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BEHAVIORAL CHARACTERIZATION OF APOLIPOPROTEIN E-KNOCKOUT MICE

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Abstract

Apolipoprotein E-knockout (apoEKO) mice were characterized behaviorally to evaluate the impact of apolipoprotein E deficiency on spatial learning and memory function at different ages. Wild type and knockout mice were tested in two tasks assessing spatial memory function, Morris water maze (MWM) and Radial arm maze (RAM). Both young and aged apoEKO mice backcrossed six generations displayed deficits in the MWM. However, young and aged 10th generation apoEKO mice did not display any deficits in the MWM or the RAM when spatial cues that could be used to solve these tasks were provided. Removal of spatial cues after training had occurred also did not result in an impairment. In contrast, apoEKO mice were impaired when spatial cues were removed from the beginning of training. This result suggests that these mice are less able to utilize non-spatial cues to solve these tasks. The impairments observed in the MWM and RAM were not the result of impaired reference memory function, but rather appeared to arise from a dysfunction in working memory. Additional tests assessing sensorimotor gating function (Prepulse inhibition), and emotionality (the Open field, the Elevated plus maze) suggested that these cognitive deficits did not arise from alterations in sensorimotor gating function or emotionality, as both young and aged apoEKO mice performed at levels similar to those observed in their aged C57BL/6J control groups.

Résumé

La souris déficiente en apolipoprotéine E (apoEKO) fut étudiée au niveau comportemental afin d'évaluer l'impact d'une déficience en apolipoprotéine E sur l'apprentissage spatial et les fonctions mnémoniques à différents âges. Des souris apoEKO et des souris contrôles ont été évaluées au moyen de deux tests mesurant les fonctions de la mémoire spatiale, soit le Morris water maze (MWM) et le Radial arm maze (RAM). Les souris apoEKO jeunes et âgées de 6ème génération ont démontré un déficit dans le MWM. Cependant, les souris apoEKO jeunes et âgées de 10ème génération n'ont démontré aucun déficit dans le MWM et le RAM, lorsque des indices spatiaux pour effectuer la tâche sont fournis. Lorsque les indices spatiaux sont retirés après la période d'entraînement, aucun déficit n'est observé. Par ailleurs, les souris apoEKO ont montré un déficit lorsque les indices spatiaux sont retirés pendant toute la durée de la tâche. Ces résultats suggèrent que les souris apoEKO sont moins habiles pour utiliser des indices non spatiaux afin d'effectuer ces tests. Les déficits observés dans le MWM et le RAM ne sont pas le résultat d'un déficit fonctionnel de la mémoire de référence, mais semble plutôt émerger d'un dysfonctionnement de la mémoire de travail. Les deux types de souris ont également été testés quant à la réactivité sensorimotrice (Prepulse inhibition) et à l'affectivité comportementale (Open field, Elevated plus maze). Les résultats suggèrent que les déficits cognitifs observés chez les souris apoEKO ne proviennent pas d'un déficit fonctionnel de réactivité sensorimotrice ou d'affectivité comportementale, puisque les souris jeunes et âgées ont performé à des niveaux comparables à ceux observés chez les groupes de souris contrôles du même âge.

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I. Introduction

A. Dementia and Alzheimer's Disease

1. Epidemiology

In Canada, the number of elderly individuals, aged 85 years and older, has increased more than six-fold since 1940 (Gautrin et al., 1990). The population aged 65 and over numbered 3.2 millions (12% of the population) in 1991 (Statistics Canada, 1992), and is expected to reach 7.8 millions 31 years from now (Statistics Canada, 1990). Dementia associated with aging represents an important obstacle to acceptable quality of life in the elderly and has important medical and economic implications (Ebly et al., 1994). Ebly et al. (1994) have reported that the prevalence of dementia in individuals aged 85 years and older was 28.5% in Canada and that the prevalence for dementia doubles every five years between age 65 and 84. Alzheimer's disease (AD) is the most common cause of dementia in the elderly accounting for 75.3% of all cases (Ebly et al., 1994). In Canada, 21.5% of those 85 and older have manifested symptoms of AD. Moreover, it has been reported that more women than men have dementia in Canada (Ebly et al., 1994) which agrees with other reports revealing an increased prevalence of AD in women versus men in Canada (ratio 2:1) (Canadian Study of Health and Aging Working Group, 1994).

2. Clinical Phenotype of Alzheimer's Disease

AD is a neurodegenerative disorder characterized by a progressive loss of memory and a general decline in intellectual functions, leading to severe debilitation and death between 4 and 12 years after onset (Schoenberg et al., 1987). AD is assumed to be a syndrome rather than a single disease with a single discrete etiology. The etiology of AD

is heterogeneous and a variety of risk factors can contribute to the occurrence of AD such as age, genetics, gender, trauma, toxins, vascular diseases and other permissive environmental factors (Cummings and Khachaturian, 1996). Clinical criteria for the diagnosis of AD have been proposed by the *National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association Work Group* (McKhann et al., 1984) the *International Classification of Diseases*, 10th revision (World Health Organization, 1992) and *the Diagnostic and Statistical manual of Mental Disorders*, 4th edition (American Psychiatric Association, 1994). Many patients diagnosed with probable AD often show memory and cognitive deficits, impaired activities of daily living, alteration in mood, delusions and illusions, progressive deterioration of language (aphasia), motor skill (apraxia), and perception (agnosia). Visual dysfunction is also very common. (Van Broeckhoven, 1998; Levy-Lahad et al., 1995; Growdon, 1995; McKhann et al., 1984). Although the diagnostic accuracy for probable AD is high when clinicians use the aforementioned diagnostic method, a definite AD diagnostic is only obtained after death, by histopathologic evidence from a biopsy or an autopsy.

3. Neuropathological Features of Alzheimer's Disease

AD was first described histopathologically by Alois Alzheimer in 1907 (Bick et al., 1987). The most prominent feature is intraneuronal accumulation of paired helical filaments (PHF) which are composed of abnormally phosphorylated tau proteins (Brion et al., 1985). PHF are involved in the formation of neurofibrillary tangles (NFT), neuropil threads and dystrophic neurites. NFT also surround neuritic or senile plaques (SP) (Kosunen et al., 1996; Hyman et al., 1984; Brion et al., 1985), which are aggregates of

extracellular beta-amyloid A (Aβ) protein derived from the amyloid precursor protein (APP) (Chartier-Harlin et al., 1991; Goate et al., 1991). Aβ deposits are found in cerebral blood vessels as well as in neuropils. While the inner core of SP is formed of Aβ, the middle is constituted of swollen axons and dendrites, and the out side part presents degenerating neuritic processes (Haass and Selkoe, 1993; Kidd, 1964; Terry et al., 1964).

SP and NFT are highly concentrated (among other areas) in the hippocampal formation, entorhinal cortex (EC) as well as in the neocortex (Bouras et al., 1994). Although the presence of SP is widely accepted as a reliable diagnostic indicator for AD (Mirra et al., 1991) the presence of such alterations have been also found in aged non-demented individuals (> 60 years). The reasons for this phenomenon are unknown, but it has been proposed that the presence of Aβ deposition in non-demented aged individuals might represent a silent stage of the disease that precedes significant neuronal pathology (Braak and Braak, 1991; Crystal et al., 1988; Morris et al., 1996). Moreover, it has been shown that non-neuritic Aβ plaques, as opposed to neuritic plaques (containing dystrophic neurites), are more commonly found in non-demented individuals and can be used to distinguish between non-demented and demented patients with cortical Aβ deposition (Mirra et al., 1991; Mountjoy et al., 1983; Tomlinson et al., 1968; Braak and Braak, 1991). Histopathological examination of AD brains also reveals loss of neurons and synapses (greater than that of neurons), cytoskeletal aberrations, localized inflammatory reaction, enlarged ventricles and cerebral atrophy (Kosunen et al., 1996; Terry, 1996). It is now believed that dementia associated with AD becomes clinically apparent when the synaptic density falls below a certain threshold.

4. Vulnerability to Degeneration in AD

Certain population of neurons localized in some specific areas are more vulnerable than others to degeneration. For instance, large neurons of association cortex as well as certain subcortical nuclei, including the cholinergic cells of the nucleus basalis complex, the serotonergic cells of the raphe and the noradrenergic cells of the locus coeruleus, are more vulnerable to degeneration (Arendt et al., 1997; Mann et al., 1983; Halliday et al., 1992; Arendt et al., 1983). Increasing evidence for cholinergic dysfunction as a major hallmark for AD and aging have stimulated the interest in the cholinergic forebrain system (Bartus et al., 1982; Whitehouse et al., 1982; Vogels et al., 1990; Deker, 1987). Acetyl choline (ACh) is the major chemical transmitter involved in that system and its action is mediated by nicotinic and muscarinic ACh receptors (Wainer et al, 1984). The synthesis of ACh takes place in axonal terminals and is catalyzed by the cytosolic enzyme choline acethyltransferase (ChAT), which is one of the most reliable markers of cholinergic neurons, fibers and synapses (Houser et al., 1985; Wainer et al., 1984). The cholinergic system has been shown to be involved in mediating learning and memory processes (Gordon et al., 1995). The memory loss in AD has been associated with a decrease of cortical acetyl choline esterase (AChE), the ACh-degradating enzyme present in cholinergic neurons, ChAT activity, choline (Nitsch et al., 1992); as well as loss and shrinkage of cholinergic neurons of the nucleus basalis of Meynert complex (Vogels et al., 1990; Bartus et al., 1982; Coyle et al., 1983; Ransmayr et al., 1989).

Other vulnerable areas such as the enthorinal cortex (EC) and the hippocampus (HIP) present many abnormalities very early in the course of AD (Lehtovirta et al., 1996). These two regions are of a great interest since a large amount of multimodal information

is transmitted to the HIP via the perforant path originating from the EC (Samuel et al., 1994), which, in turn, receives neocortical and subcortical afferents (Pirttila et al., 1996). Extensive loss of neurons in the CA1 area of the HIP and in the EC have been found in AD (Hyman et al., 1984). The loss of neurons in the EC causes the loss of the perforant projection which in turn causes the denervation of the dentate gyrus (DG) (Hyman et al., 1984; Hyman et al., 1986; De Ruiter and Uylings, 1987; Steward, 1976; Steward, 1986; Cotman and Nadler, 1978). The deafferentation of the DG as well as cell loss in CA1 area, along with cholinergic dysfunction, has been proposed to account for the memory impairment in AD (Bartus et al., 1982; Hyman et al., 1984).

5. Genetics

Genetic factors often create a predisposition and play a crucial role in the pathogenesis of AD. In fact 25-40% of patients with AD seem to have a familial history of AD (Davies, 1986). The transmission pattern of AD is fairly complicated. Families with early-onset AD (EOAD) (< 65 years) have a transmission pattern consistent with an autosomal dominant disorder with penetrance dependent on age (Heston et al., 1981; Fitch et al., 1988; Hofman et al., 1989). On the other hand, late-onset AD (LOAD) (> 65 years) families have a complex pattern of inheritance where genetic and environment factors interact in an unknown fashion (Curts and Van Broeckhoven, 1998a).

Four genetic loci have been identified as contributing to AD, including the amyloid precursor protein gene (APP) on chromosome 21, the presenilin 1 gene (PS1) on chromosome 14, the presenilin 2 gene (PS2) on chromosome 1, and the apolipoprotein E gene (APOE) on chromosome 19. However, these four loci do not account for all the genetic risks for AD indicating that there are additional unidentified AD loci. Mutations

in APP, PS1 and PS2 genes can cause EOAD. Mutation in APOE gene is mostly associated with the more common familial and sporadic form of LOAD (Pericak-Vance et al., 1997; Poirier, 1993; Corder et al., 1993), but has also been associated with EOAD with a positive family history (Van Duijn et al., 1994). APP, PS1 and PS2 will be discussed briefly while APOE will receive a greater attention in the following sections.

5.1. APP

The mutated APP gene (precursor of Aβ) was the first gene to be associated with AD (Wisniewski et al., 1985; Mann 1988). Five missense mutations have been identified and account for less than 1% of all AD cases (Van Broeckhoven, 1998) and 5% of EOAD (Van Broeckhoven, 1998; Van Broeckhoven, 1995). The relation between APP and AD came from the observation that many Downs syndrome (trisomy 21) patients also suffer from EOAD, and with a corresponding increase in Aβ deposits (Van Broeckhoven, 1998). These findings emphasized the role of Aβ in plaques formation (Van Broeckhoven, 1998; Kang et al., 1987). It is believe that an abnormal functioning of the APP gene will lead to an increased Aβ production and deposition, which in turn will contribute to SP and NFT formation (Yanker et al., 1990). In vitro studies have shown that high concentrations of the mutated Aβ protein are toxic for differentiated neurons, suggesting that Aβ could be responsible, to some extent, for neuronal death in AD death (Hardy and Higgins, 1992).

5.2. PS1 and PS2

The PS1 gene may account for up to 50% of familial EOAD (Curts and Van Broeckhoven, 1998b). The onset of the disease is very early (30 to 60 years) in patients carrying a mutated version of the gene (Sherrington et al., 1995). Approximately 43 mutations of this gene have been found (Curts and Van Broeckhoven, 1998b). Scheuner et

al. (1995) suggested that PS1 may influence APP processing and indirectly increase Aβ production and ultimately, deposition.

PS2 may account for 20% of familial EOAD (Levy-Lahad et al., 1995). Three missense mutations of this gene have been reported (Curts and Van Broeckhoven, 1998b). PS1 and PS2 show a 67% homology. The natural biological functions of both PS1 and PS2 have not been clearly defined yet. However, mutant versions of PS1 and PS2 induce a very aggressive form of AD with a rapid progression, leading to death within 3-5 years of onset (Van Broeckhoven, 1995).

5.3. APOE

In humans, APOE has three major alleles (APOE ε2, APOE ε3 and APOE ε4) giving rise to three common apoE isoforms (apoE2, apoE3 and apoE4) and six apoE phenotypes (E2/2, E2/3, E2/4, E3/3, E3/4, E4/4) (Wisniewski and Frangione, 1992). Numerous studies worldwide have now confirmed the ε4 allele as representing a major risk factor associated with sporadic (Schmechel et al., 1993; Beffert and Poirier, 1996; Poirier, 1993; Saunders, 1993a) and familial (Strittmatter, et al., 1993; Corder, et al., 1993; Saunders, 1993a; Saunders, 1993b) LOAD. Estimates indicate that more than half of the susceptibility to AD is associated with the APOE locus (Nalbantoglu et al., 1994; Roses et al., 1995).

6. APOE Allele Frequency and Alzheimer's Disease

The frequencies in eastern Canada for a control population for the ε2, ε3 and ε4 alleles are, respectively, 77.0%, 8.8% and 12.2% (Poirier, 1993). The most common phenotype in the human population is E3/3 (Davignon et al., 1988; Poirier, 1993; Farrer et

al., 1997). The ε4 allele frequency is roughly 3 fold (i.e. 40%-50%) greater in the AD population as compared to controls (Poirier, 1993; Strittmatter et al., 1993). In terms of prevalence, a total of 80% of familial and 64% of sporadic AD late-onset cases have at least one copy of the ε4 allele compared to 31% of controls subjects (Corder et al., 1993). The prevalence, as a function of age, indicated that as many 80% of AD subjects between the ages 65 and 75 were shown to carry at least one copy of the ε4 allele (Poirier, 1994; Poirier, 1993; Nalbantoglu et al., 1994). However, a sharp decline in the prevalence of the ε4 allele is observed in very old subjects (> 85 years), suggesting the presence of a late-late onset form of AD and is also consistent with the increased risk of coronary heart disease leading to death in ApoE ε4 subjects before the age of 85 years (Davignon and Roy, 1993).

7. APOE ε4 Allele and Alzheimer's Disease

The mechanisms by which the ε4 allele influences the pathology and the progression of AD are unknown. However, the number of inherited copies of ε4 allele appears to affect the progression and the severity of AD. For instance, the number of ε4 copies correlates with the number of SP (Namba et al., 1991; Rebeck et al., 1993; Richey et al., 1995) and NFT (Ohm et al., 1995; Rebeck et al., 1993; Schmechel et al., 1993). Additionally, greater synaptic cholinergic degeneration in the HIP, temporal cortex (Poirier et al., 1995; Poirier, 1994) and forebrain (Blacker et al., 1997) has been observed in ε4 AD patients as compared to non-ε4 AD patients. Several studies have shown a marked decrease in presynaptic cholinergic markers such as ChAT activity, nicotinic receptors and nerve growth factors receptors as well as AChE-positive neuron density in

the nucleus basalis of Meynert in ε4 AD individuals as compared to non ε4 AD individuals (Arendt et al., 1997; Aubert et al., 1992; Poirier et al., 1995). The presence of ε4 has also been shown to impact on drug response to cholinominetic and noradrenergic agents. Specifically, non ε4 AD patients show greater improvement after 30 weeks of treatment with the AChE inhibitor tacrine as compared to ε4 AD patients (Poirier et al., 1995; Richard et al., 1997).

APOE genenotype has also been shown to impact on the severity of neuronal degeneration and the extent of plastic dendritic remodeling in AD (Bennett et al., 1995; Arendt et al., 1997). ε4 AD patients show significantly more degeneration in the basal nucleus of Meynert, locus coeruleus, raphe magnus nucleus and medial amygdaloid nucleus and less neuronal plasticity in these same areas (Arendt et al., 1997). In addition, ε4 patients are more inclined to abnormal aggregation of cytoskeletal proteins, leading to the formation of NFT (Higgins et al., 1997; Mahley et al., 1996; Strittmatter et al., 1994). The presence of ε4 in AD patients accentuates the synaptic loss and the severity of the dementia (Miller et al., 1994). These findings suggest a profound impairment in neuronal repair and a compromised functional compensation in ε4 AD patients, which accounts to explain the earlier age of onset in these patients (Arendt et al., 1997; Corder et al., 1993). Not only is the ε4 allele associated with the biological markers of AD, it also has a strong impact on cognitive functions. A study of Blisa et al. (1996), analyzing the incidence of the ε4 allele in pre-symptomatic subjects with age-related memory decline, has shown an association between high AD assessment scale-cognitive component (ADAS-Cog) scores and the incidence of ε4 allele in these subjects. In addition, a correlation between the prevalence of the ε4 allele and deterioration in working memory was found in AD

patients (Blacker et al., 1997).

APOE genotype has also been demonstrated to impact the rate of recovery following brain damage caused by strokes. Clinical observations reveal that ε4 genotyped patients who had an intracerebral hemorrhage, have higher mortality than non-ε4 patients (Alberts et al., 1995). Another study has shown that ε4 genotyped patients who survive a stroke display more cognitive deficits than non ε4 patients (Slooter et al., 1997; Snowdon et al., 1997). These findings are in agreement with others studies showing an association with ε4 and decline of cognitive performances after a head injury (Teasdale et al., 1997), or an operation (Tardiff et al., 1997; Newman et al., 1995).

8. Role of ApoE in the Central Nervous System

Increasing interest to investigate the function of apoE in the brain followed the discovery of the association between APOEε4 and AD (Poirier, 1993; Corder et al., 1993) as well as with the detection of apoE mRNA in the brain of rat, marmoset and human (Elshourbagy et al., 1985). ApoE is a protein constituent of plasma lipoproteins and serves as a ligand for receptor mediated clearance of several classes of lipoproteins through its interaction with several receptors including the low density lipoprotein (LDL) receptor (Mahley, 1988; Goldstein et al., 1983), the LDL receptor-related protein (LRP) (Beisiegel et al., 1989), the very low density lipoprotein (VLDL) receptor (Takahashi et al., 1992) and the apoER2 receptor (Kim et al., 1996).

In the brain, apoE is produced by astrocytes and apoE immunoreactivity and mRNA have been detected throughout the whole brain. Like apoE, the apoE receptors are also expressed in the central nervous system (CNS) (Beffert et al., 1998). Although the function of apoE in the nervous system is still unclear, many studies have proposed a role

for apoE in lipid (cholesterol and phospholipids) transport and metabolism (Poirier et al., 1991; Poirier et al., 1993; Poirier, 1994; Masliah et al., 1995a; Masliah et al., 1995b; Mahley, 1988) since other peripheral apolipoproteins, such as apoAI, apoA-IV and apoB, involved in cholesterol metabolism are not synthesized in the CNS (Beffert et al., 1998; Roheim et al., 1979). It is then presumed that both lipid transport and metabolism will rely, at least in part, on apoE (Pitas et al., 1987; Poirier, 1994; Malhey, 1988).

Important contributions to the understanding of the functional role of apoE in the nervous system were suggested by findings showing that apoE mRNA was upregulated and protein production increased after peripheral nerve crush (Boyles et al., 1989; Boyles et al., 1990; Goodrum and Novicki, 1988; Gelman et al., 1987) and several CNS injuries including ischemia, excitotoxic insult, deafferentation, brain transection, seizure activity and inflammation (Passinetti et al., 1993; Poirier et al., 1991; Cheng et al., 1994; Ong et al., 1997; Schauwecker et al., 1998; Terrisse et al., 1999; Laskowitz et al., 1997). These results suggest an important role for apoE in neuronal repair in both peripheral and central nervous system (Poirier, 1994; Boyles et al., 1990).

Poirier (1994) has proposed an hypothetical model to characterize the role of apoE following injury to the CNS. ApoE has been shown to play a role in the transport of cholesterol released from breakdown of presynaptic terminals following neuronal injury to both neuron undergoing reinnervation and terminal and axonal sprouts of residual afferents present in the deafferented area (Beffert et al., 1998; Poirier et al., 1991; Poirier, 1994). The apoE-cholesterol-lipoprotein-complex is thought to be internalized by the LDL receptor and/or other apoE receptors expressed on cells in need of cholesterol and used as a precursor for the synthesis of new membrane to insure dendritic reorganization

and reactive synaptogenesis.

B. Genetic Model of Alzheimer's Disease

1. The ApoE-Knockout Mouse Model

The apoE-knockout (apoEKO) mouse model was initially created to study hypercholesterolemia (Breslow, 1996). Two different lines of apoEKO mice have been created in two different laboratories (Piedrahita et al., 1992; Plump et al., 1992). Both have been produced by inactivating the endogenous APOE gene by using gene targeting in mouse embryonic stem cells. The offspring of the homozygous apoEKO mice are backcrossed six or ten times with C57BL/6J mice, a stable inbred strain, before being used for testing. The two lines of apoEKO mice show severe arteriosclerosis with age and elevated plasma cholesterol (Piedrahita et al., 1992; Plump et al., 1992). Both lines of apoEKO mice have been used to evaluate the physiological role of apoE in the brain (Anderson and Higgins, 1997; Anderson et al., 1998; Veinbergs and Masliah, 1999). Several of the neuropathological, neurochemical, neurophysiological and behavioral abnormalities that have been detected in these mice brain will be reviewed in the next section.

1.1. Correlations between Neuropathological Alterations and Behavioral Deficits

An age-dependent 15-40% loss of synaptophysin-immunoreactivity (SYN-ir) nerve terminals and microtubules-associated protein-2-immunoreactive (MAP2-ir) dendrites was detected in the neocortex and HIP of apoEKO mice as compared to wild type mice. Ultrastructural studies using electron microscopy revealed extensive vacuolization of apical dendrites (molecular layer of the dentate gyrus) and disruption of

the endomembrane system and neuronal cytoskeleton (Masliah et al., 1995b). Dentritic alterations were absent in other areas including cerebellum, thalamus, caudo-putamen, mesencephalic pontine and bulbar nuclei, which are also some of the areas spared in AD. Cytoskeleton alterations and synaptic loss have been shown to correlate with cognitive impairments (memory deficit) in apoEKO mice (Masliah et al., 1995b; Gordon et al., 1995; Gandy et al., 1995; Masliah et al., 1997; Chen et al., 1997; Fisher et al., 1998; Gordon et al., 1996b; Gozes et al., 1997; Krzywkowski et al., 1999; Masliah et al., 1997; Oitzl et al., 1997; Veinbergs and Masliah, 1999). Cognitive impairments were partially restored with intracerebral injection of human recombinant apoE (Masliah et al., 1997). However, in contrast to these findings, Anderson et al. (1998) failed to find any memory deficits, CNS morphological changes, cytoskeleton alterations or synaptic and dendritic density reduction in apoEKO mice.

Abnormal and delayed synaptic regeneration in response to hippocampal deafferentation has been shown in apoEKO mice (Masliah et al., 1995a). However, another study has shown normal and complete reinnervation processes in apoEKO mice, although a slight delay in the onset of synaptogenesis was observed (Anderson et al., 1998). This delay might be the consequence of a persistence of degeneration products normally present in the deafferented HIP soon after a lesion (Fagan et al., 1998).

1.2. Neurochemical Alterations

In view of the role of apoE in lipid homeostasis, the absence of apoE was expected to alter the cholinergic system which is highly dependent on proper lipid (cholesterol) delivery. Supporting this contention, a reduction of 40% in ChAT activity in the HIP and frontal cortex has been shown in apoEKO mice (Gordon et al., 1995). Similar findings

have shown a decrease of 25% in ChAT activity from a total brain extract in apoEKO mice as compared to control mice (Gozes et al., 1997). These cholinergic impairments were thought to be the basis for memory impairments reported in apoEKO mice. Moreover, cholinergic impairments were shown to be restored to control levels following treatment with vasoactive intestinal peptide (VIP) or its potent analog stearyl-Nle17-VIP (Gozes et al., 1997). Similarly, a recent study has shown that 3 weeks treatment with a M1-selective muscarinic agonist totally reversed the spatial working memory deficit and restored level of AchE activity and ChAT immunoreactivity in septal, cortical and hippocampal areas in apoEKO mice (Fisher et al., 1998). However, other studies have reported no signs of alterations for ChAT activity, hippocampal ACh release, nicotinic and muscarinic (M1 and M2) receptor binding sites and AChE cells or terminal density in apoEKO mice (Krzywkowski et al., 1999; Anderson and Higgins, 1997; Fagan et al., 1998). The reasons for these discrepancies are unclear, but may reflect differences in background strain of the animals studied. It is also likely that the presence of compensatory mechanisms would maintain steady state concentrations of ACh in the apoEKO mice (Wurtman, 1992).

Synaptic densities of cholinergic, noradrenergic and serotonergic projections in specific brain areas have been reported to be lower in apoEKO mice as compared to wild type mice while dopaminergic densities were not altered. The extent of presynaptic damage was found to be more pronounced the further away the nerve terminal is from its cell body. These findings suggest that the susceptibility of neurons to the absence of apoE involve both pathway-specific differences and distance of the nerve terminals from their cell body (Chapman and Michaelson, 1998).

1.3. Stress Response

Altered stress responsiveness has been shown in apoEKO mice (Gordon et al., 1996a; Zhou et al., 1998). ApoEKO mice displayed a lower elevation of corticosterone in response to restraint stress and a slower descending rate in serum corticosterone level 30 minutes after a stress period. However, under normal conditions, similar corticosterone concentrations were observed in both apoEKO and wild type mice (Gordon et al., 1996a; Zhou et al., 1998). To our knowledge, the neuroendocrine response to other stressors has not been examined. Nor have potential differences in the behavioral response to stressful environments (i..e., measures of "anxiety" or fearfulness) been explored in these mice.

C. Cognitive Tests to Evaluate Memory

The Morris water maze (MWM), a well-validated cognitive task (Morris, 1981; Morris, 1984), is used to assess spatial memory in apoEKO mice because of its sensitivity to cholinergic manipulations (Grauer and Kapon, 1996) and hippocampal functioning (Oitzl et al., 1997). In this test, animals have to swim to a platform hidden under the surface of the water, using spatial cues (Morris, 1981; Morris, 1984).

The Radial arm maze (RAM) is an other behavioral test to assess spatial learning and memory in rodents (Levin, 1988). Mice have to choose among eight alternative paths simultaneously (Olton and Samuleson, 1976). The rodents use extra-maze spatial cues in the environment rather than intra-maze cues (Maki, 1984; Olton et al., 1977). The advantage of this test over the MWM is that it permits a more detailed analysis of the type of memory impairment caused by a specific manipulation. Specifically, it allows a distinction between reference memory (the general rules needed to solve a task) and

working memory (the information needed to on a trial by trial basis). In the RAM, reference memory consists of a "win-shift" rule (i.e., visit all 8 arms once without returning to arms already visited), whereas working memory consists of the arms that have been visited (or have yet to be visited) on any one trial. Working memory can be evaluated by looking at the distribution of errors over successive choices within trials (Olton and Samuleson, 1976). The more choices an animal makes within a trial, the more the information the animal has to remember, which imposes a greater demand on working memory. Since working memory has a limited capacity, the probability of making an error should increase with the amount of information to be retained. On the other hand, the pattern of errors observed in an animal suffering from a reference memory deficit should remain constant over successive choices. It has been showed by Olton and Samuleson (1976) that the probability of making an error within the same trial is higher as the number of choices increases, suggesting that working memory function can be assessed by the analysis of the serial forgetting curve. Finally, by analyzing the patterns of arm choices made, this test permits an analysis of the strategy used by the animal to solve the task.

The Prepulse inhibition (PPI) test is another cognitive task in which presentation of a weak pre-stimulus presented 30 to 500 ms before a loud startle-elciting stimulus reduces the degree of response elicited by the startle stimulus. This test has the advantage that the response occurs naturally, without any previous learning (Swerdlow et al., 1986), and affects pre-attentive neural processes underling sensorimotor gating (Acri et al., 1995). The prepulse stimulus acts as a filter or a gate for the startle stimulus. Although the systems mediating the inhibition of startle are not fully known, Dupuy et al. (1998) have

showed that this test can identify sensorimotor deficits in mice.

D. Scope of the Present Study

Several studies have reported significant loss of synapses and deterioration of the dendritic cytoskelton with age, as well as abnormal synaptic regeneration, limited repair capacity and functional deficits following brain injury in apoEKO mice (Masliah et al., 1995a; Masliah et al., 1995b; Veinbergs and Masliah, 1999). Based on these observations, apoE is believed to play an important role in the maintenance of cytoskelton integrity, neuronal plasticity and recovery from injury (Masliah et al., 1995a; Poirier, 1994). Normal cognitive functions are highly dependent on an intact cytoskeleton. It is then likely that the behavioral, neurophatological and neurophysiological alterations seen in apoEKO mice are related to disruption of the neuronal cytoskeleton integrity caused by the absence of apoE.

Interestingly, apoEKO mice seem to share many similarities with AD patients (as summarized above). Particularly, apoEKO mice showed cognitive impairments (spatial memory deficits). However, cognitive impairments have not been observed consistently. The reasons for this variability are unknown.

One source of variation may be the implementation of the behavioral tasks used in the assessment of cognitive impairments. One of the most widely used tasks to assess cognitive deficits (spatial memory) in rodents is the MWM. This behavioral task has been employed by all the laboratories working on memory in apoEKO mice. However, experimental protocols also vary between laboratories. The impact of these technical disparities has never really been considered in the interpretation of the data. For example, although most investigators automatically assume that the MWM forces the animal to

utilize extra-maze spatial cues to solve the task, proficient performance can be achieved with the use of non-spatial learning and memory processes (Buresova et al, 1986), and it is possible that different protocols promote differentially the use of spatial and non-spatial strategies. The possibility that apoEKO mice might adopt non-spatial learning strategies to solve the task has never been explored. Identification of the learning strategies adopted by both apoEKPO and C57BL/6J mice may resolve some of the controversy surrounding the issue of whether or not apoEKO mice display cognitive deficits in the MWM. Furthermore, these studies could provide a better understanding of which type of memory is affected in apoEKO mice.

A second variable that could contribute to the variability of results observed in apoEKO mice could be age. Assuming that apoE contributes to neuronal repair, coupled with the observation that neuronal damage likely accumulates with age, it may be that apoEKO mice are less able to compensate for age-related neuronal damage.

The present series of experiments aimed to characterize the behavioral phenotype of apoEKO mice and to evaluate the impact of apoE-deficiency on cognitive functions at different ages. We also sought to identify whether we could observe differences in the use and efficiency of spatial and non-spatial learning and memory processes at different ages. Aged apoEKO mice may use a different, and perhaps less efficient, cognitive strategy to solve the MWM. Such possibilities could explain why sometimes apoEKO mice (neuropathologicaly altered) perform as well as wild type mice (neuropathologicaly intact) and how the behavioral test can fail to detect any functional impairment associated with neuropathology in discrete areas of the brain.

In order to discriminate between spatial and non-spatial learning strategies and to

evaluate the effect of the age on cognitive functions, we have tested both apoEKO and littermate wild type mice at 3 and 10 months of age in two behavioral tasks assessing spatial learning and memory: the MWM and RAM. Two different sets of experiment were carried out to assess spatial and non-spatial learning strategies. The first one was performed with extra-maze cues available while the other was done with extra-maze cues removed (hidden by curtains around the maze) for the entire duration of the task.

Since apoEKO mice were shown to have an altered response to stress (Gordon et al., 1996a; Zhou et al., 1998), and since altered stress responses could compromise cognitive function, we wanted to further characterize emotionality and/or anxiety of the apoEKO mice in several behavioral tasks including the Open field (OF) and the Elevated plus maze (EPM). Moreover, to further investigate potential attention deficits, we assessed prepulse inhibition of the acoustic startle reflex in both groups of mice.

II. ApoE-Knockout Mice Model 6th Generation

A. Materials and Methods

1. Animals

Two strains of mice were used, male C57BL/6J (controls) mice and male heterozygous apoEKO mice. The apoEKO mice, originally produced in the laboratory of Dr. Meada (University of North Carolina, Chapel Hill, NC, USA), were purchased from Jackson Laboratories, Bar Harbor, ME. They were generated by injecting targeted clones into C57BL/6J blastocytes (Plump et al., 1992). They were backcrossed six times with C57BL/6J mice for homogeneity. Experiments were done during the light phase of a 12:12-h light-dark cycle (lights on at 8:00 am). Animals were housed 1 per cage and

maintained in a room were the temperature (22°C) and the humidity (40-60%) was controlled. All mice had *ad libitum* access to water and standard laboratory mouse chow.

C57BL/6J and apoEKO mice tested at two ages (3 month old, n = 8 per strain; and 8 month old, n = 9 per strain) were used in these experiments.

2. Behavioral Testing

2.1. Morris Water Maze

The MWM was a circular pool measuring 1.6 m in diameter and 60 cm of deep. The pool was filled with water maintained between 23°C and 25°C, to a height of 55 cm and made opaque by the addition of powdered skim milk. In the center of one of the quadrants, a platform (10 cm^2) was placed 1 cm below the water surface. An **RCA** video camera hung above the pool and a **Videomex-V** Image Motion System (Columbus Instruments, Columbus, OH) automatically recorded the latency, and distance to find the platform.

For 5 consecutive days, each mouse received four trials per day. At the start of each trial the animal was placed at a different starting point and had 120 s to find the platform which was always located in the same quadrant. If the mouse did not find the platform it was placed on it for 6 s. The intertrial interval ranged from 10 to 16 min. On day 6 of the second experiment of this section, the platform was removed from the pool for 2 trials of 1 min, the latency and the distance spent in every quadrant of the pool was recorded. The platform was then placed back to its original place for 2 other trials of 120 s. For these 2 last trials the platform was made visible by lowering the water in such way that the mouse could see the platform, 1 cm above water. Mice could solve the task by using spatial cue (pictures of different patterns and shapes) placed around the testing

room.

B. Results

1. Morris Water Maze

1.1. 3 Month Old Mice

As for most studies reporting results from testing done in the MWM (Gordon et al., 1995 ; Oitzl et al., 1997), only latency was analyzed. Data from each of the four trials within each day were averaged and used in subsequent statistical analyses.

The latency data were analyzed by a Strain x Days analysis of variance (ANOVA). Strain was a between subject factor and Days was a within subject factor. It revealed a significant Strain main effect suggesting that apoEKO mice took significantly longer to find the platform than C57BL/6J mice [$F(1, 14)= 21.36$, $p<0.01$] and a significant main effect for Days [$F(4, 56)= 4.57$, $p<0.01$] indicating that both groups showed significant changes in latency across days of training (see Figure 1).

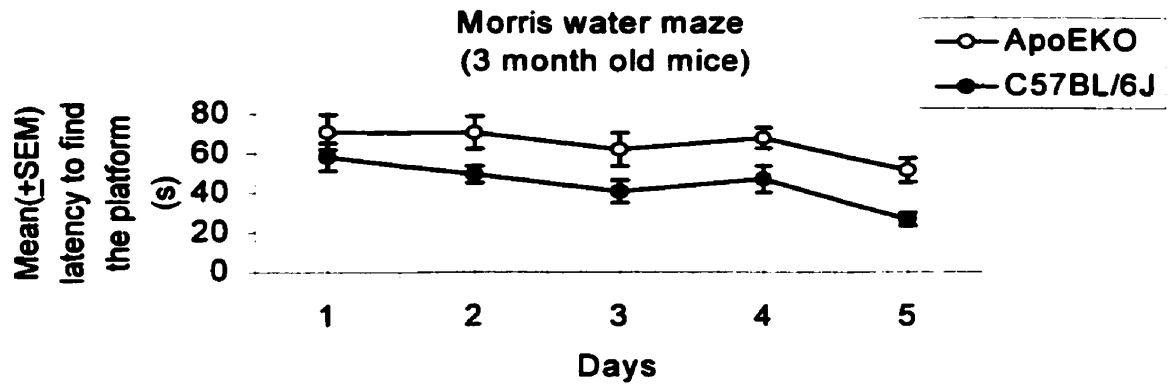

Figure 1. Latency to find the platform in the MWM with 3 month old apoEKO mice (6th generation) and C57BL/6J mice. Data represent the mean (± SEM) latency (s) to find the platform across 5 days of training (four trials per day).

1.2. 8 Month Old Mice

A Strain x Days ANOVA performed on the latency data revealed a main effect for Strain [$F(1, 16)= 86.84$, $p<0.01$] indicating that apoEKO mice took significantly longer to find the platform than C57BL/ 6J mice. A main effect for Days [$F(6, 96)= 9.96$, $p<0.01$] indicated that both groups showed significant changes in latency across days. No significant Srain x Days interaction was found [$F(6, 96)= 1.69$, $p>0.05$] (see Figure 2). To reduce the correlation of means and variances, the latency data were subject to a square root transformation before effectuating the ANOVA.

On the two probe trials when the platform was visible, apoEKO mice also took significantly longer to find the platform than C57BL/6J mice [$F(1, 112)= 6.04$, $p<0.05$]

A separate ANOVA performed on the data from the two probe trials when the platform was removed revealed a significant Strain x Quadrant interaction, [$F(3, 42)= 14.85$, $p<0.01$]. Simple main effects F-tests revealed that the mean percentage of time spent by apoEKO mice in the adjacent clockwise quadrant and the target quadrant was significantly lower than the mean percentage of time spent by C57BL/6J mice in the same quadrants, $Fs\ (1, 56) \geq 3.98$, $ps \leq 0.05$. Furthermore the mean percentage of time spent by apoEKO mice in the opposite quadrant and in the adjacent counter-clockwise quadrant was significantly higher than the mean percentage of time spent by C57BL/6J mice in the same quadrants, $Fs(1, 56) \geq 12.65$ $ps \leq 0.01$ (see Figure 3).

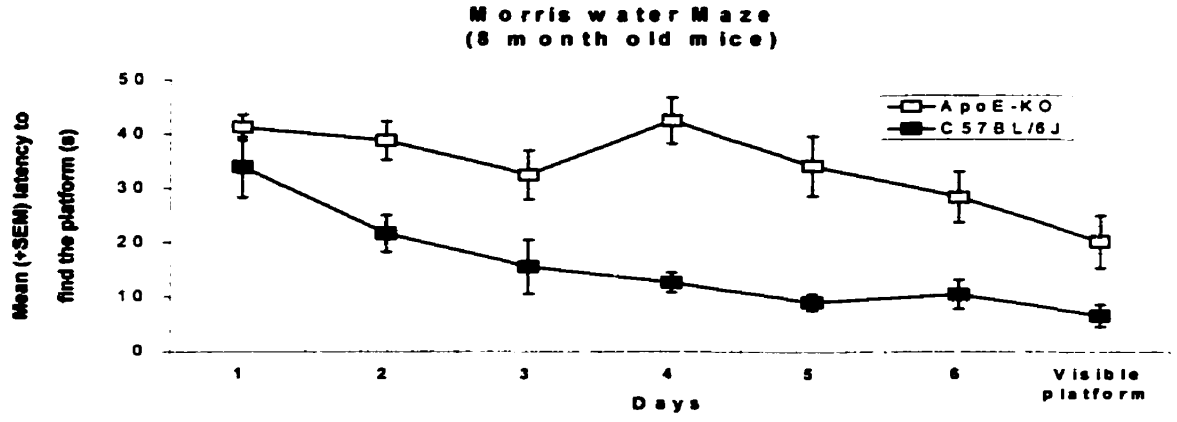

Figure 2. Latency to find the platform in the MWM with 8 month old apoEKO mice (6th generation) and C57BL/6J mice . Data represent the mean (± SEM) latency (s) to find the platform across 6 days of training (four trials per day).

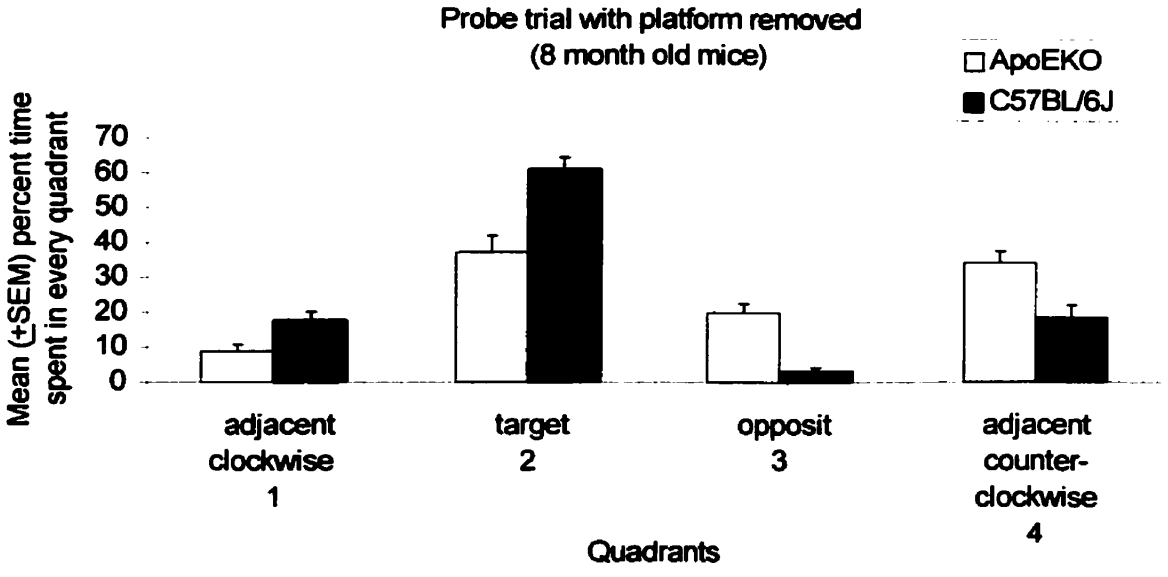

Figure 3. Percent time spent in each of the four quadrants in the MWM with 8 month old apoEKO mice (6th generation) and C57BL/6J mice . Data represent the mean (± SEM) percent time spent in every quadrant.

C. Discussion

In the present study, apoEKO mice assessed in the MWM showed a spatial memory deficit at 3 and 8 month old when compared to control mice (C57BL/6J), as indicated by longer latencies to find the platform over the course of training. ApoEKO mice spent a lower percentage of time in the target quadrant and the adjacent clockwise quadrant than controls, but spent a higher percentage of time in the opposite quadrant and in the adjacent counter-clockwise quadrant. The results we obtained in these two experiments are consistent with a recent study from Masliah et al. (1997), which found a cognitive impairment in apoEKO mice in the MWM.

Gordon et al. (1995) have suggested that the MWM impairment in apoEKO mice is due to a working memory deficit. In this study; mice were assessed two trials per day, although the location of the platform within each daily pair of trials was fixed, the platform location was changed across days. Here, within day differences between trials 1 and 2 were thought to reflect a working memory deficit (because the animal had to remember where the platform was located on that day), whereas between day differences on trial 1 latency were thought to reflect reference memory impairments (because on this trial the animal needed to remember the general rule to locate the position of the platform within the maze). Gordon et al. (1995) found that apoEKO mice showed within day deficits, but not between day impairments. Although the spatial memory deficit could be explained by a deficit in working memory caused by impairments in basal forebrain cholinergic projections (Gordon et al., 1995), it is as likely that the absence of apoE in their brain, which creates neurodegenerative changes in the HIP, frontal cortex and basal ganglia (Leblanc and Poduslo, 1990) forces these animals to adopt new strategies to

locate themselves in the environment. As observed by Morris et al. (1982), and Schenk and Morris (1985), non-spatial strategies (i.e. swimming at a given distance from the wall), are adopted by rats suffering from hippocampal lesions when spatial learning is impaired (McNamara and Skelton, 1993). This might explain why the apoEKO mice were able to find the platform but spent a significantly lower percentage of time than the controls in the target quadrant and the adjacent clockwise quadrant.

Age did not seem to predict the severity of the impairment since 3 month old apoEKO mice also displayed a deficit. In fact, it appears that the deficit in the 3 month old mice was more severe than in the 8 month old mice in that the mean latency to find the platform on the last day of training was higher in 3 month old mice (approximately 60 s) in comparison to 8 month old mice (approximately 20 s). The reason for the improved performance in older mice is difficult to explain, suffice it to say that it will be difficult to invoke age as a factor to account for the discrepant results that have appeared in the literature.

Two additional factors that were not explored in the present experiment, but that could differentially contribute to impaired function in the MWM are differences in reactivity to stress (Zhou et al., 1998), and genetic variability. In the present experiment we used apoEKO mice which were backcrossed six times with C57BL/6J mice. Six generations might not be sufficient to completely eliminate variability in the genetic background (Krzywkowski, 1999). Therefore, in the next experiments we examined apoEKO mice which were backcrossed for ten generations with C57BL/6J mice. We decided to test these animals in the MWM and RAM, a test never used in these mice to

assess memory. We also evaluated sensorimotor gating by PPI and reactivity to stress in two different tests of anxiety.

III. ApoE-Knockout Mice Model 10th Generation

A. Materials and Methods

1. Animals

C57BL/6J and apoEKO mice were obtained from the same source as described above. In this experiment the mice were backcrossed ten times with C57BL/6J mice in order to provide increased genetic homogeneity. Housing and colony room conditions were as described previously. All mice had *ad libitum* access to water and standard laboratory mouse chow, except those tested in the RAM. These later mice where fed one hour per day (after the daily RAM trial) for the duration of the experiment and were given one Kellogg's Froot Loop (which also served as the reinforcer for the RAM training) in their home cage 3 days before the experiment. A total of 93 mice (48 C57BL/6J and 45 apoEKO mice) were used these experiments. Different cohorts of 3-4 month old and 10-14 month old animals were tested sequentially in the MWM, RAM, EPM, OF and prepulse inhibition paradigm.

2. Behavioral Testing

2.1. Morris Water Maze with Spatial Cues Present

The apparatus was identical to that used previously. Training consisted of 5 consecutive days of four trials per day, as described above. On day 6 of the second experiment of this section, the platform was removed from the pool for 2 trials of 1 min, the latency and the distance spent in every quadrant of the pool was recorded. The

platform was then placed back to its original place for 2 other trials of 120 s. For these 2 last trials, the platform was made visible by lowering the water in such way that the mouse could see the platform 1 cm above water.

2.2. Morris Water Maze with Spatial Cues Removed

To assess MWM performance in which extra-maze spatial cues were removed, an opaque white curtain extending from the ceiling to the floor was placed around the pool. Two different procedural variants were examined. In the first, training over the first 5 days was conducted without the curtain as described above. On day 6, the curtain was installed and all mice were assessed under this condition for 4 trials. On day 7, the curtain was removed and two trials were conducted with the platform visible. On day 8, the platform was removed from the pool for 2 trials of 1 min and the latency and the distance spent in every quadrant of the pool was recorded.

For the second of these two water maze tests, the curtain was installed for each of the first 6 days of training. On days 1-5 (4 trials per day) the platform was invisible and on day 6, two trials were conducted with the platform visible.

2.3. Radial Arm Maze with Spatial Cues Present

The Radial arm maze (Pathfinder System, Lafayette Instruments, Lafayette, IN) consisted of a central platform (shaped as an octagon) 33 cm wide from which extended eight 10.5 cm wide, 50 cm long arms. At the end of each arm there was a food cup measuring 5 cm in diameter. Each arm was enclosed by plexiglas walls 18.5 cm high. The stainless steel floors of the central platform and of the eight arms were painted flat black. The RAM was located in a bright 3.6 m^2 room. Spatial cues (pictures of different patterns and shapes) were placed around the testing room.

Mice were first adapted to a 23 h food restriction schedule. During 5 days of habituation, the animals were individually placed in the maze and permitted to explore it freely for 8 min. Food (Kellogg's Froot Loops) was placed throughout the maze on the first day and was gradually restricted over the following four days, to the end of each arm. Subsequently, for the next 21 days, animals were given one trial per day. During a trial, animals were placed on the central platform of the maze and allowed to choose freely among the eight arms baited with a quarter piece of food in the food cups. A choice was defined as an entry of the mouse with four paws in the arm. An error were defined as a repeat entry into an arm already visited. A trial was terminated when an animal entered all eight arms (baited only once per trial), when it made 16 errors or when 8 min had elapsed. When the animal finished its daily trial, it was placed back to its home cage and the maze was cleaned and rebaited. The choices made by the animal were entered on a computer, which kept tracked of the errors and the time.

2.4. Radial Arm Maze with Spatial Cues Removed

For next two tests we used the same apparatus. In the first test, the curtain was placed around the RAM from days 22-24. In the second test, the curtain enveloped the maze for the entire 21 days of training. All other procedural details were as described above.

2.5. Prepulse Inhibition

Two ventilated, sound-attenuating startle chambers (San Diego Instruments, San Diego, Calif., USA) were used. Each chamber consisted of plexiglas tube mounted on a base connected to a piezoelectric strain meter, transmitting each animal's response. Readings of the stabilimeter were rectified and digitized on a 4095 scale and recorded by

computer. All acoustic stimuli were provided by a speaker located in the ceiling of each chamber.

All mice were placed in the startle chambers for a acclimatization period of 5 min. An average of 100, 1 ms reading, beginning at stimulus onset, was used as the measure of startle amplitude. In order to evaluate startle magnitude in the absence of the prepulse, a 120dB, 50 ms stimulus was presented alone. To assess prepulse inhibition, the startle stimulus was preceded by a short 30 ms prepulse stimulus. This prepulse ranged between 3 and 15 dB above background (70 dB) in 3 dB increments. Mice were exposed to a total of 42 trials, 12 startle trials and 5 trials at each prepulse intensity (3, 6, 9, 12, 15) but without 2 successive presentations of the same stimulus intensity. Trials were separated by randomly generated inter-trial intervals ranging between 5-30 s (average = 15 s).

2.6. Elevated Plus Maze

The EPM was made of wood and painted gray. It was elevated 50 cm above ground and had four arms of the same length (30 x 5 cm) shaped as a +. Two opposite arms were enclosed by side and end walls 12 cm high. The two other arms and the center of the maze (5 x 5 cm) were open.

Every animal was placed once into the center of the maze facing an open arm and had 5 min to explore freely. The time spent in open arms, the number of open arm entries and the total number of arm entries were recorded.

2.7. Open Field

The OF measured 60 cm long x 60 cm wide x 50 cm high, was made of wood and painted black. The floor was divided into 64 squares of 7.5 cm delimited by tape. Every animal was placed once into the right corner maze and had 5 min to explore freely. The

time spent in center squares, the number of center squares crossed and the total number of squares crossed were recorded.

B. Results

1. Morris Water Maze with Spatial Cues Present (3 month old mice)

A Strain x Days ANOVA revealed a significant Strain x Days interaction [$F(5, 90)= 3.10$, $p<0.01$]. Simple main effects F-tests indicated that apoEKO mice took significantly longer than C57BL/6J mice to find the platform on day 1 [$F(1, 108)= 7.42$, $p<0.01$] and day 3 of training [$F(1, 108)= 17.2$, $p<0.01$] (see Figure 4).

On the two probe trials when the platform was visible, there was no significant difference between apoEKO mice and C57BL/6J mice [$F(1, 108)= 0.01$, $p>0.05$].

A Quadrant x Strain ANOVA conducted on the two probe trials when the platform was removed revealed a significant Quadrant main effect [$F(3, 58)= 91.60$, $p<0.01$]. Further Tukey's pairwise comparisons revealed that all groups spent significantly more time in the target quadrant than in any other quadrants $ps\leq 0.01$ (see Figure 5).

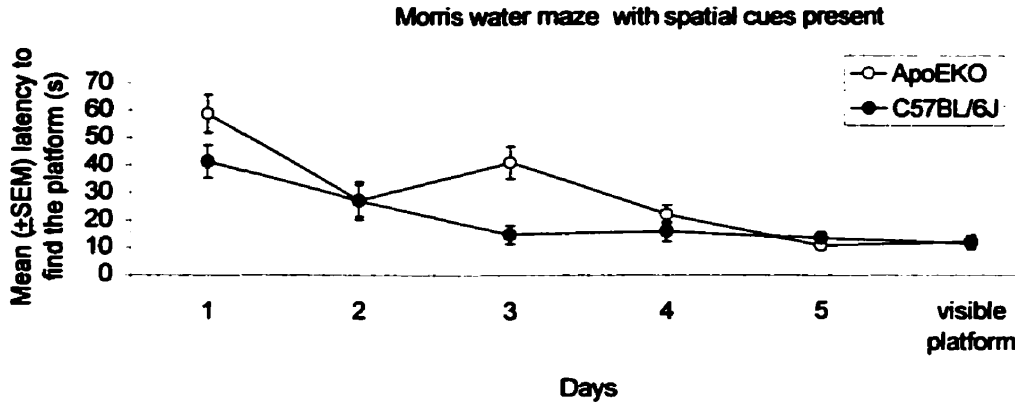

Figure 4. Latency to find the platform in the MWM with spatial cues present in 3 month old apoEKO mice (10^{th} generation) and C57BL/6J mice . Data represent the mean (± SEM) latency (s) to find the platform across 5 days of training (four trials per day).

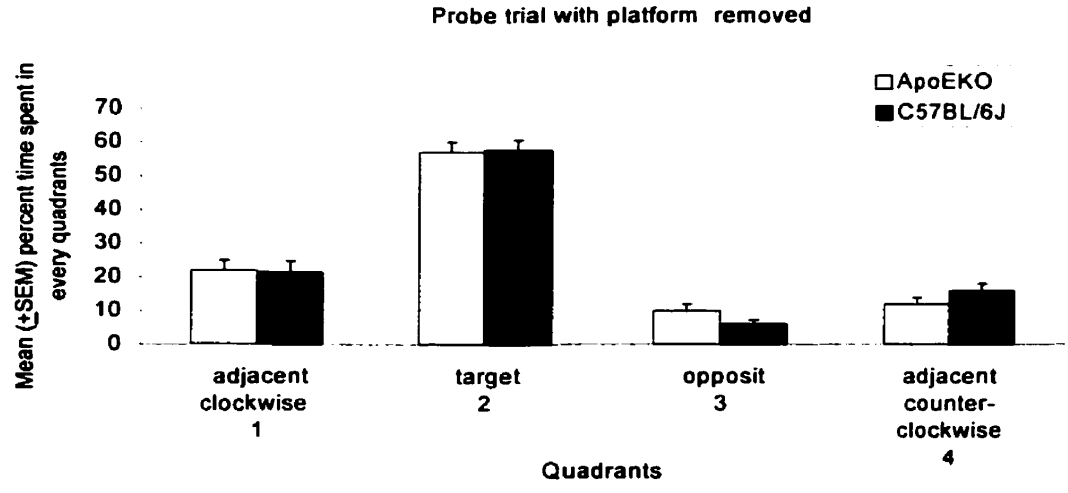

Figure 5. Percent time in each quadrant in the MWM with 3 month old apoEKO mice (10^{th} generation) and C57BL/6J mice . Data represent the mean (± SEM) percent time spent in every quadrants (for two trials).

2. Morris Water Maze (10 month old mice)

2.1. Spatial Cues Removed on Day 6

A Strain x Days ANOVA performed on the latency data revealed a main effect for Days [$F(6, 108)= 9.96$, $p<0.01$] indicating that all groups showed significant changes in latency across days of training. No significant interaction was found [$F(6, 108)= 0.98$, $p>0.05$]. There were no differences between apoEKO mice and C57BL/6J mice in their latency to find the platform across days of training, or on the two probe trials when the platform was visible, or on day 6, when the curtain enveloped the maze, [$F(1, 18)= 1.35$, $p>0.05$] (see Figure 6).

A Quadrant x Strain ANOVA conducted on data from the two probe trials with the platform removed revealed no significant Quadrant x Strain interaction [$F(3, 54)= 0.64$, $p>0.05$] and no significant Quadrant or Strain main effect, $Fs\leq 2.06$, $ps\geq 0.1686$ (see Figure 7).

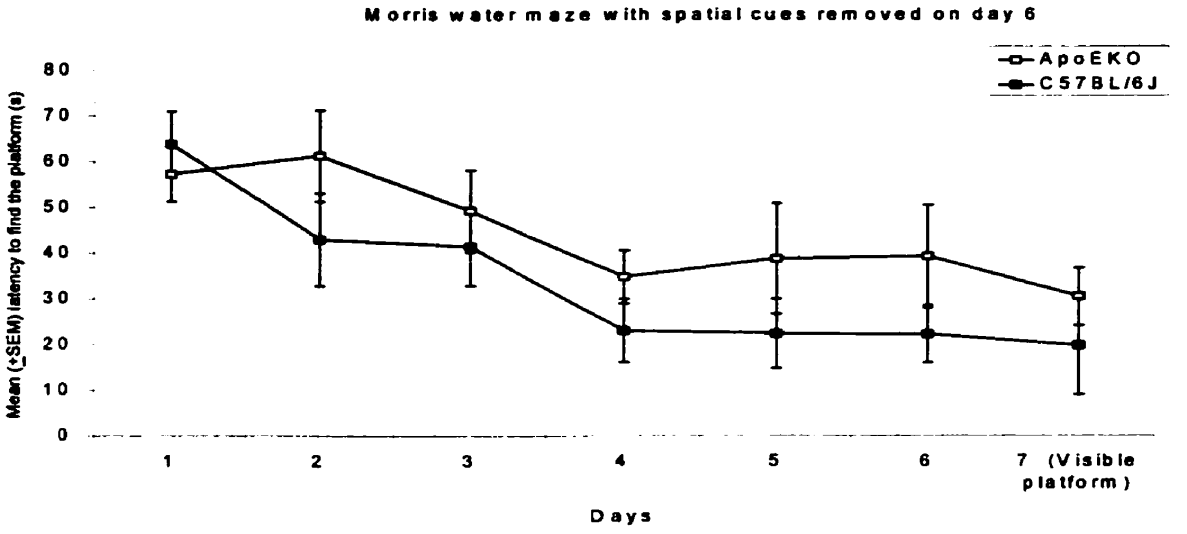

Figure 6. Latency to find the platform in the MWM with 10 month old apoEKO mice (10^{th} generation) and C57BL/6J mice . Data represents the mean (± SEM) latency (s) to find the platform across 5 days of training (four trials per day). Spatial cues were obscured by a curtain on day 6.

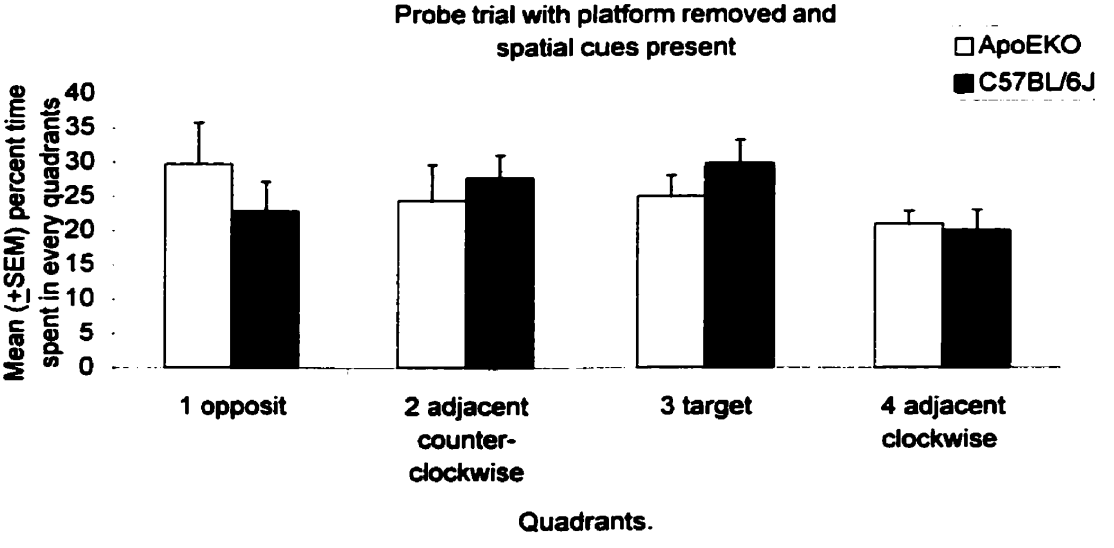

Figure 7. Percent time in each quadrant in the MWM in 10 month old apoEKO mice (10^{th} generation) and C57BL/6J mice . Data represents the mean (± SEM) percent time spent in every quadrant (for two trials).

2.2. Spatial Cues Removed Throughout Training

A Strain x Days ANOVA performed on the latency data revealed a main effect for Strain [$F(1, 18)= 5.64$, $p<0.05$] indicating that apoEKO mice took significantly longer to find the platform than C57BL/6J mice. A main effect for Days [$F(5, 90)= 7.02$, $p<0.01$] indicated that all groups showed significant changes in latency across days. No significant Strain x Days interaction was found [$F(5, 90)= 1.99$, $p>0.05$]. As we can see on Figure 8, apoEKO mice performed as well as C57BL/6J mice only when the platform was visible .

A Quadrant x Strain ANOVA performed on the two probe trials when the platform was removed revealed a significant Quadrant main effect [$F(3, 54)= 5.29$, $p<0.01$], so that both strains spent significantly more time in the target quadrant than in the opposite quadrant or the adjacent clockwise quadrant, $ps< 0.05$, using Tukey's pairwise comparisons (see Figure 9). There was no significant Quadrant x Strain interaction [$F(3, 54)= 0.61$, $p>0.05$] and no significant Strain main effect [$F(1, 18)= 0.00$, $p>0.05$].

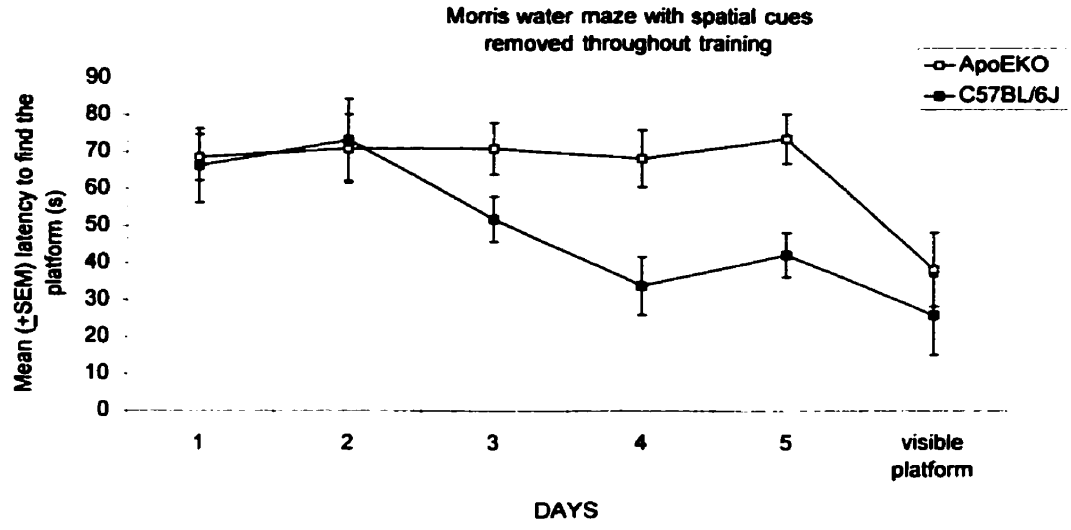

Figure 8. Latency to find the platform in MWM in 10 month old apoEKO mice (10^{th} generation) and C57BL/6J mice. Spatial cues were obscured throughout the duration of training by a curtain. Data represent the mean (± SEM) latency (s) to find the platform across 5 days of training (four trials per day).

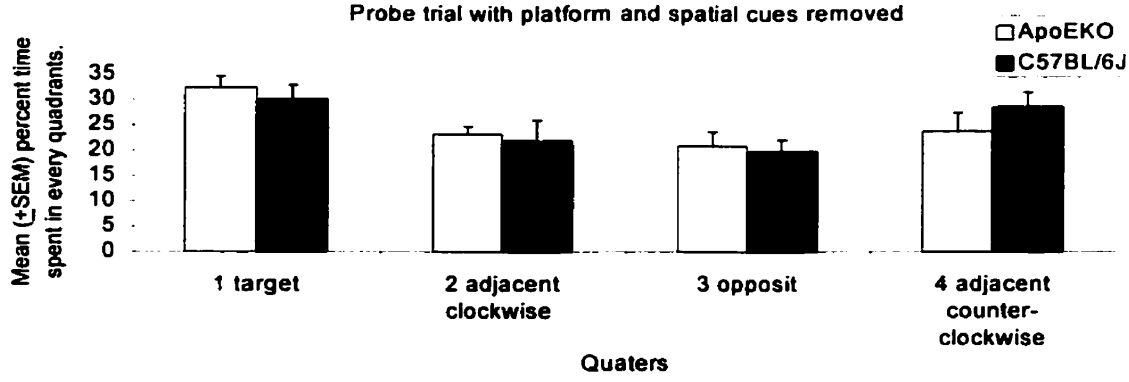

Figure 9. Percent time in each quadrant in the MWM with 10 month old apoEKO mice (10^{th} generation) and C57BL/6J mice . Data represents the mean (± SEM) percent time spent in every quadrant (for two trials).

3. Radial Arm Maze with Spatial Cues (3 and 13 month old mice)

3.1. Errors Analysis

For this analysis, the number of errors (repeat entries into visited arms) were summed over blocks of three days for each of the 21 days of training. Figure 10 shows the mean ($\pm$ SEM) errors made by 3 and 13 month old apoEKO and C57BL/6J mice over the seven training blocks. A three-way Age x Strain x Blocks ANOVA, with Age and Strain as between subject factors, and Block as a within subject factor, was performed on these data. It revealed a significant main effect for Age [$F(1, 36)= 10.80$, $p<0.01$] indicating that the mean number of errors in young mice was significantly lower than the mean number of errors for old mice, and a significant Strain x Blocks interaction effect [$F(6, 216)= 3.99$, $p<0.01$]. Simple main effect tests on the Strain x Blocks interaction revealed that apoEKO mice made the same number of errors as the C57BL/6J mice on all blocks, $Fs(1, 252) \leq 3.06$, $ps \geq 0.0821$, except block 6 where the mean number of errors for apoEKO mice was significantly lower than the mean number of errors for C57BL/6J mice, [$F(1, 252)=10.42$, $p<0.01$].

The Age x Strain interaction effect [$F(6,36)= 0.98$, $p>0.05$], the Age x Blocks interaction effect [$F(6, 216)= 1.03$, $p>0.05$], and Age x Strain x Blocks interaction effect [$F(6, 216) = 1.35$, $p>0.05$] were not significant (see Figure 10).

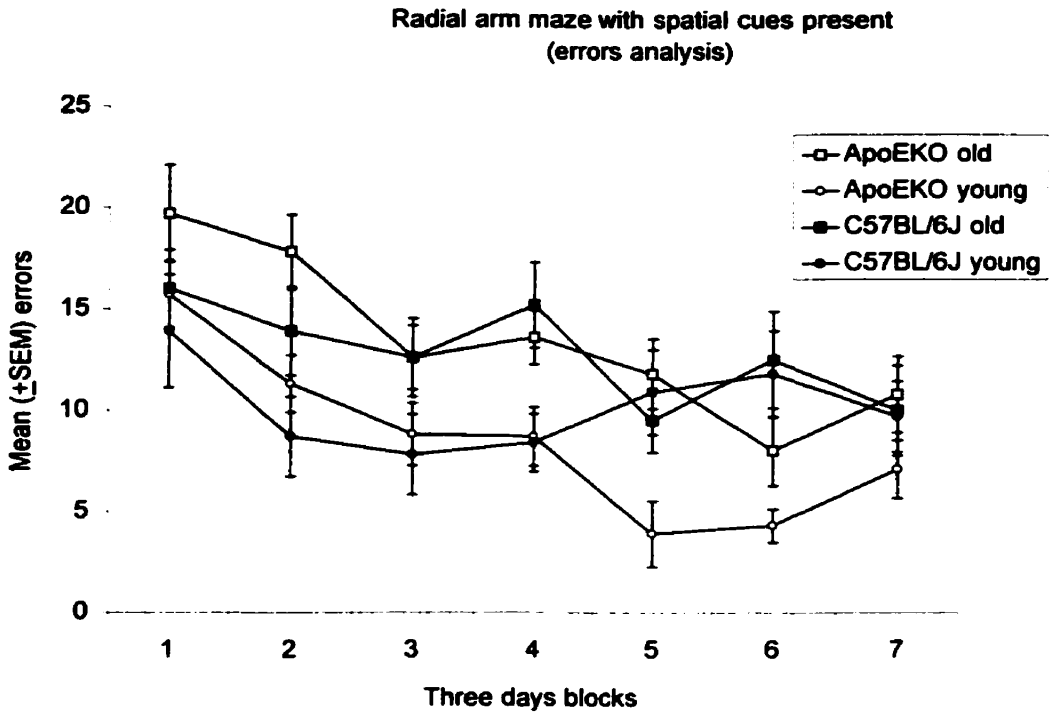

Figure 10. Mean errors in the RAM in 3 and 13 month old apoEKO mice (10^{th} generation) and C57BL/6J mice . Mean (± SEM) errors during the 21 days of training (summed over blocks of three days).

3.2. Serial Forgetting Curve Analysis

This analysis indicates the probability of making a correct response over sequential choices. However, the basal probability of a correct response fluctuates over consecutive choices, and is influenced by whether or not choices made before were correct or incorrect. Thus, the observed probability of a correct response for a given choice must be adjusted for changes in the basal probability. For this reason, Olton and Samuelson (1976) developed this following formula:

$$p(cor)transformed = \frac{p(cor)observed - p(cor)expected}{100-p(cor)expected} \times 100$$

where

p(cor)transformed = the probability of a correct response, adjusted to correct for changes in the basal rate of success for a given choice,

p(cor) observed = the observed probability of a correct response for a given choice, i.e.,

$$\frac{\text{number of correct responses}}{\text{total number of responses}} \times 100$$

and

p(cor)expected = the expected chance probability of a correct response for a given choice, i.e.,

$$\frac{\text{number of arms not chosen}}{8} \times 100.$$

Serial forgetting curves were constructed for both the first and latter half of training, and were restricted to choices 2 to 8 for every group because choice 1 was always correct, and whereas animals were required to make at least 8 choices (when no error occurred) on any given trial, the number of choices beyond choice 8 could vary, depending on the number of errors the animals made. This made interpretation of corrected probabilities beyond choice 8 difficult.

The transformed probabilities of a correct response over days 1-10 and 11-21 were analyzed by different Strain x Choice x Age ANOVAs, with Strain and Age as between subject factors, and Choice as a within subject factor.

Over days 1-10, there was a significant Strain x Choice interaction effect [$F(6, 216)= 1.74$, $p>0.05$]. Simple main effects tests suggested that apoEKO mice made significantly fewer correct responses than C57BL/6J mice from the fourth to the seventh choices, $Fs(1, 252)\geq 3.84$, $p\leq 0.05$. There was a significant Strain x Age interaction effect [$F(1, 36)= 5.21$, $p<0.05$]. Simple main effects tests suggested that young apoEKO mice made significantly fewer correct responses than young C57BL/6J mice [$F(1, 36)= 17.26$, $p<0.01$], but significantly more correct responses than old apoEKO mice [$F(1, 36)= 12.96$, $p<0.01$]. Young C57BL/6J mice made significantly more correct responses than old C57BL/6J mice [$F(1, 36)= 46.62$, $p<0.01$]. There was also a significant Choice x Age interaction [$F(6, 216)= 3.87$, $p<0.01$]. Simple main effects tests revealed that the aged animals made significantly fewer correct responses than young animals from the second to the eighth choices, $Fs(1, 252)\geq 9.22$, $p\leq 0.0027$ (see Figure 11).

The Strain x Choice x Age interaction effect [$F(6, 216)= 1.85$, $p>0.05$] was not significant.

Over days 11-21, there was a significant Choice x Age interaction [$F(6, 216)= 3.28$, $p<0.01$]. Simple main effects tests revealed that the aged animals made significantly fewer correct responses than young animals from the second to the eighth choices, $Fs(1, 252)\geq 4.40$, $ps\leq 0.03$ (see Figure 12).

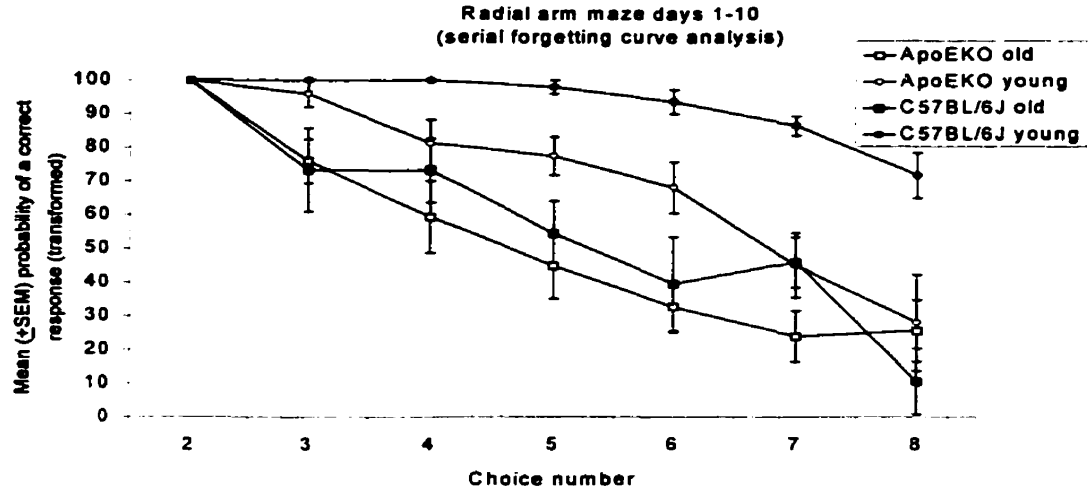

Figure 11. Mean (± SEM) probability of a correct response (transformed for changes in basal probability) for 3 (young) and 13 (old) month old apoEKO mice (10th generation) and C57BL/6J mice for the first 10 days of training.

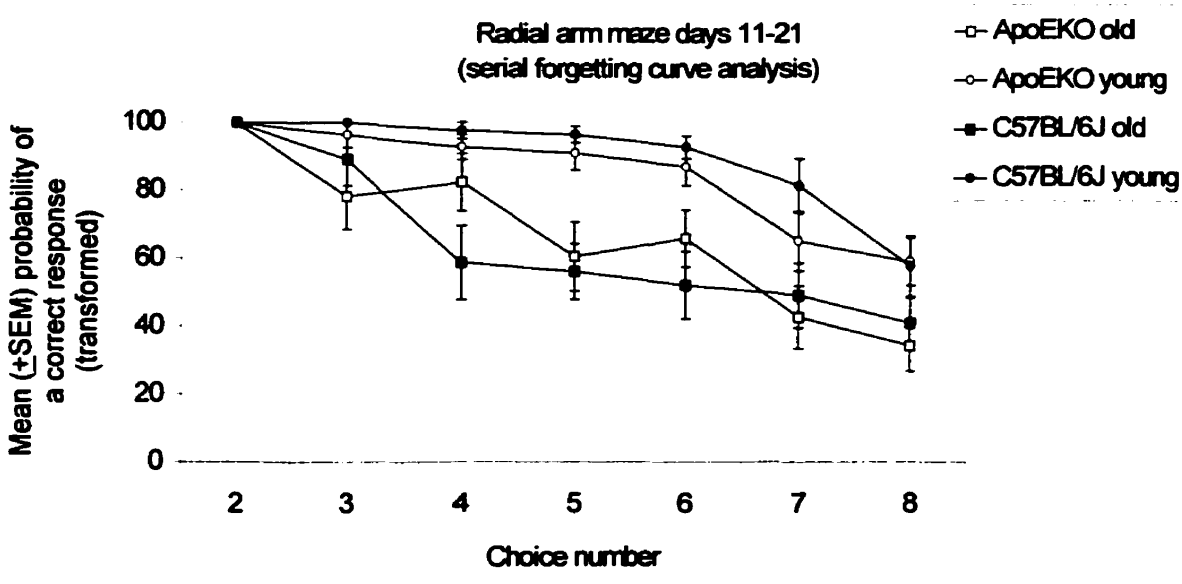

Figure 12. Mean (± SEM) probability of a correct response (transformed for changes in basal probability) for 3 (young) and 13 (old) month old apoEKO mice (10th generation) and C57BL/6J mice for the last 11 days of training.

3.3. Arm Distance Analysis

To evaluate if the different strains used alternative strategies to solve the task, we calculated the percent of total responses in which mice in the different groups selected arms immediately next to, as well as two, three and four arms distance away from the previously selected arm. Repeat entries to previously selected arms accounted for less than 1% of the total choices. Thus, these data were excluded from subsequent analyses. The percentage of one, two, three and four arms distance selections over days 1-10 and 11-21 were subjected to separate Strain x Distance x Age ANOVAs, with Strain and Age as between subject factors, and Distance as a within subject factor.

Over days 1-10 and 11-21, there were significant main effects for Distance, [$F(3, 108)= 190.44$, $p<0.01$] and [$F(3, 108)= 154.32$, $p<0.01$] respectively. All groups made more one and two arm choices in contrast to three and four arm choices. Over days 11-21, there was a significant Strain x Distance interaction [$F(3, 108)= 9.11$, $p<0.01$]. Simple main effects tests revealed that apoEKO mice made significantly more 1 arm choices than C57BL/6J mice but significantly less 2 arm choices than C57BL/6J mice (see Figure 13 and 14).

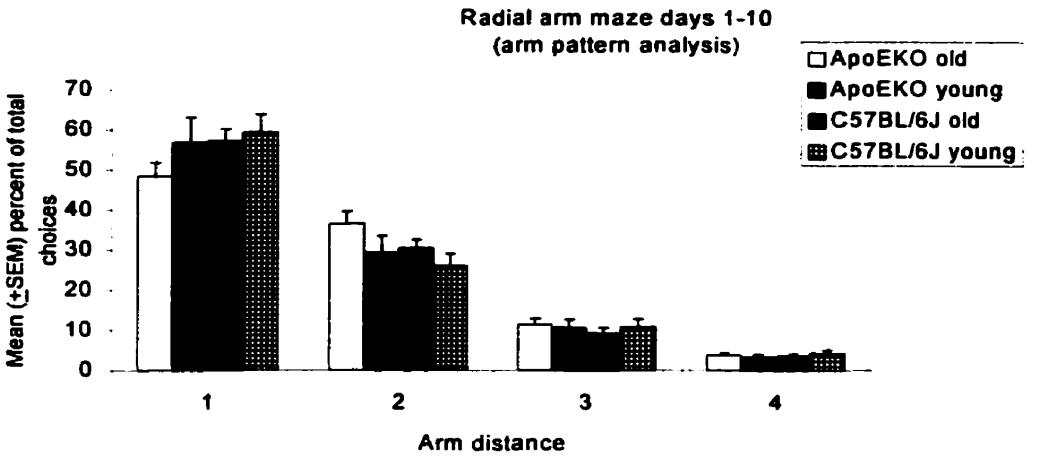

Figure 13. Mean (± SEM) percent choices that were one, two, three and four arms distance from the previous choice for 3 (young) and 13 (old) month old apoEKO mice (10^{th} generation) and C57BL/6J mice for the first 10 days of training.

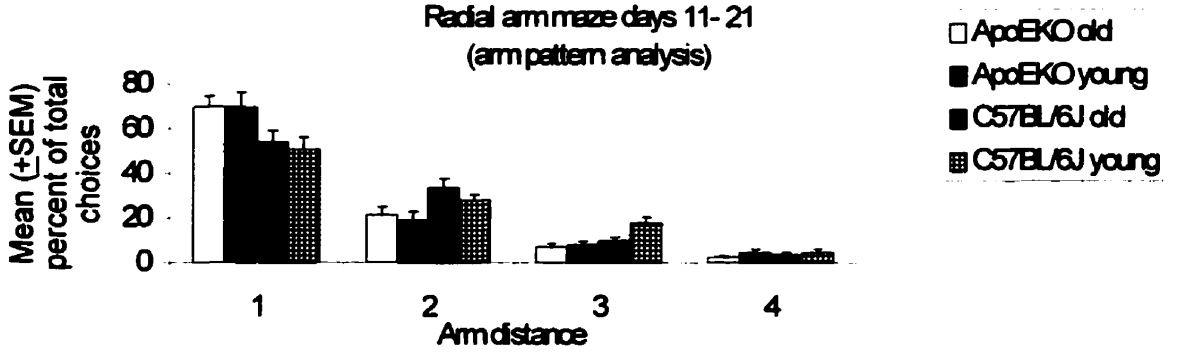

Figure 14. Mean (± SEM) percent choices that were one, two, three and four arms distance from the previous choice for 3 (young) and 13 (old) month old apoEKO mice (10^{th} generation) and C57BL/6J mice for the last 11 days of training.

4. Radial Arm Maze (12 month old mice)

4.1. Spatial Cues Removed On the Last 3 Days of Training

4.1.1. Errors Analysis

The data analysis for this experiment was done the same way as for the previous RAM. The 24 days of tests were summed-up in blocks of three days, as we can seen in Figure 15. A two-way ANOVA performed on these data revealed a significant main effect for blocks [$F(1, 105)= 2.75$, $p<0.05$] suggesting that all groups showed significant changes in errors across blocks. There was no significant main effect for Strain [$F(1, 15) = 0.34$, $p>0.05$] and no significant Strain x Block interaction effect [$F(1, 105)= 0.49$, $p>0.05$].

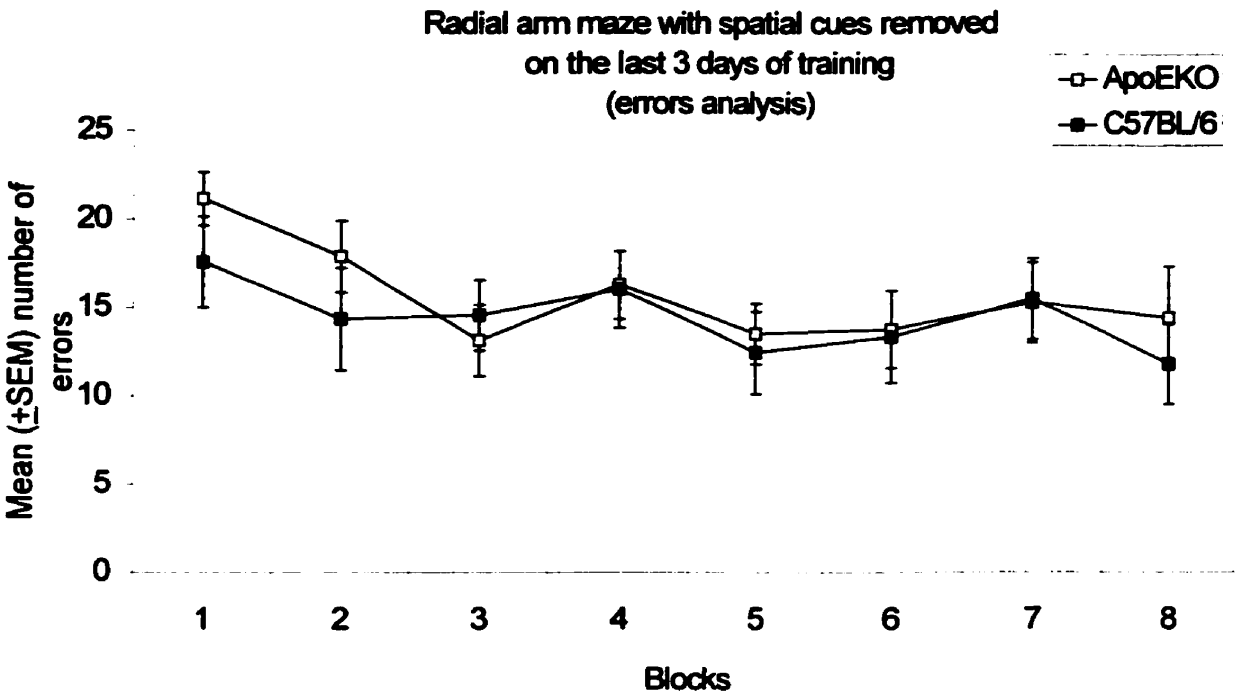

Figure 15. Mean (± SEM) number of errors (summed over blocks of 3 days) in the RAM in 12 month old apoEKO mice (10^{th} generation) and C57BL/6J mice. Spatial cues were occluded by a curtain during the last block.

4.1.2. Serial Forgetting Curve Analysis

The probabilities of a correct response were adjusted as described above. The probabilities over days 1-12 and 13-24 were analyzed by separate Strain x Choice ANOVAs, with Strain as a between subject factor, and Choice as a within subject factor.

Over days 1-12, there was a significant Strain main effect [$F(1, 15)= 3.82$, $p<0.05$] suggesting that apoEKO mice made significantly fewer correct responses than C57BL/6J mice, and a significant Choice main effect [$F(6, 90)= 6.90$, $p<0.01$] indicating that accuracy decreased over successive choices (see Figure 16).

Over days 13-24, no significant main effect or interaction was found, other than a significant Choice main effect [$F(6, 90)= 11.17$, $p<0.01$] indicating that accuracy decreased over successive choices (see Figure 17).

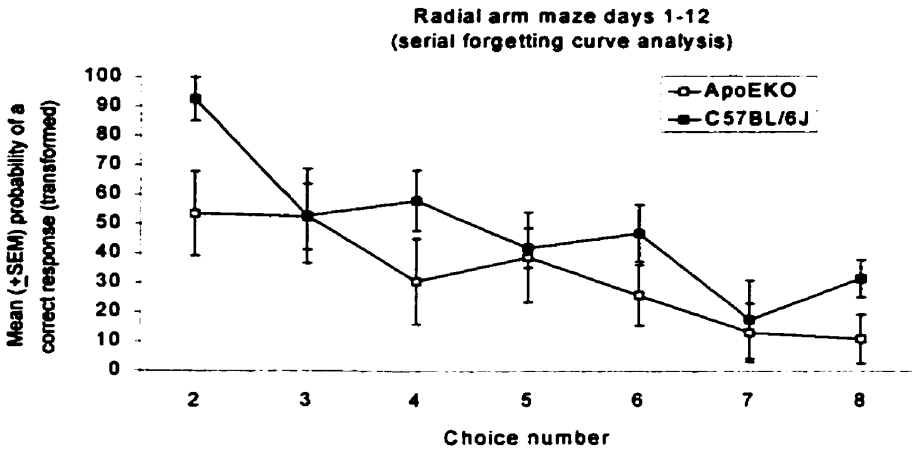

Figure 16. Mean (± SEM) probability of a correct response (transformed for changes in basal probability) for 12 month old apoEKO mice (10th generation) and C57BL/6J mice for the first 12 days of training.

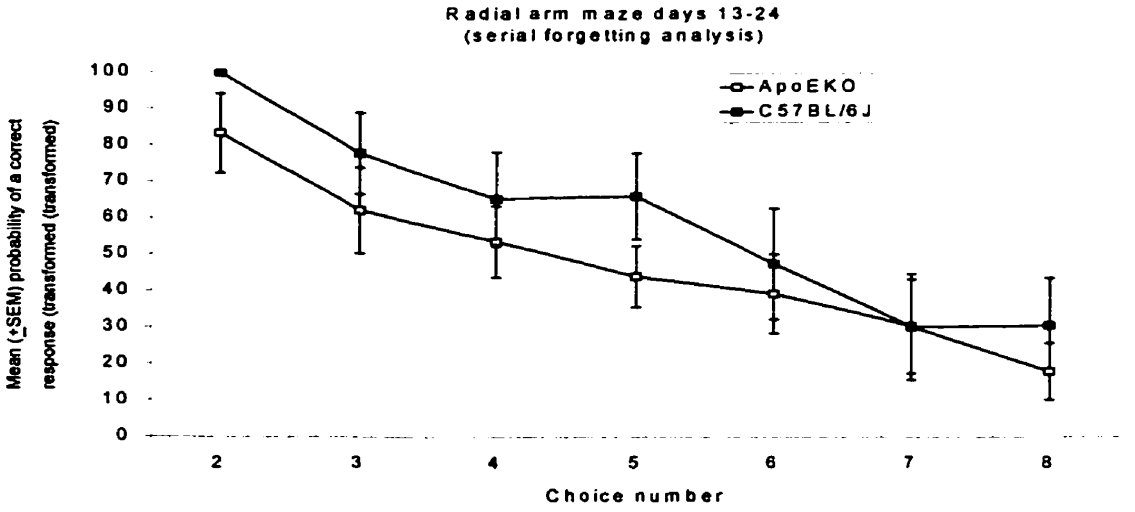

Figure 17. Mean (± SEM) probability of a correct response (transformed for changes in basal probability) for 12 month old apoEKO mice (10th generation) and C57BL/6J mice for the last 12 days of training.

4.1.3. Arm Distance Analysis

To target the strategy mice use to solve the task, the percentage of one, two, three and four arms distance selections over days 1-12 and 13-24 were submitted, as in the previous RAM, to separate Strain x Distance ANOVAs, with Strain as a between subject factor and Distance as a within subject factor.

Over days 1-12 and 13-24 there was a significant main effect for Distance [$F(3, 45) = 39.90$, $p<0.01$] and [$F(3,45) = 35.44$, $p<0.01$] respectively. As we can see in Figures 18 and 19, all groups made more one and two arm choices in contrast to three and four arm choices. There was no other main effect or significant interaction found.

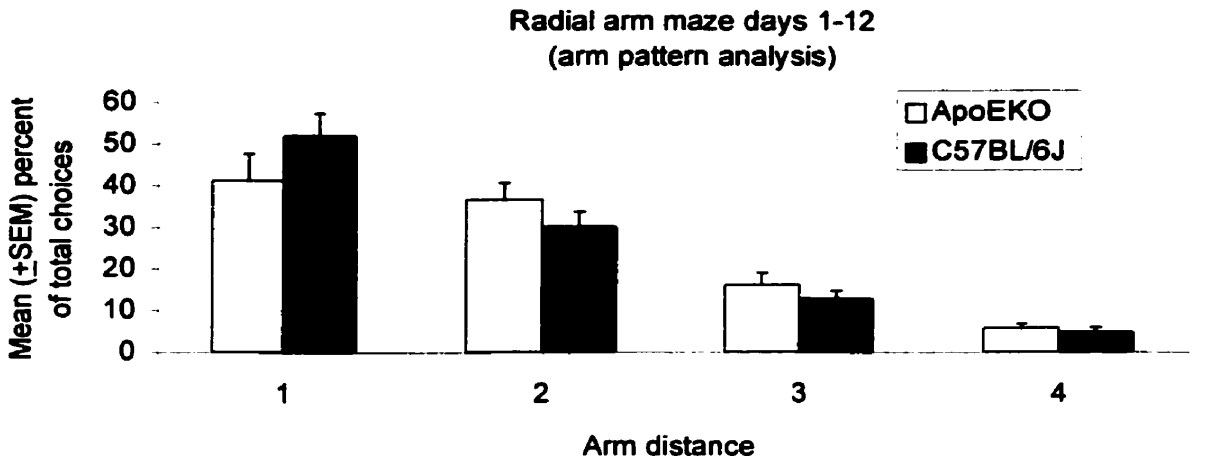

Figure 18. Mean (± SEM) percent choices that were one, two, three and four arms distance from the previous choice for 12 month old apoEKO mice (10th generation) and C57BL/6J mice for the first 12 days of training.

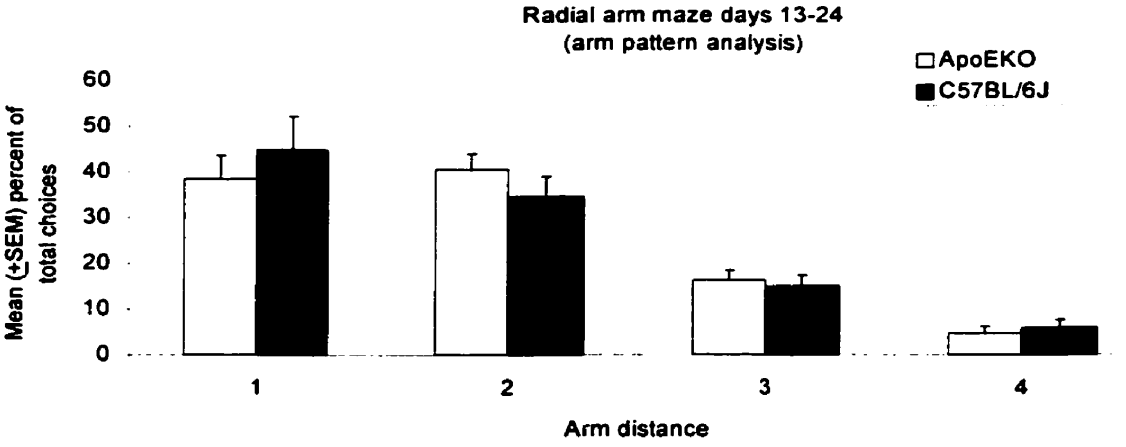

Figure 19. Mean (± SEM) percent choices that were one, two, three and four arms distance from the previous choice for 12 month old apoEKO mice (10th generation) and C57BL/6J mice for the last 12 days of training.

4.2. Spatial Cues Removed Throughout Training

4.2.1. Errors Analysis

The data analysis for this experiment was done the same way as for the first RAM. Twenty one days of tests were summed-up in blocks of three days, as we can see in Figure 20. A two-way analysis of variance (ANOVA) performed on these data revealed a significant main effect for Strain indicating that apoEKO mice mean number of errors was significantly higher than the mean number of errors for C57BL/6J mice [$F(1, 15)= 6.62$, $p<0.05$] and a main effect for Blocks [$F(6, 90)= 3.93$, $p<0.01$] suggesting that all groups showed significant changes in errors across blocks. There was no significant Strain x Blocks interaction effect [$F(6, 90)= 0.14$, $p>0.05$].

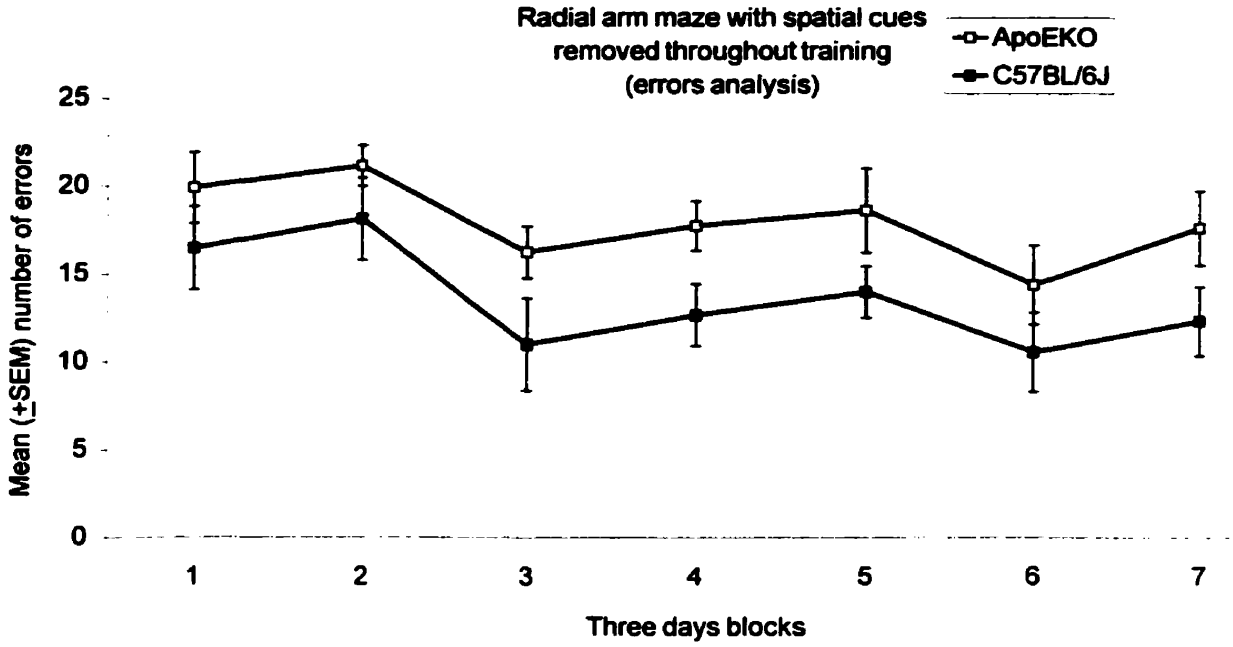

Figure 20. Mean (± SEM) errors during 21 days of training (summed over blocks of three days) in the RAM with 12 month old apoEKO mice (10^{th} generation) and C57BL/6J mice. Spatial cues were obscured throughout the duration of training.

4.2.2. Serial Forgetting Curve Analysis

These corrected probabilities of a correct response over successive arm choices over days 1-10 and 11-21 were analyzed by separate Strain x Choice ANOVAs, with Strain as a between subject factor, and Choice as a within subject factor.

Over days 1-10, there was a significant Strain main effect [$F(1, 15)= 4.33$, $p<0.05$] suggesting that apoEKO mice made significantly fewer correct responses than C57BL/6J mice and a significant Choice main effect [$F(6, 90)= 10.97$, $p<0.01$] indicating that accuracy decreased over successive choices (see Figure 21).

Over days 11-21, no significant main effect or interaction was found, other than a significant Choice main effect [$F(6, 90)= 13.77$, $p<0.01$] indicating that accuracy decreased over successive choices (see Figure 22).

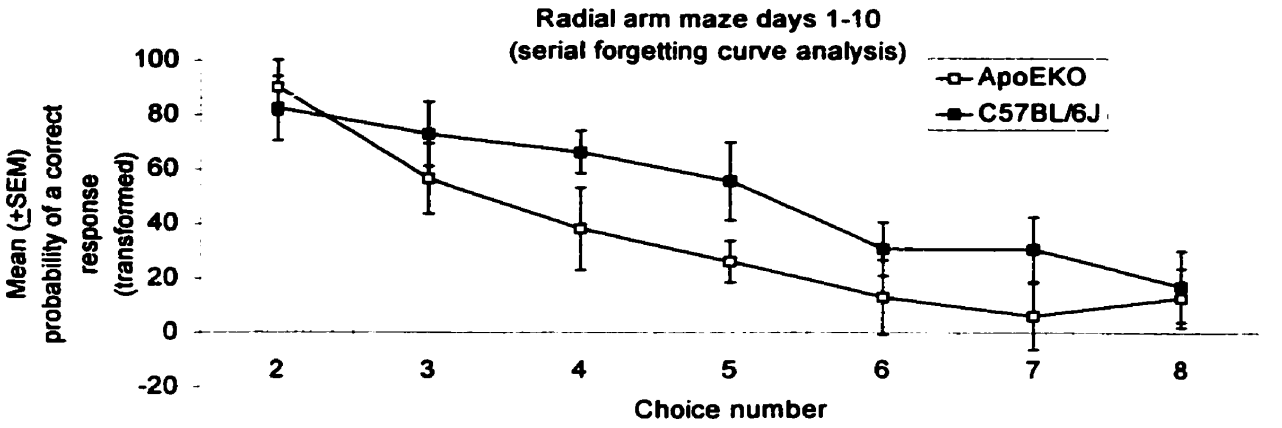

Figure 21. Mean (± SEM) probability of a correct response (transformed for changes in basal probability) for 12 month old apoEKO mice (10^{th} generation) and C57BL/6J mice for the first 10 days of training.

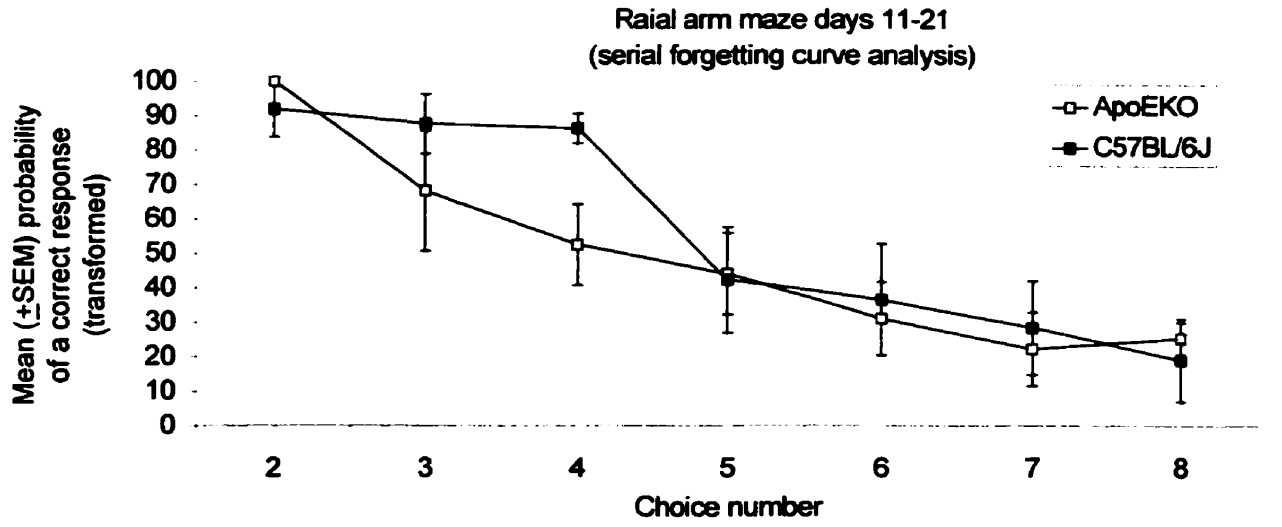

Figure 22. Mean (± SEM) probability of a correct response (transformed for changes in basal probability) for 12 month old apoEKO mice (10^{th} generation) and C57BL/6J mice for the last 11 days of training.

4.2.3. Arm Distance Analysis

The percentage of one, two, three and four arms distance selections over days 1-10 and 11-21 were analyzed by separate Strain x Distance ANOVAs to find the strategy mice use to solve the task.

Over days 1-10 and 11-21 there was a significant Strain x Distance interaction [$F(3, 45)= 4.63$, $p<0.01$] and [$F(3, 45)= 4.26$, $p<0.01$] respectively. Simple main effects tests over days 1-10 revealed that apoEKO mice made significantly fewer 1 arm choices than C57BL/6J mice [$F(1, 60)= 13.30$, $p<0.01$], and a non-significant trend of more 3 arm choices than C57BL/6J mice [$F(1, 60)= 3.75$, $p<0.06$]. Simple main effects tests over days 11-21 revealed that apoEKO mice made significantly fewer 1 arm choices than C57BL/6J mice [$F(1, 60)= 12.03$, $p<0.01$], and a non-significant trend for more 2 arm choices than C57BL/6J mice [$F(1, 60)= 2.86$, $p<0.10$] (see Figures 23 and 24).

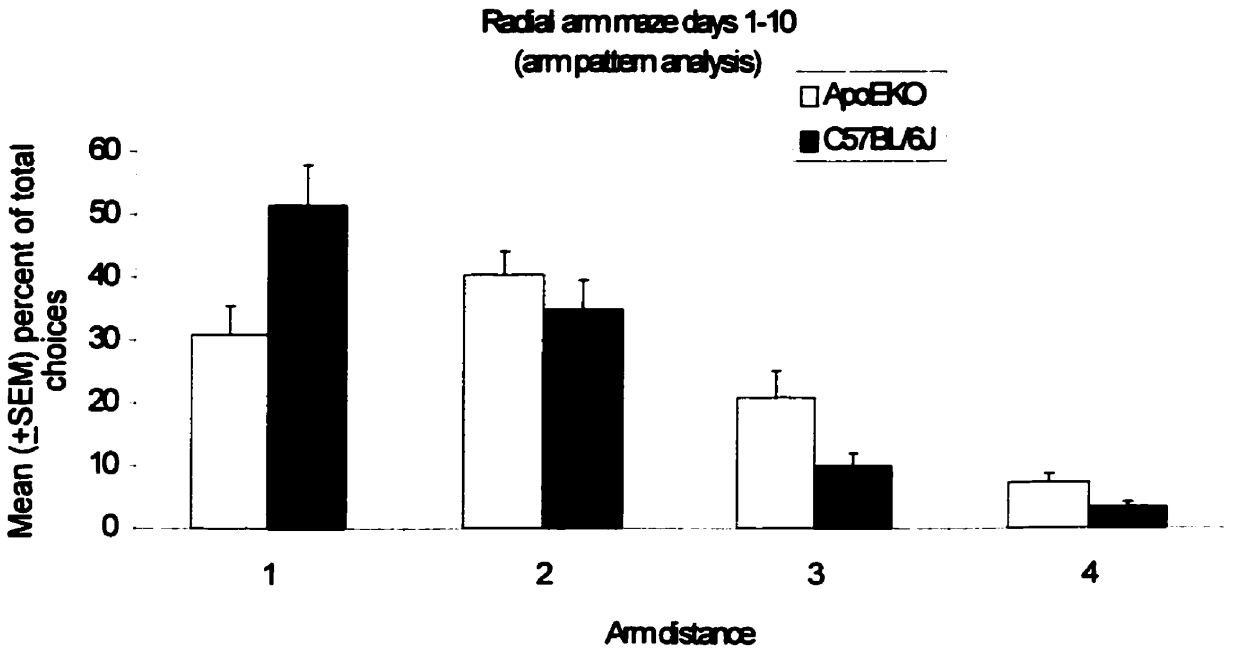

Figure 23. Mean (± SEM) percent choices that were one, two, three and four arms distance from the previous choice for 12 month old apoEKO mice (10th generation) and C57BL/6J mice for the first 10 days of training.

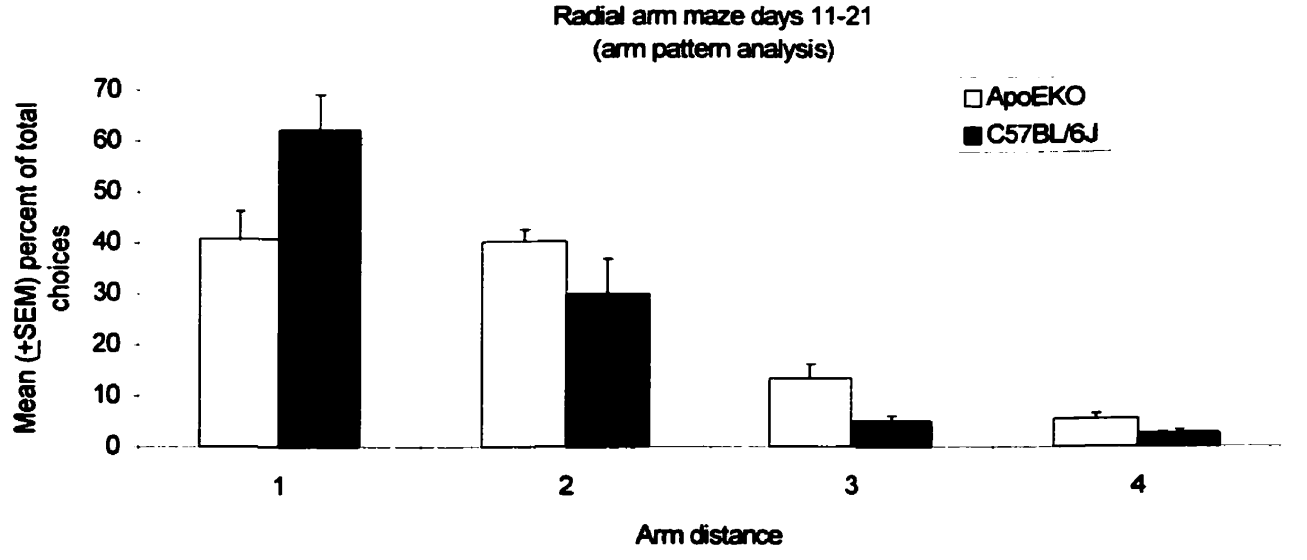

Figure 24. Mean (± SEM) percent choices that were one, two, three and four arms distance from the previous choice for 12 month old apoEKO mice (10th generation) and C57BL/6J mice for the last 11 days of training.

5. Prepulse Inhibition

5.1. 4 and 14 Month Old Mice

The degree of prepulse inhibition was calculated using the formula of Geyer et al. (1993):

$$PPI = \left(\frac{\text{startle- PP}}{\text{startle}} \right) \times 100$$

Based on this formula, a value of 0 reflects no inhibition and a value of 100 reflects total inhibition.

An Age x Strain x Prepulse intensity three-way analysis of variance (ANOVA) performed on these data revealed a significant main effect for Age [$F(1, 36)= 14.87$, $p<0.01$] indicating that the mean percent prepulse inhibition in young mice (4 month old) was significantly higher than the mean percent prepulse inhibition for old mice (14 month old), and for Prepulse intensity [$F(4, 144)= 14.64$, $p<0.01$] indicating a significant increase for all groups across the 5 prepulse intensities (see Figure 25).

The main effect for Strain [$F(1, 36)= 1.74$, $p>0.05$], the Age x Strain interaction effect [$F(4, 36)= 0.0$, $p>0.05$], the Age x Prepulse intensity interaction effect [$F(4, 144)= 1.18$, $p>0.05$], the Prepulse intensity x Strain interaction effect [$F(1, 144)= 1.05$, $p>0.05$] and Prepulse intensity x Strain x Age interaction effect [$F(4, 144)= 0.90$, $p>0.05$] were not significant.

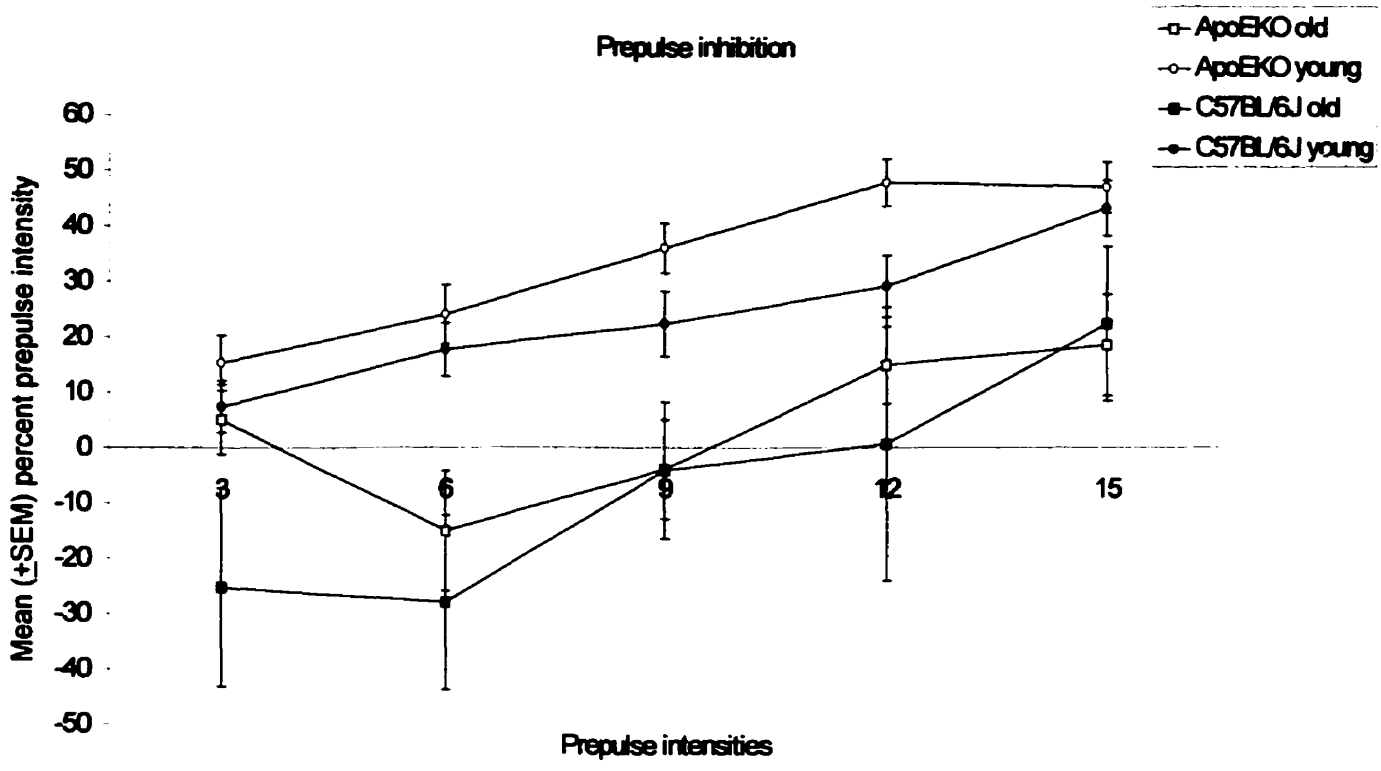

Figure 25. Mean (± SEM) percent prepulse inhibition as a function of prepulse stimulus intensity for 4 (young) and 14 (old) month old apoEKO mice (10^{th} generation) and C57BL/6J mice.

6. Elevated Plus Maze (4 and 14 month old mice)

6.1. Time in Open Arms

An Age x Strain ANOVA performed on these data revealed a significant main effect for age [$F(1, 35)= 74.80$, $p<0.01$], showing that the mean time spent in the open arms for old mice was significantly higher than the mean time in open arms for the young mice. On the other hand, there was no significant main effect for Strain [$F(1, 35)= 3.02$, $p>0.05$] and no significant Age x Strain interaction effect [$F(1, 35)= 1.89$, $p>0.05$] (see Figure 26).

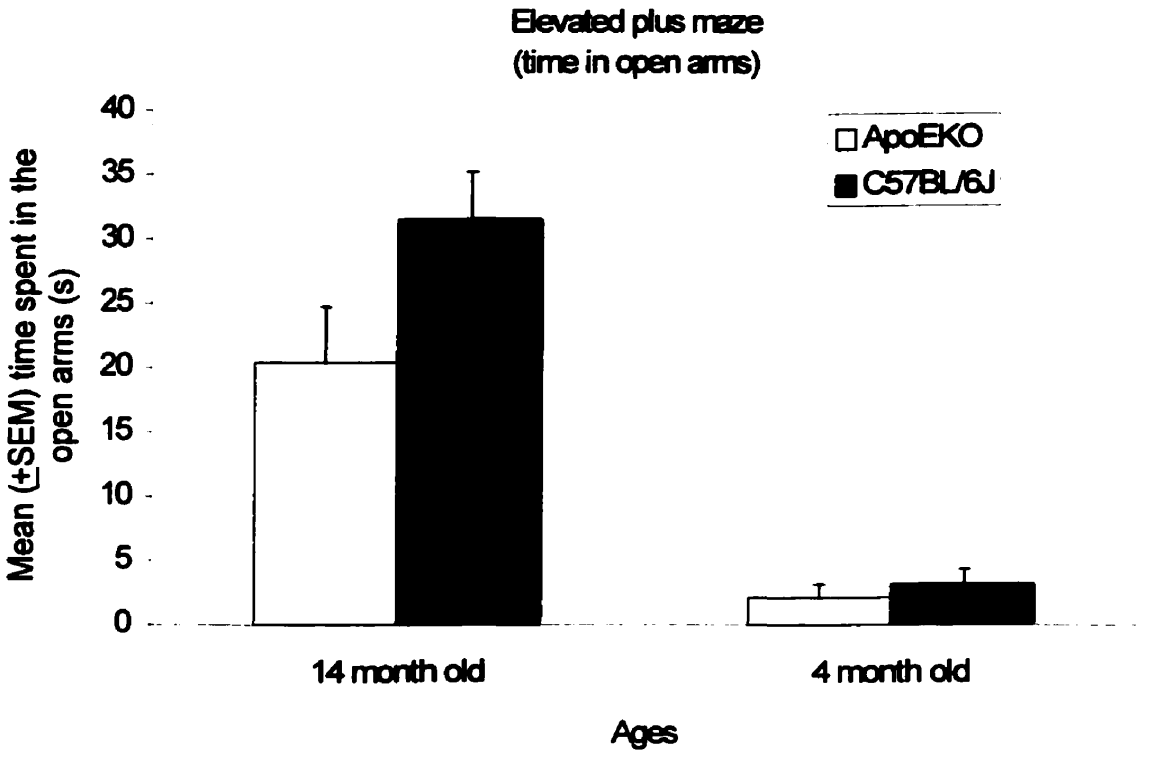

Figure 26. Mean (± SEM) time (s) spent in the open arms in the EPM in 4 and 14 month old apoEKO mice (10^{th} generation) and C57BL/6J mice .

6.2. Open/Total Arm Entries

The percentage of open to total arm entries was analyzed by an Age x Strain ANOVA. The main effect for age was significant [$F(1, 35)= 49.03$, $p<0.01$], suggesting that the mean percent open to total arm entries in old mice was significantly higher than in the young mice (see Figure 27). There was no significant main effect for Strain [$F(1, 35) = 0.58$, $p>0.05$] and no significant Age x Strain interaction [$F(1, 35)= 0.14$, $p>0.05$].

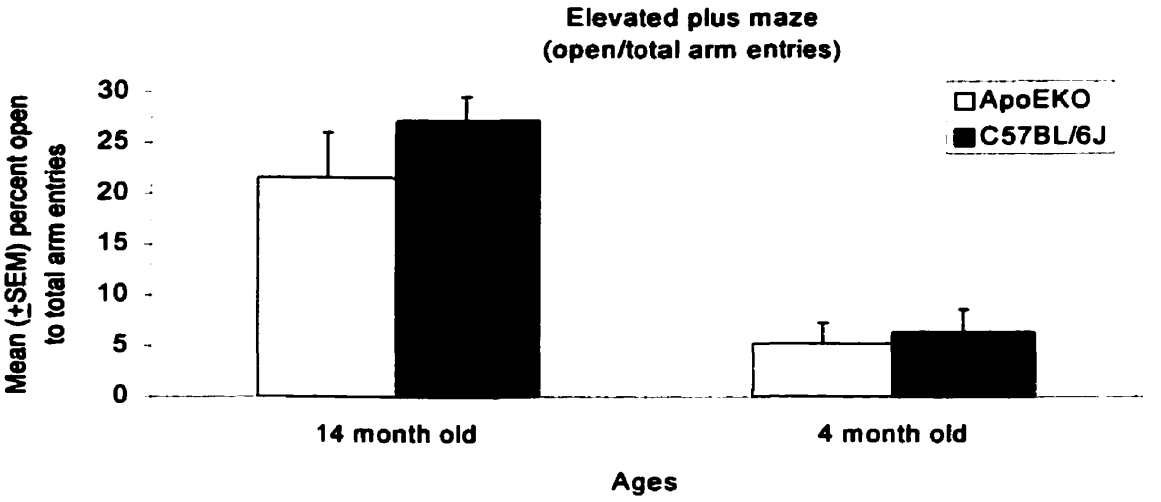

Figure 27. EPM. Mean (± SEM) percent open to total arm entries in the EPM for 4 and 14 month old apoEKO mice (10^{th} generation) and C57BL/6J mice .

6.3. Total Arm Entries

No difference was observed in the number of total arm entries when a two-way analysis of variance (ANOVA) was performed on these data. No main effect for Age [$F(1, 35)= 0.54$, $p>0.05$], no significant main effect for Strain [$F(1, 35)= 0.04$, $p>0.05$] and no significant Age x Strain interaction effect [$F(1, 35)= 3.67$, $p>0.05$] were seen (see Figure 28).

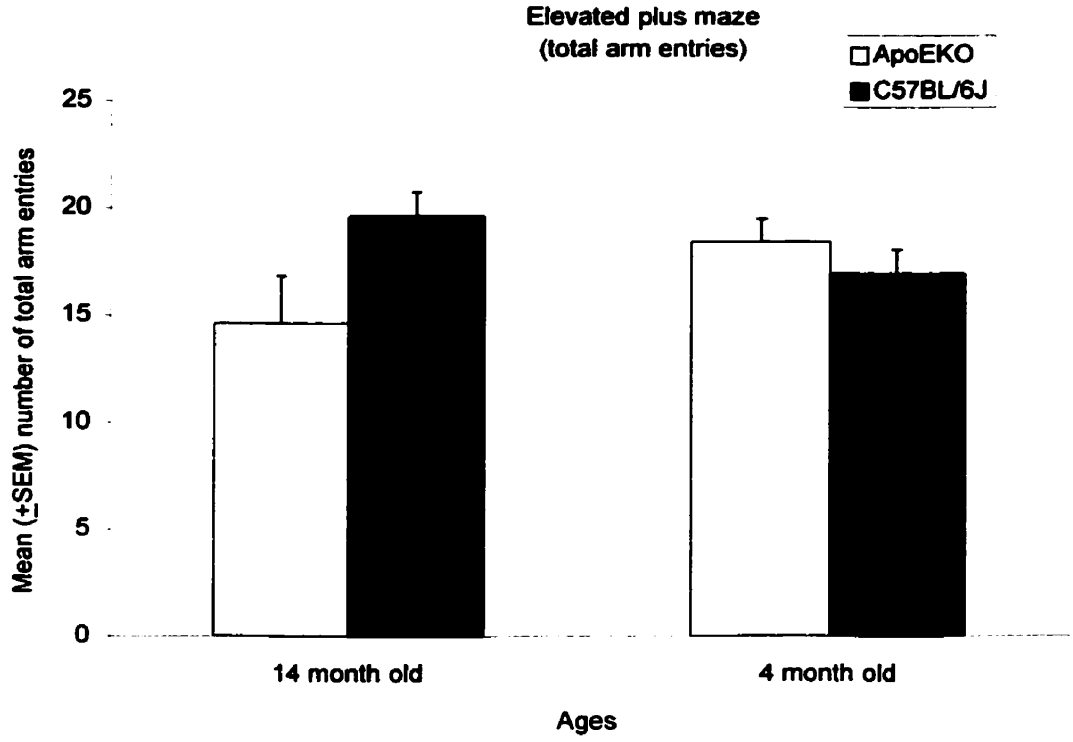

Figure 28. Mean (± SEM) number of total arm entries in the EPM for 4 and 14 month old apoEKO mice (10th generation) and C57BL/6J mice .

7. Open Field (4 and 14 month old mice)

7.1. Center Time

An Age x Strain two-way analysis of variance (ANOVA) revealed a significant main effect for Age [$F(1, 36)= 16.50$, $p<0.01$] suggesting that old mice spent significantly more time in the center than young mice. There was no significant main effect for Strain [$F(1, 36)= 0.05$, $p>0.05$], therefore apoEKO mice are not significantly different from C57BL/6J mice in the time they spend in the center. There was no significant Age x Strain interaction effect [$F(1, 36)= 3.31$, $p>0.05$] (see Figure 29).

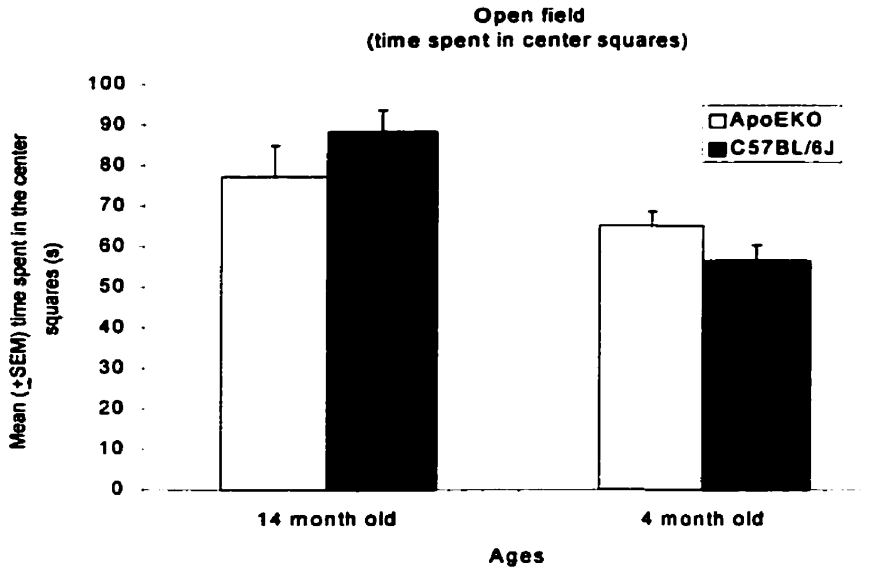

Figure 29. Mean (± SEM) time (s) spent in the center squares of the OF for 4 and 14 month old apoEKO mice (10^{th} generation) and C57BL/6J mice .

7.2. Center Squares Crossed

An Age x Strain ANOVA yielded a significant main effect for age [$F(1, 36)= 13.75$, $p<0.01$] indicating that old mice crossed significantly more center squares than young mice. There was no significant main effect for Strain [$F(1, 36)= 0.04$, $p>0.05$], therefore apoEKO mice were not significantly different from C57BL/6J mice in the number of squares crossed inside the OF. There was no significant Age x Strain interaction effect [$F(1, 36)= 2.61$, $p>0.05$] (see Figure 30).

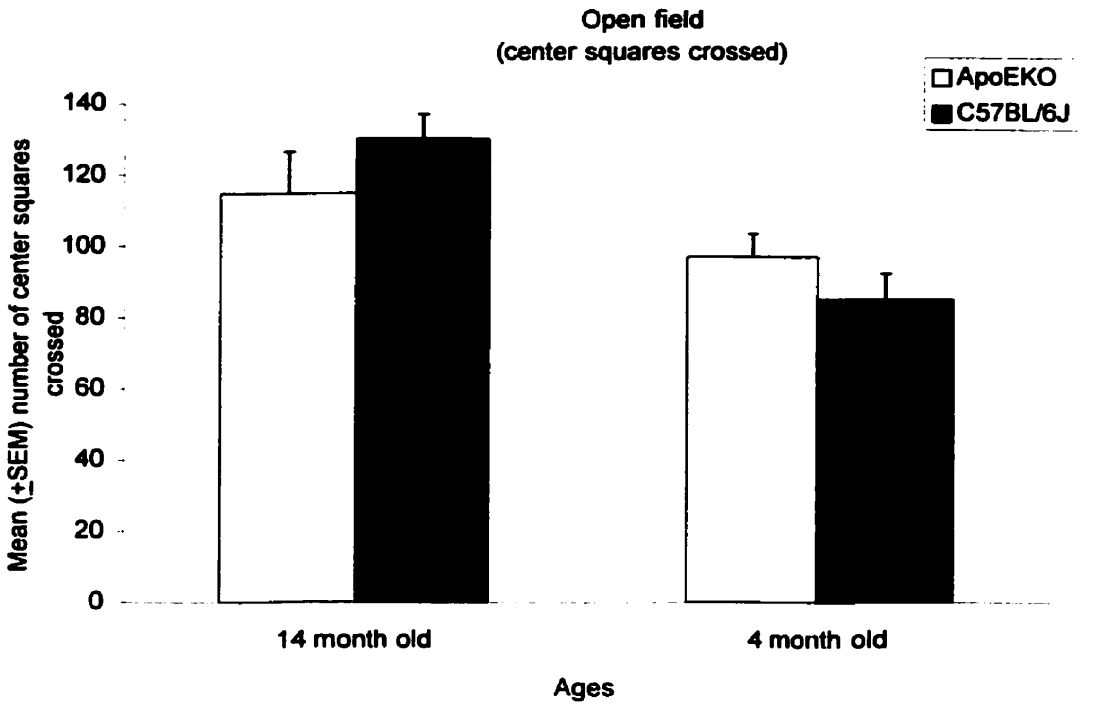

Figure 30. Mean (± SEM) number of center squares crossed in the OF for 4 and 14 month old apoEKO mice (10^{th} generation) and C57BL/6J mice .

7.3. Total Squares Crossed

No difference was observed in the number of total squares crossed as revealed by a an Age x Strain ANOVA : the main effect for Age [$F(1, 36)= 0.21$, $p>0.05$], the main effect for Strain [$F(1, 36)= 0.39$, $p>0.05$] and the Age x Strain interaction effect [$F(1, 36) = 2.61$, $p>0.05$] were all not significant (see Figure 31).

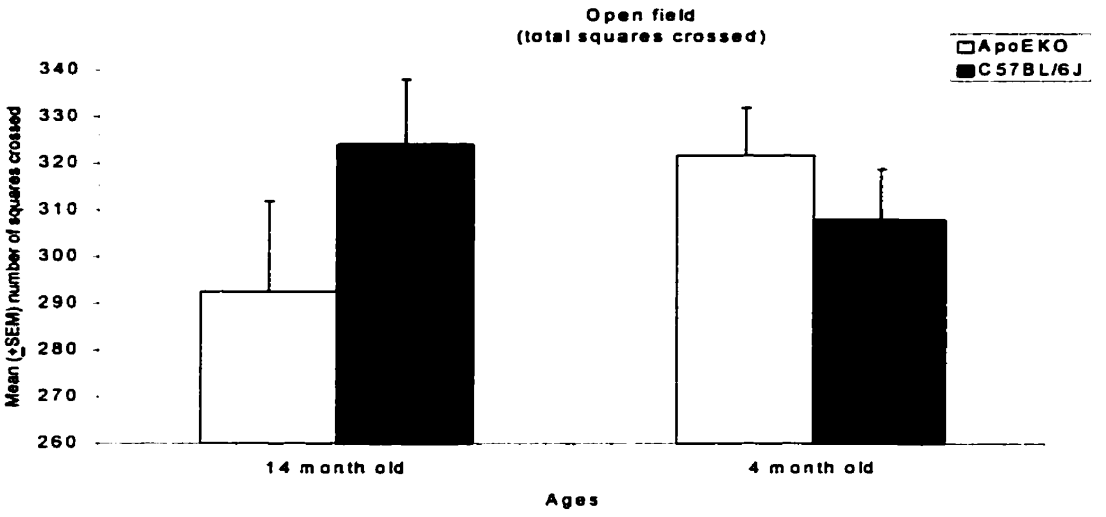

Figure 31. Mean (± SEM) number of total squares crossed in the OF for 4 and 14 month old apoEKO mice (10^{th} generation) and C57BL/6J mice .

C. Discussion

In the present study, we used two different tests to assess spatial learning and memory function in apoEKO mice and C57BL/6J mice. This was performed in apoEKO mice that were backcrossed 10 generations, in order to improve genetic homogeneity. In the MWM, apoEKO mice displayed a subtle deficit, in that the latency to find a platform on day 1 and on day 3 of training was longer than that in C57BL/6J mice. It would be tempting to suggest that these differences are due to a cognitive impairment, however, the magnitude of the differences was small and restricted to only two of the six training days. This contrasts with the more pronounced deficit observed in the 6th generation apoEKO mice, as shown in the first series of experiment. Accordingly, it is possible that the degree of cognitive impairment observed in these animals may be generation-dependent. Exactly why this effect occurred is impossible to say, but could reflect some sort of a compensatory mechanism that limits the detrimental impact of the absence of the APOE gene. The neurobiological adaptation mediating this adaptation is difficult to speculate upon, given the fact that we did not use any neurobiological measures in the present experiments.

At a behavioral level, one possible compensatory mechanism could be a shift in the strategy used to solve the MWM. As mentioned earlier, although most animals typically utilize spatial cues to master the MWM, utilization of non-spatial strategies can result in proficient performance. To assess this possibility, we examined whether removal of spatial cues would interrupt performance after acquisition had taken place. Under this condition, obscuring the extra-maze, spatial cues did not impair performance in both apoEKO and C57BL/6J mice. However, this result only demonstrates that both strains of

mice can utilize non-spatial strategies once the task has been mastered, it does not assess the strategy normally used to acquire the task. To assess this issue, we removed spatial cues from the onset of training. In this condition, we observed that apoEKO mice took significantly longer to find the platform than C57BL/6J mice. Thus, removal of spatial cues impaired performance in apoEKO mice more so than it did in C57BL/6J mice. Thus, the pattern of effects across the two testing conditions could suggest the following hypotheses: First, both apoEKO and C57BL/6J mice normally adopt a spatial strategy to solve the MWM. However, in so doing they may also acquire a non-spatial strategy that can be relied on when spatial cues are removed. This would account for the absence of an effect when spatial cues were removed *after* training has taken place. Second, acquisition of a spatial strategy facilitates acquisition of a non-spatial strategy in apoEKO mice. This would account for the impairment observed in apoEKO mice when spatial cues were removed from the start of training. Why might this be so? This may reflect the operation of "incidental" learning. Specifically, as the animal acquires the spatial information needed to solve the task, it may also acquire (incidentally) non-spatial information that could be used to locate the platform. Thus, when spatial cues are removed, the animal can rely on the non-spatial information it acquired. Without spatial cues to guide acquisition, however, this incidental learning may not occur.

In contrast to the first series of tests assessing performance in the MWM, in this series there was no correlation between the presence or absence of deficits and changes in the percent time spent in the four quadrants during the two probe trials in which the platform was removed. Indeed, in both of the experiments in this series, the amount of time spent in the target quadrant remained near chance levels (25%). Given this finding,

it is difficult to assess how the presence or absence of spatial cues might have influenced or altered search patterns.

To further investigate the importance of spatial and non-spatial information to accurate performance, we tested apoEKO and C57BL/6J mice in another test of spatial learning and memory, the RAM. The results paralleled, more or less, those observed in the MWM. Specifically, there was no impairment evoked when spatial cues were removed *after* training. In fact, apoEKO mice made significantly fewer errors on block 6 in comparison to C57BL/6J mice. Additionally, the removal of spatial cues on block 7 (by the addition of the curtain), did not produce a deficit in performance in comparison to block 6. Thus, as it was the case in the MWM, animals permitted to utilize spatial cues during acquisition are not detrimentally affected when those cues are obscured. Also similar to the results from the MWM, removal of spatial cues from the initiation of training did induce an impairment in apoEKO mice, adding further support to the conclusion that these animals cannot compensate for the absence of spatial information by utilizing possible non-spatial sources of information.

The serial forgetting curve analyses from the experiment in which spatial cues were not obtruded during training and the experiment in which spatial cues were removed on block 6 yielded three main findings. First, apoEKO mice made more errors over the fourth to the seventh choices over days 1-10 than C57BL/6J mice. Second, young apoEKO mice made fewer correct responses over successive choices over days 1 to 10 than young C57BL/6J mice. Third, aged animals made significantly fewer correct responses over successive choices than young animals from the second to the eighth choices over days 1-10 and 11-21.

If we consider that working memory store holds the information concerning what arms have been visited, the serial forgetting curve observed here supports the hypothesis of Gordon et al. (1995) suggesting that the absence of apoE creates a deficit in working memory. Specifically, the serial forgetting curve revealed that the impairments in apoEKO mice were more pronounced over the latter half of the curve, suggesting that these animals had more difficulty retaining information as the number of arms visited increased. Had the apoEKO mice suffered from a reference memory deficit, it might be expected that this would result in an impairment throughout the entire serial forgetting curve.

Additional evidence against a reference memory deficit was provided by the analyses examining the pattern of arm choices. In the present experiment, all eight arms were baited and visited arms within the same trial were not rebaited. Considering this, the best way to execute the task is to adopt a "win-shift" strategy (i.e. do not return to arms that have been visited). In fact, less than 1% of all arm choices were repeat entries into the arm previously visited, supporting the idea that all groups learned and used the win-shift strategy. Furthermore, the most parsimonious win-shift strategy would be to visit arms adjacent to the last arm visited (Hodges, 1996). The arm distance analysis showed that the number of choices that involved arms immediately adjacent to the previous choice was higher than the number of choices that were two arms distant, which was in turn higher than the number of choices that were three arms distant, and which was finally higher than the number of choices that were four arms distant in all groups over days 1-10 and 11-21.

Although no differences between apoEKO mice and C57BL/6J mice were

observed over the first 10 days, over the last 11 days, apoEKO mice made more 1 arm and fewer 2 arm choices than C57BL/6J mice. These last results might partially explain why apoEKO mice displayed less errors than C57BL/6J mice on block 6 and why the performance of C57BL/6J mice actually deteriorated somewhat over trials 11-21. The reason underlying the deterioration in C57BL/6J mice cannot be identified.

The serial forgetting curve analysis from the experiment in which spatial cues were removed throughout the duration of training yielded a pattern of results similar to those observed in the previous two RAM experiments. However, removal of spatial cues throughout training did appear to alter search patterns. Specifically, the arm distance analysis over days 1-10 showed that apoEKO mice made fewer 1 arm choices and tended to make more 3 arm choices than C57BL/6J mice. Over the latter half of testing, apoEKO mice continued to make significantly fewer 1 arm choices, and tended to make more 2 arm choices than C57BL/6J mice, although not significantly different. These results suggest that the absence of spatial cues altered search strategies in apoEKO mice. Again, this may reflect an inability of apoEKO mice to utilize alternative, non-spatial forms of information that can be used to solve the task.

It is unlikely that impairments in systems other than those responsible for spatial learning and memory function, such as visual or motor impairments, altered emotionality or sensitivity to stress, or impairment in attentional function, can account for the deficits observed in the MWM and RAM in apoEKO. In the MWM, both strains of mice were significantly faster to locate the platform when it was made visible. Nor can motor deficits account for the observed differences observed. Given that accuracy in the RAM is based on choice, rather than a temporal measure, it is difficult to see how a motor

impairment would result in deficits in RAM performance. Additionally, motor and swimming function have been assessed in other studies using the MWM, and no differences between apoEKO and C57BL/6J mice were detected (Oitzl et al., 1997; Gordon et al., 1995).

There were also no significant strain differences in PPI. To the extent that this paradigm assesses attentional function, the equal performance observed in apoEKO and C57BL/6J mice would argue against an attentional deficit. Of course, we appreciate that the suggestion that PPI assesses attentional function is debatable, in that this phenomenon can be equally conceived of as reflecting a lower level "preattentional" sensorimotor gating mechanism. It may also be possible to invoke mechanisms involved in emotion that may influence the magnitude of PPI. However, it is clear that PPI does reflect the extent to which a stimulus (the prepulse) can impede another stimulus (the startle stimulus) from subsequent cognitive processing. As such, PPI reflects the operation of one type of stimulus selection. We have proposed that the impairment in apoEKO mice observed in MWM and the RAM when spatial cues are removed throughout training may reflect an inability to utilize non-spatial cues. Consequently, the results from the PPI test would suggest that this inability does not result from a deficit in at least one process involved in stimulus selection.

The results from the OF test and the EPM would suggest that the MWM and RAM performance do not results from altered stress reactivity or emotionality. In both tests, both young and old apoEKO displayed a pattern of behavior no different from their respective C57BL/6J aged control groups.

In contrast to the MWM and RAM tests, where age did not strongly predict either

presence or the magnitude of cognitive impairment, in the PPI paradigm, the OF and the EPM there were age-related differences in behavior. Specifically, aged animals showed reduced levels of PPI, increased time and number of entries into the center squares of OF, and increased time and percent entries into the open arms of the EPM. Similar age-related changes in these paradigms have been reported in the literature (Gower and Lamberty, 1993; Ammassari-Teule et al., 1981). It is also worth noting that the age-related differences were strain-independent, in that these behavioral changes were observed in both aged apoEKO and C57BL/6J mice. Thus, the age effect is not influenced by the presence or absence of the APOE gene. Moreover, since the performance of apoEKO and C57BL/6J mice in the MWM and RAM were age-independent, whereas performance in the PPI paradigm, OF and EPM were influenced by age, this pattern of results adds further indirect support for the conclusion that the spatial memory deficits observed in apoEKO mice occurred independently of any alterations in sensorimotor gating function or emotionality.

IV. Conclusion

The present series of experiments aimed to characterize the behavioral phenotype of apoEKO mice and to evaluate the impact of apoE-deficiency on cognitive function at different ages. Whereas many previous studies reported cognitive impairments (spatial memory deficit) in this animal model, others found no differences between this model and wild type mice. To further investigate the cognitive abilities of apoEKO mice, we tested animals that had a stabilized genetic background (backcrossed at least ten times). Our results clearly support the hypothesis that apoEKO mice can perform as efficiently as C57BL/6J mice on spatially-guided tasks, but are deficient in non-spatially guided tasks.

Additionally, our analyses suggest normal reference memory, but disrupted working memory function. Finally, these memory deficits appear to occur independently of any changes in visual, motor, attentional or emotional function.

ApoEKO mice share many similarities (i.e. working memory deficit) with AD patients. Whereas it is possible that the working memory deficit seen in apoEKO mice is related to disruption of the neuronal cytoskeleton integrity caused by the absence of apoE, the present study did not confirm any neurophatological or neurophysiological alterations in these apoEKO mice.

V. References

Acri, J., Brown, K., Saah, M. and Grunberg, N. (1995). Strain and age differences in acoustics startle responses and effects of nicotine in rats. *Pharmacology Biochemistry and Behavior*, **50**, 191-198.

Alberts, M.J., Graffagnino, C., McClenny, C., DeLong, D., Strittmatter, W., Saunders, A.M. and Roses, A.D. (1995). ApoE genotype and survival from intracerebral haemorrhage. *Lancet*, **34**, 65-75.

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders*, 4th ed. Washington, DC.

Ammassari-Teule, M. Fagioli, S. and Rossi-Arnaud, C. (1994). Radial arm maze performance and open-field behaviours in aged C57BL/6 mice: further evidence for preserved cognitive abilities during senescence. *Physiology and Behaviour*, **55**, 341-345.

Anderson, R., Barnes, J.C., Bliss, T.V.P., Cain, D.P., Cambon, K., Davies, H.A., Errington, M.L., Fellows, L.A., Gray, R.A., Hoh, T., Stewart, M., Large, C.H. and Higgins, G.A. (1998). Behavioral, physiological and morphological analysis of a line of apolipoprotein E-knockout mouse. *Neuroscience*, **85**, 93-110.

Anderson, R. and Higgins, G.A. (1997). Absence of central cholinergic deficits in ApoE-knockout mice. *Psychopharmacology*, **132**, 135-144.

Arendt, T., Bigl, V., Arendt, A. and Tennstedt, A. (1983). Loss of neuron in the nucleus of meynert in Alzheimer's disease, paralysis agitans and Korsakoff's disease. *Acta Neuropathol.*, **61**, 101-108.

Arendt, T., Schindler, C., Bruckner, M.K., Eschrich, K., Bigl, V. Zedlick, D. and Marcova, L. (1997). Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein (4 allel. *J. Neurosc.*, **17**, 516-529.

Aubert, I., Araujo, D.M., Cecyre, D., Robitaille, Y., Gauthier, S. and Quirion, R. (1992). Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J. Neurochem.*, **58**, 529-541.

Bartus, R.T., Dean, R., Bear, G. and Lippa, A.S. (1982). The cholinergic hypothesis of geriatric memory dysfunction: a critical review. *Science*, **217**, 408-414.

Beffert, U., Danik, M., Krzywkowski, P., Ramassamy, C., Berrada, F. and Poirier, J. (1998). The neurobiology of apolipoproteins and their receptors in the CSN and Alzheimer's disease. *Brain Research Reviews*, 1-23.

Beffert, U. and Poirier, J. (1996). Apolipoprotein E, plaques, tangles and cholinergic dysfunction in Alzheimer's disease. *Ann. N.Y. Acad. Sci.*, **177**, 166-174.

Beisiegel, R.W., Weber, W., Ihrke, G., Herz, J. and Stanley, K.K. (1989). The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature*, **341**, 162-164.

Bennett, C., Crawford, F., Osborne, A., Diaz, P., Hoyne, J., Lopez, R., Roques, P., Duara, R., Rossor, M. and Mullam, M. (1995). Evidence that the apoE locus influences the rate of disease progression in late-onset familial Alzheimer's disease but not is causative. *Am. J. Med. Genet.*, **60**, 1-6.

Bick, K., Amaducci, L. and Pepeu, G. (1987). *The early story of Alzheimer's disease.* Padua: Livinia Press.

Blacker, D., Haines, J.L., Rodes, L., Terwedow, H., Go, R.C.P., Harrell, L.E., Perry, R.T., Bassett, S.S., Chase, G., Meyers, D., Albert, M.S. and Tanzi, R. (1997). ApoE-4 and age at onset of Alzheimer's disease: the NIMH genetics initiative. *Neurology*, **48**, 139-147.

Blisa, R., Adroer, R., Santacruz, P., Ascaso, C., Tolosa, E. and Oliva, R. (1996). High apolipoprotein E epsilon 4 allele frequency in age-related memory decline. *Ann. Neurol.*, **39**, 548-551.

Bochachio, M., Latronico, N., Zani, D.G., Mariotti, M., Morandini, L., Acquarolo, A.M. and Candiani, A. (1990). Free radical-induced lipoperoxidation and severe head injury. A clinical study. *Intensive Care Med.*, **16**, 444-447.

Bouras, C., Hof, P.R., Giannakopoulos, P., Michel, J.P. and Morrison, J.H. (1994). Regional distribution of neurofibrillary tangles and senile plaques in the cerebral cortex of elderly patients: a quantitative evaluation of one-year autopsy population from a geriatric hospital. *Cereb. Cortex*, **4**, 138-150.

Boyles, J.K., Notterpek, L.M. and Anderson, L.J. (1990). Accumulation of apolipoproteins in the regenerating and remyelinating mammalian peripheral nerve: identification of apolipoprotein D, apolipoprotein A-IV, apolipoprotein E and apolipoprotein A-I. *J. Biol. Chem.*, **265**, 17805-17815.

Boyles, J.K., Zoellner, C.D., Anderson, A.J., Kosik, L.M., Pitas, R.E., Weisgraber, K.H., Hui, D.Y., Mahley, R.W., Gebicke-Haerter, P.J., Ignatius, M.J. and Shooter, E.M. (1989). A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J. Clin. Invest.*, **83**, 1015-1031.

Braak, H. and Braak, E. (1991). Neuropathological stageing of Alzheimer's-related changes. *Acta Neuropathol.*, **82**, 239-259.

Breslow, J.L. (1996). Mouse models of atherosclerosis. *Science*, **272**, 685-688.

Brion, J.P., Passareiro, H., Nunez, J. and Flament-Durand, J., (1985). Immunological detection of tau protein in neurofibrillary tangles of Alzheimer's disease. *Arch. Biol.*, **96**, 229-235.

Buresova, O., Bolhuis, J.J. and Bures, J. (1986). Differential effects of cholinergic blockage on performance of rats in the water tank navigation task and in radial water mase. *Behav. Neurosci.*, **100**, 476-482.

Canadian Study of Health and Aging Working Group. (1994). Prevalence of dementia in Canada. *Can. Med. Assoc. J.*, **150**, 899-906.

Chapman, S. and Michaelson, D.M. (1998). Specific neurochemical derangements of brain projecting neurons in apolipoprotein E-deficient mice. *J. Neurochem.*, **70**, 708-714.

Chartier-Harlin, M.C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., Goate, A., Rossor, M., Roques, P., Hardy, J. and Mullan, M. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the (-amyloid precursor protein gene. *Nature,* **353**, 844-846.

Chen, Y., Lomnitski, L., Michaelson, D.M. and Shohami, E. (1997). Motor and cognitive deficits in apolipoprotein E-deficient mice after closed head injury. *Neurosci.*, **80**, 1255-1262.

Cheng, H.-W., Jiang, T., Brown, S.A., Pasinetti, C.E., Finch, C.E. and McNeill, T.H. (1994). Response of striatal astrocytes to neuronal deafferentation: An immunohistochemical and ultrastructural study. *Neurosci.*, **62**, 425-439.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L. and Pericak-Vance, M.A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*, **261**, 921-923.

Cotman, C.W. and Nadler, J.V. (1978). Reactive synaptogenesis in the hippocampus. *Raven Press*, 227-271.

Coyle, J.T., Price, D.L. and DeLong, M.R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science,* **219**, 1184-1190.

Crystal, H., Dickson, D., Fuld, P., Masur, D., Scott, R., Mehler, M., Masdeu, J., Kawas, C., Aronson, M. and Wolfson, L. (1988). Clinico-pathologic studies in dementia: non-demented subjects with pathologically confirmed Alzheimer's disease. *Neurology*, **38**, 1682-1687.

Cummings, J. and Khachaturian, Z. (1996). Definition and diagnostic criteria. In S. Gauthier (Ed.), *Clinical Diagnosis and Management of Alzheimer's disease* (pp.3-15). London: Martin Dunitz.

Curts, M. and Van Broeckhoven, C. (1998a). Molecular genetics of Alzheimer's disease. *Ann. Med.*, **30**, 560-565.

Curts, M. and Van Broeckhoven, C. (1998b). Presenilin mutations in Alzheimer's disease. *Human Mutation*, **11**, 183-190.

Davies, P. (1986). The Genetics of Alzheimer's disease: a review and a discussion of the implications. *Neurobiol. Aging*, **7**, 459-66.

Davignon , J., Gregg, R.E. and Sing, C.F. (1988). Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis*, **8**, 1-21.

Davignon, J. and Roy, M. (1993). Familial hypercholesterolomia in French-Canadian: taking advantage of the presence of a "founder effect". *Am. J. Cardiol.*, **72**(10), 6D-10D.

Deker, M.W. (1987). The effect of aging on hippocampal and cortical projections of the forebrain cholinergic system. *Brain Res. Rev.*, **12**, 423-438.

De Pablo, J.M., Parra, A., Segovia, S. and Guillamon, A. (1988). Learned immobility explains the behaviour of rats in the forced swimming test. *Physiology & Behaviour*, **46**, 229-237.

De Ruiter, J.P. and Uylings, H.B. (1987). Morphometric and dendritic analysis of fascia dentata granule cels in human aging and senile dementia. *Brain Res.*, **402**, 217-229.

Dupuy, J.B., Beaulieu, S., Barden, N. and Rochford, J. (1998). Effect of chronic desipramine treatment on behavioral reactivity, sensorimotor gating and spatial memory deficits in transgenic mice with impaired type II glucocorticoid receptor function. *Society for Neuroscience Abstracts*, **24**, 958.

Ebly, E.M., Parhad, I.M., Hogan, D.B.and Fung, T.S. (1994). Prevalence and types of dementia in the very old: Results from the Canadian Study of Health and Aging. *Neurology*, **44**, 1593.

Elshourbagy, N.A., Liao, W.S., Mahley, R.W. and Taylor, J.M. (1985). Apolipoprotein mRNA is abundant in the brain and adrenals as well as the liver, and is present in other peripheral tissues of rat and marmosets. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 203-207.

Fagan, A.M., Murphy, B.A., Patel, S.N., Kilbridge, J.F., Mobley, W.C., Bu, G. and Holtzman, D.M. (1998). Evidence for normal aging of the septo-hippocampal cholinergic system in apoE(-/-) mice but impaired clearance of axonal degeneration products following injury. *Exp. Neurol.*, **151**, 314-325.

Farrer, L.A., Cupples, L.A. and Haines, J.L. (1997). Effects of age, sex, and ethnicity on the association between apolipoproteinE genotype an d Alzheimer's disease. A meta-analysis. *JAMA*, **278**, 1349-1356.

Fisher, A., Brandies, R., Chapman, S., Pittel, Z. and Michaelson, D.M. (1998). M1 muscarinic agonist treatment reverses cognitive and cholinergic impairments of apolipoprotein E-deficient mice. *J. Neurochem.*, **70**, 1991-1997.

Fitch, N., Becker, R. and Heller, A. (1988). The inheritance of Alzheimer's disease: a new interpretation. *Ann. Neurol.*, **23**, 14-9.

Fordyce, D.E., and Wehner, J.M. (1993). Effects of aging on spatial learning and hippocampal protein kinase C in mice. *Neurobiology of Aging*, **14**, 309-317.

Gandy, S.E., Stafstrom, C.B.T., Sweeney, D., Breslow, J., Greengard, P., Smith, J. and Ouimet, C. (1995). Aged apoE deficient mice: Abnormal learning and glial markers but no obvious neuropathology. *Soc. Neurosci. Abstracts.*, 215.

Gautrin, D., Froda, S., Tetreault, H. and Gauvreau, D. (1990). Canadian projections of cases suffering from Alzheimer's disease and senile dementia of Alzheimer type over the period 1986-2031. *Can. J. Psychiatry*, **35**, 162-165.

Gelman, B.B., Rifia, N., Goodrum, J.F., Bouldin, T.W. and Krigman, M.R. (1987). Apolipoprotein E is released by sciatic nerve during segmental demyelination and remyelination. *J. Neurophatol. Exp. Neurol.*, **46**, 644-652.

Geyer, M., and Markou, A. (1995). Animal models of psychiatric disorders. In F.E. Bloom and D.J., Kupfer (Eds.) *Psychopharmacology: The fourth Generation of Progress.* New York: Raven Press, Ltd.

Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M.A., Roses, A.D., Williamson, R., Rossor, M., Owen, M. and Hardy, J. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature,* **349**, 704-706.

Goldstein, J.L., Basu, S.K. and Brown, M.S. (1983). Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. *Methods Enzymol.*, **98**, 241-261.

Goodrum, J.F. and Novicki, D.L. (1988). Macrophage-like cells from explant cultures of rat sciatic nerve produce apolipoprotein E. *J. Neurosci. Res.*, **20**, 457-462.

Gordon, I., Ben-Eliyahu, S., Rosenne, E., Sehayek, E. and Michaelson, D.M. (1996a). Derangement in stress response of apolipoprotein E-deficient mice. *Neurosci. Lett.*, **206**, 212-214.

Gordon, I., Grauer, E., Genis, I., Sehayek, E. and Michaelson, D.M. (1995). Memory deficits and cholinergic impairments in apolipoprotein E-deficient mice. *Neuroscience Letters,* **199**, 1-4.

Gordon, I., Graur, E., Genis, I., Sehayek, E. and Michaelson, D. M. (1996b). Memory deficits in apolipoprotein E-deficient mice. *Neurosci. Lett.*, **199**, 1-4.

Gower, A.J. and Lamberty, Y. (1993). The aged mouse as a model og cognitive decline with special emphasis on studies in NMRI mice. *Behavioural Brain Research*, **57**, 163-173.

Gozes, I., Bachar, M., Bardea, A., Davidson, A., Rubinraut, S., Fridkin, M. and Giladi, E. (1997). Protection against developmental retardation in apolipoprotein E-deficient mice by a fatty neuropeptide: implications for early treatment of Alzheimer's disease. *J. Neurobiol.*, **33,** 329-342.

Grauer, E. and Kapon, J. (1995). Differential effects of anticholinergic drugs on paired discrimination performance. *Pharmacol. Biochem. Behav.*, **53**(2), 463-467.

Growdon, J.H. (1995). Advances in the diagnosis of Alzheimer's disease. In K. Iqbal, J.A. Mortimer, B. Winblad and H.M. Wisniewski (Eds.). *Research advances in Alzheimer's disease and related disorders* (pp. 139-153). John Wiley & Sons Ltd.

Haass, C. and Selkoe, D.J. (1993). Cellular processing of (-amyloid precursor protein and the genesis of amyloid (-peptide *Cell*, **75,** 1039-42.

Halliday, G.M., McCann, H.L., Pamphlett, R., Brooks, W.S., Creasey, H., McCusker, E., Cotton, R.G.H., Broe, G.A. and Harper, C.G. (1992). Brainstem serotonin synthesizing neurons in Alzheimer's disease: a clinicopathological correlation. *Acta Neuropathol.*, **84**, 638-650.

Halliwell, B. (1992). Reactive oxygen species and the central nervous system. *J. Neurochem.*, **59**, 1609-1623.

Handley, S.L. and McBlane, J.W. (1993). An assessment of the elevated x-maze for studying anxiety and anxiety-modulating drugs. *Journal of Pharmacoloy and Toxicology Methods*, **29**, 129-138.

Hardy, J. and Higgins, G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science*, **256**, 184-185.

Hawkins, J., Hicks, R.A., Phillips, N. and Moore, J.D. (1978). Swimming rats and human depression. *Nature*, **274**, 512.

Hayek, T., Oiknine, J. Brook, J.G. and Aviram, M. (1994). Increased plasma and lipoprotein lipid peroxidation in apo E-deficient mice. *Biochem. Biophys. Res. Commun.*, **201**, 1567-1574.

Heston, L.L., Mastri, A.R., Anderson, V.E. and White, J. (1981). Dementia of the Alzheimer type. *Arch. Gen. Psychiatry*, **38**, 1085-90.

Higgins, G.A., Large, C.H., Rupniak, H.T. and Barnes, J.C. (1997). Apolipoprotein E and Alzheimer's disease: a review of recent studies. *Pharmacol. Biochem. Behav.*, **56**, 675-685.

Hodges, H. (1996). Maze procedures: the radial arm and water maze compared. *Cognitive Brain Research*, **3**, 167-181.

Hofman, A., Schulte, W., Tanja, T.A. et al. (1989). History of dementia and Parkinson's disease in 1st-degree relatives of patients with Alzheimer's disease. *Neurology*, **39**, 1589-92.

Houser, C.R., Crawford, G.D., Braber, R.P., Salvaterra, P.M. and Vaughn, J.E. (1985). Immunohistochemical localization of choline acetyl-transferase in rat cerebral cortex: a study of cholinergic neurons and synapses. *J. Comp. Neurol.*, **234**, 17-34.

Hyman, B.T., Van Hoesen, G.W., Damasio, A.R. and Barnes C.L. (1984). Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. *Science*, **225**, 1168-1170.

Hyman, B.T., Van Hoesen, G.W., Kromer, L.J. and Damasio, A.R. (1986). Perforant pathway changes and the memory impairment of Alzheimer's disease. *Ann Neurol.*, **20**, 472-481.

Jeffries, D., Boublik, J. and Funder, J.W. (1985). A "k"-selective opiodergic pathway is involved in the reaversal of a behavioural effect of adrenalectomy. *European Journal of Pharmacology*, **107**, 331-335.

Jeffries, D., Kopolov, D. and Funder, J.W. (1984). Naloxone inhibits both glucocorticoid and [D-Ala^2, Met^5] enkephalinamide reversal of behavioural effects of adrenalectomy. *European Journal of Pathology*, **103**, 205-210.

Kang, J., Lemaire, H., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K., Multhaup, G., Beyreuther, K. and Müller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*, **325**, 733-736.

Kidd, M. (1964). Alzheimer's disease: an electron microscopical study. *Brain*, **87**, 307-320.

Kim, D.H., Iijima, K., Goto, K., Sakai, J.H.I., Kim, H.J., Suzuki, H., Kondo, H., Saeki, S. and Yamamoto, T. (1996). Human apolipoprotein E receptor 2. A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in the brain. *J. Biol. Chem.*, **271**, 8373-8380.

Kosunen, O., Soininen, H., Paljarvi, L., Heinonen, O., Talasniemi S. and Riekkinen, P.J., Sr. (1996). Diagnostic accuracy of Aalzheimer's disease: a neuropathological study. *Acta Neuropathol.*, **91**, 185-193.

Krugers, H.J., Mulder, M., Korf, J., Havekes, L.M., deKloet, E.R. and Joels, M. (1997). Altered synaptic plasticity in hippocampal CA1 area of apolipoprotein E-deficient mice. *Neuroreport*, **8**, 2502-2510.

Krzywkowski, P., Ghribi, O., Gagne, J., Chabot, C., Kar, S., Rochford, J., Massicotte, G. and Poirier, J. (1999). Cholinergic systems and long-term potentiation in memory-impaired apolipoprotein E-deficient mice. *Neurosci.*, **92**, 1273-1286.

Laskowitz, D.T., Sheng, H., Bart, R., Joyner, K., Roses, A.D. and Warner, D. (1997). Apolipoprotein E deficient mice have increased susceptibility to focal cerebral ischemia. *J. Cereb. Blood Flow Metab.*, **17**, 753-758.

Leblanc, A.C. and Poduslo, J.F. (1990). Regulation of apolipoprotein E gene expression after injury of the rat sciatic nerve. *J. Neurosci. Res.*, **25**, 162-171.

Lehtovirta, M., Soininen, H., Laakso, M.P., Partanen, K., Helisalmi, S., Mannermaa, A., Ryynanen, M., Kuikka, J., Hartikainen, P.and Riekkinen, P.J. Sr. (1996). SPECT and MRI analysis in Alzheimer's disease: relation to apolipoprotein E (4 allele. *J. Neurol. Neurosurg. Psychiatry,* **60**, 644-649.

Levin, E. (1988). Psychopharmacological effects in the radial-arm maze. *Neuroscience and Behavioral Reviews,* **12**, 169-175.

Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H., Yu, C., Jondro, P.D., Schmidt, D., Wang, K., Crowley, A.C., Fu ,Y.H., Guenette, S.Y., Galas, D., Nemens, E., Wijsman, E.M., Bird, T.D., Schellenberg, G.D. and Tanzi, R.E. (1995). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*, **269**, 973-977.

Lomnitski, L., Chapman, S., Hochman, A., Kohen, R., Shohami, E., Chen, Y., Trembovler, V. and Michaelson, D.M. (1999). Antioxidant mechanisms in apolipoprotein E deficient mice prior to and following closed head injury. *Biochimica. Biophysica. Acta*, **1453**, 359-368.

Lomnitski, L., Kohen, R., Chen, Y., Shohami, E., Trembovler, V., Vogel, T. and Michaelson, D.M. (1997). Reduced levels of antioxidants in brains of apolipoprotein E-deficient mice following closed head injury. *Pharmacol. Biochem. Behav.*, **56**, 669-673.

Mahley, R.W. (1988). Apolipoprotein E: cholesterol transport protein with expending role in cell biology. *Science*, **240**, 622-630.

Mahley, R.W., Nathan, B.P. and Pitas, R.E. (1996). Apolipoprotein E: structure, function and possible roles in Alzheimer's disease. *Ann. NY Acad. Sci.*, **777**, 139-145.

Maki, W.S. (1984). Spatial memory over long retention intervals: Nonmemorial factors are not necessary for accurate performance on the radial-arm maze by rats. *Behavioral and Neural Biology,* **41**, 1-6.

Mann, D.A.M. (1988). The pathological association between Down's syndrome and Alzheimer's disease. *Mech. Aging. Dev.*, **43**, 99-136.

Mann, D.M.A., Yates, P.O. and Hawkes, J. (1983). The pathology of the human locus coeruleus. *Clin. Neuropathol.*, **2**, 1-7.

Masliah, E., Mallory, M., Alford, M., Ge, N. and Mucke, L. (1995a). Abnormal synaptic regeneration in $hAPP_{695}$ transgenic and apoE-knockout mice. In K. Iqbal, J.A. Mortimer, B. Winblad and H.M. Wisniewski (Eds.). *Research Advances in Alzheimer's Disease and related disorders* (pp. 405-414). New York: Wiley.

Masliah, E., Mallory, M., Nianfeng, G., Alford, M., Veinbergs, I. and Roses, A. (1995b). Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp. Neurol.*, **136**, 107-122.

Masliah, E., Samuel, W., Veinbergs, I., Mallory, M., Mante, M. and Saitoh, T. (1997). Neurodegeneration and cognitive impairment in apoE-deficient mice is ameliorated by infusion of recombinant apoE. *Brain Res.*, **751**, 307-314.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D. and Standlan, E.M. (1984). Clinical diagnosis of Alzhemer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. *Neurology*, **34**, 939-944.

McNamara, R.K. and Skelton, R.W. (1993). The neuropharmacological and neurochemical basis of place learning in the Morris water maze. *Brain Res. Rev.*, **18**, 33-49.

Miller, A., Alford, M., Katzman, R., Thal, I. and Masliah, E. (1994). *Ann. Neurol.*, **36**, 268-269.

Mirra, S.S., Heyman, A., Mckeel, D., Sumi, S.M., Crain, B.J., Brownlee, L.M., Vogel, F.S., Hughes, J.P., Van Bell, G. and Berg, L. (1991). The consortium to establish a registery for Alzheimer's disease (CERAD). Part II. Standardization of the neuropathological assessment of Alzheimer's disease. *Neurology*, **41**, 479-486.

Morris, J.C., Storandt, M., McKeel, D.W., Rubin, E.H., Price, J.L., Grant, E.A. and Berg, L. (1996). Cerebral amyloid deposition and diffuse plaques in normal aging: evidence from presymptomatic and very mild Alzheimer's disease. *Brain Res.*, **416**, 331-336.

Morris, R.G.M. (1981). Spatial localisation does not require the presence of local cues. *Learning Motiv.*, **12**, 239-249.

Morris, R.G.M. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Meth.*, **11**, 47-60.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P. and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, **297**, 681-683.

Mountjoy, C.Q., Roth, M., Evans, N.J.R. and Evans, H.M. (1983). Cortical neuronal counts in normal elderly controls and demented patients. *Neurobiol. Aging*, **4**, 1-11.

Nalbantoglu, J., Gilfix, B.M., Bertrand, P., Robitaille, Y., Gauthier, S., Rosenblatt, D.S. and Poirier, J. (1994). Predictive value of apolipoprotein E genotyping in Alzheimer's disease. *Ann. Neurol.*, **36**, 889-895.

Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E. and Ikeda, K. (1991). Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and Kuru plaque amyloid in Creutzfeldt-Jacob disease. *Brain Res.*, **541**, 163-166.

Newman, M.F., Croughwell, N.D., Blumenthall, J.A., Lowry, E., White, W.D., Spillane, W., Davis, R.D., Glower, D.D., Smith, L.R. and Mahanna, E.P. (1995). Predictors of cardiac decline after cardiac operation. *Ann. Thorac. Surg.*, **59**, 1326-1330.

Nitsch, R.M., Blusztajn, J.K., Pitas, A.G., Slack, B.E. and Wurtman, R.J. (1992). Evidence for a membrane defect in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA*, **89**, 1671-1675.

Ohm, T.G., Kirca, M., Bohl, J., Scharnagl, H., Gross, W. and Marz, W. (1995). Apolipoprotein polymorphysm influences not only cerebral senile plaque load but also Alzheimer-type neurofibrillary tangle formation. *Neuroscience*, **66**, 583-587.

Oitzl, M.S., Mulder, M., Lucassen, P.J., Havekes, L.M., Grootendorst, J. and Ron de Kloet, E. (1997). Severe learning deficits in apolipoprotein E-knockout mice in a water maze task. *Brain Research*, **752**, 189-196.

Olton, D.S., Collison, C. and Werz, M.A. (1977). Spatial memory and radial arm maze performance of rats. *Learning and Motivation*, **8**, 289-314.

Olton, D.S. and Samuleson, R.J. (1976). Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Processes*, **2**, 97-115.

Ong, W.-Y., Suresh, H.S. and Patel, S.C. (1997). Differential expression of apolipoprotein D and apolipoprotein E in the kainic acid-lesioned rat hippocampus. *Neurosci.*, **79**, 359-367.

Palinski, W., Ord., V.A., Plump, A.S., Breslow, J.L. Steinberg, D. and Witztum, J.L. (1994). ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis: demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler. Thromb.*, **14**, 605-616.

Pasinetti, G.M., Cheng, H.W., Lampert-Etchells, M., McNeill, T.H. and Finch, C.E. (1993). Astrocytic messenger RNA responses to striatal deafferentation in male rats. *Neurosci.*, **53**, 199-211.

Pericak-Vance, M.A., Bass, M.P., Yamaoka, L.H., Gaskell, P.C., Scott, W.K., Terwedow, H.A., Menold, M.M., Conneally, P.M., Small, G.W., Vance, J.M., Saunders, A.M. and Haines, J.L. (1997). Complete genomic screen in late-onset familial Alzheimer's disease. Evidence for a new locus on chromosome 12. *JAMA*, **278**, 1237-1241.

Perry, T.A., Torres, E., Czech, C., Beyreuther, Richards, S.J. and Dunnett, S.B. (1995). Cognitive and motor function in transgenics mice carrying excess copies of the 695 and 751 amino acid isoforms of the amyloid precursor protein gene. *Alzheimer's Research*, **1**, 5-14.

Piedrahita, J.A., Zhang, S.H., Hagaman, J.R., Oliver, P.M. and Maeda, N. (1992). Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natn. Acad. Sci. USA*, **89**, 4471-4475.

Pïrttila, T., Soininen, H., Heinover, O., Lehtimäki, T., Bogdanovic, N., Paljar, L., Kosunen, O., Winblad, B., Riekkinen, P. and Wisniewski, H.M. (1996). Apolipoprotein E (ApoE) levels in brains from Alzheimer's disease patients and controls. *Brain Research*, **722**, 71-77.

Pitas, R.E., Boyles, J.K., Lee, S.H., Hui, D. and Weisgraber, K.H. (1987). Lipoproteins and their receptors in the central nervous system. Characterization of the lipoproteins in the cerebrospinal fluid and identification of apolipoprotein B, E (LDL) receptors in the brain. *J. Biol. Chem.*, **262**, 14352-14360.

Plump, A.S., Smith, J.D., Hayek,T., Aalto-Setala, K., Walsh, A., Verstuyft, J.G., Rubin, E.M. and Breslow, J.L. (1992). Severe hypercholesterolaemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell*, **71**, 343-353.

Poirier, J. (1994). Apolipoprotein E animal models of CNS injury and in Alzheimer's disease. *Trends in Neurosciences*, **17**, 525-530.

Poirier, J. (1993). Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet*, **342**, 697-699.

Poirier, J., Baccichet, A., Dea, D. and Gauthier, S. (1993). Role of hippocampal cholesterol synthesis and uptake during reactive synaptogenesis in adult rats. *Neuroscience*, **55**, 81-90.

Poirier, J., Delise, M.-C., Quirion, R., Auberts, I., Farlow, D., Lahiri, D., Hui, S., Bertrand, P., Nalbantoglu, J. and Grefix, B.M. (1995). Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA*, **92**, 12260-12264.

Poirier, J., Hess, M., May, P.C. and Finch, C.E. (1991). Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. *Mol. Brain Res.*, **11**, 97-106.

Poirier, J., Nalbantoglu, J., Guillaume, D. and Bertrand, P. (1996). In A. Goate and F. Ashall (Eds.), *Neurofibrillary Tangles and Amyloid Protein in Alzheimer's Disease*. Academic Press.

Ransmayr, G., Hisch, E. and Agid, Y. (1989). Choline acetyltransferase-like immunoreactivity in the hippocampal formation of control subjects and patients with Alzheimer's disease. *Neuroscience,* **32**, 701-714.

Rebeck, G.W., Reiter, J.S., Strickland, D.K. and Hyman, B.T. (1993). Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron*, **11**, 575-580.

Richard, F., Helbecque, N., Neuman, E., Guez, D., Levy, R. and Amouyel, P. (1997). ApoE genotyping and response to drug treatment in Alzheimer's disease. *Lancet*, **349**, 539.

Richey, P.L., Siedlak, S.L., Smith, M.A. and Perry, G. (1995). Apolipoprotein E interaction with the neurofibrillary tangles and senile plaques in Alzheimer's disease: implication for disease pathogenesis. *Biochem. Biophys. Res. Commun.*, **208**, 657-663.

Roheim, G.W., Carey, M., Forte, T. and Vega, G.L. (1979). Apolipoproteins in human cerebrospinal fluid. *Proc. Natl. Acad. Sci. USA*, **76**, 4646-4649.

Rousse, I., Beaulieu, S., Rowe, W., Meaney, M.J., Barden, N. and Rochford, J. (1997). Spatial memory in transgenic mice with impaired glucocorticoid receptor function. *NeuroReport,* **8**, 841-845.

Roses, A.D., Delvin, B. and Conneally, P.M. (1995). Measuring the genetic contribution of APOE in late-onset Alzheimer's disease. *Am. J. Hum. Genet.*, **57**, A202.

Russell, R., Jenden, D., Booth, R., Lauretz, S., Rice, K., and Roch, M. (1990). Global in vivo replacement of choline by N-aminodeanol- Testing a hypothesis about progressive degenerative dementia: II. Physiological and behavioral effects. *Pharmacology, Biochemistry and Behavior,* **37**, 811-820.

Samuel, W., Masliah, E., Hill, L.R., Butters, N. and Terry, R. (1994). Hippocampal connectivity and Alzheimer's dementia: effects of synapse loss and tangle frequency in a two-component model. *Neurology,* **44**, 2081-2088.

Saunders, A.M. (1993a). Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology*, **43**, 1467-1472.

Saunders, A.M. (1993b). Apolipoprotein E epsilon 4 allele distributions in late-onset Alzheimer's disease and in other amyloid-forming diseases. *Lancet*, **342**, 710-711.

Schauwecker, P.E., Cogen, J.P., Jiang, T., Cheng, H.W., Collier, T.J. and McNeil, T.H. (1998). Differential regulation of astrocytic mRNAs in the rat striatum after lesions of the cortex or substantia nigra. *Exp. Neurol.*, **149**, 87-96.

Schenk, F. and Morris, R.G.M. (1985). Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Exp. Brain Res.*, **58**, 11-28.

Scheuner, D., et al. (1995). *Soc. Neurosci. Abstr.*, **21**, 1500.

Schmechel, D.E., Saunders, A.M., Strittmatter, W.J., Crain, B.J., Hulette, C.M., Joo, S.H., Pericak-Vance, M.A., Goldgaber, D. and Roses, A. (1993). Increased amyloid beta-peptide deposition in the cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 9649-9653.

Schoenberg, B.S., Kohmen, E., and Ohazaki, H. (1987). Alzheimer's disease and other dementing illness in a defined United States population: incidence rates and clinical features. *Ann. Neurol.*, **22**, 724-729.

Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Holman, K., Tsuda, T., Mar, L., Foncin, J.-F., Bruni, A.C., Montesi, M.P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R.J., Wasco, W., and Da Silva, H.A.R., Haines, J.L., Pericak-Vance, M.A., Tanzi, R.E., Roses, A.D., Fraser, P.E., Rommens, J.M. and St George-Hyslop, P.H. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*, **375**, 754-760.

Slooter, A.J., Tang, M.X., van Duijn, C.M., Stern, Y., Ott, A., Bell, K., Breteler, M.M., Van Broeckhoven, C., Tatemichi, T.K., Tycko, B., Hofman, A. and Mayeux, R. (1997). Apolipoprotein E epsilon 4 and the risk of dementia with stroke: a population-based investigation. *JAMA*, **277**, 818-821.

Snowdon, D.A. Greiner, L.H., Mortimer, J.A., Riley, K.P., Greiner, P.A. and Markesbery, W.R. (1997). Brain infarction and the clinical expression of Alzheimer's disease: the nun study. *JAMA*, **277**, 813-817.

Statistics Canada. (1992). *Age, sex anf marital status.* Ottawa ON: Supply and Services Canada, 1991 Census of Canada, Catalogue number, **93310**.

Statistics Canada. (1990). *Population projections for Canada, provinces and territories 1989-2011*. Ottawa: Statistics Canada.

Steward, O. (1986). *Lesion-induced synapse growth in the hippocampus: in search of cellular and molecular mechanisms*. Plenum.

Steward, O. (1976). Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. *J. comp. Neurol.*, **167**, 285-314.

Stokes, P.E. (1995). The potential role of excessive cortisol induced by HPA hyperfunction in the pathogenesis of depression. *Eur. Neuropsychopharmacol.*, **5**, 77-82.

Stratakis, C.A. and Chrousos, G.P. (1995). Neuroendocrinology and pathophysiology of the stress system. *Ann. NY Acad. Sci.*, **771**, 1-18.

Strittmatter, W.J., Saunders, A., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G.S. and Roses, A.D. (1993). Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. USA*, **90**, 1977-1981.

Strittmatter, W.J., Weisgraber, K.H., Goedert, M., Saunders, A.M., Huang, D., Corder, E.H., Dong, L.M., Jakes, R., Alberts, M.J., Gilbert, J.R. et al. (1994). Hypothesis: microtubule instability and paired helical filament formation in the Alzheimer's disease brain are related to apolipoprotein E genotype. *Exp. Neurol.*, **125**, 163-174.

Swerdlow, N.R., Geyer, M., Braff, D. and Koob, G. (1986). Central dopamine hyperactivity in rats mimics abnormal startle in schizophrenics. *Biological Psychiatry*, **21**, 23-33.

Takahashi, S., Kawarabayasi, Y., Nakai, T., Sakai, J. and Yamamoto, T. (1992). *Proc. Natl. Acad. Sci. USA*, **89**, 9252-9256.

Tardiff, B.E., Newman, M.F., Saunders, A.M., Strittmatter, W.J., Blumenthal, J.A., White, W.D. et al. (1997). Preliminary report of a genetic basis for cognitive decline after cardiac operations. *Ann. Thorac. Surg.*, **64**, 715-720.

Teasdale, G.M., Nicoll, J.A.R., Fiddes, H. and Murray, G. (1997). Association of apoE polymorphism with outcome after head injury. *Lancet*, **350**, 1069-1071.

Terrisse, L., Séguin, D., Bertrand, P., Poirier, J., Milne, R. and Rassart, E. (1999). Modulation of apolipoprotein D and apoE mRNA expression in rat entorhinal cortex lesion. *Brain Res. Mol. Brain,* **70**(1), 25-35.

Terry, R.D. (1996). Basis of structural Alzheimer's disease and some pathogenic concepts. In R. Becker and E Giacobini (Eds.), *Alzheimer's disease: from molecular biology to therapy*, (pp.19-23). Boston: Birkhäuser.

Terry, R.D., Gomatos, N.K. and Weiss, M. (1964). Ultrastructural studies in Alzheimer's presenile dementia. *American Journal of Pathology,* **44**, 269-297.

Tomlinson, B.E., Blessed, G. and Roth, M. (1968). Observations on the brains of non-demented old people. *J. Neurol. Sci.*, **7**, 731-356.

Utermann, G., Langenbeck, U., Beisiegel, W. and Weber, W. (1980). Genetics of the apolipoprotein E system in man. *Am. J. Hum. Genet.*, **32**, 339-347.

Utermann, G., Pruin, N. and Steinmetz, A. (1979). Apolipoprotein E polymorphism in health and disease. *Clin. Genet.*, **15**, 37-62.

Van Broeckhoven, C. (1998). Alzheimer's disease: Identification of genes and genetic risk factors. *Progress in Brain Research,* **117**, 315-325.

Van Broeckhoven, C. (1995). Presenilins and Alzheimer's disease. *Nature Genet.*, **11**, 230-232.

Van Duijn, C.M., de Knijff, P., Cruts, M., Wehnert, A., Havekes, L.M., Hofman, A. and Van Broecknoven, C. (1994). Apolipoprotein E4 allele in a population-based study of early onset Alzheimer's disease. *Nature Genet.*, **7**, 74-78.

Veinbergs, I., Jung, M. W., Young, S.J., Van Uden, E., Groves, P.M. and Masliah, E. (1998). Altered long-term potentiation in the hippocampus of apolipoprotein E-deficient mice. *Neurosci. Lett.*, **249**, 1-4.

Veinbergs, I. and Masliah, E. (1999). Synaptic alterations in apolipoprotein E-knockout mice. *Neuroscience*, **91**, 410-403.

Velhuis, H.D., De Korte, C.C.M.M. and De Korte, E.R. (1985). Glucocorticoids facilitate the retention of acquired immobility during forced swimming. *European Journal of Pharmacology*, **115**, 211-217.

Vogels, O.J.M., Broere, C.A.J., Terlaak, H.J., Ten Donkelaar, H.J., Nieuwenhuys, R. and Schulte, B.P.M. (1990). Cell loss and shrinkage in the nucleus basalis of Meynert complex in Alzheimer's disease. *Neurobiol.*, **11**, 3-13.

Wainer, B.H., Levey, A.I., Mufson, E.J. and Mesulam, M.M. (1984). Cholinergic system in mammalian brain identified with antibodies against choline acetyl-tranferase. *Neurochem. Int.*, **6**, 163-182.

Whitehouse, J.P., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T. and Delon, M.R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science*, **215**, 1237-1239.

Wisniewski, K.E., Dalton, A.J., Crapper-McLachlan, D.R., Wen, G.Y. and Wisniewski, H.M. (1985). Alzheimer's disease in Down's syndrome: clinicopathologic studies. *Neurology*, **35**, 957-961.

Wisniewski, T. and Frangione, B. (1992). Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci. Lett.*, **135**, 235-238.

World Health Organization. (1992). *The ICD-10 Classification of mental and behavioral disorders*. Geneva : World Health Organization.

Wurtman, R.J. (1992). Choline metabolism as a basis for selective vulnerability of cholinergic neurons. *Trends Neurosci.*, **15**, 117-122.

Yankner, B.A., Duffy, L.K. and Kirschner, D.A. (1990). Neurotrophic and neurotoxic effects of (amyloid protein: reversal by tachykinin neuropeptides. *Science*, **250**, 279-282.

Zhou, Y., Elkins, P.D., Howell, L.A., Ryan, D.H. and Harris, R.B.S. (1998). Apolipoprotein-E deficiency results in an altered stress responsiveness in addition to an impaired spatial memory in young mice. *Brain Research*, **788**, 151-159.