at the age of 90 weeks. They were housed in pairs in standard Makrolon™ cages and adapted to a reversed day/night cycle (light on from 20·00 to 8·00) by a 13-hours light-off, 12-hours light-on schedule on eleven consecutive days De Koning-Verest (1981, pp 14-33) showed that 3-, 12-, and 30-month-old rats adapted evenly well to a reversed day/night cycle

When the rats were 93 weeks old they were housed individually in standard Makrolon™ cages Twelve rats of each strain were randomly assigned to a choline enrichment group, the other twelve rats served as untreated controls Chronic dietary choline enrichment started when the animals were 94 weeks old

Methods Before inhibitory avoidance retention was assessed 25 weeks later, the rats were tested in the open field (see Chapter 6) and trained on a spatial discrimination task in a holeboard (see Chapter 5) The behavior in the inhibitory avoidance task was tested as described earlier However, there were two important modifications Firstly, in the shock session, the duration of the scrambled footshock was extended to 3 s. Secondly, retention was tested 9 days after the shock session

There were two reasons for these procedural changes. Firstly, the step-through latencies of middle-aged rats had been found to be rather short in both strains (compare exp 2III) Extrapolating these results one might expect an even poorer performance in aged rats Paré (1969)

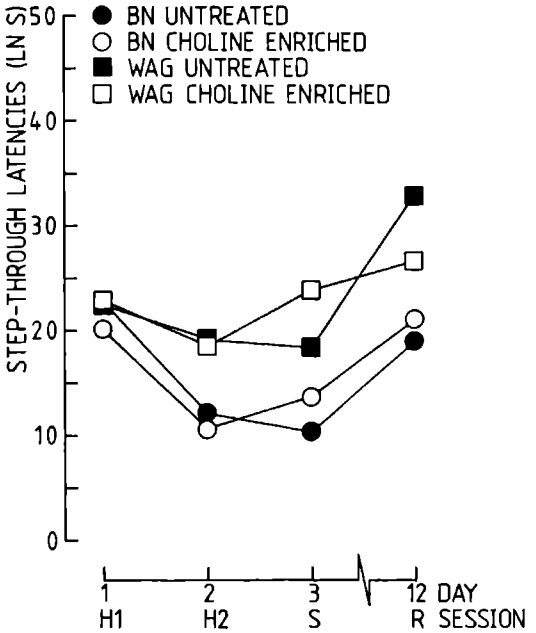


FIGURE 27 Step-through latencies (ln s) of 27-month-old untreated and chronically choline-enriched BN and WAG rats in a one-trial inhibitory avoidance task Abbreviations H1, and H2 first and second habituation session, S shock session, R retention session

reported that the aversive threshold of grid shock increases with age This effect is due to the higher body weights of the older animals De Koning-Verest (1981), on the other hand, did not find a relationship between age and shock sensitivity Because we wanted to make sure that the shock delivered to the aged rats would have clear aversive effects the shock duration was increased sixfold in this experiment compared with that used in the previous experiments Increasing the shock intensity or applying shocks of longer duration gives comparable effects, both increase the aversive property of the shock stimulus (Bolles, 1975)

Secondly, Deutsch and co-workers (Huppert & Deutsch, 1969, Deutsch, & Rogers, 1979, Deutsch, 1983) reported that retention performance might depend on the age of the memory and on changes at cholinergic synapses, which take place during the retention interval and might take several days One of the hypothesized actions of dietary choline is on the central cholinergic neurotransmission (Bartus, Dean & Beer, 1984) We therefore increased the time during which the extra dietary choline could act on cholinergic parameters after acquisition by increasing the retention interval to 9 days

RESULTS

Illness and death considerably reduced the number of subjects that completed the experiment Two BN animals of the choline-treated group and one BN from the untreated group died for unknown reasons Seven choline-treated WAG rats and six WAG rats from the untreated group also died Autopsy revealed that all except one of these WAG rats had an extended tumor of the pituitary gland Thus, the final composition of groups when tested at 119 weeks of age was 11 untreated BN rats 10 choline-enriched BN rats, 6 untreated WAG rats, and 5 choline-enriched WAG rats

The same variables used in the previous experiments were analyzed by a two factorial strain (BN vs WAG) by treatment (untreated vs choline-enriched) ANOVA (SAS GLM procedure for unequal cell sizes Freund & Littell 1985)

Step-through latency (see Fig 27) The BN rats had shorter step-through latencies than the WAG animals during the second habituation session ($F_{1\,28} = 4.69$, $p < 0.05$), during the shock session ($F_{1\,28} = 15.89$ $p < 0.01$ on the average 3.5 s for BN rats 8.5 s for WAG rats) and during the retention session ($F_{1\,28} = 6.11$ $p < 0.05$ on the average 7.5 s for BN rats, 20.5 s for WAG rats) Because strains differed in the shock session, we analyzed whether the differences in the step-through latencies of the retention session merely mirrored a tendency which had already developed in the shock session An analysis of covariance on step-through latency in the retention session with step-through latency in the shock session as covariate, confirmed that the strains showed the same retention performance ($F_{1\,27} = 1.47$ $p > 0.10$) There were no indications that the treatment affected step through latencies (all $Fs_{1\,28}$ with associated probabilities > 0.10)

Time spent in the light compartment (see Fig 28, lower panel) Neither strain nor treatment differences were found for the time spent in the light compartment (all $Fs_{1\,28}$ with associated probabilities > 0.10)

Frequency of entering the light compartment (see Fig 28, upper panel) The BN rats entered the light compartment more frequently than the WAG rats during both habituation sessions (first habituation $F_{1\,28} = 13.09$ $p < 0.01$ second habituation $F_{1\,28} = 11.72$, $p < 0.01$) and retention ($F_{1\,28} = 25.53$, $p < 0.05$) As indicated by a strain by treatment interaction ($F_{1\,28} = 4.64$, $p < 0.05$), and confirmed by a post-hoc Duncan analysis, the choline-enriched BN rats entered the light compartment more frequently than untreated BN rats on the first

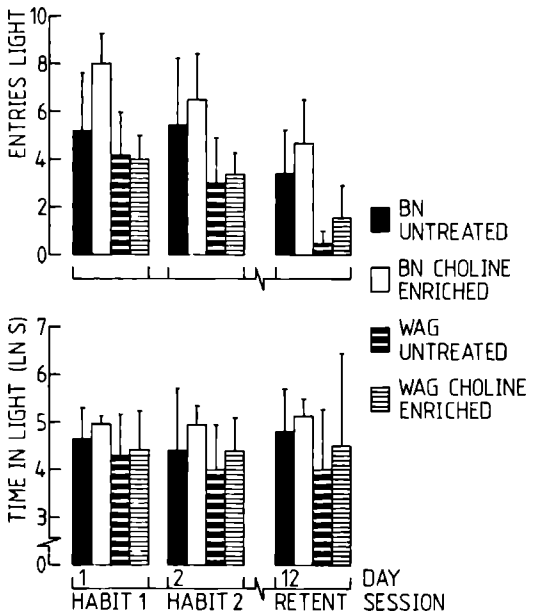


FIGURE 28 Frequency of entering the light compartment (upper panel), and time spent in the light compartment (lower panel) of 27-month-old untreated and chronically choline-enriched BN and WAG rats in a one-trial inhibitory avoidance task The vertical bars indicate standard deviations

habituation day During retention testing choline treated rats of both strains entered the light compartment more frequently than the untreated animals ($F_{1,28} = 4.34$ $p < 0.05$)

Discussion

Although retention was very poor in both strains chronic dietary choline enrichment did not improve the performance of animals of either strain Treatment effects on the frequency of entering the light compartment were only observed in the BN strain during the first habituation session A reversed interaction effect was found in experiment 2 III only the treated WAG animals showed a heightened frequency of entering the illuminated side during habituation The procedures used during the habituation sessions of the inhibitory avoidance experiments of the present study were identical However the age of the rats and the start and duration of the chronic dietary choline supplementation were different in experiments 2 III and 2 IV Whether these factors might have influenced the present results cannot be decided without evidence from additional experiments

In the retention session the choline-treated animals of both strains were more active than the untreated ones The higher incidence of entering the light compartment did not influence the time spent in the light compartment Two explanations may account for this result Firstly, the choline treatment could have reduced the emotional reactivity of the animals which might have been expressed in the BN strain during habituation and retention while this effect was only apparent in the WAG strain during the retention session The retention session might be considered more stressful than the habituation sessions This interpretation however, is inconsistent with the finding that the time spent in the light compartment a second index for emotional reactivity (Aulich, Spielhofen & Raaijmakers 1974, Aulich 1976 Aulich, & Spielhofen 1977) was not affected by the treatment

The second interpretation assumes that the choline treatment affected retention It is conceivable, for example, that the memory of the shock may have induced arousal which, in turn was expressed by a higher locomotor activity This interpretation, however is not supported by the step-through latencies and the time spent in the light compartment (Richar dson, & Riccio 1986) Both measures are indices for retention

The considerable loss of WAG rats (6 rats from the untreated control group and 7 rats from the choline-treated group) from the original groups of 12 animals each may have altered the sample characteristics and may therefore have biased the results As all rats that survived were to be used in additional studies months after the completion of the present experiment, it was impossible to evaluate the health of the WAG rats that completed the inhibitory avoidance task, especially with respect to the possible presence of pituitary tumors It is conceivable that some of the WAG rats had tumors when tested The high incidence of pituitary tumors clearly puts constraints on the value of the WAG strain for aging research

GENERAL DISCUSSION

The results of the four inhibitory avoidance experiments are summarized in Tables 2.2, 2.3, and 2.4

Contrary to Bartus et al (Bartus Dean, Goas, & Lippa, 1980), Davis and Trombetta (1984), and Mervis et al (Mervis, Horrocks Wallace, & Naber 1984), we did not find that dietary

choline supplements improved the retention performance in the inhibitory avoidance task. Since mice were used in the studies cited above one could hypothesize that the effect on retention is species dependent. Bartus and co-workers (Bartus, Dean, Sherman, Friedman, & Beer, 1981) failed to improve the retention of aged Fischer 344 rats by supplying choline (100 mg/kg, or 200 mg/kg) for one week before and during testing. Using rats, Beninger, Tighe, and Jhamandas (1984) did not find any effect of chronic dietary choline enrichment which lasted for seven months, on the acquisition and reversal of a successive discrimination task. The treatment (approximately 20-25 mg choline per g food eaten) started when the rats were about eight months old.

A closer look at the studies in which it is claimed that chronic dietary choline enrichment improves retention reveals that they suffer from experimental flaws, or are not strongly supported statistically.

In the study of Bartus et al. (Bartus, Dean, Goas, & Lippa, 1980) the performance of groups of mice fed a choline-deficient or a choline-enriched diet was compared with the retention performance of a group of mice of the same age but tested in a different experiment. One should be very careful when interpreting the results of an inhibitory avoidance experiment in which there are no appropriate control groups. Bartus and co-workers (1980) report that a second choline enrichment experiment corroborated the results of the first experiment. Although that experiment included an appropriate control group, the results were only poorly presented in footnotes. It is not clear why Bartus et al. did not fully report the second experiment instead of the first one with its experimental flaws. It is not possible to evaluate their results because of the inadequate presentation of the results of the second experiment.

Davis and Trombetta (1984) assessed the effects of choline-deficient, choline-enriched, and choline-control diets after a seven day retention interval with 24-month-old mice. The mice had been put on the diet when they were 8 to 9 months old. The diets had no effect on the retention performance in the inhibitory avoidance task. Thus the results of Bartus et al. (1980) were not confirmed. However, an additional group of mice that had been put on the diet at 18 months of age tended to perform better than mice that had been put on the diet at 8 to 9 months of age. It is not clear, however, whether this additional group belonged to the same cohort and was housed and treated identically to the other groups. It is possible that the 'weak' ($p < 0.10$) choline enrichment effect is an artifact of differences between cohorts and might have been interpreted as treatment effects.

Mervis and co-workers (Mervis, Horrocks, Wallace, & Naber, 1984) reported similar results in mice that had been fed different doses of choline (given as phosphatidylcholine or as commercial lecithin), starting at the age of eight months. The retention performance in the one-trial inhibitory avoidance task was assessed when the animals were 13 months old. As Mervis et al. stated, higher levels of dietary choline 'tended to significantly improve' retention compared to that induced by lower levels of choline. There was no improvement, however, when the retention was compared with that of animals fed a standard chow, indicating that most of the differences in retention performance might have been a consequence of the comparison with the group fed a choline-deficient diet.

It is remarkable that Bartus and co-workers have never been able to replicate their own findings. Perhaps as an attempt to tone down their earlier statements (Bartus, Dean, Goas, & Lippa, 1980) about chronic dietary choline supplementation as a preventive strategy to treat age-related memory impairments, Bartus, Dean, Pontecorvo, & Flicker (1985) gave the opinion

TABLE 22 Summary table of the *step-through latencies* in the habituation sessions (Hab 1, Hab 2) the shock session (Shock) and the retention session(s) (Reten 1 Reten 2) of experiments 2I, 2II 2III and 2IV The direction of strain and sex differences (exp 2I) or of chronic dietary choline enrichment effects (exp 2II 2III and 2IV) and their interactions are indicated *Abbreviations*: - no differences, f female m male u untreated control, c choline-enriched, < shorter than, > longer than The entries should be read as follows BN<WAG in the row 'Strain indicates that the step-through latencies of BN rats were shorter than those of WAG rats BN f<m in the row Str*Sex indicates that female rats had shorter step-through latencies than male rats in the BN strain

Exp		*Hab 1*	*Hab 2*	*Shock*	*Reten 1*	*Reten 2*
	Strain	-	-	BN<WAG	BN<WAG	
I	*Sex*	-	-	-	f<m	
	*Str*Sex*	BN f<m	BN f<m	-	-	
	Strain	BN>WAG	-	-	BN<WAG	BN<WAG
II	*Choline*	-	-	-	-	-
	*Str*Chol*	-	-	-	-	-
	Strain	-		-	-	
III	*Choline*	-	-	-	-	
	*Str*Chol*	-	-	-	-	
	Strain		BN<WAG	BN<WAG	BN<WAG*	
IV	*Choline*	-	-	-	-	
	*Str*Chol*	-	-	-	-	

* No difference on retention performance, if analyzed by analysis of covariance with step-through latencies on shock session as covariate

that the inhibitory avoidance test is a rather crude paradigm, as its results are open to multiple interpretations (such alternative interpretations are discussed by O'Keefe and Nadel, 1979, Masterson, & Crawford 1982)

However, there are some indications that chronic dietary choline enrichment might affect measures of emotional reactivity and activity We found that the choline-treated animals spent more time in the light compartment than control rats in experiment 2II In experiment 2III this effect was only found in the WAG strain (see Table 23) As indicated by the number of entries into the light compartment (see Table 24), the choline-treated animals were more active than their untreated controls during the habituation phase (in experiment 2II in both strains, in experiment 2III in the WAG strain only, and in experiment 2IV in the BN strain only) This effect was also observed during the retention session of experiment 2IV for both strains (see Table 24)

It is not clear why the choline enrichment effects for the habituation sessions were restricted to the WAG rats in experiment 2III and to the BN rats in exp 2IV It is obvious, however, that the effects of choline enrichment on both aspects of behavior *whenever they occur*, very consistently point to the same phenomena Heightened locomotor activity in a novel situation and long stays in a less preferred part of an apparatus are generally interpreted as an indication of lower emotionality (e g Aulich 1976, see also Appendix A, where the choline-enriched WAG rats were found to increase inter-trial crossings in a two-way active

TABLE 2.3 Summary table of the *time spent in the light compartment* in experiments 2I, 2II, 2III and 2IV. The direction of strain and sex differences (exp. 2I) or of chronic dietary choline enrichment effects (exp. 2II, 2III and 2IV) and their interactions are indicated. *Abbreviations* - : no differences; f: female; m: male; u: untreated control; c: choline-enriched; <: shorter than; >: longer than. In table 2.2 it is explained how the table entries should be read.

Exp		*Hab 1*	*Hab 2*	*Reten 1*	*Reten 2*
I	*Strain*	-	-	BN<WAG	
	Sex	-	-		
	*Str*Sex*	*	*	-	
II	*Strain*	BN>WAG	BN>WAG	BN<WAG	-
	Choline	u<c	u<c		-
	*Str*Chol*	-	-	-	-
III	*Strain*	BN>WAG	BN>WAG	BN>WAG	
	Choline	-	-	-	
	*Str*Chol*	WAG u<c	-		
IV	*Strain*	-	-	-	
	Choline	-		-	
	*Str*Chol*	-	-	-	

* Differences between groups were not confirmed by post hoc comparisons.

avoidance task). Thus, it might be possible that chronic dietary choline enrichment reduces emotional reactivity (this question is addressed in more detail in Chapter 6 where the open field was used; this apparatus is specifically designed to measure emotional reactivity).

In summary, we found that the choline enrichment effect was not restricted to one of the two genotypes involved, that it did not appear at a specific age and that it is not easily replicated.

We hypothesized that (young-) adult BN rats could be used as an adequate genetic model of age-related memory impairments and that these animals would profit from a dietary supplementation with choline more than WAG rats. Experiments 2I and 2II confirmed the retention deficits of the (young) adult BN rats on the inhibitory avoidance task. In experiments 2III and 2IV (see Table 2.2) the strain differences in retention performance were no longer found. Although comparisons across experiments should be made with great care, the results of experiments 2I, 2II and 2III suggest that the WAG rats show an age-related decline of the retention performance in the inhibitory avoidance task (experiment 2IV should not be considered as shock and retention interval parameters had been changed in that experiment). The retention of the BN rats was very poor in all experiments and could hardly decline any more.

The dietary choline supplementation in experiments 2II and 2III started shortly after weaning. In the fourth experiment the dietary choline treatment started when the rats were already 94 weeks (21.5 months) old. It is possible that the WAG strain already had undergone some age-associated deterioration of cognitive functions at this particular age[3]. This is

[3] For the BN strain it makes no sense to speak about age-associated impairments of retention in the inhibitory avoidance task, as they performed equally poor at all ages.

TABLE 2 4 Summary table of the *frequency of entering the light compartment* in experiments 2I 2II 2III and 2IV The direction of strain and sex differences (exp 2I) or of chronic dietary choline enrichment effects (exp 2.II 2III. and 2IV) and their interactions are indicated *Abbreviations* - no differences, f female m male, u untreated control, c choline-enriched, < less compartment entries than, > more compartment entries than In table 2 2 it is explained, how the table entries should be read

Exp		*Hab 1*	*Hab 2*	*Reten 1*	*Reten 2*
	Strain	-		BN>WAG	
I	*Sex*	-	-	f>m	
	*Str*Sex*	-	-	-	
	Strain	BN>WAG	BN>WAG	BN>WAG	BN>WAG
II	*Choline*	-	u<c	-	-
	*Str*Chol*	-		-	-
	Strain	BN>WAG	BN>WAG	BN>WAG	
III	*Choline*	-		-	
	*Str*Chol*	WAG u<c	WAG u<c	-	
	Strain	BN>WAG	BN>WAG	BN>WAG	
IV	*Choline*	-	-	u<c	
	*Str*Chol*	BN u<c	-	-	

suggested by the relatively poor retention performance of the middle-aged (15 5 months old) WAG rats in experiment 2III According to Bartus and co-workers (Bartus, Dean, Goas, & Lippa, 1980, Bartus Dean, & Beer 1984) precursor therapy would be most effective if started at an age when the cognitive impairment has not yet developed If this assumption is true, then treatment might have been initiated too late in experiment 2IV However, if the relatively poor retention performance observed in experiment 2 III mirrored age-related retention impairments to some extent, then the chronic dietary choline supplementation should have been effective in that particular experiment In experiments 2 II and 2 III the choline treatment was clearly 'prophylactic', but ineffective with respect to retention performance

It cannot be ruled out that the differences in effects of the choline treatment observed in experiments on 'emotional reactivity' and 'activity' are due to developmental factors that make the two strains susceptible to the treatment at different ages Moreover, the choline concentration was doubled in experiments 2 III and 2.IV compared with that used in the second experiment These experimental modifications preclude direct comparisons between the experiments

It is unlikely that age-associated changes in shock sensitivity were responsible for the decreased retention performance in the WAG strain De Koning-Verest (1981, pp 14-33) measured the footshock thresholds of 3-, 12-, and 30-month-old female WAG/Rij rats She found that age did not affect the flinch threshold Her data, however, are in disagreement with those presented by Paré (1969), who found that the aversive threshold for footshock is positively correlated with the body weights of the rats (older rats are normally heavier than younger ones)

Based on a growing body of evidence it can be questioned whether a relationship exists in the BN strain between central cholinergic activity and inhibitory avoidance performance

McCarty, Gilad, and co-workers (McCarty & Kopin, 1978, Gilad & Jimerson, 1981 Gilad & McCarty, 1981, McCarty, Kirby, & Garn 1984, Gilad, & Shiller, 1989) observed that BN rats show a low responsiveness to stressful stimulation, both physiologically and behaviorally This finding may be one of the reasons for the poor performance of BN rats in shock motivated tasks The retention deficit in the BN strain may depend on the motivational system involved (BN rats also showed a very poor performance in an active avoidance task compared with the performance of WAG rats see Appendix A. The retention of BN rats in appetitively motivated tasks is assessed in the Chapters 3, 4, and 5, and the question whether the retention deficits of BN rats represents a general impairment is specifically addressed in Chapter 3) As non-mnemonic factors seem to be the main cause of their poor retention performance, adult and middle-aged BN rats may not provide an adequate model of age-related impairments of learning and memory

The results obtained with the inhibitory avoidance procedure in the present study underline the necessity to investigate the effects of chronic dietary choline enrichment on tasks that are appropriate to assess cognitive and memory performance, and on tasks that are designed to measure activity and emotionality

CHAPTER 3

RETENTION OF AN INCOMPLETELY ACQUIRED APPETITIVE BAR-PRESS RESPONSE: STRAIN AND SEX DIFFERENCES, AND EFFECTS OF CHRONIC DIETARY CHOLINE ENRICHMENT

SUMMARY

In a previous study it was found that Brown Norway (BN) rats showed a very poor 24 hour retention performance in an inhibitory avoidance tasks when compared with WAG rats, and that the 24-hour retention performance of female rats of both strains was poorer than that of male rats In a first experiment we investigated whether comparable strain and sex differences were apparent for the retention of an incompletely acquired appetitively motivated bar-press response It was questioned whether BN rats when compared with WAG rats and female rats when compared with male rats, might suffer from a general impairment of their ability to retain responses over a 24-hour retention interval Neither strain nor sex differences were found for retention performance in the appetitively motivated task In a second experiment the effects of chronic dietary choline enrichment on the retention of the incompletely acquired bar-press response were studied in male BN and WAG rats Chronic dietary choline enrichment had no effect on their retention performance, and there was no difference between the strains for the acquisition and retention of the task In addition, we found that the retention interval did not influence performance, as no differences were found between the performance after the 24-hour retention interval and the performance in an uninterrupted condition (no retention interval) It was concluded 1) that neither female rats nor adult BN rats suffer from a general impairment of their ability to retain responses over a 24-hour retention interval, 2) that as a consequence the BN strain does not provide a 'genetic model' of age-related cognitive deficits, and 3) that dietary choline enrichment does not affect the retention of the incompletely acquired appetitively motivated bar-press response

INTRODUCTION

Young-adult and adult Brown Norway (BN) rats show very poor retention performance in an inhibitory avoidance task (see Chapter 2, exp 2.I and 2.II), and poor acquisition in other shock-motivated tasks (e.g. two-way active avoidance Appendix B, drinktest De Koning-Verest, Knook, & Wolthuis, 1980, runway approach avoidance conflict Koene 1988) The poor acquisition and retention in aversively motivated tasks shown by adult BN rats resembles that usually found in aged rodents (e.g. Dean, Scozzafava, Goas, Regan, Beer, & Bartus, 1981 for mice, McNamara, Benignus, Benignus, & Miller, 1977, Zornetzer, Thompson, & Rogers 1982, for rats)

Furthermore, the BN strain is characterized by a relatively low central cholinergic activity compared with the WKY (e.g. Gilad & Gilad, 1981), the WAG (see Chapter 7) and other inbred strains of rats (W. Raaijmakers, personal communication) A decrease in central cholinergic activity may be another characteristic of aging rodents (e.g. Strong, Hicks, Hsu, Bartus, &

Enna 1980 Shuman Kuster Dean Bartus, & Friedman 1981) although the experimental evidence to support this idea is not as unambiguous as that for the deficit in aversive conditioning (e g De Koning Verest 1981 compare also Chapter 7 of this thesis)

Based on the observations that a decrease of central cholinergic activity in cortical areas and an impairment of cognitive functions seem to be consistent characteristics of aging rodents (both deficits are very pronounced in patients suffering from dementias of the Alzheimer type reviewed by Kopelman, 1986) it was hypothesized (see also Chapter 2) that (young-) adult BN rats could provide a genetic model of age related impairments both behaviorally and neurochemically

However although (young-) adult BN rats have been found to have an impaired acquisition and retention in aversively motivated tasks (see Chapter 2 and Appendix A) it is not clear whether these deficits were caused by impaired cognitive functioning There are indications that the deficits might be caused by a low responsiveness of the BN rats to stressful stimulation (McCarty Kirby & Garn 1984)

In the same series of experiments (Chapter 2, exp 21) it was found that the female BN and WAG rats showed poorer retention in the inhibitory avoidance task than male rats Van Haaren and co workers (van Haaren & van de Poll, 1984 Heinsbroek van Haaren & van de Poll 1988) suggested that non-mnemonic factors (for example sex-dependent susceptibility to shock-induced behavior Heinsbroek et al 1988) might be responsible for this difference between the sexes

Therefore, the first experiment of the present study addressed the question whether BN rats show poor retention in an appetitively motivated task and whether the retention of female rats is poorer than that of male rats An impaired performance in learning and memory irrespective of the motivational system involved would greatly enhance the validity of using (young) adult BN rats as a genetic model' of age-related memory impairments which are associated with low cholinergic activity In addition, an appetitively motivated paradigm provides the opportunity to investigate whether the poor retention performance of female rats in the aversively motivated task reflects cognitive impairments of a more general nature

Based on the assumption that the low central cholinergic activity underlies -at least partly- the retention deficit observed in BN rats in the inhibitory avoidance task (see Chapter 2) we hypothesized that chronic dietary choline enrichment should improve memory performance in this strain This hypothesis was tested in the second experiment of the present study Evidence for such an effect of a dietary choline supplement on the inhibitory avoidance performance of aging mice has been reported (Bartus Dean, Goas & Lippa, 1980, Davis & Trombetta, 1984, Mervis, Horrocks, Wallace, & Naber, 1984)

The 24-hour retention performance of an incompletely acquired bar-press response on a continuous reinforcement (CRF every bar-press is reinforced) schedule was used as the behavioral paradigm in both experiments This task has some features in common with the inhibitory one-way avoidance task it consists of a very restricted acquisition period followed by a retention test 24-hours later Contrary to the one-trial inhibitory avoidance task this paradigm also provides information about the acquisition phase Retention is measured by comparing the performance at the end of the acquisition phase and at the beginning of the retention phase

Incomplete learning is achieved by interrupting a session after a predetermined low number of trials (Destrade, Soumireu-Mourat, & Cardo 1973) Besides providing an indication of forget-

ting this paradigm also gives information about what is sometimes referred to as 'reminiscence' i e an improvement in the recall of the incompletely learned material after an interval of time without intervenient formal learning or review' (McGeoch, cited in Anderson, 1940) This phenomenon was already recognized in the thirties, when Bunch and Magdsick (1933) and Anderson (1940) observed 'reminiscence in rats after incomplete acquisition of complex multiple T-mazes. A comparable phenomenon was reported by Huppert & Deutsch (1969) for the retention performance of rats trained for 15 trials to escape from shock into the lit arm of an Y-maze

In a series of studies performed during the last one and a half decades, 'reminiscence' was found to be reproducible for the retention of the incompletely acquired CRF task (Destrade, Soumireu-Mourat, & Cardo, 1973, Destrade & Cardo, 1974 Jaffard, Destrade Soumireu-Mourat, & Cardo, 1974, Soumireu-Mourat Destrade, & Cardo, 1975, Destrade, Jaffard, Deminiere, & Cardo 1976 Gauthier, Destrade, & Soumireu-Mourat, 1982, 1983) Mice from the inbred strains BALB/c, C57Bl/6 and C57BR were trained in one 15-minute session to press the lever in an operant conditioning box to obtain a food reward Training was interrupted and the retention of the bar-press response was measured 24 hours later The BALB/c mice showed 'reminiscence of responding to the CRF schedule Their rate of responding was higher at the beginning of the retention session than at the end of the original learning session On the other hand C57Bl/6 mice (Destrade, Jaffard, Deminiere, & Cardo, 1976) and C57BR mice (Jaffard, & Destrade, 1982) had not improved their rate of responding after a 24 hour interval

Post-trial treatments such as subseizure electric stimulation of the dorsal hippocampus or of the lateral entorhinal cortex (which connects the hippocampus via the perforant path) produced facilitation of retention[1] in BALB and C57BL mice (e g Destrade, Jaffard, Deminiere, & Cardo, 1976, Gauthier, & Soumireu-Mourat, 1981) In the BALB strain the extent of the improvement exceeded the reminiscence' effect found when no stimulation was applied. C57BR mice showed no facilitation of retention (Jaffard, & Destrade, 1982) Genetic factors may therefore constitute important determinants for the occurrence of 'reminiscence' and for treatment induced facilitation of retention For these reasons more than one strain was used in the present experiments

Jaffard and co-workers found that the occurrence of facilitation of retention after electric hippocampal stimulation correlated with changes in hippocampal cholinergic activity (Jaffard, Ebel, Destrade, Durkin, Mandel, & Cardo, 1977, Jaffard, & Destrade, 1982) They argued that post-trial cholinergic changes might also be responsible for the phenomenon of 'reminiscence'. In general, the results of these experiments might be interpreted as reflecting processes that are involved in the consolidation of the incompletely acquired habit (Martinez, Jensen, & McGaugh, 1983)

The apparent involvement of cholinergic processes makes the assessment of retention of the incompletely acquired bar-press response on a CRF-schedule very suitable to determine the influence of chronic dietary choline enrichment on memory performance

[1] 'Reminiscence' indicates spontaneously occurring improved performance during the retention session compared with the performance during the original learning session, while 'Facilitation of retention' refers to a similar effect which is induced by (post-trial) experimental manipulations (for example electrical stimulation of the hippocampus)

EXPERIMENT 3.I: RETENTION OF AN INCOMPLETELY ACQUIRED BAR-PRESS RESPONSE: STRAIN AND SEX DIFFERENCES

The 24-hour retention of the incompletely acquired bar-press response on a CRF schedule of adult male and female BN and WAG rats was assessed. It was found in an earlier experiment (Chapter 2, exp. 2.I) with the same animals that BN rats showed very poor retention in an inhibitory avoidance task. We investigated whether BN rats showed a general retention deficit, independent of the motivational system involved, in order to determine whether (young-) adult BN rats could serve as a 'genetic' model of age-related memory impairments. The performance of the BN rats was compared with that of animals of the WAG strain.

In addition to investigating strain differences, sex differences were also studied. This was done because female rats of both strains showed poorer 24-hour retention performance than males in an inhibitory avoidance task (Chapter 2, exp. 2.I), and because sex differences have been observed in an appetitively motivated task (e.g. van Hest, van Haaren, & van de Poll, 1988a, 1988b). It was questioned whether female rats of both strains suffered from a general impairment of their ability to retain information over a 24-hour interval.

MATERIAL AND METHODS

Apparatus: Eight identical Skinner boxes (24 cm wide, 23 cm deep, 22 cm high) were used. The left side-wall served as a control panel and included manipulanda and discriminanda. A recess (5.0 * 4.0 * 4.7 cm) was built into this panel 2 cm above the floor and contained a food tray, into which a pellet dispenser delivered 45 mg food pellets (Campden Instruments). Retractable stainless steel levers (4 cm wide) projected through the panel 2 cm into the Skinner box. The levers were located at a distance of 2.7 cm from both sides of the recess, 6.7 cm above the floor. Two 2.8 W lamps were centered 4.7 cm above both levers. A third lamp was mounted 3.9 cm below the ceiling and 8.1 cm from the right edge of the control panel. A houselight and a 6 cm speaker were attached to the roof of the conditioning chamber.

The conditioning chamber was enclosed in modified sound-attenuating housing (Campden Instruments, model 412). Its inner surface was entirely covered with acoustic plastic foam. The front wall consisted of a horizontally opening 'double-glazed' swing-door made of two layers of transparent plexiglass. An exhaust fan produced a background noise of approximately 42 dBA. A PDP 11/03 computer controlled the experimental equipment and collected the data.

A rat was placed in a transparent PVC holding cage (23.7 * 22.3 * 20.1 cm) at the beginning of a session. The entire left side wall of the holding cage was a sliding door. This cage was inserted into the conditioning chamber. After removing the sliding door, the rat had free access to the panel. At the end of a session the sliding door was put back and closed, and the rat was withdrawn from the apparatus while remaining in the holding cage.

Animals: A total of forty rats of the BN/BiRij and the WAG/Rij strains were used (see Appendix C, first experimental protocol). Ten male and ten female rats of each strain were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands, at the age of 12 weeks. When the rats were 16 weeks old they were housed individually in standard Makrolon™ cages (31 * 28 * 20 cm). The light regimen was reversed (light on between 20:00 and 8:00) when the rats were 21 weeks old.

Methods: The animals were tested in an open field at the age of 23 weeks (not reported). When they were 24 weeks old they were tested in a one-way inhibitory avoidance task

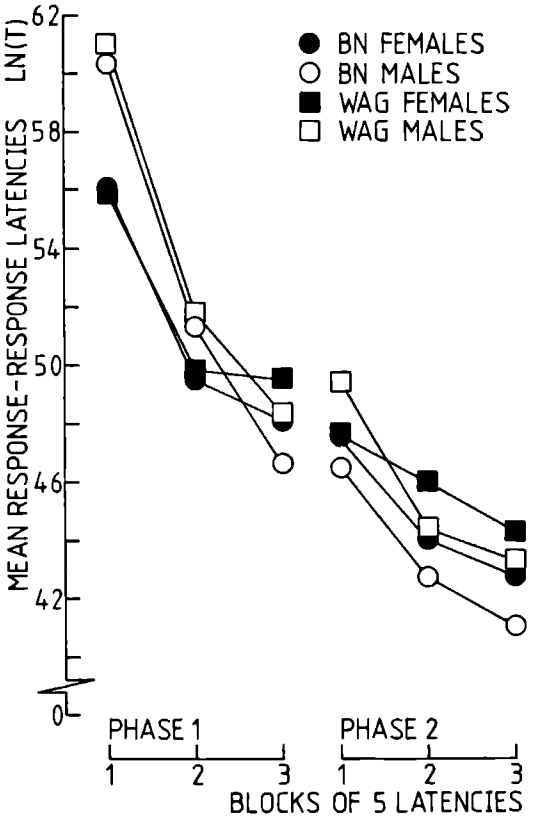


FIGURE 31 Response-response latencies (ln (T) T represents the number of 01 s clock counts) of female and male BN and WAG rats Block means of 5 latencies each of the acquisition session (phase 1) and the retention session after a 24-hour period (phase 2) are shown

(Chapter 2 exp 21) The weights of all animals were gradually reduced to 85% of their free-feeding values during the twenty-fifth week The rats were magazine trained in the Skinner box in daily 30 minute sessions until they had consumed at least twenty 45 mg food pellets in each of two sessions The pellets were randomly supplied at intervals ranging from 20 to 100 s, levers were retracted from the operant conditioning chamber during this phase

Formal training in the incompletely acquired operant conditioning task was given in week 27 two levers were presented alternately As soon as the rat pressed a lever it was retracted, a 45 mg food pellet was delivered and the other lever was presented The first session was terminated when the rat had performed 16 lever presses (8 times to the left and 8 times to the right lever) The latencies between lever presses (response-response latencies) were registered in 01 s Retention was tested twenty-four hours later The retention session was terminated after 16 lever presses

RESULTS

Response-response latencies after the retention interval (see Figure 31, and Table 31) As the response-response latencies tended to show heavily skewed distributions they were transformed to the natural logarithm (ln (T) where T represents the number of 01 s clock counts)

TABLE 3.I: Block means and standard deviations (stddev) of logarithmically transformed response-response latencies of the third block of phase 1 (acquisition session) and the first block of phase 2 (retention session). Each strain by sex group consisted of 10 animals.

Strain	*Sex*	*Last block phase 1*		*First block phase 2*	
		mean	*stddev*	*mean*	*stddev*
BN	*females*	4.81	0.30	4.75	0.11
BN	*males*	4.65	0.18	4.64	0.26
WAG	*females*	4.94	0.43	4.73	0.26
WAG	*males*	4.83	0.33	4.94	0.43

before statistical evaluation (cf. Ferguson, 1971; Kerbusch, van der Staay, & Hendriks, 1981). In order to analyze the data of the incompletely acquired operant conditioning task three block means of five response-response latencies each were calculated for phase 1 (acquisition session) and phase 2 (retention session). The effect of the 24-hour retention interval was evaluated by a strain (BN vs. WAG) by sex (females vs. males) by blocks (last block mean of phase 1 vs. the first block mean of phase 2) ANOVA with repeated measures on the last factor.

The response-response latencies after the retention interval were similar to those recorded during the last block of the acquisition phase ($F_{1,36} < 1$; ns). Neither strain ($F_{1,36} = 1.54$; ns) nor sex differences ($F_{1,36} < 1$; ns), nor strain by sex interactions ($F_{1,36} = 1.86$; ns) were found after the retention interval. Thus, there were no indications that the animals showed retention deficits or 'reminiscence'.

Learning during the two phases of the experiment: The progression of learning was evaluated separately for the acquisition and retention session by calculating the general means, and linear and quadratic trend coefficients over the three block means per phase. The general means (which represent the response-response latencies averaged over the three blocks of a particular phase of the experiment) were used to evaluate whether there was a difference in the overall level of performance. Orthogonal trend coefficients are tools used to describe the learning curves, and to assess whether the shapes of these curves were different between groups.

In both phases of the experiment learning was characterized by general linear (phase 1: $F_{1,36} = 100.3$, phase 2: $F_{1,36} = 104.2$; both p-values < 0.01) and quadratic (phase 1: $F_{1,36} = 23.22$, phase 2: $F_{1,36} = 12.78$; both p-values < 0.01) trends. In both phases more than 90% of the variation over the three successive blocks of five response-response latencies could be predicted from a linear regression equation. The quadratic component explained only minor proportions of the learning curves for the acquisition and retention phases. Latencies between responses thus decreased, and the decreases were slightly negatively accelerated. During phase 1 (acquisition) the linear component ($F_{1,36} = 8.85$, $p < 0.01$) was different between the sexes. The male rats showed a sharper decline of response latencies than female rats. This is because in the first block of the acquisition phase, the male rats had longer response-response latencies ($F_{1,36} = 4.94$, $p < 0.05$). This sex difference was no longer apparent in the next blocks. During phase 2 (retention), the response latencies of the BN rats were generally shorter than those of the WAG rats (general means: $F_{1,36} = 8.71$, $p < 0.01$).

DISCUSSION

The 24-hour delay did not affect response-response latencies in the incompletely acquired operant conditioning task. This means that the phenomenon of 'reminiscence' did not occur; on the other hand, there was no indication of forgetting over the 24-hour retention period. The failure to demonstrate an improvement of memory performance after a 24-hour delay was not due to floor effects, i.e. the rats had not yet reached the maximum speed of responding at the end of the acquisition session. Trend analysis confirmed that the speed of responding was still increasing during the retention session.

The female rats of both strains had shorter response-response latencies in the first block of phase 1 than the male rats. This might indicate that they acquired the CRF contingency faster. Van Haaren and colleagues (van Haaren, van Hest, & van de Poll, 1987) found that, in a discriminated autoshaping procedure, female rats respond less frequently to both the positive (CS^+) and the negative (CS^-) conditioned stimulus than male rats. In this autoshaping procedure the CS^+ or CS^- (left or right lever) was presented, but only the CS^+ was followed by delivery of a food pellet. Although presentation of the reinforcer is completely independent of the lever press responses, rats will eventually start to press the lever. When the CS^+ and CS^- were reversed, female rats reduced their responses to the CS^+ faster than male rats. Van Haaren interpreted this finding as an indication that female rats might be more sensitive to the contingencies that were in effect than male rats were. The same interpretation may apply to our findings.

It is equally conceivable, however, that the female rats had higher activity levels in the present experiment. This might have facilitated the initial contact with the levers, and may have caused shorter response-response latencies during the first block of phase 1. A presumed higher activity level (e.g. van Hest, van Haaren, & van de Poll, 1987) provides a non-mnemonic interpretation of the slightly more efficient learning curve of female rats during the acquisition phase.

The female and male rats did not differ in their retention performances, and both sexes responded equally well during the retention session. It may be concluded that the poorer retention performance of the female rats in the inhibitory avoidance task (Chapter 2, exp. 2.1) was not caused by a general impairment of their ability to retain responses over a 24-hour retention interval.

EXPERIMENT 3.II: INFLUENCE OF CHOLINE ON RETENTION OF AN INCOMPLETELY ACQUIRED BAR-PRESS RESPONSE

The effect of chronic dietary choline enrichment was investigated in male rats of both strains. Female rats were not used in this experiment because neither sex differences nor strain by sex interactions on retention performance had been found in the first experiment. As the BN rat is characterized by a low central cholinergic activity and by a poor performance in aversively motivated tasks, it was expected that these animals would profit more from the increased availability of choline and that they would show an improved retention performance. Although no retention deficits had been found in the first experiment, the assessment of chronic dietary choline supplementation is justified, because the treatment might improve performance above the final level reached during the acquisition phase ('reminiscence') (Jaffard, Destrade, Soumireu-Mourat, Durkin, & Ebel, 1980; Jaffard, & Destrade, 1982).

The first experiment did not investigate whether the 24-hour delay retarded the acquisition of the operant habit in comparison with a training session without a delay after the sixteenth response (an uninterrupted training) Thus in order to have an appropriate control for the effect of the 24-hour delay between acquisition and retention an uninterrupted (zero-delay) training condition was included

The aim of this experiment was to assess the effects of chronic dietary choline enrichment on the retention performance in this task In addition we compared the response-response latencies during the first block of phase 2 after interrupting the training for 24 hours with the performance in an uninterrupted training session

Material and Methods

Animals Forty male rats were used (see Appendix C second experimental protocol) Ten litters with two littermates each of the BN/BiRij and the WAG/Rij strain were supplied by the TNO Institute for Experimental Gerontology Rijswijk the Netherlands at the age of three weeks They were housed in pairs in standard Makrolon™ cages and were habituated to a reversed day/night cycle (light being on from 20 00 to 8 00)

Methods At the age of five weeks one animal from each litter was assigned randomly to the choline enrichment condition Drinking water was supplied with 2 5 mg choline chloride (Merck Art nr 500117) per ml When the rats were 26 weeks old they were housed individually in standard Makrolon™ cages The animals were tested in a one trial inhibitory avoidance task (see Chapter 2) when they were 26 weeks old

Acquisition and retention of the incompletely acquired operant conditioning task was assessed in week 37 Within strains both littermates (the choline-enriched rat and its untreated littermate) of a nest were randomly assigned to either the zero delay condition or the 24-hour delay condition The rats from the 24-hour delay condition responded 16 times during the acquisition session (phase 1) and 16 times during retention session (phase 2) one day later The rats from the zero-delay condition responded 31 times during one uninterrupted session (phase 1 and phase 2 were not separated)

Results

Response response latencies after the retention interval (see Table 3 2) In order to analyze the data of the incompletely acquired operant conditioning task six block means of five response-response latencies each were calculated Retention of the incompletely acquired operant conditioning task was analyzed by a strain (BN vs WAG) by treatment (untreated vs choline enriched) by delay condition (uninterrupted vs 24-hour retention interval) by blocks (last block mean of phase 1 vs the first block mean of phase 2) ANOVA with repeated measures on the last factor[2]

Although the data from the BN rats shown in Table 3 2 might suggest an effect of the delay condition (uninterrupted training vs 24-hour retention interval) because all response-response latencies after the 24-hour delay seemed to increase while they seemed to decrease when

[2] An ANOVA including litters as a factor would have been appropriate and was in fact performed As the special (litter) error terms however proved to be homogeneous the data were re-analyzed by a simplified ANOVA from which the factor litters was excluded

TABLE 32 Block means and standard deviations (stddev) of logarithmically transformed response-response latencies of the third block of phase 1 and the first block of phase 2 Each strain by treatment by retention interval group consisted of 5 rats

Strain	*Treatment*	*Retention interval*	*Last block phase 1*		*First block phase 2*	
			mean	*stddev*	*mean*	*stddev*
BN	*choline-enriched*	*24-hour delay*	4 93	0 33	5.04	0.34
BN	*choline-enriched*	*uninterrupted*	4 89	0 28	4 64	0 44
BN	*untreated*	*24-hour delay*	4 41	0 39	4 61	0 17
BN	*untreated*	*uninterrupted*	4 84	0.53	4 80	0 42
WAG	*choline-enriched*	*24-hour delay*	4 80	0 41	4 69	0 54
WAG	*choline-enriched*	*uninterrupted*	4 82	0 34	4 73	0.25
WAG	*untreated*	*24-hour delay*	4 87	0 17	4 83	0 44
WAG	*untreated*	*uninterrupted*	5 16	0 47	4 95	0.50

training was continued uninterrupted, this impression was not supported statistically Likewise, the WAG rats seemed to have shorter response-response latencies in the first block of phase 2, irrespective of the delay condition. The two delay conditions, however, did not affect the performance on the first block of the second phase differently (all $Fs_{1\,32} < 2\,44$, ns), as compared with the performance at the end of phase 1. Performance levels during the third block of phase 1 and the first block of phase 2 were similar ($F_{1,32} < 1$, ns) No strain differences were found with respect to retention performance (all $Fs_{1,32} < 1$, ns). Thus, the BN rats showed as good a retention performance as the WAG rats. Chronic dietary choline enrichment did not influence the performance at any stage of the experiment (all $Fs_{1,32} < 1$, ns)

Learning during the two phases of the experiment[3] Because the two delay conditions had no differential effect on performance during the first block of phase 2, this factor was omitted from further analyses. As in experiment 3.I the progression of learning was evaluated separately for both phases of the experiment by calculating general means, linear and quadratic trend coefficients over the three block means per phase They were analyzed by a strain (BN vs WAG) by treatment (untreated vs choline-enriched) ANOVA. Learning was characterized by general linear (phase 1· $F_{1\,36} = 152\,55$, phase 2 $F_{1\,36} = 26\,31$, both p-values < 0 01) and quadratic (phase 1 $F_{1,36} = 15\,33$, phase 2 $F_{1,36} = 11\,66$, both p-values < 0 01) trends in both sessions Latencies between responses decreased, slightly negatively accelerated Chronic dietary choline enrichment had no effect on the speed of learning, and both strains performed equally well (all $Fs_{1,36}$ with associated probabilities > 0 10)

DISCUSSION

The performance of the rats in the first block of phase 2 could not be distinguished from that in the last block of phase 1, irrespective of whether a 24-hour delay was introduced after the first phase or whether training was continued without delay Chronic dietary choline

[3] Experiments 3.I and 3.II produced nearly identical learning curves The curves of the male rats in Fig 3.1 thus are representative for the curves obtained in the second experiment They might therefore be consulted to get a graphical presentation of the progression of learning

enrichment had no effect on the retention of the incompletely acquired operant conditioning task Trend analysis confirmed that the speed of bar pressing increased during the first and second phase Thus the failure to demonstrate any memory modulating effects of choline enrichment was not due to floor effects at the end of the first phase of training

GENERAL DISCUSSION

Both strains retained the incompletely acquired bar-press response equally well (experiments 3I and 3II) There was also no difference between the strains for the rate of learning during acquisition (phase 1) or retention (phase 2) In the first experiment the female rats acquired the bar press response faster than the male rats during the acquisition phase Sex differences were not apparent for the retention performance and for the rate of learning during the second phase of the experiment These results show that the poorer retention performance of female rats in the inhibitory avoidance task (Chapter 2 exp 2I) does not reflect a more general impairment of their ability to retain information over a 24-hour interval As no strain by sex interactions had been found in the first experiment the second experiment was performed with male rats only Retention in both experiments was good neither forgetting nor reminiscence could be demonstrated as the animals performance in the last block of phase 1 and in the first block of phase 2 were virtually the same

Dietary choline enrichment did not influence retention after the 24 hour interval The 24 hour retention interval itself had no effect on performance The rats increased their speed of responding in phase 2 in both experiments Floor effects can thus be ruled out as explanation of our negative findings The rats had not yet reached their maximum response speed at the end of phase 1

It should be pointed out that our experiments differed from the experiments performed by Jaffard Gauthier Destrade and co workers on one major and some minor points Firstly we used rats instead of mice Secondly a session was terminated as soon as a rat had executed 16 responses whereas Destrade and co workers (Destrade & Cardo 1974 Destrade Jaffard, Deminiere & Cardo 1976) Gauthier and co workers (Gauthier & Soumireu Mourat 1981 Gauthier Destrade & Soumireu-Mourat 1982 1983) and Jaffard and co workers (Jaffard Destrade Cardo & Soumireu-Mourat 1974) trained their mice for 15 minutes Their mice responded approximately 15 times during a 15 minute session In fact they analyzed the number of responses per 5-minute blocks In their approach the latency between making the lever available and the first response could have influenced the results In our experiments the latencies to respond for the first time varied from some seconds to some ten minutes, especially in BN rats but as only the latencies between responses were analyzed an initial exploration of the apparatus or a long initial period of inactivity (which was typical for BN rats) did not affect our results Our approach has the additional advantage that the number of responses was strictly controlled A third difference concerns the complexity of the task In our experiments the left and right lever were presented in turn while Destrade Gauthier, and Jaffard used a single lever Reminiscence in rats has been described to occur in complex situations (e g Bunch & Magdsick 1933 Anderson 1940) The presentation of the two levers in alternation was meant to increase the task complexity

There is no strain of rats, as there is of mice, available which shows reminiscence in a CRF task We were unable to modulate the memory performance in this task by chronic

dietary choline enrichment. In contrast with our findings after a long-term treatment, facilitation of retention on the appetitive tasks occurs after acute, post-trial treatments such as subseizure stimulation in the dorsal hippocampus (Destrade, Soumireu-Mourat, & Cardo, 1973, Destrade, & Cardo, 1974, Destrade, Jaffard, Deminiere, & Cardo, 1976, Jaffard, Ebel, Destrade, Durkin, Mandel, & Cardo 1977, Jaffard, & Destrade, 1982) or stimulation of the lateral entorhinal cortex (Gauthier, & Soumireu-Mourat, 1981) in mice However, long-term choline enrichment may exert memory modulating effects since an improved retention of inhibitory avoidance has been observed (Bartus, Dean, Goas & Lippa, 1980, Davis, & Trombetta, 1984, Mervis, Horrocks, Wallace, & Naber, 1984, van der Staay, Raaijmakers, & Collijn, 1986)

The retention of BN rats was unimpaired in the appetitively motivated task This observation was corroborated by results from an experiment in which the animals from experiment 31 of the present study were trained in a simultaneous discrimination task in the Skinner box (see Appendix C, first experimental protocol) The BN rats showed a better discrimination performance than the WAG rats. No decrease of discrimination performance was found in either strain after a retention interval of four weeks.

It may be concluded that neither female rats nor young adult BN rats suffer from a general impairment of their ability to retain responses over a 24 hour period The poor retention performance in an inhibitory avoidance task observed earlier seems to depend upon the motivational system involved and might be caused by non-mnemonic factors (see Chapter 2) Young-adult and adult BN rats therefore do not provide an adequate model for age-related 'cognitive' impairments

CHAPTER 4

EFFECTS OF CHRONIC DIETARY CHOLINE ENRICHMENT ON TEMPORAL DISCRIMINATION IN RATS

SUMMARY

Timing behavior can be modulated by cholinergic drugs. Therefore, we hypothesized that chronic dietary choline enrichment would improve timing behavior in inbred BN and WAG rats. In addition, we expected that the BN rats would profit more than WAG rats from the choline supplementation because BN rats have the lower central cholinergic activity. In a first DRL 8" experiment, choline enrichment (2.5 mg choline chloride/ml water) apparently affected temporal discrimination performance. However, the results were not clear-cut. Therefore, a second DRL 8" experiment was performed with rats that had received a diet in which the choline concentration was doubled. Now, no indications for effects of the choline treatment on the timing behavior were found. At the age of 62 weeks the same animals were trained on a DRL 16" schedule. Contrary to expectations, the WAG rats that had received the choline-enriched diet showed a poorer temporal discrimination performance than the control animals. Choline supplementation did not affect the performance of the BN rats. Two alternative explanations are discussed. Firstly, choline enrichment might have increased the speed of memory storage, which in turn caused a systematic overestimation of the time elapsed since the last reinforcement (Meck, & Church, 1987a,b). Secondly, it has been reported recently that the WAG strain suffers from generalized absence epilepsy (van Luijtelaar, & Coenen, 1986). Choline, when available in increased amounts, might possess 'epileptogenic' properties. A heightened incidence of epileptic activity in turn might have disrupted timing behaviour. There were considerable differences between the strains on the DRL tasks.

INTRODUCTION

An age-related decrease of performance has been found on spatial discrimination tasks in a variety of infra-human species. If age-associated impairments in this kind of task reflect a more general age-dependent impairment of general cognitive functioning, then one might expect that other aspects of cognition -for example temporal discrimination- would also deteriorate with age. There is little data about the temporal regulation of behavior in rats of different ages, in contrast to spatial discrimination learning. Campbell and Haroutunian (1981) trained 6-, 12-, and 26-month-old rats in a 'fixed interval 60 s task' (FI 60"), and assessed retention 16 days later. In a FI schedule a fixed period of time must have exceeded since the last reinforced response before a new response can be reinforced. Under this contingency the response frequency increases toward the end of the interval (Church, 1978). The temporal pattern of responding establishes a scallop, with few if any responses immediately after the reinforced response (post-reinforcement pause), and a high frequency of responding at the end of the interval.

Rats of all ages acquired the FI scallop equally well in Campbell and Haroutunian's (1981) study. However, aged rats deviated from the exact temporal characteristics of the schedule

after a retention interval of 16 days They responded more in the period that followed reinforcement and already reached the maximum response rate in the fourth instead of the sixth 10-s period following the previously reinforced response

Lejeune, Jasselette, Nagy, and Peree (1986) compared the FI 60" responding of rats of three different ages Three-week-old weanlings performed better than young adult (3-month-old) and old (26-month-old) rats The old rats adapted as well as the young adults to the FI schedule These results are in agreement with the data on acquisition presented by Campbell and Haroutunian (1981) It may be concluded that acquisition of responding is not affected by age (exception very young rats), while retention may be impaired in aged rats

In a study by McKim (1974) the FI 2' performance of rats was disrupted by scopolamine, physostigmine antagonized this disruptive effect Physostigmine is a drug that inhibits the action of actylcholinesterase, the enzyme that is responsible for the breakdown of acetylcholine (ACh), and thus prolongs the action of ACh

Another type of temporal discrimination task uses the DRL schedule, in which low response rates are differentially reinforced In the DRL schedule the interval between two responses must exceed a critical delay Only responses meeting this criterion will be reinforced, whereas all responses with shorter inter-response-times (IRT) are not rewarded (Church, 1978) Hamm Knisely, and Dixon (1983) trained 6- and 24-month-old Sprague-Dawley rats on a DRL 6" task (an IRT exceeding 6 s was rewarded) for twenty 30-min sessions The old rats were not able to inhibit responses with short IRT durations during the first training sessions, but this deficiency was of a temporary nature They responded as well as the 6-month-old animals during the last sessions Hamm et al (1983) interpreted their results as reflecting a transient response bias, and not as a deficiency of short-term memory in the old rats

In a second study Hamm, Dixon, and Knisely (1984) evaluated DRL 6" as a test for age-related changes in long-term memory Results from the acquisition phase of this task corroborated the findings of their previous study Retention was tested 21 days later No differences were found between the 6 and 26 month-old Sprague-Dawley rats It should be pointed out that most DRL studies use critical delays that exceed the 6 s used in the studies by Hamm and co-workers (Hamm, Knisely, & Dixon, 1983- Hamm, Dixon, & Knisely, 1984)

The temporal regulation of behavior under a DRL schedule can be disrupted by lesions in many brain regions which are part of or are innervated by cholinergic pathways (Robinson, 1985, and Butcher, & Woolf, 1986, provide overviews of the anatomic organization of cholinergic systems) Lesions of the habenular nucleus (Evans & Thornton, 1984), the medial and lateral fornix (Gage & Evans 1981), the fimbria (Gray & Sainsbury, 1979), the medial or lateral septum (Brookes, Rawlins, Gray, & Feldon 1983), or the medial frontal cortex (Nalwa & Rao, 1985) have been reported to negatively affect DRL performance

Considerable research has focused on the role of the hippocampus in temporal discrimination (e g Johnson, Olton, Gage, & Jenko, 1977, Boitano, Dokla, Mulinski, Misikonis, & Kaluzynski, 1980, Rawlins, Winocur, & Gray, 1983) Sinden Rawlins, Gray, and Jarrard (1986) tested totally hippocampectomized rats or rats with lesions in different areas of the hippocampus, e g the CA3 region or the subiculum in DRL 12" and DRL 18" schedules and compared their performance with that of sham-lesioned rats The DRL 18" responding was impaired more than the DRL 12" responding Removal of the entire hippocampus impaired the DRL 18" performance more than smaller lesions which were restricted to particular hippocampal subregions The administration of scopolamine, an anticholinergic drug that blocks

muscarinic receptors, reduced the efficiency of responding to the same extent in all groups Sinden et al (1986) concluded that the behavioral effect of scopolamine apparently did not depend upon the decrement of activity of hippocampal cholinoceptive cells McDonough (1982) reported, that the DRL 28" responding of rhesus monkeys was disrupted by anticholinergic drugs

To summarize there is little evidence to suggest that aging disrupts the acquisition of temporal discrimination tasks The retention of temporal discrimination, however, seems to be disrupted by aging Pharmacological manipulations of the central cholinergic system modulate timing performance Drugs that reduce the level of ACh disrupt temporal discrimination performance This effect can be antagonized by physostigmine

Because it has been found that pharmacological manipulations of the central cholinergic neurotransmission affect temporal discrimination performance, it was expected that chronic dietary choline enrichment would improve the acquisition and retention of temporal discrimination behavior of rats in the DRL task The dietary administration of choline or lecithin -precursors of ACh- has been found to increase serum free choline levels (Wurtman, & Hirsch, 1977) and to accelerate ACh synthesis (Wurtman, & Fernstrom, 1976 Wurtman, 1982)

In the present study we used two inbred strains of rats BN/BiRij and WAG/Rij Gilad and co-workers compared the activity of the enzyme choline acetyl transferase (ChAT) in different brain regions of BN rats with that of the WKY strain The pigmented BN/BiRij strain is characterized by a lower central cholinergic activity, as measured by ChAT activity (hippocampus Gilad & Gilad 1981, Gilad, Rabey & Shenkman, 1983 (also lower [^{3}H] choline uptake), striatum Gilad, & McCarty, 1981, septum Gilad, & Gilad, 1981) We found similar results when we compared the cholinergic activity of BN and WAG rats (see Chapter 7) Because the normal level of central cholinergic activity is lower in BN than in WAG rats, we wondered whether BN rats would benefit more than WAG rats from the increased availability of choline

EXPERIMENT 4 1 EFFECTS OF CHOLINE (2 5 mg choline/ml water) ON THE ACQUISITION AND RETENTION OF A DRL 8" TASK

The effects of chronic dietary choline enrichment on the acquisition and retention of a DRL 8" task were assessed in adult BN and WAG rats The DRL 8" schedule is only slightly more demanding than the DRL 6" schedule used by Hamm and co-workers (Hamm, Knisely, & Dixon, 1983, Hamm, Dixon, & Knisely, 1984) Retention was measured after an interval of four weeks

MATERIAL AND METHODS

Animals A total of forty male rats was used (see Appendix C, second experimental protocol) Ten litters with two littermates each of the pigmented BN/BiRij and the albino WAG/Rij strain were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands at the age of three weeks They were housed in pairs in standard Makrolon™ cages and habituated to a reversed day/night cycle (light being on from 20-00 to 8-00)

Apparatus Eight identical operant conditioning chambers were used (for a detailed description, see Chapter 3) In short, a Skinner box (inner dimensions 24 * 25 * 22 cm) was provided with a food tray, on either side of which a retractable lever was positioned Lamps (2 8 W)

were positioned above the food tray and above both levers A house-light and a 6 cm speaker were attached to the roof of the conditioning chamber The Skinner box was enclosed in modified sound-attenuating housing (Campden Instrument model 412) An exhaust fan produced a background noise of approximately 42 dBA The equipment was controlled and data were collected by a PDP 11/03 computer

Methods At the age of five weeks one animal from each litter was assigned randomly to the choline enrichment condition Drinking water contained 2 5 mg choline chloride ($C_5H_{14}ClNO$, Merck) / ml When the animals were 26 weeks old they were housed individually in standard Makrolon™ cages The rats were tested in a one-trial inhibitory avoidance task when they were 31 weeks old

At the age of 36 weeks the weights of the animals were gradually reduced to 85% of the free-feeding weights The rats were magazine trained in a Skinner box during daily 30 minute sessions until they had consumed at least twenty 45 mg food pellets (Campden Instruments) in each of two sessions The pellets were supplied at random intervals ranging from 20 to 100 s During this phase the levers were retracted from the operant conditioning chamber

The animals were then trained in a CRF schedule until a criterion of 16 responses (left and right lever were presented in alternation) in one session was reached Retention of responding was tested 24 hours later (see Chapter 3) From previous experience with BN rats in other appetitively motivated tasks, we expected to encounter difficulties when switching from a CRF to a DRL schedule, since the density of reinforcement is drastically decreased as long as the rats do not respond according to the DRL contingency This was the main reason why a DRL 8" schedule -a relatively easy task- was selected The rats were trained in weeks 38/39 to respond to the left lever in a DRL 8" schedule on 12 consecutive days A session was ended as soon as a rat had gained 30 reinforcements After the twelfth session the rats were returned to an ad libitum feeding regimen

After three weeks the body weights of the rats were gradually reduced to 85% of their free-feeding weights within one week by restricted feeding The retention of the DRL 8" responding was assessed during one session four weeks after completion of acquisition when the animals were 43 weeks old All testing was done in a counterbalanced order between 12 00 and 17 00

RESULTS

The first step of data analysis consisted of computing a measure for the efficiency of responding (Richelle, & Lejeune, 1980) The most commonly used measure consists of the ratio

N'/N,	where
N'	number of reinforced IRTs (inter-response-times), and
N	number of responses

This measure treats all non-reinforced IRTs as equivalent An alternative formula (originally developed by Heuchenne et al, unpublished report, cited by Richelle & Lejeune 1980, p 24, formula 3) was used in the present study

$$\frac{N + \sum_{x_i < T} \frac{x_i}{T}}{N}$$ where

x_i duration of the i^{th} IRT, and

T critical delay (IRTs ≥ T are rewarded)

In this formula all non-reinforced IRTs are weighed according to their duration All reinforced IRTs, on the other hand, are considered equivalent ($x_i \geq T$ are assigned the value T) irrespective of their duration

$$\sum_{x_i \geq T} \frac{x_i}{T} = N'$$

Thus, the sum of all reinforced IRTs equals N If all $x_i \geq T$ then the efficiency of responding reaches its maximum value 1 0 (in this case the equation can be written in a simplified form as N/N)

Even when a rat gains a low number of reinforcements but extends the duration of IRTs in the course of training, the above-mentioned efficiency measure approaches the maximum value (1 0) This efficiency measure provides a useful overall index of temporal discrimination in DRL tasks, rather than a measure for the efficiency of gaining rewards Henceforth, it will be referred to as the temporal discrimination index

Where it is appropriate, additional analyses on the number of reinforcements attained, on the number of responses, and on the distributions of IRTs per opportunity (IRTs/opp) and of IRTs will be discussed According to recommendations made by Kramer and Rilling (1970, p 230), all these analyses should be included in DRL studies, "for the sake of clarity, completeness, and ease of comparison with other experiments"

In the IRT distribution responses are classified according to their IRTs Normally, the critical delay is divided into a number of equal IRT bins (or classes) In our study the bin width was a quarter of the interval necessary to gain reinforcement If the rats acquire temporal discrimination, the mode of the IRT distribution should move towards the critical delay in the course of training

The IRTs per opportunity (IRTs/opp) may provide a more sensitive measure to detect temporal discrimination (Kramer & Rilling, 1970) IRTs/opp were calculated from a formula presented by Anger (1956) In short the number of opportunities to respond in a particular IRT bin equals the number of responses elicited in that bin plus the number of responses in all bins with longer IRTs The number of responses in the bin under consideration is divided by the number of opportunities to respond in that bin Thus contrary to analyses on distributions of IRTs, an IRTs/opp analysis takes into account the inequality of the number of opportunities to respond in a given IRT bin (Kramer & Rilling, 1970)

Orthogonal polynomials of the temporal discrimination index over all acquisition trials were calculated General means and trend components were analyzed by a strain by treatment

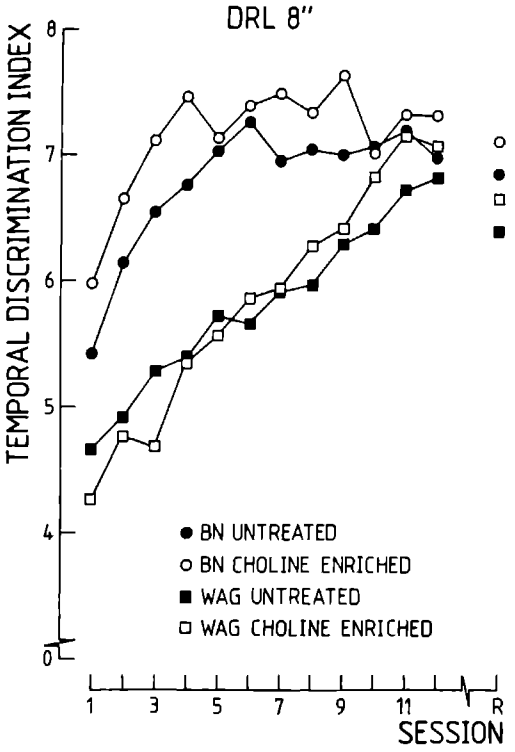


FIGURE 4.1: Temporal discrimination index of untreated and chronically choline-enriched BN and WAG rats on a DRL 8" schedule. Abbreviation: R: retention session.

ANOVA. Retention performance was analyzed by difference scores between the last acquisition session and the retention session.

Temporal discrimination index (see Fig. 4.1): Analysis of the general means revealed that temporal discrimination performance averaged over all acquisition sessions was better in BN rats than in WAG rats ($F_{1,36} = 56.97$, $p < 0.01$). The temporal discrimination index improved over the sessions, as indicated by general linear ($F_{1,36} = 171.31$, $p < 0.01$) and quadratic ($F_{1,36} = 16.63$, $p < 0.01$) trends. Of the increase over the successive sessions, 89% may be predicted from a linear regression equation. A strain by treatment interaction for the linear component ($F_{1,36} = 4.98$, $p < 0.05$) indicated that the rate of mastering the task was differently influenced by choline treatment in both strains. A Duncan post hoc analysis, however, could not confirm that there were differences between the treated and untreated groups within either strain. Pairwise comparisons only confirmed that BN rats showed less improvement over sessions than the WAG rats did (linear trend components: 0.11 vs. 0.27). Both strains showed the same temporal discrimination performance on the DRL 8" schedule by the tenth acquisition session (day 10: $F_{1,36} = 3.10$, $p > 0.10$; day 11: $F_{1,36} = 2.12$, $p > 0.10$; day 12: $F_{1,36} < 1$, ns).

The temporal discrimination index was unaffected by the retention interval, as the difference scores between the last acquisition session and the retention session did not deviate from zero ($F_{1,36} = 3.86$, $p > 0.05$). Strain ($F_{1,36} = 1.12$, ns) and treatment effects on the retention performance were not found ($F_{1,36} < 1$, ns).

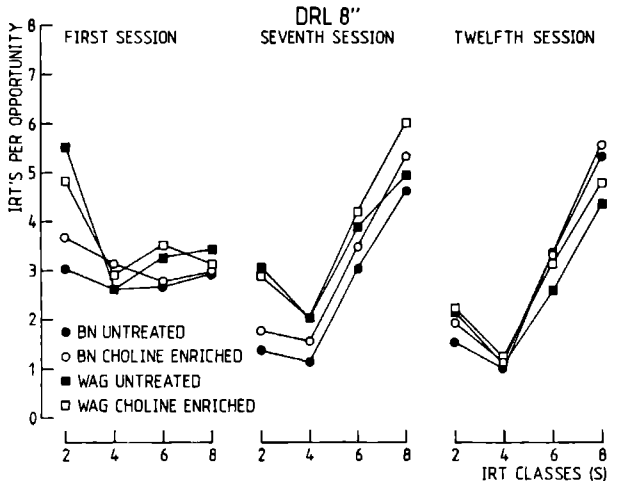


FIGURE 4.2: IRTs/opp (inter-response-times per opportunity) on the first, seventh, and twelfth session on a DRL 8" schedule. The first four (unreinforced) IRT classes are shown. The IRT classes are indicated by their upper limits.

The distribution of IRTs/opp of the first, seventh, and twelfth session are shown in Fig. 4.2. They demonstrate that the rats of both strains improved their temporal discrimination in the course of training, as the probability of responding in the higher IRT classes increased.

DISCUSSION

The strain by treatment interaction found for the temporal discrimination index points to a possible differential effect of chronic dietary choline enrichment on the acquisition of temporal discrimination over the sessions. However, within strains effects of the chronic dietary choline enrichment were not found, and the results are therefore inconclusive.

The WAG rats had a much lower efficiency level at the start of the formal training than the BN rats did. Both strains responded equally well during the last acquisition sessions as indicated by the temporal discrimination index. Contrary to what was expected, the BN rats could be shifted to a DRL 8" schedule without any problem. The finding that BN rats reached their maximum temporal discrimination performance within about two sessions and that their performance remained unchanged in the sessions which followed (the strain differences found are discussed in more detail in experiment 4.III of the present study) was quite surprising. Retention deficits were not found after the retention interval of four weeks.

EXPERIMENT 4.II: EFFECTS OF CHOLINE (5 mg choline/ml water) ON THE ACQUISITION, RETENTION, AND EXTINCTION OF A DRL 8" TASK

In order to investigate the possibility that the choline enrichment effects were weak because the dosage of choline chloride was too low, the experiment was repeated with animals that had been treated chronically with a higher concentration of choline. After a retention interval of seven weeks, the effects of chronic dietary choline enrichment on long-term memory were assessed during one retention and two extinction sessions. The rate of extinction over the sessions might provide additional information about the rate of forgetting (Alescio-Lautier, & Soumireu-Mourat, 1986).

Material and Methods

Animals. A total of sixty male rats were used (see Appendix C, third experimental protocol). Ten litters with three littermates each of the pigmented BN/BiRij and the albino WAG/Rij strain were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands, at the age of five weeks. They were housed in pairs in standard Makrolon™ cages and habituated to a reversed day/night cycle (light being on from 20·00 to 8·00).

Apparatus: The same equipment as in the first experiment was used.

Methods: At the age of seven weeks two subgroups of five litters each from both strains were formed by random assignment. Two littermates per litter from the first subgroup and one littermate per litter from the second subgroup were randomly assigned to the choline treatment condition. The remaining animals were assigned to the untreated control condition (compare Chapter 2, Table 2.1). Thus, the choline-enriched group and the control group for both strains consisted of 15 subjects. Choline enrichment consisted of supplying 2.5 mg choline chloride per ml drinking water. The rats were 28 weeks old when they were housed individually in standard Makrolon™ cages. The choline concentration was doubled (5 mg choline chloride per ml water) from the age of 33 weeks.

The body weights of the rats were reduced to 85% of their free-feeding values in week 43. Pre-training was carried out between 13:00 and 17·00. After magazine training (for procedural details see exp. 4.1) the animals were handshaped to respond to the left lever on a CRF schedule. Hand-shaping was terminated when the rats had gained fifteen pellets which were contingent on lever pressing.

The animals were 45 weeks old when training on DRL schedules started. One session on a DRL 1" schedule was given. Then, the animals were trained in a counterbalanced order on a DRL 8" schedule on 14 consecutive days (between 9·00 and 13:00). A session was terminated as soon as a rat had gained 30 food pellets. After the fourteenth session the rats were given free access to food for six weeks. The body weights of the animals were then gradually reduced to 85% of their free feeding values, again in the course of one week. Retention was tested in one session seven weeks after completion of acquisition when the rats were 54 weeks-old. Thirty-minute extinction sessions were given over the next two days.

Results

The data were analyzed as described in experiment 4.1. In addition, the effects of extinction were evaluated by the decrease of the total number of responses from the retention session to the first extinction session, and from the first to the second extinction session.

Temporal discrimination index (see Fig. 4.3): Analysis of the general mean of the temporal discrimination index over all sessions revealed that the BN rats showed better temporal discrimination than the WAG rats ($F_{1,56} = 37.57$, $p < 0.01$) (see Fig. 4.3). The learning process was characterized by an improvement of temporal discrimination which showed a general linear ($F_{1,56} = 141.81$, $p < 0.01$) and quadratic ($F_{1,56} = 25.34$, $p < 0.01$) component. Ninety-two percent of the variation of this measure over successive sessions may be predicted from a linear regression equation. Therefore, only results of the linear component will be reported. The BN rats showed less improvement over the sessions than the WAG rats did ($F_{1,56} = 29.56$, $p < 0.01$) (linear components: 0.13 vs. 0.26). Both strains responded equally well to the requirements of the DRL schedule by the twelfth session (days 12, 13, and 14: $Fs_{1,56} < 1.81$, ns).

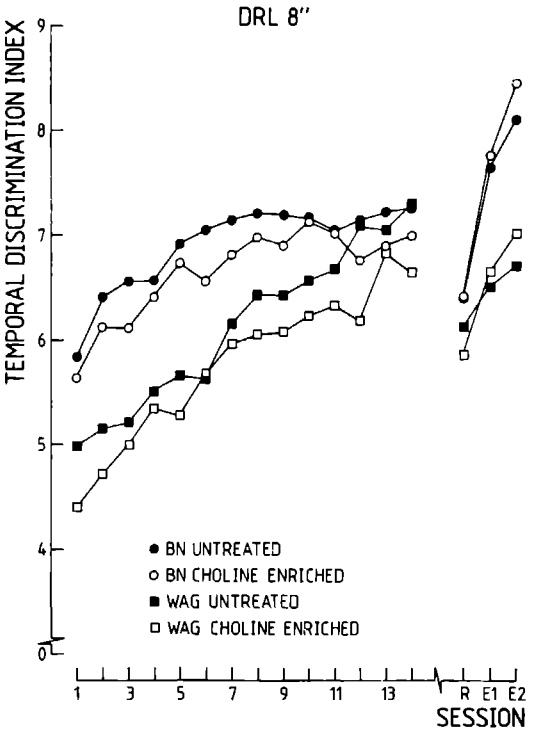


FIGURE 4.3: Temporal discrimination index of untreated and chronically choline-enriched BN and WAG rats on a DRL 8" schedule. Abbreviations: R: retention session; E1, and E2: First and second extinction session, resp.

The IRTs/opp of the first, seventh, and fourteenth session are shown in Figure 4.4, and demonstrate that the rats acquired the temporal discrimination behavior. Figure 4.5 shows the IRT distribution of the two strains over sessions and bins. It is clear that the IRT distributions were qualitatively different for both strains.

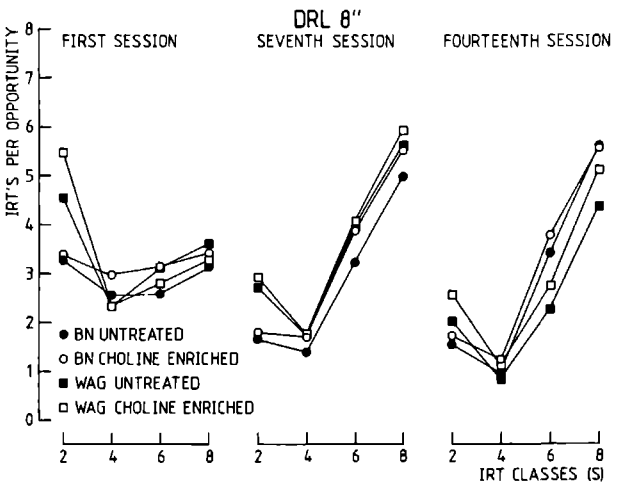


FIGURE 4.4: IRTs/opp (inter-response-times per opportunity) on the first, seventh, and fourteenth session on a DRL 8" schedule. The first four (unreinforced) IRT classes are shown. The IRT classes are indicated by their upper limits.

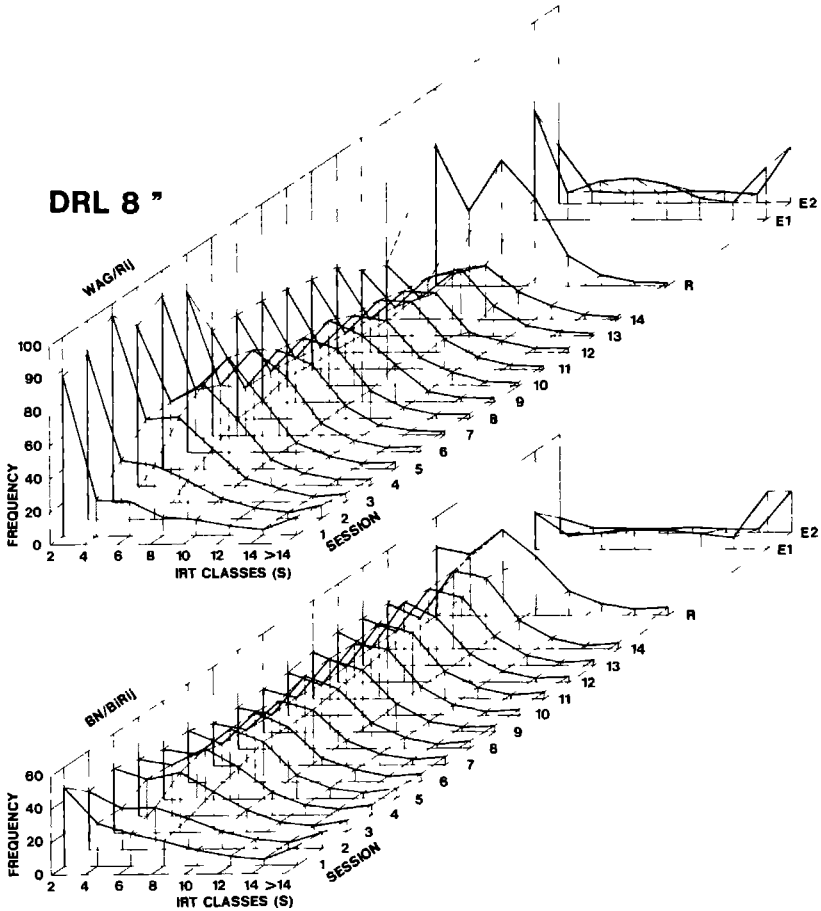


FIGURE 4.5: Response surfaces of IRTs of BN and WAG rats on a DRL 8" schedule. Data of the untreated and chronically choline-enriched animals within strains were pooled. X-axis: IRT classes (indicated by their upper limits); Y-axis: Frequencies of IRTs within classes; Z-axis: Acquisition sessions 1 to 14, retention session (R), and first and second extinction session (E1, E2).

After the retention interval of seven weeks temporal discrimination performance was slightly impaired, as measured by the difference scores between the last acquisition session and retention ($F_{1,56}$ = 49.96, $p < 0.01$), but the decrease was the same for both strains ($F_{1,56}$ = 1.13, $p > 0.10$) and treatment conditions ($F_{1,56}$ = 1.78, $p > 0.10$) (see Fig. 4.3).

The temporal discrimination index might not provide the most appropriate measure to evaluate the rate of extinction. Therefore, the extinction rate, defined as the decrease of the number of responses as a consequence of non-reinforcement, was also analyzed. The difference scores between the retention and the first extinction session, and between the first and the

second extinction session were used The frequency of responding was strongly decreased in the first extinction session ($F_{1,56} = 34.77$, $p < 0.01$) (see also Fig 4.5) There was no difference between the strains for this decrease in response rate ($F_{1,56} < 1$) However, the responses of the BN rats extinguished faster from the first to the second extinction session than the responses of the WAG rats ($F_{1,56} = 17.72$, $p < 0.01$)

Discussion

Increasing the concentration of choline chloride in the drinking water from 2.5 mg/ml to 5.0 mg/ml had no effect on the acquisition, retention, or extinction of the DRL 8" responding The results are quite clear-cut The strain by treatment interaction on the temporal discrimination index which was found in the first experiment, was not observed in the present experiment In conclusion, neither experiment supports the hypothesis that chronic dietary choline enrichment improves the temporal discrimination of rats in a DRL 8" task Moreover the hypothesis that BN rats, because of their lower central cholinergic activity, would benefit most from the extra choline on this schedule of reinforcement can be rejected The strain differences found are discussed in more detail in experiment 4.III of the present study

EXPERIMENT 4 III EFFECTS OF CHOLINE ON THE ACQUISITION OF A DRL 16" TASK

DRL schedules such as the DRL 6" or DRL 8" schedules with a relatively short critical delay are not very demanding DRL schedules with longer critical delays might be more sensitive to detect the effects of experimental manipulations (Richelle & Lejeune 1980) In order to explore this possibility, the rats were trained in a DRL 16" schedule, and the effects of chronic dietary supplementation of choline on this task were assessed

Material and Methods

Animals The animals that were trained in exp 4 II were used (see Appendix C, third experimental protocol)

Apparatus· The same equipment as in the previous experiments was used

Methods After extinction of the DRL 8" responding, the animals were trained in a spatial discrimination task in a holeboard (see Chapter 5) while remaining under the 85% food deprivation regime When the rats were 62 weeks old they were trained on a DRL 16" schedule in 30-minute sessions on 13 consecutive days All testing was done between 9.00 and 13.00 in a counterbalanced order

Results

Temporal discrimination index (see Fig 4.6) A strain by treatment interaction ($F_{1,56} = 9.47$, $p < 0.01$) for the general mean of the temporal discrimination index over all the sessions indicated that the treatment affected the two strains differently Duncan post-hoc analysis revealed that the choline-treated BN rats, their untreated controls, and the untreated WAG rats did not have different temporal discrimination indices Treated WAG rats on the other hand showed an impaired temporal discrimination performance when compared with their untreated controls

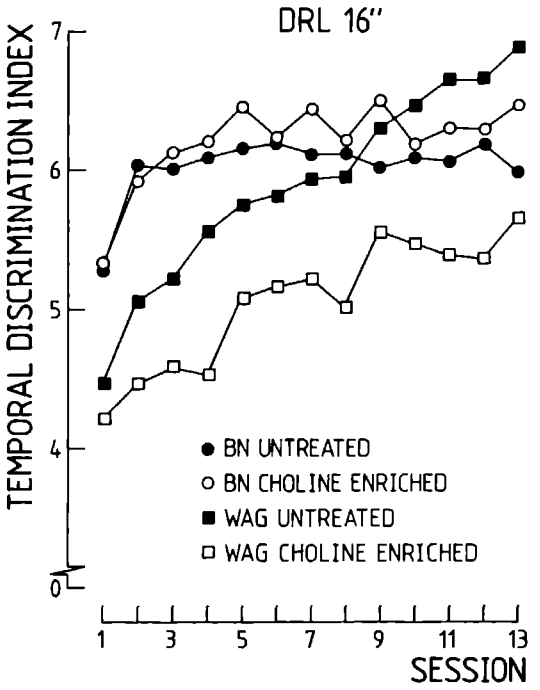


FIGURE 4 6 Temporal discrimination index of untreated and chronically choline-enriched BN and WAG rats on a DRL 16" schedule

In the first acquisition session the temporal discrimination index of the WAG rats was lower than that of the BN rats ($F_{1\,56}$ = 38 81 p < 0 01) At the end of acquisition (session 13) strain differences were no longer apparent ($F_{1\,56}$ < 1, ns) There was, however, a strain by treatment interaction ($F_{1,56}$ = 14 64, p < 0 01) Duncan post-hoc analysis revealed that the untreated and choline-enriched BN rats did not differ The temporal discrimination index of the untreated WAG rats was considerably higher than that of the choline-treated WAG rats

The learning process assessed by the temporal discrimination index was characterized by general linear ($F_{1,56}$ = 97.51, p < 0 01), quadratic ($F_{1,56}$ = 3.58, p < 0 01), and cubic ($F_{1,56}$ = 22 64, p < 0 01) trends Eighty-two percent of the variation in this measure over the successive sessions may be predicted from a linear regression equation A strain by treatment interaction was found on the linear trend component ($F_{1\,56}$ = 6 06, p < 0 05) Duncan post-hoc analysis confirmed that the treatment effect was restricted to the WAG strain temporal discrimination performance improved very slowly in the treated WAG rats as compared with the untreated controls

In order to determine the nature of the effects of the choline enriched diet in the WAG strain, additional analyses were performed

Number of reinforcements attained (see Fig 4 7) The number of reinforcements attained showed a development which quite closely paralleled that of the temporal discrimination index For the mean number of reinforcements over all sessions, a strain by treatment interaction was found ($F_{1,56}$ = 7 15, p < 0 01) Duncan post-hoc analysis revealed that the BN rats

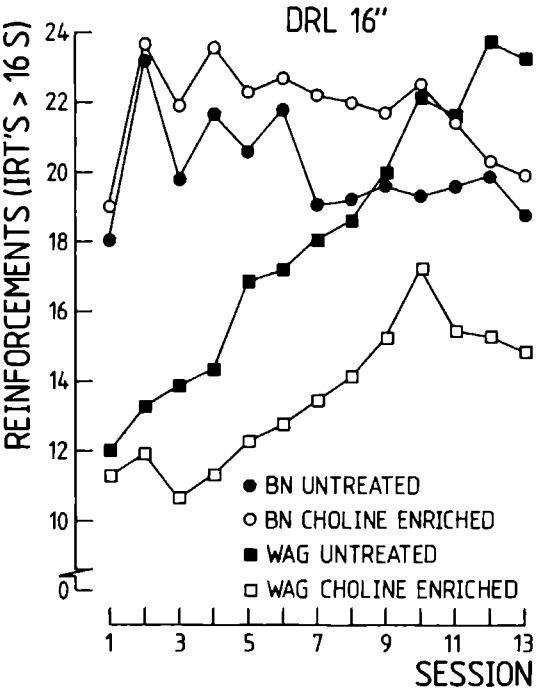


FIGURE 4.7: Reinforcements attained by untreated and chronically choline-enriched BN and WAG rats on a DRL 16" schedule.

and the untreated WAG rats gained the same amount of reinforcements, while the choline-treated WAG rats gained considerably less.

In the course of DRL 16" acquisition the rats gained an increasing number of reinforcements, as indicated by general linear ($F_{1,56}$ = 28.43, $p < 0.01$), and a number of higher order trends. Seventy-three percent of the variation in the number of reinforcements attained over successive sessions may be predicted from a linear equation. Each of the other polynomials, although contributing to the reinforcement curves, covered only minor percentages of variation. Thus, only the results of the linear component will be presented here. A strain by treatment interaction on this component ($F_{1,56}$ = 6.57, $p < 0.05$) indicated that chronic dietary choline enrichment had a differential effect on the increase of the reinforcements gained. Duncan post-hoc analysis revealed that the treated and untreated BN rats did not differ from one another, but that they showed considerably lower trend coefficients than both WAG groups. The treated WAG rats showed less progress in the rate of obtaining reinforcements than their untreated controls.

Number of responses (Fig. 4.8): For the mean number of responses over all trials, a strain by treatment interaction was found ($F_{1,56}$ = 7.15, $p < 0.01$). Duncan post-hoc analysis revealed that the mean number of responses was the same for the BN rats and the untreated WAG rats, while the treated WAG rats responded more frequently. The development of response rates over successive sessions followed general linear ($F_{1,56}$ = 57.23, $p < 0.01$) and quadratic ($F_{1,56}$ = 24.59, $p < 0.01$) trends. Eighty-four percent of the variation in response frequency over the sessions may be predicted from a linear regression equation; an additional 10 percent may

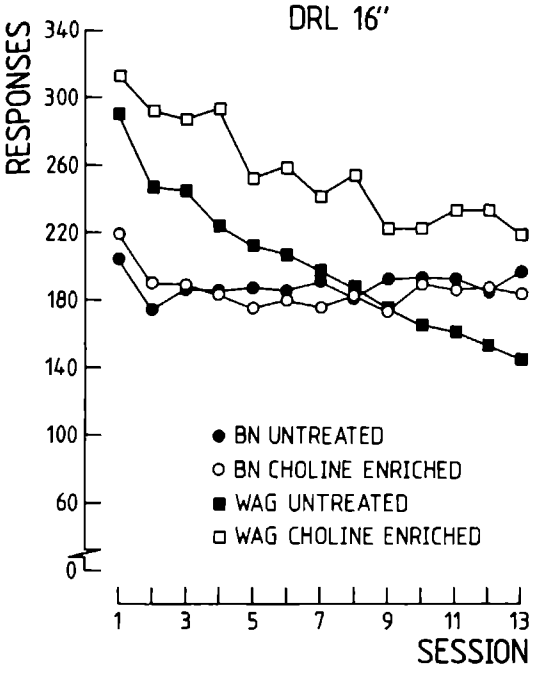


FIGURE 4.8: Total number of responses of untreated and chronically choline-enriched BN and WAG rats on a DRL 16" schedule.

be predicted from a quadratic equation. The linear component of strains was different: WAG rats showed a continuous reduction of their response frequencies over the sessions, while the BN rats did not. Choline treatment had no effect on the decrease of the responses. No differences between groups were found for the quadratic trend components.

The temporal discrimination index offers no clue as to which response class(es) were affected by the treatment. This question was addressed in an analysis on the 'response surfaces' of IRTs/opp and IRTs. The combined orthogonal polynomials over the thirteen sessions and over the bins were calculated and subjected to ANOVA (e.g. Winer, 1971, section 7.6). The analyses were restricted to the four unreinforced bins, in order to assure reliable results. As Anger (1956) pointed out, the reliability of computing IRTs/opp may decrease because of a decrease of sample size (i.e. "opportunities").

Distribution of IRTs/opp (Fig. 4.9): Linear to cubic trends over sessions by linear to quadratic trends over bins explained 83% of the variation in the IRTs/opp profile, and 68% of the variation in the IRT profile (Fig. 4.10). In the IRTs/opp profile the linear trend over sessions by quadratic trend over bins covered 52% of total variation (see Table 4.1). In the IRTs-profile none of the separate trends over sessions by trends over bins covered more than 16% of the total variation. Neither the analysis of the response profiles of IRTs/opp nor that of IRTs revealed evidence for qualitative differences in temporal discrimination between the treatment conditions, as the profiles of IRTs/opp and IRTs had equal curvatures over sessions and bins within strains. The analyses confirmed that the BN and WAG rats differed on both the profiles of IRTs/opp and of the IRTs.

TABLE 4.1: ANOVAs on the first to third order polynomials over thirteen DRL 16" sessions by the first to third order polynomials over IRT-bins of the distributions of IRTs/opp. Only the first four unreinforced 4-second bins are included. *F*-ratios (*df*s: 1,56) for strain differences, treatment effects, and strain by treatment interactions are shown. The percentage of variation which may be explained by the individual n^{th}-order session by m^{th}-order bin polynomial is indicated in the last column. It should be noted, that all polynomials contributed to the response profile of IRTs/opp over sessions (all $Fs_{1,56}$ for general means > 16.5, with associated probabilities < 0.01). +: p < 0.01; *: p < 0.05.

		IRT-BINS			
		Strain	*Treatment*	*Str.*Tr.*	*% var. expl.*
		linear			
	linear	1.2	2.6	1.3	12
	quadratic	1.4	1.1	<1.0	7
	cubic	1.6	<1.0	<1.0	6
		quadratic			
	linear	39.4 +	<1.0	2.2	52
SESSIONS	*quadratic*	1.5	3.0	2.4	4
	cubic	<1.0	2.8	<1.0	2
		cubic			
	linear	<1.0	2.3	<1.0	5
	quadratic	<1.0	<1.0	<1.0	3
	cubic	5.1 *	3.8	<1.0	1

DISCUSSION

Contrary to what was hypothesized, chronic dietary choline enrichment impaired temporal discrimination performance as measured by the temporal discrimination index and the number of reinforcements attained by the WAG rats. By analyzing the profiles of IRTs/opp and IRTs

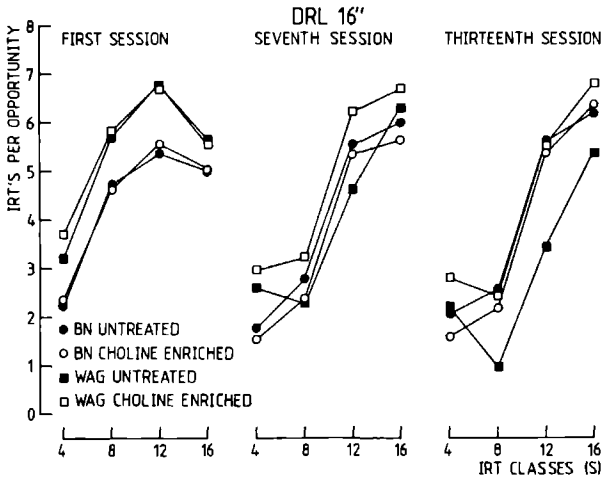


FIGURE 4.9: Inter-response-times per opportunity (IRTs/opp) on the first, seventh, and twelfth session on a DRL 16" schedule. The first four (unreinforced) IRT classes are shown. The IRT classes are indicated by their upper limits.

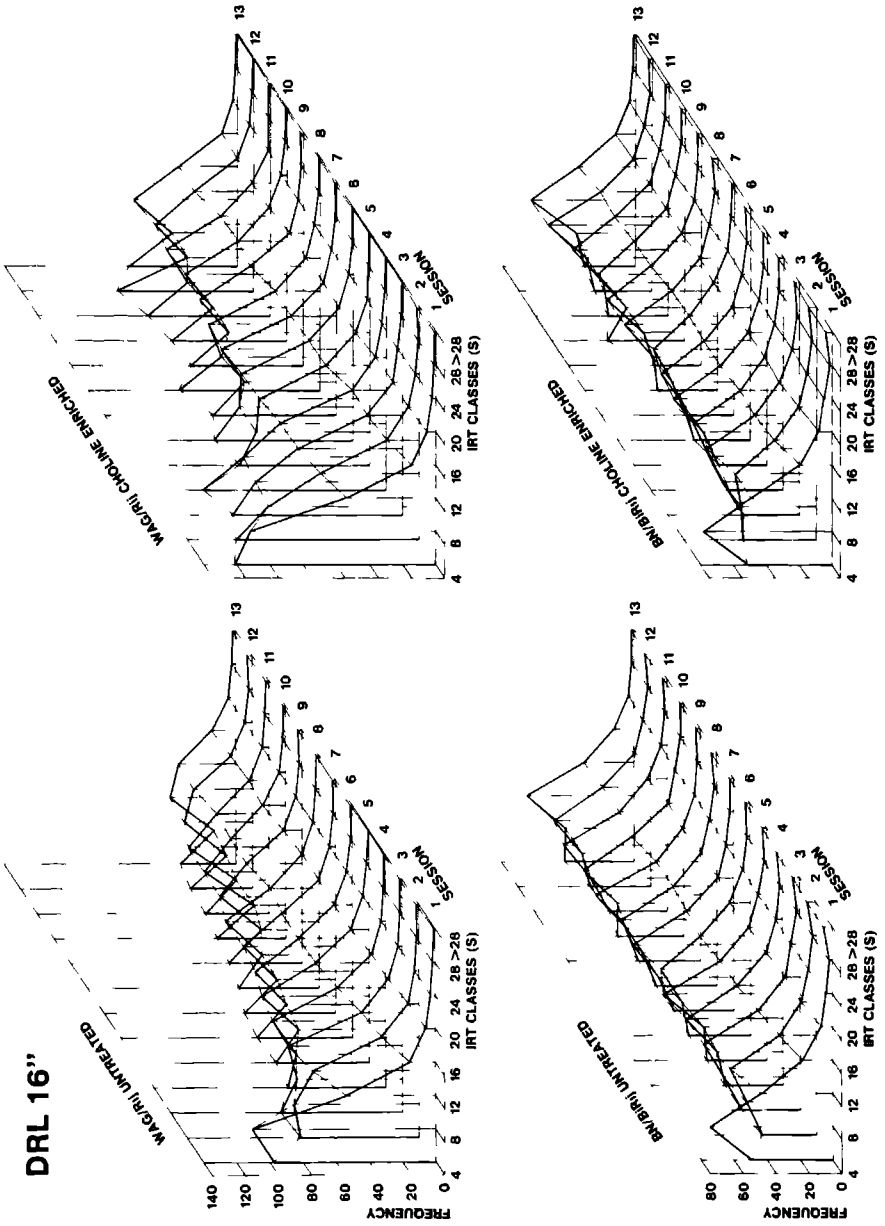


FIGURE 4.10. Response surfaces of IRTs of untreated and chronically choline-enriched BN and WAG rats on a DRL 16" schedule. X-axis. IRT classes (indicated by their upper limits); Y-axis. Frequencies of IRTs within classes. Z-axis. Acquisition sessions 1 to 13.

over sessions and non-reinforced bins, it was assessed whether a particular class of IRTs was affected by the treatment (i.e., whether there was an increase in the number of perseveration or anticipation errors In the IRT-distribution for example, these errors can be identified as increased response frequencies in the first and fourth bin, respectively)

It was found that the choline-treated and untreated WAG rats did not have different profiles of IRTs/opp and IRTs (no strain by treatment interactions were found) It can be concluded that the choline-treated WAG rats were less able to inhibit responding before the critical delay had elapsed, because they responded more frequently than the untreated WAG rats and gained less reinforcements However, all non reinforced IRT classes were equally affected As a result, the shapes of the response profiles were preserved in comparison with the response profiles of the untreated WAG rats Choline enrichment did not have any effect on the temporal discrimination performance of BN rats

The response profile of the BN rats differed from that of the WAG rats for both DRL schedules Firstly, the number of short IRTs was considerably lower for the BN than for the WAG rats Secondly, the BN rats showed almost no change in the distribution of IRTs over successive sessions Two alternative explanations might account for these observations The BN rats may have acquired the DRL responding very quickly (within one or very few sessions) In that case, the learning curves of the temporal discrimination index, the number of reinforcements attained and the number of responses elicited, and the distributions of IRTs/opp and IRTs failed to mirror the learning process because all learning occurred within the first session(s) The data obtained for the BN strain, however, might equally well be interpreted as a failure to learn, because changes in the operant responding behavior over sessions are not visible in the learning curves Acquisition of the DRL responding within a single session without further improvement over successive training sessions has, as far as we know, never been described, and is therefore considered as being very unlikely (though not impossible, it might be a strain characteristic of BN rats)

On a CRF schedule, the rats of the BN and WAG strains responded equally fast (see Chapter 3), although the BN rats showed a better simultaneous discrimination performance than the WAG rats (unpublished results, see also Appendix C, second experimental protocol, and Chapter 3) Thus, the BN rats are able to adapt their behavior to the specific requirements of different schedules of reinforcement in appetitively motivated tasks These findings contrast with the poor performance of BN rats in aversively motivated tasks

GENERAL DISCUSSION

The results of the experiments are quite puzzling and do not agree with the hypothesis that extra dietary choline improves temporal discrimination performance However the results might be explained along two other lines that are not considered as mutually exclusive Both alternative explanations will be discussed Although our experiments were not designed to provide experimental evidence for either of the two explanations, the results provide, at least partly, evidence in favour of both

Recently, Meck and Church (1987a) reported that nutrients may affect timing behavior in rats The timing behavior was assessed by a peak interval procedure (discussed in detail by Roberts, 1981) Rats were trained to respond in a discrete fixed interval 20-s schedule until the maximum response rate occurred around 20 s ('peak time the time during which the rat maximally 'expects' food) The start of each trial was signalled by the onset of white noise

The first lever-press following the critical 20-s interval was reinforced and the noise was turned off. A 130-s time-out interval was allowed between the termination of the noise signal and its onset at the beginning of the next trial. Then, no reinforcement was given for half of the trials, chosen at random, and the white noise was turned off 50 s after its onset, independently of whether the animal had responded. Responses made during these 'empty' trials were fitted to a scalar timing model (Gibbon, Church, & Meck, 1984; Meck, & Church, 1987a; Roberts, 1981). The maximal response rate might shift to a shorter or longer interval as a consequence of experimental manipulations. The shift might be due to a change in the speed of the internal clock, or be due to a change in the remembered time of reinforcement. Meck and co-worker have elaborated methods to distinguish between the effects of treatments on the internal clock and on memory processes (Meck, 1983; Meck, & Church, 1987a).

Using this approach, Meck and Church (1987a) found that rats that were prefed with a lecithin snack 20 min prior to the training sessions gradually shifted their peak time to the left. The peak time remained at this shorter interval during successive training sessions and during testing sessions, when prefeeding was discontinued. The effect was similar to that observed after the systemic administration of physostigmine (Meck, & Church, 1983, 1987b) on the peak interval procedure. Physostigmine prolongs the action of ACh by inhibiting the activity of acetylcholinesterase, the enzyme that hydrolyses ACh.

Both prefeeding with lecithin (Meck, & Church, 1987a) and systemic physostigmine administration (Meck, & Church, 1983, 1987b) influence the parameter associated with the content of the reference memory in the scalar timing model. Information about consequences of past trials (i.e. the number of clock counts that indicates the duration of the reinforced interval) is stored permanently in the reference memory (Church, 1984). The stored time interval that leads to reinforcement is compared with clock counts in an accumulator. The accumulator is reset whenever a bar-press is made. If the current value in the accumulator (also called 'working memory'; compare Gibbon, Church, & Meck, 1984) is close enough to the value stored in the reference memory, then the next response is elicited. Meck (1983) suggested that an increased storage speed might decrease the values of remembered durations, i.e. the content of the reference memory.

The presumed relationship between that parameter of the formal scalar timing model which is supposed to be affected by the treatment, and the biological (heightened level of ACh) and psychological processes (increased storage speed into the reference memory) involved (Church, 1984) might provide a useful framework for the interpretation of our own results.

The treated WAG rats might have systematically overestimated the time that had elapsed since the last response. If the overestimation of the time elapsed lies well within the width of one IRT bin, then the shape of the response profiles might be preserved while the number of responses and the number of reinforcements attained (and thereby efficiency of responding) were affected. Thus, the effects of the chronic dietary choline enrichment found with the WAG strain are consistent with the hypothesis proposed by Meck and Church (1983, 1987a, 1987b) about the role of the increased availability of choline on timing behavior. However, the DRL schedules used in our experiments are less suited to provide direct testing of treatment effects on a scalar timing model than the procedures used by Meck, Church, and coworkers (summarized in Church, 1984). The leftward shift of the peak time (Meck, & Church, 1983, 1987a, 1987b) was attributed to a change in the memory storage speed, which is a property of the reference memory (Meck, 1983). According to Meck, Church, & Olton (1984)

free-operant DRL schedules lack an external stimulus to indicate that an interval should be timed. The absence of an external stimulus means that a working memory component is added to the task, since there is no explicit reminder that the internal clock should be running. There is no way to estimate the possible contribution of the working memory in our experiments.

If we interpret our results within the context of the theory advanced by Meck, Church and co-workers, some of our findings do not fit very well. Firstly, we only observed the effect of the chronic dietary choline enrichment in one of the two strains. The performance of the BN rats, which have a lower central cholinergic activity than the WAG rats (see Chapter 7), did not change. This finding shows that our results found with the WAG strain cannot be generalized to other strains of rats.

A second problem is the lack of effects of dietary choline supplementation on DRL 8" acquisition, retention, and extinction in the BN and WAG rats. In a chronic dietary choline enrichment experiment with CPBB female rats of two different ages we found a transient effect on the frequency of short IRTs (< 2 s) during the acquisition of a DRL 8" task. The effect was restricted to the 21.5-month-old rats, while the 10.5-month-old rats were not affected. Moreover, the frequency of short IRTs decreased to the level of the control animals in the course of learning (Collijn, 1985). Meck and Church (1987a) stress that the cholinergic modulation of the speed of memory storage is a permanent effect, which continues even when the cholinergic agent is no longer supplied. Collijn's (1985) results showed only a transient effect and are thus in disagreement with an interpretation in terms of changes in the speed of memory storage.

Thus there may be a number of factors that determine whether or not choline enrichment influences DRL responding. Such factors might be the genotype of the animals involved, the complexity of the task, the age of the animals, or a combination of these factors. It is noteworthy that the effects of chronic dietary choline (or lecithin) supplementation on timing behavior were observed in animals that were at least 14 months old (Meck and Church (1987a): 18 months; Collijn (1985): 21.5 months; experiment 4.III of the present study: 14 months). No effects were found in younger rats (Collijn (1985); experiments 4.I and 4.II of the present study). However, the task was less complex (DRL 8") in those experiments; this might provide an equally acceptable explanation for the lack of effects of dietary choline supplementation. Further research is needed to identify the relevant factors that contribute to the choline enrichment effects found.

An alternative explanation for the effects of dietary choline on DRL 16" responding in the WAG strain emerges from a different line of evidence. It has been observed recently that WAG/Rij rats suffer from generalized absence epilepsy (van Luijtelaar, & Coenen, 1986; Coenen, & van Luijtelaar, 1987). Absence epilepsy is characterized by spontaneously occurring spike-wave complexes. The number and the total duration of the spike-wave complexes increases with age (Coenen, & van Luijtelaar, 1987).

In a study comparing the EEGs of five-month-old A*C, BN, CPBB, CPB-G and WAG/Rij rats (van Luijtelaar, personal communication), animals of the A*C and the BN strains were found to be nearly free of epileptic activity. A low incidence of spike-wave complexes was found in the CPBB rats (ca. 2 per hour). The frequency of spike-wave complexes was higher in CPB-G rats (ca. 7 per hour), and highest in the WAG strain (ca. 18 per hour). A clear circadian rhythm in the absence epilepsy has been observed in WAG rats (van Luijtelaar, &

Coenen, 1988). The acrophase appears during the early hours of the dark phase. This coincides with the testing period of our DRL 16" experiment.

A second line of evidence for this alternative explanation is derived from reports that cholinergic agonistic agents might possess 'epileptogenic' properties. Turski and co-workers (Turski, Cavalheiro, Schwarz, Czuczwar, Kleinrok, & Turski, 1983) reported that systemic intraperitoneal administration of different doses of pilocarpine, a potent cholinergic agonist, produced electrophysiological and behavioral phenomena of epileptic activity, predominantly in limbic structures. Pre-treatment with scopolamine prevented the occurrence of the pilocarpine-induced EEG alterations. McCann and co-workers (McCann, Cain, & Philbrick, 1983) found that chronic enrichment of the diet with choline for at least 40 days prior to electric kindling of the amygdala reduced the after-discharge threshold and the number of afterdischarges needed to provoke the first generalized seizure. They suggested that supplementation of the diet with extra choline could increase the susceptibility of the animal to epileptic seizures. However, it should be noted that contradictory results have also been reported. Blackwood, Martin, and Howe (1982) concluded that there is no fundamental relationship between changes in the cholinergic system and the development or maintenance of amygdaloid kindling in rats.

At present no information is available as to whether cholinergic neurotransmission plays a significant role in the absence epilepsy shown by WAG rats. The supplemented choline might affect the cholinergic activity as ACh agonist (e.g. Krnjevic, Reinhardt, & Ropert, 1982), or it might increase ACh synthesis (Wecker, 1986). Less direct actions of the supplemented choline have also been proposed (compare General Introduction of this thesis). Morley and Garner (1987), for example, reported that dietary choline enrichment increased the number of brain nicotinic receptors.

The possible action of choline is not restricted to cholinergic or cholinoceptive neurons. It might also affect neuronal plasticity (e.g. Bertoni-Freddari, Mervis, Giuli, & Pieri, 1985), or membrane fluidity (Farooqui, Liss, & Horrocks, 1988) of non-cholinergic neurons. It is conceivable that these neuronal changes might influence the excitability and activity of neurons and might be responsible for an increase of epileptic activity, which in turn affects timing behavior.

Taken together, the high incidence of generalized absence epilepsy in WAG rats and the possible 'epileptogenic' properties of cholinergic agents (although studied in other forms of epilepsy), or the other modes of action discussed above, might well account for the differential effects of the dietary choline enrichment found in our third experiment. No effects of the choline enrichment were found in the BN strain, which is virtually free of epilepsy. On the other hand, the WAG strain suffers from numerous spike-wave complexes (up to 25 per hour during the first half of the dark period; van Luijtelaar, & Coenen, 1988) which might disrupt ongoing timing behavior. As a consequence of the supplementation of extra choline, the number of spike-wave complexes might increase, and thus further disturb ongoing timing behavior. Further support for this interpretation of our findings is provided by the results of the experiment by Collijn (1985). The CPBB rats used in her study show a low incidence of spike-wave complexes when 5 months old. It is very likely that the incidence of spike-wave complexes increases with age. As a result of the assumed 'epileptogenic' effects of the supplemented choline, observable behavioral effects might have occurred in the 21.5-month-old animals only, and not in the 10.5-month-old rats.

The explanations of the observed effects of chronic dietary choline enrichment on timing behavior in terms of an 'increased memory storage speed' (Church, 1984; Meck, & Church, 1983, 1987a, 1987b; Meck, 1983) or in terms of possible 'epileptogenic' effects are speculative and must remain tentative. The decision as to which hypothesis is correct (if any of the two) must be based on further experimental evidence. The evidence available so far, however, might be in favor of the 'epilepsy' interpretation, because it can account for the results obtained with three different inbred strains of rats, while the 'memory speed' interpretation can only account for the results obtained with the WAG strain.

Whatever explanation is the correct one, our results support the expectation that chronic dietary choline enrichment can affect behavior. However, the hypothesis that the chronic supplementation of dietary choline improves temporal discrimination performance was not confirmed.

CHAPTER 5

AGE AND CHRONIC DIETARY CHOLINE ENRICHMENT: EFFECTS ON SPATIAL DISCRIMINATION IN A HOLEBOARD TASK

SUMMARY

In a previous study we observed that chronic dietary choline enrichment improved the spatial working and reference memory in a complex holeboard discrimination task and improved the retention of an inhibitory avoidance task of one- and two-year-old female CPBB rats. This suggests that the effect of this treatment might not be restricted to aged animals. In the first experiment of the present study we investigated whether the effects of the chronic dietary choline treatment would affect the spatial discrimination performance of about one-year-old rats of the Brown Norway (BN) and the WAG strains. The animals were put on the choline-enriched diet when they were weanlings. We hypothesized that BN rats would profit more from the treatment than WAG rats would because the BN strain is characterized by a low central cholinergic activity. The treatment did not affect holeboard discrimination. Thus, our earlier results with CPBB rats may not be generalized to other strains of rats. In the second experiment the relationship between age and the decline in spatial memory performance in BN rats was assessed. Animals of five ages (4, 13, 19, 25, and 30 months) were tested. A clear age-related decline in spatial working and reference memory performance was found, which was most profound between 19 and 25 months of age. The speed of visiting the holes and the development of a preferred pattern of hole visits did not influence spatial discrimination performance. In a third experiment 21.5-month-old BN and WAG rats were put on a choline supplemented diet for 4 months. They were then tested in the holeboard. No effects of the treatment were found, indicating that chronic dietary choline supplementation does not prevent the age-associated decline in spatial discrimination performance.

INTRODUCTION

Age-associated decreases of learning ability and memory performance have been found in man and in a variety of other species, including rats and mice. It has been hypothesized that one of the major causes of the decrease of cognitive functioning that occurs with aging is a reduction in central cholinergic activity (Perry, 1980). In its most dramatic form the relationship between central cholinergic dysfunction and severe loss of cognitive capacities can be found in elderly people suffering from Alzheimers' disease (Coyle, Price, & DeLong, 1983).

Although other neurotransmitter systems may also be involved in age-related cognitive impairments, it has been the 'cholinergic hypothesis of geriatric memory' (Bartus, Dean, Beer, & Lippa, 1982) that has received considerable attention and has triggered a great number of studies. As this hypothesis emphasizes the role of cholinergic dysfunction, there have been attempts to pharmacologically modulate cholinergic activity. One approach is to try to improve memory by enhancing the availability of the acetylcholine precursor choline or lecithin. It has been reported that an increased availability of the precursor increases cholinergic activity

(Haubrich, Wang, Clody, & Wedekind 1975) Enhanced cholinergic activity in its turn should be accompanied by a reduction of cognitive impairment (Bartus, Dean, & Beer, 1984)

A few studies have demonstrated an amelioration of age-associated retention deficits in a (one-way) inhibitory (passive) avoidance paradigm after a 'precursor therapy', which consisted of chronic dietary choline enrichment in mice (Bartus Dean, Goas, & Lippa 1980, Davis, & Trombetta, 1984 Mervis, Horrocks, Wallace, & Naber, 1984)

Most studies on 'cholinergic precursor therapy', however, have yielded disappointing results (Bartus, Dean, Pontecorvo, & Flicker, 1985) Many different factors may determine whether or not precursor therapy is effective Therefore, the psychological and behavioral consequences of supplying extra dietary choline should be understood in more detail The variables taken as measures of cognitive functions should reflect psychological processes and should not be confounded by other factors

The inhibitory avoidance paradigm was used in the majority of dietary choline supplemen tation experiments The index of memory performance step through latency, has been criticized as it might be confounded by other non-cognitive factors such as locomotor activity, shock sensitivity, or light aversion (O Keefe, & Nadel, 1978, Masterson & Crawford 1982) Appetitively motivated complex spatial discrimination tasks, however, offer the advantage to distinguish between cognitive (i e different aspects of spatial memory) and non-cognitive (e g speed of visiting places where a reinforcement might be found) factors that might affect the acquisition and retention of a discrimination

In their natural environment it is of utmost importance for small rodents to acquire precise knowledge of their environment in order to obtain food and water and to escape from predators Rats are capable of finding their way through very complex mazes, as has been known since the early beginnings of experimental animal psychology (cf Munn, 1950)

Besides the 'sequential choice' (alley) mazes, which consist of a fixed starting point, and one correct route (with many blind alleys to avoid) to a fixed goal position, 'free choice' spatial discrimination tasks (Crannell, 1942, Lachman, & Brown, 1957) have been developed Examples are the 'radial maze', which has been used during the last decade, in particular by D.S Olton and co-workers (Olton & Samuelson, 1976; Olton, 1977, Olton, Collison, & Werz, 1977), and the holeboard (Oades, & Isaacson, 1978)

In this type of task food can be found in different places, and the rat is free to visit these places in whatever order it wishes Once a rat has visited a place and consumed the food, its revisits to the same location will remain unreinforced, as the place will not be rebaited within a trial Thus the most efficient behavior is to visit every baited location once, and to remember the list of places already visited in order to avoid revisits This list of visits is held in the 'working memory' (Olton & Samuelson, 1976)

Contrary to the working memory, which holds information that is relevant only within a specific trial, the 'reference memory' (Olton, Becker, & Handelmann, 1979) holds trial independent information, for example about the localization of the food This information is of special relevance when only a fixed subset of places is baited with food Thus, if food can be found only in one subset of all the potential places which the foraging rat can visit, then two memory components can be distinguished working and reference memory

It is unclear whether the two memory components are associated with different neuronal substrates. Working and reference memory have been conceived as operational definitions of presumably different memory processes throughout this study.

Two main questions were addressed in the experiments of the present study Firstly, it was questioned whether chronic dietary choline enrichment affects spatial discrimination performance as measured by the working and reference memory performance in the holeboard In addition, the speed of visiting the different locations where food could be found was analyzed Spatial memory performance should be independent of this measure Secondly, it was asked how age influences spatial discrimination performance in the holeboard Three experiments were performed to study these questions

In the first experiment it was investigated whether chronic dietary choline enrichment, starting after weaning, ameliorates the spatial discrimination of about one-year-old rats from the inbred pigmented BN and albino WAG strains in the holeboard Based on previous findings (van der Staay, Raaijmakers, & Collijn, 1986) it was expected that the dietary choline enrichment would already affect spatial discrimination performance at this relatively young age Rats of the BN strain are characterized by a low central cholinergic activity compared with rats of the WAG strain Therefore, it was hypothesized that BN rats would profit most from the choline enrichment

The use of two different genotypes in the present study was also indicated because we had found in previous studies (reported in Chapters 2 and 4) that the effects of chronic choline enrichment were strain-dependent during the habituation phase of an inhibitory avoidance task (Chapter 2), and in a temporal discrimination experiment (Chapter 4) Moreover the effects of cholinergic drugs on spatial discrimination in mice were found to be strain-dependent (e g Ammassari-Teule & Caprioli, 1985, Upchurch & Wehner, 1987)

In the second experiment of the present study the age at which BN rats show age-related impairments of spatial discrimination in the holeboard was assessed In addition, the relationship between alternative operational definitions of working and reference memory was evaluated We specifically addressed the question of whether working memory and reference memory represent different aspects of spatial memory The speed of visiting the holes and the relationship with the measures of spatial memory was studied It is conceivable that the speed of visiting the holes in the holeboard might interfere with response accuracy (as measured by working and reference memory) The last question to be addressed in this experiment was whether there is an age difference with respect to the development of a response strategy for visiting the holes that contain food Goodrick (1975) reported that behavioral rigidity can serve as a mechanism of problem-solving in old rats Adult and senescent rats might adopt different 'strategies' to solve spatial discrimination problems Therefore, we analyzed the similarity of the sequences of rewarded food-hole visits over subsequent trials

In the third experiment aging rats were treated with dietary choline in order to assess whether the treatment could ameliorate the age-related impairments of spatial discrimination performance It was expected that the choline-treated rats of both strains would improve their spatial discrimination performance For the rest, the aims of this experiment were the same as those of the first experiment

Old rodents show impaired performance in 'sequential choice' mazes such as the Stone 14-unit maze (Goodrick, 1968, 1975, Michel & Klein, 1978) 'Free choice'-type mazes, such as the circular platform and the radial maze (Barnes, 1979, Barnes, Nadel, & Honig, 1980, Wallace, Krauter, & Campbell, 1980, Davis, Idowu, & Gibson, 1983, van Gool, Mirmiran, & van Haaren, 1985), the Morris water maze (Gage, Dunnett, & Bjorklund, 1984, Rapp, Rosenberg, & Gallagher, 1987, but Lindner, & Schallert, 1988) and the holeboard (van der Staay, Raaijmakers, & Collijn,

1986; van der Staay, Raaijmakers, Sakkee, & van Bezooijen, 1988) are also sensitive to age-related impairments.

We chose to use the holeboard as a representative of the 'free choice' mazes because this type of tests has been found to be sensitive to age-related impairments, and because discrimination performance can be influenced by cholinergic drugs

Performance in spatial discrimination tasks has been reported to be affected by pharmacological treatment with cholinergic drugs. Davis, Idowu, and Gibson (1983) reported that treatment with 3,4-diaminopyridine -a drug that stimulates acetylcholine release- attenuated the age-related impairments in the working memory performance of 24-month-old Fischer 344 rats However, muscarinic cholinergic antagonists such as atropine and scopolamine have been found not only to impair the acquisition but also the performance of an already acquired spatial discrimination in radial mazes (Ammassari-Teule, & Caprioli, 1985, Eckerman, Gordon, Edwards, MacPhail, & Gage, 1980, Godding, Rush, & Beatty, 1982, Levy, Kluge, & Elsmore, 1983; Okaichi, & Jarrard, 1982, Stevens, 1981, Watts, Stevens, & Robinson, 1981, Wirsching, Beninger, Jhamandas, Boegman, & El-Defrawy, 1984)

Cholinergic antagonists also have disruptive effects on spatial discrimination in other spatial discrimination tasks such as the shock motivated 14-unit T-maze (Spangler, Rigby, & Ingram, 1986), and the Morris water-maze (Burešová, Bolhuis, & Bureš, 1986, Hagan, Tweedie, & Morris, 1986) In these tasks as well as in the radial maze, however, the precise nature and the specificity of cholinergic involvement in spatial behavior remains unclear (Godding, Rush, & Beatty, 1982, Hagan, Tweedie, & Morris, 1986, Okaichi, & Jarrard, 1982, Wenk, Sweeney, Hughey, Carson, & Olton, 1986) Although some authors report that working memory is selectively affected by cholinergic antagonists (Wirsching, Beninger, Jhamandas, Boegman, & El-Defrawy, 1984, Levy, Kluge, & Elsmore, 1983) while reference memory remains unaffected, others have not found evidence for specificity of the disruption of one of the memory components (Okaichi, & Jarrard, 1982) The apparent selective effects of cholinergic treatments on either the working or reference memory component in complex spatial discrimination tasks again emphasizes the importance to distinguish between these different memory components and to use a paradigm that can measure both components simultaneously

As early as 1929, Stone concluded that it is a prerequisite in experimental aging research that measures of cognitive performance are not confounded by factors such as speed of responding (cited by Goodrick, 1980). Analyses of holeboard performance are entirely based on error scores. Performance does not depend on particular locomotor abilities which might be impaired in senescent rats (e.g Gage, Dunnett, & Bjorklund, 1984), as long as rats are able to move around in the holeboard apparatus. Thus, this test seems to be especially suited to compare the performance of animals of all ages

Material and General Methods

Apparatus: Two identical holeboards (70*70*45 cm) were constructed according to the descriptions given by Oades (1981) and used in the present study All walls were made of transparent PVC, the floor consisted of grey PVC. There were 16 holes (diameter of 3.5 cm) in the floor. The distance between the holes was 10 cm. The bottom of each hole (see Fig. 5.1) consisted of a flattened cone of perforated aluminum, turned upside down A cup filled with about twenty 45 mg food pellets (Campden Instruments) was placed under each aluminum bottom. The rat could not reach the pellets in these cups. They masked potential odor cues which

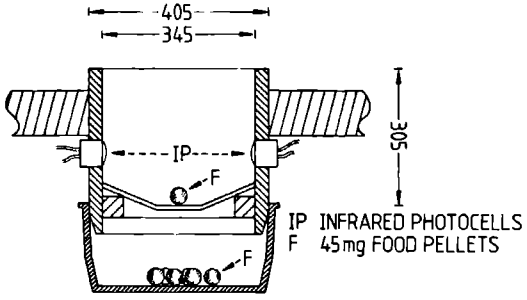


FIGURE 5.1: Cross-section of a hole in the holeboard apparatus. The measurements are in cm.

emanated from the reward in the baited holes. Thus, rats were unable to discriminate between baited and unbaited holes by olfactory cues (Willner, Wise, & Ellis, 1986). The holeboards were situated in two identical rooms which were equipped with a radial maze, two tables (one with the control equipment and the micro computer), and two chairs. Posters were hanging on the walls. Both rooms were dimly illuminated by two red fluorescent tubes.

General method: After the rats had gradually been deprived of food to achieve 85% of their free-feeding weights, they were habituated to the holeboard during 10-min sessions on five consecutive days. During habituation all holes were baited with one 45-mg food pellet. Some pellets were scattered randomly on the floor of the holeboard on the first two days.

The rats were then trained to collect pellets from a fixed set of four holes. A rat was placed in a clear plexiglass start-box which could be connected with the holeboard in the middle of one side wall. A trial was initiated by raising the guillotine door between start-box and the holeboard; it was terminated when the rat had found all the food pellets or when ten minutes had elapsed, whichever event occurred first. Hole visits were registered manually using a keyboard with 16 keys (representing the 16 holes), which was connected to an Apple//e microcomputer. A hole visit was scored when the nose of a rat turned to the edge of a hole, moved over it or was placed in it (Oades, 1981). In addition, infrared photocells detected automatically whether a rat poked its nose into a hole.

Three measures were analyzed statistically: working memory, reference memory, and mean inter-visit interval.

Working memory was defined by the ratio: (number of food-rewarded visits)/(number of visits and revisits to the baited set of holes). Thus, this measure represents the percentage of all visits to the baited set of holes that had been reinforced with food. (van der Staay, Raaijmakers, & Collijn, 1986; van der Staay, Raaijmakers, Sakkee, & van Bezooijen, 1988).

Reference memory was defined by the ratio: (Number of visits and revisits to the baited set of holes)/(number of visits and revisits to all holes). This measure expresses the number of visits to the baited set of holes as a percentage of the total number of visits to all the holes. As Olton and co-workers (Olton, Becker, Handelmann, 1979; Olton, & Papas, 1979) point out, both measures of spatial memory may be considered to be independent of each other because visits to the unbaited holes are not considered in the working memory measure (van der Staay, Raaijmakers, & Collijn, 1986; van der Staay, Raaijmakers, Sakkee, & van Bezooijen, 1988).

The mean inter-visit interval was determined by dividing the time elapsed between the first and last visit in a trial by (number of visits - 1). This variable provides a measure for the speed of visiting the holes.

EXPERIMENT 5I EFFECTS OF CHOLINE ON THE SPATIAL DISCRIMINATION OF MIDDLE-AGED RATS

The aim of this experiment was to evaluate the effects of chronic dietary choline enrichment on the acquisition and retention of a spatial discrimination, and on the acquisition of a second problem in the holeboard The rats were tested at the relatively young age of 13 months because of our findings in a previous study (van der Staay, Raaijmakers, & Collijn, 1986) in which one- and two-year-old chronically choline-treated female CPBB were tested in the hole-board The performance of the older animals was clearly inferior to that of the younger ones on both acquisition and retention of the working and reference memory components It had been hypothesized that choline treatment would only improve the age-related decrease in performance of the two year old animals Unexpectedly however, the choline-treated animals of both ages showed improved reference memory performance when compared with the untreated controls

These results suggest that choline did not specifically ameliorate the age-related memory impairments, as the treatment affected rats of both ages We determined the generality of the results obtained with the CPBB rats by using about one-year-old rats of the BN and WAG strains We hypothesized that choline enrichment would improve the acquisition and retention of the first problem It was expected that choline treatment would impair the acquisition of the second problem, due to the stronger conflicting memory of the first problem As BN rats are characterized by a lower central cholinergic activity than WAG rats (compare Chapter 7), it was expected that the dietary choline supplementation would affect the spatial discrimination performance of BN rats more than that of WAG rats

MATERIAL AND METHODS

Animals A total of sixty male rats were used (see Appendix C, third experimental protocol) Ten litters with three littermates each of both the pigmented BN/BiRij and the albino WAG/Rij strains were supplied by the TNO Institute for Experimental Gerontology, Rijswijk the Netherlands, at the age of five weeks They were housed in pairs in standard Makrolon™ cages and habituated to a reversed day/night cycle (light being on from 20·00 to 8·00)

Methods At the age of seven weeks, two subgroups of five litters each from both strains were formed by random assignment. Two of the three littermates from the first subgroup, and one littermate per litter from the second subgroup were randomly assigned to the choline treatment condition The remaining animals were assigned to the untreated control condition The result of this operation was that the choline enriched group and the control group of both strains consisted of 15 subjects (see also Chapter 2, Table 2 1) Choline enrichment consisted of supplying 2 5 mg choline chloride ($C_5H_{14}ClNO$, Merck) per ml drinking water When the animals were 28 weeks old, they were housed individually in standard Makrolon™ cages The concentration of choline was doubled (5mg choline chloride per ml water) from the age of 33 weeks

After the retention of temporal discrimination (DRL 8", see Chapter 4) had been tested, the rats remained on the food deprivation schedule (85% of free-feeding weight) and were habituated to the holeboard apparatus The rats were 56 weeks old at the start of formal training They were trained on the first configuration of baited holes (problem A, see Fig 5 2) with massed trials on 12 consecutive days (4 trials/day, except on day 1, when 2 trials were given)

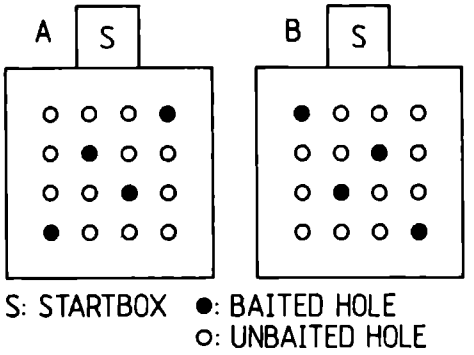


FIGURE 5.2: Map of the holeboard. The holes that were baited in problem A (left panel) and in problem B (right panel) are indicated by filled circles.

After completion of the forty-sixth trial the rats were returned to an ad libitum feeding regimen for two weeks. Then the body weights of all animals were again gradually reduced to 85% of their free-feeding values within one week. After a retention interval of three weeks rats were tested on problem A (10 trials/day on two consecutive days). The rats were then trained to collect their food from a different set of holes (problem B, see Fig. 5.2) with massed trials (10 trials/day on four consecutive days).

All testing was done between 13:00 and 16:00. Tests were performed simultaneously in two rooms by two experienced experimenters. Each experimenter trained half of the animals.

RESULTS

Mean block scores of ten trials each (exception: first block of the acquisition phase of problem A with six trials only) were calculated for working and reference memory performance, and for mean inter-visit interval. Changes in the course of training were evaluated separately for the acquisition and retention phase of problem A, and the acquisition phase of problem B by a two-factorial analysis of variance (ANOVA) (strain by treatment) on the the scores averaged over all the trialblocks (general mean) and orthogonal trend coefficients over successive trialblocks. The general mean evaluated whether there is a difference in the overall level of

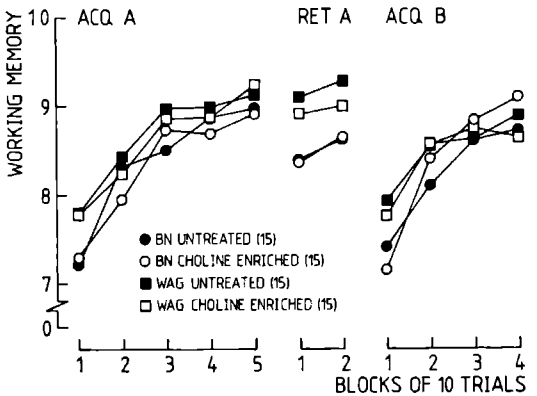


FIGURE 5.3: Working memory performance (number of food-rewarded visits)/(number of visits and revisits to the baited set of holes) of untreated and chronically choline-enriched BN and WAG rats. The number of animals per strain by treatment group is indicated between parentheses. Note that trialblock 1 of the acquisition of problem A represents the mean of 6 trials only. Abbreviations: ACQ. A: Acquisition of problem A; RET.A: Retention of problem A; ACQ. B: Acquisition of problem B.

performance. Orthogonal trend coefficients are tools to describe the learning curves and to assess whether the shapes of these curves are different between groups. These analyses were supplemented by ANOVAs on individual trialblocks. In case a particular measure showed a parallel increase or decrease over sessions between age groups, the result of the analyses on general means are reported. If measures diverged or converged between age groups in the course of training, then ANOVAs on individual block means are discussed to indicate in which phase of training differences between age groups appeared or disappeared.

The effect of the retention interval on the three measures was evaluated by a two-factorial ANOVA (strain by treatment) on the difference scores between the last block of the acquisition of problem A and the first trialblock of the retention of this problem. In the same way, the effects of switching to a new problem were evaluated by an ANOVA on the difference scores between the last trialblock of the retention phase on problem A and the first trialblock of the acquisition of problem B.

Working memory (see Fig. 5.3): Analysis of the general mean, i.e. mean performance over all trialblocks of problem A, revealed that the WAG rats performed better than the BN rats ($F_{1,56}$ = 11.51, $p < 0.01$). The better performance shown by the WAG rats was confirmed for the first block only by the ANOVAs on the individual trialblocks ($F_{1,56}$ = 8.89, $p < 0.01$). The improvement of working memory performance in the course of acquisition was characterized by general linear ($F_{1,56}$ = 296.22, $p < 0.01$) and quadratic ($F_{1,56}$ = 25.16, $p < 0.01$) trends. Eighty-nine percent of the variation over successive trialblocks may be predicted from a linear regression equation, an additional 10% of the variation may be explained by the quadratic trend component. This means that the learning curve increased predominantly in a linear fashion. The quadratic trend component indicated that the rate of improvement of the performance slowed down somewhat as training progressed. The strains did not differ on the trend components (linear: $F_{1,56}$ = 2.97, ns; quadratic: $F_{1,56}$ = 1.72, ns). Chronic dietary choline enrichment did not influence working memory.

The WAG rats were less affected by the retention interval than the BN rats ($F_{1,56}$ = 4.65, $p < 0.05$). The treatment did not affect retention performance ($Fs_{1,56} < 1.0$, ns).

During the retention phase the working memory performance improved (general linear trend: $F_{1,56}$ = 5.41, $p < 0.05$), and the WAG rats continued to perform better than the BN rats (first trialblock: $F_{1,56}$ = 19.39, $p < 0.01$; second trialblock: $F_{1,56}$ = 11.44, $p < 0.01$, for strain differences). Again, chronic dietary choline enrichment did not affect performance.

Presentation of a new problem (B) did not differentially disrupt working memory performance (all $Fs_{1,56} < 1.0$, ns).

The BN rats performed less well than the WAG rats in the first trialblock of problem B, ($F_{1,56}$ = 13.05, $p < 0.01$), as had been the case during the acquisition and retention of problem A. The improvement of working memory performance was characterized by general linear ($F_{1,56}$ = 187.16, $p < 0.01$) and quadratic ($F_{1,56}$ = 36.20, $p < 0.01$) trend components. The linear trend component covered 86.5% of variation over successive trialblocks, the quadratic trend component accounted for additional 12.5%. The BN rats improved their working memory performance faster than the WAG rats (strain differences for linear trends: $F_{1,56}$ = 16.76, $p < 0.01$). Both strains performed equally well in the last two trialblocks of acquisition of problem B (third trialblock: $F_{1,56} < 1.0$, ns; fourth trialblock: $F_{1,56}$ = 1.79, $p > 0.10$).

Reference memory (see Fig. 5.4): In the first trialblock both strains performed at about chance level and did not differ ($F_{1,56}$ = 3.29, ns). Reference memory performance during

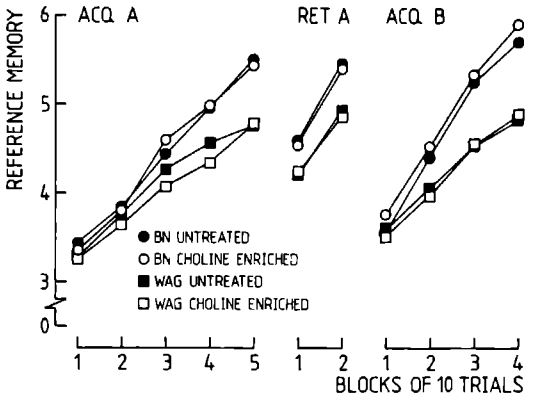


FIGURE 5.4: Reference memory performance (number of visits and revisits to the baited set of holes)/(number of visits and revisits to all holes) of untreated and chronically choline-enriched BN and WAG rats. For further details see Fig. 5.3.

acquisition of problem A was characterized by a general linear trend ($F_{1,56}$ = 714.60, $p < 0.01$). This trend component explained 99% of the variation over successive trialblocks. The strains differed on the linear trend ($F_{1,56}$ = 20.65, $p < 0.01$). The reference memory performance of the BN rats improved faster than that of the WAG rats.

Although both strains performed worse after the retention interval, the decrease in performance was not different for the BN and WAG rats ($F_{1,56}$ = 2.75, ns).

Reference memory performance improved during the retention phase of problem A, as indicated by a general linear trend ($F_{1,56}$ = 196.55, $p < 0.01$). The BN rats again improved faster than the WAG rats ($F_{1,56}$ = 9.08, $p < 0.01$).

Presentation of the new problem (B) disrupted reference memory performance of both strains, but the BN rats were more affected than the WAG rats ($F_{1,56}$ = 9.52, $p < 0.01$).

The performance of both strains was near chance level in the first trialblock of the acquisition of problem B, and there was no difference between the strains ($F_{1,56}$ = 1.30, ns). Both

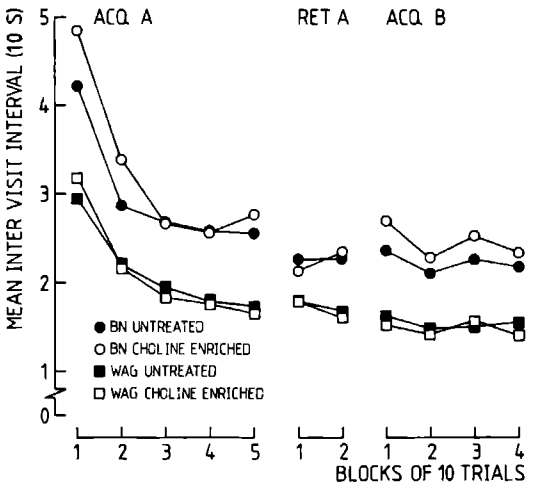


FIGURE 5.5: Mean inter-visit intervals of untreated and chronically choline-enriched BN and WAG rats. For further details see Fig. 5.3.

strains improved their reference memory performance in the course of training in problem B The improvement was characterized by a general linear trend ($F_{1,56} = 476.26$, $p < 0.01$) Ninety-nine percent of the variation over successive trials blocks may be predicted from a linear regression equation The strains differed on the linear trend component ($F_{1,56} = 30.51$ $p < 0.01$) The performance of the BN rats improved faster than that of the WAG rats Dietary choline enrichment did not affect reference memory performance in any phase of the experiment

Mean inter-visit interval (see Fig 5 5) The mean inter-visit interval became shorter during the acquisition of problem A. This decrease followed a general linear ($F_{1,56} = 79.95$, $p < 0.01$), quadratic ($F_{1,56} = 45.19$, $p < 0.01$), and cubic ($F_{1,56} = 8.99$, $p < 0.01$) trend Seventy-three percent of the variation in the decrease of the interval between visits over successive trialblocks was explained by the linear trend component, and 24 5% was explained by the quadratic component The cubic trend thus did not cover any variation of importance (2%) The strains differed on the quadratic trend component ($F_{1,56} = 4.24$, $p < 0.05$) The decrease of the inter-visit interval of the BN rats showed a stronger quadratic curvature than that of the WAG rats. The mean inter-visit interval of the BN strain, however, was longer on all trialblocks (all $Fs_{1,56} > 11.0$, with p-values < 0.01) indicating that the BN rats needed more time to visit the holes

The retention interval affected the mean inter-visit interval of the two strains differently ($F_{1,56} = 5.86$ $p < 0.05$) The BN rats chose faster after the interval than before, while the mean inter-visit intervals of the WAG rats seemed to be unaffected The strain difference on the speed of visiting the holes remained during retention of problem A (first trialblock $F_{1,56} = 10.35$, $p < 0.01$, second trialblock $F_{1,56} = 15.83$, $p < 0.01$)

Analysis of the difference scores between the last trialblock of retention of problem A and the first trialblock of acquisition of problem B revealed that the transition to the new problem (B) did not affect the inter-visit intervals of the strains in a different way ($F_{1,56} = 1.89$ ns) The mean inter-visit interval during acquisition of problem B was again longer for the BN than for the WAG rats (for strain differences on all trialblocks $Fs_{1,56} > 17.35$, all associated probabilities < 0.01) Trend analyses indicated that the speed of visiting the holes did not change during this phase of the experiment Chronic dietary choline enrichment did not affect the mean inter-visit intervals in any phase of the experiment

Discussion

Chronic dietary choline enrichment had no effect on behavior in the spatial discrimination task in the holeboard The rats of both strains and treatment conditions improved their spatial orientation in the course of training Even at the end of the acquisition phase on problem A, however the performance of all animals was far from a faultless level and had not yet stabilized at an asymptote, while the speed of visiting holes had reached an asymptotic level The failure to demonstrate improvement of spatial memory performance after chronic dietary choline enrichment therefore cannot be due to ceiling effects The present results contrast with earlier findings (van der Staay Raaijmakers, & Collijn, 1986) in which one- and two-year-old CPBB rats showed improved holeboard performance after dietary choline supplementation Thus, the effect of the treatment on relatively young animals cannot be generalized to other strains of rats

On the working memory measure, the WAG rats were better than the BN rats in all phases of the experiment (acquisition and retention of problem A, acquisition of problem B) The rate of improvement of the working memory performance was equal for both strains An exception

was the acquisition of problem B, where the BN rats improved faster than the WAG rats, and eventually reached the same level of working memory performance as the WAG rats.

On the reference memory measure, both strains performed almost at a chance level during the first block of acquisition of problems A and B. The BN rats, however, improved their reference memory performance faster than WAG rats. Thus, while the WAG rats showed a better working memory performance than the BN rats, the reverse holds true for the reference memory performance.

Following a retention interval of three weeks, the working memory performance of the WAG rats was unimpaired, while the BN rats showed a decreased performance. Reference memory performance was equally decreased for both strains after the retention interval. These findings suggest that forgetting in the WAG strain was restricted to the reference memory component. The BN rats showed a loss of accuracy on both memory measures.

As a result of switching from problem A to the acquisition of problem B, working memory performance decreased to an equal degree for both strains. The BN rats showed a greater loss of accuracy of discriminating between holes of the baited and unbaited set (reference memory) than the WAG rats. The reference memory performance of both strains dropped to near chance level. The stronger decline observed with the BN rats was thus a consequence of the fact that they had reached a higher level of reference memory performance before they were switched to the new problem.

The steeper learning curves of the BN rats during acquisition of problem B indicate, that the rats of this strain may be less sensitive to conflict of the memory of a previously acquired problem than the WAG rats. Taken together, our expectation that the BN rats would show poorer memory performance than the WAG rats was not supported. The results underline the necessity to distinguish between the two components of spatial memory. The fact that BN rats performed better than the WAG rats in the reference memory component, while the reverse was true for the working memory component provides experimental evidence that the memory measures might be considered to be independent (Olton, & Papas, 1979).

The mean inter-visit interval of the BN rats was longer throughout all phases of the experiment. Asymptotic levels were reached within approximately 40 trials. It can be hypothesized that a higher speed of visiting holes is a potential source of errors on both memory components. Our results, however, do not support this hypothesis, as the WAG rats performed better in the working memory measure and worse in the reference memory measure than the BN rats. It is not clear why the speed of visiting the holes should affect working and reference memory performance differently.

EXPERIMENT 5II: AGE-RELATED CHANGES OF SPATIAL DISCRIMINATION IN A HOLEBOARD TASK

Experiment 5I did not reveal any evidence for an effect of the chronic dietary choline enrichment on acquisition and retention in the holeboard task. It is possible that the rats were still too young (ca. 61 weeks by the end of the experiment) for an age-dependent decrease of memory to occur.

The aim of the present experiment was threefold. Firstly, it was determined at what age the decline in spatial discrimination performance in the holeboard was most prominent. Secondly, the relationship between alternative ways of operationally defining measures of

working and reference memory, and the relationship between these measures were investigated. Thirdly, two potential causes of the age-related differences in spatial discrimination performance were evaluated: the speed of visiting the holes, and the development of a preferred food-search pattern.

Because the development of a preferred food-hole-visiting sequence may facilitate improvement of food search behavior in the holeboard, the choice correspondence of reinforced visits from trial to trial was determined. Goodrick (1968, 1975) reported that aged rats make more repetitive errors in a complex maze. Based on this finding he characterized aged rats as being behaviorally rigid. In a holeboard study by van der Staay et al. (van der Staay, Raaijmakers, & Collijn, 1986) involving one- and two-year-old female CPBB rats, it was found that it was mainly the younger rats that developed a preferred pattern of visiting the holes. Oades and Isaacson (1978) found that normal rats acquired their individual strategies in finding food-containing holes. In the same study hippocampus lesioned rats were found to be impaired in the development of a search strategy and to make more erroneous visits. Similar impairments were also found in rats with lesions of the ventral tegmentum (Oades, 1982).

We wondered whether the differences in holeboard performance between the age groups could be paralleled by differences in increasing the choice correspondence of reinforced visits from trial to trial. This measure is of special importance since the development of a preferred sequence of visiting the baited holes might interfere with the interpretation of the rat's spatial discrimination performance in terms of working and reference memory.

Material and Methods

Animals: Male inbred BN/BiRij rats were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands (see Appendix C, fourth experimental protocol). They were 3, 12, 18, 24, or 29 months old. All groups consisted of 10 animals, except the oldest group, which consisted of 5 individuals.

The rats were housed individually in standard Makrolon™ cages. Within 11 days the rats were adapted to a reversed day/night cycle by a 13-hours dark/12-hours light scheme. The rats were then returned to a 24-hour circadian cycle (lights on from 8:00 to 20:00) and remained on this schedule throughout the testing.

Methods: About three weeks after their arrival at our laboratory, the body weights of the rats were gradually reduced to 85% of their free-feeding values within one week. The rats were then habituated to the holeboard apparatus on five consecutive days. A total of 80 acquisition trials in problem A was given (day 1: 2 trials; days 2-10: 4 trials; days 11-17: 6 trials/day).

Differential deprivation was applied in the course of the 17 days of training to try to reduce the motivational differences between age groups (Goodrick, 1968, 1980; Ingram, London, & Goodrick, 1981). The 4-month-old rats were kept at 85% of their free-feeding weights (corrected for normal growth, as measured in undeprived peers). The 13-month-old rats were kept at 85% of their free-feeding weights without any correction. The three older groups were gradually deprived further until they had reached 82.5% (19-month-old rats), 80% (24-month-old rats), or 77.5 (30-month-old rats) of their free-feeding weights on the last day of acquisition. All testing was done between 9:00 and 12:30. The tests were carried out by two experienced experimenters simultaneously in two identical rooms. Each experimenter trained half of the animals.

RESULTS

Block means of 10 trials each were calculated for the working and reference memory, the mean inter-visit intervals and the choice correspondence of reinforced visits. Changes in the course of training were analyzed by a one-factorial (age) ANOVA (SAS GLM-procedure for unequal cell sizes; Freund, & Littell, 1985) on the general mean, and the orthogonal trend components calculated over all trialblocks.

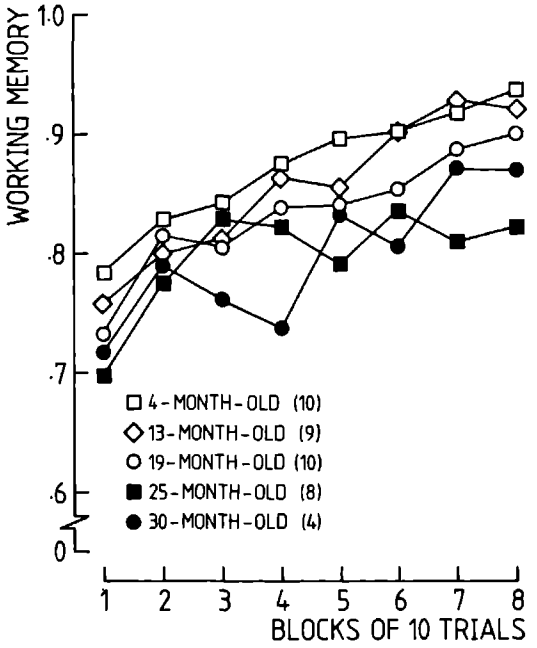


FIGURE 5.6: Working memory performance (number of food-rewarded visits)/(number of visits and revisits to the baited set of holes) of BN rats of five different ages. The rats acquired problem A. The number of animals per strain by treatment group is indicated between parentheses.

Working memory (see Fig. 5.6): Analysis of the general means revealed that working memory performance averaged over all blocks of ten trials, was consistently impaired in the older age groups ($F_{4,36} = 11.04$, $p < 0.01$). The rats of all ages, however, improved in the course of training. The improvement was characterized by general linear ($F_{1,36} = 107.70$, $p < 0.01$) and quadratic ($F_{1,36} = 5.14$, $p < 0.05$) trends. The linear trend covered 93% of the total variation over successive trialblocks. The age groups did not differ on the trend components (linear trend: $F_{4,36} = 1.25$, ns; quadratic trend: $F_{4,36} = 1.46$, ns). The results indicate that the improvement of working memory performance in the course of training was equal in all age groups, but that the overall level of performance showed an age-related decrease. Duncan post-hoc analysis on the trialblocks revealed that the 25- and 30-month-old rats performed less well than the younger groups.

Reference memory (see Fig. 5.7): The age groups did not differ in their reference memory performance during the first ($F_{4,36} = 1.50$, ns) and second ($F_{4,36} = 2.43$, ns) trialblock. The development of reference memory performance over successive trialblocks was characterized by general linear trends ($F_{1,36} = 606.73$, $p < 0.01$). Ninety-nine percent of the variation of

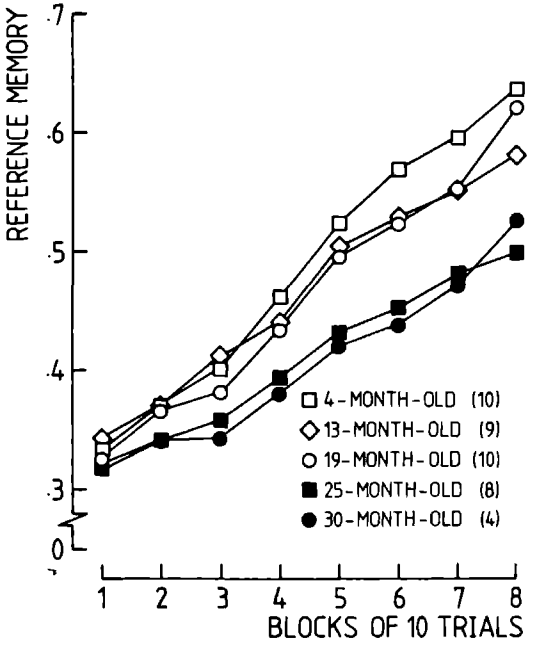


FIGURE 5.7: Reference memory performance (number of visits and revisits to the baited set of holes)/(number of visits and revisits to all holes) of BN rats of five different ages. The rats acquired problem A. The number of animals per strain by treatment group is indicated between brackets.

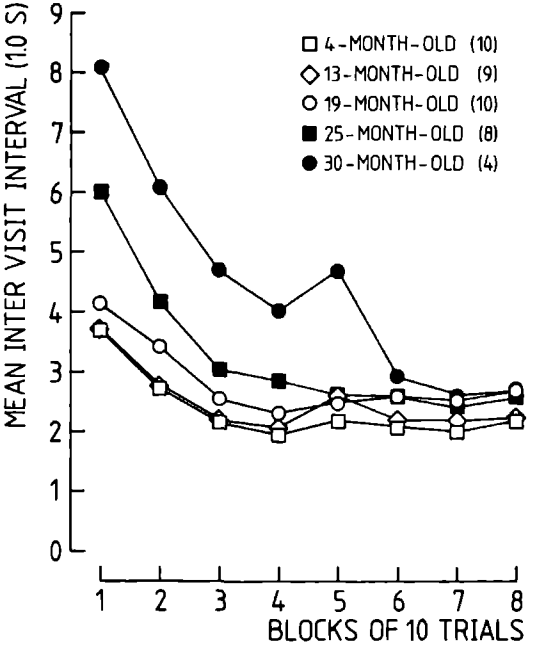


FIGURE 5.8: Mean inter-visit intervals of BN rats of five different ages. The rats acquired problem A. The number of animals per strain by treatment group is indicated between brackets.

reference memory performance in the course of training may be predicted from a linear regression equation. The ages differed on this trend component ($F_{4,36} = 6.19$, $p < 0.01$). Duncan post-hoc analysis revealed that the linear trend components of the 25- and 30-month-old groups were lower than those of the younger groups. Thus, reference memory performance of the age groups diverged in the course of training.

Mean inter-visit interval (see Fig. 5.8): The inter-visit interval decreased over the successive blocks of ten trials, as indicated by general linear ($F_{1,36} = 48.50$, $p < 0.01$), quadratic ($F_{1,36} = 62.79$, $p < 0.01$), and cubic ($F_{1,36} = 35.21$, $p < 0.01$) trend components. Sixty-five percent of the variation over successive trials blocks may be predicted from a linear regression equation, an additional 25% was covered by the quadratic trend component. The cubic trend component explained only 5% of variation. The age groups differed on the linear trend component ($F_{4,36} = 4.11$, $p < 0.01$). Post-hoc Duncan analysis revealed that the 25- and 30-month-old rats decreased their inter-visit interval faster than the other age groups. There was no difference between the ages from the fifth trialblock on (block 5: $F_{4,36} = 2.33$; block 6: $F_{4,36} = 1.22$; block 7: $F_{4,36} = 1.32$, block 8: $F_{4,36} = 1.88$, all associated probabilities > 0.05). Thus the speed of visiting holes converged in the course of training, and all age groups stabilized at the same speed level.

Choice correspondence of reinforced visits (see Fig. 5.9): This measure reflects the comparison of the sequences of reinforced choices of two subsequent trials. The longest common sequence was taken as the measure of correspondence. This measure could range from 1 to 4. A score of one was given when sequences were completely different, and a four was scored whenever the sequences of all four reinforced choices were identical. This measure reflects the variability of the spatial pattern of obtaining rewards, but it neglects all erroneous choices (visits to the unbaited holes and revisits to holes of the baited set).

The age groups differed in their choice correspondence of reinforced visits when averaged over trialblocks ($F_{4,36} = 2.78$, $p < 0.05$). Post-hoc Duncan analysis confirmed a difference between the 4- and 25-month-old rats. The choice correspondence increased linearly ($F_{1,36} = 86.48$, $p < 0.01$) over trialblocks, but the age groups did not differ on their rate of increase

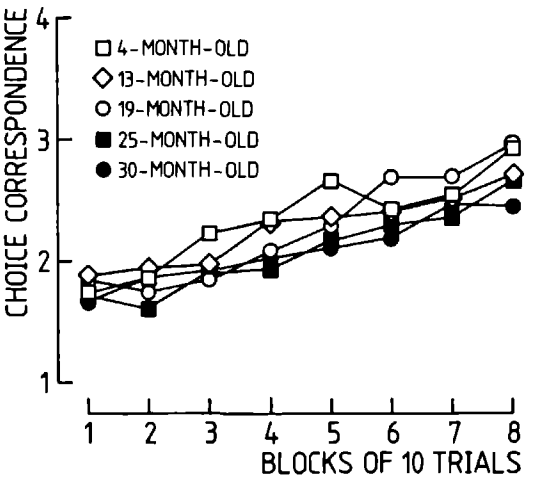


FIGURE 5.9: Choice correspondence of reinforced visits of BN rats of five different ages.

($F_{4,36} < 1.0$, ns). Ninety-seven percent of the variation in choice correspondence over successive trialblocks may be predicted from a linear regression equation. It should be pointed out that the increase over successive trialblocks was rather moderate, and that differences between age groups on the general level of choice correspondence were quite small (in fact, differences between age groups were not confirmed statistically when analyses were performed on individual trialblocks).

Correlation Analyses

Besides the operational definition of working and reference memory measures as ratios of different types of hole visits, the working and reference memory performance can both be expressed in terms of the number of specific errors (i.e. revisits to the baited set of holes for working memory; visits to the unbaited set of holes for reference memory) (Beatty, Bierley, & Boyd, 1984; Wirsching, Beninger, Jhamandas, Boegman, & El-Defrawy, 1984). It is questioned whether the alternative measures of working and reference memory are correlated. The correlations should be high and negative, as the ratio measures increase where the error measures decrease.

In Tables 5.1 and 5.2, the product-moment correlation coefficients between the alternative operational definitions are shown per block mean of 10 trials for each of the five age groups. As eight tests per age group were performed on the hypothesis that the correlations deviate from zero, a Bonferroni multistage multiple correlation procedure was used, which adjusts the alpha-levels of significance to a family-wise alpha level of 0.05 (Crosbie, 1986).

High correlations were found, indicating that the two alternative measures cover the same concept with respect to both working and reference memory. The poorest correlations were found in the first trialblock(s) between the two operational definitions of reference memory. In that phase of acquisition the rats performed more or less at a chance level.

A second correlation analysis addressed the question whether working and reference memory are independent measures; that is, whether they cover different aspects of spatial discrimination performance. The product-moment correlation coefficients between the ratio-

TABLE 5.1: Product-moment correlation coefficients between working memory errors (revisits to holes of the baited set) and a working memory measure defined as the ratio of: (reinforced visits to the baited set of holes)/(all visits to this set). Coefficients per trialblock for five different age groups are presented. A Bonferroni multistage multiple correlation procedure was used which adjusts the family-wise alpha levels of significance to 0.05. *: $p < 0.05$

Trial-block	*4 months (n = 10)*	*13 months (n = 9)*	*19 months (n = 10)*	*25 months (n = 8)*	*30 months (n = 4)*
1	-0.96 *	-0.91 *	-0.99 *	-0.98 *	-0.99
2	-0.92 *	-0.99 *	-0.87 *	-0.98 *	-0.99
3	-0.99 *	-1.00 *	-0.99 *	-0.99 *	-0.98
4	-0.99 *	-0.99 *	-0.99 *	-0.99 *	-0.97
5	-0.99 *	-0.95 *	-0.99 *	-0.92 *	-0.99
6	-0.98 *	-0.98 *	-0.88 *	-0.99 *	-0.26
7	-0.99 *	-0.99 *	-0.96 *	-0.97 *	-0.98
8	-0.95 *	-0.99 *	-0.98 *	-0.94 *	-0.99

TABLE 5.2: Product-moment correlation coefficients between reference memory errors (visits to holes of the unbaited set) and a reference memory measure defined as the ratio of: (visits to the baited set of holes)/(total number of hole visits). Coefficients per trialblock for five different age groups are presented. A Bonferroni multistage multiple correlation procedure was used which adjusts the family-wise alpha levels of significance to 0.05. *: $p < 0.05$

Trial-block	*4 months (n = 10)*	*13 months (n = 9)*	*19 months (n = 10)*	*25 months (n = 8)*	*30 months (n = 4)*
1	-0.57	-0.24	-0.74 *	-0.46	-0.74
2	-0.77 *	-0.69	-0.91 *	-0.88 *	-0.17
3	-0.70	-0.83 *	-0.66 *	-0.66	-0.97
4	-0.90 *	-0.86 *	-0.77 *	-0.74	-0.74
5	-0.95 *	-0.92 *	-0.93 *	-0.97 *	-0.58
6	-0.90 *	-0.95 *	-0.96 *	-0.92 *	-0.93
7	-0.95 *	-0.84 *	-0.90 *	-0.81 *	-0.98
8	-0.94 *	-0.77 *	-0.93 *	-0.90 *	-0.96

TABLE 5.3: Product-moment correlation coefficients between working memory (number of food rewarded visits)/(number of visits and revisits to the baited set of holes) and reference memory (number of visits to the baited set of holes)/(total number of hole visits). Coefficients per trialblock for five different age groups are presented, together with correlations calculated over the subjects of all age groups. *: $p < 0.05$; +: $p < 0.01$.

Trial-block	*4 months (n = 10)*	*13 months (n = 9)*	*19 months (n = 10)*	*25 months (n = 8)*	*30 months (n = 4)*	*all subj. (n = 41)*
1	-0.08	-0.61	0.18	-0.22	-0.88	-0.11
2	0.42	-0.23	-0.38	0.17	-0.58	0.05
3	-0.04	0.38	-0.02	-0.63	0.71	0.06
4	0.18	0.10	-0.16	0.08	0.42	0.36 *
5	0.43	0.26	0.43	0.54	0.79	0.56 +
6	0.26	-0.36	-0.68 *	-0.12	0.66	0.23
7	0.37	-0.02	-0.05	-0.02	0.78	0.43 +
8	0.51	0.03	-0.42	-0.08	0.81	0.44 +

measures of working and reference memory for blocks means of ten trials per age group are presented in table 5.3.

Only one of the correlation coefficients had an associated probability < 0.05, thus the measures may be considered as independent. Moreover, the signs of the correlation coefficients (21 times '+', 19 times '-'; binomial test: z = - 0.16, ns) seem to be evenly distributed over trialblocks. The two measures (working memory and reference memory) seem to become more related as training progresses only when the product-moment correlations are calculated over the animals of all age groups. These correlations, however, were at best moderate.

DISCUSSION

An age-related decrease in spatial discrimination performance in the holeboard task was found. Both the working and reference memories of older rats were impaired, corroborating

findings obtained with other 'free choice' type mazes (e g Barnes, 1979, Barnes, Nadel, & Honig, 1980, Gage, Dunnett, & Björklund 1984 Wallace, Krauter, & Campbell, 1980) The performance of the 25- and 30-month-old rats was decreased when compared with that of the younger age groups It may be concluded that there is a considerable decrease of accuracy between the ages of 19 and 25 months in BN rats A comparable age-related decrease was reported by Wolthuis and co-workers (Wolthuis, Knook, & Nickolson, 1976) for female WAG/Rij rats in a conditioned suppression task of drinking They found that these rats showed an acquisition deficit only after 18 months of age

As in experiment 5I, the speed of responding can be ruled out as a factor contributing to the differences between the working and reference memory performances While the 25- and 30-month old rats initially visited holes much slower than the younger ones, the age groups did no longer differ in the duration of the inter-visit intervals from the fifth trialblock on Moreover, the duration of the inter-visit intervals reached a plateau within fifty trials The difference in reference memory performance between the groups, on the other hand, continued to increase

A second potential source for the differences between the age groups could depend on whether the rats develop a food search strategy (Oades & Isaacson, 1978, Oades 1981, 1982) In the present experiment a slight reduction in the variability in visiting the baited holes (i e an increase in the choice correspondence of reinforced visits) was found for all age groups The differences between the age groups, however, were very small Therefore, it is very unlikely that the differences in choice correspondence are a major source for the age differences found in the memory measures[1]

A third potential source for the differences between the age groups in appetitively motivated tasks has been signalled by Goodrick and co-workers (Goodrick, 1968 Ingram, London, & Goodrick, 1981) When their body weights are reduced to the same percentage of their free-feeding values, older rats are less motivated than younger rats In order to reduce the possibility that differences in the motivational state are responsible for the differences observed between the performance of the different age groups, we applied a differential deprivation technique the older the rats were, the more their body weights were reduced in the course of training

To summarize, it is not likely that factors unrelated to 'cognitive' processes caused the differences in the working and reference memory performances between the age groups Nevertheless, it cannot completely be ruled out that other factors such as the speed of responding, development of a fixed food-search strategy, or the motivational state could have contributed to the differences found

This experiment was also intended to throw light upon two questions concerning the interdependencies of the measures of spatial memory.

Firstly, we examined the relationship between the alternative operational definitions of working and reference memory The measures of working memory, based on two alternative operational definitions, were strongly correlated The same was true for the two alternative measures of reference memory, except for the first trialblock(s) The finding that the alter-

[1] In order to cope effectively with this problem, we have recently developed a cone-field task (which is based on the holeboard) in which the development of a preferred food search pattern does not occur (van der Staay, F.J., Raaijmakers, W.G.M., Lammers, A.J.J.C., & Tonnaer, J.A.D.M. (1989) *Behavioural Brain Research, 32*, 151-161)

native working memory measures were correlated more consistently than the two reference memory measures may have been caused by the fact that rats show a strong unlearned tendency to avoid revisits to places just visited (FitzGerald, Isler, Rosenberg, Oettinger, & Bättig, 1985), even if they still don't know where to find the food. For reference memory, on the other hand, rats rely on experience with the testing apparatus, and they perform at a chance level during the first trials of the acquisition phase. The 'random' variation on reference memory performance in the first trialblocks thus may be responsible for the (few) low correlations found.

Alternatively, the few trials that were terminated before the rats had visited all the baited holes (because 10 minutes had elapsed), which occurred in the first phase of training only, might have induced an extra variation in the error measure. In that case, the error measure overestimates the reference memory performance. The ratio measure, on the other hand, is clearly less biased in incomplete trials. As soon as all trials were completed (which occurred in all but the very first trialblocks), correlations between the two alternative measures were very high.

To summarize, the two alternative operational definitions of working and reference memory cover the same traits since they produce highly correlated measures. In this case, one may well dispense with one of the alternative measures (Walsh, & Cummins, 1976). As they reduce the bias induced by incomplete trials, the ratio-measures were favored.

We also investigated whether the working and reference memory measures are uncorrelated, i.e. whether they cover different aspects of spatial memory, as was proposed by Olton and co-workers (Olton, Becker, & Handelmann, 1979). Within the age groups, the working and reference memory performances appeared to be uncorrelated. A weak relationship between the two memory components arises in the course of training if the correlations are calculated over all the animals. These correlations may be caused by the fact that the older rats performed less well than the younger animals on both measures. The underlying variable responsible for such gradually arising correlations may thus be aging. In conclusion: working and reference memory measures represent distinct aspects of spatial memory.

EXPERIMENT 5.III: EFFECTS OF CHOLINE ON THE SPATIAL DISCRIMINATION OF AGED RATS

Experiment 5.II revealed that both the working and reference memory were affected by age. The decrease in reference memory performance was most prominent between 19 and 25 months. The aim of the present experiment was to study whether in rats that were passing the age at which the clearest age-related decline in spatial memory performance occurred, chronic dietary choline enrichment would prevent the development of this age-associated impairment. We hypothesized that rats from both strains would profit from chronic dietary choline supplementation.

MATERIAL AND METHODS

Animals: A total of 48 male rats were used (see Appendix C, fifth experimental protocol). Twenty-four rats of the pigmented BN/BiRij and 24 rats of the albino WAG/Rij strains were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands, at the age of 20.5 months. They were housed in pairs in standard Makrolon™ cages and habitu-

ated to a reversed day/night cycle by a 13 hours light-off, 12 hours light-on schedule on eleven consecutive days. The rats were then returned to a 24-hour circadian cycle (light on from 20:00 to 8:00) and remained on this schedule throughout all testing.

Methods: The rats were housed individually in standard Makrolon™ cages when they were 21.5 months old. Twelve rats of each strain were randomly assigned to the choline enrichment condition (5 mg choline chloride/ml tap water); the other twelve rats served as untreated controls. Chronic dietary choline enrichment started when the subjects were 94 weeks old. When the animals had been on the choline-enriched diet for 3.5 months, their body weights were gradually reduced to 85% of their free-feeding values. The rats were then habituated to the holeboard apparatus. A total of 46 acquisition trials were given for problem A (first day: two trials; days 2-12: 4 trials/day). Two days after the end of training on problem A, the rats were trained to collect pellets from a different configuration of baited holes (problem B: four days with 10 trials/day). The rats were about 25.5 months old at the start of formal spatial discrimination learning.

Results

Illness and death considerably reduced the number of animals during the experiment. One BN of the choline-treated group died for unknown reasons. Seven choline-treated WAG rats and four WAG rats of the untreated group died. Autopsy revealed that all except one of these WAG rats had suffered from an extended tumor of the pituitary gland. Thus the final composition of groups was: 12 untreated BN rats, 11 choline-enriched BN rats, 8 untreated WAG rats, and 5 choline-enriched WAG rats (total n = 36).

Mean block scores of ten trials each were calculated for the working and reference memory performance, and for the mean inter-visit intervals (exception: first block of the acquisition of problem A with 6 trials only). Changes in the course of training were evaluated separately for the acquisition phase of problem A, and the acquisition phase of problem B by a strain (BN vs. WAG) by treatment (untreated vs. choline-enriched) analysis of variance (ANOVA) on the general mean and orthogonal trend coefficients over successive trialblocks. These analyses were supplemented by ANOVAs on individual trialblocks. The effect of switching to a new problem on the three measures was evaluated by a strain by treatment ANOVA on the difference scores between the last block of the acquisition of problem A and the first trialblock of the acquisition of problem B.

Working memory (see Fig. 5.10): During the acquisition of problem A the working memory performance of the WAG rats was better than that of the BN rats, averaged over all trialblocks (general mean: $F_{1,32} = 13.55$, $p < 0.01$). The improvement of performance was characterized by general linear ($F_{1,32} = 56.75$, $p < 0.01$) and quadratic trend components ($F_{1,32} = 5.13$, $p < 0.05$). Ninety-one percent of the variation of working memory performance over successive blocks may be predicted from a linear regression equation; the quadratic trend component covered an additional 8%. Neither strain differences nor effects of the chronic dietary choline enrichment were found for the trend components.

The presentation of a new problem (B) did not differentially disrupt working memory performance (all $Fs_{1,32}$ had associated probabilities > 0.10).

The improvement of working memory performance during the acquisition of problem B followed general linear ($F_{1,32} = 33.95$, $p < 0.01$) and quadratic trends ($F_{1,32} = 6.20$, $p < 0.05$). The linear trend component explained 80% of the variation over successive trialblocks, the

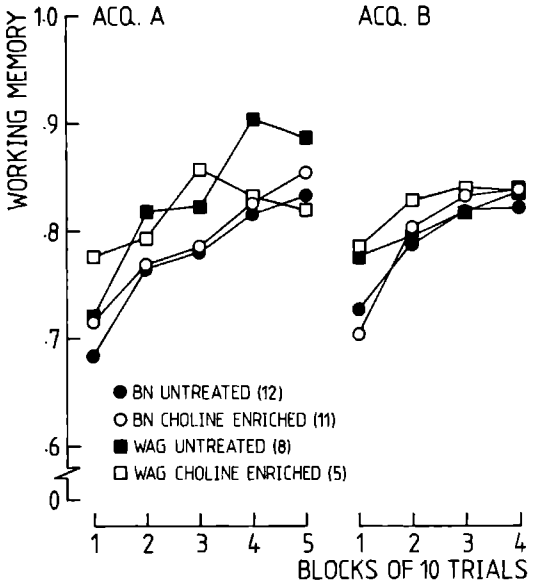


FIGURE 5.10: Working memory performance (number of food-rewarded visits)/(number of visits and revisits to the baited set of holes) of aged untreated and chronically choline-enriched BN and WAG rats. The number of animals per strain by treatment group is indicated between brackets. Note that trialblock 1 of the acquisition of problem A represents the mean of 6 trials only. Abbreviations: ACQ. A: Acquisition of problem A; ACQ. B: Acquisition of problem B.

quadratic trend component covered 14% of the systematic variation. Neither strain differences nor influences of the treatment were found on the trend components (though strains differed on the first trialblock: $F_{1,32} = 6.61$, $p < 0.05$).

Reference memory (see Fig. 5.11): Strain differences ($F_{1,32} = 6.44$, $p < 0.05$) and a strain by treatment interaction ($F_{1,32} = 4.18$, $p < 0.05$) were found for reference memory performance,

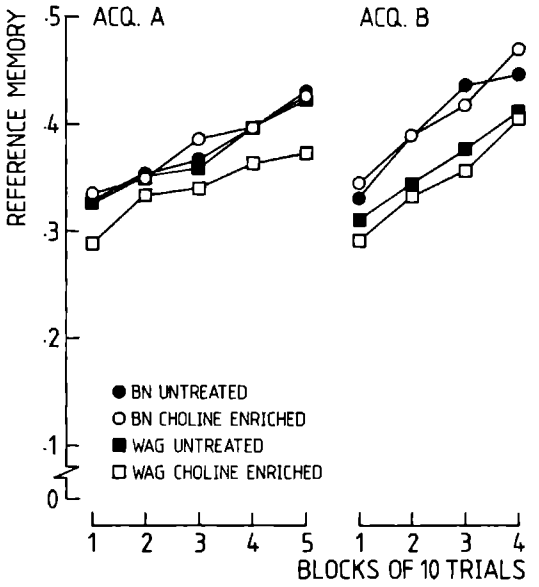


FIGURE 5.11: Reference memory performance (number of visits and revisits to the baited set of holes)/(number of visits and revisits to all holes) of aged untreated and chronically choline-enriched BN and WAG rats. For further details see Fig. 5.10.

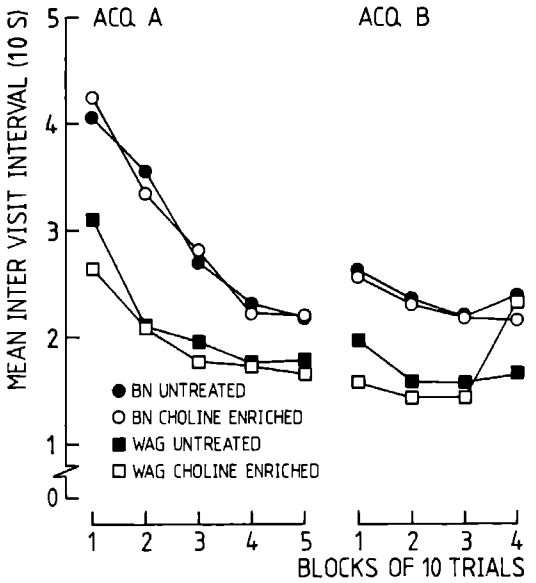


FIGURE 5 12 Mean inter-visit interval of aged untreated and chronically choline-enriched BN and WAG rats For further details see Fig 5 10

averaged over all trialblocks of the acquisition of problem A. The BN rats performed better in this measure than the WAG rats. The strain by treatment interaction may be caused by a difference between the treated and control WAG rats The choline-enriched rats of this strain seemed to perform less well than the untreated controls A post-hoc Duncan analysis, however, failed to confirm this The improvement of performance during training was characterized by a general linear trend ($F_{1\,32}$ = 98.53, $p < 0.01$) Ninety-nine percent of the variation over successive trialblocks may be predicted from a linear regression equation Neither strain differences nor treatment effects were found on the improvement of reference memory performance in the course of training There was a similar drop in performance after confrontation with the new problem (all $Fs_{1\,32} < 1.89$, ns) in all groups

During acquisition of problem B the BN rats performed consistently better than WAG rats ($Fs_{1\,32}$ for successive block means 11 24, 12 50, 8 00, and 4 27, resp., all associated probabilities < 0 05) The improvement of reference memory performance followed a general linear trend ($F_{1,32}$ = 91 74, $p < 0.01$) Virtually all the variation over successive trialblocks (99.5%) may be predicted from a linear regression equation Neither strain differences nor choline enrichment effects were found on the linear trend component

Mean inter-visit interval (see Fig 5 12)- During acquisition of problem A, the mean inter-visit interval was consistently shorter for the WAG than for the BN rats ($Fs_{1\,32}$ of successive block means 19 80, 5 82, 10.17, 9 57, and 16.39, resp., with associated probabilities < 0 05) The inter-visit intervals became progressively shorter in the course of training, as indicated by general linear ($F_{1,32}$ = 111 19, $p < 0.01$) and quadratic trend components ($F_{1,32}$ = 9 82, $p < 0.01$) The linear trend component accounted for about 89% of the variation over successive trialblocks, the quadratic trend component added about 10%. The strains differed on the linear trend ($F_{1,32}$ = 11 73, $p < 0.01$) The BN rats decreased their inter-visit intervals faster than the WAG rats did Chronic dietary choline enrichment did not affect this measure

The mean inter-visit interval was not differentially affected by the confrontation with the new problem (B), as neither strain nor treatment effects were found on the difference scores between the last trialblock of acquisition of problem A and the first trialblock of acquisition of problem B.

As noted during acquisition of problem A, the BN rats showed longer inter-choice intervals than the WAG rats during acquisition of problem B, except in the last trialblock ($Fs_{1,32}$ for block means are 9.22, 21.71, 28.01, and 1.26 resp.; all associated probabilities < 0.01, except for the last trialblock: $p > 0.05$). The change in the duration of intervals over trialblocks followed a general quadratic trend ($F_{1,32} = 7.94$, $p < 0.01$). Ninety-five percent of the variation over successive trialblocks may be predicted from a quadratic regression equation. The strains did not differ on this trend component, and it was unaffected by chronic dietary choline enrichment (all $Fs_{1,32} \leq 1.0$, ns).

Discussion

Although the performance of rats declines with age in complex spatial discrimination tasks, dietary choline enrichment could not prevent the development of age-related impairments. On the whole, experiments 5.I and 5.III yielded comparable results. The BN rats performed less well than the WAG rats on the working memory aspect of the holeboard task, but better on the reference memory aspect of this task. If the learning curves for the working and reference memory of the BN rats are compared with the curves of the corresponding age group from experiment 5.II (25-month-old rats), then a perfect match is found.

Differences between the results of experiments 5.I and 5.III may mainly be caused by the considerable loss of WAG rats (4 rats of the untreated control group, and 7 rats from the chronically choline-enriched group) from the original sample which contained 12 subjects each. The losses probably altered the sample characteristics and may have biased the results to some extent. It is conceivable, for example, that the fittest animals are also those with better memory performance or higher motivational states.

It can be excluded that the present results are due to extreme values, as the variances were homogeneous, and the loss of animals did not affect the within group variations. As non-orthogonal ANOVAs (i.e. factorial designs with different n's per cell) are not robust and can lead to biased hypothesis testing (Milligan, Wong, & Thompson, 1987), the treatment effects within strains were also evaluated by Student *t*-tests. These tests, like the ANOVAs, did not support the hypothesis that chronic dietary choline enrichment influenced behavior in the holeboard.

GENERAL DISCUSSION

Neither spatial working nor reference memory was affected by chronic dietary choline enrichment in the first and third experiment. These results contrast with those of van der Staay, Raaijmakers, and Collijn (1986) who used one- and two-year-old female CPBB rats. They reported that the reference memory performance in the holeboard improved in both age groups after chronic dietary choline enrichment. The results of experiment 5.III also contrast with those in which the age-related impairments of retention in one-way inhibitory avoidance tasks were improved by chronic dietary choline enrichment (Bartus, Dean, Goas, & Lippa, 1980; Davis, & Trombetta, 1984; Mervis, Horrocks, Wallace, & Naber, 1984), and with our DRL 16"

experiment (DRL differential reinforcement of low-rate responding, a rat receives a food reward if two successive bar-press responses are executed at least 16 s apart, see Chapter 4) in which the temporal discrimination performance of WAG rats was decreased by the treatment

Experiment 5.II clearly showed that spatial discrimination performance in the holeboard declined with age This observation is in good agreement with results obtained in other free choice mazes (e g Morris water maze Gage, Dunnett & Bjorklund, 1984, radial maze Barnes, Nadel, & Honig, 1980)

Correlation analyses of the data from this experiment confirmed that the working memory and the reference memory can be considered to be independent components of spatial memory (Olton, Becker, & Handelmann, 1979) Additional experimental evidence was obtained from experiments 5.I and 5.III to support this notion With respect to the working memory performance, it was found that the BN rats generally performed less well than the WAG rats The reverse was true for reference memory performance Such a reversed relationship would not have been found if the spatial memory components were related

The rats used in the experiments 5.I and 5.III had also been tested in a working memory version of a radial maze task (all arms baited) (see Appendix C, third and fifth experimental protocol) The results from both experiments completely corroborated those obtained in the holeboard for the working memory The WAG rats performed better than the BN rats in this task Chronic dietary choline enrichment, however had no effect on this memory component Because the holeboard task allows the simultaneous assessment of both working and reference memory and because the radial maze task did not add new information, we decided to only present the results from the holeboard experiments

To summarize We hypothesized firstly that chronic dietary choline enrichment ameliorates the spatial memory performance of adult BN rats, which are characterized by a low central cholinergic activity and a very poor retention in passive avoidance tasks, and secondly that this treatment ameliorates age-related impairments of spatial memory The two hypotheses were not confirmed Chronic dietary choline supplementation did not affect the behavior of rats in the holeboard task Age-related impairments on both working and reference memory performance, however, were found in this complex spatial discrimination task It is unlikely that non-mnemonic factors such as speed of responding or the development of a preferred food search pattern caused these age-associated differences

CHAPTER 6

EFFECTS OF CHRONIC DIETARY CHOLINE ENRICHMENT ON ADAPTATION TO A NOVEL ENVIRONMENT, CIRCADIAN DRINKING PATTERNS, AND 'EMOTIONAL REACTIVITY'

SUMMARY

We assessed whether chronic dietary choline enrichment affects basal aspects of behavior. In the first experiment the effects of the chronic dietary choline treatment on the speed of adaptation to a novel environment (an activity alley) and on the circadian drinking pattern of male BN and WAG rats were studied. The choline supplementation had no effect on the speed of adaptation. The BN rats adapted slower to the activity alley than the WAG rats. The circadian drinking pattern was not affected by dietary choline enrichment. While WAG rats consumed water almost exclusively during the dark phase of the 24-hour cycle, the BN rats distributed their drinking bouts more evenly over the entire day. The hypothesis that chronic dietary choline enrichment reduces 'emotional reactivity' in an open field was tested in the second experiment. The hypothesis was based on previous findings with choline-treated female CPBB rats in an open field, and on the observation that choline-supplemented BN and WAG rats spent more time and entered the light compartment of an inhibitory avoidance apparatus during adaptation sessions more frequently than untreated rats. Contrary to expectations, it was found that choline enrichment did not affect the open field behavior of the BN and WAG rats. The BN rats scored higher than WAG rats on several indices of 'emotional reactivity'. These results show that previous findings with CPBB rats cannot be generalized to other strains. No evidence was found that chronic dietary choline enrichment affects basal aspects of behavior.

INTRODUCTION

Besides an age-associated reduction of cholinergic activity (Strong, Hicks, Hsu, Bartus, & Enna, 1980; Sherman, Kuster, Dean, Bartus, & Friedman, 1981), other changes of the cholinergic system that occur with age are shifts in the circadian rhythms of acetylcholine, acetylcholinesterase, and choline acetyl transferase (ChAT) levels (Mohan & Radha, 1978). Thus there appears to be a change in the regulatory mechanisms that control acetylcholine levels in the central nervous system during the day and throughout the life-span of an animal.

Cholinergic dysfunctions may be a major cause of age-associated behavioral impairments (Bartus, Dean, Beer, & Lippa, 1982; Collerton, 1986). By enhancing the availability of the acetylcholine precursor choline or lecithin, several investigators have tried to increase cholinergic activity with the aim to ameliorate age-related impairment of cognitive functions (Bartus, Dean, & Beer, 1984). Some of these studies have been successful (e.g. Bartus, Dean, Goas, & Lippa, 1980; Davis, & Trombetta, 1984; Leathwood, Heck, & Mauron, 1982; Van der Staay, Raaijmakers, & Collijn, 1986).

It is conceivable that besides cognitive functions chronic manipulations of dietary choline might affect basal aspects of behavior e g the diurnal patterns of locomotor activity or consummatory behavior, or emotional reactivity It was the aim of the present study to assess the effects of chronic dietary choline enrichment on these basal aspects of behavior

Diurnal patterns

There is experimental evidence for a cholinergic involvement in the regulation of the circadian rhythm of activity in rats (see Meijer, 1989 pp 16-18) Mistlberger and Rusak (1986) for example found that intraventricular injections of carbachol, a cholinergic agonist, produced a phase shift in the activity of rats

Beninger and co-workers (Beninger, Tighe, & Jhamandas, 1984) assessed the effects of chronic dietary choline manipulations on the locomotor activity of Fischer 344 rats When the rats were 35 weeks old they were put on a choline-enriched, a choline-deficient, or control diet for about 23 weeks Then, the locomotor activity of the rats was tested in a running wheel for 21 5 hours No differences were seen during the first 90 minutes of testing, which were considered the acute phase of the experiment In the subsequent 20-hour period, which was treated as the chronic phase dietary choline enrichment reduced locomotor activity during the second and third 4-hour period, which corresponded with the first eight hours of the dark phase

Sandberg and co workers (Sandberg, Sanberg, & Coyle, 1984) investigated the effects of bilateral striatal cholinergic lesions induced by the neurotoxin AF65A. The lesions caused an increase in spontaneous nocturnal activity, an effect opposite to that observed by Beninger et al (1984) after chronic dietary choline enrichment These results seem to fit the hypothesis of a cholinergic involvement in the regulation of activity very well treatments that presumably heighten cholinergic activity reduce locomotor activity in the dark phase, while a treatment that presumably reduces cholinergic activity increases nocturnal activity In Beninger s (Beninger, et al., 1984) study, however, the choline-deficient animals showed even lower locomotor activity than the choline-enriched animals With respect to the behavioral consequences of reducing cholinergic parameters (though by different techniques), the results of Beninger and Sandberg are conflicting The discrepancy might partly have been caused by the manner of inducing the choline deficiency and the choline enrichment in Beninger s experiment (discussed in detail in Appendix B)

Because of these conflicting data our first experiment was designed to investigate the effects of chronic dietary choline enrichment on the diurnal patterns of locomotor activity Choline enrichment was given via the drinking water In addition it was questioned whether choline in the drinking water would alter the diurnal drinking pattern Such a change might be induced by an altered taste of the water (i e the supplemented choline might add aversive or appetitive properties to the drinking water) or might be mediated by alterations of central cholinergic activity, as cholinergic mechanisms may be involved in the control of drinking (Levitt, 1971)

Emotional reactivity

There is some evidence that another basal aspect of behavior -emotional reactivity- can also be influenced by drugs that might affect the cholinergic system

In an experiment of Collijn (1985) it was found that chronic dietary choline enrichment (5 mg choline chloride / ml tap water), administered to female CPBB rats over a period of six

months, reduced the time spent in the corner squares and increased the occupancy of the center of an open field The animals were 29 or 77 weeks old when they were tested These results could be interpreted as indicating reduced 'emotional reactivity' This effect was observed with both age groups

Further indications that chronic dietary choline enrichment might be able to reduce 'emotional reactivity have been found in our inhibitory and active avoidance experiments (compare Chapter 2, and Appendix B) The generality of these results, however, was severely hampered by the fact that the effects were restricted to either the BN or the WAG strain in some of these experiments The effects found in our avoidance experiments and in the open field study of Colliin (1985), however, are consistent with the notion that chronic dietary choline enrichment might reduce emotional reactivity', as all the effects were in the same direction (lengthened occupancy of the aversive part of the apparatus, and heightened number of entries into the aversive region)

Additional evidence that the manipulation of central cholinergic activity might reduce emotional reactivity was presented by Flicker and Geyer (1982) They observed that rats that had received bilateral chronic infusions of carbachol into the dentate gyrus spent considerably more time in the center of a holeboard box than untreated control animals Carbachol is a muscarinic and nicotinic receptor agonist Unfortunately, however, studies in which cholinergic parameters are pharmacologically manipulated have not led to an agreement about the role of cholinergic mechanisms with respect to activity and reactivity in novel environments (Hughes 1982)

The aim of the second experiment was to assess whether the effects of the diet on the emotionality of BN and WAG rat could be demonstrated more clearly in the open field test, this test is specifically designed to measure this trait The results will indicate whether the effect found by Colliin (1985) in CPBB rats can be generalized to other strains of rats

EXPERIMENT 6.1. EFFECTS OF CHOLINE ON THE SPEED OF ADAPTATION IN AN ACTIVITY ALLEY, AND ON THE CIRCADIAN DRINKING PATTERN

The first experiment was designed to investigate the effects of chronic dietary choline enrichment on the diurnal patterns of locomotor activity and drinking The rats were tested in an activity alley In accordance with Beninger and co-workers (1984), the first two hours were treated as the 'acute' phase of the experiment The rate of decrease of locomotor activity during this phase was taken as a measure of adaptation to the novel environment (Gentsch, Lichtsteiner, Kraeuchli, & Feer, 1982, Geyer, Russo, & Masten, 1986)

Three successive 24-hour day/night cycles were treated as the chronic phase of the experiment The circadian patterns of locomotor activity and of drinking behavior were determined by cosinor analysis Based on the data presented by Beninger et al (1984) we expected that chronic dietary choline enrichment reduces locomotor activity in the dark phase of the day/night cycle

MATERIAL AND METHODS

Animals Seventeen male BN/BiRij rats and 17 male WAG/Rij rats (8 choline-enriched, 9 untreated controls) were used These animals were taken from a group of 60 animals (see also Appendix C, third experimental protocol) that had been supplied by the TNO Institute for

Experimental Gerontology, Rijswijk, the Netherlands, at the age of five weeks. The rats were housed in pairs in standard Makrolon™ cages and were adapted to a reversed day/night cycle (lights on: 20:00 to 08:00). Choline enrichment (2.5 mg choline chloride ($C_5H_{14}ClNO$, Merck) / ml tap water) was started when the animals were seven weeks old (see Chapter 2, Table 2.1 for details on assignment to experimental conditions). When the rats were 28 weeks old they were housed individually. The choline chloride concentration in the drinking water was doubled (5 mg / ml) when the rats were 33 weeks old. The effects of choline enrichment were assessed in a series of tests, including those of temporal (Chapter 4) and spatial (Chapter 5) discrimination learning.

Apparatus: Three identical activity alleys were used. The apparatus consisted of a straight alley (length 90 cm, width 10 cm). Boxes measuring 25 * 25 cm were connected to either end. The alley and boxes were made of grey PVC and had a stainless steel grid floor (bars 1.4 cm apart). The alley was equipped with a total of 24 infrared photocells which were attached 4 cm above the grid floor at regular distances of 3.5 cm along the whole length of the alley. Each interruption of an infrared beam activated a counter. The number of counts was taken as a measure of locomotor activity. The ceiling of the apparatus was 22.5 cm above the grid floor and was made of clear plexiglass except for the ceiling of one end box, which was completely covered with a black PVC lid. The darkened end box was empty, and it was expected that rats would prefer to sleep in this part of the apparatus.

The second end box was provided with a drinking tube and a food rack on the wall facing the entrance into the alley. A food container was attached to the outside of the wall. Food could be reached through a grid in the wall, measuring 9 * 6 cm (heights * width), 6 cm above the grid floor and 4 cm from the right edge of the wall.

A water nipple protruded about 1 cm into the box. An infrared photo-detection system was fixed equidistant on either side of the nipple (0.9 cm apart) in such a way that the photo-beam was interrupted when the rat drank. Every interruption (drinking bout) activated a counter. The system was embedded in small blocks of PVC (2.5 * 2.0 * 0.8 cm; height * depth * width) to protect it. The detection system, including the drinking nipple, was fastened 9 cm above the grid floor and 4 cm from the left edge of the wall.

The detection systems of the alley and the drinking nipple were connected to an interface which collected data per 30-s period (during the initial 2 hours of the experiment) or per 5-min period (throughout three successive 24-hour cycles). At the end of each 24-hour cycle all the data were transferred to an APPLE//e microcomputer in an adjacent room, and were stored on floppy disk.

The activity counts and the number of drinking bouts recorded during the 'acute' phase (first two hours) were cumulated over 10-min periods. The counts recorded during the 'chronic' phase (the three successive 24-hour cycles) were cumulated over periods of one hour.

Methods: Between the age of 89 and 104 weeks, the reaction of the rats to a novel environment was recorded for two hours, and the activity in that environment was recorded for 72 hours (covering three complete day/night cycles).

Three rats were tested at the same time in three activity alleys in the same room. The rats were randomly assigned to one of these alleys. A new group of rats was tested every four days. The rats were taken from their homecages and put into the activity alleys at 14:00 h. Locomotor activity and the number of drinking bouts were registered for 2 hours (seventh

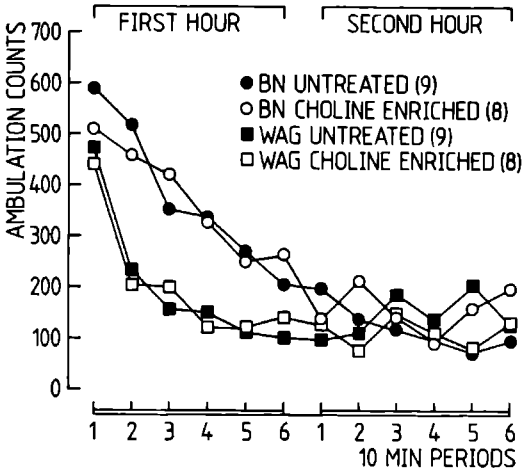


FIGURE 6.1: Ambulation counts of untreated and chronically choline-enriched BN and WAG rats during the first two hours ('acute' phase) in the activity alley.

and eighth hour of the dark period). At 20:00 the registration of locomotor activity and the number of drinking bouts started for an uninterrupted period of 72 hours. The alleys were cleaned thoroughly between groups.

RESULTS

One photosensor of the alley proved to be defective and to produce unreliable, self-triggered counts at irregular time intervals. It was possible to detect those erroneous counts with great certainty during the first two hours by using a statistical test on extreme values (Dixon, 1950, 1951). The unreliable count was replaced by the mean of the 30-s period preceding and following this period[1].

Attempts to detect and remove the unreliable activity counts of the 'chronic' phase proved to be unsuccessful. It cannot be excluded that some of the activity counts collected during the chronic phase had been affected by the defect. Therefore, the activity counts of this phase were not submitted to statistical analyses.

Adaptation to the activity alley during the 'acute' phase:

Activity counts: Orthogonal polynomials and general means of the activity counts over the six 10-minute periods of the first two hours were calculated separately and subjected to a strain (BN vs. WAG) by treatment (untreated vs. choline-enriched) analysis of variance (ANOVA). In addition, ANOVAs were performed on the individual 10-min periods. The activity scores for all groups during the two hours of the 'acute' phase are shown in Figure 6.1.

The process of adaptation was restricted to the first hour. Neither between nor within subject effects were found for the second hour of the adaptation period when the locomotor activities of all groups had stabilized at the same low level.

All groups exhibited the same high locomotor activity during the first 10-min period of the first hour. The rate of adaptation was characterized by general linear ($F_{1,30} = 143.92$, $p < 0.01$)

[1] It should be noted that only 3 of 8160 30-second periods (240 30-second periods per rat, 34 rats) had to be replaced.

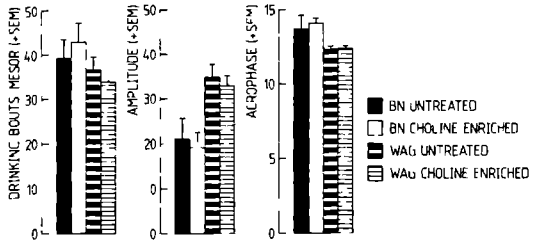


FIGURE 62 Mesor, amplitude and acrophase (+ SE_M) of the circadian rhythm of drinking bouts of untreated and chronically choline-enriched BN and WAG rats in the activity alley (chronic phase)

and quadratic trends ($F_{1\,30}$ = 18 79, p < 0 01) The linear trend covered 90% of the variation in locomotor activity over successive 10-min periods Neither strain differences ($F_{1\,30}$ < 1 0, ns) nor treatment effects ($F_{1\,30}$ = 2 66 ns) were found on this trend component An additional 9% of the decrease of locomotor activity during the first hour could be predicted from a quadratic regression equation The strains differed on this trend component ($F_{1\,30}$ = 7 07, p < 0 05) The locomotor activity of the WAG rats decreased faster during the first 10 min periods followed by a slowing down of this process in the later 10-min periods The decline of locomotor activity was a more gradual and steady process in the BN rats The chronic dietary choline enrichment did not affect the rate of adaptation

Drinking bouts The rats hardly contacted the water nipple during the two hours of the adaptation period The scores were very low and did not permit meaningful analyses

Diurnal drinking pattern during the chronic phase

A cosine curve fitting procedure was used (Monk & Fort, 1983) to estimate mesor, amplitude, and acrophase of the drinking bouts of each individual rat over the three consecutive 24 hour cycles The mesor reflects the mean of drinking bouts per hour (the mesor differs from the mean if observations are not equally spaced, see Monk & Fort, 1983) The acrophase reflects the point of maximum activity in decimal hours after midnight, that is, the point where the sinus curve reaches its maximum deviation from the mesor, as defined by its amplitude

The three measures of the sinoid were submitted to a strain (BN vs WAG) by treatment (untreated vs choline-enriched) ANOVA

The results are summarized in Figures 62 and 63 There were no strain or treatment effects on the mean number (mesor) of drinking bouts The strains did, however, differ on the amplitude ($F_{1\,30}$ - 16 60, p < 0 01) and acrophase ($F_{1\,30}$ = 8 37 p < 0 01) of the drinking bouts The WAG rats hardly contacted the drinking nipple in the light period BN rats showed a less clear distribution of drinking bouts over the light/dark cycle The acrophase of the WAG rats occurred approximately in the middle of the dark phase while the acrophase of the BN rats was shifted nearly 90 min towards the end of the dark phase Chronic dietary choline enrichment did not affect any of the three parameters of the sinoid (mesor, acrophase, and amplitude $Fs_{1\,30}$ < 1 0, ns)

In order to determine the precise location of the differences between the groups within the 24-hour cycle, ANOVAs were performed on the number of drinking bouts per hour for the data aggregated over all 24-hour cycles

The BN rats had more drinking bouts than the WAG rats in the first seven hours of the light phase, and in the first hour of the dark phase, while the reverse was true in the second, third and fifth hour of the dark phase Although a strain by treatment interaction was found

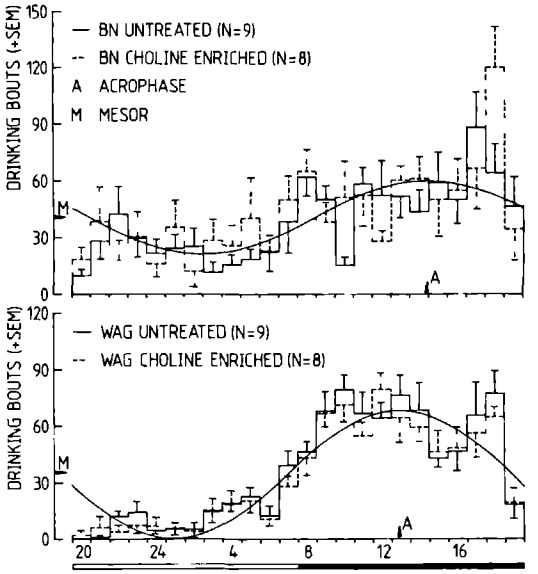


FIGURE 63· Mean drinking bouts per hour (+ SE_M) based on three successive 24-hour cycles of untreated and chronically choline-enriched BN and WAG rats Sinus curves based on estimates from the 17 rats per strain are depicted Arrows show the estimates of mesor (M) and acrophase (A) within strains.

in the third hour of the dark phase, we feel that such a result can be expected by chance in a series of 24 ANOVAs.

DISCUSSION

'Acute' phase· No effects of chronic dietary choline enrichment were found during the acute phase of the experiment, corroborating the results reported by Beninger et al (1984). The BN rats adapted to the novel situation slower than the WAG rats.

'Chronic' phase· Due to technical problems only the effects of the diet on the diurnal drinking pattern in the chronic phase of the experiment were analyzed

Drinking behavior, as expressed by the number of drinking bouts per hour, was unaffected by the diet. This behavior (Greenwood, Armstrong, & Coleman, 1981; Spiteri, 1982, Stephan, & Zucker, 1972), like feeding behavior (Peng, & Kang, 1984; Rosenwasser, Boulos, & Terman, 1981; Siegel, 1961; Spiteri, 1982) or locomotor activity (Greenwood, Armstrong, & Coleman, 1981; Büttner, & Wollnik, 1984; Wollnik, Gärtner, & Büttner, 1987), shows strong circadian rhythms in rats. We found, that the rhythmicity of the circadian drinking pattern of the BN strain was less pronounced compared with that of the WAG strain. A possible cause for the strain difference observed may be hydronephrosis which caused kidney dysfunctions in aging BN rats. The incidence of this disease in BN rats is high (Burek, 1978). By distributing their drinking bouts over the whole 24-hour cycle, BN rats might regulate their fluid balance. This hypothesis, however, needs to be addressed in further studies.

Comparing the circadian rhythms of wheel-running activity, of feeding, and of drinking behavior in young and old rats, Peng and Kang (1984) reported that half of the aged animals lost circadian rhythms in wheel running, while the feeding and drinking rhythms were well preserved. The circadian pattern of drinking may be less susceptible to age-related disturbances

than that of locomotor activity but Mosko and co-workers (Mosko, Erickson, & Moore 1980) reported that both the activity and drinking rhythms became progressively less pronounced with increasing age in rats

Beninger et al (1984) found that choline enrichment affected running wheel activity, but it should be kept in mind that they only tested rats of about 13 months of age Despite the suggestive misnomer adult aging, their sample can hardly be considered to represent an age, at which age-related disturbances should be expected We used rats ranging from 20.5 to 24 months of age for our study For male WAG rats, 24 months is the age of 50% survival The 50% survival age of the BN strain was estimated to fall between 28 to 32 months (Burek, 1978 p 24)

Due to the absence of young controls, we do not know whether the drinking rhythms found in the two rat strains had already undergone age-related shifts We can conclude however, that dietary choline enrichment did not affect the daily drinking patterns

EXPERIMENT 6.II EFFECTS OF CHOLINE ON OPEN FIELD BEHAVIOR

Unpublished results from an open field experiment with female CPBB rats performed at our laboratory indicated, that chronic dietary choline enrichment might reduce emotional reactivity (Collijn, 1985) The aim of this experiment was thus to determine whether chronic dietary choline enrichment affects the 'emotional reactivity of BN or WAG rats, and whether the previous findings could be generalized to other strains of rats The open field test provides various measures which may be used as indices of 'emotional reactivity' A lower number of squares entered (e g Broadhurst, 1957 Aulich, 1976), high defecation scores (Broadhurst, 1957, Gentsch, Lichtsteiner, & Feer, 1981), increased occupancy of side squares (thigmotaxis, Valle 1970), or more particular of corner squares (Morrison, & Thatcher, 1969) and decreased time spent in the center (Valle, 1971) of the open field are considered to indicate higher 'emotional reactivity'

MATERIAL AND METHODS

Animals A total of 48 male rats were used (see Appendix C, fifth experimental protocol) Twenty-four rats of the pigmented BN/BiRij and 24 rats of the albino WAG/Rij strains were supplied by the TNO Institute of Experimental Gerontology, Rijkswijk, the Netherlands, at the age of 90 weeks (about 20 5 months) They were housed in pairs in standard Makrolon™ cages and adapted to a reversed day/night cycle (lights on from 20·00 to 8·00) by a 13 hours light-off/12 hours light-on schedule on eleven consecutive days The animals were housed individually when they were 94 weeks old Twelve rats of each strain were assigned randomly to the choline enrichment condition (5 mg choline chloride/ml tap water), the remaining 12 animals served as untreated controls

Apparatus. The open field consisted of a square base (100 * 100 cm) divided into 36 equal squares by black lines The walls were 30 cm high The base and three walls were made of wood painted white The front wall was made of transparent plexiglass to facilitate observation The open field was placed 30 cm above the floor of the experimental room Two red fluorescent tubes provided an illumination of two lux (Metrux, Metrawatt AG, Nuremberg, West Germany) on the floor of the apparatus

Methods: A rat was placed into the open field for five minutes on five consecutive days (Vossen, 1966, p. 78). As soon as the rat was released in the center of the apparatus, its behavior was registered. The rat was returned to its homecage at the end of a trial. The whole apparatus was then cleaned with a damp sponge. The order of testing was randomized, and this order was used on each of the five days of testing.

Entering a square was scored when a rat moved into it with both hindlegs. If a rat moved parallel to a side wall without entering a square with both hindlegs, its entry into the square pointing towards the side wall was scored when the animal put one or two forelegs and one hindleg into that square. The number of squares entered in the different areas and the total time (in seconds) spent in these areas was recorded

Three different areas were distinguished in the open field (Vossen, 1966, p. 78):

1) a corner area, consisting of the four corner squares,
2) a side area, consisting of all squares which lie along the side walls and between the corner squares, and
3) a center area, consisting of all squares without contact to the side walls.

Besides horizontal movements, the frequency of vertical movements (rearings and leanings combined) and defecation scores (number of boli) were registered and stored in an AIM-65 microcomputer by the experimenter, who sat in front of the open field.

RESULTS

Illness and death considerably reduced the number of animals that participated in the open field testing. One BN of the choline-enriched group died for unknown reasons. Seven choline-treated WAG rats and four WAG rats of the untreated group were lost. Autopsy revealed that all except one of these WAG rats suffered from an extended tumor of the pituitary gland. Thus, the final composition of the groups was: 12 untreated BN rats, 11 choline-enriched BN rats, 8 untreated WAG rats, and 5 choline-enriched WAG rats[2].

All measures were subjected to a strain (BN vs. WAG) by treatment (untreated vs. choline-enriched) ANOVA (SAS GLM-procedure for unequal cell frequencies: Freund & Littell, 1985)[3]. Data from the five consecutive days were combined and averaged to enhance the reliabilities (Ossenkopp, & Mazmanian, 1985, Tachibana, 1985). The averaged time scores were transformed to the natural logarithm before statistical analyses in order to remove any lack of homogeneity.

[2] All rats that survived were used for additional studies several months after the completion of this experiment. Therefore, it is impossible to evaluate the health of the WAG rats that completed the open field task, especially with respect to possible presence of pituitary tumors at the time of testing. It is conceivable, however, that some of the WAG rats had tumors when tested. The high incidence of pituitary tumors clearly puts constraints on the value of the WAG strain for aging research.

[3] Because defecation scores in the WAG strain were very low, and some animals of that strain did not defecate at all (floor effects), non-parametric statistics were applied whenever this measure was involved (strain and treatment effects and correlational analyses). The results of these analyses did not deviate from the outcomes of the parametric analyses. Therefore, only the results of the parametric analyses are presented.

The results are summarized in Figure 6 4 In the three areas of the open field the BN rats were less active than the WAG rats as far as horizontal activity is concerned (frequency of entering corner squares $F_{1\,32}$ = 44.54 p < 001, side squares $F_{1\,32}$ = 5519, p < 001, center squares $F_{1\,32}$ = 1364, p < 001, Fig 6 4, panels A, B, and C) For vertical activity the reverse was true the BN rats reared and leaned more than the WAG rats ($F_{1\,32}$ = 1390 p < 001 Fig 6 4, panel D) Correlations over all 36 animals revealed that the horizontal and vertical activity scores were unrelated (the product-moment correlations (r_{PM}) between the frequency of leaning and rearing on one hand, and the frequency of entering the corner, side, and center squares, on the other, were -010, - 011, and 006, resp) The BN rats defecated more than the WAG rats ($F_{1\,32}$ = 3335 p < 001, Fig 6 4 panel H) The defecation scores correlated negatively with the frequency of entering the corner side, and center squares (r_{PM}s -051, -051, and -039, resp, associated probabilities < 005) The less active rats were the more they defecated With respect to the time the rats spent in the different areas of the open field the strains did not differ (Fig 6 4, panels E, F, and G) Chronic dietary choline enrichment did not affect any of the measures in the open field (for treatment, and strain by treatment interactions all Fs$_{1\,32}$ < 165, ns)

Unequal cell frequencies might affect the robustness of the ANOVAs (Milligan Wong, & Thompson, 1987) In the present study many rats of the WAG strain died Variances within strain by treatment groups, however, remained homogeneous (as evaluated by F-statistics)

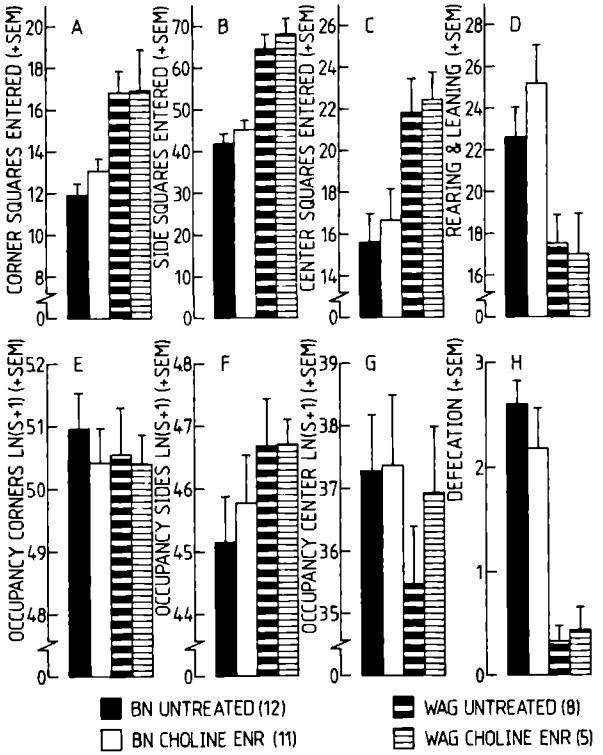


FIGURE 6 4 Number of entries into the corner, side, and center squares of untreated and chronically choline-enriched BN and WAG rats in the open field, averaged over five daily five-minute sessions (panels A, B, and C) The corresponding averaged times of occupying the three areas of the open field were transformed to the natural logarithm They are depicted in panels E, F, and G Panels D and H show the mean number of rearings and leanings, and of the number of fecal boli, resp.. Vertical lines indicate SE_Ms

and indicate that the failure to find treatment effects was not caused by the occurrence of extreme scores in the WAG rats (e.g. due to the possible poorer health of some of the WAG rats). In addition to the ANOVAs, the effects of the chronic dietary choline enrichment were evaluated separately by *t*-tests within strains. These analyses confirmed the outcome of the ANOVAs, namely that the treatment did not affect open field behavior.

Discussion

No effects of chronic dietary choline enrichment on 'emotional reactivity' in the open field were found in this experiment. These results thus contrast with those reported by Collijn (1985), and with our findings in the avoidance tasks which have been reported in Chapter 2 and Appendix A.

The BN rats showed less horizontal activity (as measured by the number of squares entered), and they defecated more than WAG rats. The data might be interpreted as showing that the BN strain has higher 'emotional reactivity' (e.g. Aulich, 1976; Broadhurst, 1957; Gentsch, Lichtsteiner, & Feer, 1981). However, on a third index of emotionality, the amount of time spent in the three distinct areas of the open field (Valle, 1970; Morrison, & Thatcher, 1969), the BN and WAG rats did not differ. These measures thus do not support the interpretation, that BN rats are more emotionally reactive than WAG rats. However, before a final conclusion can be drawn, more information about the cross-validities of the various measures of 'emotional reactivity' in the open field test is needed. A complicating factor for the assessment of the validity of open field measures, however, might be the deviant reaction of BN rats to stressful stimulation (e.g. McCarty, Kirby, & Garn, 1984).

GENERAL DISCUSSION

No evidence was found that chronic dietary choline enrichment influenced the speed of adaptation to a novel environment, the circadian pattern of drinking behavior, or 'emotional reactivity'.

Diurnal patterns

The only study which reported that choline supplementation had effects on locomotor activity in the dark phase of the day/night cycle was performed by Beninger and co-workers (1984). A closer look at their study makes an interpretation in terms of specific effects on cholinergic systems questionable.

The manner of providing the different diets in Beninger's study was a source of problems[4]. The choline-enriched diet proved to be so unpalatable or unattractive that the rats did not consume enough to keep their body weights at the pre-experimental level. As a consequence, the choline-enriched food was supplemented with normal Purina rat chow to prevent further loss of weight. Sanders and her co-workers (1984) compared the effects of different purified diets on the growth and food intake of weanling rats. They found that some purified diets retarded normal growth, probably because the rats on these diets failed to consume sufficient casein.

[4] Appendix B provides information about the growth curves and liquid consumption of the chronically choline-enriched BN and WAG rats of our own studies. Our data are contrasted with those presented by Beninger et al. (1984).

The rats on the choline-deficient diet, on the other hand, gained considerable weight in the study by Beninger et al. They suggest that the rats might have eaten more in order to compensate for the deficit or alternatively, because the food may have been more palatable. To equalize the body weights of the three groups, the choline-deficient group and the control group were put on a restricted feeding schedule, beginning 15 weeks after the start of dietary manipulation. When activity in the running wheel was assessed eight weeks later, the control group had approximately reached the body weight of the choline-enriched rats; the choline-deficient rats were still loosing weight (but had already lost at least 25% as compared with their initial weights when restricted feeding started!).

Goodrick (1966) reported that food deprivation can have a great effect on locomotor activity, depending on age and the level of deprivation. He showed that the relationship between these two variables is rather complex. Unfortunately, Goodrick's study did not include rats of approximately the same age as those used in the study by Beninger et al. It is quite plausible that differential food deprivation may have, at least partially, contributed to the differences in activity reported.

Another reason why the 'cholinergic' interpretation of their data raises doubts has been put forward by Beninger himself. The normal Purina chow and the choline-enriched and choline-deficient diets may have had considerably different levels of other ingredients beside choline chloride. "Thus, differences between rats fed Purina versus those fed Bio-Serv diets may not be attributed solely to differences in choline content" (Beninger, et al., 1984, p. 30).

Our method of administering the choline chloride in the drinking water has the advantage that the problems reported by Beninger were not encountered (see also Appendix B). This ensures that the choline-enriched and the untreated control rats received the same food, and only differed in the quantity of choline consumed.

Emotional reactivity

Based on the results reported by Collijn (1985) and on our results from the behavior of rats in the inhibitory and active avoidance task (described in Chapter 2 and Appendix A) we hypothesized that chronic dietary choline enrichment would reduce 'emotional reactivity' in rats. This hypothesis is not supported by the results from this open field experiment.

The effects of choline enrichment on the circadian pattern of activity and on the measures of 'emotional reactivity' that have been reported previously were found in rats of different ages, and were not restricted to aged rats. The rats in the study by Beninger et al. (1984) were about 13 months old when they were tested. Collijn (1985) used rats that were approximately 7 and 18 months old. The effect of the diet on the frequency of entering the light compartment and on time spent in the light compartment during the habituation sessions of the inhibitory avoidance tests (see Chapter 2) were found with rats that were 7 to 27 months old.

These effects surely cannot be interpreted as an amelioration of age-related impairments. In fact, only the WAG rats used in the present open field experiment (and in the fourth inhibitory avoidance experiment of Chapter 2; both experiments used the same animals) had reached an age that justifies the qualification 'aged'. Choline enrichment failed to affect the behavior of these 'aged' rats (except for the increased frequency of the BN rats to enter the light compartment of the inhibitory avoidance apparatus in Chapter 2, experiment 2.IV).

The results of the present study may have been influenced by particular characteristics of the two strains used: the incidence of pituitary tumors is quite high in the 24-month-old

males of the WAG strain. This may have changed the sample characteristics of this strain, especially in the open field experiment. The BN strain differs from most other strains with respect to their behavioral and neurochemical reactions to stressful stimuli (McCarty, & Kopin, 1978; Gilad, & Jimerson, 1981; Gilad, & McCarty, 1981; McCarty, Kirby, & Garn, 1984; Gilad, & Shiller, 1989). These reactions might have overruled possible treatment effects on the BN rats in the open field, which is a stress inducing environment for rats.

Finally, it can be questioned whether the effect of the choline treatment, which was observed in other experiments, possesses practical significance. It is worthwhile, however, to further explore the potential 'emotionality'-reducing property of choline enrichment in tests that have specifically been designed to assess emotionality.

CHAPTER 7

CENTRAL CHOLINERGIC ACTIVITY IN BN/BiRij AND WAG/Rij RATS: INFLUENCE OF CHRONIC DIETARY CHOLINE ENRICHMENT AND AGE

In collaboration with W.G.M. Raaijmakers and E. Willems-van Bree

SUMMARY

The adult Brown Norway (BN) rat is supposed to provide a 'genetic model' of age-associated changes in central cholinergic activity and of the concomitant impairments of cognitive functions. This hypothesis is based upon reports that indicate that the central cholinergic activity of adult BN rats is low compared with that of other strains of rats. Moreover, the retention performance of BN rats in inhibitory avoidance tasks resembles the impaired performance that is normally seen in aged rats. In the present study we assessed whether the cholinergic activity of BN rats is lower than that of WAG rats (exp. 7.I and 7.II) (we used both strains in a series of behavioral experiments), whether the sexes differ in their central cholinergic activity (exp. 7.I), and whether chronic dietary choline enrichment influences cholinergic activity (exp. 7.II). BN rats showed a lower central cholinergic activity than WAG rats in the posterior cortex (about 30%), the corpus striatum (about 20%), and the hippocampus (about 13%), as measured by the activity of the enzyme choline acetyltransferase (ChAT) and of the 'sodium-dependent high-affinity choline uptake (SDHACU) system'. Sex differences were not found. Chronic dietary choline enrichment slightly reduced the hippocampal ChAT activity in both strains (about 4%). In a third experiment it was assessed whether BN rats show an age-related decrease of hippocampal and cortical ChAT activity and SDHACU. No age-related changes were found in the cortical samples. The hippocampal SDHACU decreased with age in BN rats while ChAT activity increased. Thus, there is no general decrease of cholinergic markers with age in BN rats. Taking the results provided by others into account, it seems unlikely that the decline in cholinergic activity is a consistent characteristic of aging rats.

INTRODUCTION

Cholinergic activity can be assessed neurochemically by measuring one or more components of cholinergic transmission. The synthesis of acetylcholine (ACh) is catalyzed by the enzyme choline-acetyltransferase (ChAT). This enzyme is found in the nervous system, and specifically in cholinergic neurons. The rate-limiting step in the synthesis of ACh appears to be the size of the intra-neuronal pool of choline, although under certain circumstances the availability of acetyl-CoenzymeA (acetyl-CoA) may also be rate-limiting (reviewed by Tuček, 1985).

Neurons cannot synthesize choline *de novo*. Brain neurons are, however, able to synthesize choline from phosphatidylethanolamine or ethanolamine plasmogens through sequential methylation. The resulting phospholipids are then hydrolyzed (Blusztajn, & Wurtman, 1983). The cholinergic nerve endings are primarily dependent on the extraneuronal supply of choline for the synthesis of ACh. The uptake of choline is facilitated by a carrier-mediated process

that is characterized by a high affinity for choline and a dependence on extra-cellular sodium and energy However, the 'low-affinity choline uptake system is not dependent on sodium or energy Its affinity for choline is only one thousandth of that of the 'high-affinity choline uptake (SDHACU) system', and it is associated with different types of cells The changes in SDHACU induced *in vivo* by the inhibition or stimulation of the cholinergic cells are also observed, to a comparable extent, *in vitro* (Atweh, Simon, & Kuhar, 1975) It appears that changes of impulse flow activity are reflected by changes in SDHACU activity *in vitro*. It is therefore of interest to establish whether chronic dietary choline supplementation influences SDHACU activity in selected brain areas

Age-associated decreases in cholinergic activity or efficiency have been established in aged humans and primates Conflicting data have been reported for rats (Strong, Hicks, Hsu, Bartus & Enna, 1980, Sherman, Kuster, Dean, Bartus & Friedman, 1981, Briggs, Petersen, & Cook, 1982, Meyer, Onge, & Crews, 1984)

Age-related impairments of the retention performance in inhibitory (passive) avoidance tasks in rodents can be ameliorated by dietary choline or lecithin (Bartus, Dean, Goas, & Lippa, 1980, Davis, & Trombetta, 1984 Mervis, Horrocks, Wallace, & Naber, 1984 Muma, & Rowell, 1984) These early findings were related to the supposed facilitative effect of choline or lecithin on ACh synthesis and release Dietary administration of choline or lecithin increases serum free choline levels (Wurtman, & Hirsch, 1977), and brain ACh synthesis (Wurtman, & Fernstrom, 1976- Wurtman, 1982) For some time there was considerable controversy about whether brain ACh synthesis is affected by increased plasma levels of choline It is now established that an increase in plasma choline per se does not lead to an increase in the synthesis and release of ACh An increased exogenous supply of choline will only lead to an increase in ACh synthesis and release under conditions in which there is an enhanced demand for choline due to an increased release of ACh or to previous depletion of ACh stores (Bierkamper & Goldberg, 1979, Jope, 1982, Trommer, Schmidt, & Wecker, 1982) The early findings of alleviation of age-associated impaired inhibitory avoidance performance have been tremendously important for the formulation and experimental verification of the 'cholinergic precursor hypothesis This hypothesis proposes that an increased choline availability increases ACh synthesis and release and alleviates memory impairments associated with aging and dementia of the Alzheimer-type

Three different experiments were performed in the present study.

- In the first experiment differences in cholinergic activity between the inbred BN/BiRij and WAG/Rij rat strains and between female and male rats were assessed
- In the second experiment the effects of chronic dietary choline enrichment on ChAT and SDHACU activities in the hippocampus, striatum and cortex of male rats of both strains were estimated
- The third experiment explored the age-related changes of the hippocampal and cortical central cholinergic activity in male BN rats

Both the pigmented BN/BiRij and the albino WAG/Rij strains are well characterized with respect to the incidence of age-associated pathologies (Burek, 1978) Behaviorally, rats of the BN strain have been described as poor learners in shock-motivated tasks (de Koning-Verest, Knook, & Wolthuis, 1980, de Koning-Verest, 1981, see also Chapter 2, and Appendix A)

Gilad and co-workers compared the central cholinergic activity in different brain regions of BN rats with that of animals of the WKY strain The BN strain is characterized by a lower

cholinergic activity as measured by ChAT, SDHACU or AChE activities in various brain regions such as the hippocampus (Gilad, & Gilad, 1981, Gilad, Rabey, & Shenkman, 1983), striatum (Gilad, & McCarty, 1981) and septum (Gilad, & Gilad, 1981). On the basis of this information we formulated the following working hypothesis at the start of the research reported in this thesis: Young-adult BN rats provide an adequate genetic model of age-related decreases in cholinergic controlled memory performance, given their general low cholinergic activity as well as their poor performance in certain learning tasks. Here we report additional neurochemical data on the influence of strain, chronic choline-enriched diet and age on cholinergic activity in the neocortex, hippocampus and striatum

General Methods

After decapitation, the brain was rapidly cooled by dipping the head in liquid nitrogen for about 5 sec. The brain samples were rapidly dissected in an open refrigerator (4-10°C) and weighed on a cooled, pre-weighed aluminium block They were then homogenized in 50 vol (2%, w/v) ice-cold 0.32 M sucrose containing 5 mM Tris-HCl (pH 7.4) (6 strokes at 1200 rpm and 100N force, centrifuge-compatible tube, clearance 0.40 mm) with a teflon-in-glass homogenizer designed and built at the workshop of the Psychological Laboratory. The homogenate was centrifuged twice at 4°C for 10 min at 1000 x g. The pellet was washed between the centrifugation steps with 1.0 ml buffered sucrose Then, the combined supernatants were centrifuged at 4°C at 17000 x g for 15 min. The pellet was resuspended in 20 vol (5%, w/v) buffered sucrose (with respect to fresh weight); this crude mitochondrial suspension is referred to as the synaptosomal suspension P_2

Sodium-dependent, high-affinity choline uptake (SDHACU) (experiments 7.I, 7 II, and 7 III):

Synaptosomal uptake activities were measured in freshly prepared Tris-buffered Krebs-Ringer solution (TKR, pH 7.4) composed of (mM): NaCl, 128; Na_2HPO_4, 0.9, KCl, 6.2; $MgCl_2$, 1.3, $CaCl_2$, 2.7, Tris-HCl, 25.9; and glucose, 2 mg/ml SDHACU was determined by a modification of the method of Simon, Atweh, & Kuhar (1976, see also Raaijmakers, 1982), the uptake of choline in sodium-free medium (as normal TKR, except that NaCL and Na_2HPO_4 were replaced by sucrose (256 mM) and Tris (0.9 mM)) was subtracted from the uptake in normal TKR. The uptake in sodium-free medium represents the low-affinity uptake as well as the non-specific binding of choline.

Uptake was measured as follows. A 0.1 ml aliquot of the synaptosomal suspension was added to 0.8 ml of incubation medium (37°C) and kept at 37°C Two min later, 0.1 ml of [methyl-^{3}H] choline chloride solution (Amersham, 0.4 µM, 0.4 µCi) was added. The incubation was stopped after 4 min by the addition of 2 ml medium at room temperature. The preparation was then immediately filtered through mixed cellulose acetate and nitrate filters (Millipore HAWP, 0.45 µM pore size) under vacuum (Amicon manifold). The filters were washed once with 2 ml medium. They were then transferred to a scintillation vial (Packard, picovial), and 4 ml of a scintillation fluid (Packard, Filtercount) was added. The vial was shaken for 20 min (200 rpm) to transfer the material from the filter into the scintillation fluid. Radioactivity was measured by liquid scintillation spectrometry (Packard TriCarb). The counting efficiency was 30%, as determined by external standardization.

All uptake activities, both in normal and sodium-free media, were determined in triplicate. The SDHACU values were calculated as picomoles choline taken up per 4 min per mg protein.

Protein was measured in every sample according to the method of Lowry and co-workers (Lowry, Rosebrough, Farr, & Randall, 1951) as modified by Miller (1959)

Choline acetyltransferase activity (ChAT) (exp 7II, and 7III)

ChAT activity was measured according to the radiochemical method of Fonnum (1975). ChAT is the enzyme that catalyzes the formation of acetylcholine from acetyl-CoA and choline·

[1-^{14}C]Acetyl-CoA + Choline -> Acetylcholine (labeled) + CoA.

The frozen (-60°C) crude mitochondrial P_2 suspension was used. Twenty microliters of this suspension was mixed with 30 µl sodium phosphate buffer (50 mM, pH 7.4) and 50 µl of a solution of EDTA (20 mM) and Triton X-100 (0.2%) Aliquots (20 µl) of this mixture were taken in triplicate.

The incubation medium contained in its final concentration: choline chloride, 8 mM (Calbiochem), EDTA, 20 mM (pH 7.4); NaCl, 300 mM; Neostigmine, 0.1 mM and [1-^{14}C]Acetyl-CoA (57.6 mCi/mmol, Amersham) diluted with the unlabeled Acetyl-CoA (Boehringer) (0.2 mM). All solutions were made in sodium phosphate buffer (50 mM, pH 7.4)

The P_2 mixture (20 µl) was placed in a tube on ice and 50 µl of freshly made incubation medium was added. The tube contents were mixed and incubated for 30 min at 37°C. The incubation was stopped by the addition of 10 µl 14 % trichloroacetic acid (TCA). The tubes were placed on ice for 10 min The content of the tubes was then transferred to a scintillation vial containing 1 ml sodium phosphate buffer (10 mM, pH 7.4), the tubes were rinsed with 4 ml of this buffer. Two milliliters of acetonitrile containing 10 mg tetraphenylboron (Kalignost) and 5 ml toluenescintillator (Packard) was added to the scintillation vials (the toluene-scintillator contained 5 g PPO and 0.1 g POPOP per liter toluene). After the vials had been gently shaken by hand for 5 s, the contents were allowed to separate into two layers for 10 min The aqueous phase contained acetyl-CoA. ACh was extracted into the toluene phase. A liquid scintillation spectrometer (Packard TriCarb) was used to measure the radioactivity The counting efficiency was 92%, as determined by external standardization.

The ChAT activity was calculated as the nanomoles acetylcholine formed per hour per mg protein Protein was measured in every sample according to the method of Lowry et al. (1951) as modified by Miller (1959).

EXPERIMENT 7I. CENTRAL CHOLINERGIC ACTIVITY: STRAIN AND SEX DIFFERENCES

The BiRij substrain of BN rats may differ from the substrain used by Gilad and co-workers. Likewise, although both the inbred WKY and the WAG strains are derived from Wistar rats, differences between the BN and the WAG strain are not necessarily in the same direction and of the same magnitude as those observed between the BN the WKY strains in the studies performed by Gilad Moreover, heterogeneity between different Wistar-derived inbred strains (Bender, et al., 1984), and even between different WKY lines (Kurtz, & Morris, 1987) has been reported BN lines from different sources are remarkably homogeneous (Bender et al., 1984). Both female and male rats were included in this experiment, because ChAT activity might show sex differences. For example, sex differences (apart from age differences) in ChAT activity were reported recently for some of the 'basal forebrain nuclei' in Fischer-344 rats (Luine, Renner, Heady, & Jones, 1986).

Material and Methods

Animals: A total of 40 rats was used. Ten female and 10 male rats of the inbred BN/BiRij and of the inbred WAG/Rij strain were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands, at the age of 13 weeks (an overview of the experimental history of the rats is given in Appendix C, first experimental protocol).

Methods: The rats were decapitated without anesthesia when they were about 35 weeks old The severed head was kept about 5 s in liquid nitrogen to cool the brain The brain was then rapidly dissected into different parts. The temperature was kept at 4-10°C by performing the dissection in an open refrigerator.

Three samples were dissected, the posterior neocortex, hippocampus and striatum. In the coronal plane the posterior cortical sample was delimitated with a calibrated plastic T-square (see Figure 7.1, top right; after Rosenzweig, Bennett, & Diamond, 1972, see also Raaijmakers, 1978) In the lateral orientation, the sample was delimitated by the fissura rhinalis. The tangent plane with the cerebellum delimitated the sample in the rostro-caudal orientation.

The remaining neocortex and corpus callosum were removed. The hippocampus was dissected free The striatum (caudate putamen and globus pallidus) was dissected free from the capsula interna and the capsula externa (or septal area and ventral cortex), ventrally delimitated at the level of the anterior commissure. Adhering white matter was removed SDHACU was assessed according to the procedure described in 'General methods'

Results

Sodium-dependent, high-affinity choline uptake (SDHACU), wet weight, and P_2 protein concentration were analyzed by a strain (BN vs. WAG) by sex (females vs. males) analysis of variance (ANOVA). Due to technical problems, the SDHACU values from one subject in each group were lost. Thus, the SDHACU values of nine animals per group were subjected to statistical analysis. The means and standard errors of the means (SE_M) are shown in Table 7.1.

Compared with the values of the WAG strain, the SDHACU values of the BN rats were 13% lower in the hippocampus ($F_{1,32}$ = 7.61, $p < 0.01$), 23% lower in the corpus striatum ($F_{1,32}$ = 13.71, $p < 0.01$), and 30% lower in the posterior cortex ($F_{1,32}$ = 9.59, $p < 0.01$).

There were no strain differences for wet weight of the brain samples (all $Fs_{1,36}$ with associated probabilities > 0.05). The weights of the brain samples were higher for male rats than for female rats (hippocampus: $F_{1,36}$ = 30.65, $p < 0.01$; corpus striatum: $F_{1,36}$ = 7.20, $p < 0.05$;

TABLE 7.1: High-affinity choline uptake (SDHACU) in the hippocampus, corpus striatum and posterior cortex of male and female BN and WAG rats. Means and standard errors of the means (SE_M) are shown.

SDHACU: pmole choline/4 min/mg protein

	hippocampus		*corpus striatum*		*posterior cortex*	
	mean	SE_M	*mean*	SE_M	*mean*	SE_M
BN females	18.37	0.74	40 90	2 30	9.08	1 31
BN males	17.21	1.34	43.81	3.16	8 90	1 16
WAG females	21 83	1.00	55.31	4.11	13 35	1.14
WAG males	19.14	0.79	54.26	3.82	12.39	1.38

posterior cortex: $F_{1,36} = 5.30$, $p < 0.05$). The protein contents of the P_2 samples did not differ between strains or sexes (all Fs with associated probabilities > 0.05).

Discussion

The data clearly show that the BN rats had a lower SDHACU activity than the WAG rats. This lower activity of the SDHACU system may reflect either a less dense cholinergic innervation or a lower state of activity of the cholinergic neurons. No differences in the activity of the SDHACU system between female and male rats in any of the brain regions were found.

EXPERIMENT 7II: EFFECTS OF CHOLINE ON THE CENTRAL CHOLINERGIC ACTIVITY IN RATS

The effects of chronic dietary choline enrichment on cholinergic activity in the hippocampus, the striatum and the posterior cortex were studied. Since no sex differences had been found in the previous experiment, only male rats were used. It was hypothesized that the effects of chronic choline supplementation on the activity of ChAT and on the activity of the SDHACU system, as markers of central cholinergic activity, would be more pronounced in the BN than the WAG rats. This expectation was based on the finding, observed in the previous experiment, that BN rats had lower cholinergic activities than WAG rats, especially in the cortical sample.

Material and Methods

Animals: A total of 40 rats was used. Twenty male rats of the inbred BN/BiRij and of the inbred WAG/Rij strain were supplied by the TNO Institute for Experimental Gerontology, Rijswijk, the Netherlands, at the age of 3 weeks (an outline of the experimental history of the animals is given in Appendix C, second experimental protocol).

Methods: At the age of five weeks 10 rats per strain were put on a chronic dietary choline-enriched diet (2.5 mg choline chloride ($C_5H_{14}ClNO$; Merck) / ml water) until the rats were decapitated at the age of 46 weeks. The brain samples were dissected using the procedure that was described in experiment 7.I. High-affinity choline uptake and choline acetyltransferase activity were assessed according to the procedure described in 'General methods'.

Results

SDHACU, ChAT activity, wet weight and P_2 protein were analyzed for the hippocampus, corpus striatum, and posterior cortex by a strain (BN vs. WAG) by treatment (untreated vs. choline-enriched) ANOVA. As a result of Dixon's analysis of extreme values (Dixon, 1950, 1951), a total of four ChAT essays were excluded from the analysis (see Table 7.2).

The activity of the SDHACU system was similar to that found in the first experiment. Compared with the values of the WAG strain, the SDHACU values of the BN rats were 13% lower in the hippocampus ($F_{1,36} = 5.03$, $p < 0.05$), 16% lower in the corpus striatum ($F_{1,36} = 10.62$, $p < 0.01$), and 33% lower in the posterior cortex ($F_{1,36} = 39.83$, $p < 0.01$).

Similar strain differences were found for ChAT activity. Hippocampal ChAT activity was 14% lower in the BN than in the WAG rats ($F_{1,35} = 55.23$, $p < 0.01$). For striatal ChAT activity, the difference was 17% ($F_{1,35} = 45.75$, $p < 0.01$). Again, the difference between the two strains

was most pronounced for the posterior cortex. In that brain area the ChAT activity of BN rats was 36% lower than that of the WAG rats ($F_{1,34}$ = 317.15, $p < 0.01$). Chronic dietary choline-enrichment reduced hippocampal ChAT activity (by approximately 4%; $F_{1,35}$ = 4.50, $p < 0.05$). None of the cholinergic measures in the other brain regions were affected by chronic dietary choline enrichment.

Neither strain differences nor treatment effects were found for the wet weights and P_2 protein contents of the three brain samples (all Fs with associated probabilities > 0.05).

TABLE 7.2: High-affinity choline uptake (SDHACU) and choline acetyltransferase activity (ChAT) in the hippocampus, corpus striatum and posterior cortex of untreated and chronic dietary choline-enriched BN and WAG rats. Number of animals per group, means and standard errors of the means (SE_M) are presented.

SDHACU: pmol choline/4 min/mg protein

	hippocampus			*corpus striatum*			*posterior cortex*		
	n	*mean*	SE_M	*n*	*mean*	SE_M	*n*	*mean*	SE_M
BN untreated	10	15.71	1.03	10	40.28	1.76	10	6.32	0.39
BN choline-enriched	10	15.93	0.88	10	42.41	1.64	10	7.20	0.43
WAG untreated	10	18.32	1.14	10	48.55	2.56	10	10.17	0.42
WAG choline-enriched	10	18.12	1.20	10	49.51	3.15	10	9.95	0.76

ChAT: nmol choline/hour/mg protein

	hippocampus			*corpus striatum*			*posterior cortex*		
	n	*mean*	SE_M	*n*	*mean*	SE_M	*n*	*mean*	SE_M
BN untreated	10	135.7	3.44	10	381.7	11.40	10	61.07	2.02
BN choline-enriched	10	131.7	2.15	9*	356.8	5.75	9*	59.13	1.08
WAG untreated	9*	159.7	2.16	10	437.0	13.31	9*	91.84	1.15
WAG choline-enriched	10	151.2	3.45	10	451.9	11.28	10	96.03	2.55

*: one observation excluded as a result of Dixon's analysis of extreme values.

DISCUSSION

Chronic dietary choline enrichment reduced the hippocampal ChAT activity by approximately 4%. However, it can be questioned whether a change of only 4% has any functional significance. It can be speculated that the ability of choline to act as an acetylcholine agonist may have caused this effect. According to Bartus and co-workers (Bartus, Dean, & Beer, 1984) it may be predicted that, under conditions in which the cholinergic system is not stimulated, the synthesis of acetylcholine is inhibited so as to maintain a steady state (probably via the inhibition of the activity of the acetylcholine synthesizing enzyme ChAT). This interpretation, however, must be considered as highly speculative, since there are no relevant experimental data to support this idea.

We found that the SDHACU of the BN rats was lower than that of the WAG rats. These results fully corroborate the findings obtained in the first experiment.

In addition to SDHACU, ChAT activity was used as a second marker of central cholinergic activity. In accordance with the results presented by Gilad and co-workers, the BN rats were

found to have a low cholinergic activity The magnitude of the differences between the BN and WAG rats however, was considerably smaller than the differences between the BN and WKY rats reported by Gilad and co workers We found a difference of 13% between the hippocampal ChAT activity of BN and WAG rats, while Gilad and colleagues (Gilad, & Gilad, 1981, Gilad, Rabey, & Shenkman, 1983) reported a difference of about 50% between their BN and WKY strains The difference between the striatal ChAT activity in BN and WAG rats was similar in magnitude to the difference observed in BN and WKY rats (Gilad, & McCarty, 1981)

In our experiment cortical ChAT activity showed the largest difference (30%) between the BN and WAG rats Gilad and co workers do not provide data for this brain area It is not clear whether the smaller differences between the cholinergic activities of the BN and WAG rats were due to differences between the BN rats used by Gilad et al and the substrain of BN rats which was used in our experiments Likewise differences between the WKY rats used by Gilad et al and the WAG rats used in the present study may be responsible for the present findings It should be kept in mind, however, that BN substrains from different sources are very homogeneous Nine sublines of BN rats have been compared for biochemical markers at 28 genetic loci by Bender et al (1984) The genetic profiles of these strains differed at only one locus Thus, the smaller differences in ChAT activity found in the present study were most likely due to differences between the WKY and the WAG strains

The results can be summarized as follows the central cholinergic activity of BN rats is lower than that of WAG rats as indicated by both markers (SDHACU and ChAT) However, there are clear regional differences, the strain difference is most pronounced in the cortex and is less pronounced in the striatum and hippocampus

EXPERIMENT 7 III AGE RELATED CHANGES OF THE CENTRAL CHOLINERGIC ACTIVITY IN BN RATS

In the third experiment the effects of age on the central cholinergic activity in the hippocampus and two cortical samples were explored in the BN strain The additional cortical sample included the frontal and parietal cortices ChAT activity in post-mortem brain samples has been found to be reduced throughout the whole neocortex in normal elderly people (Perry, 1980) and, more pronouncedly in patients suffering from Alzheimer s disease (Procter et al, 1988) By inclusion of the additional cortical sample, most of the neocortex of the rat was represented in the present experiment

MATERIAL AND METHODS

Animals Twenty-eight male BN rats were used All rats had been supplied by the TNO Institute of Experimental Gerontology, Rijswijk, the Netherlands, but they were delivered at different ages and on different dates Most of the rats had already been studied in a number of behavioral tests The 16-week-old rats (n = 5) had not been tested The 52-week-old (n = 4) and 96 week-old rats (n = 2) had been trained previously in a food-motivated task in a Skinner-box All other rats (age in weeks, n between parentheses 98 (4), 100 (3), 103 (3), 105 (2), and 141 (5)) had been trained in spatial discrimination tasks (holeboard, radial maze, and/or Morris water maze)

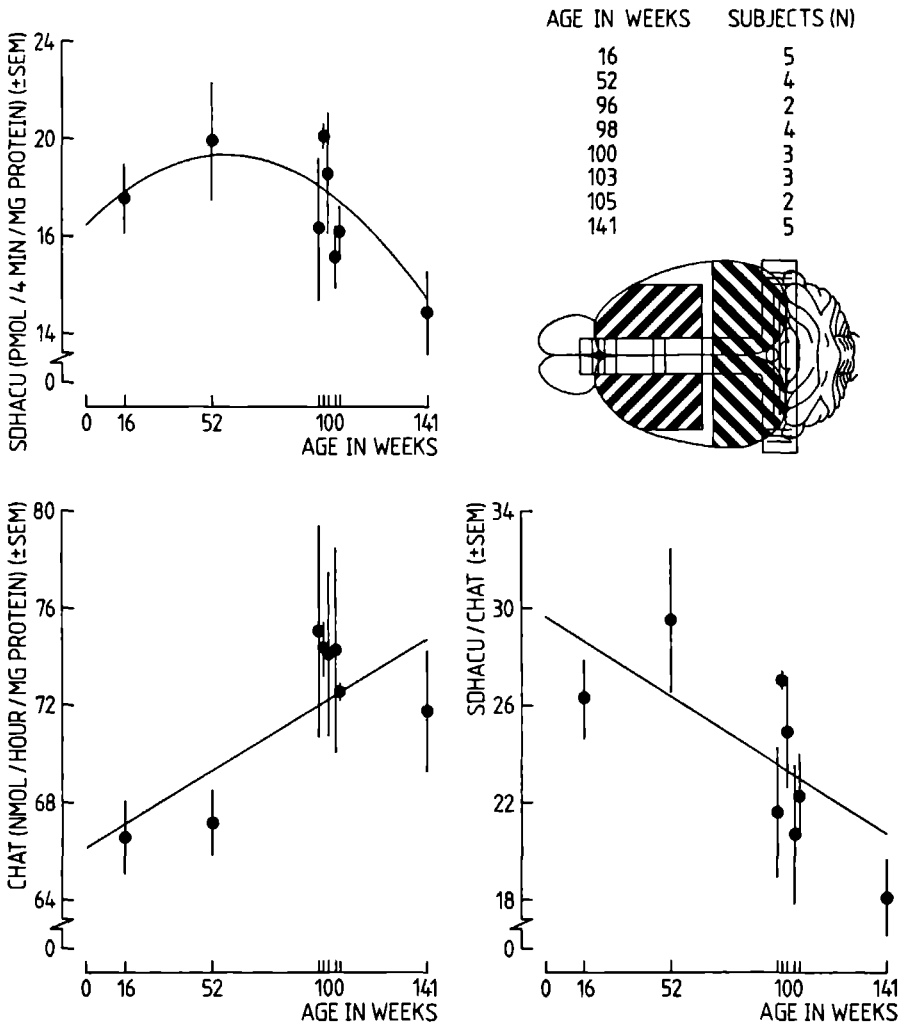


Figure 7.1· Means and standard errors of the mean (SE_M) and the estimated regression lines of hippocampal sodium-dependent high-affinity choline uptake (SDHACU; top left), ChAT activity (bottom left), and the ratio between SDHACU and ChAT (bottom right) of male BN rats from 16 to 141 weeks of age are depicted. The composition of the age groups is shown at the top right The limits of the the cortical samples as determined by a calibrated plastic T-square (after Rosenzweig, Bennett, & Diamond, 1972) are shown on the dorsal view of a rat brain: ///-hatched: frontal and parietal cortex; \\\-hatched posterior cortex

All the animals had been housed in standard Makrolon™ cages under a reversed day/night cycle (lights on between 20:00 and 08:00), and had been returned to an ad libitum feeding regimen at least one month before they were killed.

Methods: The procedure for decapitation and dissection followed was the same one as described for experiments 7.I and 7.II with one modification: the corpus striatum was not used. Instead, a second cortical sample, including the frontal and parietal cortices, was dissected free with the help of a calibrated plastic T-square (see Figure 7.1, top right).

Sodium-dependent high-affinity choline uptake and choline acetyltransferase activity were assessed according to the procedure described in 'General Methods'.

Results

SDHACU, ChAT activity, wet weight and P_2 protein of the hippocampus, frontal/parietal cortex, and posterior cortex were analyzed by regression analysis, with the age in weeks as the independent variable (Freund, & Littell, 1985). First- and second-degree polynomials were fitted to the data, and the intercept and regression parameters were estimated. The results for the wet weight and P_2 protein content of a particular brain sample are presented and discussed only when there are age-related changes in SDHACU or ChAT activity.

Neither the first nor the second order regression model indicated any age-related changes in the activity of the SDHACU system in the two cortical samples. Hippocampal SDHACU, on the other hand, fitted a second order regression model (SDHACU = 0.098 * age - 0.00084 * age^2 + 16.47; $F_{2,25} = 3.87$, $p < 0.05$; see Figure 7.1, top left), suggesting an inverted U-shaped change with age.

The ChAT activity in the two cortical samples did not change with age. Again, age-related changes were found in the hippocampus. The activity of the enzyme increased linearly with age (ChAT activity = 0.061 * age + 66.143; $F_{1,26} = 8.19$, $p < 0.01$; see Figure 7.1, bottom left).

There were no age-related changes in the P_2 protein content of the brain samples. The same was true for the wet weight of the two cortical samples. The wet weight of the hippocampus, however, increased linearly with age (wet weight of hippocampus = 0.21 * age + 99.75; $F_{1,26} = 36.27$, $p < 0.01$).

Discussion

An age-related inverted U-shaped change in the activity of the hippocampal SDHACU system was found in the BN rats. A decline in SDHACU activity was observed in rats older than one year. In the oldest age group the activity declined to 75% of the SDHACU activity of the one-year-old animals. De Koning-Verest (1981) observed an increase in hippocampal ChAT activity in 14-month-old female WAG/Rij rats when compared with 3-month-old animals. She did not observe a further increase in older (30-month-old) rats. Our results indicate that hippocampal synaptosomal ChAT activity increased beyond one year of age. However, the results can not be compared because not only did the strain and sex of the animals differ from those in the study of De Koning-Verest (1981), but the fraction in which the ChAT activity was assayed was also different (total homogenate versus P_2 fraction).

Cooper and Schmidt (1980) suggested that ChAT activity in the P_2 fraction can be used as an index of the number of (intact) cholinergic synaptosomes. Therefore, the ratio between the hippocampal SDHACU and ChAT activities was calculated and submitted to regression analysis. This was done to obtain a measure of SDHACU activity based on an index of intact cholinergic synaptosomes, instead of protein content. The ratio measure showed an age-

related linear decrease (SDHACU/ChAT = - 0.00063 * age + 0.297; $F_{1,26} = 9.92$, $p < 0.01$; see Figure 7.I, bottom right). This means that SDHACU decreased with age, irrespective of whether the protein content or ChAT activity was used as the basis of the calculations. However, the use of ChAT activity as an index of the number of cholinergic synaptosomes, as proposed by Cooper and Schmidt (1980), is of limited value. When a treatment changes synaptosomal ChAT activity, it is generally impossible to decide whether this is based on a change in the number of cholinergic synaptosomes or whether the amount of ChAT activity per synaptosome has changed.

An age-related decrease in cortical cholinergic activity in the BN strain was not found. It cannot be ruled out, however, that the different (though similar) experimental histories of the age groups involved introduced additional variation which affected the power of the analyses performed.

GENERAL DISCUSSION

Strain and sex differences: The first and second experiment confirmed that BN rats showed a lower cholinergic activity (measured by SDHACU in experiment 7.I, and by SDHACU and ChAT in experiment 7.II) than WAG rats in the three brain samples (hippocampus, corpus striatum, and posterior cortex) studied. The most pronounced difference was found for the cortical area where the cholinergic markers (SDHACU, ChAT) of the BN rats were at least about 30% lower than those of the WAG rats. In the hippocampus and the corpus striatum the differences between the strains were smaller (hippocampus: about 13% for both markers; corpus striatum: between 16% and 23% for SDHACU, and about 17% for ChAT). Sex differences were not found.

Effects of chronic dietary choline supplementation: The chronic dietary choline supplementation reduced ChAT activity in the hippocampus by about 4%. This finding indicates that the treatment affected a central cholinergic marker, although the precise mechanism of action remains unclear. This reduction might have little or no functional significance. It should be kept in mind, however, that the effects of supplemented choline on ACh synthesis might depend upon neuronal demands (e.g. Schmidt, & Wecker, 1981; Jenden, Weiler, & Gundersen, 1982; Wecker, 1986), i.e. the synthesis is increased only when ACh release is increased.

Age-associated changes of cholinergic activity in the BN rat: SDHACU per mg protein in the hippocampus showed a curvilinear relationship with age (exp. 7.III). SDHACU increased during the first year of life and then decreased. When ChAT activity instead of the protein content was used as the basis of the calculations, SDHACU decreased linearly with age. Cooper and Schmidt (1988) suggest that the ChAT activity serves as an index for the number of cholinergic synaptosomes. When this measure is used, one implicitly assumes that the cholinergic activity per synaptosome is not affected by age. This assumption, however, cannot be substantiated. Thus it remains unclear whether hippocampal SDHACU shows an inverse U-shaped change or a linear decrease with age. It can be concluded from both measures that SDHACU decreases in the aging rat, thus corroborating results reported by Sherman and co-workers (Sherman, Kuster, Dean, Bartus, & Friedman, 1981).

Hippocampal ChAT activity increased with age in the BN rat. An increased hippocampal ChAT activity was also observed by De Koning-Verest (1981) with female WAG rats. Others, however, have not observed age-associated changes of hippocampal ChAT activity (e.g. Strong, et al, 1980; Sherman, et al., 1981; Lowy, Ingram, Olton, Waller, Reynolds, & London, 1985).

ChAT activity in the cortical brain regions did not change This finding corroborates the results reported by Lowy et al (1985) but contrasts with results presented by Strong et al (1980), who reported a decrease of cortical ChAT activity with age Direct comparisons between the results from different studies are complicated by the fact that a different strain of rat was used in the different studies It is conceivable that age-associated changes of cholinergic markers are strain dependent, and that a decrease of central cholinergic activity is not a general characteristic of aging rats

In conclusion, it can be questioned whether the adult Brown Norway rat provides a genetic model for the aged animal, as age-related decreases in cholinergic activity are apparently not a consistent characteristic of the aged rat Based on behavioral observations similar reservations with respect to this model have been discussed in Chapters 2 and 3

Bartus and co-workers (Bartus, Dean, Goas, & Lippa, 1980, Lippa, Pelham, Beer, Critchett, Dean, & Bartus, 1980, Sherman, et al, 1981) have suggested that there is a direct link between central cholinergic activity and cognitive functions The results of the present study, in which an age-related increase of ChAT activity was found combined with our finding that BN rats show an age-related decrease of spatial discrimination performance (see Chapter 5) challenge this idea

CHAPTER 8

SUMMARY AND CONCLUSIONS

INTRODUCTION

This thesis describes a series of experiments which were performed to assess the behavioral consequences of chronic dietary choline enrichment in rats. Chronic supplementation with the acetylcholine precursor choline or lecithin, the so-called precursor therapy, has been suggested as a therapy for alleviating the impairments of cognitive functioning observed in the geriatric elderly (Bartus, Dean & Beer, 1984). The rationale behind this approach is based on the observation that central cholinergic activity is severely reduced in patients suffering from Alzheimer's disease. The extent of this reduction correlates with the severity of cognitive disturbances in patients with Alzheimer's disease (Katzman, 1986) but also in patients with Parkinson's disease (Perry, 1986).

Thus, as Bartus and co-workers put it in its most simple and direct form, the 'cholinergic hypothesis of geriatric memory' assumes that the reduction of cholinergic activity in the brain is the main cause of the decline in cognitive functions (Bartus, Dean, Beer, & Lippa, 1982). It was expected that increasing the availability of the precursors of acetylcholine would restore cholinergic function (Cohen, & Wurtman, 1975, 1976). This in turn would ameliorate cognitive functioning (Bartus, Dean, Pontecorvo, & Flicker, 1985).

In experiments with aging mice it was found that semi-chronic or chronic dietary choline or lecithin supplementation improved the performance of these animals in shock-motivated avoidance tasks (Bartus, Dean, Goas, & Lippa, 1980; Leathwood & Heck, & Mauron, 1982; Davis, & Trombetta, 1984; Mervis, Horrocks, Wallace, & Naber, 1984; Muma, & Rowell, 1984). However, most experiments with animals and most clinical trials with patients have yielded disappointing results (Bartus, Dean & Beer, 1984).

We investigated whether the effects of chronic dietary choline supplementation on the performance of mice in shock-motivated tasks could be reproduced with rats. Therefore, the effects of the choline treatment on the retention in an inhibitory (passive) avoidance task and on the acquisition of shuttle-box avoidance behavior were studied.

The effects of dietary choline enrichment on learning and memory in appetitively motivated tasks were assessed in a series of experiments. We studied the acquisition and 24-hour retention of an incompletely acquired bar-press response, and the acquisition and retention of complex temporal and spatial discrimination tasks.

In addition, we studied whether choline supplementation would affect basal behaviors such as the speed of adaptation to a novel environment, the circadian drinking pattern, and 'emotional reactivity'.

Two animal models were used in the experiments: an 'aging model' and a 'genetic model'. Both are models which make use of naturally occurring deficits, as opposed to models in which deficits are induced by experimental manipulations such as lesions or pharmacological interventions (Gamzu, 1985). In the aging model', the old rat served as a model for aging humans.

The results in the literature suggest that the (young) adult Brown Norway (BN) rat might provide a model for the age related decreases of learning and memory performance and of central cholinergic activity observed in humans For example De Koning and colleagues found that BN rats performed less well than WAG rats in a shock-motivated drink-test (De Koning-Verest, Knook, & Wolthuis, 1980) Compared with WKY rats (Gilad, & McCarty 1981, Gilad Rabey, & Shenkman, 1983) and other strains of rats (Raaijmakers, unpublished results), BN rats have a low central cholinergic activity Poor learning and a low cholinergic activity have been found in aged mice and rats (Strong, Hicks, Hsu, Bartus, & Enna, 1980)

In the experiments performed either the 'genetic model' or the 'aging model' or a combination of both was used The inbred WAG/Rij rats served as the control strain for the BN/BiRij strain in the genetic model We hypothesized that (young-) adult BN rat would profit more from dietary choline supplementation than WAG rats because BN rats have a lower central cholinergic activity Senescent BN and WAG rats were used in a number of experiments We expected that the treatment would ameliorate the age-related impairments of cognitive functioning in both strains The rate of occurrence of age-related behavioral and neurochemical changes is different in animals with a different genetic constitution (Goodrick, 1975) Because age by strain interactions may determine at which age impairments do occur, it is advisable to use more than one genotype in studies on aging

SUMMARY OF THE EXPERIMENTS

Chapter 2 Behavior in an inhibitory avoidance task

The retention of BN and WAG rats in an inhibitory avoidance task (Gold, 1986) was assessed In the first experiment we compared the performance of young-adult BN rats in this task with that of WAG rats and the performance of female rats with that of male rats We expected that female rats would show a poorer retention performance than male rats, as sex differences in inhibitory avoidance tasks have been observed earlier (e g van Oyen, van de Poll, & de Bruin, 1979, Drago, Bohus, Scapagnini, & de Wied, 1980) The main objective of the experiment was to evaluate whether the BN rat might provide a genetic model' for age-related cognitive impairments

The effects of chronic dietary choline enrichment on retention performance in this task were assessed in the second third and fourth experiment The treatment started at weaning in experiments 2II and 2III The rats were tested when they were about 7 (exp 2II) or 15 5 (exp 2III) months old In the fourth experiment 27-month-old rats were tested after a five month treatment with choline

Adult BN rats (exp 2I and 2II) performed very poorly when compared with WAG rats Female rats (exp 2I) performed less well than male rats The middle-aged (exp 2III) and senescent rats (exp 2IV) of both strains showed a very poor retention performance Comparisons across experiments suggest that, while the BN rats performed poorly at all ages, the WAG rats probably showed an age-related decline of retention performance Chronic dietary choline enrichment did not have any effect on retention performance in any of the experiments Thus, the poor performance predicted for the BN rats was found, whereas the hypothesized beneficial effects of choline treatment on the performance of adult BN rats and on the aged rats of both strains were not observed

We found that the choline-treated rats spent more time in the light compartment of the inhibitory avoidance apparatus and that they entered this compartment more frequently than

their controls during the habituation sessions. A heightened number of entries into the aversive part of a novel environment and long stays in this part of the apparatus are generally interpreted as indicating lowered 'emotional reactivity' (e.g. Aulich, 1976). These effects, however, were not consistent and were found in both strains (exp. 2.II), or were restricted to the WAG (exp. 2.III) or the BN strain (exp. 2.IV). The rats that were used in experiment IV had also been tested in the open field, a test designed to assess 'emotional reactivity' (described in Chapter 6). The results of both tests (open field, and adaptation in the inhibitory avoidance apparatus) were compared to shed light on the inconclusive data obtained in the adaptation sessions of the inhibitory avoidance experiments.

Chapter 3: Retention of an incompletely acquired bar-press response

In the first experiment we investigated whether the impaired retention performance of BN rats compared with that of WAG rats, and of female rats compared with that male rats, was due to a general impairment of their ability to retain responses. The 24-hour retention of an appetitively motivated incompletely acquired bar-press response in the Skinner box was used as the behavioral test (Destrade, Soumireu-Mourat, & Cardo, 1973). In the second experiment the effects of chronic dietary choline enrichment on the retention of the bar-press response were investigated. Neither strain (exp. 3.I and 3.II) nor sex differences (exp. 3.I) were found for the retention performances; chronic dietary choline supplementation did not have any effect on retention (exp. II). We conclude that neither female rats nor BN rats suffer from a general impairment in the retention of responses over a 24-hour interval. This finding questions the validity of the 'genetic model'.

Chapter 4: Temporal discrimination

The strain differences and the effects of chronic dietary choline supplementation were studied in a series of three experiments. In the first and second experiment a DRL 8" schedule was used (DRL 8": differential reinforcement of low rate responding; A Skinnerbox is used, and each bar-press that is separated from the previous bar-press by at least eight seconds produces a food reward; Church, 1978). A more complex DRL 16" schedule (bar-presses must be separated by at least 16 seconds) was introduced in the third experiment. The choline treatment had no effect on the performance of rats in the DRL 8" task. Chronic dietary choline enrichment, however, affected the rate of responding of the WAG rats on the DRL 16" schedule. The treated WAG rats showed a poorer timing performance than their untreated controls.

There were considerable differences between the performances of animals from both strains. The BN rats reached their final performance level within two or three training sessions. The WAG rats showed poorer timing behavior than the BN rats in the first session. The performance of WAG rats steadily improved and eventually reached the same level as that of the BN rats.

Chapter 5: Spatial discrimination

The effects of the treatment on spatial discrimination performance in a holeboard task (Oades, & Isaacson, 1978) are described in this Chapter. BN and WAG rats that had been treated from weaning with choline were tested at the age of 13 months in experiment 5.I. Dietary choline supplementation did not improve their performance in spatial discrimination tasks. In the second experiment the age-related decrease in the spatial working and reference memory was assessed in the BN strain. Rats of five ages (4, 13, 19, 25, and 30 months) were used. An age-related decline in the working and the reference memory was found. Correlation analyses revealed that the spatial working memory and the reference memory can be con-

sidered as independent measures The most notable decline in the spatial discrimination performance occurred between 19 and 25 months of age

Based on this result, aging (21 5-month-old) BN and WAG rats were put on a choline-enriched diet for 4 months They were then tested in the holeboard at an age of 25 5 months This was the age at which the BN rats had shown clear age-related impairments in experiment 5 II Chronic dietary choline supplementation did not prevent the age-associated decline in the performance of these animals on the spatial discrimination in the holeboard task

Chapter 6 Adaptation to a novel environment, diurnal drinking pattern, and emotional reactivity'

Whether the chronic choline treatment affects basal aspects of behavior such as the speed of adaptation to a novel environment, the diurnal pattern of drinking, and 'emotional reac tivity' was assessed In the first experiment, strain differences and the effects of the treatment on the speed of adaptation to a novel environment were measured in an activity alley The WAG rats adapted to the novel environment faster than the BN rats Chronic dietary choline enrichment did not affect the speed of adaptation

In the same experiment and with the same apparatus, the 24-hour drinking pattern was measured While WAG rats consumed water almost always during the dark phase of a 24-hour cycle the BN rats distributed their drinking sessions over the entire day The drinking pattern of the rats was not affected by the choline treatment

In a second experiment, 20.5-month old BN and WAG rats were tested in an open field on five consecutive days The open field is designed to measure emotional reactivity' The treatment did not affect the behavior of the rats in this apparatus The BN rats had higher scores on some of the measures for 'emotional reactivity' The results were somewhat inconclusive with regard to whether BN rats are more 'emotionally reactive than WAG rats and do not support the suggestive results from the adaptation sessions of the inhibitory avoidance task (Chapter 2) that choline enrichment might reduce 'emotionality'

Chapter 7 Cholinergic activity

In the first experiment we studied whether adult BN rats provide a 'genetic model' for the age-related decrease of central cholinergic activity observed in humans The central cholinergic activity of BN rats was compared with that of WAG rats In addition, the effects of chronic dietary choline enrichment were assessed in experiment 7.II The central cholinergic activity of the BN rats was lower than that of the WAG rats in the posterior cortex (about 30%), the corpus striatum (about 20%), and in the hippocampus (about 13%), as measured by the activity of the enzyme choline acetyltransferase (ChAT) and the activity of the 'sodium-dependent high-affinity choline uptake' (SDHACU) system Chronic dietary choline supplementation slightly reduced ChAT activity in the hippocampus (about 4%)

In a third experiment we explored whether the BN strain shows an age-related decrease in the activity of ChAT and SDHACU in the cortex and hippocampus No age-related changes were found in the cortical samples SDHACU activity in the hippocampus showed an inverted U-shaped change with age, and ChAT activity in the hippocampus increased. These results indicate that there is no general decrease of cholinergic markers with age in the BN strain

Appendices A and B Active-avoidance learning, growth curves and water consumption

The active avoidance experiment in Appendix A confirmed that BN rats show a general impairment in the acquisition of shock-motivated tasks This result extends the findings of

the inhibitory avoidance experiments described in Chapter 2 The growth curves and the water consumption of untreated and choline-treated BN and WAG rats are compared in Appendix B The chronic supplementation of the drinking water with choline did not affect the growth of the animals or the amount of water consumed

CONCLUSIONS

Effects of chronic dietary choline enrichment on behavior

Chronic dietary choline supplementation did not improve learning and memory nor did it affect the speed of adaptation to a novel environment or the circadian drinking pattern of (young-) adult BN rats or of aging BN and WAG rats

We found some indications in the behavior of the rats during the habituation sessions of the inhibitory avoidance task that the treatment reduced emotional reactivity This effect was not observed consistently, because it was found either in the BN or the WAG rat or in both strains, depending on the experiment In the open field test which is specifically designed to assess 'emotional reactivity (Chapter 6, exp 6II) choline supplementation was found not to have an effect This result contrasts with the finding that the same BN rats that had participated in the open field study showed a higher frequency of entering the light compartment in the first adaptation session of the inhibitory avoidance task (compare Chapter 2 exp 2IV) The rats were used in this test three months after they had been tested in the open field Thus, the results are inclonclusive with respect to the effect of chronic dietary choline supplementation on 'emotional reactivity'

Choline enrichment did not affect the performance of BN rats in the DRL 8" and DRL 16" task, which measures temporal discrimination performance The timing behavior of the choline-treated WAG rats, however, was impaired on the DRL 16" schedule Two alternative explanations for this finding are discussed The first one concerns an explanation within the framework of the 'scalar timing model (Church, 1984) This interpretation is based on the assumption that the speed of storage of a memory is increased by supplemented choline This in turn decreases the remembered durations of intervals Comparable results have been reported by Meck and Church (1987a) They prefed rats with a 'lecithin snack' before they were trained in a modified fixed-interval task

The second explanation is based on the observation that WAG rats suffer from generalized absence epilepsy, which is characterized by a high incidence of spike-wave complexes (van Luijtelaar, & Coenen, 1986) The BN strain is free of this aberrant EEG activity It is assumed that dietary choline supplementation increases the number of spike-wave complexes in the WAG rats and that the increased epileptic activity impairs their temporal discrimination performance Both explanations are, however, highly speculative and deserve further study

The aging rat as a model for behavioral and neurochemical aging in humans

Along with the 'genetic model', aging rats of both strains were used as models for age-related neurochemical and behavioral changes in the elderly This model has a higher face-validity than the genetic model', because aging rats show impaired performances in a great variety of behavioral tests We studied the age-related decline of spatial discrimination performance of BN rats in a holeboard task The results from this experiment confirmed that there is an age-associated decline in the spatial working and reference memory of rats

The results from the different inhibitory avoidance experiments in which young-adult, adult, middle-aged and aged BN and WAG rats were used suggest that the performance of the animals in this task also declines with age This interpretation is based on comparisons across the different experiments In each of these experiments, only one age group was tested Therefore our results on the inhibitory avoidance task are suggestive but not conclusive They are, however in agreement with findings reported by others (e g Sprott, & Stavnes, 1975, McNamara, Benignus, Benignus, & Miller, 1977, Zornetzer, Thompson, & Rogers, 1982) that rats show an age-related decline in inhibitory avoidance tasks

We could not confirm that there is an age-related decline in cholinergic activity in the central nervous system Cholinergic activity, as measured by the activity of the acetylcholine synthesizing enzyme choline acetyltransferase (ChAT) and the activity of the sodium-dependent high-affinity choline uptake (SDHACU) system in the cortex of BN rats did not show an age related decline We found an inverted U-shaped change in the activity of SDHACU in the hippopcampus and an increase in hippocampal ChAT activity with age These results contrast with the decline of cognitive functions observed in aging BN rats The data question whether there is a direct relationship between cholinergic activity and the performance of aging rats in learning and memory tasks

Thus, the value of the aging rat as a model for the age-related decline of cognitive functions which are assumed to be related to a decrease in central cholinergic activity, is doubtful Our results do not mirror the correlations between cholinergic parameters and cognitive functions that are found in aging humans, and even more pronouncedly in demented humans (most clearly in dementia of the Alzheimer type, Katzman, 1986, Perry, 1986)

The (young-) adult BN rat as a 'genetic model' for behavioral and neurochemical aging

We suggested that the (young-) adult BN rat might be used as a genetic model for the age-related impairments of cognitive functions and of decreased central cholinergic activity observed in humans Evidence from the literature indicates that BN rats have a poor performance in aversively motivated tasks (e g De Koning-Verest, Knook, & Wolthuis, 1980, Koene, 1988, van Luijtelaar, Kerbusch, & Coenen, 1988, Gilad, & Shiller, 1989) and that the cholinergic activity in the central nervous system is lower in this strain than in other inbred strains of rats (e g Gilad Rabey, & Shenkman, 1983) Our data confirm that BN rats perform poorly in aversively motivated tasks and that they have a lower cholinergic activity than rats from other strains

The results of additional experiments in which we tested BN rats in appetitively motivated tasks, however, question whether the (young-) adult BN rat provides a valid model for age-associated cognitive impairments BN rats acquire and retain complex spatial discrimination and simultaneous discrimination tasks as well as WAG rats Moreover, their 24-hour retention in an incompletely acquired appetitively motivated bar press task was unimpaired This contrasts with the severely impaired 24-hour retention of the inhibitory avoidance response of BN rats

The poor performance of BN rats in aversively motivated tasks is most likely caused by non-mnemonic factors BN rats differ from other strains of rats in their reaction to stressful stimuli. Our results thus raise doubt as to the validity of the (young-) adult BN rat as a genetic model for age-related changes of cognitive functions

The current state of the cholinergic precursor therapy'

There is agreement about the notion that lecithin or choline consumption increase serum-free choline levels (e g Cohen & Wurtman, 1975, 1976 Jope, Domino, Mathews, Sitaram, Jenden, & Ortez, 1982) The assumption that a heightened level of choline in turn increases the synthesis of ACh is a matter of dispute (e g Consolo, Ladinsky, & Gomeni, 1979, Leathwood, & Schlosser, 1986) There is some experimental evidence that the increase of ACh synthesis occurs as a reaction to increased neuronal demands (e g Schmidt, & Wecker, 1981, Jenden, Weiler, & Gundersen, 1982, Wecker, 1986)

Recently, Wecker (1988) published a series of experiments in which she tested the hypothesis that the bound pool of choline is enriched by the chronic administration of choline, and that under conditions of heightened neuronal demand, choline from this pool is used to increase the synthesis of ACh Although choline supplementation was found to increase the pool of bound choline, the synthesis of ACh in cortical and hippocampal slices was unaltered, both under resting conditions and under conditions of an increased demand (K^+-evoked condition) Therefore, the enhancement of cholinergic activity might not be an efficient treatment to improve cognitive functions even though the decline of cholinergic activity and impaired cognition are correlated

These results challenge an important assumption underlying the 'precursor therapy', namely that choline supplementation can ameliorate cognitive impairment via stimulation of ACh synthesis In view of these recent results (Wecker, 1988), it is less surprising that the 'precursor therapy' has not yielded the expected beneficial effects in most studies The 'precursor therapy has not been very effective in humans (Bartus, Dean, Pontecorvo, & Flicker, 1985), and evidence for positive effects in animal studies is scarce From discussions with other investigators in the field of aging we learned that many studies on the 'precursor therapy' with negative results have not been published, and this makes the experimental evidence in favor of this treatment strategy even weaker

The lack of treatment effects does not invalidate the 'cholinergic hypothesis of geriatric memory dysfunction' The involvement of cholinergic neurotransmission in learning and memory is well established (reviewed by Spencer, & Lal, 1985, Wauquier, & Clincke, 1985 Hagan, & Morris, 1988), and a dysfunction of cholinergic neurotransmission is clearly found in dementias (Coyle, Price, & DeLong, 1983)

Alternative approaches

The animal models used in the present thesis appear to be less suited to investigate the interrelationships between central cholinergic neurotransmission, aging, some types of dementias, and cognitive impairments Other models are needed Recently, lesions in cholinergic brain areas, for example the basal forebrain nuclei, by neurotoxins such as kainic or ibotenic acid or by AF64A, which seems to be a more specific cholinergic neurotoxin, have been used to produce animal models for Alzheimer-type dementia (Smith, 1988) The value of these new models remains to be determined.

An interesting, but not yet confirmed, observation reported by Arendash and co-workers (Arendash, Millard, Dunn, & Meyer, 1987) would make the animal models in which lesions are made in the nucleus basalis magnocellularis especially suitable for this type of research They found that neuronal atrophy and neuronal loss, neuritic-plaque-like and neurofibrillary changes developed months after lesioning These pathological changes resemble those found in Alzheimer patients The nucleus basalis magnocellularis provides the animal analogue of

the human nucleus basalis of Meynert, which degenerates in patients with Alzheimer's disease (Davison, 1987).

In conclusion, experimental aging research is crucially dependent on the quality and adequacy of the animal models used. More efforts should be made to develop animal models for this line of research. The present thesis contributes to this line of research by assessing the 'aging model' and a 'genetic model' of the age-related decline of central cholinergic neurotransmission and the concomitant behavioral impairments. The cholinergic 'precursor therapy' does not provide an effective treatment strategy to ameliorate the cognitive impairments that accompany aging and dementia.

Choline is, evenals fosfatidylcholine (lecithine) een normaal bestanddeel van het voedsel. Uit beide stoffen worden fosfolipiden gevormd, die belangrijke bouwstenen van biologische membranen zijn, en beide stoffen zijn voorloper moleculen (precursor) van de boodschapperstof (neurotransmitter) acetylcholine (ACh).

ACh is, naast andere neurotransmitters, verantwoordelijk voor de signaaloverdracht of communicatie tussen zenuwcellen in de hersenen. Bij Alzheimer patiënten is de functie van systemen in de hersenen waarin ACh als neurotransmitter wordt gebruikt ernstig verstoord. Cholinerge systemen (systemen met ACh als neurotransmitter) vervullen een belangrijke rol bij cognitieve functies zoals leren en geheugen. De mate van functie-vermindering van cholinerge systemen en de mate van cognitieve stoornissen bij Alzheimer patiënten lijken nauw met elkaar samen te hangen (Katzman, 1986). De 'cholinerge hypothese van geriatrische geheugen dysfunctie' veronderstelt dat de afname van de functie van cholinerge systemen de oorzaak is voor de achteruitgang van cognitieve functies (Bartus, Dean, Beer, & Lippa, 1982).

Al gauw werd derhalve gesuggereerd dat door een zogenaamde 'precusor therapie', die bestaat uit een chronisch verhoogde toevoer van een precursor van ACh, de cognitieve stoornissen zouden kunnen worden verminderd (Bartus, Dean, & Beer, 1984). De verwachting was dat een verhoogde beschikbaarheid van een precursor van ACh de cholinerge functies herstelt, doordat meer ACh in de zenuwcellen wordt aangemaakt (Cohen, & Wurtman, 1975, 1976). Hierdoor zou dan ook het cognitief functioneren worden verbeterd (Bartus, Dean, Pontecorvo, & Flicker, 1985).

De klinische toepassing van de 'precursor therapie' en de meeste experimenten met proefdieren leverden niet de verwachte positieve resultaten op (Bartus, Dean, & Beer, 1984). Doel van ons onderzoek was de effecten van chronisch (enkele maanden tot 2 jaar) toegediende choline op leren en geheugen en op enkele basale gedragingen te bestuderen.

Twee diermodellen werden op hun bruikbaarheid getest: een 'genetisch model' en een 'verouderings model'. In het 'genetisch model' werden (jong-) volwassen Brown Norway (BN) ratten als model voor de leeftijdsafhankelijke afname van cholinerge activiteit en cognitieve functies bij de mens beschouwd. Van de BN rat was bekend dat hij op jonge leeftijd een lagere cholinerge activiteit vertoont dan ratten van andere stammen (b.v. Gilad, Rabey, & Shenkman, 1983) en dat hij een zeer slechte prestatie in taken vertoont die gebruik maken van straf (lichte schok). Beide kenmerken werden ook bij veroudering bij ratten gevonden, en de (jong-) volwassen BN rat lijkt derhalve met betrekking tot deze twee kenmerken op een oud dier.

Op het eerste gezicht lijken oude ratten eerder te voldoen als model voor de effecten van veroudering bij de mens. Het is echter zo dat de evidentie voor leeftijdsgerelateerde veranderingen in de hersenen van oude ratten niet eenduidig is, terwijl oude ratten meestal wel een leeftijdsafhankelijke afname van leer- en geheugenprestaties vertonen. Als controlestam voor beide modellen gebruikten we de WAG stam.

Hoofdstuk 2

In een klein aantal experimenten met muizen van verschillende leeftijden werden positieve effecten van een semi-chronische of chronische toediening van choline of lecithine op geheugenprestaties gevonden (Bartus, Dean, Goas, & Lippa, 1980, Leathwood, Heck, & Mauron, 1982, Davis, & Trombetta, 1984 Mervis, Horrocks, Wallace, & Naber, 1984, Muma, & Rowell, 1984)

In deze experimenten moesten dieren leren een donker compartiment te vermijden waarin ze 24 uur eerder een lichte electrische schok toegediend hadden gekregen (inhibitoir vermijden), of om op een signaal naar de andere kant van een uit twee compartimenten bestaande box over te lopen, om zo een lichte schok te vermijden (actief vermijden, beschreven in Appendix A) Wij onderzochten, of gelijksoortige effecten van een chronisch choline-verrijkt dieet ook bij ratten konden worden gevonden

We vonden dat langdurige choline verrijking geen enkel effect had op het vermijdingsleren van BN en WAG ratten Jonge BN ratten presteerden echter veel slechter dan WAG ratten

Hoofdstuk 3

Vervolgens werden de effecten van de choline-behandeling op leren en geheugen in taken bestudeerd, die geen gebruik maken van straf (een lichte schok), maar van een voerbeloning In de eerste van deze appetitieve taken leerden ratten, om voor het verkrijgen van een voerbeloning op een hefboom te drukken De aanleerfase was erg kort en werd beëindigd, zodra een dier 16 keer een beloning had behaald Vierentwintig uur later werd gekeken of de cholinebehandeling invloed had op het onthouden van het gedrag (drukken op een hefboom) waarmee het voer kon worden verdiend Wederom had de behandeling met choline geen enkel effect op het onthouden over een 24-uur interval Zowel BN als WAG ratten bleken na 24 uur niets vergeten te zijn er was geen verschil in prestatie tussen beide stammen

Hoofdstuk 4

Het aanleren en onthouden van complexe discriminatietaken werd in een volgende serie experimenten bestudeerd In tijddiscriminatie' taken moesten de proefdieren leren dat alléén een wachttijd van tenminste acht seconden (eerste twee experimenten) of 16 seconden (derde experiment) tussen twee drukken op een hefboom leidt tot een voerbeloning Iedere druk op een hefboom die minder dan acht of 16 seconden na de vorige wordt uitgevoerd levert geen voerbeloning op Enkele weken na het einde van de aanleerfase werd gekeken of de cholinebehandeling positieve effecten op het onthouden van deze tijddiscriminaties had De chronische cholineverrijking verbeterde noch de leer- noch de geheugenprestatie van de ratten op deze taken

De cholinebehandeling had bij de WAG ratten een sterk verstorend effect op het aanleren van de moeilijkere van de twee tijddiscriminatie taken (waarin een wachttijd van tenminste 16 seconden na de laatste druk op de hefboom leidt tot een voerbeloning)

Twee alternatieve, maar elkaar niet uitsluitende verklaringen voor dit resultaat worden besproken De eerste verklaring veronderstelt dat door de beschikbaarheid van extra choline de opslagsnelheid in het geheugen voor tijdsintervallen wordt verhoogd, waardoor de tijdsintervallen systematisch te kort worden ingeschat (Meck, & Church, 1987a, 1987b) Deze verklaring gaat dus uit van een in principe positief effect op de geheugenfunctie (hogere opslagsnelheid),

die door haar specifieke werking tot een negatief resultaat op de tijdsdiscriminatie leidt De vraag blijft onbeantwoord waarom dit effect beperkt is tot de WAG stam

Recentelijk werd ontdekt (van Luijtelaar & Coenen, 1986) dat WAG ratten een hoge frequentie van een electrofysiologische abnormaliteit, zogenaamde spike-wave complexen, vertonen Deze treden op bij absence epilepsie De BN rat vertoont een dergelijk fenomeen niet

De tweede verklaring voor het slechte leren van choline-behandelde WAG ratten in de tijd discriminatie taak veronderstelt dat choline op een ons niet bekende manier de frequentie en/of de ernst van de toevallen verhoogt De betrokkenheid van acetylcholine bij absence epilepsieen is onduidelijk Naast een verhoging van de synthese van acetylcholine, of door directe werking als cholinerge agonist, kan de toegediende choline ook via een niet farmacologisch effect, b v door invloed op de samenstelling en vloeibaarheid van celmembranen (Farooqui, Liss, & Horrocks, 1988) de electrofysiologische abnormaliteiten bij de WAG rat hebben beinvloed Het is denkbaar dat de behandeling met choline de exciteerbaarheid van de membranen in zenuwcellen verhoogt en daardoor de frequentie en ernst van de absences beinvloedt

Beide verklaringen zijn zeer speculatief Zij zijn echter experimenteel toetsbaar

Hoofdstuk 5

In een ruimtelijke discriminatie taak moesten de proefdieren leren om in een apparaat *met 16 potentiele vindplaatsen van voer (een holeboard) onderscheid te maken tussen de* vier plaatsen waar voer kon worden gevonden en de overige twaalf plaatsen waar nooit voer lag (referentiegeheugen) Bovendien leerden ze tijdens een zoektocht de plaatsen waar ze het voer hadden gevonden maar één keer te bezoeken (werkgeheugen) We vonden een leeftijdsafhankelijke afname van het ruimtelijke werk- en referentiegeheugen in een cross-sectioneel onderzoek met 4, 13, 19, 25, en 30 maanden oude ratten Een behandeling met choline kon echter deze leeftijdsafhankelijke verslechtering van spatieel discriminatieleren niet voorkomen

Hoofdstuk 6

De vraag of een chronische behandeling met choline een effect op meer basale gedragingen had werd in Hoofstuk 6 aan de orde gesteld Onderzocht werd de reactie van de proefdieren wanneer ze in een hen onbekende nieuwe omgeving werden geplaatst en de 24-uurs ritmiek van hun drinkgedrag Op deze gedragingen werden geen effecten van de choline behandeling gevonden

Hoofdstuk 7

Tenslotte bestudeerden we, welke effecten de choline-behandeling had op indicatoren voor de activiteit van het cholinerge systeem Gekeken werd naar de activiteit van het enzym choline acetyltransferase dat betrokken is bij de synthese van de neurotransmitter ACh en naar de activiteit van het natrium-afhankelijke transportsysteem voor choline dat verantwoordelijk is voor het transport van choline van buiten de cholinerge zenuweindiging door de membraan naar binnen (het zogenaamde sodium-depent high affinity choline uptake (SDHACU) systeem)

We deden bovendien een exploratief onderzoek naar de leeftijdsafhankelijke veranderingen van deze indicatoren bij de BN rat, door beide maten bij ratten te meten die in leeftijd varieerden van 16 tot 141 weken

We vonden dat de BN rat een duidelijk lagere cholinerge activiteit had, met name in de cortex Leeftijdsafhankelijke veranderingen in de cholinerge activiteit van de cortex werden niet gevonden De activiteit van het SDHACU-systeem in de hippocampus vertoonde een omgekeerde U-vormige verandering met de leeftijd Na een toename tot de leeftijd van een jaar nam de activiteit van dit systeem af De activiteit van het enzym choline acetyltransferase vertoonde een leeftijdsafhankelijke toename

Samenvattend We vonden dat de BN rat inderdaad een sterke deficientie bij het leren en onthouden in de taken vertoonde, waarin straf werd toegepast Bovendien was de cholinerge activiteit van de BN rat lager dan die van de WAG rat In de taken echter waarin voerbeloning werd toegepast, vertoonden de BN ratten geen deficienties van leren en geheugen We concluderen dan ook, dat de BN rat niet beschouwd kan worden als een 'genetisch model' voor de leeftijdsafhankelijke verslechtering van cognitieve functies

Chronische verrijking van het dieet met choline had geen positief effect op leren en geheugen Er werden aanwijzingen gevonden, dat de choline-behandeling kan leiden tot een zekere verlaging van 'emotionele reactiviteit' De evidentie voor een dergelijk effect is echter niet erg overtuigend, omdat dit effect, afhankelijk van het experiment helemaal niet, óf alléén bij de WAG, óf alléén bij de BN, of bij beide stammen werd gevonden

We vonden een duidelijke leeftijdsgerelateerde verslechtering in de ruimtelijke discriminatietaak, en sterke aanwijzingen voor een dergelijke afname in de inhibitoire vermijdingstaak Er was echter geen sprake van een algehele achteruitgang van de cholinerge activiteit bij de door ons onderzochte BN ratten Gelijksoortige resultaten werden door De Koning-Verest (1981) voor WAG ratten gerapporteerd

De achteruitgang in leren en geheugen blijkt in de door ons gebruikte diermodellen niet parallel te lopen met veranderingen in het cholinerge systeem Deze resultaten zetten vraagtekens bij de veronderstelde samenhang tussen de cholinerge neurotransmissie en leren en geheugen Uiteraard kan uit onze resultaten geen argument worden ontleend tegen de 'cholinerge hypothese van geriatrische geheugen dysfuncties' bij de mens Andere diermodellen voor het toetsen van deze hypothese zullen moeten worden ontwikkeld

De toegepaste 'precusor therapie' had niet het beoogde effect De ontmoedigende resultaten in de klinische praktijk, gevoegd bij de zeer schaarse ondersteuning vanuit dierexperimenteel onderzoek, maken duidelijk dat de precusor therapie' als een onbruikbare behandelingsstrategie in de strijd tegen de afname van cognitieve functies ten gevolge van veroudering en van dementieen moet worden beschouwd

APPENDIX A

EFFECTS OF CHRONIC DIETARY CHOLINE SUPPLEMENTATION ON THE BEHAVIOR OF BN/BiRij AND WAG/Rij RATS IN A TWO-WAY ACTIVE AVOIDANCE TASK

In collaboration with J.N.F. van Betteray

SUMMARY

Ninety-seven weeks old BN and WAG rats were trained in a two-way active avoidance task for 50 trials after having been on a choline-enriched diet for 90 weeks. We investigated whether choline enrichment would improve the acquisition of this task, whether the treatment would affect the 'emotional reactivity' of the rats, and whether the poor performance of BN rats in the inhibitory avoidance task, which was found in a previous study, would reflect a more general impairment in shock motivated tasks. The dietary choline enrichment did not improve the acquisition of the active avoidance response. The treatment affected the number of inter-trial crossings in the WAG strain: this behavior increased in the course of training in this group while it remained unchanged in the other groups. This finding might be considered to indicate a reduced 'emotional reactivity' of the treated WAG rats. As the BN rats performed very poorly in the two-way active avoidance task, it is concluded that this strain shows a general impairment in shock-motivated tasks.

INTRODUCTION

Leathwood and co-workers (Leathwood, Heck, & Mauron, 1982) reported that 8% phosphatidylcholine added to a casein based diet for four days prior to training increased the active avoidance performance of 17-month-old, but not of 6-month-old SEC/1ReJ mice. Phosphatidylcholine is a substance from which choline can be liberated in neurones (Leathwood, & Schlosser, 1986).

The aim of the present experiment was, firstly, to investigate whether chronic dietary choline supplementation would influence two-way active avoidance (shuttle-box avoidance) learning in the inbred BN/BiRij and WAG/Rij strains of rats. Secondly, we investigated whether, apart from effects on learning, chronic dietary choline enrichment would affect traits associated with 'emotionality'. Results that could be considered to indicate that choline enrichment has an effect on the reduction of 'emotional reactivity' had been obtained in the passive avoidance experiments, which are described in Chapter 2. Thirdly, we questioned whether Brown Norway rats show a general impairment of their performance in shock-motivated tasks, or whether the poor performance observed in the inhibitory avoidance task (see Chapter 2) was caused by an inability of these rats to inhibit the step-through response.

Material and Methods

Animals: A total of 32 male rats from the two inbred strains were used (see Appendix C, third experimental protocol). Two littermates from eight litters of both strains were used. One of the littermates had been on a choline-enriched diet for 92 weeks before shuttle-box avoidance behavior was tested. The other littermate served as untreated control. The animals were tested when they were 97 weeks old.

Apparatus: Two shuttle-boxes (40 * 25 * 45 cm) were used (Van Hulzen & Coenen, 1979). Thus, two rats could be trained simultaneously and independently. The boxes were placed in sound-attenuating cubicles. A three cm high barrier separated the two shuttle compartments.

Methods: All testing was done between 14:00 and 18:00. The rats were put in the shuttle-box and were allowed to habituate to the apparatus for five minutes. 'Pre-session' crossings were recorded during this period. During the training session, which immediately followed the habituation period, the inter-trial intervals ranged at random from 40 to 64 s. The UCS (0.25 mA scrambled footshock) started eight seconds after onset of the CS (a 4000 Hz tone). Both stimuli were terminated when the animal moved to the other compartment or when ten seconds had elapsed from the start of the UCS if no avoidance or escape response had been made. The animals received fifty trials in one session (cf. van der Staay, Raaijmakers, & Kerbusch, 1983).

The mean number of avoidances and inter-trial crossings per block of ten trials was calculated. The general means (scores averaged over the five trialblocks) and orthogonal trend coefficients were calculated on these trialblocks. The 'pre-session' crossings, the total number of boli produced during the whole session (including the habituation period), and the general means and trend components of the number of avoidances and inter-trial crossings were then analyzed by a strain (BN vs. WAG) by treatment (untreated vs. choline-enriched) ANOVA.

Results

No differences between groups were found for the number of 'pre-session' crossings (all $Fs_{1,28}$ with associated probabilities > 0.10). The WAG rats produced more boli than the BN rats (mean ± SE_M for WAG: 5.69 ± 0.47, for BN: 3.81 ± 0.43; $F_{1,28} = 8.27$, $p < 0.01$).

Analysis of the general means of avoidances over the five trialblocks revealed that the WAG rats avoided better than the BN rats ($F_{1,28} = 28.65$, $p < 0.01$) (see Fig. A1, upper panel). The increase in the number of avoidances over the blocks was characterized by a general linear trend ($F_{1,28} = 6.02$, $p < 0.01$). Ninety-one percent of the variation over blocks may be predicted from a linear regression equation. The rate of avoidance learning was higher in the WAG rats than in the BN rats ($F_{1,28} = 17.38$, $p < 0.01$). Dietary choline enrichment had no effect on the acquisition of avoidance responses.

Analysis of the inter-trial crossings revealed somewhat puzzling results. The number of inter-trial crossings changed linearly ($F_{1,28} = 5.91$, $p < 0.01$), and this particular trend component explained 79% of the variation over the trialblocks. Although inspection of the lower panel of Fig. A1 gives the impression that the choline enrichment might have predominantly caused an increase of inter-trial crossings in the treated WAG rats, the effect was not confirmed statistically by the ANOVA (strain by treatment interaction for the linear trend component: $F_{1,28} = 2.89$, $p > 0.10$). Post-hoc Duncan tests on the linear trend coefficients, however, confirmed that the number of inter-trial crossings over the successive blocks in the chronically

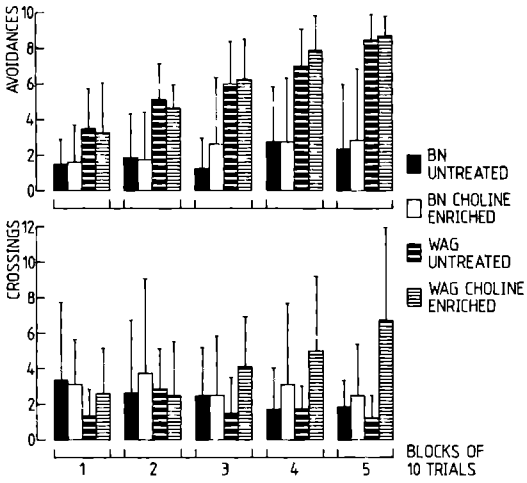


FIGURE A.1: Number of avoidances (upper panel) and number of inter-trial crossings (lower panel) of untreated and dietary choline-enriched BN and WAG rats in an active avoidance test. The vertical bars indicate standard deviations.

choline-enriched WAG rats differed from that of the other groups. For the individual strain by treatment groups, *t*-tests on the linear trend coefficients revealed that the inter-trial crossings did not change in the untreated WAG rats or in either of the BN groups (no individual linear trend coefficient differed from zero). However, a positive linear trend component was found for the choline-treated WAG rats ($t_7 = 3.12$, $p < 0.05$), indicating that their inter-trial crossings increased in the course of training.

DISCUSSION

Chronic dietary choline enrichment had no effect on active avoidance learning. The results obtained by Leathwood and co-workers with mice (Leathwood, Heck, & Mauron, 1982) were thus not observed with rats.

Choline supplementation, however, enhanced the number of inter-trial crossings of the WAG rats in the course of training. This treatment effect was not found in the BN strain. A heightened activity in the shuttle-box apparatus might be considered to indicate lower 'emotional reactivity', because the rat re-entered the 'unsafe' part of the apparatus. The increasing number of inter-trial crossings was not accompanied by poorer learning in the choline-enriched rats, as the treated and untreated WAG rats did not differ on the number of avoidance responses. The number of boli, which may be used as an additional index for 'emotional reactivity' (Broadhurst, 1957, Gentsch, Lichtsteiner, & Feer, 1981) was not affected by chronic dietary choline enrichment. A more direct test of the possible effects of chronic dietary choline enrichment on 'emotional reactivity' is needed, as the results of the present experiment concerning this question were not conclusive.

Our results confirm that BN rats show a more general impairment in shock-motivated tasks. Thus, there is no simple explanation for the results of the passive avoidance experiments described in Chapter 2. If the inability to inhibit the step-through response, perhaps caused by shock-induced activity, would have been the main reason for the poor inhibitory avoidance performance (described in Chapter 2), then active avoidance learning should have been

facilitated High locomotor activity is incompatible with the freezing' response, which retards or completely prevents acquisition of the active avoidance response (Driscoll, & Battig, 1982) The level of activity per se, however, might be a trait relatively independent from avoidance behavior Brush and co-workers (Brush, Froehlich, & Sakellaris, 1979) demonstrated that both traits respond independently to genetic selection

Although the BN rats showed a poorer performance in inhibitory and active avoidance tasks these deficiencies probably do not reflect a general impairment of their ability to acquire or retain avoidance behavior There is growing evidence that these results are caused by a reduced responsiveness to stressful stimuli This is a characteristic of the BN strain The reduced responsiveness has been found for both physiological and behavioral characteristics (e g McCarty, & Kopin, 1978, Gilad, & Jimerson, 1981, Gilad, & McCarty, 1981, McCarty, Kirby, & Garn, 1984)

APPENDIX B

GROWTH CURVES AND WATER CONSUMPTION OF UNTREATED AND CHRONICALLY DIETARY CHOLINE-ENRICHED BN AND WAG RATS

SUMMARY

Beninger and co-workers reported that manipulation of the amount of choline given to rats in their food affected their activity in a running wheel. Both the choline-enriched and the choline-deficient rats were fed the same purified choline-deficient chow which was supplemented with choline in the enrichment condition. The choline-enriched and the choline-deficient rats showed reduced locomotor activity during the dark phase of the light/dark cycle compared with control rats which were fed standard rat chow. The results of Beninger et al. might have been caused by an effect of the diets on the growth of the rats and not by diet-induced changes in central cholinergic activity In our own experiments enriched and control animals received the same standard rat chow. Choline enrichment was achieved by adding choline chloride to the drinking water Contrary to the method used by Beninger et al., our method of manipulating the levels of choline in the diet did not influence the growth of the treated rats. Moreover, the presence of choline chloride in the drinking water did not affect liquid consumption Thus, the effects of chronic dietary choline enrichment found in our own study are most likely not induced by non-specific effects of the treatment, but reflect direct effects of the supplemented choline.

INTRODUCTION

Beninger and co-workers (Beninger, Tighe, & Jhamandas, 1984) studied the effects of chronic dietary choline manipulations on the locomotor activity of rats in a running wheel and reported conflicting results. A chronically choline-enriched group of rats received a choline chloride supplemented purified diet (70 mg per gram). A choline-deficient group received a purified diet that contained about 0.5 mg choline chloride per gram. The control group was fed standard rat chow (Purina), which contained about 1.6 mg choline chloride per gram. Compared with the control rats, the rats of the choline-enriched and choline-deficient groups showed reduced locomotor activity during the first eight hours of the dark phase of the light/dark cycle. However, the body weights of the rats belonging to the different treatment conditions were considerably different in Beninger's study. These non-specific treatment effects may, at least partially, have been responsible for the conflicting results found in his study The animals of the choline-enriched group lost weight as soon as the choline treatment started. In order to stop the weight loss, the choline-enriched food of this group had to be supplemented with normal rat chow. Even then, the choline-enriched animals did not regain weight to the level of the normal controls. The choline-deficient group, on the other hand, gained more weight than the normal control animals.

Contrary to Beninger et al. (1984), we supplied choline chloride in the drinking water. All the rats had access to the same standard laboratory chow (Hope Farms).

The aim of the present study was to examine whether choline enrichment of the drinking water would influence the growth curves of the BN or the WAG rats In addition we investigated, whether the amount of water drunk would be affected by the presence of choline chloride in the drinking water

Material and Methods

The data of the animals from two shipments were analyzed

First shipment A total of forty male rats were used (see Appendix C second experimental protocol) Ten litters with two littermates each of the pigmented BN/BiRij and of the albino WAG/Rij strains were supplied by the TNO Institute for Experimental Gerontology Rijswijk The Netherlands at the age of three weeks They were housed in pairs in standard Makrolon™ cages and were accustomed to a reversed day/night cycle (lights on from 20·00 to 08·00) When the animals were five weeks old one animal of each litter was randomly assigned to the choline enrichment condition Animals from two different litters within the same treatment condition were housed in pairs in one cage, when dietary choline supplementation started Choline chloride ($C_5H_{14}ClNO$, Merck, 2·5 mg per ml) was added to the drinking water of these animals The rats were housed individually in standard Makrolon™ cages at the age of 26 weeks Behavioral testing started when the rats were 31 weeks old The animals were weighed once a week from week 5 to 32 From week 27 to 32, the 24-hour liquid consumption was measured once a week

Second shipment Thirty male BN/BiRij and thirty WAG/Rij rats (ten litters of three pups for both strains) were supplied by the TNO Institute for Experimental Gerontology at the age of five weeks (see also Appendix C third experimental protocol) They were housed in pairs in standard Makrolon™ cages and were accustomed to a reversed day/night cycle (lights on from 20·00 to 08·00) One or two rats per litter were randomly assigned to the choline enrichment condition (2·5 mg choline chloride/ml tap water) at the age of seven weeks Animals from two different litters within the same treatment condition were housed in pairs in one cage when choline supplementation started (in four cages, one BN and one WAG rat were housed together) The choline-enriched and untreated control groups consisted of 15 animals each The rats were housed individually in standard Makrolon™ cages when they were 28 weeks old The animals were weighed once a week from week 5 to 32

Results

Growth curves were analyzed using the data obtained from both shipments of rats by a four factorial ANOVA (shipment by strain by treatment by age) with repeated measures on the last factor As no differences between shipments were found (all $Fs < 1·0$), data from animals of both shipments were pooled and subjected to a three factorial ANOVA (strain by treatment by age) with repeated measures on the last factor Each strain by treatment group consisted of 25 animals The growth curves, starting at five weeks of age, covered a period of 28 successive weeks (see Fig B.1) The two strains had different growth rates (strain by age interaction $F_{27,2592} = 10·39$, $p < 0·01$) Choline enrichment, however, had no effect on growth rate ($Fs_{27,2592} < 1·0$, ns)

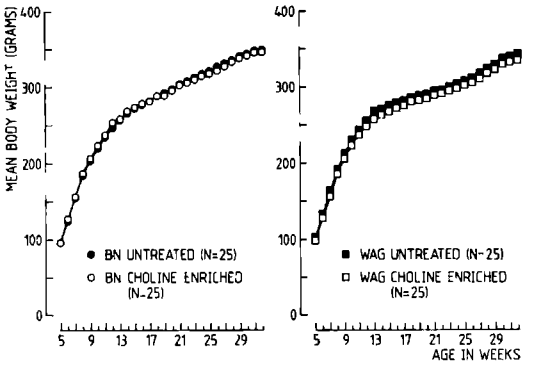


FIGURE B.1: Growth curves of untreated and dietary choline-enriched male BN (left panel) and WAG rats (right panel) from 5 to 32 weeks of age.

The consumption of water was measured in the rats from the first shipment. The average volume of water drunk (in ml) during 24 hours over a period of six weeks was determined for each animal. The average volumes were then subjected to a strain (BN vs. WAG) by treatment (untreated vs. choline-enriched) ANOVA. The BN rats drank more (volume ± standard deviation: 26.8 ± 1.5 ml) during 24 hours than animals from the WAG strain (21.4 ± 3.3 ml) ($F_{1,36} = 42.20$, $p < 0.01$). Chronic dietary choline enrichment did not affect the volume of water drunk ($Fs_{1,36} < 1.0$, ns).

DISCUSSION

Contrary to the data presented by Beninger et al. (1984), choline-enriched drinking water in our experiments neither influenced the growth of BN and WAG rats nor did it affect the consummatory behavior of these rats.

It should be pointed out, that we did not find any evidence for differential effects of chronic dietary choline enrichment on these basal parameters, even after extended periods (up to approximately 2 years) of treatment. For all experiments involving food deprivation (for example appetitively motivated learning in operant conditioning paradigms and spatial discrimination, see Chapters 3, 4, and 5) the ad lib weights of choline-enriched and untreated rats are available. Choline enrichment effects on the free-feeding body weights were never found.

Thus, it is very unlikely that non-specific effects of the diet, for example on growth and liquid consumption, may have been responsible for the treatment effects found in our study (compare Chapters 2, 4, and 7, and Appendix A).

APPENDIX C

EXPERIMENTAL PROTOCOLS

FIRST EXPERIMENTAL PROTOCOL

Age (weeks)	Events
13	arrival at our laboratory: female and male BN/BiRij and WAG/Rij rats (n = 10 per strain by sex group):
16	individual housing
21	reversed day/night cycle (lights on from 20:00 to 8:00)
23	open field test
24	one-way inhibitory avoidance (24-hour retention interval)
25	gradual reduction to 85% of ad libitum body weight
26	operant conditioning: magazine training
27	operant conditioning: retention of incompletely acquired bar-press response
28	operant conditioning: training on a CRF (continuous reinforcement) schedule on three consecutive days operant conditioning: training on a simultaneous discrimination schedule on five consecutive days
29-31	ad libitum access to food
32	gradual reduction to 85% of ad libitum body weight operant conditioning: retention of simultaneous discrimination
35/36	decapitation for biochemical assays

SECOND EXPERIMENTAL PROTOCOL

Age (weeks)	Events
3	arrival at our laboratory: male BN/BiRij and WAG/Rij rats (n = 20 per strain) reversed day/night cycle (lights on from 20:00 to 8:00)
5	start choline enrichment (n = 10 per strain; 2.5 mg choline chloride/ml water)
26	individual housing
31	one-way inhibitory avoidance: 24-hour retention
32	one-way inhibitory avoidance: second retention eight days later
36	gradual reduction to 85% of ad libitum body weight
37	operant conditioning: magazine training operant conditioning: retention of incompletely acquired bar-press response
38/39	operant conditioning: DRL 8" training (differential reinforcement of low-rate responding) on 12 consecutive days
40-42	ad libitum access to food
43	gradual reduction to 85% of ad libitum body weight operant conditioning: retention of simultaneous discrimination
46	decapitation for biochemical assays

THIRD EXPERIMENTAL PROTOCOL

Age (weeks)	Events
5	arrival at our laboratory: male BN/BiRij and WAG/Rij rats (n = 30 per strain)
	reversed day/night cycle (light on from 20:00 to 8:00)
7	start choline enrichment (n = 15 per strain; 2.5 mg choline chloride/ml water)
28	individual housing
33	choline concentration doubled (5 mg choline chloride / ml water)
43	gradual reduction to 85% of ad libitum body weight
44	operant conditioning: magazine training
45	operant conditioning: hand-shaping on a CRF schedule
45/46	operant conditioning: one session DRL 1" training
	operant conditioning: DRL 8" training on 14 consecutive days
47-52	free access to food
53	gradual reduction to 85% of ad libitum body weight
54	operant conditioning: one retention session of DRL 8" responding
	operant conditioning: two extinction sessions on DRL 8" schedule
55	holeboard: adaptation sessions (5 consecutive days)
56/57	holeboard: acquisition of problem A on 12 consecutive days
58/59	ad libitum access to food
60	gradual reduction to 85% of ad libitum body weight
61	holeboard: retention and re-acquisition of problem A on 2 consecutive days
	holeboard: acquisition of problem B on 4 consecutive days
62/63	operant conditioning: DRL 16" training on 13 consecutive days
64-79	ad libitum access to food
68	one-way inhibitory avoidance: retention interval of 24 hours
80	food deprivation to 85% of ad libitum weight
81	radial maze: adaptation sessions (5 consecutive days)
82/83	radial maze: acquisition (all arms baited) on 12 consecutive days
84-114	ad libitum access to food
86-88	perfusion of 21 animals for histology
89-104	activity alley: adaptation; 24-hour drinking pattern on 3 consecutive days
97	two-way active avoidance: 50 massed trials
114	gradual reduction to 85% of ad libitum body weight
115	cone-field: adaptation sessions (5 consecutive days)
116/117	cone-field: acquisition (all cones baited)
120	perfusion for histology

FOURTH EXPERIMENTAL PROTOCOL

Age (months)	Events
3,12,18,24,29	arrival at our laboratory: male BN/BiRij rats (n's = 10, 10, 10, 10, and 5, resp. for age groups) adaptation to reversed day/night cycle within 11 days (schedule: 12 hours light, 13 hours dark)
4,13,19,25,30	gradual reduction to 85% of ad libitum body weight within one week holeboard: adaptation sessions (5 consecutive days) holeboard: acquisition of problem A on 12 consecutive days Subjects were deprived differentially during training: • 4-month-old: 85% plus correction for growth • 13-month-old: 85% without correction for growth • 19-month-old: from 85% to 82.5% • 25-month-old: from 85% to 80% • 30-month-old: from 85% to 77.5%
5,14,20,26,31	free access to food

FIFTH EXPERIMENTAL PROTOCOL

Age (weeks)	Events
90	arrival at our laboratory: male BN/BiRij and WAG/Rij rats (n = 24 per strain)
93	individual housing adaptation to reversed day/night cycle within 11 days (schedule: 12 hours light, 13 hours dark)
94	start choline enrichment (n = 12 per strain; 5 mg choline chloride / ml water)
106	open field test: 5-min sessions on 5 consecutive days
109	gradual reduction to 85% of ad libitum body weight
110	holeboard: adaptation sessions (5 consecutive days)
111/112	holeboard: acquisition of problem A on 12 consecutive days
113	holeboard: acquisition of problem B on 4 consecutive days
113/114	radial maze: adaptation sessions (5 consecutive days)
114	radial maze: acquisition (all arms baited) on 12 consecutive days
115-120	ad libitum access to food
119	one-way inhibitory avoidance: retention interval of 9 days

Alescio-Lautier, B., & Soumireu-Mourat, B. (1986). Comparison of retention and extinction of a visual discrimination as an index of forgetting in mice. *Animal Learning & Behavior, 14*(2), 197-204.

Altman, P.L., & Katz, D.D. (Eds.), (1979). *Inbred and genetically defined strains of laboratory animals, Part I: Mouse and rat.* Bethesda: Federation of American Societies for Experimental Biology.

American Psychiatric Association (1987). *DSM-III-R: Diagnostic and statistical manual III* (3rd ed., revised). Washington D.C.: APA Press.

Ammassari-Teule, M., & Caprioli, A. (1985). Spatial learning and memory, maze running strategies and cholinergic mechanisms in two inbred strains of mice. *Behavioural Brain Research, 17,* 9-16.

Anderson, A. (1986). Japan faces the old old problem. *Nature, 321,* 553.

Anderson, A.C. (1940). Evidences of reminiscence in the rat in maze learning. *Journal of Comparative Psychology, 30,* 399-412.

Anger, D. (1956). The dependence of interresponse times upon the relative reinforcement of different interresponse times. *Journal of Experimental Psychology, 52,* 145-161.

Arendash, G.W., Millard, W.J., Dunn, A.J., & Meyer, E.M. (1987). Long-term neuropathological and neurochemical effects of nucleus basalis lesions in the rat. *Science, 238,* 952-956.

Arendt, T., Bigl, V., & Arendt, A. (1984). Neurone loss in the nucleus basalis of Meynert in Creutzfeldt-Jakob disease. *Acta Neuropathologica, 65,* 85-88.

Arendt, T., Bigl, V., Arendt, A., & Tennstedt, A. (1983). Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's disease. *Acta Neuropathologica (Berlin), 61,* 101-108.

Armstrong, D.M., Hersh, L.B., & Gage, F.H. (1988). Morphologic alterations of cholinergic processes in the neocortex of aged rats. *Neurobiology of Aging, 9,* 199-205.

Atweh, S., Simon, J.R., & Kuhar, M.J. (1975). Utilization of sodium-dependent high affinity choline uptake in vitro as a measure of cholinergic neurons in vivo. *Life Sciences, 17,* 1535-1544.

Aulich, D. (1976). Escape versus exploratory activity: An interpretation of rats' behaviour in the open field and a light-dark preference test. *Behavioural Processes, 1,* 153-164.

Aulich, D., & Spielhofen, J. (1977). Effects of situational complexity and repeated testing on rats' behaviour in a light-dark preference situation. *Zeitschrift für Tierpsychologie, 44,* 148-153.

Aulich, D.K., Spielhofen, J., & Raaijmakers, W.G.M. (1974). The influence of adult handling and social isolation on dark preference in albino rats. *Animal Behavior, 22,* 987-990.

Bammer, G. (1982). Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: A review and some new results. *Neuroscience & Biobehavioral Reviews, 6,* 247-296.

Barnes, C.A. (1979). Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *Journal of Comparative and Physiological Psychology, 93,* 74-104.

Barnes, C.A., Nadel, L., & Honig, W.K. (1980). Spatial memory deficit in senescent rats. *Canadian Journal of Psychology/Review of Canadian Psychology, 34,* 29-39.

Bartus, R.T., Dean, R.L., III, & Beer, B (1984) Cholinergic precursor therapy for geriatric cognition Its past its present and a question of its future In J.M Ordy D Harman & R. Alfin-Slater (Eds), *Nutrition in gerontology* (pp 191-225) New York Raven Press

Bartus, R.T., Dean, R.L., III, Beer, B., & Lippa, A.S (1982) The cholinergic hypothesis of geriatric memory dysfunction *Science 217*, 408-417

Bartus R.T., Dean, R.L., Goas, J.A., & Lippa, A.S (1980) Age-related changes in passive avoidance retention Modulation with dietary choline *Science, 209* 301-303

Bartus, R.T., Dean, R.L., Pontecorvo, M.J., & Flicker, C (1985) The cholinergic hypothesis A historical overview current perspective and future directions In D.S Olton, E Gamzu, & S Corkin (Eds), Memory dysfunctions An integration of animal and human research from preclinical and clinical perspectives *Annals of the New York Academy of Sciences, 444*, 332-358

Bartus R.T., Dean R.L., III, Sherman, K.A., Friedman, E., & Beer, B (1981) Profound effects of combining choline and piracetam on memory enhancement and cholinergic function in aged rats *Neurobiology of Aging, 2*, 105-111

Beatty, W.W Bierley R.A., & Boyd, J (1984) Amphetamine disrupts both working and reference memories of rats trained in a radial maze *Behavioral and Neural Biology, 42*, 169 176

Beatty, W.W., Butters, N., & Janowsky D.S (1986) Patterns of memory failure after scopolamine treatment Implications for cholinergic hypotheses of dementia *Behavioral and Neural Biology, 452*, 196-211

Bell J.M., & Lundberg P.K (1985) Effects of a commercial soy lecithin preparation on development of sensorimotor behavior and brain biochemistry in the rat *Developmental Psychobiology, 18* 59-66

Bell J.M & Slotkin, T.A. (1985) Perinatal dietary supplementation with a commercial soy lecithin preparation Effects on behavior and brain biochemistry in the developing rat *Developmental Psychobiology, 18*, 383-394

Bender, K., Adams, M., Baverstock, P.R., den Bieman, M., Bissbort, S., Brdicka, R., Butcher, G.W., Cramer, D.V., von Deimling, O., Festing, M.F.W., Günther, E., Guttmann, R.D., Hedrich, H.J., Kendall, P.B., Kluge R., Moutier, R Simon, B., Womack, J.E., Yamada, J., & van Zutphen, B (1984) Biochemical markers in inbred strains of the rat (Rattus norvegicus) *Immunogenetics 19* 257-266

Beninger, R.J., Tighe, S.A. & Jhamandas, K (1984) Effects of chronic manipulations of dietary choline on locomotor activity, discrimination learning and cortical acetylcholine release in aging adult Fischer 344 rats *Neurobiology of Aging, 5*, 29-34

Bertoni-Freddari, C., Mervis, R.F., Giuli, C., & Pieri, C (1985) Chronic dietary choline modulates synaptic plasticity in the cerebellar glomeruli of aging mice *Mechanisms of Ageing and Development 30*, 1-9

Bierkamper, G.G., & Goldberg, A.M (1979) The effect of choline on the release of acetylcholine from the neuromuscular junction In A. Barbeau, J.H Growdon, & R.J Wurtman (Eds), *Nutrition and the brain, 5*, 243-251 New York Raven Press

Bjorklund, A., & Gage, F.H (1986) Transplantation of basal forebrain cholinergic neurons in the aged rat brain In D.F Swaab, E Fliers, M Mirmiran, W.A. van Gool, & F van Haaren (Eds), Aging of the brain and Alzheimer's disease *Progress in brain research, 70*, 499-512

Blackwood, D.H.R., Martin, M.J., & Howe, J.G (1982) A study of the role of the cholinergic system in amygdaloid kindling in rats *Psychopharmacology, 76*, 66-69

Blusztajn, J.K., Maire, J.C., Tacconi, M.T., & Wurtman, R.J (1984) The possible role of neuronal choline metabolism in the pathophysiology of Alzheimer s disease A hypothesis In R.J Wurtman, S.H Corkin, & J.H Growdon (Eds), *Alzheimer's disease Advances in basic research and therapies* (pp 183-198) Cambridge, Mass., Center for Brain Sciences and Metabolism Charitable Trust

Blusztajn, J.K., & Wurtman, R.J. (1983). Choline and cholinergic neurons. *Science, 221,* 614-620.

Boitano, J.J., Dokla, C.P.J., Mulinski, P., Misikonis, S., & Kaluzynski, T. (1980). Effects of hippocampectomy in an incremental-step DRL paradigm. *Physiology & Behavior, 25,* 273-278.

Bolles, R.C. (1975). *Theory of motivation* (2nd ed.), (p. 381). New York: Harper & Row.

Bond, N.W. (1984). Avoidance, classical, and pseudoconditioning as a function of species-specific defence reactions in high- and low-avoider rat strains. *Animal Learning & Behavior, 12,* 323-331.

Briggs, R.S., Petersen, M.M., & Cook, P.J. (1982). Muscarinic agonist receptor subtypes in aging rat brain. *Neurobiology of Aging, 3,* 259-261.

Brizzee, K.R., & Ordy, J.M. (1979). Age pigments, cell loss and hippocampal function. *Mechanisms of Ageing and Development, 9,* 143-162.

Broadhurst, P.L. (1957). Determinants of emotionality in the rat. I. Situational factors. *British Journal of Psychology, 48,* 1-12.

Brody, J.A. (1985). Prospects for an ageing population. *Nature, 315,* 463-466.

Broks, P., Preston, G.C., Traub, M., Poppleton, P., Ward, C., & Stahl, S.M. (1988). Modelling dementia: Effects of scopolamine on memory and attention. *Neuropsychologia, 26,* 685-700.

Brookes, S., Rawlins, J.N.P., Gray, J.A., & Feldon, J. (1983). DRL performance in rats with medial or lateral septal lesions. *Physiological Psychology, 11,* 178-184.

Brunello, N., Cheney, D.L., & Costa, E. (1982). Increase in exogenous choline fails to elevate the content or turnover rate of cortical, striatal, or hippocampal acetylcholine. *Journal of Neurochemistry, 38,* 1160-1163.

Brush, F.R., Froehlich, J.C., & Sakellaris, P.C. (1979). Genetic selection for avoidance behavior in the rat. *Behavior Genetics, 9,* 309-315.

Bunch, M.E., & Magdsick, W.K. (1933). The retention in rats of an incompletely learned maze solution for short intervals of time. *Journal of Comparative Psychology, 16,* 385-409.

Burek, J.D. (1978). *Pathology of aging rats.* West Palm Beach: CRC Press, Inc.

Burešová, O., Bolhuis, J.J., & Bureš, J. (1986). Differential effects of cholinergic blockade on performance of rats in the water tank navigation task and in a radial water maze. *Behavioral Neuroscience, 100,* 476-482.

Butcher, L.L., & Woolf, N.J. (1986). Cholinergic systems in the brain and spinal cord: Anatomic organization and overview of functions. In: A. Fisher, I. Hanin, & C. Lachman (Eds.), Alzheimer's and Parkinson's diseases: Strategies for research and development. *Advances in behavioral biology, 29,* 5-16. New York: Plenum Press.

Büttner, D., & Wollnik, F. (1984). Strain-differentiated circadian and ultradian rhythms in locomotor activity of the laboratory rat. *Behavior Genetics, 14,* 137-152.

Campbell, B.A., & Haroutunian, V. (1981). Effects of age on long-term memory: Retention of fixed interval responding. *Journal of Gerontology, 36,* 338-341.

Candy, J.M., Perry, R.H., Perry, E.K., Irving, D., Blessed, G., Fairbairn, A.F., & Tomlinson, B.E. (1983). Pathological changes in the nucleus of Meynert in Alzheimer's and Parkinsons's diseases. *Journal of the Neurological Sciences, 54,* 277-289.

Church, R.M. (1978). The internal clock. In: S.H. Hulse, H. Fowler, & W.K. Honig (Eds.), *Cognitive processes in animal behavior* (pp. 277-310). Hillsdale, N.J.: Lawrence Erlbaum.

Church, R.M. (1984). Properties of the internal clock. In: J. Gibbon, & L. Allan (Eds.), Timing and time perception. *Annals of the New York Academy of Sciences, 423,* 566-582.

Coenen, A.M.L., & van Luijtelaar, E.L.J.M. (1987). The WAG/Rij rat model for absence epilepsy: Age and sex factors. *Epilepsy Research, 1,* 297-301.

Cohen, E.L., & Wurtman, R.J. (1975). Brain acetylcholine: Increase after systemic choline administration. *Life Sciences, 16,* 1095-1102.

Cohen, E L., & Wurtman, R J (1976) Brain acetylcholine Control by dietary choline *Science, 191,* 561-562

Collerton, D (1986) Cholinergic function and intellectual decline in Alzheimer's disease *Neuroscience, 19,* 1-28

Colljn, T H (1985) *Effecten van chronische choline verrijking op leren en geheugen bij CPBB ratten van twee verschillende leeftijden* [Effects of chronic choline enrichment on learning and memory in CPBB rats of two different ages] Unpublished master thesis, University of Nijmegen, The Netherlands

Consolo, S, Ladinsky, H., & Gomeni, R. (1979) Effect of high plasma choline on brain area acetylcholine content Drug intervention *Pharmacological Research Communications, 11*(10) 903-919

Cooper, D O., & Schmidt, D E (1980) The use of choline acetyltransferase as a cholinergic marker in the determination of high-affinity choline uptake *Journal of Neurochemistry, 34,* 1553-1556

Coss, R.G., & Perkel D H (1985) The function of dendritic spines A review of theoretical issues *Behavioral and Neural Biology, 44,* 151-185

Coyle, J T., Price, D L., & DeLong, M.R. (1983) Alzheimer's disease· A disorder of cortical cholinergic innervation *Science 219,* 1184-1190

Craik, F I.M (1977) Age differences in human memory In J.E Birren, & K W Schaie (Eds), *Handbook of the psychology of aging* (pp 384-420) New York Van Nostrand Reinhold Company

Crannell, C W (1942) The choice point behavior of rats in a multiple path elimination problem. *The Journal of Psychology, 13,* 201 222

Crosbie, J (1986) A PASCAL program to perform the Bonferroni multistage multiple-correlation procedure *Behavior Research Methods, Instruments, & Computers, 18,* 327-329

Curcio, C A., & Hinds, J W (1983) Stability of synaptic density and spine volume in dentate gyrus of aged rats *Neurobiology of Aging, 4,* 77-87

Davidson, M., Haroutunian, V, Mohs, R.C, Davis, B.M, Horvath, T B., & Davis, K.L. (1986) Human and animal studies with cholinergic agents How clinically exploitable is the cholinergic deficiency in Alzheimer's disease? In A. Fisher, I Hanin, & C Lachman (Eds), Alzheimer s and Parkinson s disease Strategies for research and development *Advances in behavioral biology, 29,* 531-537 New York Plenum Press

Davies, P (1985) A critical review of the role of the cholinergic system in human memory and cognition In D.S Olton, E Gamzu, & S Corkin (Eds), Memory dysfunctions An integration of animal and human research from preclinical and clinical perspectives *Annals of the New York Academy of Sciences, 444,* 212-217

Davis, H P, Idowu, A., & Gibson, G.E (1983) Improvement of 8-arm maze performance in aged Fischer 344 rats with 3,4-Diaminopyridine *Experimental Aging Research, 9,* 211-214

Davis, H P, & Trombetta, L (1984) Behavioral and neuropathological effects of differential diets in 24 month old C57Bl/6j mice *Society for Neuroscience Abstracts, 10*(2), 776

Davison, A.N (1987) Pathophysiology of ageing brain *Gerontology, 33,* 129-135

Dean, R.L., III, Scozzafava, J., Goas, J A., Regan, B, Beer, B., & Bartus, R.T (1981) Age-related differences in behavior across the life span of the C57Bl/6J mouse *Experimental Aging Research, 7,* 427-451

De Koning-Verest, I.F. (1981) *Observations on the central nervous system of the rat Functional changes in relation to ageing* Doctoral dissertation, University of Utrecht, The Netherlands

De Koning-Verest, I.F, Knook, D L., & Wolthuis, O.L (1980) Behavioral and biochemical correlates of aging in rats In D G Stein (Ed), *The psychobiology of aging Problems and perspectives* (pp 177-199) New York Elsevier North Holland, Inc

Destrade, C, & Cardo, B (1974) Effects of post-trial hippocampal stimulation on time-dependent improvement of performance in mice *Brain Research, 78,* 447-454

Destrade, C., Jaffard, R., Deminiere, J M., & Cardo, B (1976) Effets de la stimulation de l hippocampe sur la réminiscence chez deux lignées de souris *Physiology & Behavior, 16,* 237-243

Destrade, C., Soumireu-Mourat, B., & Cardo, B (1973) Effects of posttrial hippocampal stimulation on acquisition of operant behavior in the mouse *Behavioral Biology, 8,* 713-724

Deutsch, J A. (1983) The cholinergic synapse and the site of memory In J A. Deutsch (Ed), *The physiological basis of memory* (2nd ed), (pp 367-386) New York Academic Press

Deutsch J A., & Rogers, J B (1979) Cholinergic excitability and memory Animal studies and their clinical implications In K.L Davis, & P A. Berger (Eds), *Brain acetylcholine and neuropsychiatric disease* (pp 175-204) New York Plenum Press

Dimitrieva, N., Gozzo, S., Dimitriev, Y., & Ammassari-Teule, M (1984) Mossy fiber distribution in four lines of rats A correlative study with avoidance abilities and excitability thresholds *Physiological Psychology, 12,* 30-34

Dixon, W.J (1950) Analyses of extreme values *Annals of Mathematical Statistics, 21,* 488-506

Dixon, W.J (1951) Ratios involving extreme values *Annals of Mathematical Statistics, 22,* 68-78

Drachman, D A. (1977) Memory and cognitive function in man Does the cholinergic system have a specific role? *Neurology, 27,* 783-790

Drachman, D A., Glosser, G., Fleming, P., & Longenecker, G (1982) Memory decline in the aged Treatment with lecithin and physostigmine *Neurology, 32,* 944-950

Drachman, D A. & Leavitt, J (1974) Human memory and the cholinergic system *Archives of Neurology, 30,* 113-121

Driscoll, P., & Battig, K (1982) Behavioral, emotional and neurochemical profiles of rats selected for extreme differences in active, two-way avoidance performance In I Lieblich (Ed), *Genetics of the brain* (pp 95-123) Amsterdam Elsevier Biomedical Press

Drago, F., Bohus, B, Scapagnini, U, & de Wied, D (1980) Sexual dimorphism in passive avoidance behavior of rats Relation to body weight, age, shock intensity and retention interval *Physiology & Behavior, 24,* 1161-1164

Eckerman, D A., Gordon, W A., Edwards, J D., MacPhail, R.C., & Gage, M.L (1980) Effects of scopolamine, pentobarbital, and amphetamine on radial arm maze performance in the rat *Pharmacology Biochemistry & Behavior, 12,* 595-602

Elias, P.K., & Elias, M.F (1976) Effects of age on learning ability Contributions from the animal literature *Experimental Aging Research, 2,* 165-186

Evans, J A.C., & Thornton, E W (1984) Impaired acquisition of DRL operant responding following lesion of the habenular nucleus *Physiological Psychology, 12,* 220-226

Farooqui, A.A., Liss, L., & Horrocks, L A. (1988) Neurochemical aspects of Alzheimer's disease Involvement of membrane phospholipids *Metabolic Brain Disease, 3,* 19-35

Ferguson, G A. (1971) *Statistical analysis in psychology & education* (3rd ed) (p 220 ff). Tokyo McGraw-Hill Kogakusha, Ltd

Festing, W.M F (Ed) (1980) *International index of laboratory animals* (4th ed) Medical Research Council, Laboratory animals centre, United Kingdom

FitzGerald, R.E., Isler, R., Rosenberg, E, Oettinger, R, & Battig, K (1985) Maze patrolling by rats with and without food reward *Animal Learning & Behavior, 13,* 451-462

Flentge, F., & van den Berg, C.J (1979). Choline administration and acetylcholine in brain *Journal of Neurochemistry, 32,* 1331-1333

Flicker, C., & Geyer, M A. (1982) Behavior during hippocampal microinfusions II. Muscarinic locomotor activation *Brain Research Reviews, 4,* 105-127

Flood, J.F., & Cherkin, A. (1986) Scopolamine effects on memory retention in mice A model of dementia? *Behavioral and Neural Biology 45* 169-184

Flood, J.F., & Cherkin, A. (1988) Effect of acute arecoline, tacrine and arecoline+tacrine post-training administration on retention in old mice *Neurobiology of Aging, 9* 5-8

Fonnum, F (1975) A rapid radiochemical method for the determination of choline acetyl transferase *Journal of Neurochemistry, 24,* 407-409

Freund, G (1980) Cholinergic receptor loss in brains of aging mice *Life Sciences, 26,* 371-375

Freund, R.J., & Littell, R.C (1985) *SAS for linear models.* Cary, N.C SAS Institute Inc

Friedman, E., Lerer, B., & Kuster, J (1983) Loss of cholinergic neurons in the rat neocortex produces deficits in passive avoidance learning *Pharmacology Biochemistry & Behavior, 19* 309-312

Gage F.H Dunnett, S.B., & Bjorklund, A. (1984) Spatial learning and motor deficits in aged rats *Neurobiology of Aging,* 5, 43-48

Gage, F.H., & Evans, S.H (1981) Exploratory analysis of deficits in DRL performance induced by medial and lateral fornix damage *Physiological Psychology 9* 49 53

Gallagher, M., & Pelleymounter, M.A. (1988) Spatial learning deficits in old rats A model for memory decline in the aged *Neurobiology of Aging, 9* 549-556

Gamzu, E (1985) Animal behavioral models in the discovery of compounds to treat memory dysfunction In D.S Olton E Gamzu & S Corkin (Eds.) Memory dysfunctions An integration of animal and human research from preclinical and clinical perspectives *Annals of the New York Academy of Sciences, 444,* 370-393

Gauthier, M., Destrade, C., & Soumireu-Mourat, B (1982) Late post-learning participation of entorhinal cortex in memory processes *Brain Research, 233,* 255-264

Gauthier, M., Destrade, C., & Soumireu Mourat, B (1983) Functional dissociation between lateral and medial entorhinal cortex in memory processes in mice *Behavioural Brain Research, 9* 111-117

Gauthier M., & Soumireu-Mourat, B (1981) Behavioral effects of bilateral entorhinal cortex lesions in the BALB/c mouse *Behavioral and Neural Biology, 33,* 419-436

Gentsch, C., Lichtsteiner, M., & Feer, H (1981) Locomotor activity, defecation score and corticosterone levels during an open-field exposure A comparison among individually and group-housed rats, and genetically selected rat lines *Physiology & Behavior, 27,* 183-186

Gentsch, C., Lichtsteiner, M., Kraeuchli, K., & Feer, H (1982) Different reaction patterns in individually and socially reared rats during exposure to novel environments *Behavioural Brain Research, 4* 45-54

Geyer M.A. Russo, P.V., & Masten, V.L (1986) Multivariate assessment of locomotor behavior Pharmacological and behavioral analyses *Pharmacology Biochemistry & Behavior, 25,* 277-288

Gibbon, J., Church, R.M., & Meck, W.H. (1984) Scalar timing in memory In J Gibbon, & L Allan (Eds.), Timing and time perception *Annals of the New York Academy of Sciences, 423,* 52-77

Gilad, G.M., & Gilad, V.H (1981) Strain-dependent differences between the septo-hippocampal cholinergic system and hippocampal size *Brain Research, 222,* 423-427

Gilad, G.M., & Jimerson, D.C (1981) Modes of adaptation of peripheral neuroendocrine mechanisms of the sympatho-adrenal system to short-term stress as studied in two inbred rat strains *Brain Research, 206,* 83-93

Gilad, G.M., & McCarty, R. (1981) Differences in choline acetyltransferase but similarities in catecholamine biosynthetic enzymes in brains of two rat strains differing in their response to stress *Brain Research, 206,* 239-243

Gilad, G.M., Rabey, J.M., & Shenkman, L (1983) Strain-dependent and stress-induced changes in rat hippocampal cholinergic system *Brain Research, 267,* 171-174

Gilad, G.M., & Shiller, I (1989) Differences in open-field behavior and in learning tasks between two rat strains differing in their reactivity to stressors *Behavioural Brain Research, 32* 89-93

Gleiser, C A., & Shain, S A (1986) The aging AXC/SSh rat Assessment of longevity and prevalence of neoplastic and nonneoplastic diseases in necropsied rats *Journal of Gerontology, 41,* 590-598

Godding, P.R., Rush, J.R., & Beatty, W W (1982) Scopolamine does not disrupt spatial working memory in rats *Pharmacology Biochemistry & Behavior, 16,* 919-923

Gold, P.E (1986) The use of avoidance training in studies of modulation of memory storage *Behavioral and Neural Biology 46,* 87-98

Gold, P.E , & McGaugh, J.L (1975) Changes in learning and memory during aging In J.M Ordy, & K.R Brizzee (Eds), *Neurobiology of aging* (pp 145-158) New York Plenum Press

Gold, P.E., McGaugh, J.L , Hankins, L.L., Rose, R.P., & Vasquez, B.J (1981) Age dependent changes in retention in rats *Experimental Aging Research, 8,* 53-58

Goodrick, C L (1966) Activity and exploration as a function of age and deprivation *The Journal of Genetic Psychology, 108,* 239-252

Goodrick, C L (1968) Learning, retention, and extinction of a complex maze habit for mature-young and senescent Wistar albino rats *Journal of Gerontology, 23* 298 304

Goodrick, C L (1975) Behavioral rigidity as a mechanism for facilitation of problem solving for aged rats *Journal of Gerontology, 30* 181 184

Goodrick, C.L (1980) Problem solving and age A critique of rodent research In R.L Sprott (Ed), *Age learning ability and intelligence* (pp 5-25) New York Van Nostrand Reinhold

Gottfries, C -G., Adolfsson, R., Aquilonius, S -M., Carlsson, A., Eckernas, S -Å , Nordberg, A., Oreland, L., Svennerholm L Wiberg, Å, & Winblad, B (1983) Biochemical changes in dementia disorders of Alzheimer type (AD/SDAT) *Neurobiology of Aging, 4,* 261-271

Gray, D.S., & Sainsbury, R.S (1979) The effects of fimbria lesions on cue utilization in the rat *Physiology & Behavior, 22,* 325-329

Greenwood, K., Armstrong, S., & Coleman, G (1981) Failure of periodic presentation of palatable diet to entrain feeding, drinking and activity rhythms under constant conditions *Physiology & Behavior, 27,* 1057-1066

Growdon, J.H (1979) Neurotransmitter precursors in the diet Their use in the treatment of brain diseases In R.J Wurtman, & J.J Wurtman (Eds), *Nutrition and the brain, 3,* 117-181 New York Raven Press

Hagan, J.J , & Morris, R.G.M (1988) The cholinergic hypothesis of memory A review of animal experiments In L L Iversen, S D Iversen, & S.H Snyder (Eds), Pharmacology of the aging nervous system *Handbook of psychopharmacology, 20,* 237-323 New York Plenum Press

Hagan, J.J., Tweedie, F., & Morris, R.G.M (1986) Lack of task specificity and absence of post-training effects of atropine on learning *Behavioral Neuroscience 100,* 483-493

Hamm, R.J., Dixon, C.E., & Knisely, J.S (1984) Long-term memory of a DRL task in mature and aged rats *Experimental Aging Research, 10,* 39-42

Hamm, R.J., Knisely, J.S , & Dixon, C.E (1983) An animal model of age changes in short-term memory The DRL schedule *Experimental Aging Research, 9,* 23-25

Haubrich, D.R., Wang, P.F.L., Clody, D.E., & Wedeking, P W (1975) Increase in rat brain acetylcholine induced by choline or deanol *Life Sciences, 17,* 975-980

Heinsbroek, R.P W., van Haaren, F., & van de Poll, N.E (1988) Sex differences in passive avoidance behavior of rats Sex-dependent susceptibility to shock-induced behavioral depression *Physiology & Behavior 43,* 201-206

Hirsch, M.J , & Wurtman, R.J (1978) Lecithin consumption increases acetylcholine concentrations in rat brain and adrenal gland *Science, 202,* 223-225

Hornberger, J C, Buell, S J, Flood, D G, McNeill, T H, & Coleman, P D (1985) Stability of numbers but not size of mouse forebrain cholinergic neurons to 53 months *Neurobiology of Aging, 6,* 269-275

Hughes, R N (1982) A review of atropinic drug effects on exploratory choice behavior in laboratory rodents *Behavioral and Neural Biology, 34,* 5-41

Huppert, F A, & Deutsch, J A (1969) Improvement in memory with time *Quarterly Journal of Experimental Psychology 21,* 267-271

Ingram, D K (1985) Analysis of age related impairments in learning and memory in rodent models In D S Olton E Gamzu, & S Corkin (Eds), Memory dysfunctions An integration of animal and human research from preclinical and clinical perspectives *Annals of the New York Academy of Sciences, 444,* 312 331

Ingram, D K (1988) Complex maze learning in rodents as a model of age-related memory impairment *Neurobiology of Aging, 9* 475-485

Ingram, D K, London, E D, & Goodrick, C L (1981) Age and neurochemical correlates of radial maze performance in rats *Neurobiology of Aging, 2,* 41-44

Jaffard, R, & Destrade, C (1982) Learning and memory processes as related to genotypic or experimental variations of hippocampal cholinergic activity in inbred strains of mice In I Lieblich (Ed), *Genetics of the brain* (pp 299-322) Amsterdam Elsevier Biomedical Press

Jaffard, R, Destrade, C, Soumireu-Mourat, B, & Cardo, B (1974) Time-dependent improvement of performance on appetitive tasks in mice *Behavioral Biology, 11* 89-100

Jaffard, R, Destrade, C, Soumireu-Mourat, B, Durkin, T, & Ebel, A (1980) Changes in hippocampal cholinergic activity following learning in mice *Neuroscience Letters, 19* 349-352

Jaffard, R, Ebel, A, Destrade, C, Durkin, T, Mandel, P, & Cardo, B (1977) Effects of hippocampal electrical stimulation on long-term memory and on cholinergic mechanisms in three inbred strains of mice *Brain Research, 133,* 277-289

Jenden, D J, Weiler, M H, & Gundersen, C B (1982) Choline availability and acetylcholine synthesis In S Corkin, K L Davis, J H Growdon, E Usdin, & R J Wurtman (Eds), Alzheimer's disease A report of progress *Aging, 19,* 315-326 New York Raven Press

Johnson, C T, Olton, D S, Gage, F H, Gage, F H, III, & Jenko, P G (1977) Damage to hippocampus and hippocampal connections Effects on DRL and spontaneous alternation *Journal of Comparative and Physiological Psychology, 91,* 508-522

Jolles, J (1986a) Cognitive, emotional and behavioral dysfunctions in aging and dementia In D F Swaab, E Fliers, M Mirmiran, W A van Gool, & F van Haaren (Eds), *Progress in brain research, 70,* 15-39

Jolles, J (1986b) Neuropeptides and the treatment of cognitive deficits in aging and dementia In D F Swaab, E Fliers, M Mirmiran, W A van Gool, & F van Haaren (Eds), *Progress in brain research 70,* 429-441

Jope, R S (1982) Effects of phosphatidylcholine administration to rats on choline in blood and choline and acetylcholine in brain. *The Journal of Pharmacology and Experimental Therapeutics, 220,* 322-328

Katzman, R (1986) Alzheimer's disease *The New England Journal of Medicine, 314,* 964-973

Kerbusch, J M L (1974) *Genetic analysis of exploratory behaviour, simple learning behaviour and cerebral AChE and ChE activities in mice by means of the diallel method* Doctoral dissertation, University of Nijmegen, the Netherlands

Kerbusch, S, van der Staay, F J, & Hendriks, N (1981) A searching procedure for transformations and models in a classical Mendelian cross breeding study *Behavior Genetics, 11,* 239-254

Koene, P (1988) *Approach-avoidance conflict and speed of conflict resolution* Doctoral dissertation, University of Nijmegen, The Netherlands

Kopelman, M.D. (1986). The cholinergic neurotransmitter system in human memory and dementia: A review *The Quarterly Journal of Experimental Psychology, 38A,* 535-573.

Kordower, J.H., & Gash, D.M. (1988). Animal models of age- and disease-related cognitive decline: Perspectives on the models and therapeutic strategies *Neurobiology of Aging, 9,* 685-688

Kramer, T.J., & Rilling, M. (1970) Differential reinforcement of low rates: A selective critique. *Psychological Bulletin, 74,* 225-254.

Krnjević, K., & Reinhardt, W. (1979) Choline excites cortical neurons. *Science, 206,* 1321-1323.

Krnjević, K., Reinhardt, W., & Ropert, N. (1982) Choline as an acetylcholine agonist in the mammalian neocortex and hippocampus. In. S. Corkin, K.L. Davis, J.H. Growdon, E Usdin, & R.J Wurtman (Eds.), Alzheimer's disease: A report of progress. *Aging, 19,* 331-344 New York: Raven Press

Kubanis, P., & Zornetzer, S.F. (1981). Age-related behavioral and neurobiological changes: A review with an emphasis on memory *Behavioral and Neural Biology, 31,* 115-172.

Kurtz, T.W., & Morris, R.C., Jr (1987). Biological variability in Wistar-Kyoto rats. *Hypertension, 10,* 127-131

Lachman, S.J., & Brown, C.R. (1957). Behavior in a free choice multiple path elimination problem. *The Journal of Psychology, 43,* 27-40.

Landfield, P.W., Braun, L.D., Pitler, T.A., Lindsey, J.D., & Lynch, G. (1981). Hippocampal aging in rats: A morphometric study of multiple variables in semithin sections *Neurobiology of Aging, 2,* 265-275.

Landfield, P.W., Rose, G., Sandles, L., Wohlstadter, T.C., & Lynch, G. (1977). Patterns of astroglial hypertrophy and neuronal degeneration in the hippocampus of aged, memory-deficient rats *Journal of Gerontology, 32,* 3-12.

Leathwood, P.D., Heck, E &, Mauron, J. (1982). Phosphatidyl choline and avoidance performance in 17 month-old SEC/1ReJ mice *Life Sciences, 30,* 1065-1071.

Leathwood, P.D., & Schlosser, B (1986) Phosphatidylcholine, choline and cholinergic function. *International Journal for Vitamin and Nutrition Research* (Suppl 29), 49-67

Lejeune, H., Jasselette, P., Nagy, J., & Peree, F. (1986). Fixed interval performance in weanling rats: A comparison with adult and senile subjects. *Physiology & Behavior, 38,* 337-343

Levitt, R.A. (1971). Cholinergic substrate for drinking in the rat. *Psychological Reports, 29,* 431-448.

Levy, A., Kluge, P.B., & Elsmore, T.F (1983). Radial arm maze performance of mice: Acquisition and atropine effects. *Behavioral and Neural Biology, 39,* 229-240

Lieberman, H.R., & Abou-Nader, T.M. (1986). Possible dietary strategies to reduce cognitive deficits in old age In: D.F Swaab, E Fliers, M Mirmiran, W.A. van Gool, & F. van Haaren (Eds.), *Progress in brain research, 70,* 461-471.

Lindner, M.D., & Schallert, T. (1988). Aging and atropine effects on spatial navigation in the Morris water task. *Behavioral Neuroscience, 102,* 621-634.

Lipp, H.P., & Schwegler, H. (1982). Hippocampal mossy fibers and avoidance learning. In: I. Lieblich (Ed.), *Genetics of the brain* (pp. 325-364) Amsterdam: Elsevier Biomedical Press.

Lippa, A.S., Pelham, R.W., Beer, B., Critchett, D.J., Dean, R.L., & Bartus, R.T. (1980). Brain cholinergic dysfunction and memory in aged rats. *Neurobiology of Aging, 1,* 13-20

Lowry, O.N., Rosebrough, N.J., Farr, A.L., & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193,* 265-275

Lowy, A.M., Ingram, D.K., Olton, D.S., Waller, S.B., Reynolds, M.A., & London, E.D. (1985). Discrimination learning requiring different memory components in rats: Age and neurochemical comparisons. *Behavioral Neuroscience, 99,* 638-651.

Luine, V.N., Renner, K.J., Heady, S., & Jones, K.J. (1986). Age and sex-dependent decreases in ChAT in basal forebrain nuclei. *Neurobiology of Aging, 7,* 193-198.

Mann, D.M.A. (1988). Neuropathological and neurochemical aspects of Alzheimer's disease. In: L.L. Iversen, S.D. Iversen, & S.H. Snyder (Eds.), Psychopharmacology of the aging nervous system. *Handbook of psychopharmacology, 20,* 1-67. New York: Plenum Press.

Martinez, J.L., Jensen, R.A., & McGaugh, J.L. (1983). Facilitation of memory consolidation. In: J.A. Deutsch (Ed.), *The physiological basis of memory* (pp. 49-70). New York: Academic Press.

Martinez, J.L., & Rigter, H. (1983). Assessment of retention capacities in old rats. *Behavioral and Neural Biology, 39,* 181-191.

Masoro, E.J. (1980). Mortality and growth characteristics of rat strains commonly used in aging research. *Experimental Aging Research, 6,* 219-233.

Masterson, F.A., & Crawford, M. (1982). The defense motivation system: A theory of avoidance behavior. *The Behavioral and Brain Sciences, 5,* 661-696.

McCann, K., Cain, D.P., & Philbrick, D.J. (1983). Facilitation of kindled seizures in rats fed choline-supplemented diets. *The Canadian Journal of Neurological Sciences, 10,* 47-49.

McCarty, R., Kirby, R.F., & Garn, P.G. (1984). Strain differences in sympathetic-adrenal medullary responsiveness and behavior. *Behavioral and Neural Biology, 40,* 98-113.

McCarty, R., & Kopin, I.J. (1978). Sympatho-adrenal medullary activity and behavior during exposure to footshock stress: A comparison of seven rat strains. *Physiology & Behavior, 21,* 567-572.

McDonough, J.H., Jr. (1982). Effects of anticholinergic drugs on DRL performance of rhesus monkeys. *Pharmacology Biochemistry & Behavior, 17,* 85-90.

McGeer, E.G., McGeer, P.L. (1975). Age changes in the human for some enzymes associated with metabolism of catecholamines, GABA, and acetylcholine. In: J.M. Ordy, & K.R. Brizzee (Eds.), Neurobiology of aging. *Advances in Behavioral Biology, 16,* 287-305. New York: Plenum Press.

McGeer, P.L., & McGeer, E.G. (1978). Aging and neurotransmitter systems. In: C.E. Finch, D.E. Potter, & A.D. Kenny (Eds.), Parkinson's disease II. *Advances in experimental medicine and biology, 113,* 41-47. New York: Plenum Press.

McKim, W.A. (1974). The effects of scopolamine and physostigmine on fixed-interval behaviour in the rat. *Psychopharmacology (Berl.), 39,* 237-244.

McNamara, M.C., Benignus, V.A., Benignus, G., & Miller, A.T., Jr. (1977). Active and passive avoidance in rats as a function of age. *Experimental Aging Research, 3,* 3-16.

Meck, W.H. (1983). Selective adjustment of the speed of internal clock and memory processes. *Journal of Experimental Psychology: Animal Behavior Processes, 9,* 171-201.

Meck, W.H., & Church, R.M. (1983). The cholinergic nervous system and temporal memory in rats. *Society for Neuroscience Abstracts, 9,* 478.

Meck, W.H., & Church, R.M. (1987a). Nutrients that modify the speed of internal clock and memory storage processes. *Behavioral Neuroscience, 101,* 465-475.

Meck, W.H., & Church, R.M. (1987b). Cholinergic Modulation of the content of temporal memory. *Behavioral Neuroscience, 101,* 457-464.

Meck, W.H., Church, R.M., & Olton, D.S. (1984). Hippocampus, time, and memory. *Behavioral Neuroscience, 98,* 3-22.

Meijer, J.H. (1989). *Neuropharmacological and photic manipulation of the circadian pacemaker.* Doctoral dissertation, University of Leiden, the Netherlands.

Mervis, R. (1981). Cytomorphological alterations in the aging animal brain with emphasis on Golgi studies. In: J.E. Johnson, Jr. (Ed.), *Aging and cell structure, 1,* 143-186. New York: Plenum Press Corporation.

Mervis, R., Horrocks, L., Demediuk, P., Wallace, L., Meyer, D.R., Beall, S., Caris, K., & Naber, E. (1985). Neurobehavioral effects of chronic choline-containing diets on the adult and aging C57Bl/6NNIA mouse brain. In : D.S. Olton, E. Gamzu, & S. Corkin (Eds.), Memory dysfunctions: An integration of animal and human research from preclinical and clinical perspectives. *Annals of the New York Academy of Sciences, 444,* 469-470.

Mervis, R.F., Horrocks, L.A., Wallace, L.J., & Naber, E. (1984). Influence of chronic choline-containing diets on neurobehavioral parameters in the C57Bl mouse. *Society for Neuroscience Abstracts, 10,* 976.

Mervis, R.F., Parker, K.L., El-Yabroudi, J., Byler, C.L., Scherer, J.A., McLaughlin, M.M., Makely, M.J., Dvorak, R., & Wierdl, M. (1987). Chronic dietary choline and phosphatidylcholine induces dendritic growth in pyramidal cells of aging mouse neocortex. Unpublished laboratory report, Ohio State University, USA.

Meyer, E.M., Onge, E.S., & Crews, F.T. (1984). Effects of aging on rat cortical presynaptic cholinergic processes. *Neurobiology of Aging, 5,* 315-317.

Michel, M.E., & Klein, A.W. (1978). Performance differences in a complex maze between young and aged rats. *Age, 1,* 13-16.

Miller, G.L. (1959). Protein determination for large numbers of samples. *Analytical Chemistry, 31,* 964.

Milligan, G.W., Wong, D.S., & Thompson, P.A. (1987). Robustness properties of nonorthogonal analysis of variance. *Psychological Bulletin, 101,* 464-470.

Mistlberger, R.E., & Rusak, B. (1986). Carbachol phase shifts circadian activity rhythms in ovariectomized rats. *Neuroscience Letters, 72,* 357-363.

Miyamoto, M., Kiyota, Y., Yamazaki, N., Nagaoka, A., Matsuo, T., Nagawa, Y., & Takeda, T. (1986). Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). *Physiology & Behavior, 38,* 399-406.

Mohan, C., & Radha, E. (1978). Circadian rhythms in the central cholinergic system in aging animals. In: H.V. Samis, & S. Capobianco (Eds.), Aging and biological rhythms. *Advances in experimental medicine and biology, 108,* 275-299.

Monk, T.H., & Fort, A. (1983). 'Cosina': A cosine curve fitting program suitable for small computers. *International Journal of Chronobiology, 8*(4), 193-224.

Morley, B.J., & Garner, L.L. (1987). Increases in the concentration of brain nicotinic receptors by dietary choline. In: R.J. Wurtman, S.H. Corkin, & J.H. Growdon (Compilers), *Alzheimer's disease: Advances in basic research and therapies* (pp. 493-498). Campbridge, MA: Center for Brain Sciences and Metabolism Cheritable Trust.

Morrison, B.J., & Thatcher, K. (1969). Overpopulation effects on social reduction of emotionality in the albino rat. *Journal of Comparative and Physiological Psychology, 69,* 658-662.

Mosko, S.S., Erickson, G.F., & Moore, R.Y. (1980). Dampened circadian rhythms in reproductively senescent female rats. *Behavioral and Neural Biology, 28,* 1-14.

Muma, N.A., & Rowell, P.P. (1984). Behavioural effects of long-term choline and lecithin treatment in aged mice. *IRCS Medical Science, 12,* 1090-1091.

Munn, N.L. (1950). *Handbook of psychological research on the rat.* Boston: Houghton Mifflin Company.

Nalwa, V., & Rao, P.S. (1985). DRL responding under uncertain reinforcement in rats after medial frontal cortical lesions. *Behavioral Brain Research, 17,* 73-76.

Oades, R.D. (1981). Impairments of search behaviour in rats after Haloperidol treatment, hippocampal or neocortical damage suggest a mesocorticolimbic role in cognition. *Biological Psychology, 12,* 77-85.

Oades, R.D. (1982). Search strategies on a hole-board are impaired in rats with ventral tegmental damage: Animal model for tests of thought disorder. *Biological Psychiatry, 17,* 243-258.

Oades, R.D., & Isaacson, R.L. (1978) The development of food search behavior by rats: The effects of hippocampal damage and Haloperidol *Behavioral Biology, 24,* 327-337.

Okaichi, H., & Jarrard, L.E. (1982). Scopolamine impairs performance of a place and cue task in rats *Behavioral and Neural Biology, 35,* 319-325

O'Keefe, J., & Nadel, L (1978). *The hippocampus as a cognitive map* (pp. 291-315). Oxford: Clarendon Press

Olton, D.S. (1977). Spatial memory. *Scientific American, 236*(6), 82-98.

Olton, D.S. (1985). Strategies for the development of animal models of human memory impairments. In: D.S. Olton, E Gamzu, & S Corkin (Eds.), Memory dysfunctions An integration of animal and human research from preclinical and clinical perspectives *Annals of the New York Academy of Sciences, 444,* 113-121

Olton, D.S., Becker, J.T., & Handelmann, G.E. (1979). Hippocampus, space, and memory. *The Behavioral and Brain Sciences, 2,* 313-365.

Olton, D.S., Collison, C., & Werz, M.A. (1977). Spatial memory and radial arm maze performance of rats. *Learning and Motivation, 8,* 289-314

Olton, D.S., & Papas, B.C (1979). Spatial memory and hippocampal function. *Neuropsychologia, 17,* 669-682.

Olton, D.S., & Samuelson, R.J. (1976). Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology Animal Behavior Processes. 2*(2), 97-116.

Ordy, J.M., Brizzee, K.R., Kaack, B., & Hansche, J. (1978). Age differences in short-term memory and cell loss in the cortex of the rat *Gerontology, 24,* 276-285

Ossenkopp, K.-P., & Mazmanian, D.S. (1985). The principle of aggregation in psychobiological correlational research: An example from the open-field test. *Animal Learning & Behavior, 13,* 339-344.

Overstreet, D.H., Russell, R.W., Crocker, A.D., & Schiller, G.D. (1984). Selective breeding for differences in cholinergic function: Pre- and postsynaptic mechanisms involved in sensitivity to the anticholinesterase, DFP *Brain Research, 294,* 327-332.

Paré, W.P. (1969). Age, sex and strain differences in the aversive threshold to grid shock in the rat. *Journal of Comparative and Physiological Psychology, 69,* 214-218.

Peng, M.T., & Kang, M. (1984). Circadian rhythms and patterns of running-wheel activity, feeding and drinking behaviors of old male rats. *Physiology & Behavior, 33,* 615-620.

Perry, E.K. (1980). The cholinergic system in old age and Alzheimer's disease. *Age and Ageing, 9,* 1-8.

Perry, E.K. (1986). The cholinergic hypothesis - Ten years on. *British Medical Bulletin, 42,* 63-69

Perry, E.K., Tomlinson, B.E., Blessed, G., Bergmann, K., Gibson, P.H., & Perry, R.H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *British Medical Journal, 2,* 1457-1459.

Perry, R.H., Candy, J.M., Perry, E.K., Irving, D., Blessed, G., Fairbairn, A.F., & Tomlinson, B.E. (1982). Extensive loss of choline acetyltransferase activity is not reflected by neuronal loss in the nucleus of Meynert in Alzheimer's disease *Neuroscience Letters, 33,* 311-315.

Peters, A., Feldman, M.L., & Vaughan, D.W. (1983). The effect of aging on the neuronal population within area 17 of adult rat cerebral cortex. *Neurobiology of Aging, 4,* 273-282.

Plotkin, D.A., & Jarvik, L.F. (1986). Cholinergic dysfunction in Alzheimer disease: Cause or effect? In: J.M. van Ree, & S. Matthysse (Eds) *Progress in brain research, 65,* 91-103.

Pontecorvo, M.J., Clissold, D.B., & Conti, L.H. (1988). Age-related cognitive impairments as assessed with an automated repeated measures memory task: Implications for the possible role of acetylcholine and norepinephrine in memory dysfunction. *Neurobiology of Aging, 9,* 617-625.

Procter, A.W., Lowe, S.L., Palmer, A.M., Francis, P.T., Esiri, M.M., Stratmann, G.C., Najlerahim, A., Patel, A.J., Hunt, A., & Bowen, D.M. (1988) Topographical distribution of neurochemical changes in Alzheimer's disease. *Journal of the Neurological Sciences, 84*, 124-140

Raaijmakers, W.G.M. (1978) *Brain cholinesterase activity*. Doctoral dissertation, University of Nijmegen, the Netherlands

Raaijmakers, W.G.M. (1982) High-affinity choline uptake in hippocampal synaptosomes and learning in the rat. In C. Ajmone-Marsan, & H. Matthies (Eds.), *Neuronal plasticity and memory formation* (pp. 373-385). New York: Raven Press.

Rapp, P.R., Rosenberg, R.A., & Gallagher, M. (1987) An evaluation of spatial information processing in aged rats. *Behavioral Neuroscience, 101*, 3-12.

Rawlins, J.N.P., Winocur, G., & Gray, J.A. (1983) The hippocampus, collateral behavior, and timing. *Behavioral Neuroscience, 97*, 857-872.

Richardson, R., & Riccio, D.C. (1986) An examination of a contextual component of memory following recovery from anterograde amnesia in rats. *Physiological Psychology, 14*, 75-81.

Richelle, M., & Lejeune, H. (1980) *Time in animal behaviour*. Oxford: Pergamon Press.

Rigter, H., Martinez, J.L., Jr., & Crabbe, J.C., Jr. (1980) Forgetting and other behavioral manifestations of aging. In D.E. Stein (Ed.), *The psychobiology of aging: Problems and perspectives* (pp. 161-175). Amsterdam: Elsevier North Holland, Inc.

Roberts, S. (1981) Isolation of an internal clock. *Journal of Experimental Psychology: Animal Behavior Processes, 7*(3), 242-268.

Robinson, S.E. (1985) Cholinergic pathways in the brain. In M.M. Singh, D.M. Warburton, & H. Lal (Eds.), *Central cholinergic mechanisms and adaptive dysfunctions* (pp. 37-61). New York: Plenum Press.

Roderick, T.H. (1960) Selection for cholinesterase activity in the cerebral cortex of the rat. *Genetics, 45*, 1123-1140.

Rosenwasser, A.M., Boulos, Z., & Terman, M. (1981) Circadian organization of food intake and meal patterns in the rat. *Physiology & Behavior, 27*, 33-39.

Rosenzweig, M.R., Bennett, E.L., & Diamond, M.C. (1972) Cerebral changes in response to experience. *Scientific American, 226*(2), 22-29.

Russell, E.S. (1972) Genetic considerations in the selection of rodent species and strains for research in aging. In D.C. Gibson (Ed.), *Development of the rodent as a model system of aging* (pp. 33-53). Bethesda: DHEW publication no. (NIH) 72-121.

Rüthrich, H.-L., Wetzel, W., & Matthies, H. (1982) Acquisition and retention of different learning tasks in old rats. *Behavioral and Neural Biology, 35*, 139-146.

Sandberg, K., Sanberg, P.R., & Coyle, J.T. (1984) Effects of intrastriatal injections of the cholinergic neurotoxin AF64A on spontaneous nocturnal locomotor behavior in the rat. *Brain Research, 299*, 339-343.

Sanders, S., Ackroff, K., Collier, G.H., & Squibb, R. (1984) Purified diets: Some cautions about casein. *Physiology & Behavior, 33*, 457-463.

Scheff, S.W., Anderson, K.J., & DeKosky, S.T. (1985) Strain comparison of synaptic density in hippocampal CA1 of aged rats. *Neurobiology of Aging, 6*, 29-34.

Schmidt, D.E., & Wecker, L. (1981) CNS effects of choline administration: Evidence for temporal dependence. *Neuropharmacology, 20*, 535-539.

Schwegler, H., & Lipp, H.P. (1983) Hereditary covariations of neuronal circuitry and behavior: Correlations between the proportions of hippocampal synaptic fields in the regio inferior and two-way avoidance in mice and rats. *Behavioural Brain Research, 7*, 1-38.

Sherman, K.A., Kuster, J.E., Dean, R.L., Bartus, R.T., & Friedman, E. (1981) Presynaptic cholinergic mechanisms in brain of aged rats with memory impairments. *Neurobiology of Aging, 2*, 99-104.

Shinitzky, M (1986) The lipid regimen In A Fisher, I Hanin, & C Lachman (Eds), Alzheimer s and Parkinson s disease Strategies for research and development *Advances in behavioral biology, 29,* 593-602

Siegel, P S (1961) Food intake in the rat in relation to the dark-light cycle *Journal of Comparative and Physiological Psychology, 54,* 294-301

Simon, J R., Atweh, S., & Kuhar, M J (1976) Sodium-dependent high affinity choline uptake A regulatory step in the synthesis of acetylcholine *Journal of Neurochemistry, 26,* 909-922

Sinden, J D., Rawlins, J N P., Gray, J A., & Jarrard, L E (1986) Selective cytotoxic lesions of the hippocampal formation and DRL performance in rats *Behavioral Neuroscience 100,* 320-329

Smith, G (1988) Animal models of Alzheimer's disease Experimental cholinergic denervation *Brain Research Reviews, 13,* 103-118

Soumireu-Mourat, B., Destrade, C., & Cardo, B (1975) Effects of seizure and subseizure post-trial hippocampal stimulation on appetitive operant behavior in mice *Behavioral Biology, 15,* 303-316

Spangler E L., Rigby, P., & Ingram, D K (1986) Scopolamine impairs learning performance of rats in a 14-unit T-maze *Pharmacology Biochemistry & Behavior, 25,* 673-679

Spencer, D G., Jr., & Lal, H (1985) Central cholinergic involvement in learning and memory In M M Singh D M Warburton & H Lal (Eds), *Central cholinergic mechanisms and adaptive dysfunctions* (pp 141 159) New York Plenum Press

Spiteri, N J (1982) Circadian patterning of feeding, drinking and activity during diurnal food access in rats *Physiology and Behavior, 28,* 139-147

Sprott, R L., & Stavnes, K (1975) Avoidance learning, behavior genetics, and aging A critical review and comment on methodology *Experimental Aging Research, 1,* 145-168

Squire, L R., & Zola-Morgan, S (1985) The neuropsychology of memory New links between humans and experimental animals In D S Olton, E Gamzu, & S Corkin (Eds), Memory dysfunctions An integration of animal and human research from preclinical and clinical perspectives *Annals of the New York Academy of Sciences, 444,* 137-149

Stephan, F K., & Zucker, I (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions *Proceedings of the National Academy of Sciences, USA 69,* 1583-1586

Stevens, R (1981) Scopolamine impairs spatial maze performance in rats *Physiology & Behavior, 27,* 385-386

Strong, R., Hicks, P., Hsu, L., Bartus, R T., & Enna, S J (1980) Age-related alterations in the rodent brain cholinergic system and behavior *Neurobiology of Aging, 1,* 59-63

Swaab, D F., & Fliers, E (1986) Clinical strategies in the treatment of Alzheimers's disease In D F Swaab E Fliers, M Mirmiran, W A van Gool, & F van Haaren (Eds), *Progress in brain research, 70,* 413 427

Tachibana, T (1985) Higher reliability and closer relationship between open-field test measures on aggregation data *Animal Learning & Behavior, 13,* 345-348

Tagliavini, F., & Pilleri, G (1983) Basal nucleus of Meynert A neuropathological study in Alzheimer's disease, simple senile dementia, Pick's disease and Huntington's chorea *Journal of the Neurological Sciences, 62,* 243-260

Tagliavini, F., Pilleri, G., Bouras, C., & Constantinidis, J (1984) The basal nucleus of Meynert in idiopathic Parkinsons's disease *Acta Neurologica Scandinavica, 69,* 20-28

Trommer, B A., Schmidt, D E., & Wecker, L (1982) Exogenous choline enhances the synthesis of acetylcholine only under conditions of increased cholinergic neuronal activity *Journal of Neurochemistry, 39* 1704-1709

Tuček, S (1985) Regulation of acetylcholine synthesis in the brain *Journal of Neurochemistry, 44,* 11-24

Turski, W.A., Cavalheiro, E.A., Schwarz, M., Czuczwar, S.J., Kleinrok, Z., & Turski, L. (1983). Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. *Behavioural Brain Research, 9,* 315-335.

Uhl, G.R., Hilt, D.C., Hedreen, J.C., Whitehouse, P.J., & Price, D.L. (1983). Pick's disease (lobar sclerosis): Depletion of neurons in the nucleus basalis of Meynert. *Neurology, 33,* 1470-1473.

Upchurch, M., & Wehner, J.M. (1987). A mechanistic and genetic analysis of spatial learning in mice. *Behavior Genetics, 17,* 641-642.

Valle, F.P. (1970). Effects of strain, sex, and illumination on open-field behavior of rats. *American Journal of Psychology, 83,* 103-111.

Valle, F.P. (1971). Rats' performance on repeated tests in the open field as a function of age. *Psychonomic Science, 23,* 333-335.

Van der Staay, F.J., Raaijmakers, W.G.M., & Collijn, T.H. (1986). Spatial discrimination and passive avoidance behavior in the rat: Age-related changes and modulation by chronic dietary choline enrichment. In: A. Fisher, I. Hanin, & C. Lachman (Eds.), Alzheimer's and Parkinson's diseases: Strategies for research and development. *Advances in behavioral biology, 29,* 603-608. New York: Plenum Press.

Van der Staay, F.J., Raaijmakers, W., & Kerbusch, S. (1983). Rapid acquisition of two-way active avoidance in inbred Roman Low Avoidance rats. *Behavior Genetics, 13,* 435-440.

Van der Staay, F.J., Raaijmakers, W.G.M., Sakkee, A.N., & van Bezooijen, C.F.A. (1988). Spatial working and reference memory in senescent rats after thiopental anaesthesia. *Neuroscience Research Communications, 3,* 55-61.

Van Gool, W.A., Mirmiran, M., & van Haaren, F. (1985). Spatial memory and visual evoked potentials in young and old rats after housing in an enriched environment. *Behavioral and Neural Biology, 44,* 454-469.

Van Haaren, F., & van de Poll, N.E. (1984). The effect of a choice alternative on sex differences in passive avoidance behavior. *Physiology & Behavior, 32,* 211-215.

Van Haaren, F., & van de Poll, N.E. (1986). Effects of light intensity on passive avoidance behavior of male and female Wistar rats. *Physiology & Behavior, 36,* 123-125.

Van Haaren, F., van Hest, A., & van de Poll, N.E. (1987). Acquisition and reversal of a discriminated autoshaped response in male and female rats: Effects of long or short and fixed or variable intertrial interval durations. *Learning and Motivation, 18,* 220-233.

Van Hest, A., van Haaren, F., & van de Poll, N.E. (1987). Behavioral differences between male and female Wistar rats on DRL schedules: Effect of stimuli promoting collateral activities. *Physiology & Behavior, 39,* 225-261.

Van Hest, A., van Haaren, F., & van de Poll, N.E. (1988a). The behavior of male and female Wistar rats pressing a lever for food is not affected by sex differences in food motivation. *Behavioural Brain Research, 27,* 215-221.

Van Hest, A., van Haaren, F., & van de Poll, N.E. (1988b). Delayed response alternation: Effects of stimulus presentations during the delay interval on response accuracy of male and female Wistar rats. *Bulletin of the Psychonomic Society, 26,* 141-144.

Van Hulzen, Z.J.M., & Coenen, A.M.L. (1979). Selective deprivation of paradoxical sleep and consolidation of shuttle-box avoidance. *Physiology & Behavior, 23,* 821-826.

Van Luijtelaar, E.L.J.M., & Coenen, A.M.L. (1986). Two types of electrocortical paroxysms in an inbred strain of rats. *Neuroscience Letters, 70,* 393-397.

Van Luijtelaar, E.L.J.M., & Coenen, A.M.L. (1988). Circadian rhythmicity in absence epilepsy in rats. *Epilepsy Research, 2,* 331-336.

Van Luijtelaar, E.L.J.M., Kerbusch, J.M.L., & Coenen, A.M.L. (1988). Paradoxical sleep, learning, and memory processes in rats: A genetic approach. In: W.P. Koella, H. Schulz, F. Obál, & P. Visser (Eds.), *Sleep '86* (pp. 379-381). Stuttgart: Gustav Fischer Verlag.

Van Oyen, HG., van de Poll, NE, & de Bruin, JPC (1979) Sex, age and shock-intensity as factors in passive avoidance *Physiology & Behavior, 23* 915-918

Van Oyen, HG., van de Poll, NE, & de Bruin, JPC (1980) Effects of retention interval and gonadectomy on sex differences in passive avoidance behavior *Physiology & Behavior, 25,* 859-862

Vossen, JMH (1966) *Exploratief gedrag en leergedrag bij de rat* [Exploratory behavior and learning behavior in the rat] Amsterdam Swets & Zeitlinger

Wallace, JE, Krauter, EE & Campbell, BA (1980) Animal models of declining memory in the aged Short-term and spatial memory in the aged rat *Journal of Gerontology, 35* 355-363

Walsh RN & Cummins, RA (1976) The open-field test A critical review *Psychological Bulletin, 83,* 482-504

Watts, J, Stevens, R., & Robinson, C (1981) Effects of scopolamine on radial maze performance in rats *Physiology & Behavior, 26,* 845-851

Wauquier, A., & Clincke, GHC (1985) Functions of central cholinergic systems in the brain-behavior In MM Singh DM Warburton & H Lal (Eds), *Central cholinergic mechanisms and adaptive dysfunctions* (pp 63-103) New York Plenum Press

Wecker, L (1986) Neurochemical effects of choline supplementation. *Canadian Journal of Physiology and Pharmacology, 64* 329-333

Wecker, L (1988) Influence of dietary choline availability and neuronal demand on acetylcholine synthesis by rat brain *Journal of Neurochemistry 51,* 497-504

Weinstock M, Razin, M, Chorev, M., & Tashma, Z (1986) Pharmacological activity of novel anticholinesterase agents of potential use in the treatment of Alzheimer's disease In A Fisher I Hanin, & C Lachman (Eds), Alzheimer s and Parkinson s diseases Strategies for research and development *Advances in behavioral biology 29,* 539-549 New York Plenum Press

Wenk, G., Sweeney, J., Hughey, D, Carson, J., & Olton, D (1986) Cholinergic function and memory Extensive inhibition of choline acetyltransferase fails to impair radial maze performance in rats *Pharmacology Biochemistry & Behavior 25,* 521-526

Whitehouse PJ Hedreen, JC, White, CL III, & Price, DL (1983) Basal forebrain neurons in the dementia of Parkinson disease *Annals of Neurology, 13,* 243-248

Wilcock GK, Esiri, MM., Bowen, DM., & Smith, CCT (1982) Alzheimer's disease Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities *Journal of the Neurological Sciences, 57,* 407-417

Willner, P, Wise D, & Ellis, T (1986) Specific disruption of spatial behaviour in rats by central muscarinic receptor blockade *Psychopharmacology 90,* 229-235

Winblad, B., Hardy, J, Backman, L, & Nilsson, L-G (1985) Memory function and brain biochemistry in normal aging and in senile dementia In DS Olton E Gamzu, & S Corkin (Eds), Memory dysfunctions An integration of animal and human research from preclinical and clinical perspectives *Annals of the New York Academy of Sciences, 444* 255-268

Winer, BJ (1971) *Statistical principles in experimental design* (2nd ed) New York McGraw-Hill Book Company

Wirsching, BA., Beninger, RJ., Jhamandas, K, Boegman, RJ., & El-Defrawy, SR (1984) Differential effects of scopolamine on working and reference memory of rats in the radial maze *Pharmacology Biochemistry & Behavior 20,* 659-662

Wollnik, F, Gartner, K., & Büttner, D (1987) Genetic analysis of circadian and ultradian locomotor activity rhythms in laboratory rats *Behavior Genetics, 17,* 167-178

Wolthuis, OL., Knook, DL., & Nickolson, VJ (1976) Age-related acquisition deficits and activity in rats *Neuroscience Letters, 2,* 343-348

Wurtman, J.J. (1979). Sources of choline and lecithin in the diet. In: A. Barbeau, J.H. Growdon, & R.J. Wurtman (Eds.), Choline and lecithin in brain disorders. *Nutrition and the brain, 5,* 73-81. New York: Raven Press.

Wurtman, R.J. (1982). Nutrients that modify brain function. *Scientific American, 246*(4), 42-51.

Wurtman, R.J., & Fernstrom, J.D. (1976). Control of brain neurotransmitter synthesis by precursor availability and nutritional state. *Biochemical Pharmacology, 25,* 1691-1696.

Wurtman, R.J., Hefti, F., & Melamed, E. (1981). Precursor control of neurotransmitter synthesis. *Pharmacological Reviews, 32*(4), 315-335.

Wurtman, R.J., Hirsch, M.J., & Growdon, J.H. (1977). Lecithin consumption raises serum-free-choline levels. *The Lancet,* July 9, 68-69.

Zornetzer, S.F., Thompson, R., & Rogers, J. (1982). Rapid forgetting in aged rats. *Behavioral and Neural Biology, 36,* 49-60.

DANKWOORD

Een proefschrift is de afsluiting van een belangrijke periode van de wetenschappelijke vorming. Uiteraard kan men een dergelijk werk niet als solist voltooien. Velen hebben in meerdere of mindere mate een bijdrage geleverd aan de tot stand koming van dit boekje.

Allereerst zijn dit de medewerkers van de vakgroep Vergelijkende en Fysiologische Psychologie van de Katholieke Universiteit Nijmegen. Als student-assistenten hebben mijn huidige collegae Marja Abbenhuis en Jan van Nies geholpen bij het testen van de proefdieren.

Technische ondersteuning was er altijd, zowel voor het ontwikkelen en bouwen van de benodigde testopstellingen, als ook bij kalamiteiten. Bijzondere dank hiervoor gaat uit naar Frits Dexel, Huub Willems, Jos Wittebrood, Mary Wansink en Lee Sallows.

Uiteraard werd gepoogd dit boekje van fouten te ontdoen. Het Engels is gecorrigeerd door Jane Sykes. Hanneke Receveur, Marja Abbenhuis, John van Betteray en Anke Schintz hebben mij op veel (tik-) fouten gewezen die ik zelf nooit zou hebben ontdekt. Voor fouten die de oplettende lezer desondanks in dit proefschrift aantreft ben alleen ik verantwoordelijk.

Tenslotte wil ik mijn ouders Josef en Trudi van der Staay hartelijk bedanken. Zij vonden het vanzelfsprekend dat hun kinderen een goede opleiding zouden genieten. Op deze manier hebben zij aan het begin van de weg gestaan die uiteindelijk heeft geleid tot dit proefschrift. Helaas heeft mijn vader dit boekje niet meer mogen zien. Hij overleed enkele maanden geleden.

De schrijver van dit proefschrift werd op 7 juni 1951 te Goch, West Duitsland, geboren. Na de studie psychologie aan de Katholieke Universiteit Nijmegen (KUN) is hij in tijdelijke dienst werkzaam geweest aan de KUN, bij de Nederlandse Stichting voor Wetenschappelijk Onderzoek (NWO) en aan de Rijksuniveriteit Limburg. In dit proefschrift zijn de resultaten beschreven van het door de universitaire onderzoekpool van de KUN gesubsidieerde project 'Effect van choline verrijkte voeding op cholinerge transmissie en geheugen' (projectleiders: dr. W.G.M. Raaijmakers, en dr. J.M.L. Kerbusch). Het onderzoek werd verricht op de vakgroep Vergelijkende en Fysiologische Psychologie van de KUN.

STELLINGEN BEHOREND BIJ HET PROEFSCHRIFT

BEHAVIORAL CONSEQUENCES OF CHRONIC DIETARY CHOLINE ENRICHMENT

1 Ons staat thans nog geen goed diermodel voor dementie bij de mens ter beschikking

Dit proefschrift

2 De door Meck en Church voorgestelde interpretatie dat lecithine[a] en de anticholinesterase fysostigmine[b] in hun 'peak interval' procedure leiden tot een efficientere geheugenopslag, waardoor de dieren uiteindelijk slechter presteren, weerspiegelt een nogal afwijkende opvatting over leer- en geheugenprocessen Immers, uiteindelijk leidt een verbetering van de geheugenopslag tot minder adaptief gedrag en dit gedrag is niet te corrigeren door ervaring

[a] Meck, W H, & Church, R M (1987) Nutrients that modify the speed of internal clock and memory storage processes *Behavioral Neuroscience, 101,* 465-475

[b] Meck, W H, & Church, R M (1987) Cholinergic modulation of the content of temporal memory *Behavioral Neuroscience, 101,* 457-464

3 Bij het onderzoek naar leren verdient de analyse van oplosstrategieen speciale aandacht

Dit proefschrift

4 Bij het onderzoek naar de effecten van interventies die tot doel hebben het leren en geheugen te verbeteren verdienen het 'holeboard' en het 'conusveld' de voorkeur boven het 'radiale doolhof'

5 Het gebruik van 'experimenteel naïeve' dieren bij onderzoek naar de effecten van veroudering op leren en geheugen beperkt de validiteit en generaliseerbaarheid van de resultaten

6 Onderzoeksresultaten die een hypothese niet bevestigen hebben in het algemeen een minder grote kans door een tijdschrift voor publicatie te worden geaccepteerd dan resultaten, die een hypothese bevestigen Het gevolg hiervan is dat spectaculaire, maar empirisch mager onderbouwde hypothesen een lang leven kunnen leiden en een aandacht blijven houden die ze niet verdienen

7 De duur van het verblijf in de hoeken van het 'open veld' is een valide maat voor 'emotionele reactiviteit'

van der Staay, F J, Kerbusch, S, & Raaijmakers, W G M Genetic correlations in validating emotionality Ter publicatie aangeboden aan *Behavior Genetics*

8 De operationaliseringen van de theoretische concepten werk- en referentiegeheugen vertonen een zodanige verscheidenheid dat daardoor de experimentele verdieping van deze concepten in ernstige mate wordt bemoeilijkt

9 Conclusies van onderzoek gebaseerd op het vergelijken van resultaten van experimentele manipulaties met een controle groep afkomstig uit een ander experiment zouden niet voor publicatie mogen worden geaccepteerd

Bartus, R.T., Dean, R.L., Goas, J.A., & Lippa, A.S. (1980) Age-related changes in passive avoidance retention: Modulation with dietary choline. *Science, 209* 301-303

10 Het gebruik van het bijvoeglijk naamwoord 'seniel' voor ratten van 26 maanden (Lejeune, et al., 1986) of van 28 maanden (Peng, & Lee, 1979) maakt de oude rat nog niet tot een valide diermodel voor dementie.

Lejeune, H., Jasselette, P., Nagy, J., & Peree, F. (1986) Fixed interval performance in weanling rats: A comparison with adult and senile subjects. *Physiology & Behavior, 38.* 337-343

Peng, M.T., & Lee, L.R. (1979) Regional differences of neuron loss of rat brain in old age. *Gerontology, 25.* 205-211

11 De in de farmaceutische industrie gevolgde screeningsprocedure voor psycho-actieve stoffen zijn met het oog op de kosten vaak simpel en snel bij een groot aantal proefdieren toe te passen. De vraag is echter, of de mazen van dit screeningsnet niet zo grof zijn dat waardevolle verbindingen te snel als oninteressant terzijde worden geschoven.

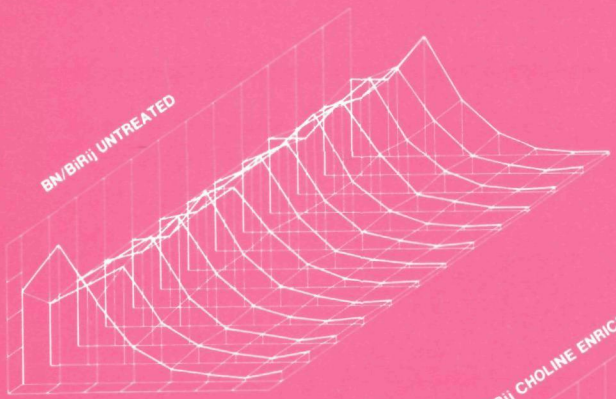
12 De sterke oriëntatie van het hoger en het wetenschappelijk onderwijs op amerikaanse voorbeelden doet geen recht aan de grote rijkdom van de europese wetenschappelijke traditie.

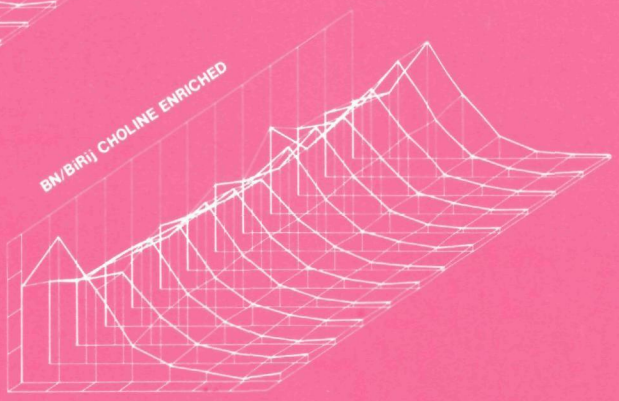
13 Het verzorgen van post-doctoraal onderwijs hoort niet thuis bij de zogenaamde beroepsorganisaties (b.v. het Nederlands Instituut voor Psychologen) maar bij de universiteiten en dient daar een structurele voorziening te zijn.

14 Weglaten van het woord 'significant' in een proefschrift of artikel leidt tot een significante verkorting van de tekst, zonder dat significante (betekenisvolle) informatie verloren gaat.

15 Het door graffiti-kunstenaars vertoonde gedrag, een uitgebreid territorium te markeren, is een fraai voorbeeld van een atavisme. Opmerkelijk is dat voornamelijk mannelijke exemplaren van de species homo sapiens dit gedrag vertonen.

Maastricht, mei 1989
F. Josef van der Staay


BN/BiRij UNTREATED


BN/BiRij CHOLINE ENRICHED