2003

Behavioral Aspects of Apolipoprotein E Knockout Mice

Melissa T. Litherland

Eastern Illinois University

This research is a product of the graduate program in Biological Sciences at Eastern Illinois University. Find out more about the program.

Recommended Citation

THESIS REPRODUCTION CERTIFICATE

TO: Graduate Degree Candidates (who have written formal theses)

SUBJECT: Permission to Reproduce Theses

The University Library is receiving a number of request from other institutions asking permission to reproduce dissertations for inclusion in their library holdings. Although no copyright laws are involved, we feel that professional courtesy demands that permission be obtained from the author before we allow these to be copied.

PLEASE SIGN ONE OF THE FOLLOWING STATEMENTS:

Booth Library of Eastern Illinois University has my permission to lend my thesis to a reputable college or university for the purpose of copying it for inclusion in that institution's library or research holdings.

██████████████████████   ██████████████████████

Author's Signature   Date

I respectfully request Booth Library of Eastern Illinois University **NOT** allow my thesis to be reproduced because:

_____

_____

_____

_____   _____

Author's Signature   Date

This form must be submitted in duplicate.

BEHAVIORAL ASPECTS OF APOLIPOPROTEIN E KNOCKOUT MICE

(TITLE)

BY

Melissa T. Litherland

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY

CHARLESTON, ILLINOIS

2003

YEAR

I HEREBY RECOMMEND THAT THIS THESIS BE ACCEPTED AS FULFILLING

THIS PART OF THE GRADUATE DEGREE CITED ABOVE

DATE

DATE

THESIS DIRECTOR

Acknowledgments

Table of Contents

Abstract

Apolipoprotein E (apoE) is a lipid transporting protein that has been shown to play a vital role in nerve repair and remodeling. Previous studies have shown that apoE is highly expressed in human and mouse olfactory bulbs. ApoE deficiency in apolipoprotein E knockout (apoE-KO) mice leads to considerable delay in olfactory nerve repair and deficits in olfactory functioning. Olfactory function is necessary for a number of social behaviors in mice. Loss of olfaction can greatly reduce social behaviors. Since apoE-KO mice display olfactory dysfunction, this deficit may result in alterations in social behavior. Olfactory function was assessed in apoE-KO mice and wild type (WT) mice by using the odor cued taste avoidance test (OCTA). ApoE-KO mice were significantly less effective than WT mice in avoiding the odorant cued tastant solution. In order to determine if olfactory dysfunction disrupted social behavior, we used two standard social behavior tests: 1) pup retrieval test and 2) resident-intruder test. ApoE-KO mice performed poorly on both behavior tests. ApoE-KO mice display maternal behavior deficits such as fewer litters retrieved and lower nest quality scores. Furthermore, apoE-KO mice were less aggressive than WT individuals. These studies suggest that mice rely heavily upon olfactory communication for modification of social behaviors. ApoE is necessary for normal olfactory function in mice and may play a role in regulating social behaviors in mice.

Introduction

Apolipoprotein E

      Apolipoprotein E (apoE) is a plasma protein that participates in the transport of cholesterol and other lipids among many cells in the body. ApoE has a molecular weight of 34-kDa and is a 299 amino acid component of lipoproteins. Regulation of lipid transport and lipid redistribution among target sites is mediated by apoE interaction with the lipoprotein receptors (Mahley, 1988). Cellular uptake and degradation of the lipoproteins is initiated by the receptor-lipoprotein binding. The lipid portion becomes available for intracellular metabolism. ApoE, hence serves as a ligand for the receptor-mediated clearance of lipoproteins from the plasma (Rall et al., 1982).

The apolipoprotein E gene

      The apoE gene is located on chromosome 19 and contains 3597 nucleotides and four exons. ApoE is encoded by a 1163 nucleotide mRNA (Mahley, 1988). Three common isoforms of apoE (apoE2, apoE3, and apoE4) are found in humans. These are the products of three alleles denoted ε2, ε3, and ε4, respectively (Zannis, et al., 1982). The molecular basis of this polymorphism of the apoE gene results from cysteine-arginine interchanges at two positions (112 and 158) in the protein. ApoE3 is the most common isoform and contains cysteine and arginine at positions 112 and 158 respectively (Mahley, 1988). The apoE2 isoform contains cysteine at both positions, while the apoE4 isoform

contains arginine at both positions (Mahley, 1988). Mice have one form of apoE, which is similar to human apoE3 in function (Weisgraber, 1994). These amino acid substitutions result in dramatic biological differences of apoE. ApoE3 is the most common of the apoE alleles; representing approximately 78% of all alleles whereas apoE4 and apoE2 are less common representing 15% and 7%, respectively (Strittmatter and Roses, 1996). The proportion of different apoE alleles varies between racial and ethnic groups.

ApoE synthesis and function

ApoE is primarily synthesized in the liver, but it is also made in significant amounts in the brain (Elshourbaby et al., 1985). A wide variety of cell types are capable of producing apoE and include, oligodendrocytes (Stroll et al., 1989), astrocytes (Pitas, 1987), and macrophages (Mahley, 1988). ApoE has also been implicated in a wide range of physiological processes throughout the body : namely, lipid metabolism, immunoregulation (Cuthbert and Lipsky, 1984), nerve growth, repair, and regeneration in both the central nervous system (CNS) and the peripheral nervous system (PNS) (Snipes et al., 1986; Boyles et al., 1989), steroidogenesis in adrenal cells (Elshourbagy et al., 1985), modulation of intracellular cholesterol utilization (Reyland et al., 1991), and as an activator of hepatic lipase (Ehnholm et al., 1984). Additionally, apoE is believed to participate in the regeneration of peripheral nerves after injury (Boyles et al., 1989) and in the redistribution of lipids during normal development of the nervous system (Pitas et al., 1987).

ApoE in the nervous system

ApoE is the major apolipoprotein found in the brain and cerebrospinal fluid (CSF) (Pitas et al., 1987). ApoE is primarily produced and secreted by astrocytes and microglia (Boyles et al., 1985) While the exact function of apoE in the nervous system eludes researchers, studies have shown that injury in either the CNS or PNS results in an increase in apoE levels (Ignatius et al., 1986). Following peripheral nerve injury in rats, the synthesis of apoE increases by 250- to 350-fold within three weeks (Boyles et al., 1989). Moreover, macrophages synthesize and release apoE following peripheral nerve lesion, accounting for 5% of the total extracellular protein (Skene and Shooter, 1983). It has been proposed that the purpose of this accumulation of apoE is to scavenge cholesterol from the degenerating myelin and recycle it to the growth cones of sprouting axons for membrane biosynthesis (Mahley, 1988). ApoE is the only apolipoprotein in the CNS that is able to interact with lipoprotein receptors (Pitas et al., 1987). Subsequently, apoE and apoE-containing lipoproteins are present within the brain where they can interact with neurons.

Lipoprotein transport by apoE is important for normal functioning of adult neurons. Studies have shown that apoE4 addition to a culture inhibits neurite extension, while apoE3 stimulates neurite outgrowth in transformed murine neuroblastoma (Neuro-2a) cells (Bellosta et al., 1995). Furthermore, apoE-KO mice display disruption of the dendritic cytoskeleton, significant synaptic loss with age, and a reduced recovery following perforant pathway lesioning (Masliah et al., 1995; Masliah et al., 1996; Masliah et al., 1997).

Alzheimer's disease and apoE

The apoE genotype has been associated as a risk factor for AD. Previous studies have shown that there is an increased apoE immunoreactivity present in the brains of patients with AD, Creutzfeld-Jakob disease, and Down's syndrome (Namba et al., 1991). Recent studies also suggest that apoE4 allele may be involved with the onset of Parkinson's disease (Zareparsi et al., 2002). AD is characterized by the presence of senile plaques, neurofibrillary tangles, and widespread loss of neuronal synapses (Honing and Mayeux, 2001). ApoE immunoreactivity is associated with the pathological structures present in AD patient brains including neurofibrillary tangles and neuritic plaques (Namba et al., 1991; Strittmatter et al., 1993).

There are two types of AD: familial Alzheimer's disease (FAD) and late-onset sporadic. FAD represents approximately 5 % of AD patients, usually occurring before the age of 65, while late-onset AD accounts for a majority of AD cases and occurs after the age of 60 (Digivanna et al., 2000). The apoE gene is associated with variations in the age of onset and risk for Alzheimer's disease.

ApoE4 is associated with late-onset familial and sporadic AD (Corder et al., 1993; Rocchi et al., 2003). The apoE4 allele increases the probability of AD at an earlier age, while apoE3 and apoE2 alleles decrease the probability of AD and increase the age of onset (Strittmatter and Roses, 1996). Moreover, the apoE4 gene dose is also a major risk factor for late-onset AD (Corder et al., 1993). The frequency of the apoE4 allele is over-represented in late-onset AD (52 % of the subjects) versus controls (16 %). The risk of AD in individuals

homozygous for the apoE4 allele is over five times that of homozygous apoE3 individuals (Corder et al., 1993).

ApoE, AD, and cognition

One of the hallmark features and diagnosis of AD involves a progressive decline in cognitive function, especially related to memory and executive function (Albert, 1996). Difficulty with acquisition of new information is the first and most common symptom to emerge in AD patients (Albert, 1996). The most frequent cause of senile dementia is AD (Price et al., 1998). Many AD patients experience a decline in cognitive performance including difficulties with memory, learning, recall accuracy, problem solving, language, calculation, visuospatial perceptions, judgment, and behavioral problems such as irritability, agitation, verbal and physical aggression, wandering, and disinhibition (Albert, 1996; Price et al., 1998; Honing and Mayeux, 2001). With the decline of cognitive function, the activities of daily living become gradually impaired. Psychopathological features such as delusions and hallucinations are pertinent characteristics of AD and likewise present serious problems for caregivers of demented AD patients (Honing and Mayeux, 2001; Holtzer et al., 2003). Wandering/agitation and delusions are common problems throughout the course of AD and increase with time (Honing and Mayeux, 2001; Holtzer et al., 2003). Physical aggression is less prevalent appearing in only the more severely impaired patients, and increases as a function of cognitive decline (Holtzer et al., 2003). While physical aggression occurs infrequently in early stages of AD, it appears to persist only in late stages

(Holtzer et al., 2003). Psychopathological aspects of AD are important to determine and predict the stage of the disease so that proper treatment can be administered. Furthermore, since there is no cure for AD and with the rapid decline of cognitive function, many AD patients rely on caregivers for support of everyday activities. Caregivers also need to be educated on the variety of behavioral dysfunction that can manifest over the course of the disease.

Many animal studies involving the apoE gene have demonstrated a decline in cognition and suggest that apoE is involved in learning and memory (Masliah et al., 1997; Oitzl et al., 1997; Lancashire et al., 1998; Raber et al., 1998). ApoE-KO mice exhibit severe learning deficits in water maze tasks (Gordon et al., 1995; Oitzl et al., 1997; Zhou et al., 1998). Raber, et al (1998) showed that that female apoE-KO and apo-E4 mice have impairments in spatial learning and the degree of impairment increases with age. Additionally, Grootendorst et al (*In Press*). demonstrated that aged female apoE-KO mice had impaired water maze performance than WT individuals. ApoE-KO mice also display impaired long-term potentiation (Krzywkowski et al., 1999). These studies strongly suggest that the apoE gene is involved with learning and memory.

ApoE, AD, and olfaction

There has been an increased interest in olfactory dysfunction due to the realization that anosmia is a common feature of AD dementia. Odorant recognition and odor identification losses are prominent in the early stages of AD

and olfactory dysfunction in AD patients has been consistently reported in numerous studies (Warner et al., 1986; Mesholam et al., 1998; Nordin and Murphy, 1998; Murphy, 1999; Schiffman et al., 2002). AD patients with moderate dementia also have deficits in odor identification, odor threshold, and odor memory (Doty et al., 1987; Murphy et al., 1990; Serby et al., 1991; Nordin and Murphy, 1998). Likewise, AD patients also show neuropathological changes in parts of the brain associated with olfactory processing (Talamo et al., 1989; Struble and Clark, 1992; Davies et al., 1993; Braak and Braak, 1994; Braak and Braak, 1997). One of the first signs of AD is olfactory deficit and has been proposed as a predictor of AD (Morgan et al, 1995; Nordin and Murphy, 1996; Bacon et al., 1998; Graves et al., 1999; Devanand et al., 2000; Morgan and Murphy, 2002).

In the early stages of AD, patients have a deficit in olfactory processing, and AD individuals have deficits in odor recognition when compared to elderly controls (Hughes et al., 2002). Christen-Zaech and others (2003) have recently demonstrated that there is a close relationship between the olfactory and cortical degenerative changes, indicating that the olfactory bulb is on of the earliest events in the degenerative process of the CNS in AD. Thus, olfactory dysfunction prior to cognitive impairment suggests that well-designed neuropsychological testing for olfactory memory and discrimination may provide an accurate preclinical diagnoses of AD (Weiss, 2003).

Further studies have also indicated that apoE4 allele status is associated with olfactory dysfunction (Bacon et al., 1998; Murphy et al., 1998; Graves et al.,

1999). A number of authors have demonstrated that normal elderly persons with one apoE4 allele showed significantly poorer odor identification than those who lacked the apoE4 allele (Murphy et al.,1998; Wetter and Murphy, 2001). Additionally, individuals who had mild cognitive impairment and also had the apoE4 allele displayed poorer odor threshold than those without the apoE4 allele (Bacon et al., 1998; Murphy, 1999) and had a decrease in odor identification ability (Wang et al., 2002). ApoE4 positive individuals exhibited delays in processing olfactory information when compared to individuals who were apoE4 negative (Wetter and Murphy, 2001). Olfactory dysfunction is frequent and severe in AD patients and in individuals with anosmia who have at least one apoE4 allele have a 5-fold increased risk of AD (Hawks, 2003).

In previously reported studies, apoE is known to be expressed at high levels in human and mouse olfactory bulbs, especially in the olfactory nerve layer and around the glomeruli (Struble et al., 1999). Additional studies have also shown that apoE deficiency in apoE-KO mice leads to considerable delay in olfactory nerve repair (Nathan et al., 2000) and deficits in olfactory functioning (Nathan et al, *In press*). ApoE-KO mice performed poorly on three olfaction tests (buried food pellet, odor choice test, and the odor cued taste avoidance test), as compared to WT mice (Nathan et al, *In press*). Thus, apoE is required for normal functioning of the olfactory system. These data indicate that apoE deficiency in apoE-KO mice leads to olfactory deficits, which suggests that apoE may play an important role in the olfactory system. Furthermore, these data suggest that apoE is associated with the continuous degeneration and regeneration processes that

occur in the olfactory nerve, thus resulting in decreased olfactory function and potential consequences on social behavior.

Olfaction and maternal behavior

Olfactory function is important in many behavioral processes including foraging, mate selection, sexual behavior, territorial formation, and maternal behaviors (Doty, 1986). Adult mice are able to discriminate between their own and alien offspring and this discrimination seems to be based upon olfactory and gustatory cues rather than auditory cues (Ostermeyer and Elwood, 1982). Several brain areas in rats that may inhibit maternal behavior have been identified (Sheehan, 2000). The primary olfactory and vomeronasal systems have been shown to mediate avoidance behavior in virgin female rats exposed to pup odor cues (Flemming, 1998). In mice, maternal care is impaired in anosmic individuals. Gandelman et al (1971) found that removal of the olfactory bulb in both lactating and virgin mice results in consistent and severe deficits in maternal behavior. Female mice bulbectomized during gestation fail to build nests or display maternal care (Zarrow et al., 1971). Vandenbergh (1973) showed that the sense of smell is essential for normal ovarian rhythm and that bulbectomy and anosmia (via $ZnSO_4$) both produce disruption in nest building and maternal care. Likewise, bilateral removal of the olfactory bulbs produced deficits in maternal behavior in primiparous females rats, while multiparous female maternal behavior was unaffected by bulbectomy (Schwartz, 1976). In contrast, removal of the vomeronasal organ (accessory olfactory system) does not

interfere with the expression of maternal processes in the mouse or rat (Lepri et al.,1985). These studies suggest that the olfactory bulb is important for maternal behavior in mice, and olfactory dysfunction will lead to maternal behavior deficits.

In addition to chemosensory regulation of maternal behavior in mice, hormonal influences on maternal behavior have also been observed. For instance, prolactin has been shown to trigger maternal behaviors in rats and exposure to prolactin during pregnancy may initiate the immediate onset of maternal behavior at parturition (Bridges, 1985; Bridges, 1990). Mice carrying the null mutation of the prolactin receptor gene (PRLR) also result in a deficiency in pup-induced maternal care (Lucas, 1998). Shingo et al. (2003) reported that pregnancy stimulated neurogenesis in the adult female forebrain subventricular zone is mediated by prolactin. Moreover, these progenitors migrate to produce new olfactory interneurons (Shingo, 2003). Increasing the amount of new olfactory interneurons by pregnancy or prolactin can have significant functional effects on maternal behavior (Shingo, 2003). Prolactin, which is produced in high levels just prior to parturition and subsequently during lactation, is believed to stimulate the establishment of maternal care and stimulate the immediate onset of maternal behavior at parturition (Bridges et al, 1985). On the other hand, maintenance of maternal behavior appears to be related to the cues given off by the offspring.

Olfaction and aggression

Fighting behavior in rats and mice is closely related to olfaction. The combination of smells from a male conspecific triggers an offense mechanism in the territory owner (Adams, 1976). To study the olfactory influences on aggression, researchers have used a variety techniques that suppress the sense of smell including bilateral bulbectomy, lateral olfactory tract lesions, and intranasal $ZnSO_4$ (Brain and Benton, 1983). Chronic or acute induction of $ZnSO_4$ causes anosmia and eliminates social aggression (Benton et al., 1979; Brain and Al-Maliki, 1978). Furthermore, olfactory loss has been found to reduce aggression in both feral and laboratory rats, golden hamsters, and gerbils (Alberts and Galef, 1973; Devor and Murphy, 1973; Alberts, 1974; Luciano and Lore, 1975; Flannelly and Thor, 1976). Similarly, olfactory bulbectomized mice elicit attack behavior from normal males, and would never initiate an attack themselves (Denenberg et al, 1973). Male conspecific odors also appear to be able to elicit aggressive acts in many mammals (Bronson, 1971; Brain, 1981).

Olfactory signals are involved in most aspects of mammalian social communication. There are two groups of social odors found in mammals: *identifier odors*- produced by the body's normal metabolic processes and are stable for long periods of time; *emotive odors*- produced or released only in special circumstances (Donat et al., 1994). The odor of male mice has functionally distinct components. One component is of general nature and elicits attacks from other males, while the other component is specific to that individual, allowing for identification by other members of its species (Archer, 1968; Donat et

al., 1994). The production of pheromones, which stimulates aggression and causes aversion in other males, has been shown to be androgen dependent (Mugford and Nowell, 1970; Brain and Evans, 1974; Donat et al., 1994). An individual male's odor contains information about its individual identity as well as dominance status. Dominant and subordinate animals are clearly distinguished by olfactory cues in mice (Brown, 1979; Parmigiani et al., 1982).

Anosmia affects both aggressive and defensive agnostic behavior in mice (Donat et al., 1994). Surgical removal of the olfactory bulb abolishes aggressive behavior (Ropartz, 1968; Bean, 1982). Perfusion of the olfactory epithelium with $ZnSO_4$ solution damages the olfactory epithelium, though not all receptors are necessarily destroyed by the treatment and there is usually some regeneration (Donat et al., 1994). After anosmia treatment there was a significant reduction in the number of attacks observed in aggressive males, which was also accompanied by a significant increase in soc al investigation (Donat et al., 1994). Thus, these results show the importance of olfactory signals in the modulation of aggression in mice. Prior assessment of opponents in scent-marking can reduce the energetic costs and risk of injury in agnostic encounters (Donat et al., 1994). Olfactory signals are means of two-way communication. They provide the opponent with an assessment of the quality of the donor, as well as give important information to the recipient which will lead to the decision regarding the appropriate behavioral strategy to utilize (Payne et al., 1992; Donat et al., 1994).

Resident-Intruder Paradigm

Fighting and threat behavior may be employed for a variety of reasons including: subduing prey species, predator avoidance, defending a territory, obtaining a potential mate, advancing one's status in social hierarchy, and defending young or nesting site (Brain, 1981). There are several types of aggression classifications and for this purpose only intermale aggression will be addressed. Intermale aggression is defined as fighting occurring between two male conspecifics which have not become habituated to each other (Brain, 1981). One technique used to study intermale aggression involves isolation-induced aggression tests (resident-intruder paradigm). Isolation-induced aggression tests have been used as a tool in the study of molecular mechanisms underlying aggressive behavior (Simler et al., 1980; Maxon, 1992). The resident-intruder paradigm is based upon the establishment of a territory by a male and its defense against unfamiliar male intruders (Martinez et al., 1998). The test consists of introducing an intruder into the home cage of a resident male and the former being defeated by the latter (Martinez et al., 1998). The following manipulations are widely used to ensure that this scenario will occur: the resident is usually of a more aggressive strain than the intruder, is heavier, paired with a female or isolated, or has previous experience of victory (Martinez et al., 1998). There are two advantages for this the resident-intruder test for genetic analysis. First, the aggressive behavior is polarized; secondly, this test has been widely used in pharmacological and endocrinological research on agnostic behaviors (Maxon, 1992).

In the resident-intruder test, resident males are housed individually for four weeks and then a group housed intruder is introduced into the resident's home cage (Rodgers, 1981). Typically, the intruder is rendered anosmic via nasal irrigation of either $ZnSO_4$ or a triton solution (Brain, 1981). Anosmic males display defensive or submissive behaviors and have been shown to elicit fighting in isolated males while never initiating aggression themselves (Ropartz, 1968; Brain et al., 1981; Navarro et al., 2000). The number of attacks, defense, and offensive behaviors are typically observed within this test.

Intermale fighting in mice consists of a well-defined sequence of acts and postures (Grant and Mackintosh, 1963). A number of authors have postulated a sequence of behaviors that lead up to aggressive behavior in rats and mice, suggesting that investigative (sniffing) or exploratory (rearing) behaviors usually preludes any type of aggressive behavior in rodents (Brain and Poole, 1974; Miczek and Krsiak 1981). These initial behaviors are a way for the resident to assess the intruder prior to engaging in fighting behavior. Maintenance behavior (self-grooming) is also observed within both resident and the intruder, especially following an aggressive bout (Brain and Nowell, 1970).

Goal and Hypotheses

Experiment I: Olfactory function

This study was conducted to determine how apoE-KO and apoE4 mice would perform in the olfactory-cued taste avoidance (OCTA) test. We hypothesized that both apoE-KO and apoE4 mice would have olfactory deficits when compared to WT individuals since apoE is required for normal olfactory function.

Experiment II: Maternal behavior

In this study we looked at the functional aspect of apoE on maternal behavior. Since maternal behavior is associated with olfactory cues emitted by the offspring, we wanted to determine if there were any deficits in maternal care in apoE-KO female mice in comparison to WT controls. We predicted that apoE-KO female mice would have maternal deficits when compared to WT controls.

Experiment III: Aggressive behavior

For this experiment we observed the functional aspects of apoE in regards to aggressive behavior. Processing of pertinent olfactory cues is important in determining social status among many mammalian species, and thus we wanted to determine if the olfactory dysfunction observed in apoE-KO mice would have any effects on social behavior. We hypothesized that apoE-KO would be less aggressive than WT controls.

EXPERIMENT I: OLFACTORY FUNCTION IN APOLIPOPROTEIN E

KNOCKOUT MICE

Methods

Animals

Sixteen male WT (C57BL/6J) mice, eleven apoE-KO mice (C57BL/6J-Apoe ^{tm1Unc}), ten apoE-KO littermates (KOL) (C57BL/6J-Apoe), and nine apoE4 (C57BL/6J-Apoe), were obtained from The Jackson Laboratory (Bar Harbor, ME). We looked at both pure breeding apoE-KO mice in comparison to apoE-KOL (apoE-KO littermates of apoE4 transgenic breeder pairs) to determine if there were behavioral differences. Pure breeding knockout mice were defined as apoE-KO mice born to homozygous apoE-KO parents, while apoE-KOL mice are defined as offspring of a heterozygous apoE4 (apoE-KO/apoE4) parent paired with a apoE-KO (apoE-KO/apoE-KO) parent. Male mice, ages two to four months old, were used in this experiment. The mice were housed in a sound attenuated room, with a light cycle from 08:00- 18:00 and a constant temperature was maintained at 22°C. Each mouse was tested at the same time of day to avoid diurnal variation. Each mouse was housed individually and had access to food *ad libitum*. A water deprivation schedule (0.4ml/day) was initiated twenty-four hours prior to testing, and continued throughout the 6-day test schedule.

Odor Cued Taste Avoidance

The odor cued taste avoidance (OCTA) test was a modification of previously published procedures (Darling & Slotnick, 1994). The test chamber

consisted of a metal-floored box (20 cm x 30 cm x 12 cm) with a PVC tube attached near the base of the box. A stainless steel drinking nozzle fitted with a 10 ml syringe was placed inside the PVC tube such that the nozzle could be placed at variable distances from the opening. One syringe contained tap water (S+) and the other syringe (S-) contained either a 1% vanillin + 0.05% quinine (QHCL) solution or 0.001% vanillin: + 0.05 % QHCL. A touch circuit was established were the nozzles of the syringes were connected to a Powerlab (ADI Instruments) by an electrical cable (CB Sciences), while the metal floor was connected to the power lab via a cable. The aluminum foil flooring of the test chamber was changed between each individual mouse trial. The computerized touch circuit precisely measured the latency of first lick contact and the latency was recorded via Chart software (ADI Instruments).

Behavioral testing

Each experiment consisted of 2 periods: 1) sampling period of 30 seconds in which the nozzle was recessed 1.5 cm into the PVC tube, so that the mice could sniff the odor, but not make contact with the nozzle and 2) a drinking period of 60 seconds in which the nozzle was moved forward so that the mice could contact the drinking nozzle. A positive contact score was awarded upon any contact with the nozzle. A total of 6 trials per day were conducted. Trials were separated by a 60 second rest period, where the mouse was removed from the test chamber and placed in a separate cage without access to food or water. At

the commencement of the 6th trial for the day the mouse was returned to its home cage and it was given its ration of water (0.4ml) a ' ʳ ' *ad libitum*.

The trial consisted of 6 consecutive days where each mouse received 6 trials/day. Days 1-3 were a training period, where mice were trained to drink tap water from the nozzle. Days 4 and 5 the animals were offered three trials with the S+ solution (tap water) and three trials with the S- solution, which consisted of 1% vanillin plus 0.05% quinine monohydrocloride dihydrate (QHCL, Acros Organics). On day 6, the S- concentration of vanillin was reduced from 1% to 0.001%, while the QHCL concentration remained at 0.05%. The order of S+ and S- was randomized such that each individual received the same random order within a day but the sequence of S+ and S- differed between the days. Vanillin was used as an odorant because it was an olfactory nerve stimulant, and thus the experimenter was assured that odor cued taste avoidance was solely due to the ability to detect an odor, rather than a trigeminal reflex.

Statistical analysis

All data analyses were performed using SPSS statistical software. Repeated measures ANOVA was used to compare the average latency to lick the water nozzle for day, genotype, and day x genotype interaction for all six of the S+ trials. Mean treatment differences between S- and S+ latencies, for days 4, 5, and 6, were analyzed by using repeated measures ANOVA and taking into account the day, genotype, and day x genotype interaction. Since data did not follow assumptions for days 1-6 S+ only, Univariate Greenhouse-Geisser was

used to test the within subject test (Table 2). Univariate Sphericity Assumed was used to test the within subject effects for the mean treatment differences for days 4-6 (Table 2). Post hoc tests were conducted using least significant differences (LSD) test.

Results

Odor cued taste avoidance

In order to determine whether WT, apoE-KO, apoE-KOL, and apoE4 differed in their ability to perform the OCTA test, we used repeated measures ANOVA, with day and genotypes as factors on the water (S+ only) results for days 1-6 (Table 2). WT, apoE-KO, apoE-KOL, and apoE4 mice did not differ in their S+ latencies during the trial ($F_{3,42}$ = 0.79, p =0.50) (Fig 1). However, S+ latencies did decrease over the days for all 3 genotypes ($F_{5,210}$ = 88.43, p < 0.05) (Fig. 1).

To investigate how WT, apoE-KO, apoE-KOL, and apoE4 mice responded to the vanillin-cued quinine, we used repeated measures ANOVA, to analyze the mean treatment differences between S- and S+ (Table 2). ApoE-KO, apoE-KOL, and apoE4 mice had significantly smaller mean treatment differences than WT mice ($F_{3,42}$= 4.24, p = 0.01) (Fig 2). For the S- contact latencies, no significant interaction existed between genotype and day ($F_{6,84}$ = 0.70, p= 0.64), but there was significant difference in latencies for day ($F_{2,84}$= 10.66, p < 0.05) (Fig 3). Thus, apoE-KO, apoE-KOL, and apoE4 genotypes showed significant

differences in their ability to detect S- when compared to WT individuals. Furthermore, there were no differences observed between apoE-KO and apoE-KOL during the trial (Figs 1-3), suggesting that these two genotypes could be grouped together in future studies regardless of how the apoE-KO mice were derived.

Discussion

The results from this study indicate that apoE deficiency in apoE-KO and apoE-KOL mice, as well as human expressed apoE4 in mice, leads to a deficit in olfactory function. ApoE-KO, apoE-KOL, and apoE4 mice performed poorly on the odor-cued taste avoidance task, and were unable to detect a difference in tap water verses an odorant-cued with a bitter tasting solution. While these results can be interpreted as olfactory dysfunction in apoE-KO, apoE-KOL, and apoE4 mice, possibly other alternative interpretations need to be addressed. Motivational properties of the mice may influence the performance on thirst tests used in this study. There was no significant difference in latency between WT, apoE-KO, apoE-KOL, and apoE4, to drink water during the OCTA test. In another study, Raber, et al (2000), found that apoE-KO mice did not differ significantly in water or food intake from WT mice. Together, these studies suggest that lack of motivation or thirst is not a primary cause for the olfactory dysfunction found in apoE-KO, apoE-KOL, and apoE4 mice.

Another possible explanation for the results was an inability to learn a task, which could have attributed to the poor performance in the OCTA test.

However, we did not observe a cognitive defect where the latencies for S+ in WT, apoE-KO, apoE-KOL, and apoE4 mice all decreased over the 6-day trial, which suggests that all genotypes did not significantly differ in their ability to learn the OCTA task. These results revealed that apoE-KO, apoE-KOL, and apoE4 mice exhibit olfactory dysfunction, which suggests that apoE likely plays an important role in the olfactory system.

While the function of apoE in the olfactory system remain unclear, recent studies had demonstrated that apoE is found in high levels within the olfactory bulb (Struble, et al., 1999). Other studies have examined the effects of the individual apoE isoforms in humans. The results revealed that AD patients who were positive for the apoE4 allele were impaired in odor identification (Murphy, et al., 1998). These studies suggest that apoE4 may not support the continuous neuronal remodeling in the olfactory system as efficiently as the other apoE isoforms, which in turn may lead to olfactory dysfunction.

Further research comparing the human expressed apoE3 and apoE4 mouse genotypes olfactory ability, using the OCTA test, need to conducted. Other social behavioral studies also need to be addressed, such as aggression and maternal behavior, in order to determine the behavioral consequences of human apoE expression in mice. This may help to clarify the role of apoE in dementing disorders such as AD.

EXPERIMENT II: APOLIPOPROTEIN E AND MATERNAL BEHAIVOR

Materials and methods

Animals

The apoE KO (C57BL/6J-Apoe ^{tm1Unc}) and wild-type (WT) C57BL/6J mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME) and housed and breed within the animal care facility at Eastern Illinois University *(Charleston, IL 61920).* Mice were housed in a sound attenuated room under constant temperature (22 °C), light from 07:00 h to 19:00 h, and access to food and water ad libitum. All animals were housed in clear Plexiglas cages. In order to control for possible effects of previous breeding experience, all females were tested with first their litter. Sexually mature female mice, 2-6-months-old, were used in this experiment. Retrieval tests (n=48) and nest scores were conducted between 1300h and 1800h to minimize any diurnal variation in behaviors.

Breeding

Females were placed 3-5 animals per cage and a sexually mature male of the same genotype was introduced into the cage. Obviously pregnant females were removed from group cages 4-7days prior to parturition and placed in an individual cage. Total offspring and sex of each pup was noted for a subset of litters (WT n=12; KO n=12) at 21 days (i.e. prior to separation).

Nesting

Each cage was provided with shredded wood bedding and one square of Nestlet (virgin cotton, Ancare) for nesting material. Typically, pregnant females shred all of the bedding and Nestlet and construct a compact nest with walls and a ceiling (Slotnick & Nigrosh, 1975; Leckman & Herman, 2002). Nests were rated using a scale similar to that of Slotnick and Nigrosh (1975). The nests were scored on a 0-4 point scale: 0 = no nest constructed with shredded or un-shredded Nestlet scattered around the cage; 1 = shredded Nestlet, but no obvious nest constructed; 2 = most Nestlet shredded in small cup-like nest with low walls; 3 = most Nestlet shredded in a cup-like nest with high walls; 4 = all Nestlet shredded in a ball shaped nest with high walls and a ceiling (Slotnick & Nigrosh, 1975). Scores were taken one day prior to testing (D-1), day of testing (D 0), and one day after testing (D+1). On retrieval test day (D 0) scores were taken prior to pup removal.

Retrieving test

The retrieval test and behavioral definitions were similar to tests conducted by Slotnick and Nigrosh (1975) and Pardon, et al (2000). Observations were recorded 24 ± 10 hours after parturition. The experimenter wore latex gloves for each trial to prevent extraneous transmission of human odors onto the pups. To begin the test, the dam was removed and placed in holding cage. The pups were removed, weighed, and the presence or absence of milk bands in the abdomen was noted. The pups were then placed together

approximately 15cm away from the nest. The metal cage lid was replaced with a clear Plexiglas lid to permit an unobstructed view of the cage. Observations began once the observer had placed the female back into the nest side of the cage. The dam's behavior was recorded for 15 minutes using a video recorder (Sony, Hi8) placed approximately 60 cm above the cage. After testing, the Plexiglas lid was replaced with the original metal lid. If any pups remained outside of the nest they were returned to the nest at the end of the testing period.

The videos were scored later for the following behaviors: retrieving latency (number of seconds from the beginning of the test till first pup was picked up or carried) retrieval time (time to gather all pups into nest from the time the first pup was picked up) carry time (duration dam spent carrying pups) drops (frequency of times dam dropped pups outside nest) pup sniff (pups contacted with nose with no oral contact) rearing (raise up on hind legs) nest re-build (duration spent digging, moving, or shredding bedding around and toward nest).

Statistical analysis

All behaviors were analyzed using SPSS (ver. 11.0). Nonparametric tests were used because data did not conform to parametric assumptions; these tests took tied values into account when appropriate. Statistical significance was accepted at $P < 0.05$. Data are presented as mean $\pm$ S.E.M.

Ethical considerations

All trials were conducted according to the guidelines governing animal research at Eastern Illinois University. Females who had difficulty in labor and appeared to be in distress were monitored closely. If they did not appear to be improving, they were humanely euthanized in accordance with the animal care and use guidelines. Additionally, pups left outside the nest at the end of the test were placed back into the nest with their siblings.

Results

Pregnancy, litter size, and pup care

There were no significant differences between WT and apoE-KO mice in the number of pups at the time of testing (WT= 6.63 ± 0.39, N= 24; apoE-KO= 6.75 ± 0.43, N= 24; Mann-Whitney, U=279, z = 0.19, *p* = 0.85) or mean pup weight at test (WT= 1.34 g ± 0.03, N=24; apoE-KO = 1.32 g ± 0.04, N=24; Mann-Whitney, U=260.5, z = 0.57, *p* = 0.57). Furthermore, there were no significant differences between WT and apoE-KO in the total number of pups at weaning (WT= 5.83 ± 0.46, N=12; apoE-KO= 6.75 ± 0.57, N= 12; Mann-Whitney; U= 51.5, z = 1.22, *p* = 0.22), or the sex ratio at weaning (WT = 0.54 ± 0.08, N= 12; apoE-KO= 0.52 ± 0.05, N=12; Mann-Whitney, U= 64, z = 0.47, *p* = 0.64). All pups in both genotypes did have milk bands present prior to testing.

Nesting behavior

On average, apoE-KO mice had lower quality nest scores compared to WT mice for day on all three days (Fig. 4). Nest scores were significantly higher for D-1 (apoE-KO = 2.71 ± 0.15, N= 24; WT= 3.17 ± 0.13, N=24; Mann-Whitney, U= 198.5, z = 2.11, *p* = 0.04) and D +1 (apoE-KO = 3.09 ± 0.17, N= 24; WT= 3.71 ± 0.10, N=24; Mann-Whitney, U = 152, z = 2.75, *p* = 0.006), but not D0 (apoE-KO = 2.71 ± 0.15, N= 24; WT= 3.17 ± 0.13, N=24; Mann-Whitney, U= 208, z = 1.65, *p* = 0.06) (Fig 4). Nests for WT individuals were generally better constructed than apoE-KO mice, with high walled nests and ceilings which covered the pups completely. Both genotypes, however, showed an increase in nest scores over the 3-day scoring period (Fig 4). During the retrieval test there was no difference in duration of nest rebuilding behavior (Table 3).

Retrieval test

WT mice retrieved a higher percentage of pups at the end of the trial than apoE-KO mice (WT= 97.40 +/- 2.60, N=24; apoE-KO= 84.97 +/- 5.09, N=24; Mann-Whitney, U=194.5, z= 2.72, *p*= 0.0066) (Fig 5). Indeed, WT individuals were more likely to pick up their entire litter; 23 / 24 WT individuals retrieved all pups compared to 15 / 24 apoE-KO dams(χ^2 = 8.08, df = 1, *p* = 0.004). In order to take into account variation in litter size, we standardized by dividing by the number of pups for some behaviors. There were no significant differences in carry time/pup, retrieval time/pup, total # of pup sniffs/pup, total # drops/pup, latency to pick up first pup, or # sniffs before first pup pick up (Table 3). There

was a non-significant trend for apoE-KO mice to have more pup sniffs than WT (Table 3).

Discussion

Pup retrieval

Previous researchers have demonstrated that pup removal studies are an accurate marker of maternal care in mice (Carlier, 1982; Pardon, 2000) and suggest a strong correlation between maternal care and pup survival (Cohen-Salmon, 1985). If pups are not retrieved quickly when separated from the dam, they may suffer consequences such as a decrease in body temperature or an increased chance of predation. Survival of rodent offspring is therefore dependent upon the initiation of a specific set of maternal behaviors. (Leckman & Herman, 2002). Dams must be able to recognize their own pups, be able to protect them from the elements and predation, and be able to account for all of their offspring. Somatosensory and maternal olfactory processing is necessary in order for the dam to retain responsiveness to pups over long periods of separation (Flemming et al., 1999). A number of studies have demonstrated that prolactin is the initial trigger for maternal behavior (Bridges et al, 1985; Lucas et al, 1998), while pup cues (namely olfactory and auditory cues emitted by offpring) are important for maintaining maternal behavior (Rosenblatt and Lehrman, 1963; Harper, 1981; Rosenblatt, 1990)

In our study, apoE-KO mice when compared to WT mice were less likely to retrieve and carry all of their pups back to their nest. Three possible explanations exist for this pattern. First, WT mice may have been better at carrying the pups and were therefore more likely to complete the task in the time allotted. No difference existed between average pup weight, carry time/pup, or retrieval time/pup which suggests that the apoE-KO mice were just as capable as WT mice in carrying their pups. Additionally, there were no difference in the number of drops per pup for WT and apoE-KO mice, which indicates that both genotypes drop a similar proportion of pups per trial. Thus, apoE-KO mice are just as able to carry pups as WT mice and can be ruled out as an explanation.

Second, apoE-KO mice may have been less likely to investigate the pups outside of the nest when first introduced into the home cage than WT mice. We can reject this possibility as no difference was shown between the latency of first sniff (first pup contact) between WT and apoE-KO. In addition, there was no difference in rearing behavior, which has previously been described as an exploratory motivational behavior (Mitchell, 1994). Thus, at least at the initiation of the trial, WT and apoE-KO mice were both physically and motivationally able to explore their environment and to determine that their pups had been moved from their previous location in the nest.

Third, apoE-KO mice may have chemosensory deficits that impair maternal behavior. Olfaction is important in maternal behavior in mice. Studies have suggested that olfactory cues emitted from the offspring stimulate the area in hypothalamus responsible for maternal behavior expression and without the

olfactory stimulation, maternal behavior may not be evoked (Gandelman et al., 1971; Gandelman et al., 1972) or maintained. Additionally, females who have undergone olfactory bulbectomy after parturition only show a reduction in maternal behavior and bulbectomy does not completely eliminate maternal behavior (Cowley and Cooper, 1977). Further studies show that If new mothers are rendered anosmic and have no taste cues due to application of anesthetic onto either the mouth or ventrum, then pups are not retrieved or licked (Flemming et al, 1999). Lepri et al. demonstrated that normal maternal behavior in mice is expressed even in the absence of an intact vomeronasal organs (Lepri et al., 1985). Other researchers also have suggested that olfactory stimuli from pups was more important than ultrasonic emissions from the pups and thus recognition is dependent upon olfactory cues (Ostermeyer and Elwood, 1982).

Olfactory function is needed in order to retrieve pups. A female must be able to use her senses to locate the pups either initially or after a pup has been dropped. Olfaction appears to be the key sense utilized in retrieval ability. Since we did not see a difference in latency of initial pup sniff, we suggest that apoE-KO mice were able to determine the location of the group of pups in the cage. However, we suggest that apoE-KO females were unable to locate the pups once dropped away from the group and outside the nest. Considering that apoE-KO and WT mice dropped the same proportion of pups as WT mice it is not that apoE-KO mice had problems carrying the mice back to the nest, but rather an inability to find the scattered pup or pups after dropping them. We suggest that this is due to their inability to detect the pup odor of the dropped pups. When the

pups are together in a group, their odor is stronger than a scattered pup's odor. Thus we do not see a difference in latency to first sniff the pups, but we do see a difference in retrieval behavior. Nathan et al. has previously shown that apoE-KO mice have olfactory dysfunction when compared to WT individuals (Nathan et al., 2003) and apoE deficiency in apoE-KO mice leads to delay in olfactory nerve repair. Since apoE-KO mice have only impaired olfaction rather than complete olfactory destruction, their behavior can be compared to mice that have had slight olfactory impairments who also display maternal behavior deficits.

Nest building

Nest construction is also important in protecting and rearing healthy offspring. Previous studies in female mice have shown that with olfactory bulb removal (Gandelman et al, 1971) or sepal lesions (Slotnick & Nigrosh, 1975) mice will exhibit profound deficits in nest construction and maternal care. In our study we found that apoE-KO mice displayed significantly lower nest quality scores than WT mice. This difference cannot be due to apoE-KO females being physically incapable of constructing nests, because both genotypes did construct nests and both genotypes showed an increase in nest scores over the 3-day observation period. We attribute the decrease in nest building quality in apoE-KO mice to olfactory dysfunction. If the female is not receiving or has a decrease in the ability to receive cues from her pups then maternal behavior, namely nest buliding, will be decreased as well. We believe that prolactin, the trigger for maternal and nest building behavior, is not what is causing the decrease in

maternal care since females were observed to be lactating and the pups had milk bands present. Prolactin, which is produced in high levels just prior to parturition and subsequently during lactation, is believed to stimulate the establishment of maternal care and stimulate the immediate onset of maternal behavior at parturition (Bridges et al, 1985). Other studies have shown that prolactin also induces neurogenesis within the olfactory bulb, which has important implications in mating and offspring recognition (Shingo et al, 2003). Thus, while the trigger for maternal care is not likely to be different in our two genotypes, maintaining the behavior appears to be the likely cause. If a female has an olfactory deficit, her ability to process olfactory information will mostly likely be decreased as well. The olfactory deficit that has been previously reported in apoE-KO mice will inevitably cause problems in the ability to maintain maternal care when compared to WT individuals.

Overall, apoE deficiency results in olfactory deficits in apoE-KO mice and those deficits in turn have consequences on maternal behavior in apoE-KO mice. However, the olfactory deficits exhibited in apoE-KO mice are not likely to eliminate maternal behavior completely, suggesting that apoE in concert with other factors are contributing to the presence of maternal behavior deficits observed in apoE-KO mice.

EXPERIMENT III: APOLIPOPROTEIN E AND AGGRESSION

Methods

Subjects

Male ApoE KO (C57BL/6J-Apoe ^{tm1Unc}) and wild-type (WT) C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in transparent Plexiglas cages with access to food and water *ad libitum,* except during behavioral testing. The floors of the cages were covered with wood shavings and nestlet squares were provided for each. Cages remained undisturbed, except for weekly cleaning. The mice were maintained under constant temperature (22 ^{O}C) and a 12L: 12D light cycle (lights on at 0700 h).

Procedure

Resident mice (KO n=16, WT n=15) were separated at approximately 10 weeks of age and were housed individually in cages for 4 weeks prior to behavioral testing. Intruders were WT male mice, 4-6 weeks of age at the time of testing, and housed in groups of 4-6 since weaning. Each intruder was rendered temporarily anosmic one day prior to testing, via nasal irrigation with a Triton-X solution (0.7% Triton-X dissolved in 0.9% NaCl). Prior to intruder introduction, the resident's cage was moved to the viewing platform (located within the same housing room) and allowed a 5-minute habituation period. Food and water were removed from the home cage and a transparent Plexiglas lid was placed over the

top of the cage. In order to distinguish between the mice, the intruders were marked on the tail prior to introduction, via black, non-toxic permanent marker.

The resident-intruder test consisted of placing a randomly chosen intruder into the resident's home cage for 10 minutes. Each session was video recorded directly above the cage by a JVC VHS-C recorder and reviewed at a later date by an independent experimenter who was unaware of the genotype of the animals. All tests were conducted between 1600 and 1900 (last 3 hours of light cycle). The following behaviors were recorded: (1) attack = rapidly to a partly over the conspecific and biting (2) aggressive groom = aggressive posture with nibbling / licking of the conspecific's fur while holding down conspecific and preventing escape (3) anal-genital sniff = orientation to the conspecific's anal-genital region (4) rear = exploratory behavior involving static up right locomotion (5) body care = maintenance behavior involving scratching, licking, or wiping of own body parts (Mitchell, 1994).

All trials were conducted according to the guidelines governing animal research at Eastern Illinois University. To minimize any stress to the animals during the test, trials were halted if an animal appeared to be in distress. An observer was always present during each encounter and if the aggression escalated too far, the observer was prepared to terminate the trial. All mice however, were checked for wounds directly after each encounter and again 24 hours later and there were no visible wounds observed for any trials conducted and no encounters had to be terminated.

Statistical analysis

All behaviors were analyzed using SPSS version. 11.0. Nonparametric tests were used because the data did not conform to parametric assumptions; these tests took tied values into account when appropriate. Statistical significance was accepted at $p < 0.05$. Data are presented as mean ± SEM.

Results

ApoE-KO mice were less aggressive than WT mice. ApoE-KO mice attacked significantly fewer times than WT mice (apoE = 0/16 attacking ; WT = 6/15 attacking; fisher's exact test, $p = 0.007$) and had lower aggressive groom frequencies than WT mice (WT= 23.1 ± 2.07 grooms, n=15; apoE-KO = 16.3 ± 1.30 grooms, n=16; Mann-Whitney, U = 54.5, z = 2.59, $p = 0.009$) (Fig 6). ApoE-KO mice had longer aggressive groom latencies than WT mice, but this trend was not significant (apoE-KO = 87.0 ± 13.72, n = 16; WT = 68.3 ± 9.87, n = 15; Mann-Whitney, U= 91, z = 1.15, $p = 0.25$). While WT mice had more aggressive grooms than apoE-KO mice, apoE-KO mice spent a longer duration per groom (WT = 10.0 ± 1.32 s/groom, n = 15; apoE-KO = 14.6 ± 1.36 s/groom, n = 16; Mann-Whitney, U = 63.0, z = 2.25, $p= 0.024$) (Fig 7). ApoE-KO mice also had more body care occurrences than WT mice (apoE-KO = 15.1 ± 2.18, n = 16; WT = 7.5 ± 1.55, n = 15; Mann-Whitney, U = 56.5, z = 2.51, $p= 0.012$) (Fig 8) and had a shorter latency to exhibit body care than WT individuals (apoE-KO = 110.0 ± 28.51 s, n = 16; WT = 191.6 ± 22.63 s , n = 15; Mann-Whitney, U= 40.0, z = 3.16, $p= 0.002$) (Fig 9).

There were no significant differences in total number of sniffs for either genotype (WT = 44.1 ± 3.43 sniffs, n = 15; apoE-KO = 43.2 ± 2.34 sniffs, n = 16; Mann-Whitney, U= 113, z = 0.26, p= 0.80), the number of sniffs prior to display of aggressive groom (WT = 14.3 ± 1.52 sniffs, n = 15; apoE-KO = 15.6 ± 1.89 sniffs, n = 16; Mann-Whitney, U= 117.5, z = 0.01, p= 0.92), or the latency for first sniff (WT = 11.5 ± 3.23 s , n = 15; apoE-KO = 8.5 ± 2.19 s, n = 16; Mann-Whitney, U= 104 z = 0.63, p= 0.53). Additionally there were no differences observed in total number of rears (WT = 17.6 ± 2.68, n = 15; apoE-KO = 17.6 ± 2.27, n = 16; Mann-Whitney, U= 114, z = 0.24, p = 0.81) or in latency of first rear (WT = 81.4 ± 9.76, n = 15; apoE-KO = 79.6 ± 9.04, n = 16; Mann-Whitney, U= 114, z = 0.198, p= 0.84).

Both apoE-KO and WT mice displayed sniffing behavior prior to exhibiting other behaviors (apoE-KO = 15/16 sniffed first; WT = 15/15). However, following sniffing, the next behavior performed by WT was typically aggressive groom, while apoE-KO had a more uniform likelihood of aggressive groom, body care, or rearing (Fig 10).

Discussion

Numerous studies have shown that olfactory dysfunction leads to altered aggressive states. Olfactory bulbectomy (Ropartz, 1968; Denenberg et al., 1973; Alberts, 1974; Liebenauer and Slotnick, 1996) and anosmia via nasal irrigation of $ZnSO_4$ (Benton et al., 1979; Brain and Al-Maliki, 1978) eliminates olfactory function and aggression in mice. Olfactory dysfunction has also been shown to

reduce social aggression in feral rats (Alberts and Galef, 1973), laboratory rats (Luciano and Lore, 1975; Flannelly and Thor, 1976), Golden hamsters (Devor and Murphy, 1973), and gerbils (Alberts, 1974). We propose that the olfactory dysfunction documented in apoE-KO mice (Nathan et al., *In Press*) is responsible for the decrease in aggression levels observed in our study. Lacking the ability to properly process the olfactory cues emitted by the intruder, apoE-KO mice will behave similarly to that of bulbectomized or nasal irrigated anosmic mice. Without the functioning apoE gene, apoE-KO mice display deficits in olfaction, which can have potential consequences on aggressive behavior.

Intermale fighting consists of a well-defined sequence of acts and postures that lead up to an attack (Brain and Poole, 1974). Usually residents will display assessment behaviors such as sniff and aggressive grooming prior to attacking. In our study the assessment behaviors that we looked at were sniff and aggressive groom, which were chosen based on preliminary observations of our two genotypes. Both genotypes exhibited sniffing behavior as the first behavior, which suggests that both mice are aware of the change within their environment and are capable of investigating the intrusion. In the case of the second behavior exhibited, we saw a different picture emerge. ApoE-KO mice had a more uniform likelihood of displaying either aggressive groom, body care, or rear as the 2nd behavior exhibited, while WT mice predominantly displayed aggressive groom behavior. Aggressive groom behavior is the next behavior that usually occurs within the sequence of agnostic encounters, while body care and rear are not likely to be displayed in mice that are going to attack. This trend

suggests that apoE-KO mice may not be displaying the sequence of agnostic behavior early on in the encounter with the conspecific.

Since we observed a difference in sequence of behavior we next looked at aggressive groom behavior overall. WT individuals had more frequencies of aggressive grooms, while apoE-KO mice spent a longer duration per groom than WT mice. We propose that the longer duration per grooming was necessary for apoE-KO mice to access the status of the intruder. Due to olfactory deficits associated with the apoE-KO males (Nathan et al, *In Press*) we propose that the apoE-KO mice must use other senses (i.e. taste) to access social status or perhaps are actually unable to properly assess at all. Allogrooming consists of forcibly holding down the conspecific and nibbling on their fir. Thus, valuable sensory information (i.e. olfactory and taste) can be gained by being is close proximity to the conspecific. Perhaps apoE-KO mice are unable to obtain and process the olfactory sensory information as quickly as the WT mice and thus are unable to determine the social state of the intruder as quickly as WT mice.

ApoE-KO mice were less aggressive by displaying few aggressive behaviors, in fact they did not attack at all. WT mice also showed few attacks with only 40% of WT individuals engaging in attack behavior. Other studies involving the C57 genetic background, namely the C57/BL6N (Parmigiani et al., 1999), C57Bl/6 (Simler et al., 1982), C57BL/10 and C57BL/6Bg (Selmanoff and Ginsburg, 1981) backgrounds, have displayed low aggression levels. Several explanations have been postulated including low testosterone, higher GABA and higher serotonin levels, all of which lead to lower aggression levels when

compared to other strains of mice. In a similar background strain of our WT mice, Selmanoff and Ginsburg (1981) observed lower levels of testosterone in the blood of adult C57BL/10Bg males, suggesting that lower testosterone levels results in decreased aggression.

The tendency to exhibit aggressive responses is inversely related to GABA concentration (Poshivalov, 1981; Simler et al., 1982). Simler et al., (1982) demonstrated that C57Bl/6 mice have higher levels of GABA, an inhibitory neurotransmitter, in the olfactory bulb and striatum and lower aggression levels when compared to other mouse strains. Thus it is highly likely that GABA plays a major role in the control of aggressive behavior.

Serotonin has also been implicated in aggressive behaviors. Low levels of serotonin results in higher levels of aggressive behavior (Chiavegatto et al., 2001). Chiavegatto et al. (2001) found similar levels of aggression as our WT strain of mice, however the fact that our apoE-KO mice had significantly lower aggression levels suggests that there must be another explanation for the difference in aggression between WT and apoE-KO mice. Since our WT results are similar to numerous other studies conducted on similar background strains, we conclude that the lack of aggression displayed in apoE-KO mice is due to the absence of a functioning apoE gene, rather than the less aggressiveness of our C57 background strain. Further studies need to be conducted to determine if there is also a difference in apoE-KO mice in the testosterone, serotonin, and/or GABA levels.

Conclusions

ApoE is necessary for normal olfactory function in mice. In the absence of apoE, apoE-KO mice show impairments in olfactory function and social behaviors. Since social behaviors in mice are highly regulated by chemosenory stimulation, impairment in olfactory function inevitably causes an alteration in social behaviors. Maternal and aggressive behaviors are reduced in apoE-KO mice most notably due to olfactory deficits. To our knowledge, this is one of the first studies to link olfactory dysfunction in apoE-KO mice with a decrease in social behaviors. How apoE leads to decrease in olfaction, however, remains elusive. ApoE animal models may be of importance in bringing clarity to not only AD, but also bring clarity to how apoE plays a role within the olfactory system.

Table 1. Definitions of behavioral elements observed in the maternal behavior and in the aggressive behavior tests.

Behavior	Definition & Motivational category
Maternal test behaviors	
Retrieval[7, 8]	Pick up and carry pup with mouth
Carry[7, 8]	Pup in mouth during locomotion of dam
Drop[8]	Deposit or drop pup outside nest
Pup sniff	Sniff pup
Nest re-build[1]	Push or pull bedding/nestlet toward nest from outside or inside nest
Rear[5]	Static, upright locomotion; raise up on rear legs; Exploration
Aggression test behaviors	
Rear[5]	Static, upright locomotion; raise up on rear legs; Exploration
Anal-genital sniff[3]	Sniff anal-genital region of conspecific; Investigation
Aggressive groom[3, 1]	Social grooming, involves nibbling of conspecific's fur while forcibly holding the conspecific down; Aggression
Attack[4]	Bite directed at back or flanks of the opponent ; Aggression
Body Care[2, 6]	Abbreviated groom, self-groom, wash shake, scratch; Maintenance

[1]Boccia and Pedersen, 2001
[2]Felip et al., 2000
[3]Grant and Mackintosh, 1968
[4]Miczek et al., 2001
[5]Mitchell et al., 1994
[6]Navarro et al., 2000
[7]Pryce et al., 2001
[8]Slotnick and Nigrosh, 1975

Table 2. Repeated measures ANOVA comparing variation in latency of 2 trial periods : a) Days 1-6, water (S+) trials only and b) testing period, days 4-6, where the mean treatment differences [(S-) − (S+)] were analyzed. Within subject tests were reported using univariate Greenhouse-Geisser for a) days 1-6 analysis. For b) days 4-6 within subject effects were reported using univariate sphericity assumed .

Source of Variation	dF	SS	MS	F	P
A) Days 1-6 : S+ only					
Within Subject Effects					
Day	3.7	35902.1	9819	88.43	P < 0.05
Day x genotype	11	1407.0	128.3	1.16	P = 0.32
Between Subject Effects					
Genotype	3	100.1	33.4	0.79	P = 0.50
B) Days 4-6 : Mean treatment difference					
Within Subject Effects					
Day	2	2393.4	1196.7	10.66	P < 0.05
Day x genotype	6	473.2	86.5	0.70	P = 0.64
Between Subject Effects					
Genotype	3	726.4	242.119	4.24	P = 0.01

Table 3. Representative means (± SEM) of behaviors observed in female mice during maternal 15-minute retrieval test.

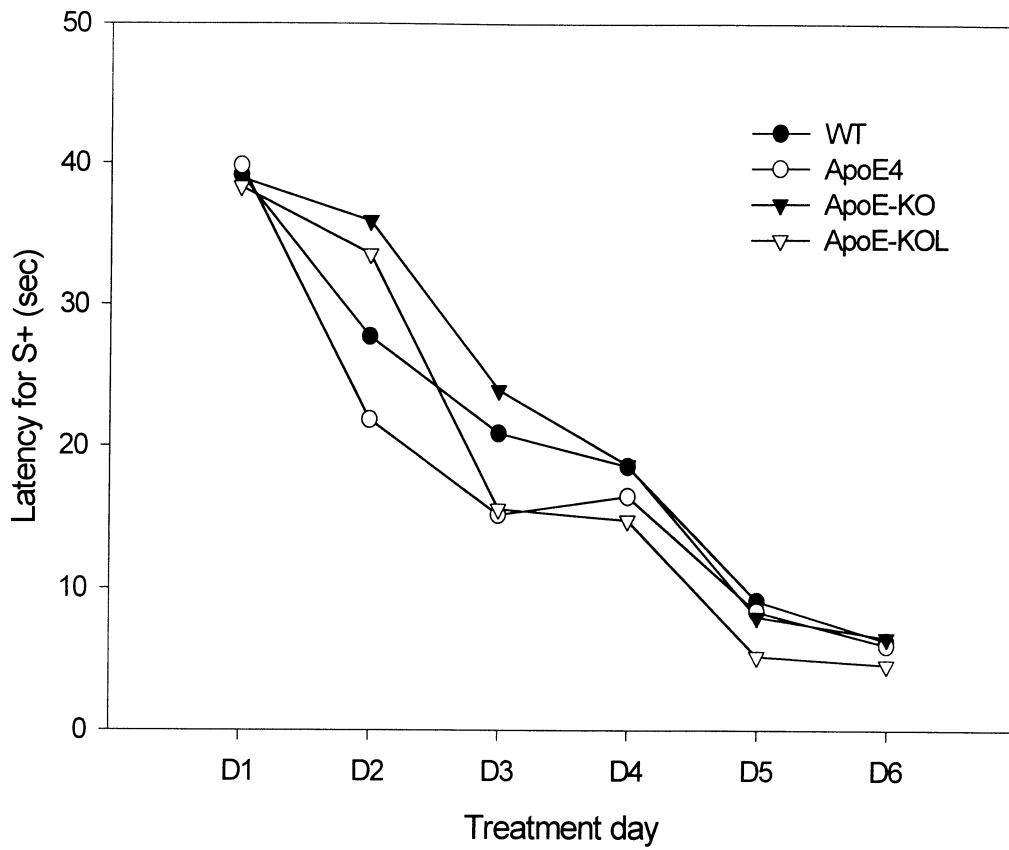|	WT	ApoE-KO	U	z	p
Carry time / pup (s)	3.47±0.21	3.54±0.26	285.0	0.062	0.951
Duration spent nest rebuilding (s)	391.79±17.83	394.11±23.50	271.0	0.351	0.726
Retrieval time duration / pup (s)	47.17±13.62	67.32±14.08	237.0	1.052	0.293
Total # Drops / pup	0.36±0.05	0.40±0.08	250.5	0.779	0.436
Latency 1st pup pick up (s)	44.81±14.69	63.29±18.02	237.5	1.041	0.298
Total # pup sniffs / pup	4.09±0.67	5.28±0.66	209.5	1.619	0.105
# Sniffs before 1st pup pick up / pup	1.12±0.22	1.49±0.33	237.0	1.052	0.293
Total # pup sniffs	34.67±4.13	24.75±3.56	197.5	1.868	0.062

43

Fig 1. Performance of WT, apoE4, apoE-KO, and apoE-KOL mice on odor cued taste avoidance test. There were no differences observed between genotypes for latency to contact water (S+) and day.
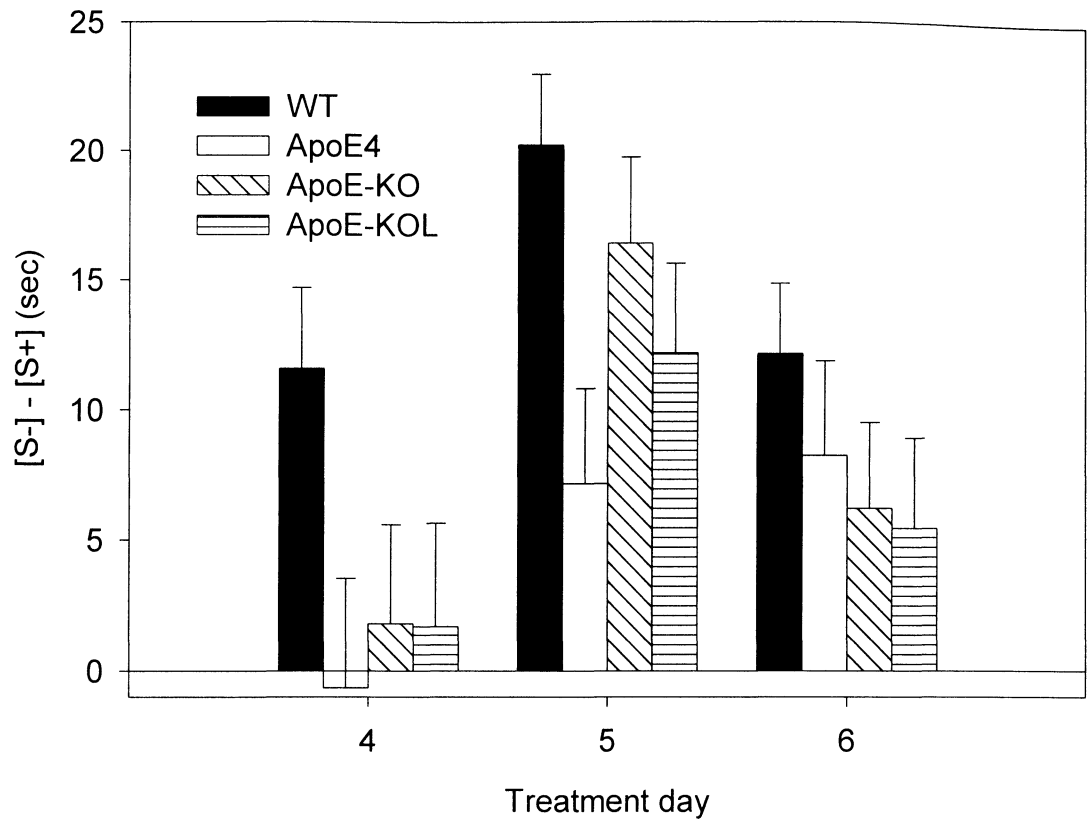
Fig 2. Mean treatment differences between latency to contact S- and S+ in the OCTA test. Latency to contact S- was subtracted by the latency to contact S+. ApoE4, apoE-KO, and apoE-KOL mice had significantly lower mean treatment differences compared to WT individuals for the 3-day testing period ($P < 0.05$).
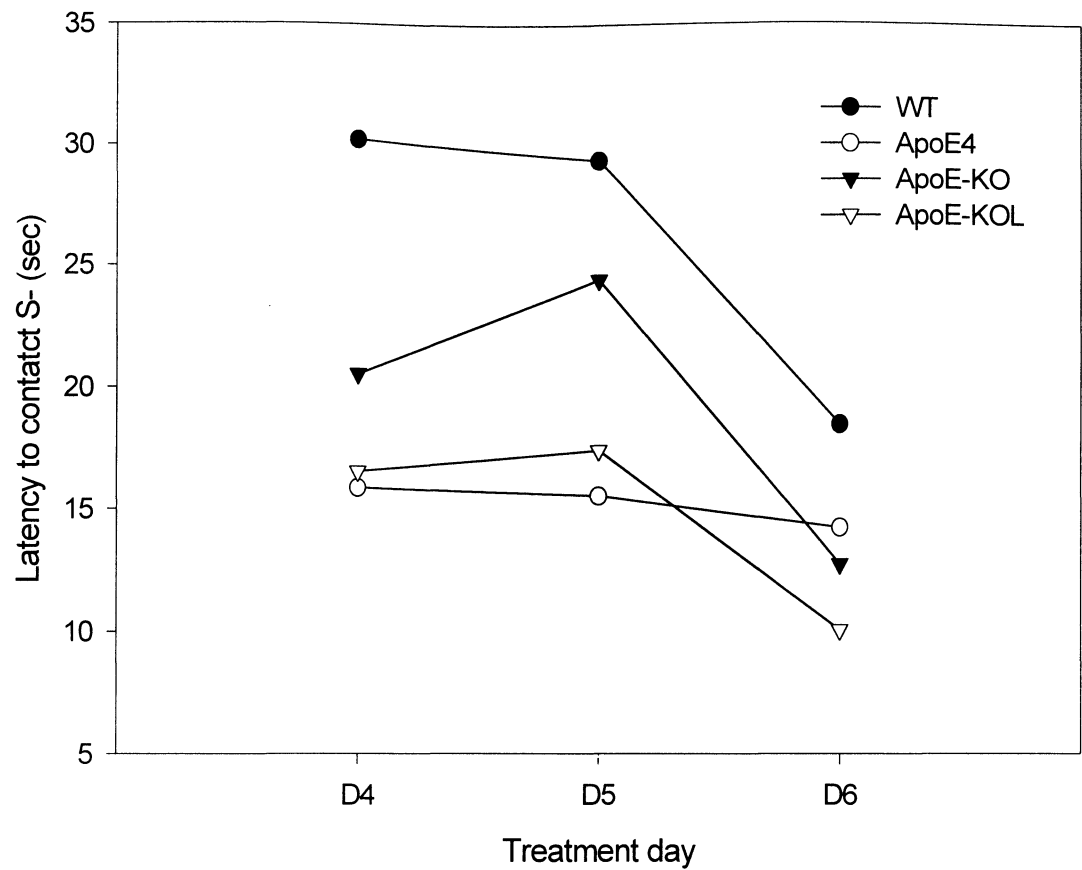
Fig 3. Performance of WT, apoE4, apoE-KO, apoE-KOL mice on odor cued taste avoidance test for S- treatment. WT mice had significantly longer latencies to contact S- than apoE4, apoE-KO, and apoE-KOL mice for all three testing days. However, there was no difference between apoE4, apoE-KO, and apoE-KOL mice for either treatment day.
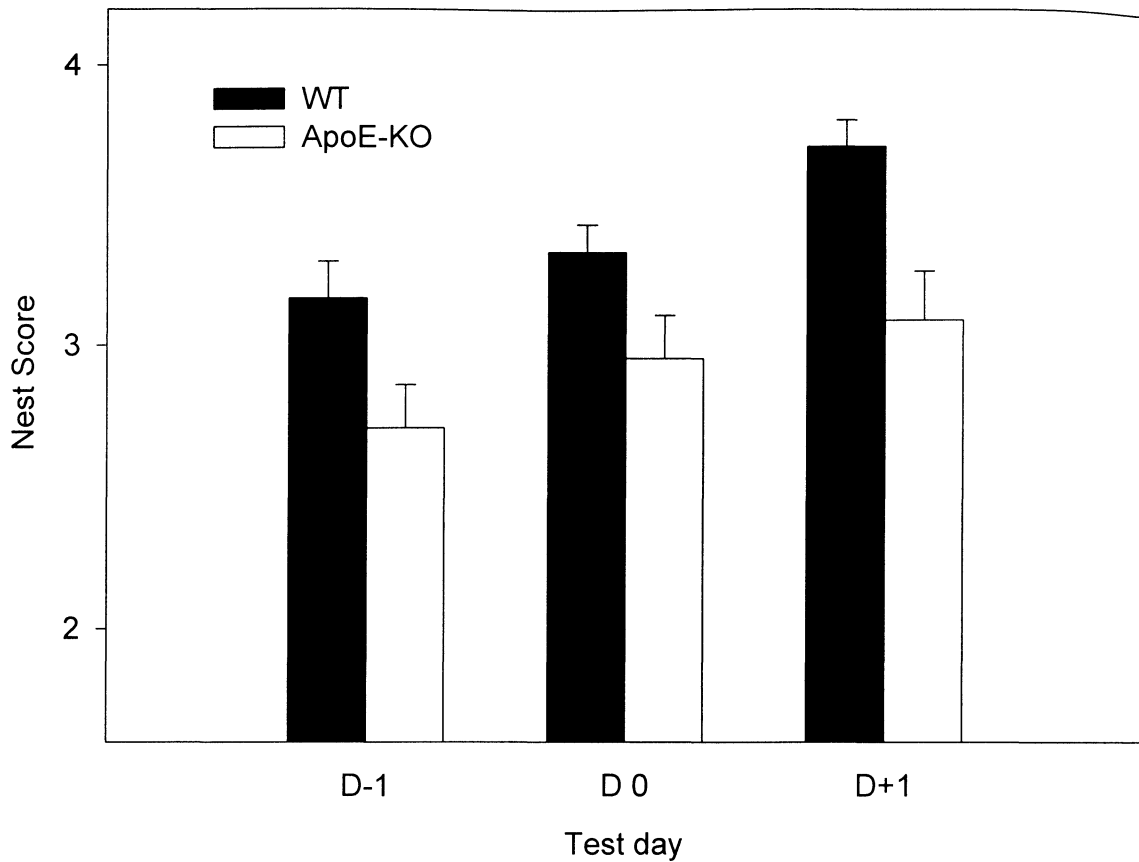
Fig 4. Nest scores of WT (n= 24) and apoE-KO (n=24) observed for 1 day prior to maternal behavior test (D-1), at pup retrieval test (D0) and 1 day after testing (D+1). Scores ranged from 0-4 (see text for details). Nest scores increased for both the WT and apoE-KO mice over the 3-day testing period. ApoE-KO mice had significantly lower quality nest scores for days D-1 and D+1. All scores were conducted between 13:00 h and 18:00 h. On D0, scores were recorded prior to testing.
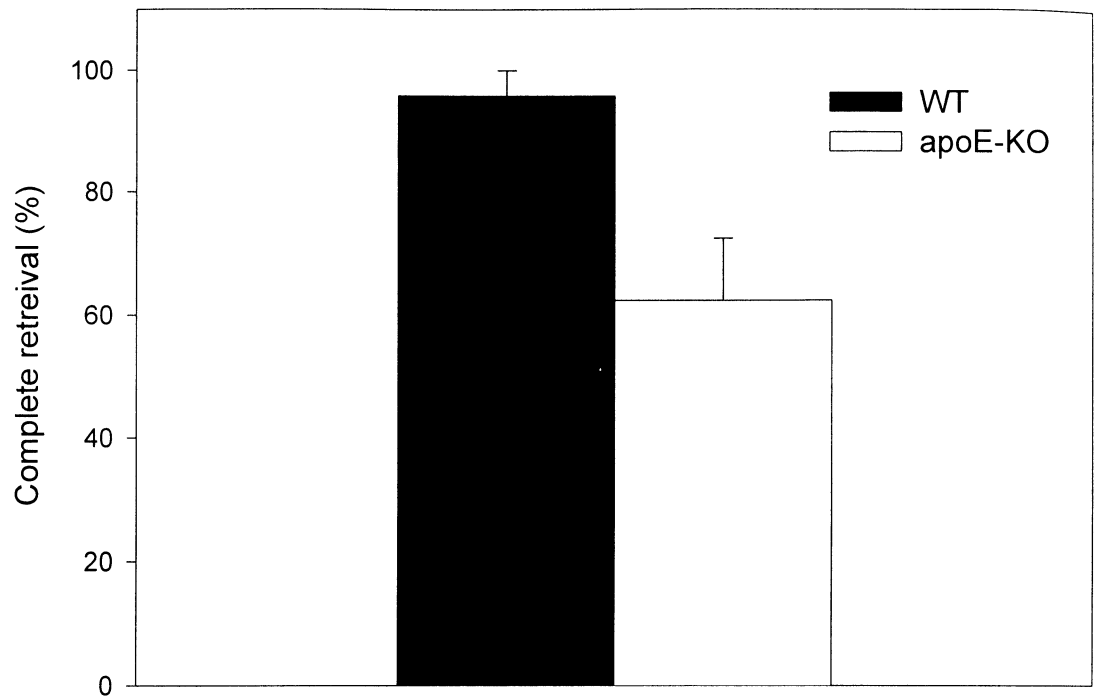
Fig 5. Percentage of dams that retrieved all pups during the 900 s retrieval test. ApoE-KO dams (n=24) retrieved significantly fewer complete litters than WT individuals (n=24). A complete retrieval consisted of the dam picking up all of her pups and carrying the pups back to the nest.
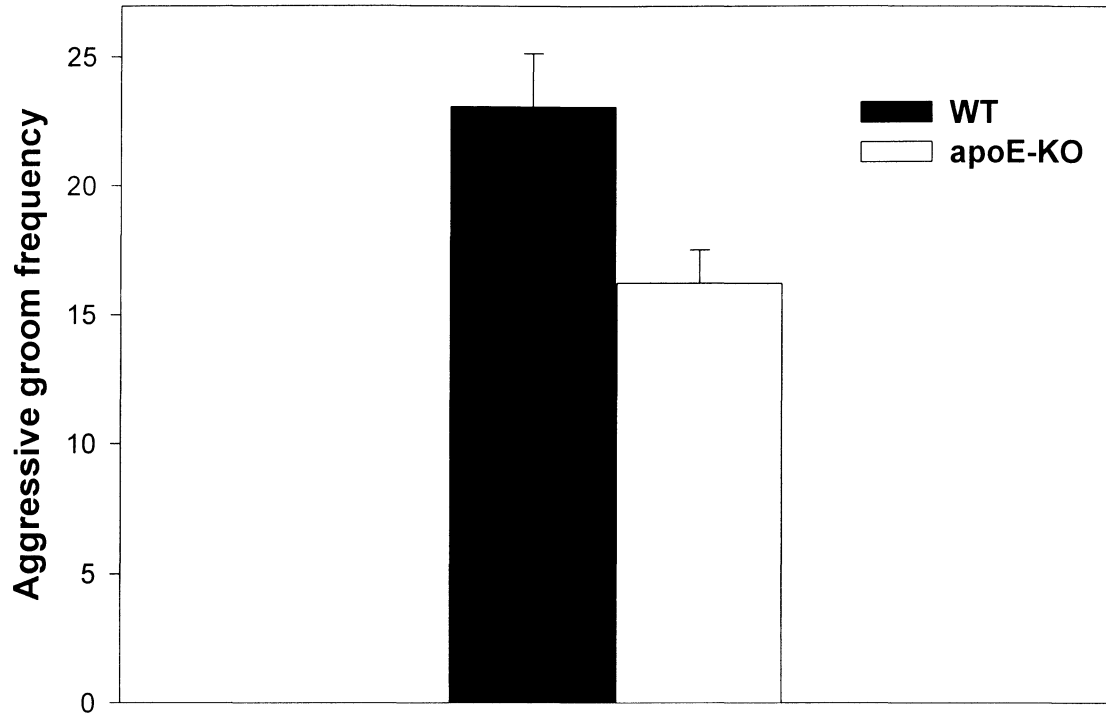
Fig 6. Frequency of aggressive grooms for ApoE-KO (n=16) and WT (n=15) in the 10-minute resident intruder test. ApoE-KO mice had significantly fewer aggressive grooms than compared to WT individuals.

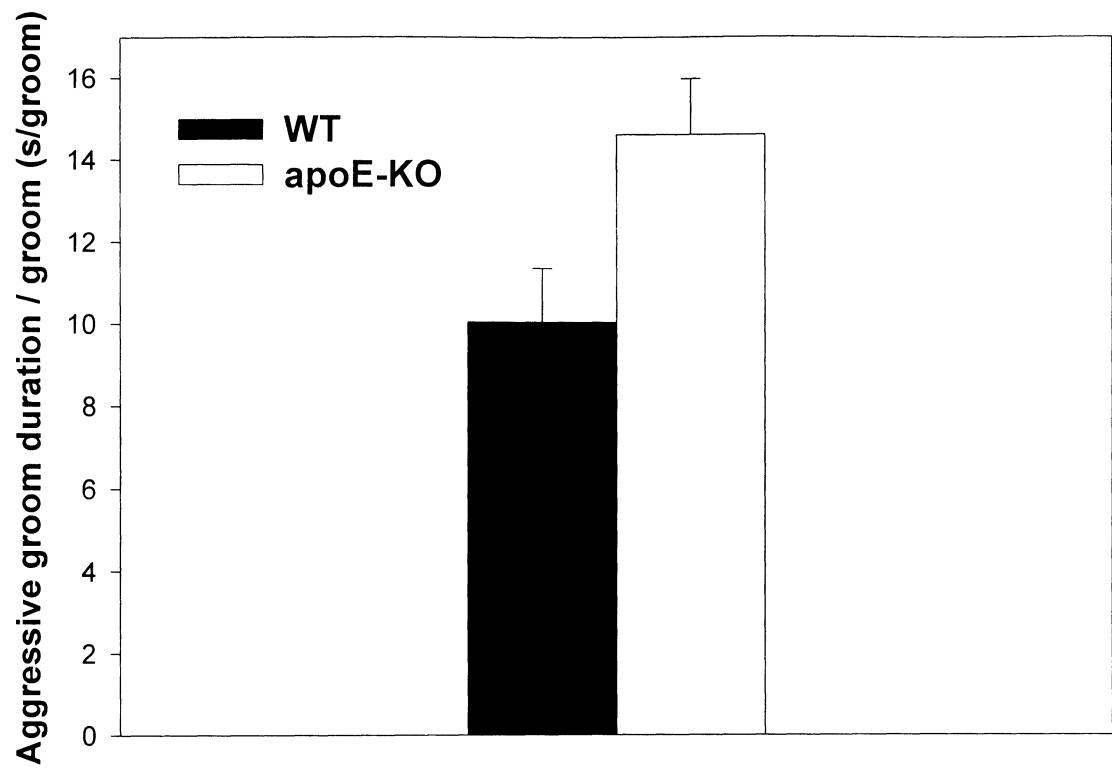Fig 7. Average duration of each aggressive groom (s/groom). ApoE-KO mice (n=16) spent a significantly longer time per groom than compared to WT individuals (n=16).
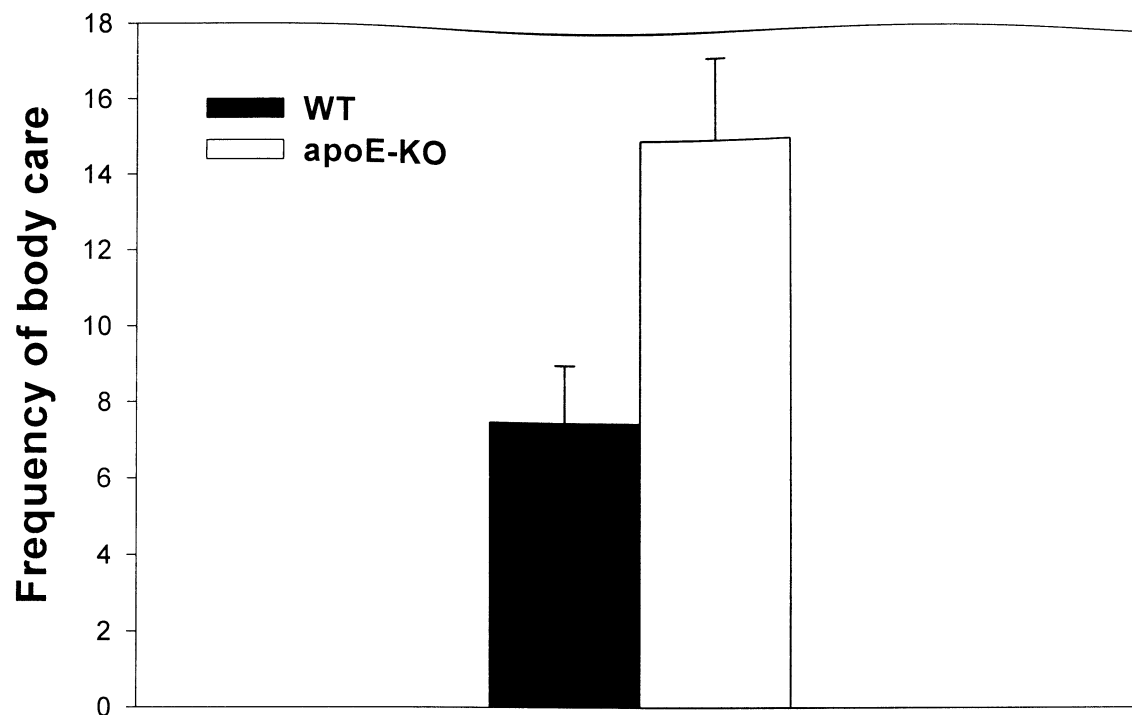
Fig 8. Frequency of body care behavior. WT mice (n=15) displayed significantly fewer episodes of body care behavior than apoE-KO individuals (n=16) during the 10-minute observation period. Body care was defined as scratching, licking, or grooming oneself.
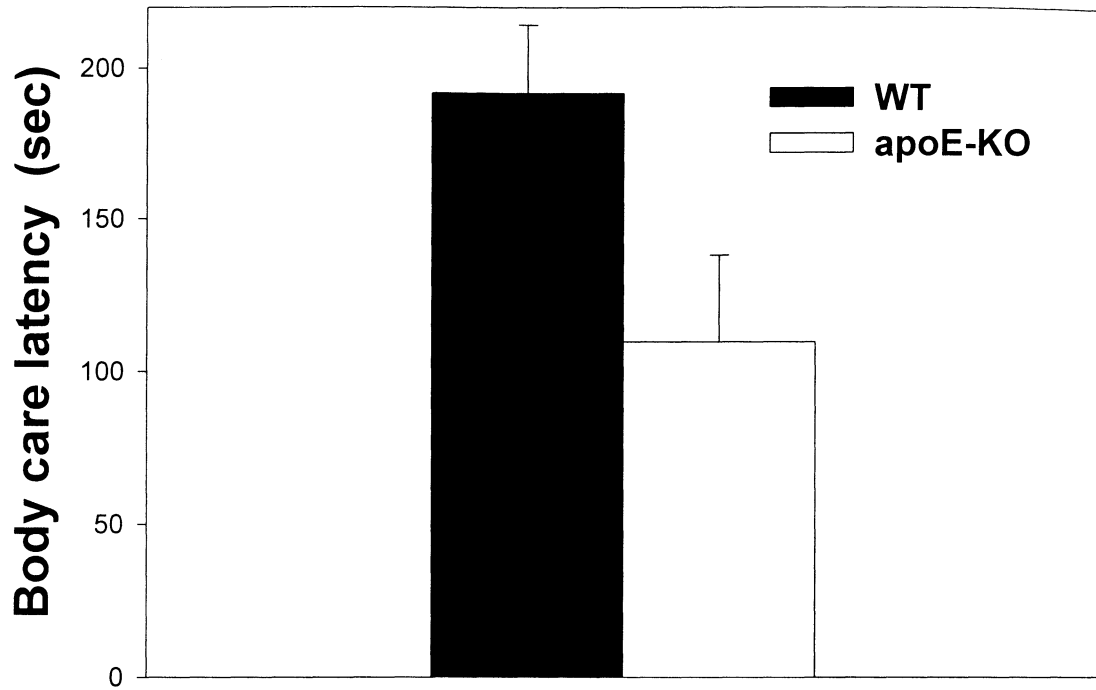
Fig 9. Average latency to display body care behavior during the 10-minute observation period. ApoE-KO mice (n=16) had shorter latencies than WT individuals (n=15) for initial body care display.
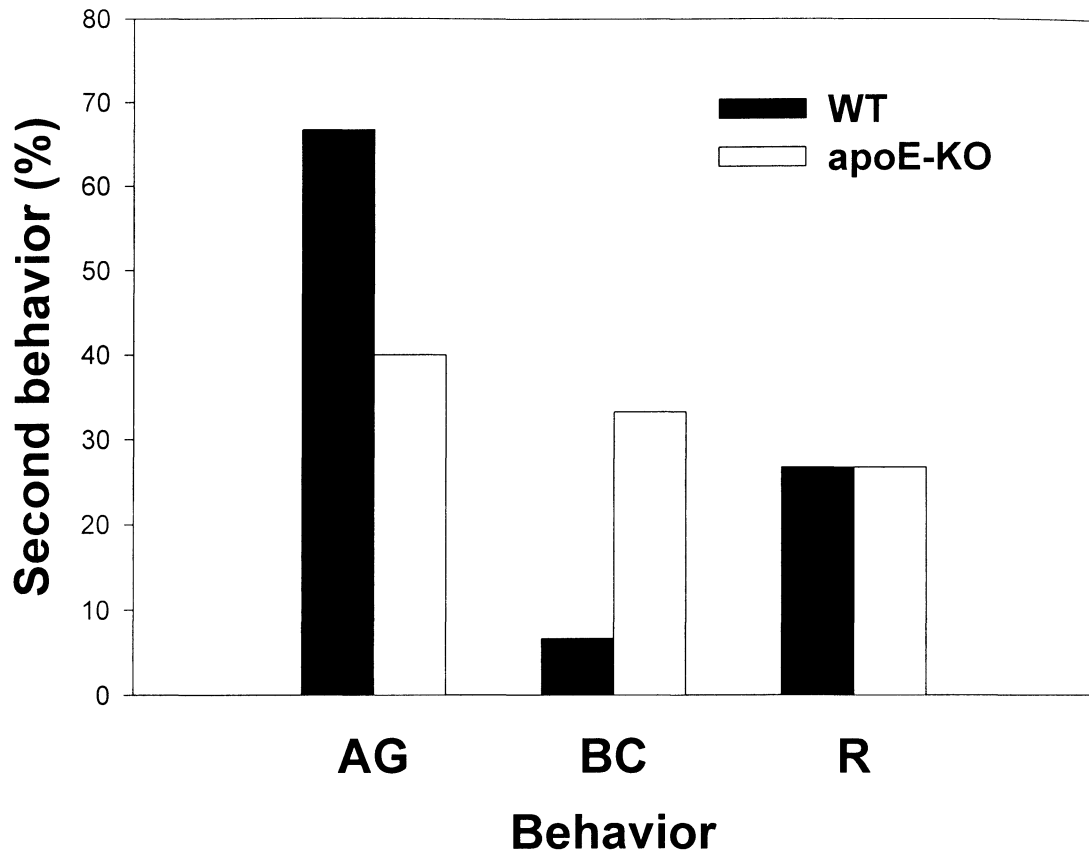
Fig 10. Percentage of behaviors occurring after initial sniffing behavior for WT (n=15) and apoE-KO (n=15) mice. The three behaviors observed after sniffing were aggressive groom (AG), body care (BC), or rear (R). WT mice were more likely to engage in AG than apoE-KO mice, while apoE-KO mice had a more uniform likelihood of AG, BC, or R. Only mice who exhibited sniffing behavior first were included in this analysis.

References

Adams, D.B. (1976) The relation of scent-marking, olfactory investigations, and specific postures in isolation-induced fighting of rats. Behavior 56, 289-297.

Albert, M. (1996) Cognitive and neurobiologic markers of early Alzheimer's disease. *Proc Natl Acad Sci USA* 93, 13547-13551.

Alberts, J.R., (1974) Producing and interpreting experimental olfactory deficits. *Physiology and Behavior* 12, 657-670.

Alberts, J.R. and Galef, B.C. (1973) Olfaction and movement: stimuli mediating intra specific aggression in wild rats. *Journal of Comparative and Physiological Psychology.* 85, 233-242.

Archer, J. (1968) The effects of strange male odors on aggressive behavior in male mice. *J Mammal* 49, 572-575.

Bacon, A.W., Bondi, M.W., Salmon, D.P., Murphy, C. (1998) Very early changes in olfactory functioning due to Alzheimer's disease and the role of apolipoprotein E in olfaction. *Ann NY Acad Sci* 855, 723-731.

Bean, N.J. (1982) Modulation of agnostic behavior by the dual olfactory system in male mice. Phsyiol Behav 29, 433-437.

Bellosta, S., Nathan, B.P., Orth, M., Dong, L.M., Mahley, R.W., Pitas, R.E. (1995) Stable expression and secretion of apolipoprotein E 3 and E4 in mouse neuroblastoma cells produces differential effects on neurite outgrowth. *J Biol Chem* 270, 27063-27071.

Benton, D., Brain, P.F., and Goldsmith, J.F., (1979) The involvement of various sensory modalities and isolation-induced aggression in laboratory mice. *Aggressive Behaviour*, 5, 198-199.

Boccia, M.L., Pedersen, C.A. (2001) Brief vs. long maternal separations in infancy: contrasting relationships with adult maternal behavior and lactation levels of aggression and anxiety. *Psychoneuroendocrinology* 26, 657-672.

Boyles, J.K., Pitas, R.E., Wilson, E., Mahley, R.W. (1985) Apolipoprotein E associated with atrocytic glia in the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J Clin Invest* 76, 1501-1513.

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

Braak, H., Braak, E. (1994) Morphological criteria for the recognition of Alzheimer's disease and the distribution pattern of cortical changes related to this disorder. *Neurobiol Aging* 15, 355-356; discussion 379-380.

Braak, H., Braak, E. (1997) Frequency of stages of Alzheimer's-related lesions in different age categories. *Neurobiol Aging* 18, 351-357.

Brain, P.F. (1981) Hormones and aggression in infra-human vertebrates. In: P.F.Brain, D. Benton (Eds.) *The biology of aggression.* Sijthoff-Noordhoff, Alpen aan den Rijn. The Netherlands.

Brain, P.F. and Al-Maliki ,S. (1978) A comparison of Intermale fighting in standard opponent tests and attack directed towards locusts by TO strain mice: Effects of simple experimental manipulation. *Animal Behaviour* 26, 723-737.

Brain, P.F., Benton (1983) Condition of housing, hormones, and aggressive behavior. In: B.Savare (Ed.), *Hormones and Aggressive Behavior.* Plenum Press, New York, pp. 351-372.

Brain, P.F., Benton, D., Childs, G., Parmigiani, S. (1981) The effects of opponent in tests of murine aggression. *Behav Processess* 6, 319-328.

Brain, P.F., Evans, C.M. (1974) Effects of androgens on the attackability of gonadectomized mice by TO trained fighter individuals: confirmatory experiments. *Endocr Neurobiol Physiol Psychol* 2, 1730.

Brain, P.F., Nowell, N.W. (1970) Some observations on intermale aggression testing in albino mice. *Communications in Behavioral Biology* 5, 7-17.

Brain, P.F., Poole, A.E. (1974) The role of endocrines in isolation-induced intermale fighting in albino laboratory mice. *Aggressive Behavior* 1, 39-69.

Bridges, R.S., DiBiase, R., Loundes, D.D., Doherty, P.C. (1985) Prolactin stimulation of maternal behavior in female rats. *Science* 227, 782-784.

Bridges, R.S., Numan, M., Ronsheim, P.M., Mann, P.E., Lupini, C.E. (1990) Central prolactin infusions stimulate maternal behavior in steroid-treated nulliparous female rats. *Proc Natl Acad Sci USA* 87, 8003-8007.

Bronson, F.H. (1971) Rodent pheromones. *Biology of Reproduction,* 4, 344-357.

Brown, R.E. (1979) Mammalian social odors: a critical review. In: *Advances in the Study of Behavior.* Academic Press, New York

Brown, M.S., Goldstein, J.L., (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* 232, 34-47.

Carlier, M., Roubertoux, P., Cohen-Salmon, C. (1982) Differences in patterns of pup care in *Mus Musculus Domesticus* : comparisons between eleven inbred strains. *Behavioral and Neural Biology* 35, 205-210.

Chan, A., Tam, J., Murphy, C., Chiu, H., Lam, L. (2002) Utility of olfactory identification test for diagnosing Chinese patients with Alzheimer's disease. *J Clin Exp Neuropsychol* 24, 251-9.

Chiavegatto, S., Dawson,V., Mamounas, L., Koliatsos, V., Dawson, T., Nelson, R. (2001). Brain serotonin dysfunction accounts for aggression in male mice lacking neuronal nitric oxide synthase. *Proc. Natl. Acad. Sci.* USA 98, 1277-1281.

Christen-Zaech, S. Kraftsik, R., Pillevuit, O., Kiraly, M., Martins, R., Khalili, K., Miklossy, J. (2003) Early olfactory involvement in Alzheimer's disease. *Can J Neurol Sci* 30, 20-25.

Cohen-Salmon, C. Carlier, M., Roubertoux, P., Jouhaneau, J., Smal, C., Paillette, M.(1985) Differences in patterns of pup care in mice. V: pup ultrasonic emissions and pup care behavior. *Physiol Behav* 35, 167-174.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, G.W., Small, G.W., Roses, A.D., Haines, J.L., 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.

Cuthbert, J.A., Lipsky, P.E. (1984) Modulation of human lymphocyte response by low density lipoprotein (LDL):enhancement by not immunosuppression is mediated by LDL receptors. *Proc Natl Acad Sci USA*, 81, 4539-4543.

Darling F.M., Slotnick, B.M. (1994) Odor-cued taste avoidance: a simple and efficient method for assessing olfactory detection, discrimination, and memory in the rat. *Physiol Behav* 55, 817-822.

Das, H.K., McPherson, J., Bruns, G.A.P., Karathanasis, S.K., Breslow, J.L.. (1985) Isolation, characterization , and mapping to chromosome 19 of the human apolipoprotein E gene. *J Biol Chem* 260, 6240-6247.

Davies, D.C., Brooks, J.W., Lewis, D.A. (1993) Axonal loss from the olfactory tracts in Alzheimer's disease. Neurobiol Aging 14, 353-357.

Denenberg, V.H., Gaulin-Kremer, E., Gandelman, R., Zarrow, M.X. (1973) The development of standard stimulus animals for mouse (Mus musculus) aggression testing by means of olfactory bulbectomy. Animal Behaviour 21, 590-598.

Devanand, D.P., Michaels-Marston, K.S., Liu, X., Pelton, G.H., Padilla, M., Marder, K., Bell, K., Stern, Y., Mayeux, R. (2000) Olfactory deficits in patients with mild cognitive impairment predict Alzheimer's disease at follow-up. Am J Psychiatry 157, 1399-1405.

Devor, M., and Murphy, M.R. (1973) The effect of peripheral olfactory blockage on the social behavior the male golden hamster. Behavioral Biology, 31-42.

Donat, P., Krsiak, M., Sulcova, A. (1994) The involvement of pheromones in offensive and defensive agnostic behavior in laboratory mice. In: Cooper S.J., Hendrie, C. A. (eds.) Ethology and Psychopharmacology. Wiley. England.

Doty, R.L. (1986) Odor-guided behavior in mammals. Experientia 42, 257-271.

Doty, R.L., Reyes, P.F., Gregor, T., (1987) Presence of both odor identification and detection deficits in Alzheimer's disease. Brain Res Bull 18, 597-600.

Ehnholm, C., Mahley, R.W., Chappell, D.A., Weisgraber, K.H., Ludwig, E., Witztum, J.L. (1984) Role of apolipoprotein E in athe lipolytic concersion of beta-very low density lipoproteins to low-density lipoproteins in type III hyperlipoproteinemia. Proc Natl Acad Sci USA 81, 5566-5570.

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

Felip, C.M., Rodriquez-Arias, M., Espejo, E.F., Minarro, J., Stinus, L. (2000) Naloxone-Induced opiate withdrawl produces long-lasting and context-Independent changes in aggressive and social behaviors of postdependent male mice. Behavioral Neuroscience 114, 424-430.

Flannelly, K.J, and Thor, D.H. (1976) Territorial behavior of laboratory rats under conditions of peripheral anosmia. Animal Learning and Behaviour 337-340.

Flemming, A.S. (1992) Effects of transections to the vomeronasal nerves or to the main olfactory bulbs on the initiation and long term retention of maternal behavior in primiparous rats. *Behavioral and Neural Biology* 57, 177-188.

Flemming, A.S. (1998) Factors influencing maternal responsiveness in humans: Usefulness of an animal model. *Psychoneuroendocrinolgy* 13, 189-212.

Flemming, A.S., O'Day, D.H., Kraemer, G.W. (1999) Neurobiology of mother-infant interactions: experience and central nervous system plasticity across development and generations. *Neuroscience and Biobehavioral Reviews* 23, 673-685.

Flemming, A.S., Vaccarino, F., Luebke, C. (1980) Amygdaloid inhibition of maternal behavior in the nulliparous female rat. *Physiology and Behavior* 25, 731-743.

Flemming, A.S., Vaccarion, F., Tambosso, L., Chee, P. (1979) Vomeronasal and olfactory system modulation of maternal behavior in the rat. *Science* 203, 372-374.

Gandelman, R., Zarrow, M.Z., Denenberg, V.H., Myers, M. (1971) Olfactory bulb removal eliminates maternal behavior in the mouse. *Science* 171, 210-211.

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

Grant, E.C., Mackintosh, J.H. (1968) A comparison of the social postures of some common laboratory rodents. *Behaviour* 21, 246-259.

Graves, A.B., Bowen, J.D., Rajaram, L., McCormick, W.C., McCurry, S.M., Schellenberg, G.D., Larson, E.B. (1999) Impaired olfaction as a marker for cognitive decline: Interaction with apolipoprotein E e4 allele status. *Neurology* 53, 1480-1487.

Grootendorst, J., Enthoven, L., Dalm, S., de Kloet, R., Oitzl, M. (*In Press*) Increased corticosterone secretion and early-onset of cognitive decline in female apolipoprotein E-knockout mice. *Behavioural Brain Research*

Harper, LV (1981) Offspring effects on parents. IN DJ Gubernick, PH Kloper (Eds) *Parental Care in Mammals* New York: Plenum Press.

Hawks, C. (2003) Olfaction in Neurodegenerative Disorder. *Movement Disorders* 18, 364-372.

Holtzer, R., Tang, M., Devanand, D.P., Albert, S., Wegesin, D., Marder, K., Bell, K., Albert, M., Brandt, J., Stern, Y. Psychopathological features in Alzheimer's disease: course and relationship with cognitive status. *J Am Geriatr Soc* 51, 953-960.

Honing, L.S., Mayeux, R. (2001) Natural history of Alzheimer's disease. *Aging Clin Exp Res* 13, 171-182.

Hughes , L.F., Struble, R.G., Shaffer, C.L. (2002) Olfaction in elderly and patients with Alzheimer's disease: Difference in feedback conditions. *J Alzheimer's Dis* 3, 367-375.

Ignatius, M.J., Gebicke-Haerter, P.J., Skene, J.H., Schilling, J.W., Weisgraber, K.H., Mahley, R.W., Shooter, E.M. (1986) Expression of apolipoprotein E during nerve degeneration and regeneration. *Proc Natl Acad Sci USA* 83, 1125-1129.

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

Lancashire, C., Mileusnic, R., Rose, S.P. (1998) Apolipoprotein E antibodies affect the retention of passive avoidance memory in the chick. *Neural Plast* 6, 29-40.

Leckman, J., Herman, A. (2002) Maternal behavior and developmental psychopathology. *Biol Psychiatry* 51, 27-43.

Lepri, J., Wysock, C., Vandenberg, J. (1985) Mouse vomeronasal organ: effects on chemosensory production and maternal behavior. *Physiology and behavior* 35, 809-814.

Liebnaur, L., Slotnick, B., (1996) Social organization and aggression in a group of olfactory bulbectomized male mice. *Physiology and Behavior* 60, 403-409.

Lucas, B.K., Ormandy, C.J., Binart, N., Bridges, R.S., Kelly, P.A. (1998). Null mutation of the prolactin receptor gene produces a defect in maternal behavior. *Endocrinology* 139, 4102-4107.

Luciano, D. and Lore, R. (1975) Aggression and social experience in domesticated rats. *Journal of Comparative and Physiological Psychology* 88, 917-923.

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

Martinez, M., Calvo-Torrent, A., Pico-Alfonso, M.A. (1998) Social defeat and subordination as models of social stress in laboratory rodents: a review. *Aggressive Behaivor* 24, 241-256.

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

Masliah, E., Mallory, M., Veinbergs, I., Miller, A., Samuel, W. (1996) Alterations in apolipoprotein E expression during aging and neurodegeneration. *Prog Neurobiol* 50, 493-503.

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

Maxson, S., Methodological issues in genetic analyses of an agnostic behavior (offense) in male mice. In Goldowitz, D., Wahlsten, D., and Wimer, R. (eds.) *Techniques for the genetic analysis of brain and behavior: focus on the mouse.* Elsevier. Amsterdam, 1992.

Mena, F. and Grosvenor, C.E. (1971) Release of prolactin in rats by exteroceptive stimulation: sensory stimuli involved. *Hormones and Behavior* 2, 107-116.

Mesholam, R., Moberg, P., Mahr, R., Doty, R. (1998) Olfaction in neurodegenerative diseases: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Archives of Neurology* 55, 84-90.

Miczek, K.A., Krsiak, M. (1981) Pharmacological analysis of attack and flight. In: *Multidisciplinary approaches to aggression research* (eds. P.F. Brain and D. Benton) Elsevier/North-Holland Biomedical Press. Amsterdam.

Miczek, K. A., Maxson, S. C., Fish, E.W., Faccidomo, S. (2001) Aggressive behavioral phenotypes in mice. *Behavioral Brain Research* 125, 167-181.

Mitchell, P.J. (1994) Prediction of antidepressant activity from ethological analysis of agnostic behavior in rats. In: Cooper S.J., Hendrie, C. A. (eds.) *Ethology and Psychopharmacology.* Wiley. England.

Morgan, C.D., Murphy, C. (2002) Olfactory event-related potentials in Alzheimer's disease. *J Int Neuropsychol Soc* 8, 753-763.

Morgan, C.D., Nordin, S., Murphy, C. (1995) Odor identification as an early marker for Alzheimer's disease: impact of lexical functioning and detection sensitivity. *J Clin Exp Neuropsychol* 17, 793-803.

Mugford, R., Nowell, N. (1971) The preputial glands as a source of aggression-promoting odors in mice. Physiology and Behavior 6, 247-249.

Murphy, C. (1999) Loss of olfactory function in dementing disease. *Physiology and Behavior* 66, 177-182.

Murphy, C., Gilmore, M.M., Seery, C.S., Salmon, D.P., Lasker, B.R. (1990) Olfactory thresholds are associated with degree of dementia in Alzheimer's disease. *Neurobiol Aging* 11, 465-469.

Murphy, C., Bacon, A.W., Bondi, M.W., Salmon, D.P., (1998) Apolipoprotein E status is associated with odor identification deficits in nondemented older persons. *Ann NY Acad Sci* 855, 744-50.

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-Jakob disease. *Brain res* 541, 163-166.

Nathan, B.P., Bellosta, S., Sanan, D.A., Weisgraber, K.H., Mahley, R.W., Pitas, R.E. (1994) Differential effects of apolipoprotein E3 and E4 on neuronal growth in vitro. *Science* 264, 850-852.

Nathan, B.P., Chang, K.C., Bellosta, S., Brisch, E., Ge, N., Mahley, R.W., Pitas, R.E. (1995) The inhibitory effect of apolipoprotein E on neurite outgrowth is associated with microtuble polymerization. *J Biol Chem* 270, 19791-19799.

Nathan, B.P., Nisar, R., Randall, S., Short, J., Sherrow, M., Wong, G.K., Struble, R.G. (2001) Apolipoprotein E is upregulated in olfactory bulb glia following peripheral receptor lesion in mice. *Experimental Neurology* 172, 128-136.

Nathan, B.P., Yost, J., Litherland, M.T., Struble, R.G., Switzer, P.V. (in press) Olfactory function in apoE knockout mice. *Behavioural Brain Research*.

Navarro, J.F., Romero, C., and Maldonado, E. (2000) Effects of N6-cyclohexl adenosine (CHA) on isolation-induced aggression in male mice. *Methods Find Exp Clin Pharmacol* 22, 43-46.

Nordin, S., Murphy, C. (1996) Impaired sensory and cognitive olfactory function in questionable Alzheimer's disease. *Neuropsychology* 10, 113-119.

Nordin, S., Murphy, C. (1998) Odor memory in normal aging and Alzheimer's disease. Annals *New York Academy of Sciences* 855, 686-693.

Numan, M., Sheehan, T.P. (1997) Neuroanatomical circuitry for mammalian maternal behavior. *Ann NY Acad Sci* 807, 101-125.

Ohm, T.G., Braak, H. (1987) Olfactory bulb changes in Alzheimer's disease. ACTA Neuropathol (Berl). 73, 365-369.

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

Ostermeyer, M., Elwood, R. (1982) Pup recognition in Mus musculus: parental discrimination between their own and alien young. *Dev Psychobiol* 16, 75-82.

Pardon, M., Gerardin, P., Jouber, C., Perez-Diaz, F. (2000) Influence of prepartum chronic stress on maternal pup care behavior in mice. *Biol Psychiatry* 47, 858-863.

Parmigiani, S., Brunoni, V., Pasquali, A. (1982) Socio-sexual preferences of female mice (*Mus musculus domesticus):* the influence of social aggressive capabilities of isolated or grouped males. *Bool Zool* 49, 73-78.

Parmigiani, S., Palanza, P., Rodgers, J., Ferrari, P. (1999) Selection, evolution of behavior and animal models in behavioral neuroscience. *Neuroscience and Biobehavioral Reviews* 23, 957-970.

Payne, J.W., Bettman, J.R., Johnson, E.J., (1992) Behavioral decision research: a constructive processing perspective. *Annu Rev Psychol* 43, 87-131.

Price, D.L., Tanzji, R.E., Borchelt, D.R., Sisodia, S.S. (1998) Alzheimer's disease: genetic studies and transgenic models. *Annu Rev Genet* 32, 461-93.

Pitas, R.E., Boyles, J.K., Lee, S.H., Foss,D., Mahley, R.W. (1987) Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. *Biochem Biophys ACTA* 917, 48-61.

Poirier, J., Baccichet, A., Dea, D., Gauthier, S., (1993) Cholesterol synthesis and lipoprotein reuptake during synaptic remodeling in hippocampus in adult rats. *Neuroscience* 55, 81-90.

Poshivalov, V. (1981) Pharmaco-Ethological analysis of social behavior of isolated mice. *Pharmacology Biochemistry and Behavior* 14, 53-59

Pryce, C.R., Bettschen, D., Feldon, J. (2001) Comparison of effects of early handling and early deprivation on maternal care in the rat. *Dev Psychobiol* 38, 239-251.

Raber, J., Akana, S.F., Bhatnager, S., Dallman, M.F., Wong, D., Mucke, L. (2000) Hypothalamic-pituitary-adrenal dysfunction in Apoe(-/-) mice: possible role in behavioral and metabolic alterations. *J Neurosci* 20, 2064-2071.

Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R.E., Mahley, R.W., Mucke, L. (1998) Isoform-specific effects of human apolipoprotein E on brain function revealed in apoE knockout mice: increased susceptibility of females. *Proc Natl Acad Sci USA* 95, 10914-10919.

Rall, S.C., Jr., Weisgraber, K.H., Mahley, R.W. (1982) Human apolipoprotein E. The complete amino acid sequence. *J Bio. Chem* 257, 4171-4178.

Reyland, M.E., Gwynne, J.T., Forgez, P., Prack, M.M., Williams, D.L., (1991) Expression of the human apolipoprotein E gene suppresses steroidogenesis in mouse Y1 adrenal cells. *Proc Natl Acad Sci USA* 88, 2375-2379.

Rocchi, A., Pellegrini, S., Siciliano, G., Murri, L. (2003) Causative and susceptibility genes for Alzheimer's disease: a review. *Brain Res Bull* 61, 1-24.

Ropartz, P. (1968) The relation between olfactory stimulation and aggressive behavior in mice. *Animal Behaviour* 16, 97-100.

Rosenblatt, JS. (1990) Landmarks in the physiological study of maternal behavior with special reference to the rat. IN: *Mammalian Parenting*. NA Krasnegor, RS Bridges (Eds). New York: Oxford University Press.

Rosenblatt, JS, Lehrman, DS. (1963) Maternal behavior of the laboratory rat. IN: *Maternal Behavior in Mammals.* H.L.Rheingold (Ed). New York: John Wiley & Sons, Inc.

Roses, A.D. (1997) Apolipoprotein E, a gene with complex biological interactions in the aging brain. *Neurobiology of Disease* 4, 170-186.

Schiffman, S., Graham, B., Sattely-Miller, E., Zervakis, J., Welsh-Bohmer, K. (2002) Taste, Smell and neuropsychological performance of individuals at familial risk for Alzheimer's disease. *Neurobiology of Aging* 23, 397-404.

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

Schwartz, E., Rowe, F.A. (1976) Olfactory bulbectomy: influences on maternal behavior in primiparous and multiparous rats. *Physiology and Behavior* 17, 879-883.

Selmanoff, M.K., Ginsburg, B.E. (1981) Genetic variability in aggression and endocrine function in inbred strains of mice. IN P.F. Brain, D. Benton (Eds.) *Multidisciplinary approaches to aggression research.* Amsterdam: Elsevier/ North-Holland Biomedical Press.

Serby, M., Larson, P., Kalkstein, D. (1991) The nature and course of olfactory deficits in Alzheimer's disease. *Am J Psychiatry* 148, 357-60.

Sheehan, T.P., Cirrito, J., Numan, M.J., Numan, M. (2000) Using c-fos immunocytochemistiry to identify forebrain regions that may inhibit maternal behavior in rats. *Behav Neurosci* 114, 337-352.

Shingo, T., Gregg, C., Enwere, E., Fujikawa, H,., Hassam, R., Geary, C., Cross, J.C., Weiss, S. (2003) Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science* 299, 117-120.

Simler, S., Puglisi-Allegra, S., Mandel, P., (1982) γ-Aminobutyric acid in brain areas of isolated aggressive or non-aggressive inbred strains of mice. *Pharmacology Biochemistry and Behavior* 16, 57-61.

Skene, J.H.P., Shooter, E.M. (1983) Denervated sheath cells secrete a new protein after nerve injury. *Proc Natl Acad Sci USA* 83, 1130-1134.

Slotnick, B.M. (1969) Maternal behavior following forebrain lesions in the rat. *American Zoologist* 9, 40 (abstract)

Slotnick, B.M., Nigrosh, B.J. (1975) Maternal behavior of mice with cingulate cortical, amygdala, or septal lesions. *J Comp Physiol Psychol* 88, 118-127.

Snipes, G.J., McGuire,C.B., Norden, J.J., Freeman, H.A., (1986) Nerve injury stimulates the secretion of apolipoprotein E by nonneuronal cells. *Proc Natl Acad Sci USA* 83, 1130-1134.

Strittmatter, W.J., Roses, A.D. (1996) Apolipoprotein E and Alzheimer's disease. *Annu Rev Neurosci* 19, 53-77.

Strittmatter, W.J., Wiesgraber, H.H., Huang, D.Y., Dong, L.M., Salvesen, G.S., Pericak-Vance, M., Schmechel, D., Saunders, A.M., Goldgaber, D., Roses, A.D. (1993) Binding of human apolipoprotein E to synthetic amyloid beta-peptide isoform-specific effects and implications for late-onset Alzheimer's disease. *Proc Natl Acad Sci USA* 90, 8098-8102.

Stroll, G., Mueller, H.W., Trapp, B.D., Griffith, J.W. (1989) Oligodendrocytes but not astrocytes express apolipoprotein E after injury of rat optic nerve. *Glia* 2, 170-176.

Struble, R.G., Clark, HB. (1992) Olfactory bulb lesions in Alzheimer's disease. *Neurobiol Aging* 13, 469-73.

Struble, R.G., Short, J., Ghobrial, M., Nathan, B.P. (1999). Apolipoprotein E immunoreactivity in human and mouse olfactory bulb. *Neuroscience Letters* 267, 137-141.

Talamo, B.R., Rudel, R., Kosik, K., Lee, V.M., Neff, S., Adelman, L., Kauer, J.S., (1989) Pathological changes in olfactory neurons in patients with Alzheimer's disease. *Nature* 337, 736-739.

Vandenberg, J. (1973) Effects of central and peripheral anosmia on reproduction of female mice. *Physiology and Behavior* 10, 257-267.

Wang, Q.S., Tian, L., Huang, Y.L., Qin, S., He, L.Q., Zhou, J.N. (2002) Olfactory identification and apolipoprotein E epsilon 4 allele in mild cognitive impairment. *Brian Res* 951, 77-81.

Warner, M., Peabody, C., Flattery, J., Tinkleberg, J. (1986) Olfactory deficits in Alzheimer's disease. *Biol Psychiatry* 21, 116-118.

Weisgraber, K.H. (1994) Apolipoprotein E: Structure-function relationships. *Adv Protein Chem* 45, 249-302.

Weiss, S. (2003) What can our nose tell us about possible treatments for Alzheimer's disease? *Can J Neurol Sci* 30, 3.

Wetter, S. Murphy, C. (2001) Apolipoprotein E e4 positive individuals demonstrate delayed olfactory event-related potentials. *Neurobiol Aging* 22, 439-447.

Zannis, V.I., Breslow, J.L., Utermann, G., Mahley, R.W., Weisgraber, K.H., Havel, R.J., Goldstein, J.L., Brown, M.S., Schonfeld, G., Hazzard, W.R.,

Blum, C. (1982). Proposed nomenlature of apoE isoproteins, apoE genotypes, and phenotypes. *Journal of Lipid Research* 23, 911-914.

Zareparsi, S., Camicioli, R., Sexton,G., Bird, T., Swanson, P., Kaye, J., Nutt, J., Payami, H. (2002) Age at onset of Parkinson's disease and apolipoprotein E genotypes. *Am J Med Genet* 2, 156-161.

Zarrow, M., Gandelman, R., Deneberg, V., (1971) Lack of nest building and maternal behavior in the mouse following olfactory bulb removal. *Horm Behav.* 2, 227-238.

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