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AN EXPERIMENTAL STUDY ON THE CHOLINERGIC MODULATION OF CORTICAL AROUSAL AND COGNITIVE FUNCTIONS

With special emphasis on apolipoprotein E

Doctoral dissertation

To be presented with the assent of the Medical Faculty of the University of Kuopio for public examination in the Auditorium L1 of Canthia Building, University of Kuopio, on 17th November, 2000, at 12 noon.

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ABSTRACT

The brain cholinergic system is involved in a number of behaviors including arousal and cognitive processes. In Alzheimer's disease (AD) patients, the function of the cholinergic system is severely compromised. AD patients with apolipoprotein E (apoE) ε4 allele(s) suffer more pronounced cholinergic deficits which are associated with severe cognitive impairments, pronounced cortical electroencephalogram (EEG) slowing, impaired synaptic plasticity, and decreased responsiveness to cholinomimetic treatment compared to AD patients without apoE ε4 allele. The purpose of this study was to investigate the function of the brain cholinergic system and its interaction with apoE in the regulation of cortical arousal and cognitive functions. First, a rat EEG model of thalamocortically generated high-voltage spindles (HVS) was used to study the cholinergic modulation of neocortical arousal. The effects of intrathalamic (reticular nucleus of thalamus (NRT) and ventroposteromedial nucleus of thalamus (VPM)) infusions of a muscarinic M_1 receptor agonist, McN-A-343, a muscarinic M_2 receptor antagonist, methoctramine, and a muscarinic receptor agonist, oxotremorine, on HVSs were studied. Second, the role of apoE in the regulation of biochemical, cognitive and electrophysiological functions was investigated. The effects of quisqualic acid induced nucleus basalis (NB) -lesion and ageing on cholinergic activity, spatial navigation in water maze (WM), cortical EEG activity and EEG response to a muscarinic receptor antagonist, scopolamine, were investigated in apoE-deficient and control mice. The major findings of this study were: 1) intrathalamic NRT and/or VPM infusions of McN-A-343, methoctramine, and oxotremorine, decreased HVSs in rat, indicating increased neocortical arousal. These results suggest that intrathalamic infusions of cholinergic muscarinic drugs modulate neocortical arousal via muscarinic M_1 and M_2 receptors in thalamus. Furthermore, these results suggest that intracerebroventricularly and systemically administered cholinergic drugs may also modulate neocortical arousal via the thalamus. 2) The apoE-deficient mice did not suffer any apparent impairment in their cortical and hippocampal choline acetyltransferase (ChAT) -activity, number of ChAT-positive neurons in NB, or in WM spatial navigation during ageing. Furthermore, apoE-deficiency did not increase the sensitivity to cholinergic, WM spatial navigation or EEG deficits induced by NB-lesion. However, the apoE-deficient mice had slightly altered cortical EEG activity during ageing and blunted EEG response to scopolamine treatment. These results suggest that apoE does not have to be present to preserve the viability of cholinergic neurons and that spatial navigation and behavioral recovery during ageing or after NB-lesion is not affected by apoE-deficiency. However, apoE-deficiency might alter the regulation of cortical arousal during ageing and after cholinergic drug (scopolamine) manipulation. Taken together, this study provides new information about the function of the brain cholinergic system and its interaction with apoE. This may have relevance in the development of new experimental models and therapeutic treatments for clinical disorders such as AD.

National Library of Medicine Classification: WL 150, WL 155
Medical Subject Headings: Alzheimer's disease; apolipoproteins E; arousal; cholinergic agents; electroencephalography; maze learning; memory; nucleus basalis of Meynert

To my parents

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Kuopio, September 2000

Jukka Puoliväli

ABBREVIATIONS

Aβ	β-amyloid
AChE	acetylcholinesterase
AD	Alzheimer's disease
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	analysis of variance
ApoE	apolipoprotein E
APP	amyloid precursor protein
ChAT	choline acetyltransferase
CNS	central nervous system
EEG	electroencephalogram
GABA	gamma-aminobutyric acid
HACU	high affinity choline uptake
HDB	horizontal diagonal band of Broca
HVS	high-voltage spindle
i.c.v.	intracerebroventricular
IgG	immunoglobulin G
i.p.	intraperitoneal
LC	locus coeruleus
LDT	laterodorsal tegmental nucleus
LTP	long-term potentiation
MANOVA	multivariate analysis of variance
MAP-2	microtubule-associated protein 2
MS	medial septum
NB	nucleus basalis
NRT	reticular nucleus of thalamus
PPT	pedunculopontine tegmental nucleus
PS-1	presenilin-1
PS-2	presenilin-2
THA	tetrahydroaminoacridine
VDB	vertical diagonal band of Broca
VPM	ventroposteromedial nucleus of thalamus
WM	water maze

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications that are referred to in the text by the Roman numerals I-IV.

I Puoliväli J, Jäkälä P, Koivisto E, and Riekkinen P Jr.: Muscarinic M_1 and M_2 receptor subtype selective drugs modulate neocortical EEG via thalamus. NeuroReport 9: 1685-1689, 1998.

II Puoliväli J, Jäkälä P, Koivisto E, and Riekkinen P Jr.: Oxotremorine suppresses thalamocortical oscillations via thalamic muscarinic acetylcholine receptors. Psychopharmacology 140: 285-292, 1998.

III Puoliväli J, Pradier L, and Riekkinen P Jr.: Moderate cortical EEG changes in apolipoprotein E-deficient mice during ageing and scopolamine treatment but not after nucleus basalis lesion. Psychopharmacology 145: 386-392, 1999.

IV Puoliväli J, Miettinen R, Pradier L, and Riekkinen P Jr.: Apolipoprotein E-deficient mice are not more susceptible to the biochemical and memory deficits induced by nucleus basalis lesion. Neuroscience 96: 291-297, 2000.

CONTENTS

1. INTRODUCTION

Dementia is defined as the development of multiple cognitive deficits that include memory impairment and at least one of the following: aphasia, apraxia, agnosia, or a disturbance in executive functioning (American Psychiatric Association, 1994). The prevalence of Alzheimer's disease (AD), the most common cause of dementia, doubles every five years after the age of 60, rising from a prevalence of 1 % among 60-years-olds to greater than 40 % among 85-years-olds. AD is characterized by unknown etiology and a progressive course of the disease (Cummings et al. 1998). Neuritic plaques and neurofibrillary tangles are typical histopathological findings in AD (Cummings et al. 1996, Arendt et al. 1998). These lesions are predominantly located in the cerebral cortex, although they are also observed in certain subcortical structures. In addition to these two classical markers, massive loss of synapses (Terry et al. 1991) and neurons (Gomez-Isla et al. 1997) in the hippocampal formation and association regions of the neocortex is observed in AD patients.

Both genetic and environmental factors are associated with AD. Mutations in amyloid beta precursor protein (APP), presenilin-1 (PS-1) and presenilin-2 (PS-2) genes result in increased production of β-amyloid (Aβ) peptide which is deposited in the brain. These gene defects are associated with early-onset familial AD (Cummings et al. 1998). In addition, several other genes, including the ε4 allele of apolipoprotein E (apoE) gene, have been found to increase the risk for AD (Strittmatter and Roses 1996). Other risk factors for AD include advanced age and history of head trauma. On the other hand, higher levels of education, the apoE ε2 allele, and the use of nonsteroidal anti-inflammatory drugs and estrogen replacement therapy in post-menopausal women, might work as "protective" factors and decrease the risk for and delay the onset of AD (Breitner et al. 1995, Cummings et al. 1998).

AD is also associated with dysfunction of several neurotransmitter systems, including the cholinergic (Whitehouse et al. 1982), noradrenergic (Mann 1983, Marcyniuk et al. 1986) and serotonergic systems (Palmer et al. 1987, Nazarali and Reynolds 1992). The brain cholinergic system is involved in a number of behaviors, including arousal, attention, sleep-wake cycles as well as learning and memory. The basal forebrain and brainstem cholinergic systems play also an important role in the

regulation of cortical and thalamic electrical activity (McCormick 1990, McCormick 1992b). In AD, the loss of cortical cholinergic markers and the degeneration of basal forebrain cholinergic neurons are the most consistent and severe neurochemical deficits. The severity of the cholinergic deficits has been shown to correlate with the degree of cognitive decline (Bartus et al. 1982) and electroencephalogram (EEG) slowing in AD patients (Soininen et al. 1992). Those AD patients carrying the apoE ε4 allele suffer more profound cholinergic pathology (Poirier 1994, Soininen et al. 1995) which is associated with more severe cognitive impairment (Lehtovirta et al. 1996b), more pronounced EEG slowing (Lehtovirta et al. 1996a, Lehtovirta et al. 2000), and decreased responsiveness to cholinesterase inhibitor therapy (Poirier et al. 1995) when compared to AD patients without the apoE ε4 allele. Furthermore, AD patients with the apoE ε4 allele have an earlier onset of the disease (Strittmatter and Roses 1996) and impaired neuronal remodelling capacity (Arendt et al. 1997).

The *in vivo* rodent models of cholinergic hypofunction have been useful in clarifying the role of the brain cholinergic system in cognitive and electrophysiological functions (Wenk 1997). Recently, transgenic animal models of AD that have modifications in APP, PS and apoE genes have been developed (Hsiao et al. 1996, Holcomb et al. 1999, Hamanaka et al. 2000). These models make it possible to determine the role of specific molecules in the pathogenesis and neurodegenerative processes of AD, and, most importantly, to test novel therapies in the treatment of AD. In the present study, the effects of cholinergic manipulation on cortical arousal and cognitive functions were investigated. Moreover, the role of apoE in the regulation of biochemical, behavioral, and electrophysiological functions were studied.

2. REVIEW OF THE LITERATURE

2.1 THE BRAIN CHOLINERGIC SYSTEM

2.1.1 Anatomy of the brain cholinergic system

The distribution of cholinergic cells and processes in the rat brain is well characterized (Mesulam et al. 1983, Wainer and Mesulam 1990, Butcher 1995). However, there are fewer anatomical studies on the mouse brain cholinergic system (Hohmann and Ebner 1985, Kitt et al. 1994). The brain cholinergic system has two major anatomically different regions: the cholinergic nuclei in the basal forebrain and the cholinergic nuclei in the brainstem. The cholinergic nuclei of the basal forebrain consist of large magnocellular cholinergic cells situated in the medial septum (MS), the vertical and horizontal branches of the diagonal band of Broca (VDB and HDB, respectively) and the nucleus basalis (NB). The brainstem cholinergic cell groups are situated in the pedunculopontine tegmental nucleus (PPT), the laterodorsal tegmental nucleus (LDT), the medial habenular nucleus and the parabigeminal nucleus (Wainer and Mesulam 1990). In addition, striatal intrinsic cholinergic neurons can be found in both rodents and primates. Although cortical ChAT-positive neurons have been found in the rat, those neurons are not associated with ChAT mRNA. This finding has indicated that intrinsic cholinergic neurons do not exist in rat cortex, which is consistent with findings in other mammals (Butcher 1995).

Both mice and rats show regional variations in the distribution and densities of cholinergic innervation. The major source of cholinergic innervation of the hippocampus derives from the MS and VDB. The patterns of cholinergic innervation of the hippocampus seem to be quite similar in rats and mice (Wainer and Mesulam 1990, Kitt et al. 1994). HDB neurons project mainly to olfactory bulb, and the NB neurons project to the entire cerebral cortex and to amygdala (Wainer and Mesulam 1990). In mice, the cholinergic axons in the somatosensory cortex are prominent in layers I and IV, and within the lower portion of layer V and the upper segment of layer VI (Kitt et al. 1994). A similar cholinergic innervation pattern in rat sensory cortex has also been reported (Lysakowski et al. 1986). However, Eckenstein et al. (1988) reported that in rat sensory cortex the layers I-III and the upper portion of layer V have the highest densities of cholinergic innervation. The potential

differences in the laminar structure of cortical innervation between mice and rats suggest that there might be some differences in the ways in which acetylcholine modulates cortical activity in these two species (Kitt et al. 1994). Moreover, there is a strong hereditary variability within the cholinergic system in mouse strains. For example, the number of cholinergic neurons in MS and VDB, and the density of cholinergic fibers in hippocampus differ significantly between different inbred mouse strains (Ingram and Corfman 1980, Schwegler et al. 1996b), which might explain some of the behavioral variation observed between strains (Schwegler et al. 1996a).

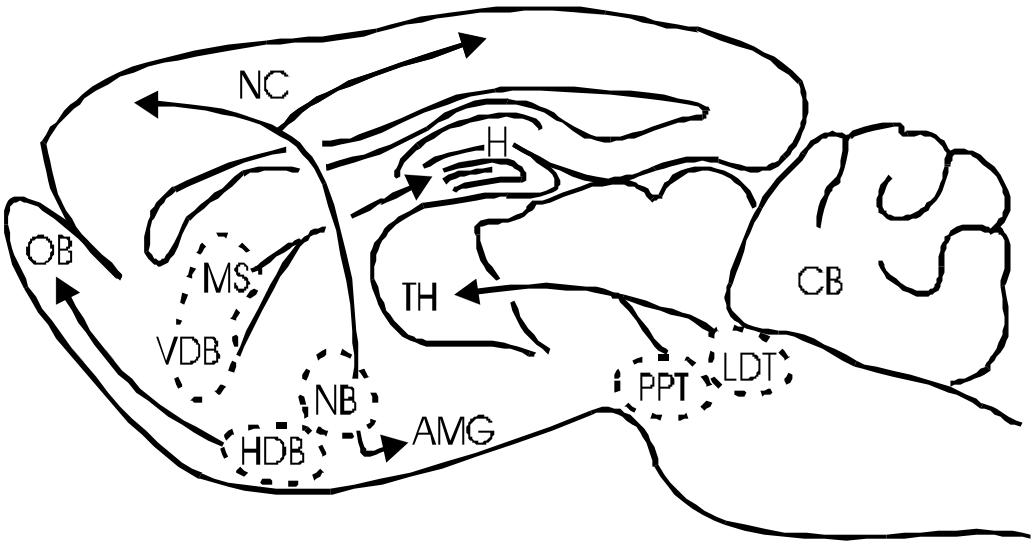

Figure 1. Sagittal view of basal forebrain and brainstem ascending cholinergic pathways in rat (modified from Mesulam et al. 1983). Abbreviations: AMG = amygdala; CB = cerebellum; H = hippocampus; HDB = horizontal diagonal band of Broca; LDT = laterodorsal tegmental nucleus; MS = medial septum; NB = nucleus basalis; NC = neocortex; OB = olfactory bulb; PPT = pedunculopontine tegmental nucleus; TH = thalamus; VDB = vertical diagonal band of Broca.

The basal forebrain cholinergic neurons also provide innervation to the thalamus. In rats, over one third of the total number of basal forebrain neurons projecting to reticular nucleus of thalamus (NRT) are cholinergic (Levey et al. 1987). However, the major source of cholinergic innervation to the thalamus derives from the cholinergic neurons of the PPT and LDT in the brainstem (Wainer and Mesulam 1990). The cholinergic innervation of the thalamus is virtually identical in mice and rats. In both species, the anteroventral, intralaminar and reticular nuclei show intense choline acetyltransferase (ChAT) immunoreactivity (Levey et al. 1987, Kitt et al. 1994). There are also less dense connections from the brainstem cholinergic nuclei to parts of the cerebral cortex, basal forebrain, and a number of extrapyramidal structures, such as the striatum, globus pallidus, and substantia nigra (Butcher 1995).

Although the basal forebrain cholinergic neurons project to many cortical areas, they receive projections from only a very limited set of limbic and paralimbic cortical areas (Wainer and Mesulam 1990). There is input to basal forebrain cholinergic neurons from the amygdala, the hypothalamus and the brainstem nuclei, including the PPT, the LDT, the substantia nigra, the ventral tegmental area, the raphe nuclei, and the locus coeruleus (LC) (Koliatsos et al. 1990, Wainer and Mesulam 1990).

2.1.2 Pharmacology of the cholinergic synapse

The synthesis of acetylcholine from choline and acetyl coenzyme A takes place in nerve terminals of cholinergic cells. The formation of acetylcholine is catalysed by ChAT, which is produced in the neuronal soma and transported by slow axonal transport down the axon to the synapses. In the cholinergic nerve terminals, the acetylcholine is stored either in the cytosol or in the synaptic vesicles from where it is released. The immediate source of the calcium dependent, evoked release of acetylcholine is the vesicular rather than the cytosolic fraction. The hydrolysis of acetylcholine into choline and acetate is catalysed by acetylcholinesterase (AChE) and takes place in the synaptic cleft. Specific acetylcholinesterase inhibitors like donepezil, galanthamine, tetrahydroaminoacridine (THA), physostigmine, rivastagmine and metrifonate, can be used to increase the amount of available acetylcholine in the cholinergic synapse (Francis et al. 1999).

The acetylcholine receptors are divided into muscarinic and nicotinic acetylcholine receptors. Different subtypes of muscarinic acetylcholine receptors have been identified by using both pharmacological and molecular techniques. On the basis of pharmacological properties, four subtypes of muscarinic acetylcholine receptors (M_1-M_4) have been identified, whereas molecular cloning has revealed five different muscarinic acetylcholine receptor subtypes (m_1-m_5). Muscarinic acetylcholine receptors transduce their signals by coupling with G-proteins, which then modulate the activity of a number of effector enzymes and ion channels. The stimulation of muscarinic receptor M_1, M_3 and m_5 subtypes can activate multiple signalling effectors simultaneously including phospholipases C, D and A_2 as well as different ion channels. The receptor-mediated activation of phospolipases results in activation of several different second messenger systems. For example, the activation of phospholipase C results in the release of inositol trisphosphate and diacylglycerol. The main cellular response to muscarinic M_2 and M_4 receptor stimulation is the inhibition of adenylate cyclase, which reduces the levels of cyclic AMP (Ehlert et al. 1995, Felder 1995).

In the rat brain, the M_1 receptor is most abundant in cortex and gradually declines in number caudally towards spinal cord. For example, the thalamus contains fewer M_1 receptors (Wall et al. 1991, Wei et al. 1994). The M_2 receptor is most abundant in cerebellum, pons/medulla, and thalamus/hypothalamus. In contrast, forebrain regions (cortex, hippocampus, striatum and olfactory bulb) contain markedly fewer M_2 receptors (Li et al. 1991, Wei et al. 1994). The highest levels of the M_4 receptors are found in the striatum and in lower amounts in cortex and hippocampus (Waelbroeck et al. 1990, Wei et al. 1994). Also in mouse brain, the M_1 receptors are abundant in cortex and hippocampus, M_2 receptors in thalamus, and M_4 receptors in striatum (Hohmann et al. 1995). In hippocampus and several regions of neocortex in human brain, the M_1 receptor constitutes from 35-60 % of all muscarinic receptors, whereas M_2 and M_4 receptors each account for about 15-25 % of the muscarinic receptors in the same areas. In contrast, the M_2 receptor is the predominant subtype in the basal forebrain, and M_4 receptor is the most abundant in the caudate and putamen (Flynn et al. 1995, Levey 1996). The M_3 and m_5 receptors are found consistently throughout the brain, although for M_3 there is a modest rostral-caudal decline (Wei et al. 1994). Muscarinic receptors can be located on both pre- and post-synaptic sides. The M_1 receptors are largely post-synaptic and their activation increases the cholinergic neurotransmission. In contrast, the M_2 receptors are predominantly presynaptic autoreceptors, which control the cholinergic tone in

negative manner. Much less is known about the precise location of muscarinic M_3, M_4 and m_5 receptor subtypes (Levey 1996).

Nicotinic acetylcholine receptors belong to the group of ligand-gated receptors. Nicotinic receptors are composed of several combinations of α- and β-subunits with considerable molecular diversity, suggesting that many functional subtypes of neuronal nicotinic receptors are possible. Nicotinic receptors are abundant in cerebral cortex, thalamus, hippocampus and hypothalamus (Arneric et al. 1995).

Table 1. Acetylcholine receptor subtypes: main locations in the brain and examples of second messenger systems and biochemical mediators. Abbreviations: cAMP = cyclic adenosine monophosphate; CNS = central nervous system; DAG = diacylglycerol; IP_3 = inositol trisphosphate (Arneric et al. 1995, Ehlert et al. 1995, Felder 1995).

Receptor subtype	Brain region	Second messenger system	Biochemical mediator
M_1	Hippocampus, cortex, olfactory bulb, striatum	Phospholipase C activation	DAG IP_3
M_2	Cerebellum, pons/medulla, thalamus/hypothalamus	Adenylate cyclase inhibition	cAMP
M_3	Cortex, hippocampus, striatum, cerebellum	Phospholipase C activation	DAG IP_3
M_4	Striatum, cortex, hippocampus	Adenylate cyclase inhibition	cAMP
m_5	Evenly distributed throughout the CNS	Phospholipase C activation	DAG IP_3
Nicotinic	Cortex, hippocampus, thalamus, hypothalamus	Ligand-gated ion channel	Na^+, K^+, Ca^{2+} conductance

2.1.3 Neurophysiology of the brain cholinergic system

The brain cholinergic system is involved in the generation of various EEG patterns, and pharmacological manipulations or pathological changes in the cholinergic system affect neocortical EEG activity. Different EEG rhythms are related to distinct behavioral states. The slow-waves (delta waves) with a frequency below 4 Hz prevail during the deep stage of normal sleep, whereas the normal theta activity within the frequency range 4-8 Hz occurs during activated behavioral states. Other EEG patterns include for example alpha rhythm that occurs during wakefulness and EEG fast-waves (beta activity) that occur during epochs of increased alertness (Steriade et al. 1990). Also different patterns of synchronized spindle oscillations, which take place during the early stages of sleep or low arousal and vigilance can be found in cortical EEG activity of humans and experimental animals (Buzsáki et al. 1988a, Steriade et al. 1990, Riekkinen et al. 1991b). In pathological situations, such as AD, the regulation of EEG activity is altered. The decline of cholinergic markers is correlated with EEG slowing observed in patients with AD (Soininen et al. 1992). The slowing of EEG is characterized by enhanced theta power in patients with mild and moderate AD, and delta waves increase in more advanced stages of AD. Moreover, the EEG slowing in AD is also manifested by a decrease of alpha and beta activities (Coben et al. 1983, Penttilä et al. 1985).

The ascending cholinergic systems of basal forebrain and brainstem regulate the neocortical and thalamic electrical activity (McCormick 1992b). The frequency changes in NB neurons correlate well with changes in behavioral and neocortical arousal of the animal. In rats, the highest frequency activity of NB neurons has been observed during running, followed by drinking, and immobility (Buzsáki et al. 1988a). NB neurons are also active when the animal is waiting to respond to visual or auditory stimuli that are associated with the expectation of a food reward (Richardson and DeLong 1991). The increase in activity of NB neurons results in neocortical desynchronization, whereas the decrease in activity results in increased slow-wave activity in the neocortex (Wenk 1997, Détári et al. 1999). In a recent study, it was also shown that the discharge pattern of rat basal forebrain cholinergic neurons shifted from an irregular tonic discharge pattern during EEG slow irregular activity to a rhythmic burst discharge pattern during EEG rhythmic slow activity (Manns et al. 2000).

These results indicate that the cholinergic basal forebrain neurons have the capacity to modulate their cortical target areas rhythmically during cortical activation (Manns et al. 2000).

The electrophysiological effects of acetylcholine are mediated via cholinergic receptors. Muscarinic receptor activation results in a variety of hyperpolarizing and depolarizing responses, dependent upon the cell type or the tissue under investigation (Jones 1993). For example, in cortex, the extracellular application of acetylcholine to cortical neurons has been found to result in both excitation as well as inhibition. In cortical pyramidal cells, acetylcholine induces a slow excitatory response associated with an increase in neuronal excitability. This is due to suppression of potassium current, which appears to be mediated through muscarinic M_1 receptors (McCormick 1990, McCormick 1992b). On the other hand, the activation of muscarinic M_2 receptors in cortical GABAergic inhibitory interneurons by acetylcholine results in depolarization of the neurons through activation of cation conductance. This can lead to inhibition of cortical pyramidal neurons (McCormick 1990, McCormick 1992b). Thus, the cholinergic system regulates the excitability of cortical pyramidal neurons both directly and indirectly via cortical GABAergic interneurons. This is in accordance with the anatomical findings showing that basal forebrain cholinergic neurons have synapses both with cortical pyramidal neurons and GABAergic interneurons (Beaulieu and Somogyi 1991).

The thalamic neurons can exhibit two basic modes of action potential generation: single spike activity and rhythmic burst generation (McCormick 1990). The activity mode of thalamic neurons correlates with changes in behavior and arousal of the animal. Typically, rhythmic activity occurs only during states of low arousal and vigilance, being virtually absent during high vigilance states. When the animal is aroused, the increased activity of the ascending systems, such as the basal forebrain and brainstem cholinergic systems, is thought to suppress rhythmic burst firing, and the typical cortical electrical activity is characterized by low-voltage fast-waves. Whereas in the drowsy or sleeping animal, large-amplitude slow-waves are observed in the delta band (Buzsáki et al. 1988b). Furthermore, in waking-immobile rats, a highly synchronized EEG pattern, characterized by large-amplitude rhythmic waves in theta/alpha band is observed. It is believed that the loop between thalamocortical

relay neurons and GABAergic neurons in NRT is responsible for the generation of rhythmic high-voltage spindle (HVS) wave activity (Figures 2 and 3). During the spindle activity, rhythmically active GABAergic neurons in NRT can phasically hyperpolarize their thalamocortical target neurons. In the absence of other depolarizing inputs, voltage- and time- dependent rebound Ca^{2+} spike bursts occur in thalamocortical relay neurons in a phase-locked manner. The rhythmical bursts of the thalamocortical relay neurons are transferred to the cortex, where they cause excitatory post-synaptic potentials in cortical pyramidal neurons, thereby generating the EEG neocortical HVSs. The corticothalamic neurons may also participate in the generation of HVSs by having an excitatory influence on NRT and thalamocortical neurons (Steriade and Deschenes 1984, Buzsáki et al. 1988b, Steriade and Llinás 1988, McCormick 1990, McCormick and Bal 1997).

The basal forebrain and brainstem cholinergic projection cells innervate thalamic nuclei and cortical areas, and active functioning of these neurons inhibits HVSs and neocortical slow-waves (Buzsáki et al. 1988b, Steriade and Buzsáki 1990). Systemically administered muscarinic and nicotinic acetylcholine receptor agonists (Jäkälä et al. 1997) as well as intrathalamic infusion of nicotine into a relay nucleus, ventoposteromedial nucleus (VPM) (Riekkinen et al. 1995), suppress neocortical HVSs in adult rats. Furthermore, a partial excitotoxic lesion of NB in adult rats can increase neocortical HVSs and slow-waves, and this can be alleviated by administering an anticholinesterase drug, THA (Riekkinen et al. 1991e). In aged rats, the increase in HVSs has also been shown to correlate with the decrease in the number of ChAT-positive cells in the NB (Riekkinen et al. 1992).

The response of a particular thalamic neuron to acetylcholine depends on both the location and type of the neuron as well as the species of the animal being studied. In thalamic slices, the application of acetylcholine in cat or guinea pig lateral geniculate nuclei relay neurons results in a pronounced inhibition of rhythmic burst firing and the appearance of single spike activity (McCormick 1990, McCormick 1992b). This change in firing mode is achieved by depolarization of the neurons through activation of both nicotinic and muscarinic receptors. The nicotinic response typically results in a rapid depolarization associated with an increase in

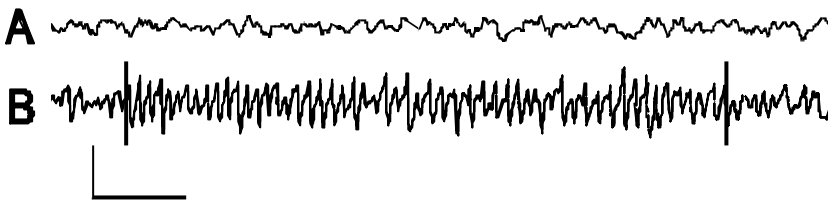

Figure 2. A) Example of non-spindling background EEG. **B)** Example of a typical neocortical HVS episode (separated from background EEG by vertical bars) recorded above the frontal cortex during a period of relaxed behavioral waking-immobility (eyes open, head held up) in rat. The amplitude scale (vertical bar) indicates 300 μV and time scale (horizontal bar) indicates 1 s.

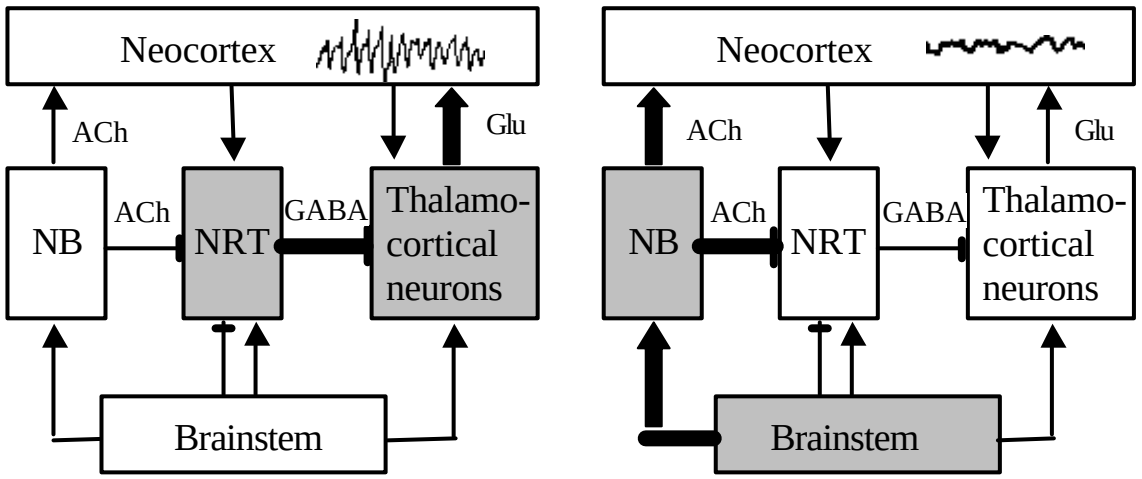

Figure 3. Schematic circuitry of the hypothesized mechanisms of HVS activity (**left**) and non-spindling EEG activity (**right**) (modified from Buzsáki et al. 1988b). Pathways and structures playing an important role in the HVS activity and non-spindling EEG activity are indicated by bold lines and shading. Arrows, excitatory pathways; bars, inhibitory pathways. When the NRT-thalamocortical system is released from the suppressive effect of the NB and brainstem nuclei, rhythmic activity of the NRT neurons phasically inhibits thalamocortical neurons and induces rhythmic rebound burst firing in these neurons, resulting in rhythmic depolarization of cortical neurons.

membrane cationic conductance, while the muscarinic response is slower, being related to a reduction in potassium conductance. In addition to these two depolarizing responses, a muscarinic hyperpolarizing response, due to an increase in potassium conductance, can occur between the fast nicotinic and slow muscarinic depolarizing responses (McCormick 1990, McCormick 1992b, McCormick and Bal 1997). Furthermore, *in vitro* application of acetylcholine in cat NRT results in marked inhibition of neuronal discharge mediated by muscarinic M_2 receptors via a post-synaptic increase in potassium conductance. This inhibition of action potential activity in NRT GABAergic neurons can prevent the hyperpolarization in thalamic relay neurons and may inhibit the rhythmic burst firing (McCormick 1990, McCormick 1992b).

2.1.4 Cognitive and attentional effects of cholinergic manipulations

The findings from experimental animals, and ageing and AD research have provided an empirical foundation for the cholinergic hypothesis of learning and memory. The cholinergic hypothesis asserts that significant, functional disturbances in brain cholinergic activity play an important role in the memory loss and related cognitive problems associated with old age and AD. Furthermore, the prediction of the cholinergic hypothesis is that the enhancement or restoration of central cholinergic function should reduce the severity of the cognitive loss (Bartus et al. 1982, Francis et al. 1999).

Several pharmacological studies have investigated the effects of cholinergic receptor antagonists and cholinomimetic drugs on cognitive functions. The administration of the muscarinic antagonist, scopolamine, induces a performance deficit in several kinds of learning and memory tasks (Blokland 1995, Ebert and Kirch 1998). However, the impairments in cognitive functions observed after scopolamine treatment reflect more likely worsening in sensory and attentional processes than in learning and memory processes *per se* (Blokland 1995, Stanhope et al. 1995, Ebert and Kirch 1998). The cholinomimetic drugs are used to enhance the cholinergic transmission and thus improve the cognitive functions. Recent evidence from clinical studies shows that the treatment of AD patients with acetylcholinesterase inhibitors (e.g. THA, donezepil, rivastagmine, and galanthamine) can improve cognitive functioning and decrease neuropsychiatric problems (Francis et al. 1999, Perry et al. 1999). Similarly, in aged rats and MS-lesioned rats and mice, the acetylcholinesterase inhibitors

stimulate spatial navigation in water maze (WM) task (Riekkinen et al. 1991a, Riekkinen et al. 1996, Ikonen et al. 1999). More recently, specific muscarinic M_1 agonists and M_2 antagonists, which might be useful in the treatment of cognitive and non-cognitive symptoms of AD, have been developed (Francis et al. 1999, Levy et al. 1999).

The early reports showing a correlation between dysfunction of the basal forebrain cholinergic system and the cognitive deficits in normal ageing and AD suggested that the basal forebrain projections to cortex and hippocampus were particularly important in learning and memory functions (Bartus et al. 1982, Francis et al. 1999). The findings that lesions in the basal forebrain with excitatory amino acid analogues, such as ibotenic acid, result in cognitive impairments and reduction of cholinergic markers in experimental animals, supported the role of the basal-cortical system in learning and memory (Dekker et al. 1991, Dunnett et al. 1991, Blokland 1995, Wenk 1997). However, the results from studies showing that the injection of other neurotoxins, such as quisqualic acid and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) into the same site caused a greater reduction in cholinergic activity but less learning and memory deficits, did not support this hypothesis (Dekker et al. 1991, Dunnett et al. 1991, Blokland 1995, Wenk 1997). Only recently, has it become possible to destroy cholinergic neurons selectively in rats by using immunoglobulin (IgG)-saporin (Wiley et al. 1991). The antibody attaches to the low-affinity nerve growth factor (NGF) receptors, which are enriched only in the cholinergic neurons, and is internalized into the neurons allowing the cytotoxin saporin to destroy the neurons (Winkler et al. 1998). The direct infusion of IgG-saporin into the NB has only minor or no effects on spatial learning and memory, despite the fact that there is extensive loss of cortical ChAT-activity. These results suggest that deficits in learning and memory tasks after basal forebrain lesions are not well correlated with the degree of cholinergic loss (Torres et al. 1994, Wenk et al. 1994, Dornan et al. 1996, Wenk 1997, Winkler et al. 1998). Compared to the infusion of IgG-saporin into the NB, the intracerebroventricular (i.c.v.) IgG-saporin administration impairs cognitive functions in several tasks. However, it is possible that the damage to striatal cholinergic neurons and cerebellar Purkinje cells can explain some of the behavioral deficits produced by i.c.v. IgG-saporin (Leanza et al. 1995, Leanza et al. 1996, Winkler et al. 1998).

Recently, it has been proposed that the neuronal networks between distinct basal forebrain cholinergic nuclei and their target areas are active during different mnemonic and attentional processes. The septohippocampal projection is thought to be involved in the modulation of short-term spatial memory, and the projections from VDB to cingulate cortex may impact on the ability to utilize response rules through conditional discrimination (Everitt and Robbins 1997). Rather than being involved in learning and memory processes *per se*, the NB-neocortical cholinergic system contributes greatly to visual attentional function (Everitt and Robbins 1997). Non-selective lesions of NB in rat have been shown to result in performance deficits in 5-choice serial reaction time task, which measures attentional abilities (Robbins et al. 1989). Some of these deficits can be ameliorated by anticholinesterase treatment or by transplantation of cholinergic-rich embryonic cells into the cortex of lesioned animals (Muir et al. 1992). More recently, it has been shown that the selective lesioning of NB cholinergic neurons with immunotoxin IgG-saporin in rats also results in impairment of certain aspects of attentional processes (Wenk 1997). For example, McGaughly et al. (1999) found that infusion of IgG-saporin into the area of the NB/substania innominata in rats produced small but significant impairments in a 5-choice serial reaction time task under baseline conditions, and even more severe impairments under conditions of increased attentional demands. In addition to behavioral data, also the electrophysiological evidence implicates NB cholinergic neurons in attentional processes. An increase of activity of basal forebrain neurons is observed when the animal is waiting to respond to visual or auditory stimuli that are associated with the expectation of a food reward (Richardson and DeLong 1991). Whereas the basal forebrain cholinergic nuclei are involved in attentional, and learning and memory functions, the brainstem cholinergic nuclei play an important role in regulation of arousal and sleep-wake cycle (McCormick 1990, Steriade and Buzsáki 1990). It seems likely that the brainstem cholinergic nuclei are also involved in behavioral activation but at a more basic level than the modulation of cognitive functioning (Everitt and Robbins 1997).

2.1.5 Brain cholinergic system during ageing and in Alzheimer's disease

Ageing in rodents. Studies on the effects of age on the integrity of the basal forebrain cholinergic system in rats and mice have revealed inconsistent findings. Some rat studies have reported significant neuron loss in NB (Riekkinen et al. 1992, De Lacalle et al. 1996) while others have found no loss at all (Armstrong et al. 1993). Moreover, both increases and decreases during ageing in the size of neurons and in the overall size of the region of cholinergic cell bodies in basal forebrain in rats, have been reported (Sarter and Bruno 1998). In one study, the number of basal forebrain cholinergic neurons was found to be the same in young and aged mice, though these neurons became substantially smaller with increasing age (Mesulam et al. 1987). Also the data on cortical ChAT-activity in rodents during ageing is inconclusive: both decreases and no changes in cortical ChAT-activity in rats (Sarter and Bruno 1998), and increase (Sherman and Friedman 1990) and no change (Bernstein et al. 1985) in cortical ChAT-activity in mice, have been reported. The results from studies measuring cortical sodium-dependent high affinity choline uptake (HACU), which is the rate-limiting step in the synthesis of acetylcholine, also do not reveal any consistent changes in aged rodents. In one study, no changes in cortical HACU were found between 5- to 6- and 23- to 25-month-old rats (Meyer et al. 1984), whereas in another study, rats aged 10-11 months had lower cortical HACU than 3-4 months old rats but no further decline in HACU was found in 24- to 25-month-old rats (Sirviö et al., 1988). Moreover, Lebrun et al. (1990) did not find differences in HACU between young and aged mice, whereas in the study of Sherman et al. (1990), HACU was significantly decreased in aged mice. The differences can partly be explained by the variability in the age, strains and methods between the studies. Taken together, the above data does not consistently support the hypothesis that normal ageing in rodents extensively affects the basal forebrain cholinergic system.

Ageing in humans. Biochemical measurements of cortical cholinergic enzymes have revealed inconsistent age-related changes in non-demented individuals. Some studies have shown significant decreases in cortical ChAT- and AChE-activities (Davies 1979, Perry et al. 1981), while others have reported no loss at all (Wilcock et al. 1982). Also the data on basal forebrain cholinergic neuron loss during normal ageing is inconsistent: some investigations have reported up to 50 %

decrease in the number of NB cholinergic neurons (De Lacalle et al. 1991) while others have found no age-related changes in neuron numbers (Chui et al. 1984). These findings suggest that there might be a slight age-related impairment in cholinergic system during normal ageing in humans. However, this decline is at best modest when compared to the cholinergic loss in AD.

Alzheimer's disease. The loss of cortical cholinergic markers and the degeneration of basal forebrain cholinergic neurons are the most consistent and severe biochemical deficits in AD. Both biochemical measurements of ChAT- and AChE-activities as well as ChAT immunohistochemistry and AChE histochemistry have demonstrated severe but regionally variable loss of cholinergic activity in AD brain (Bowen et al. 1976, Davies and Maloney 1976, Davies 1979, Reinikainen et al. 1990, Geula and Mesulam 1996). The loss of cholinergic innervation is greatest in cortical structures within the temporal lobe (Geula and Mesulam 1996). In AD, the number of cholinergic neurons is markedly decreased in all basal forebrain cholinergic cell groups. For example, in NB, neuronal loss ranging from 30 to 95 % has been reported (Whitehouse et al. 1982, Rinne et al. 1987, Geula and Mesulam 1999). The density of muscarinic receptors in AD brain has been reported to be slightly decreased or unchanged depending on the receptor subtype and brain region studied. In hippocampal formation, the loss of muscarinic M_2 receptors has been well documented (Rinne et al. 1989, Aubert et al. 1992, Rodriguez-Puertas et al. 1997). Some studies have also reported a decrease in the numbers of hippocampal M_1 and M_3 receptors, whereas the markers for M_4 receptors remained unchanged (Rodriguez-Puertas et al. 1997). In cortex, a decrease in the numbers of M_1 (Rodriguez-Puertas et al. 1997) and M_2 (Quirion et al. 1989, Aubert et al. 1992) receptors has also been documented. However, there are several reports showing no changes in cortical M_1 (Quirion et al. 1989, Rinne et al. 1989, Aubert et al. 1992) and M_2 (Rinne et al. 1989, Rodriguez-Puertas et al. 1997) or hippocampal M_1 (Quirion et al. 1989, Rinne et al. 1989, Aubert et al. 1992) receptor numbers in AD. In contrast, several studies suggest that the number of cortical nicotinic receptors is decreased in AD brain (Rinne et al. 1991, Svensson et al. 1997). In addition, the coupling of residual muscarinic receptors to G protein might be affected in AD, implying alterations in signalling pathways (Ferrari-DiLeo et al. 1995). Taken together, the cholinergic loss in AD is very extensive, involving the degeneration of cholinergic neurons, processes and receptors as well as changes in cholinergic signal transduction.

2.2 APOLIPOPROTEIN E

2.2.1 ApoE structure and function

Apolipoprotein E (ApoE) is a 35-kD glycoprotein containing 299 amino acids encoded by a four-exon gene on chromosome 19. In humans, the three isoforms of apoE, apoE2, E3 and E4 are encoded by respective apoE alleles (ε2, ε3 and ε4). These three apoE alleles give rise to six different genotypes: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4. The apoE isoforms differ in the presence of cysteine/arginine residues in the receptor-binding domain: apoE2 isoform has cysteine at residues 112 and 158, apoE3 has cysteine at site 112 and arginine at site 158, while apoE4 has arginine at both sites (Table 2) (Beffert et al. 1998).

Table 2. The polymorphism in apoE.

Isoform	Allele	Amino acids
ApoE2	ApoE ε2	Cys-112; Cys-158
ApoE3	ApoE ε3	Cys-112; Arg-158
ApoE4	ApoE ε4	Arg-112; Arg-158

The major sites for apoE synthesis are the liver and the brain. In the brain, apoE is synthesized in both astrocytes and microglia, but not in neurons. The apoE protein has two major functional domains: the carboxy- and amino-terminal domains. The binding of apoE to lipoproteins occurs via the carboxy-terminal domain and the binding of apoE to the receptors occurs via the amino-terminal domain. After being released from the liver into plasma, the apoE is complexed to three classes of lipoproteins: very large lipoproteins, such as chylomicron remnants, very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). In the CNS, only HDL-like lipoprotein particles are found. The apoE-containing lipoproteins are bound and internalized by an apoE-dependent receptor mediated mechanism (Beffert et al. 1998). In the plasma, apoE regulates the lipid and cholesterol metabolism by mediating the transport of lipids to and from the bloodstream. In humans, some of the allelic variations of apoE are associated with lipid metabolism changes and increased susceptibility to atherosclerosis and coronary heart disease. For example, the apoE ε4 allele increases the risk of coronary artery and cerebrovascular diseases and ischemic stroke (Wilson et al. 1996).

In the peripheral nervous system, apoE is involved in the mobilization and redistribution of cholesterol during development and after injury. Following sciatic nerve injury in rats, large amounts of lipids are released from degenerating myelin fibers. At the same time, apoE synthesis and release from macrophages are increased by 100- to 200-fold (Ignatius et al. 1986). At the injury site, the apoE is needed for the scavenging of cholesterol from cellular and myelin debris and for delivery of the lipids for storage in macrophages. Much of the stored cholesterol appears to be reused during axonal regeneration and remyelination (Boyles et al. 1989).

In the CNS, the apoE plays a pivotal role in the redistribution of cholesterol for sprouting neurons. Compared to the peripheral nervous system, the regenerative capabilities of CNS neurons are very limited. However, specific brain areas, such as hippocampal formation, have the ability to induce proliferation of presynaptic extensions from axons or terminals derived from undamaged neurons in order to compensate for the loss of specific input (Poirier 1994). For instance, a lesion to the rat entorhinal cortex removes the cortical connections to the hippocampus and causes a loss of the synaptic input to the granule cell layer. However, when over half of the synaptic input is destroyed, almost complete restoration of synapses can be observed within two months (Matthews et al. 1976). The lesion in the entorhinal cortex is followed by increased synthesis of apoE. It is thought that the apoE transport system and neuronal apoE receptors are involved in the release of lipids from the degenerating synaptic terminals and their storage in astrocytes, as well as in the transportation of lipid components to synaptic terminals for the synthesis of new synapses and terminals (Poirier et al. 1991, Poirier et al. 1993).

2.2.2 ApoE and Alzheimer's disease

Several research groups have recently studied the frequency distribution of different apoE alleles in AD patients. These studies show that the prevalence of the apoE ε4 allele is increased in both sporadic and familial late-onset AD, whereas the frequency of apoE ε2 allele is decreased in familial late-onset AD (Corder et al. 1994, Strittmatter and Roses 1996). In addition to being an important risk factor for AD, the apoE ε4 allele is also associated with several other characteristics of the disease, like the age of onset, severity of pathology and cognitive impairment.

The AD patients with apoE ε4 allele have an earlier average age of onset than patients not carrying an apoE ε4 allele (Strittmatter and Roses 1996). In a recent study, it was found that the inheritance of two copies of apoE ε4 alleles decreased the average age of onset by about seven years (Gomez-Isla et al. 1996). In contrast, the apoE ε2 allele might function as a protective factor and decrease the risk for AD and delay the clinical appearance of its symptoms (Corder et al. 1994, Hyman et al. 1996). The relation between the apoE ε4 allele and the presence of amyloid-containing senile plaques or intraneuronal tangles has also been examined in detail. It has been shown that the amyloid burden is increased in AD patients with the apoE ε4 allele (Schmechel et al. 1993, Gomez-Isla et al. 1996). On the other hand, a lack of correlation between apoE genotype and senile plaques has been demonstrated (Heinonen et al. 1995). The data showing the effect of apoE genotype on accumulation of neurofibrillary tangles in AD brain is more controversial. A positive correlation between neurofibrillary tangles and the apoE ε4 allele has been reported (Nagy et al. 1995, Ohm et al. 1995), whereas there are also reports showing no relationship between neurofibrillary tangles and the apoE ε4 allele (Gomez-Isla et al. 1996). The apoE genotype is also associated with the severity of hippocampal damage and memory impairment. The AD patients homozygous for apoE ε4 allele have the most prominent volume loss in the medial temporal lobe structures and more severe memory loss than those subjects without the apoE ε4 allele (Lehtovirta et al. 1996b, Lehtovirta et al. 1996c).

2.2.3 ApoE, the cholinergic system and synaptic plasticity in Alzheimer's disease

The function of brain cholinergic system is largely dependent on the production of acetylcholine from membrane lipid components. Since the apoE is the major lipoprotein involved in lipid transport and metabolism in the brain, it has an important role in cholinergic transmission. It has been shown that AD patients with apoE ε4 allele suffer more pronounced cholinergic deficits compared to AD patients without the apoE ε4 allele. For example, the cortical and hippocampal ChAT-activity is reduced in AD patients carrying the apoE ε4 allele when compared to patients without the apoE ε4 allele (Poirier 1994, Soininen et al. 1995). Furthermore, the number of cholinergic neurons in NB in AD patients with apoE ε4 is decreased (Arendt et al. 1997). Recently, it was also demonstrated that

AD patients with the apoE ε4 allele tended to have more pronounced cortical EEG slowing when compared to patients without an apoE ε4 allele, which may reflect the more severe cholinergic deficit in these patients (Lehtovirta et al. 1996a, Lehtovirta et al. 2000). The data from clinical drug trial studies suggest that there are also differences in the efficacy of cholinomimetic treatments between AD patients with or without the apoE ε4 allele. The anticholinesterase drug, THA, suppressed EEG slowing less effectively in AD patients with the apoE ε4 allele (Riekkinen et al. 1997b). Poirier et al. (1995) also found that the treatment with THA resulted in a marked improvement of cognitive symptoms in more than 80 % of apoE ε4 negative AD patients, whereas 60 % of apoE ε4 carriers performed worse compared to the baseline situation. These results suggest that the limited efficacy of anticholinesterase treatment to stimulate the cholinergic system might be due to the more severe cholinergic hypofunction observed in AD patients with the apoE ε4 allele.

In AD, the compensatory growth of fibers taking place in subcortical neurons undergoing degeneration is very limited when compared to regular dendritic growth seen during ageing (Arendt et al. 1995). Since apoE is involved in neuronal growth and synaptic remodelling after peripheral and CNS injuries, it has been suggested that apoE genotype might also have an effect on dendritic plasticity in AD patients. Indeed, Arendt et al. (1997) showed that in AD patients carrying one apoE ε4 allele, the increase in dendritic length of subcortical neurons was much less pronounced when compared to apoE ε3/ε3 individuals, and that these plastic dendritic changes were reduced further in patients homozygous for the apoE ε4 allele. Also the data from *in vitro* studies supports the idea that synaptic remodelling and plasticity might be more compromised in AD patients with apoE ε4 allele than in ε4 allele non-carriers. Holtzman et al. (1995) showed that in a CNS-derived neuronal cell line, apoE3, but not apoE4, increased neurite extension. Similarly, in cultured dorsal root ganglion neurons, apoE3 increased but apoE4 decreased neurite outgrowth (Nathan et al. 1994).

2.2.4 ApoE-mutant mice

The importance of apoE in lipid metabolism was first associated with etiology of atherosclerosis. The apoE-deficient mice were created to understand the role of apoE in lipid metabolism *in vivo* and in the genetic and environmental determinants of atherosclerosis. The common features for the apoE-

deficient mice, irrespective of the source and genetic background, are an elevated plasma cholesterol level (5-8 times higher total cholesterol level compared to controls) and atherosclerotic lesions in the aorta, and coronary and pulmonary arteries (Plump et al. 1992, Zhang et al. 1992). Only recently, have apoE-deficient mice been used to study the role of apoE in nervous system and AD.

Following a sciatic nerve crush, the apoE-deficient mice revealed a similar peripheral nerve regeneration as the control mice (Popko et al. 1993, Goodrum et al. 1995). These results suggest that in the peripheral nervous system other lipoproteins may compensate for the loss of apoE. However, in the brain, the apoE is the main apolipoprotein and it is largely responsible for the neuronal plasticity and repair. Masliah et al. (1995b) found that there was an age-dependent 15 to 40 % loss of synaptophysin-immunoreactive nerve terminals and microtubule-associated protein 2-immunoreactive (MAP-2) dendrites in the neocortex and hippocampus of apoE-deficient mice when compared to controls. This suggests that apoE-deficient mice might display age-dependent disruption of the synaptic and dendritic organization of the neocortex and limbic system. Further testing showed that apoE-deficient mice also had abnormal neuronal and synaptic regeneration after perforant pathway transection and closed head injury (Masliah et al. 1995a, Chen et al. 1997). However, Anderson et al. (1998) failed to detect any differences in MAP-2 or synaptophysin immunoreactivity between the apoE-deficient and control mice, and showed similar reactive synaptogenesis in apoE-deficient and control mice after entorhinal cortex lesion. Electrophysiologically, apoE-deficient mice show either reduced (Krugers et al. 1997, Veinbergs et al. 1998, Krzywkowski et al. 1999) or normal (Anderson et al. 1998) long-term potentiation (LTP) in hippocampus. Several reports have also demonstrated cognitive impairments in WM (Gordon et al. 1995, Oitzl et al. 1997) and cholinergic deficits in cortex and hippocampus (Gordon et al. 1995, Chapman and Michaelson 1998) as well as impairments in other neurotransmitter systems, such as the noradrenergic (Chapman and Michaelson 1998, Puoliväli et al. 2000a) and serotonergic systems (Chapman and Michaelson 1998). However, other groups have not found cholinergic (Anderson and Higgins 1997), neuropathological (Anderson et al. 1998, Fagan et al. 1998) or behavioral (Anderson and Higgins 1997, Anderson et al. 1998) alterations in apoE-deficient mice.

More recently, transgenic mice expressing different human apoE isoforms have been developed. Raber et al. (1998 and 2000) showed that mice expressing human apoE4 isoform were impaired in

WM when compared to apoE3 and control mice. Moreover, apoE4 transgenic mice showed a similar age-dependent degeneration of synaptophysin-positive presynaptic terminals and MAP-2 positive neuronal dendrites in neocortex and hippocampus as apoE-deficient mice, whereas apoE3 mice did not differ from controls (Buttini et al. 1999). In the same study, it was also reported that expression of apoE3, but not of apoE4, protected against kainic-acid induced neuronal damage (Buttini et al. 1999). These findings demonstrate that human apoE isoforms have different effects on brain functions *in vivo* and that transgenic mice expressing apoE4 develop cognitive impairments and neurodegenerative changes that resemble those observed in AD patients with the apoE ε4 allele (Raber et al. 1998, Buttini et al. 1999).

3. AIMS OF THE STUDY

The present study was designed to investigate the cholinergic modulation of neocortical arousal and the interaction between the brain cholinergic system and apoE in the modulation of cortical EEG activity, cognitive processes and neuronal plasticity. The specific aims of this study were:

To study the action site of cholinergic drugs to modulate neocortical arousal by investigating the effects of thalamic (NRT and VPM) infusions of a muscarinic M_1 receptor agonist, McN-A-343, a muscarinic M_2 receptor antagonist, methoctramine, and a muscarinic receptor agonist, oxotremorine, on thalamocortically generated HVSs in rat. The hypothesis was that thalamic muscarinic M_1 and M_2 receptors are involved in the regulation of neocortical arousal and HVS activity (I and II).

To study the role of apoE in modulation of cortical EEG activity by investigating the EEG effects of ageing, NB-lesion and scopolamine treatment in apoE-deficient mice. The hypothesis was that the lack of apoE would affect cortical EEG activity during ageing and after scopolamine treatment, and aggravate EEG defects induced by NB-lesion (III).

To study the role of apoE in modulation of cognitive processes and neuronal plasticity by investigating the effects of ageing and NB-lesion on spatial navigation and cholinergic activity in apoE-deficient mice. The hypothesis was that the lack of apoE would disrupt spatial navigation and the integrity of cholinergic system during ageing, and aggravate behavioral and biochemical defects induced by NB-lesion (IV).

4. MATERIALS AND METHODS

4.1 ANIMALS

Male Han:Wistar rats (n=83), and male apolipoprotein E-deficient and control mice (n=180) were used in this study. The rats were 16-18 months old (I and II) and the mice 3-21 months old when tested (III and IV). The animals were singly housed in a controlled environment (National Animal Center, Kuopio, Finland; temperature 22 °C, humidity 50-60 %, lights on from 0700 to 1900 hours) with water and food freely available. The apoE-deficient mice were initially obtained from a colony produced by Dr. N. Maeda and colleagues (University of North Carolina, Chapel Hill, USA) (Piedrahita et al. 1992, Zhang et al. 1992). The apoE gene was inactivated in mouse embryonic stem (ES) cells of 129/Ola origin by homologous recombination. The targeted ES cells were injected into C57BL/6J blastocysts. Chimeras were mated to C57BL/6J to obtain (C57BL/6J x 129/Ola) F1 animals heterozygous for the disrupted apoE gene. These hybrid mice were backcrossed to C57BL/6J mice for nine generations. Homozygous mice were obtained by intercrossing. Progeny from this homozygous line was transferred to Transgenic Alliance, L'Arbresle Cedex, France. The colony was maintained at Transgenic Alliance from which the experimental groups were transferred to Finland. Age-matched C57Bl/6J mice (National Animal Center, Kuopio, Finland) were used as controls. A summary of the experimental design is presented in table 3.

Table 3. The summary of the experimental design: species, animal number, operation and measurements conducted in each publication (I-IV). Abbreviations: ChAT = choline acetyltransferase; n = animal number; NB-lesion = NB-lesion or sham-lesion; WM = water maze; X = measurement/operation conducted; - = measurement/operation not conducted.

Publication	Species	n	NB-lesion	EEG	WM	ChAT-activity	Histology/ Immunohistochemistry
I	rat	41	-	X	-	-	X
II	rat	42	-	X	-	-	X
III	mouse	34	X	X	-	X	-
IV	mouse	97	X	-	X	X	X
IV	mouse	17	-	-	X	X	-
IV	mouse	32	X	-	-	-	X

4.2 IMPLANTATION OF EEG ELECTRODES AND INFUSION CANNULAS

The rats and mice used for EEG recordings were anaesthetized with Equithesin (3 or 5 ml/kg, i.p., respectively) and placed in a stereotaxic frame. Two active EEG recording electrodes (stainless steel screws 0.5 mm in diameter) were implanted symmetrically on both sides above the frontal cortex for both rats and mice. For the rats, the guide cannulas were bilaterally implanted in the NRT, VPM, and ventral hippocampus or unilaterally in the lateral ventricle during the same operation when the EEG electrodes were being implanted. The implantation of EEG electrodes and infusion cannulas is described in detail in publications I-III.

4.3 NUCLEUS BASALIS LESION

The mice used for NB-lesion studies were anaesthetized with Equithesin (5 ml/kg, i.p.) and placed in a stereotaxic frame. The NB-lesion was performed with intracerebral quisqualic acid (Sigma, USA) infusions when the mice were 4 months old. In publication III, the quisqualic acid was infused unilaterally into NB (7.6 μg/0.2 μl/lesion site; two lesion sites per hemisphere; volume of 0.2 μl was delivered in 3 min) and the other hemisphere was sham-lesioned. In publication IV, the mice were either NB-lesioned with bilateral quisqualic acid infusion (3.8 μg/0.2 μl/lesion site; two lesion sites per hemisphere; volume of 0.2 μl was delivered in 3 min) or bilaterally sham-lesioned. The sham-lesion was performed identically to the NB-lesion, but no infusions were made. In publication III, the unilateral NB-lesion was performed during the same operation as the EEG electrodes were implanted. The NB-lesion procedure is described in detail in publications III and IV.

4.4 EEG RECORDINGS

Publications I and II. The IBM-compatible software separated HVSs from background EEG. A cumulative incidence of HVSs or HVS total duration (incidence * mean duration) during a 20-min cumulative waking-immobility (eyes open, head held up) period was used as a marker for HVS activity. The EEG epochs were considered as spike-and-wave HVS periods if they fulfilled the

following criteria: 1. the amplitude of the EEG was more than twice that of the background EEG, 2. the duration of each epoch was > 0.5 s, 3. the frequency of HVS epoch was 6-12 Hz, 4. the time between two separate spindles was > 0.5 s (if the time between two spindles was < 0.5 s, it was considered as one HVS), 5. no movement registered by the magnetic coil binding on the head except vibrissal or head tremor was allowed 1 s before or during each HVS epoch. The total recording time needed to achieve a 20 min period of behavioral waking-immobility related EEG was recorded to monitor the possible motor effects of drug treatments.

Publication III. Five 4 s long artifact-free EEG episodes were recorded from both NB-lesioned and sham-lesioned hemispheres during each recording session in awake and not moving mice. EEG samples were converted to a digital form using a 250 Hz sample rate and Fast Fourier Transformation. The EEG spectrum was divided into the following frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz) and beta (12-20 Hz). The relative amplitudes of these EEG bands were used. For example, the relative delta amplitude = absolute value of delta amplitude/sum of absolute values of all the frequency bands (= delta, theta, alpha and beta). To change the data into a Gaussian distribution, a natural logarithm transformation was applied for relative amplitude values prior to the statistical analyses.

The EEG recording systems are described in detail in publications I-III.

4.5 DRUG STUDIES

Publication I. McN-A-343, a muscarinic M_1 agonist (Micheletti and Schiavone 1990, Lambrecht et al. 1993) (RBI, USA), was administered into the NRT (bilaterally; 2.0 and 20.0 μg/0.25 μl per hemisphere), i.c.v. (unilaterally; 10 and 100 μg/5.0 μl) and for control purposes intrahippocampally (bilaterally; 4.0 μg/0.5 μl per hemisphere). Methoctramine tetrahydrochloride, a muscarinic M_2 antagonist (Giraldo et al. 1988, Michel and Whiting 1988) (RBI, USA), was administered into the NRT (bilaterally; 5.0 and 20.0 μg/0.25 μl per hemisphere) and for control purposes intrahippocampally (bilaterally; 40.0 μg/0.5 μl per hemisphere). Intrathalamic, intrahippocampal or i.c.v. administrations of vehicle were used as the control treatments. The infusion speed in

intrathalamic and intrahippocampal administrations was 0.2 µl/min and in i.c.v. administrations 1.0 µl/min. The effects of McN-A-343 and methoctramine on neocortical HVSs and total recording time were studied.

Publication II. Oxotremorine, a muscarinic agonist (Tayebati et al. 1999) (RBI, USA), was administered into the NRT or VPM (bilaterally; 3.0 and 15.0 µg/0.5 µl per hemisphere), i.c.v. (unilaterally; 10, 30 and 100 µg/5.0 µl) or for control purposes intrahippocampally (bilaterally; 3.0 µg/0.5 µl per hemisphere). Methoctramine tetrahydrochloride, a muscarinic M_2 antagonist (Giraldo et al. 1988, Michel and Whiting 1988) (RBI, USA), was administered i.c.v. (unilaterally; 10, 30 and 100 µg/5.0 µl). THA, an anticholinesterase drug (Freeman and Dawson 1991, Soares and Gershon 1995) (Sigma, USA), was administered systemically (3 mg/kg). Intrathalamic, intrahippocampal, i.p or i.c.v. administrations of vehicle were used as the control treatments. The infusion speed in intrathalamic and intrahippocampal administrations was 0.2 µl/min and in i.c.v. administrations 1.0 µl/min The effects of oxotremorine, methoctramine and THA, either alone or in combination, on HVSs and total recording time were studied.

Publication III. Scopolamine hydrocloride, a muscarinic antagonist (0.05 and 0.2 mg/kg; Sigma, USA), was administered systemically. I.p. injections of vehicle were used as control treatments. In NB-lesion study, the effects of NB-lesion and scopolamine, and in the ageing study, the effects of ageing and scopolamine on cortical EEG activity were examined in control and apoE-deficient mice. The NB-lesioned mice were tested 40 days after the lesion at the age of 5 months, and the mice included in the ageing study were tested at the age of 3, 12 and 21 months.

The methods for drug testing are described in detail in publications I-III.

4.6 BEHAVIOR

The Morris WM was used to study the spatial learning in control and apoE-deficient mice. In standard use of the WM task, the animal is placed into the pool at one of the four randomly ordered start locations (e.g. north, south, west and east 'poles') near the wall and allowed to swim to a submerged platform (e.g. centre of the north-west quadrant) maintained in a fixed position throughout the training. In this study, the platform location was kept constant for five days (initial acquisition) after which the platform was placed in the opposite quadrant for one more day (reversal learning). The percentage of found platforms and the latency to find the platform were measured during training days. The probe trial accuracy was tested after initial acquisition and reversal learning training. During the probe trial, the animal is permitted to swim freely in the pool without any platform present. The use of a place strategy is inferred if the animal crosses the old platform position more often than the equivalent positions in the other three quadrants. A computer connected to an image analyser (HVS Image Ltd., Hampton, UK) monitored the swim pattern. The timing of the latency to find the submerged platform was started and ended by the experimenter. Variables used in the data analysis were: the percentage of found platforms, the latency to find the platform and the number of counter crossings during the spatial probe test. The NB-lesioned mice were lesioned at the age of four months and tested 25 (short-term recovery) or 106 (long-term recovery) days after the lesion at the age of 5 or 8 months, respectively, and the mice included in the ageing study were tested at the age of 16 months. The training paradigm and testing system are described in detail in publication IV.

4.7 BIOCHEMISTRY

The mice in publication III were decapitated at the age of 5 or 21 months, and in publication IV at the age of 5, 8 or 16 months. The brain was quickly removed; the hippocampus and/or frontal cortex were dissected on ice and frozen in liquid nitrogen. Tissue samples were stored at -75 °C until the hippocampal and cortical ChAT-activity were measured according to the method of Fonnum (1975).

4.8 HISTOLOGY AND IMMUNOHISTOCHEMISTRY

In publications I and II, the rats were decapitated after the last recordings and the brains were rapidly removed, dissected on ice and immersed in 4 % formalin. Coronal sections were stained with cresyl violet to verify the accurate placement of the infusion cannulas.

In publication IV, the NB-lesioned mice included in behavioral studies were decapitated one day after the completion of testing at the age of 5 or 8 months. After removal of frontal cortex for biochemical analyses, the remaining brain tissue including the NB-lesion area was immersed in 4 % formalin. Coronal sections were stained with cresyl violet to verify the accurate placement of the lesions. The subgroup of the NB-lesioned and sham-operated mice in publication IV was not behaviorally tested but was used for more accurate histology. These mice were transcardially perfused, and the brains were used for cresyl violet and AChE stainings and ChAT immunohistochemistry at the age of 5 months. The ChAT immunostaining and the calculation of ChAT-positive cells in the area of NB are described in detail in publication IV.

4.9 STATISTICS

The statistical analyses were made by using the SPSS for Windows program. Multivariate analysis of variance (MANOVA), one-way analysis of variance (ANOVA), Scheffe's post-hoc multiple group comparison, post-hoc Wilcoxon signed ranks test and t-test were used to analyse the treatment effects and the group and treatment interactions on different variables. Values of $P < 0.05$ were considered significant. Methods for statistical analyses are described in detail in publications I-IV.

5. RESULTS

5.1 EEG RECORDINGS IN RATS

5.1.1 High-voltage spindle activity

The NRT infusion of methoctramine 20 µg, McN-A-343 2 and 20 µg, and oxotremorine 3 and 15 µg as well as VPM infusion of oxotremorine 15 µg decreased the HVS activity (I, Fig. 2A,B; II, Fig. 4A,B). Intrahippocampal infusions of methoctramine 40 µg, McN-A-343 4 µg, and oxotremorine 3 µg had no effect on HVS activity (I, Fig. 2C; II, Fig. 4C). The i.c.v. administered McN-A-343 100 µg, and oxotremorine 30 and 100 µg decreased the HVS activity (I; II, Fig. 2A). However, the i.c.v. administered methoctramine 100 µg increased the HVS activity (II, Fig. 2B). The i.c.v. administered methoctramine 100 µg blocked the decrease in HVS activity induced by THA 3 mg/kg treatment or i.c.v. administered oxotremorine 100 µg (II, Fig. 3A,B). A summary of the drug effects on HVS activity is presented in table 4.

5.1.2 Total recording time

The NRT infusion of methoctramine 20 µg decreased the total recording time but the NRT infusion on McN-A-343 (2 and 20 µg), and NRT (3 and 15 µg) or VPM (3 and 15 µg) infusions of oxotremorine had no effect on total recording time (I; II, Table 1). Intrahippocampal infusions of methoctramine 40 µg, McN-A-343 4 µg or oxotremorine 3 µg had no effect on total recording time (I; II, Table 1). The i.c.v. administered McN-A-343 100 µg and oxotremorine 30 µg decreased total recording time, whereas the i.c.v. administered methoctramine (10, 30 and 100 µg) had no effect on total recording time (I; II, Table 1). The i.c.v. administered methoctramine 100 µg blocked the decrease in total recording time induced by i.c.v. administered oxotremorine 100 µg but failed to block the decrease in total recording time induced by THA 3 mg/kg treatment (II, Table 1).

Table 4. The effects of methoctramine (METHO), McN-A-343 (McN), oxotremorine (OXO), and tetrahydroaminoacrine (THA) on high-voltage spindle (HVS) activity during waking-immobility in rats. Abbreviations: ↑ = increase in HVS activity; ↓ = decrease in HVS activity; 0 = no change in HVS activity; - = not measured.

Drug	NRT	VPM	Hippo-campus	i.c.v.	i.p. THA 3 mg/kg + i.c.v. METHO	i.c.v. OXO 100 µg + i.c.v. METHO
METHO						
0 µg	0	-	0	0	↓	↓
5 µg	0	-	-	-	-	-
10 µg	-	-	-	0	↓	↓
20 µg	↓	-	-	-	-	-
30 µg	-	-	-	0	↓	↓
40 µg	-	-	0	-	-	-
100 µg	-	-	-	↑	0	0
McN						
2 µg	↓	-	-	-	-	-
4 µg	-	-	0	-	-	-
10 µg	-	-	-	0	-	-
20 µg	↓	-	-	-	-	-
100 µg	-	-	-	↓	-	-
OXO						
3 µg	↓	0	0	-	-	-
10 µg	-	-	-	↓	-	-
15 µg	↓	↓	-	-	-	-
30 µg	-	-	-	↓	-	-
100 µg	-	-	-	↓	-	-

5.2 EEG RECORDINGS IN APOE-DEFICIENT MICE

5.2.1 Nucleus basalis lesion study

NB-lesion decreased the relative amount of delta activity and increased the relative amount of alpha activity similarly in control and apoE-deficient mice (III, Table 3). Scopolamine 0.2 mg/kg decreased the relative amount of theta activity similarly in sham-lesioned and NB-lesioned hemispheres in both strains (III, Fig. 1B). A summary of the effects of NB-lesion and scopolamine treatment on cortical EEG activity is presented in table 5.

Table 5. Cortical baseline EEG activity and the effects of scopolamine (SCOP) on cortical EEG activity 40 days after unilateral quisqualic acid induced NB-lesion in 5 month-old awake and not moving control and apoE-deficient mice. Abbreviations: ↑ = increase in the relative amplitude; ↓ = decrease in the relative amplitude; 0 = no change in the relative amplitude; D = drug effect; L = lesion effect.

Group	Delta 1-4 Hz	Theta 4-8 Hz	Alpha 8-12 Hz	Beta 12-20 Hz
Baseline				
control mice	L↓	0	L↑	0
apoE-deficient mice	L↓	0	L↑	0
SCOP 0.05 mg/kg				
control mice	0	0	0	0
apoE-deficient mice	0	0	0	0
SCOP 0.2 mg/kg				
control mice	0	D↓	0	0
apoE-deficient mice	0	D↓	0	0

5.2.2 Ageing study

Ageing decreased the relative amount of alpha activity similarly in both strains. However, at 3 months of age, the relative amount of alpha activity and at 21 months of age, the relative amounts of alpha and beta activities were higher in apoE-deficient than in control mice (III, Table 3). Scopolamine 0.05 and 0.2 mg/kg increased the relative amount of delta activity and decreased the relative amount of theta activity more effectively in control than in apoE-deficient mice (III, Fig. 2A,B and 3A,B). Scopolamine 0.05 mg/kg more effectively decreased the relative proportion of theta activity in aged than in young mice (III, Fig. 2B). On the other hand, scopolamine 0.2 mg/kg increased the relative proportion of delta activity more effectively in aged than in young control mice, while it had almost no effect in apoE-deficient mice at any age (III, Fig. 3A). Scopolamine 0.2 mg/kg increased the relative proportion of alpha activity more effectively in apoE-deficient mice (III, Fig. 3C). A summary of the effects of ageing and scopolamine treatment on cortical EEG activity is presented in table 6.

Table 6. Cortical baseline EEG activity and the effects of scopolamine (SCOP) on cortical EEG activity in 3- to 21-month-old awake and not moving control and apoE-deficient mice. Abbreviations: ↑-↑↑↑ = increase in the relative amplitude; ↓-↓↓↓ = decrease in the relative amplitude; 0 = no change in the relative amplitude; A = ageing effect; D = drug effect; GM = genetic manipulation effect.

Group	Delta 1-4 Hz	Theta 4-8 Hz	Alpha 8-12 Hz	Beta 12-20 Hz
Baseline				
control mice	0	0	A↓, GM[1]	GM[2]
apoE-deficient mice	0	0	A↓, GM[1]	GM[2]
SCOP 0.05 mg/kg				
control mice	D↑↑	D↓↓	0	0
apoE-deficient mice	D↑	D↓	0	0
SCOP 0.2 mg/kg				
control mice	D↑↑↑	D↓↓↓	D↑	0
apoE-deficient mice	D↑	D↓	D↑↑	0

GM[1,2] = at 3 and 21 months of age, the relative alpha[1] activity, and at 21 months of age, the relative beta[2] activity were higher in apoE-deficient mice

5.3 BEHAVIOR

There were no differences in escape latency or in the number of found platforms between the control and apoE-deficient mice during the initial acquisition stage or in reversal learning at the age of 5, 8 or 16 months (IV, Fig. 1-3). Furthermore, the probe trial accuracy, measuring the number of crossings over the old platform position, was similar in both strains at the age of 5 or 8 months (IV). The NB-lesion had no effect on the escape latency or on the number of found platforms during the initial acquisition after the short- (25 days) or long-term (106 days) recovery in control and apoE-deficient mice. The NB-lesion increased the escape latency and decreased the number of found platforms during reversal learning similarly in both strains after short-term recovery. However, NB-lesion had no effect on the escape latency or on the number of found platforms during reversal learning after long-term recovery (IV, Fig. 1 and 2). NB-lesion decreased the probe trial accuracy similarly in both strains after the short-term recovery but had no effect on probe trial accuracy after long-term recovery (IV). A summary of the effects of NB-lesion on WM behavior is presented in table 7.

5.4 BIOCHEMISTRY

The control and apoE-deficient mice had similar levels of cortical ChAT-activity at the age of 5, 8, 16 and 21 months and hippocampal ChAT-activity at the age of 21 months (III, Table 4; IV, Table 1). The NB-lesion decreased cortical ChAT-activity equally in both strains after recovery of 34, 46 or 115 days (III, Table 4; IV, Table 1). A summary of the effects of NB-lesion on cortical ChAT-activity is presented in table 7.

5.5 HISTOLOGY AND IMMUNOHISTOCHEMISTRY

In publications I and II, the histological analysis revealed that the tips of the infusion cannulas were correctly located in all of the rats with intrathalamic, intrahippocampal and i.c.v. implantations (I, Fig. 3).

In publication IV, the mice included in behavioral studies were decapitated 34 or 115 days after the NB-lesion at age of 5 or 8 months, respectively. The histological analysis revealed that lesions were correctly located in the NB area in all mice. The mice that were used for more accurate histology were killed 34 days after the NB-lesion at the age of 5 months. The gliosis around the needle tract confirmed the correct placement of the NB-lesion (IV, Fig. 4B). The decrease of acetylcholine-containing fibers in cortex of NB-lesioned mice was confirmed by AChE staining (IV, Fig. 4C,D). There were no differences in the number of ChAT-positive cells between the control and apoE-deficient mice and the NB-lesion decreased the number of ChAT-positive cells similarly in both strains (IV, Fig. 4E,F). A summary of the effects of NB-lesion on the number of ChAT-positive cells in NB is presented in table 7.

Table 7. The effects of unilateral or bilateral quisqualic acid induced NB-lesion on cortical ChAT-activity, number of ChAT-positive cells in NB, and WM initial acquisition or reversal learning in control and apoE-deficient mice after different recovery periods. Abbreviations: ↓ = decrease/impairment in ChAT-activity (%), number of ChAT-positive cells (%) or WM spatial navigation in NB-lesioned hemisphere/mice compared to sham-lesioned hemisphere/mice; 0 = no changes in WM behavior; * = unilateral NB-lesion. NB-lesion decreased ChAT-activity, ChAT-positive cells and WM reversal learning similarly in both strains. No strain differences or strain by lesion interaction effects were found in ChAT-activity, number of ChAT-positive cells or WM behavior.

	ChAT-activity in cortex (%)			ChAT-positive cells in NB (%)	WM behavior			
					Initial		Reversal	
Recovery (days)	34	46*	115	34	25	106	25	106
Control mice	21 %↓	27 %↓	14 %↓	17 %↓	0	0	↓	0

ApoE-deficient mice	28 %↓	38 %↓	15 %↓	20 %↓	0	0	↓	0

6. DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Nature of transgenic and control mice

The transgenic and gene-targeted knockout mice have provided valuable models of human genetic disorders and increased our knowledge about the function of different genes in the CNS. However, there are a number of limitations and pitfalls that have to be taken into consideration when interpreting the results obtained from mutant mice. Perhaps the most important factors that may contribute to the observed phenotype are the nature of the transgenic and control lines. The differences in the transgene construct, embryonic stem cell line, host blastocyst strain, background strain and number of backcrossings may affect the phenotype as suggested by Anderson et al. (1998). For example, often the targeted mutation is made in embryonic stem cells derived from the mouse strain '129' which shows neurological anomalies and poor learning in many behavioral tasks (Gerlai 1996, Crawley et al. 1997). It is possible that the targeted locus remains flanked by the '129' mouse strain alleles, and that this piece of chromosome is transmitted to many subsequent generations of mice because of the genetic linkage. Thus, it can be argued that the particular phenotype may not be attributable to the transgene in the targeted locus but to flanking '129' mouse strain alleles (Gerlai 1996, Lipp and Wolfer 1998). In the present study, wild-type littermates were not available. Therefore, the control mice were obtained from a different colony than the apoE-deficient mice, and only matched with age and background strain. This methodological disadvantage may partly explain the lack of major behavioral, neuropathological and neurophysiological alterations observed in the apoE-deficient mice in this study. However, the results from the present study are in many respects very similar compared to the earlier studies conducted with the very same line of apoE-deficient mice (Piedrahita et al. 1992) which did have appropriate wild-type littermates (Anderson and Higgins 1997, Anderson et al. 1998). These results suggest that the behavioral phenotype of apoE-deficient mice of this strain is attributable to the nature of the transgenic line and the use of wild-type littermates vs. age-matched C57Bl/6J mice as controls may not affect the observed phenotype.

6.1.2 Quisqualic acid induced nucleus basalis lesion

The cholinergic neurons in NB are one of the most prominent systems implicated in AD-related cognitive deficits and EEG slowing. The rodent models of cholinergic hypofunction in NB-neocortex system have been widely used to examine the role of the NB in the regulation of cognitive processes and cortical electrical activity. The lesioning of NB electrolytically and with different excitotoxins (e.g. AMPA, ibotenic acid and quisqualic acid) (Winkler et al. 1998) is known to produce behavioral deficits and slowing of neocortical EEG in experimental animals (Riekkinen et al. 1990, Dekker et al. 1991, Wenk 1997). In this study, the NB of control and apoE-deficient mice was lesioned with quisqualic acid, a potent agonist for glutamate sites, which causes the influx of Ca^{2+} resulting in cell dysfunction and death. However, quisqualic acid induced NB-lesion is not selective for cholinergic neurons. More recently, the NB-lesion studies have been repeated using IgG-saporin, an immunotoxin that selectively destroys cholinergic neurons and induces marked depletion of cortical ChAT-activity. It has been shown that the selective loss of cholinergic neurons after an IgG-saporin induced NB-lesion is not associated with memory deficits and EEG slowing in rats (Wenk et al. 1994). These results have indicated that the memory impairments and EEG deficits are not well correlated with the cholinergic loss in basal forebrain and cortex. Instead, the behavioral and EEG deficits observed after NB-lesion are more likely due to destruction of non-cholinergic NB neurons, e.g. GABAergic neurons, and nearby structures involved in cognitive processes and control of cortical EEG, such as striatal and pallidal neurons (cortico-striato-pallidal loops), amygdala, and the NRT (Dunnett et al. 1991, Muir et al. 1993).

6.1.3 EEG recordings in rats and mice

In this study, a model of age-related EEG phenomenon observed in rats was used. In rats, the number of thalamocortically generated HVSs, which occur during states of low arousal and vigilance, has been shown to correlate with cholinergic deficits in aged and NB-lesioned rats (Buzsáki et al. 1988b, Riekkinen et al. 1991e, Riekkinen et al. 1992). Importantly, also in AD, the EEG slowing has been shown to correlate with loss of cholinergic markers (Soininen et al. 1992). The HVS model has been validated in our laboratory during recent years. The research has mainly focused on the

cholinergic system. In addition, several reports relating to other neurotransmitter systems, such as serotonergic (Jäkälä et al. 1995, Jäkälä et al. 1996b) and noradrenergic systems (Riekkinen et al. 1991c, Jäkälä et al. 1992), which are also impaired in AD (Mann 1983, Palmer et al. 1987), have been published. Previously, it has been shown that systemically administered cholinergic drugs modulate HVS activity (Danober et al. 1993, Jäkälä et al. 1996a, Jäkälä et al. 1996b, Jäkälä et al. 1997). However, the site of action of systemically administered drugs to modulate cortical arousal is difficult to pinpoint. Therefore, in the present study, the effects of intrathalamic (NRT and VPM) infusions of cholinergic drugs on neocortical HVSs were investigated. It is possible that the NRT and VPM administered cholinergic drugs may diffuse to the adjacent thalamic nuclei, and that the activation of these nuclei may also contribute to the modulation of the HVS activity. In this model the effects of drug treatments on locomotor activity could also be measured. It was found that the intrathalamically, i.c.v and i.p administered cholinergic drugs not only affected HVS activity but also increased or decreased motor/behavioral activity as indicated by the respective changes in the recording times, suggesting that central cholinergic muscarinic receptors also regulate locomotor activity. However, it should be noted that the effects of the drugs on HVSs truly reflect a behavioral waking-immobility state in the animals, because all the movement related EEG epochs from the HVS recordings were excluded. Therefore, the effects of the drugs on neocortical HVSs during quiet waking-immobility behavior and on behavioral/motor activity can be differentiated.

Previously, in our laboratory, the mouse EEG recording system has been used to measure the effects of $alpha_2$ agonist and antagonist on cortical arousal in $alpha_{2C}$ adrenoceptor overexpressing mice (Björklund et al. 1998). In this study, the effects of NB-lesion, age and drug treatments on cortical quantitative EEG activity were measured in control and apoE-deficient mice. In the NB-lesion EEG study, the number of mice was small, four apoE-deficient and five control mice were tested. This may lower the power of the results, and thus mask or cause misinterpretation of some of the findings. In the ageing study, the same mice were tested at 3, 12 and 21 months of age. Those animals, which had lost their EEG electrodes during the 18 months of ageing, were removed from the analyses. The proper attachment of EEG electrodes to skull of the mice was confirmed by measuring the baseline EEG activity on two consecutive days before the recordings at different ages were started. The mice that had poor connection of EEG electrodes and apparent EEG abnormalities were not included in the analyses. The relative amplitude of different frequency bands was used to reduce the variation

between the mice due to the small group size (NB-lesion study) and to permit the comparisons of EEG recordings at different ages (ageing study). It is possible that some of the EEG activity changes observed in relative amplitude of certain frequency bands might hide the changes occurring in other frequency bands. However, in the present study, the EEG activity changes were not correlated with behavioral or physiological functions of mice but used to differentiate various experimental conditions (strain, age, NB-lesion and drug treatments). This enables the use of relative amplitude as a variable for changes in cortical EEG arousal after different manipulations.

6.1.4 Behavioral testing in water maze

The WM was originally developed for studying spatial learning in the rat (Morris 1984). Today, the WM is also widely used to study learning and memory functions in normal and mutant mice (Björklund et al., 1998; Ikonen et al., 1999; Rissanen et al., 1999; Wilson et al., 1999). The main advantage of the WM task when compared to some other tasks, e.g. radial-arm-maze, is the speed of training. No pretraining is required and the acquisition is rapid. This permits the use of a large number of animals, and different pharmacological and/or lesion treatments can be assessed in short periods of time (Hodges 1996). In the WM task, it is also possible to dissociate different aspects of learning and memory functions (e.g. reference vs. working memory) (Frick et al. 1995), and to assess motor (swimming speed) and visuospatial functions (Hodges 1996, Dudchenko et al. 1997). However, the WM task has been criticised for several reasons. First of all, the WM is an aversively motivated task, in which the immersion of the animal into water may cause endocrinological or other stress effects, which may interact with lesion or pharmacological manipulations in uncontrolled ways (Morris et al. 1982, Hodges 1996). It is also possible that the observed impairment in WM, especially in mice which are not as good swimmers as rats, is attributable to the use of noncognitive behavioral strategies such as passive floating and thigmotaxis (a term used to describe animal's inclination to maintain contact with the walls of the pool) (Lipp and Wolfer 1998). Moreover, the WM procedures vary between laboratories, which makes the comparison of results difficult. For example, the pool and platform sizes, the water temperature and training schedule (e.g. duration of training trial and inter-trial-interval) may affect the acquisition and performance in the WM task (Puoliväli et al. 2000b).

In this study, both reference and working memory components of the spatial navigation were tested. The mice were first trained to locate the platform, which was held in a constant position for five days (reference memory), after which the platform position was reversed for one day (working memory). The retention of spatial memory was tested with a probe trial after both initial acquisition and reversal learning. During the probe trial, the platform was removed and the number of crossings over the previous platform position was measured. The reversal of the platform to a new position requires the animal to adapt new rules to perform successfully in this phase of the task. The results from this study showing rapid learning curves during the initial acquisition phase and impaired performance of NB-lesioned mice during reversal learning are in good agreement with earlier studies using a similar kind of WM procedure in NB-lesioned mice (Sweeney et al. 1988, Sweeney et al. 1989).

6.2 CHOLINERGIC MODULATION OF CORTICAL AROUSAL IN RATS

Previous electrophysiological studies have shown that systemic administration of both muscarinic agonists and anticholinesterases can decrease HVSs (Danober et al. 1993, Riekkinen et al. 1993, Jäkälä et al. 1997, Riekkinen et al. 1997a). The results from this study show that i.c.v. administered oxotremorine (a muscarinic agonist) and McN-A-343 (a muscarinic M_1 selective agonist), also decreased HVSs, and that i.c.v. administered methoctramine (a muscarinic M_2 selective antagonist) increased HVSs and dose-dependently blocked the effect of both i.c.v. oxotremorine and i.p. THA (an anticholinesterase drug) to decrease HVSs. The previous and present results from systemic and i.c.v. administration of muscarinic acetylcholine active drugs suggest that central muscarinic acetylcholine receptors play an important role in the regulation of thalamocortical oscillations and their related neocortical HVSs. However, when the drugs are administered systemically or i.c.v., it is difficult to interpret the exact site of location.

To pinpoint the possible action site of systemically and i.c.v. administered drugs on HVS activity, the effects of intrathalamically administered cholinergic muscarinic drugs on thalamocortical oscillations were studied. The results show that intrathalamically (NRT and/or VPM) administered oxotremorine, McN-A-343 and methoctramine decreased HVS activity, demonstrating that thalamic muscarinic receptors are involved in the regulation of thalamocortical oscillations and neocortical arousal. The results from this study are supported by the anatomical findings showing that muscarinic M_2 receptors are abundant and that M_1 receptors do also exist in the thalamus (Wang et al. 1989, Wall et al. 1991, Wei et al. 1994). Also the earlier *in vitro* electrophysiological studies are in line with the results from this study. Namely, in thalamic slice preparations, the application of the muscarinic agonist acetyl-beta-methylcholine to guinea pig lateral geniculate relay nuclei neurons resulted in a slight hyperpolarization followed by a muscarinic slow depolarization, which inhibited the generation of neuronal burst discharges and promoted the generation of single spike activity in thalamic relay neurons (McCormick 1992a). Furthermore, application of acetylcholine to GABAergic interneurons in the lateral geniculate nucleus or NRT resulted in their inhibition and desynchronization of the EEG via an increase in membrane potassium conductance, this effect being mediated by the M_2 subclass of acetylcholine receptors (McCormick 1990, McCormick 1992b).

The synaptic site of action of muscarinic cholinergic drugs in modulating thalamocortically generated HVSs in thalamus may involve both pre- and post-synaptic muscarinic receptors. Muscarinic M_1 receptors are predominantly post-synaptic, whereas M_2 receptors are both post- and presynaptic in nature (Levey 1996). The presynaptic M_2 receptors are autoreceptors, and their activation results in an inhibition of acetylcholine release, whereas their blockade with antagonists increases the release of acetylcholine. The results from this and earlier *in vitro* studies suggest that administration of oxotremorine and McN-A-343 into the NRT decreases HVSs by activating post-synaptic muscarinic receptors. This in turn inhibits the generation of action potentials in GABAergic interneurons and prevents the thalamocortical relay neurons from hyperpolarizing to burst firing mode. Furthermore, the VPM administered oxotremorine may decrease the hyperpolarization of thalamocortical relay neurons and prevent the activation of Ca^{2+} -mediated spiking and the generation of oscillatory burst firing. The effects of methoctramine on HVS activity, instead, may be mediated via increased release of acetylcholine as a consequence of the blockade of the presynaptic muscarinic M_2 inhibitory autoreceptors in the NRT, leading to an increased activation of post-

synaptic muscarinic and nicotinic receptors. Indeed, it has been previously shown that intrathalamic infusion of the nicotinic acetylcholine receptor agonist, nicotine, dose-dependently decreased HVS activity (Riekkinen et al. 1995).

In the present study, both i.c.v and intrathalamically administered oxotremorine and McN-A-343 decreased HVS activity. These results are in agreement with previous studies showing that systemic administration of muscarinic agonists suppressed HVSs (Riekkinen et al. 1993, Jäkälä et al. 1997). These results suggest that systemically or i.c.v administered muscarinic agonists may modulate neocortical HVS via the thalamus. Interestingly, however, the effects of i.c.v. and intrathalamic administration of methoctramine on HVS activity were different: the i.c.v. infusion of methoctramine, at the highest dose used (100 μg), increased whereas the intrathalamic methoctramine decreased HVS activity. It is possible that the i.c.v. administered methoctramine suppresses the activity of NB and brainstem cholinergic neurons innervating the thalamus. This releases the pacemaker neurons of NRT from the control of NB and brainstem ascending cholinergic system and thereby evokes the appearance of the HVSs in the neocortex. Indeed, it has been shown that both NB-lesion and treatment with low doses of muscarinic antagonist, scopolamine, decrease the activity of cholinergic system and increase the neocortical HVS activity in rats (Buzsáki et al. 1988b, Riekkinen et al. 1991e). On the other hand, the intrathalamic methoctramine may block the presynaptic muscarinic M_2 receptors in NRT, and thus decrease the HVS activity by increasing the available acetylcholine in the synapse. These results suggest that methoctramine regulates HVS activity at least partly via thalamic muscarinic M_2 receptors. However, muscarinic M_2 receptors in other brain structures, such as NB, brainstem and cortex, may also be involved in the regulation of thalamocortical arousal.

The present findings may have some relevance for clinical disorders, such as AD that are associated with cholinergic loss, EEG slowing and cognitive decline. Drugs that inhibit acetylcholinesterase activity are currently being used to compensate for cholinergic loss and to facilitate cognitive functioning and neuropsychiatric symptoms in AD patients (Francis et al. 1999, Levy et al. 1999). Recently, it has also been shown that neocortical EEG slow-wave activity (delta and theta) in AD patients was significantly reduced after chronic acetylcholinesterase inhibitor, THA, treatment (Jelic et al. 1998). The present findings provide further evidence that muscarinic M_1 agonists and M_2

antagonists may facilitate the functioning of cortical and thalamocortical systems, and compensate for damaged cholinergic activating input in AD.

6.3 NEUROPHYSIOLOGICAL FINDINGS IN APOE-DEFICIENT MICE

The present study investigated the effects of quisqualic acid induced NB-lesion and ageing on cortical EEG activity and the EEG response to a muscarinic antagonist, scopolamine, in apoE-deficient and control mice. The effects of NB-lesion on cortical EEG activity in mice were previously not known. The main finding in the NB-lesion study was that the NB-lesion affected EEG activity similarly in control and apoE-deficient mice, indicating that the apoE-deficiency does not increase the vulnerability of NB neurons involved in the regulation of cortical EEG activity. In rats, it has been previously shown that the excitotoxic lesions of NB increase the neocortical slow-waves and HVSs (Buzsáki et al. 1988b, Riekkinen et al. 1991e). Similarly to the situation in NB-lesioned rats, the slowing of EEG in AD has been shown to correlate with the loss of ChAT-activity in frontal cortex reflecting reduced cholinergic input (Soininen et al. 1992). In the present study, the quisqualic acid induced NB-lesion did not induce the slowing of the EEG activity in either control or apoE-deficient mice. In fact, a slight decrease in the relative amount of delta activity in both strains was found in the NB-lesioned hemisphere. It is possible that the 27-38 % cortical ChAT-activity depletion observed in the present study in NB-lesioned hemisphere was not extensive enough to induce shifting to lower EEG frequencies. Indeed, the recent findings in rats showing that IgG-saporin induced NB-lesion producing about 60 % depletion in cortical ChAT-activity also failed to induce cortical EEG slowing, suggest that the selective destruction of NB cholinergic neurons may not be sufficient to slow the overall EEG activity (Wenk et al. 1994). Furthermore, in AD patients the cortical EEG slowing may also be partly related to decreased cerebral blood flow and reduced glucose metabolism (Passero et al. 1995, Bartenstein et al. 1997, Claus et al. 2000). However, it is likely that also the species difference partly accounts for the contradictory findings from this study when compared to the findings in rat studies. Moreover, it has to be stated that the group size in NB-lesion study was small (n = 4-5), which may also complicate the interpretation of the results.

In the ageing study, the effects of muscarinic antagonist, scopolamine, on cortical EEG activity were measured in control and apoE-deficient mice. The scopolamine treatment of mice was associated with a significant shift to lower EEG frequencies. This result suggests that the EEG recording system used in this study was sensitive to shifts in the EEG power spectrum to lower frequencies, a finding that was also to be expected from the NB-lesion study. The results from this study that show EEG slowing in mice after scopolamine treatment are in agreement with previous rats studies showing that scopolamine could induce an increase in the neocortical slow-waves and HVSs (Buzsáki et al. 1988b, Riekkinen et al. 1991e). The main finding in the ageing study was that the scopolamine induced EEG slowing was clearly blunted in apoE-deficient mice, and the difference between the strains became slightly clearer with ageing. Previously, it has been shown that the hypothermic response to a muscarinic agonist, oxotremorine, was also reduced in apoE-deficient mice (Gordon et al. 1995). At the same time, there were no differences in hypothalamic ChAT-activity, suggesting that the reduced thermal response of these mice might be due to the deficiencies in post-synaptic hypothalamic muscarinic receptors (Gordon et al. 1995). In the present study, no differences were found in cortical ChAT-activity between control and apoE-deficient mice, suggesting that the activity of presynaptic cholinergic neurons in cortex is normal.

It can be argued that the blunted EEG slowing in apoE-deficient mice after scopolamine treatment might be due to the reduced number or function of cortical muscarinic receptors. However, in a recent study it was reported that the receptor binding sites for muscarinic M_1 and M_2 receptors as well as for nicotinic receptors were relatively intact in frontal and parieto-temporal cortex and basal forebrain cholinergic nuclei (MS and VDB) of apoE-deficient mice (Krzywkowski et al. 1999). Therefore, it is possible that the reduced EEG slowing in apoE-deficient mice after scopolamine treatment might not be due to the altered number of cortical or basal forebrain muscarinic receptors. It is likely that the alterations in muscarinic receptors in other brain structures, such as brainstem cholinergic nuclei and thalamus, which are also involved in the regulation of cortical arousal, may result in blunted scopolamine response in apoE-deficient mice. Unfortunately, no muscarinic receptor binding studies on these brain structures have been done in apoE-deficient mice.

Interestingly, in AD patients the slowing of EEG activity after scopolamine treatment is also less prominent than in controls, probably reflecting the reduced cholinergic tone in AD (Neufeld et al.

1994). Indeed, in AD patients, decreases in the number of cortical M_1 (Rodriguez-Puertas et al. 1997) and M_2 (Quirion et al. 1989) receptors and alterations in signalling pathways after muscarinic receptor activation (Ferrari-DiLeo et al. 1995) as well as cortical ChAT-activity depletion have been documented (Bowen et al. 1976). Moreover, in AD, the apoE ε4 allele is associated with altered cortical arousal and EEG response to cholinergic therapy. It has been reported that the AD patients carrying the apoE ε4 allele have more pronounced EEG slow-wave activity (Lehtovirta et al. 1996a, Lehtovirta et al. 2000) and decreased responsiveness to the increase in cortical arousal induced by THA (Riekkinen et al. 1997b). These and the present results further indicate that the cholinergic system and its interaction with apoE have an important role in the modulation of cortical arousal.

6.4 BEHAVIORAL FINDINGS IN APOE-DEFICIENT MICE

One of the aims of the present study was to investigate the effects of apoE-deficiency on spatial navigation in WM during ageing. The results show that both young and aged apoE-deficient mice could learn the location of a hidden platform equally well as the control mice. Furthermore, the spatial retention during the probe trial was also similar in both strains. The results from this study that show no differences in spatial navigation in WM between control and apoE-deficient mice are in agreement with previous studies (Anderson and Higgins 1997, Anderson et al. 1998) carried out with apoE-deficient mice derived from the same transgenic line (Piedrahita et al. 1992). These results indicate that the spatial learning of apoE-deficient mice of this strain is not affected during ageing.

However, there are a number of studies showing severe cognitive deficits in WM in apoE-deficient mice of other strains (Masliah et al. 1997, Fisher et al. 1998, Krzywkowski et al. 1999). The apoE-deficient mice have been shown to be impaired especially in the working memory component of the WM task (Gordon et al. 1995, Gordon et al. 1996). Recently, Oitzl et al. (1997) also showed that apoE-deficient mice were not able to learn the WM task at all. In that study, the apoE-deficient mice developed neither spatial nor other strategies to locate the platform, but rather an unusual repetitive behavioral pattern of wall bumping (Oitzl et al. 1997). The learning and memory deficits observed in these studies cannot be explained by motor impairment of apoE-deficient mice since their swimming speed and general motor activity are indistinguishable from those of controls (Gordon et al. 1995,

Krzywkowski et al. 1999). Furthermore, control and apoE-deficient mice performed equally well in a non-spatial version of the WM task in which the animals were tested with a visible platform (Krzywkowski et al. 1999). This result suggests that apoE-deficient mice do not suffer from visual impairment, which would have explained their poor performance in the spatial version of the WM task.

Instead, the behavioral alterations are usually considered to be attributable to biochemical, neurophysiological and neuropathological alterations observed in the apoE-deficient mice. In the study of Gordon et al. (1995), the apoE-deficient mice were cognitively impaired in WM and had markedly decreased cholinergic activity in hippocampus and cortex. Interestingly, the cholinergic and memory deficits observed in apoE-deficient mice could be ameliorated back to control levels by treatment with a M_1 selective agonist (Fisher et al. 1998). On the other hand, Krzywkowski et al. (1999) found that cognitively impaired apoE-deficient mice had markedly reduced LTP in hippocampus, but no changes in their cholinergic system. Moreover, apoE-deficient mice display synaptic and dendritic alterations in hippocampus and neocortex associated with cognitive impairment in WM (Masliah et al. 1997). In the same study, it was reported that apoE-deficient mice treated with apoE showed a significant improvement in WM learning, which was associated with restoration of neuronal structure in hippocampus and neocortex (Masliah et al. 1997). These results suggest that apoE-deficient mice could provide a model for development of therapeutic treatments based on cholinergic replacement therapy or for understanding the neurotrophic effect of apoE in human neurodegenerative diseases such as AD. However, the conflicting results between studies showing normal or severely impaired cognitive functioning in apoE-deficient mice complicate the interpretation of function of apoE in humans.

The inconsistent results between studies can be attributable to a variety of factors. First, the housing conditions and diet might vary between the laboratories, which may influence the results. Second, the handling of mice before the experiment and the experimental procedures of WM task itself were different, making the comparison of the results between the studies difficult. Finally, and perhaps most importantly, the differences in the nature of transgenic and control lines (Piedrahita et al. 1992, Plump et al. 1992) may contribute to the behavioral phenotype (Gerlai 1996, Gerlai 2000). It is possible that in this and earlier studies (Anderson and Higgins 1997, Anderson et al. 1998, Fagan et al. 1998) conducted with very same strain of apoE-deficient mice (Piedrahita et al. 1992), the lack of major biochemical, neurophysiological or neuropathological alterations resulting in normal cognitive functioning in WM, is due to the different genetic background of the apoE-deficient mice.

The present study also investigated whether quisqualic acid induced NB-lesion was associated with more severe WM performance deficits and impaired behavioral recovery in apoE-deficient mice. The results show that the initial acquisition after short- and long-term recovery was similar in sham- and NB-lesioned control and apoE-deficient mice. However, the NB-lesioned mice from both strains were similarly impaired in the reversal learning and subsequent spatial retention after short-term recovery. Furthermore, the impairment in the reversal learning that was observed after short-term recovery was similarly reversed in both strains after long-term recovery. These results suggest that apoE-deficiency does not increase the sensitivity to spatial navigation deficits induced by the NB-lesion and does not impair the behavioral recovery.

A variety of different kinds of NB-lesion models have been used to study the role of the basal forebrain cholinergic system in learning and memory functions in rats (Dekker et al. 1991, Wenk 1997). However, there are only a few studies where the effects of NB-lesion on spatial navigation in mice have been studied (Sweeney et al. 1988, Sweeney et al. 1989). The results from this study are consistent with previous studies in which the NB-lesioned mice were significantly impaired in reversal learning, but not in the acquisition stage of the WM task (Sweeney et al. 1988, Sweeney et al. 1989). However, in rats, impairments both in acquisition and reversal learning or only in reversal learning after NB-lesion have been reported (Riekkinen et al. 1991c). Furthermore, in this study, the NB-lesioned mice showed no impairment in reversal learning after long-term recovery. In contrast, the quisqualic acid NB-lesioned rats were similarly impaired in reversal learning after short- and

long-term recovery (Riekkinen et al. 1991c). The inconsistent behavioral findings in WM can partly be explained by differences in WM paradigms and in the extent of NB-lesions (e.g. different NB-lesion methods and coordinates) as well as by strain and species differences.

The finding that apoE-deficient and control mice were similarly impaired (short-term recovery) and recovered (long-term recovery) after the NB-lesion in WM spatial navigation, indicate that the apoE-deficiency does not predispose NB neurons to quisqualic acid induced excitotoxic trauma and that apoE is not required in the functional modification of those neuronal circuits responsible for the behavioral recovery after NB-lesion. These results differ from the findings of Chen et al. (1997) who found that apoE-deficient mice had more severe behavioral deficits than control mice in the WM task and neuronal cell death in hippocampus after closed head injury. Thus, it can be argued that the recovery of brain structures other than NB, such as hippocampal formation, which are also involved in spatial learning and memory, is more seriously affected by apoE-deficiency. However, it is also possible that other apolipoproteins can compensate for the loss of apoE (Popko et al. 1993).

6.5 BIOCHEMICAL AND NEUROPATHOLOGICAL FINDINGS IN APOE-DEFICIENT MICE

In the present study, the effects of apoE-deficiency on cholinergic activity during ageing and on biochemical recovery after quisqualic acid induced NB-lesion were investigated. Both young and aged apoE-deficient and control mice had similar cortical and/or hippocampal ChAT-activity levels and an equal number of ChAT-positive neurons in NB. Furthermore, the NB-lesion decreased cortical ChAT-activity and the number of ChAT-positive neurons in NB similarly in both strains. Finally, the recovery of cortical ChAT-activity levels after the NB-lesion was not affected in apoE-deficient mice. These results suggest that apoE-deficient mice do not have impairments in their cholinergic activity during ageing and that the apoE-deficiency does not increase the sensitivity to cholinergic deficits induced by NB-lesion nor does it affect the biochemical recovery.

In agreement with this study, it has been shown that apoE-deficient mice derived from the same colony (Piedrahita et al. 1992) do not have any significant alterations in their brain cholinergic activity

during ageing compared to controls (Anderson and Higgins 1997), and that the number and size of cholinergic neurons in MS during ageing and after fimbria-fornix lesion induced axotomy were similar in control and apoE-deficient mice (Fagan et al. 1998). However, the findings from this study are in contrast to a number of previous reports performed with different strains of apoE-deficient mice that show markedly decreased ChAT- and AChE-activities in cortex and hippocampus (Gordon et al. 1995, Chapman and Michaelson 1998, Fisher et al. 1998). Interestingly, these cholinergic deficits in the apoE-deficient mice could be reversed with M_1 selective agonist treatment (Fisher et al. 1998). ApoE-deficient mouse strains may also differ in their response to cholinergic drugs. Gordon et al. (1995) demonstrated a blunted hypothermic response to oxotremorine in apoE-deficient mice, whereas Anderson & Higgins (1997), with the same transgenic line as used in this study, found a similar hypothermic response in control and apoE-deficient mice after treatment with the anticholinesterase donepezil.

The importance of apoE in the regulation of lipid metabolism in the brain is particularly important for the function of the cholinergic system because it is largely dependent on production of acetylcholine from membrane lipid components. In AD, the patients with apoE ε4 allele(s) have more severe neuronal loss and less extensive sprouting of NB neurons (Arendt et al. 1997) associated with a greater cortical and hippocampal ChAT-activity decline (Soininen et al. 1995) compared to patients lacking the apoE ε4 allele. In one study, it was shown that AD patients with the apoE ε4 allele have decreased hippocampal and frontal cortical apoE levels (Bertrand et al. 1995). It could be argued that the decreased level of apoE in AD patients with the apoE ε4 allele compromises the neuronal reorganisation and plasticity in response to NB degeneration. However, the results from this study showing that apoE-deficiency does not influence the function of cholinergic neurons after NB-lesion, support the idea that some mechanism other than 'loss of function' (i.e. decreased levels of apoE) is responsible for the severe cholinergic deficits and impaired compensatory sprouting of NB cholinergic cells seen in AD patients who carry the apoE ε4 allele. It is possible that the different function of apoE4 compared to other apoE isoforms is responsible for the reduced synaptic plasticity in AD patients with the apoE ε4 allele. Indeed, both *in vitro* cell culture studies showing that apoE3 but not apoE4 increased neurite extension (Nathan et al. 1994, Holtzman et al. 1995) and *in vivo* transgenic model studies indicating that apoE3 protected more effectively from excitotoxin induced neuronal damage than apoE4 (Buttini et al. 1999), support this conclusion.

AD is also associated with decreased activity of other neurotransmitter systems such as the noradrenergic (Mann 1983, Marcyniuk et al. 1986, Reinikainen et al. 1990) and serotonergic systems (Palmer et al. 1987, Nazarali and Reynolds 1992, Reinikainen et al. 1990) as well as synaptic loss (Terry et al. 1991) and disruption of the cytoskeleton (McKee et al. 1991). AD patients with the apoE ε4 allele(s) show less plastic dendritic changes and more severe degeneration of LC and raphe magnus nucleus neurons (Arendt et al. 1997). Similarly, it has been shown that the apoE-deficient mice have decreased noradrenergic activity in the brain regions situated distal to LC, such as hippocampus and parietal cortex (Chapman and Michaelson 1998, Puoliväli et al. 2000a), and a decreased number of serotonergic nerve terminals in their parietal cortex (Chapman and Michaelson 1998). Moreover, apoE-deficient mice have impaired recovery of cortical and hippocampal noradrenaline levels after lesioning of LC with a noradrenergic neurotoxin (Puoliväli et al. 2000a). Several reports have suggested that apoE-deficient mice also suffer from alterations in synaptic structure and have decreased synaptic regeneration after CNS injuries. Masliah et al. (1995a and 1995b) found that MAP-2 immunoreactive dendrites and synaptophysin-immunoreactive nerve terminals were decreased in hippocampus and neocortex of apoE-deficient mice and that apoE-deficient mice displayed poor reparative ability after lesioning of the perforant pathway, which results in a loss of synaptic input to the hippocampus. Similarly, apoE-deficient mice also had more pronounced hippocampal cell death and impaired ability to recover after closed head injury (Chen et al. 1997). However, Anderson et al. (1998) showed similar reactive synaptogenesis in apoE-deficient mice after entorhinal cortex lesion compared to control mice, although the apoE-deficient mice initially showed a greater synaptic loss.

In summary, apoE-deficient mice may provide a useful model for investigation of some features of the biochemical and neuropathological changes observed in AD. However, the discrepancy in the results between different studies suggest that factors other than apoE-deficiency, such as the nature of the transgenic line and housing conditions, may be responsible for the differences observed between control and apoE-deficient mice in other studies. More recently, transgenic mice with AD-like neuropathology (e.g. mice expressing extracellular β-amyloid deposits, neuritic plaques, and human apoE4) and cognitive deficits have also been developed (Hsiao et al. 1996, Borchelt et al. 1997, Buttini et al. 1999, Raber et al. 2000). These transgenic mice that mimic several features of

AD, offer further models to examine the neuropathological events that lead to the deterioration of cognitive function in AD and will provide new ways of testing potential therapeutic treatments.

7. CONCLUSIONS

The intrathalamic (NRT and VPM) infusions of a muscarinic M_1 receptor agonist, McN-A-343, a muscarinic M_2 receptor antagonist, methoctramine, and a muscarinic receptor agonist, oxotremorine, increased neocortical arousal as indicated by the decrease in thalamocortically generated HVSs. These results suggest that intrathalamic infusions of cholinergic muscarinic drugs modulate neocortical arousal via muscarinic M_1 and M_2 receptors in thalamus. Furthermore, these results also indicate that i.c.v. and systemically administered cholinergic drugs may modulate cortical EEG activity via the thalamus.

The apoE-deficient mice do not have any apparent impairment in their cholinergic activity (ChAT-activity, the number of ChAT-positive neurons in NB) or in WM spatial navigation during ageing. Furthermore, apoE-deficiency does not increase the sensitivity to cholinergic, behavioral or EEG deficits induced by NB-lesion. However, the apoE-deficient mice have slightly altered cortical EEG activity during ageing and blunted EEG response to scopolamine treatment. These results suggest that apoE does not have to be present to preserve the viability of cholinergic neurons and that spatial navigation and behavioral recovery during ageing or after NB-lesion are not affected by apoE-deficiency. However, apoE-deficiency might alter the regulation of cortical arousal during ageing and after cholinergic drug (scopolamine) manipulation.

In summary, the present studies provide new information about the function of the cholinergic system and its interaction with apoE. This may have relevance in the development of new experimental models and therapeutic treatments for clinical disorders such as Alzheimer's disease.

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