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AR, apoE, and cognitive function

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Abstract

Reduced androgen levels in aged men and women might be risk factors for age-related cognitive decline and Alzheimer's disease (AD). Ongoing clinical trials are designed to evaluate the potential benefit of estrogen in women and of testosterone in men. In this review, we discuss the potential beneficial effects of androgens and androgen receptors (ARs) in males and females. In addition, we discuss the hypothesis that AR interacts with apolipoprotein (apoE)4, encoded by ε4 and a risk factor for age-related cognitive decline and AD, and the potential consequences of this interaction.

Introduction

Increasing age and female sex are risk factors for developing mild cognitive impairments (MCI) and Alzheimer's disease (AD) (Farrer, Cupples, Haines, Hyman, Kukull, Mayeux, Myers, Pericak-Vance, Risch, and van Duijn, 1997; Fratiglioni, Viitanen, von Strauss, Tontodonati, Herlitz, and Winblad, 1997; Gao, Hendrie, Hall, and Hui, 1998; Katzman, Aronson, Fuld, Kawas, Brown, Morgenstern, Frishman, Gidez, Eder, and Ooi, 1989; for review (Raber, Huang, and Ashford, 2004)). Therefore, evaluating risk factors and therapeutic strategies for these age-related impairments become increasingly important. We will discuss the potential roles of androgens and androgen receptors (ARs) in cognitive function in humans and mice and potential interactions of androgens and ARs with apolipoprotein E.

Sex differences in spatial learning and memory

There are sex differences in spatial learning and memory in humans (Berger-Sweeney, Arnold, Gabeau, and Mills, 1995; Reinisch, Ziemba-Davis, and Sanders, 1991; Roof and Havens, 1992) and rodents (Beatty, 1979; van Haaren, van Hest, and Heinsbroek, 1990). Spatial learning and memory is relevant, as it is impaired in Alzheimer's disease (AD). Some, but not all (Bucci, Chiba, and Gallagher, 1995), studies of spatial learning and memory in rodents have shown that males learn more quickly than females and exhibit superior performance in a variety of mazes (Bucci et al., 1995; Einon, 1980; Frye, 1995; Joseph and Gallagher, 1980; Luine, Renner, and McEwen, 1986; McEwen, 1988; McNemar and Stone, 1932; Means and Dent, 1991; Roof, 1993). Spatial learning and memory have been attributed to the hippocampus (Kesner, Bolland, and Dakis, 1993; Olton, Branch, and Best, 1978) and sex differences in hippocampal structure may contribute to sex differences in spatial learning and memory (McEwen, Alves, Bulloch, and Weiland, 1997). Different strategies used by males and females to solve spatial tasks may contribute to these sex differences. Females tend to rely more on local cues and landmarks,

while males rely more on the spatial relationship between fixed points. Rodent lesion and pharmacological studies support that females may be more susceptible to spatial memory impairments than males (Hörtnagl, Hansen, Kindel, Schneider, Tamer, and Hanin, 1993; Kolb and Cioe, 1996).

Androgens, the hippocampus, and sex differences in learning and memory

Sex steroids, which cause sex differences in brain organization (organizational effect) (Beatty, 1979) and in behaviors in adulthood (activational effect) (Joseph, Hess, and Birecree, 1978), might contribute to sex differences in spatial learning and memory (Joseph et al., 1978).

Androgens might contribute to the sex differences in spatial learning and memory. In adulthood, testosterone enhances spatial memory (Alexander, Packard, and Hines, 1994; Flood and Roberts, 1988; Ishunina, Fisser, and Swaab, 2002; Janowsky, Oviatt, and Orwoll, 1994; Raber, Bongers, LeFevour, Buttini, and Mucke, 2002; Vazquez-Pereyra, Rivas-Arancibia, and Castillo, 1995) and androgens also enhance both short-term and long-term emotional learning and memory (Vazquez-Pereyra et al., 1995). In addition, post-training administration of androgens to ovariectomized rats enhanced spatial and emotional learning and memory (Frye and Lacey, 2001). However, in some studies androgens impaired spatial learning and memory (Galea, Kavaliers, Ossenkopp, and Hampson, 1995; Gouchie and Kimura, 1991; Goudsmit, Van De Poll, and Swaab, 1990; Hampson, 1995; Naghdi, Nafisy, and Majlesi, 2001).

AR signaling

Androgens such as dihydrotestosterone (DHT) initiate many of their effects by binding to AR (Barley, Ginsberg, Greenstein, MacLusky, and Thomas, 1975). The human AR gene contains 8 exons encoding a 110 kDa member of the nuclear receptor superfamily (Simental, Sar, and Wilson, 1992). The first exon encodes the N-terminal domain containing the major transactivation function (AF-1), which interacts with the glutamine-rich region of steroid receptor coactivator-1 (SRC-1) (Robyr, Wolffe, and Wahli, 2000). SRC-1 in turn can interact with the global activator CBP/p300, which can directly interact with ARs and together with SRC-1 synergistically induce AR transactivation (McKenna, Lanz, and O'Malley, 1999). CBP/p300. The second and third exons encode the DNA binding domain (DBD), which enable ARs to bind the regulatory part of target genes (Umesono and Evans, 1989). Exons 4–8 encode the ligand binding domain (LBD) and a minor transactivation function (AF-2). AF-2 also interacts with SRC-1 (Umesono and Evans, 1989). The activity of SRC-1 is regulated by phosphorylation by mitogen-activated protein kinase (MAPK), which is stimulated by androgens. In the absence of hormone, the LBD of AR prevents the transactivation of AF-1 and the required three-dimensional structures for SRC-1 interaction at AF-2. Hormone binding enables AF-1 and AF-2 to associate with a multi-subunit complex of coactivator proteins. This complex is proposed to associate additional proteins required for interaction of AR to RNA polymerase II. AR can also be activated in a ligand-independent fashion. For example, growth factors and Interleukin-6 (IL-6) activate ARs in the absence of androgen. Point mutations at different sites in exons 2–8 have been reported for partial and complete forms of androgen insensitivity. In human prostate cancer cells, the ligand-independent activation of ARs by IL-6 involves phosphorylation of SRC-1 by MAPK (Ueda, Mawki, Bruchovsky, and Sadar, 2002). However, it should be emphasized that SRC-1 phosphorylation requires the presence of IL-6 and by itself is not sufficient for ligand-independent transactivation of ARs (Ueda et al., 2002).

AR not bound to hormone is localized in the cytoplasm as a complex with heat-shock proteins and immunophilins. When androgens bind, AR changes its confirmation, dissociates from the complex, forms homodimers, and unmasks its nuclear localization signal. This signal can then bind importins, which transport the hormone-bound AR into the nucleus. ARs can modulate

gene transcription by binding to specific androgen response elements in the DNA, but are also able to trans-activate and trans-repress without directly interacting with specific DNA elements. The calcium-binding protein calreticulin is able to dissociate AR from the DNA by competing for the DBD of the AR and may export AR back to the cytoplasm. Hormone-dissociated AR moves back to the cytoplasm ready for another nuclear translocation. In addition to nuclear receptor pathways (genomic effects), androgens might also mediate their effects by mechanisms not involving nuclear receptors (nongenomic effects).

ARs in cognitive areas in the brain

Increasing evidence supports an important role for androgens and ARs in hippocampal function. The levels of AR mRNA and AR binding in the hypothalamus and hippocampus of male rats are similar (Burgess and Handa, 1993; Kerr, Allore, Beck, and Handa, 1995). In addition, androgens increase spine-synapse density in the CA1 of ovariectomized female rodents (Leranth, Hajszan, and MacLusky, 2004; Woolley, Gould, Frankfurt, and McEwen, 1990). While there are no absolute sexual differences in AR mRNA (Simerly, Chang, Muramatsu, and Swanson, 1990) and AR binding (Handa, Reid, and Resko, 1986) in adult rat brain, differences in circulating androgen levels could modulate AR function in a sex-dependent fashion. Neonatal castration of males or prenatal treatment of DHT to females reverses the sex difference in CA3 pyramidal cell layer volume and neuronal cells soma sizes, which correlates with water maze performance in adulthood (Isgor and Sengelaub, 1998). Circulating androgens may also be important in sustaining neuron synapses in CA3 later in life. The decline in synaptic density in CA3 neurons in aged male rats is associated with age-related deficits in spatial ability (Geinisman, Detoledo-Morrell, Morrell, and Heller, 1995). Further, DHT selectively modulates the induction of the mRNA for the cellular immediate early gene c-fos in the CA1 region of the hippocampus following exposure to a novel open field for 20 min (Kerr, Beck, and Handa, 1996). Finally, DHT attenuates the binding of the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 in CA1 (Kus, Handa, Hautman, and Beitz, 1995) and modulates NMDA-mediated depolarization in CA1 pyramidal cells (Pouliot, Handa, and Beck, 1996).

Apolipoprotein E (apoE) and AR

ApoE plays an important role in the metabolism and redistribution of lipoproteins and cholesterol. The three major human apoE isoforms are encoded by distinct alleles (ε2, ε3, and ε4). They differ in having cysteine (Cys) or arginine (Arg) at positions 112 and 158 and differ in metabolic properties (Mahley and Huang, 1999). ApoE2 (Cys-112 and Cys-158) binds defectively to low-density lipoprotein (LDL) receptors. ApoE3 (Cys-112, and Arg-158) binds normally to LDL receptors and is associated with normal lipid metabolism. ApoE4 (Arg-112, Arg-158) binds normally to LDL receptors but is associated with elevated cholesterol levels. While apoE3 shows a lipoprotein preference for high-density lipoprotein (HDL), apoE4 show a lipoprotein preference for very low-density lipoprotein (VLDL).

In the brain, apoE has been implicated in development, regeneration, neurite outgrowth, and neuroprotection (Mahley, 1988). The effects of androgens on spine-synapse density (Leranth et al., 2004; Woolley et al., 1990) described above and the effects of apoE on neurite outgrowth might be one pathway involving both apoE and AR. Following injury, apoE in the nervous system has been implicated in efforts to restore neuronal function by remodeling (Laskowitz, Horsburgh, and Roses, 1998; Poirier, 1994; Weisgraber, Pitas, and Mahley, 1994), which may involve effects of apoE on cholesterol and phospholipid metabolism (Boyles, Zoellner, Anderson, Kosik, Pitas, Weisgraber, Hui, Mahley, Gebicke-Haerter, Ignatius, and Shooter, 1989; Mahley, 1988), on lipid efflux, on its cellular trafficking (Ji, Pitas, and Mahley, 1998; Nathan, Chang, Bellosta, Brisch, Ge, Mahley, and Pitas, 1995), on microtubule function. For example, glia, a major source of apoE (Boyles et al. 1985; Pitas et al., 1987), might recycle

cholesterol from neuronal membranes by packaging it with apoE for neuronal uptake to promote neurite outgrowth (Boyles et al., 1989; Laskowitz et al., 1998; Poirier, Baccichet, Dea, and Gauthier, 1993). The ability to remodel neurons is apoE isoform-dependent, as it is impaired in AD patients with an ε4 allele who show reduced dendritic remodeling of pyramidal and subcortical neurons, while AD patients with two ε4 alleles show a shift towards proximal branching and lack a relationship between dendritic growth in response to neuronal loss (Arendt, Bruckner, Gertz, and Marcova, 1998; Arendt, Schindler, Brückner, Eschrich, Bigl, Zedlick, and Marcova, 1997). The differential ability of apoE isoforms to remodel neurons by supporting neurite outgrowth (either modulating the length of the longest neurite or the total length of all neurites of a neuron) has been reported in Neuro2A cells (Bellosta, Mahley, Sanan, Murata, Newland, Taylor, and Pitas, 1995), dorsal root ganglion cells (Nathan, Bellosta, Sanan, Weisgraber, Mahley, and Pitas, 1994), primary cortical neurons (Nathan et al., 2002), and in mouse organotypic hippocampal slice cultures (Teter et al., 1999, 2002) using recombinant apoE in the presence or absence of lipoproteins, apoE derived from transfected or apoE derived from human apoE transgenic mice. These effects of apoE on neurite outgrowth are mediated by binding of apoE to the low-density lipoprotein receptor-related protein (Holtzman et al., 1995; Nathan et al, 2002). While apoE3 increased neurite outgrowth, in most, but not all, studies apoE4 either had no effect or decreased neurite outgrowth, as compared to no apoE, and its effect dominated over that of apoE3 when both were present (Holtzman, Pitas, Kilbridge, Nathan, Mahley, Bu, and Schwartz, 1995). The lack of an effect of apoE4, as compared to no apoE, and a dominant effect of apoE4 over apoE3 was also seen *in vivo* when age-dependent reductions in synaptophysin-immunoreactive presynaptic terminals were quantified in apoE transgenic mice (Buttini et al., 2000) and when neuritic sprouting was quantified in organotypic hippocampal slice cultures of apoE transgenic mice (Teter, Xu, Gilbert, Roses, Galasko, and Cole, 1999). The differential effects of apoE isoforms on neurite outgrowth might be due to a stronger interaction of apoE4 than apoE3 with AR, resulting in reduced AR-mediated neuritic sprouting in the presence of apoE4. However, it should be pointed out that at this moment it is not clear whether interaction of apoE4 with AR would be harmful because as a result of this interaction less AR is available for AR-mediated signaling (loss of AR function) and therefore this interaction interferes with the regular AR function or whether this interaction would be harmful as the apoE4-AR complex causes detrimental effects by itself (gain of AR misfunction).

Compared with ε2 and ε3, ε4 increases the risk of developing Alzheimer's disease (AD) (Corder, Robertson, Lannfelt, Bogdanovic, Eggertsen, Wilkins, and Hall, 1998; Corder, Saunders, Pericak-Vance, and Roses, 1995; Strittmatter, Saunders, Schmechel, Pericak-Vance, Engchild, Salvesen, and Roses, 1993). ApoE is associated with the pathological hallmarks of AD. The severity of AD pathology is influenced by ApoE genotype. ApoE genotype also has a detrimental impact on AD pathology when it occurs in patients with other conditions, including progressive supranuclear palsy (PSP) (Tsuboi, Joseph, Cookson, and Dickson, 2003) and Down Syndrome (DS) (Del Bo, Comi, Bresolin, Castelli, Conti, Degiuli, Ausenda, and Scarlato, 1997). While the ε4 allele frequency is similar in control subjects with no neurodegenerative condition and in PSP patients with minimal or no AD pathology, it is significantly higher in PSP patients who have concomitant AD pathology or the pathological signs of normal aging. The detrimental effects of ε4 are not limited to AD. Compared with ε2 and ε3, ε4 also increases the risk of developing cognitive impairments following neurotrauma (Nathoo, Chetty, van Dellen, and Barnett, 2003), ischemia (Guangda, Bangshun, Xiujian, and Yangzhong, 1999; Roses and Saunders, 1997), cardio-pulmonary bypass surgery (Tardiff, Newman, Saunders, Strittmatter, Blumenthal, White, Croughwell, Davis, Roses, and Reves, 1997), and human immunodeficiency virus (HIV) infection (Corder et al., 1998), as well as cognitive impairments that occur with normal aging (Howieson, Camicioli, Quinn, Silbert, Care, Moore, Dame, Sexton, and Kaye, 2003) and in the context of Parkinson Disease (Tang, Xie, Xu, Lin, and Ren, 2002; Zareparsi, Camicioli, Sexton, Bird, Swanson, Kaye, Nutt, and

Payami, 2002). In multiple sclerosis patients, ε4 is associated with a worsened disease progression (Enzinger, Ropele, Strasser-Fuchs, Kapeller, Schmidt, Poltrum, Schmidt, Hartung, and Fazekas, 2003; Fazekas, Strasser-Fuchs, Kolleger, Berger, Kristoferitsch, Schmidt, Enzinger, Schiefermeier, Schwarz, Kornek, Reindl, Huber, Grass, Wimmer, Vass, Pfeiffer, Hartung, and Schmidt, 2001; Masterman, Zhang, Hellgren, Salter, Anvret, Lilius, Lannfelt, and Hillert, 2002; Schmidt, Barcellos, DeSombre, Rimmler, Lincoln, Bucher, Saunders, Lai, Martin, Vance, Oksenberg, Hauser, Pericak-Vance, Haines, and Group, 2002). In contrast, apoE4 might be protective against liver damage caused by the hepatitis C virus (Wozniak, Itzhaki, and Faragher, 2002). These results indicate that apoE affects fundamental biological processes not unique to AD.

Various mechanisms have been proposed to mediate the differential effects of human apoE isoforms on brain function. We hypothesize that apoE4 has detrimental effects on androgen- and AR-mediated pathways.

Mice deficient in mouse apoE (*Apoe*$^{-/-}$) with or without transgenically expressing human apoE isoforms in brain under the control of neuron-specific or astrocyte-specific promoters and mice expressing human apoE isoforms under the control of the endogenous mouse apoE promoter have been used to define the physiological and pathological role of apoE. In brain, *Apoe*$^{-/-}$ mice show no obvious alterations in brain development but show age-dependent structural and functional alterations. Expression of apoE4 in *Apoe*$^{-/-}$ mice leads to age- and sex-dependent impairments in spatial learning and memory (Raber, Wong, Buttini, Orth, Bellosta, Pitas, Mahley, and Mucke, 1998; Raber, Wong, Yu, Buttini, Mahley, Pitas, and Mucke, 2000). At 6 months of age, apoE4 female showed impairments in hippocampus-dependent spatial learning and memory in the water maze. These impairments were observed in mice that express apoE4 in neurons (Raber et al., 1998; Raber et al., 2000) or astrocytes (Van Meer, Acevedo, and Raber, 2007) and are therefore independent of the cellular source of apoE. They require apoE4 and are not seen in *Apoe*$^{-/-}$ female mice, consistent with a pathogenic gain of function of apoE4. Effects of apoE4 on AR function might contribute to these cognitive impairments. Treatment of 6-month apoE4 female mice with testosterone or dihydrotestosterone antagonized these impairments (Raber et al., 2002). Further support for a role of AR in these effects comes from recent studies showing protective effects of two tissue selective androgen receptor modulators (SARMs, AC-262536 or SARM AC-264184) against detrimental effects of 6-month-old apoE4 on water maze performance (Acevedo, Gardell, Bradley, Piu, and Raber, 2008). The treatments were administered subcutaneously daily, starting one week prior to cognitive testing. These data demonstrate that, like testosterone and DHT, SARMs, antagonize cognitive deficits in 6-month-old apoE4 female mice. SARMs are tissue specific and do not have side effects associated with the use of conventional androgens. Effects of SARMs outside the brains have been reported. For example, in rats SARMs restored levator ani muscle mass to levels expressed in intact controls (Sun, Robl, Wang, Huang, Kuhns, Lupisella, Beehler, Golla, Sleph, Seethala, Fura, Krystek, An, Malley, Sack, Salvati, Grover, Ostrowski, and Hamann, 2006), muscle strength and body composition and prevented bone loss in orchidectomized animals (Gao, Reiser, Coss, Phelps, Kearbey, Miller, and Dalton, 2005), prostate tumor growth, and orchidectomy-induced bone loss (Allan, Lai, Sbriscia, Linton, Haynes-Johnson, Bhattacharjee, Dodds, Fiordeliso, Lanter, Sui, and Lundeen, 2007). Our data indicate that SARMs are also promising for developing treatments in the brain.

Compared to age-matched wild-type and *Apoe*$^{-/-}$ mice, expression of apoE4, but not apoE3, reduces cytosolic AR binding to androgens in the neocortex of female and male mice (Raber et al., 2002). As there was no sex differences in cytosolic AR binding in any mouse genotype and female mice have lower circulating androgen levels than males, apoE4 female mice might be more susceptible to the effects of reduced AR binding on cognitive function. ApoE4 males were only relatively protected. When apoE3 and apoE4 male mice were treated with the AR

antagonist hydroxyflutamide (Malgor, Valsecia, Verges, and De Markowsky, 1998), spatial learning and memory impairments were seen only in apoE4 male mice (Raber et al., 2002). Male apoE3 and apoE4 mice have similar plasma testosterone levels, suggesting that differences in plasma testosterone are not required for the differential effects of hydroxyflutamide on cognitive performance in male apoE3 and apoE4 mice. In contrast to the situation with mice, in humans, low serum testosterone concentrations and the interaction of testosterone with apoE4, might each increase one's risk of developing AD (Hogervorst, Williams, Budge, Barnetson, Combrinck, and Smith, 2001).

Some of the biological effects of testosterone require aromatization of testosterone to estrogen. Such effects might be partially or completely mediated by ERs and, in the presence of apoE4, increased ER function compensate for reduced AR function. ApoE gene expression is upregulated by 17β-estradiol (Srivastava, Srivastava, and Averna, 1997). Thus in the context of apoE4, stimulating AR-related pathway might be a valuable therapeutic target, as ARs might be more responsive to androgens (Raber et al., 2002) than ERs are to estrogens (Yaffe, Haan, Byers, Tangen, and Kuller, 2000).

The lower circulating concentrations of endogenous androgens in females than males might contribute to their increased susceptibility to the detrimental effects of apoE4 on cognitive function. To test this hypothesis, the influence of chronic reduced circulating androgens by castration on the cognitive performance of 3-4-month-old *Apoe*$^{-/-}$, apoE3, and apoE4 male mice was assessed (Pfankuch, Rizk, Olsen, Poage, and Raber, 2005).

When exploratory activity in the open field was assessed, there were no effects of genotype, treatment, or genotype x treatment interactions that could influence performance in cognitive tests.

The mice were also tested for object recognition. After habituation to an open field, the mice were trained in three consecutive trials and than tested in two consecutive trials with a 5–min inter-trial interval. For both the training and testing sessions, three objects were placed in the open field, and the animal was allowed to explore for 10 min. All objects were only used once and replicas were used in subsequent trials. Five min after the training trials, the animals were tested for recognition of the novel location of one of the familiar objects. Five min after the novel location test, the animals were tested for novel object recognition. The time spent exploring each object during the training and testing sessions was recorded by an observer. There was no genotype difference in the total time the mice spent exploring the three objects over the five trials. When a familiar object was moved to a novel location, sham *Apoe*$^{-/-}$, apoE3, and apoE4 mice spent significantly more time exploring it in the novel location (Table 1). However, while castrated *Apoe*$^{-/-}$ and apoE3 male mice spent significantly more time exploring the familiar object in the novel location, castrated apoE4 male mice did not (Table 1). Five min after the novel location trial, mice were tested for novel object recognition. While there was no genotype difference in novel object recognition, there was a significant effect of castration (Table 1); castrated *Apoe*$^{-/-}$, but not apoE3 or apoE4, mice spent less time exploring the novel object than genotype-matched sham mice. The object recognition data support that apoE4 increases the susceptibility for hippocampal dysfunction, as following hippocampal lesions novel location recognition is impaired but novel object recognition is not.

In the visible sessions of the water maze test, all groups showed comparable swim speeds and there were no effects of genotype, treatment, or genotype x treatment interactions. All groups learned to locate the visible and hidden platform locations and there were no effects of genotype, treatment, or genotype x treatment interactions on time to locate the visible or hidden platform or on cumulative distance to the visible or hidden platform. However, genotype and treatment effects were identified in the probe trials (platform removed) following the second

(Probe 1) and third day (Probe 2) of hidden platform training. While already in Probe 1 sham-treated *Apoe*$^{-/-}$ and apoE3 male mice spent more time in the target quadrant than in any other quadrant, sham-treated apoE4 mice did not (Table 2). Compared to sham genotype-matched controls, castration impaired spatial memory retention in Probe 1 in *Apoe*$^{-/-}$, but not apoE3 or apoE4, mice. In Probe 2, all groups showed spatial memory retention.

Next, we tested emotional learning and memory using the passive avoidance test. On day 1, apoE4 mice required significantly more training than *Apoe*$^{-/-}$ and apoE3 mice (Table 3), indicating that the apoE4-induced cognitive impairments are not limited to spatial learning and memory. Although the mice were all trained to criterion, this might not be required to see comparable memory retention 24 later. Twenty-four hours later, all groups showed passive avoidance memory retention and there were no effects of genotype or treatment or genotype x treatment interactions (Table 3).

These data show that castration impaired novel location recognition in apoE4, but not apoE3 or *Apoe*$^{-/-}$, mice. In contrast, castration impaired novel object recognition and spatial memory retention in the water maze in *Apoe*$^{-/-}$, but not apoE3 or apoE4, mice. While apoE4 mice required more trials than apoE3 or *Apoe*$^{-/-}$ mice to reach the criterion during passive avoidance training, castration did not modulate passive avoidance learning or memory retention in any genotype. These data support that while circulating androgen levels might have differential roles in specific cognitive tests, they protect *Apoe*$^{-/-}$ male mice against the detrimental effects of apoE4 on novel location recognition.

Castration, which lowers androgen levels and androgen receptor levels, had no detrimental effect on the hidden water maze learning curve or spatial memory retention of apoE4 mice. In contrast, acute androgen receptor blockade with hydroxyflutamide caused striking impairments in the ability of apoE4 male mice to locate the hidden platform. Chronically reduced circulating androgen levels might be more appropriate than acutely reduce circulating androgen levels to test the hypothesis that lower circulating concentrations of endogenous androgens in females than males might contribute to their increased susceptibility to the detrimental effects of apoE4. However, it is possible that in apoE4 mice the time period of 3 months between the castration and behavioral testing allowed for compensatory changes not present following acute androgen receptor blockade and that these changes protected against behavioral deficits in the water maze. Alternatively, in the presence of apoE4, lowering the androgen receptor levels might be beneficial for spatial learning and memory in the water maze. In support of this possibility, castrated, but not sham-castrated, apoE4 mice showed spatial memory retention in the first probe trial.

To further explore the role of AR in effects of apoE4 on brain function we crossed apoE4 mice with mice lacking functional ARs. Mutant mice with a naturally occurring defect in the AR gene (testicular feminization mutant or *tfm*) are available on the C57/BL6J background(Couse and Korach, 1998; Tabibnia, Cooke, and Breedlove, 1999). A single point mutation in the N-terminal region of the AR gene results in a premature stop codon and the expression of nonfunctional truncated androgen receptors (*tfm*-AR). Because the trait is X-linked, only males are androgen insensitive. The lack of functional androgen receptors results in complete infertility of the *tfm* male mice. Female *tfm* mice are heterozygous, carrying one wild-type and one *tfm* copy of the androgen receptor. Therefore, *tfm* male, but not female, mice are suitable for conclusively determining the role of AR in the effects of apoE4 on cognition. *Tfm* male mice have lower testosterone levels than controls (Jones, Pugh, Hall, Channer, and Jones, 2003), so there is no concern that due to the lack of functional AR testosterone and estradiol levels are elevated. To determine the role of AR in the cognitive effects of apoE4, we backcrossed *tfm* mice onto the *Apoe*$^{-/-}$ background to eliminate mouse apoE and crossed the *tfm/Apoe*$^{-/-}$ female mice with apoE4 transgenic male mice and the sex (Mroz, Carrel, and Hunt,

1999), presence of apoE4 (Raber et al., 2002), and *tfm* mutation (Rizk-Jackson, Robertson, and Raber, 2007) of the offspring was determined. The presence of the *tfm* mutation was determined by DNA sequencing and by confirming the *tfm* male phenotype (feminine exterior). Only male mice were behaviorally tested. We behaviorally compared *Apoe*$^{-/-}$, apoE4, *tfm*, and *tfm*/apoE4 male mice. As no effort was made to equalize circulating androgen levels across the groups, *tfm* and *tfm*/apoE4 male mice would be expected to have lower circulating androgen levels than the *Apoe*$^{-/-}$ and apoE4 male mice (Jones, Pugh, Hall, Channer, and Jones, 2003).

In the object recognition test, Apoe$^{-/-}$, apoE4, and tfm mice spent significantly more time exploring the familiar object in the novel location. In contrast, tfm/apoE4 mice did not. Five min after the novel location trial, mice were tested for novel object recognition. All groups of mice spent more time exploring the novel object than the familiar objects, but there were no group differences. In the water maze test, all groups learned to locate the visible and hidden platform and there were no group differences in ability to locate the visible or hidden platform. However, group differences were identified in the probe trial following the second day of hidden platform training. While Apoe$^{-/-}$ and apoE4 mice spent more time in the target quadrant than any other quadrant, tfm and tfm/apoE4 mice did not. Following one additional day of hidden platform training, tfm mice still did not show spatial memory retention. In contrast, all other groups showed spatial memory retention in the probe trial following the third day of hidden platform training.

ApoE4 lowers androgen binding to cytosolic AR (Raber et al., 2002). Rather than decreasing cytosolic AR levels, apoE4 might reduce androgen binding to cytosolic AR by interacting with AR. Our preliminary data indicate that mouse apoE colocalizes with AR in astrocytes. In addition, co-immunoprecipitation on extracts of cortex and hippocampus with an anti-apoE antibody and probing the resultant pull-downs with an anti-AR antibody reveals AR in pull downs of cortical and hippocampal extracts from NSE-apoE4 and NSE-apoE3, but not Apoe$^{-/-}$, mice. In cortical extracts, more AR was pulled down from extracts of NSE-apoE4 than NSE-apoE3 mice. Finally, our preliminary data indicate that GST-apoE proteins pull down ARs from cortical extracts of Apoe$^{-/-}$ mice and that GST-apoE4 might pull down more ARs than GST-apoE3 or GST-apoE2. Castration lowers androgen and AR levels (Apostolinas, Rajendren, Dobrjansky, and Gibson, 1999; Beatty, 1979; Kerr et al., 1995; Lu, McKenna, Cologer-Clifford, Nau, and Simon, 1998) and this interaction might also contribute to the lower sensitivity of AR-mediated signaling in testosterone-treated stimulated peritoneal macrophages of castrated apoE4 than apoE3 transgenic male mice (Brown, Colton, and Vitek, 2003). The impairments of tfm/apoE4, but not apoE4, mice in novel location recognition and spatial memory retention in the first water maze probe trial indicate that AR might protect against the detrimental effects of apE4 by interacting with it. Alternatively, AR might antagonize the effects of apoE4 on cognitive function through AR-mediated signaling.

Potential effects of apoE-AR interactions on anti-inflammatory effects of androgens

AR expression in microglia increases after injury, suggesting a role for AR in the immune response (Garcia-Ovejero, Veiga, Garcia-Segura, and Doncarlos, 2002). Consistent with such a role, DHT dose-dependently decreases production of nitric oxide and TNF-α (Brown, Xu, Okhubo, Vitek, and Colton, 2007). Strikingly, this protective effect of DHT was significantly lower in primary cultures of microglia from apoE4 than apoE3 mice. This was not due to potential differences in AR mRNA or protein expression. Potential apoE4-AR interactions could account for this result. Consistent with this hypothesis, DHT-mediated AR signal transduction was less robust in microglia from apoE4 than apoE3 mice. The DHT inhibition of LPS-induced activation of the MAPK pathway (including phosphorylation of p38 MAPK and p54/p56 Janus kinase) was much less profound in microglia from apoE4 than apoE3 mice

(Brown et al., 2007). MAPK might be important for the AR-apoE interactions. As described above in the section on AR signaling, the activity of SRC-1 is regulated by phosphorylation of MAPK by androgens. In contrast to androgens, apoE reduced phosphorylation of MAPK in human embryonic kidney cells (McGarvey, Nguyen, and Malkowicz, 2005) and in response to serum growth factors in rat embryonic fibroblasts(Ho, Deckelbaum, Chen, Vogel, and Talmage, 2001) and.

ApoE4 might also have detrimental effects on anti-inflammatory effects of androgen and AR function outside the brain. Peritoneal macrophages, isolated from transgenic mice that expressed either the human apoE3 or apoE4 under the control of the mouse *ApoE* promoter and stimulated with interferon-γ either alone or in combination with synthetic double-stranded RNA (Poly I:C) or lipolysaccharide, from male apoE4 homozygous mice showed a greater release of the inflammatory mediators nitric oxide and tumor necrosis factor-α (TNF-α) than those from male apoE3 homozygous mice (Brown et al., 2003; Brown, Wright, Colton, Sullivan, Laskowitz, and Vitek, 2002). This effect was sex-dependent, as it was not seen in peritoneal macrophages from female apoE3 and apoE4 homozygous mice, and involved androgens, consistent with the increase in nitric oxide synthase (NOS) activity in the brains of rats following castration and decrease of NOS activity following administration of the dihydrotestosterone (Sing, Pervin, Shryne, Gorski, and Chaudhuri, 2000). After IFN-γ + LPS stimulation, TNF-α release was higher in peritoneal macrophages from castrated apoE3 homozygous mice than in macrophages from sham-treated or castrated + testosterone-treated apoE3 homozygous mice; these effects were not seen in stimulated peritoneal macrophages from castrated and castrated + testosterone-treated apoE4 homozygous mice (Brown et al., 2003). A potential stronger interaction between apoE4 and AR than apoE3 and AR might require a higher androgen dose to see a protective effect of AR in the context of apoE4 than apoE3. In that case, a lower dose on androgen might be able to displace apoE3, but not apoE4, from the AR complex. Such effects of apoE on peripheral AR functions might have consequences for the brain. Increased levels of inflammatory proteins have been found in the plasma and brains of patients with dementia. Plasma levels of inflammatory proteins are increased before clinical onset of dementia and high levels of a1-antichymotrypsin and IL-6 were associated with increased risk to develop dementia (Engelhart, Geerlings, Meijer, Kiliaan, Ruitenberg, van Swieten, Stijnen, Hofman, Witteman, and Breteler, 2004). Importantly, peripheral inflammation was shown to activate brain microglia and produce chronically elevated pro-inflammatory cytokines (Qin, Wu, Block, Liu, Breese, Hong, Knapp, and Crews, 2007).

ApoE and ARs in humans

ApoE4 also has effects on AR function in the cholinergic basal forebrain, participating in behavioral processes such as attention and memory associated with aging and AD (Ishunina et al., 2002). In the vertical limb of the diagonal band of Broca, a major cholinergic nucleus in the basal forebrain affected in AD, the presence of AD pathology or an ε4 allele negatively correlates with the percentage of AR-positive neurons in women, but not in men.

Altered AR function resulting from a polymorphism in the glutamine (CAG) repeats in exon 1 of the AR gene might increase AD risk; 20 or fewer CAG repeats are associated with increased AD risk in men, but not women, particularly in those lacking the ε4 allele (Lehmann, Butler, and Warden, 2003). As polymorphisms in the CAG repeats correlate with altered AR transcription (Tut, Ghadessi, Trefiro, Pinsky, and Yong, 1997) and translation (Choong, Kemppaines, Zhou, and Wilson, 1996)(the more CAGs the less active AR), these data indicate that enhanced AR function might be beneficial for ε4, but not for non-ε4, carriers. However, it should be emphasized that although testosterone levels are associated with cognitive function in the elderly (see below), the potential effects of polymorphisms in CAG repeats on circulating

androgen levels are not clear. Therefore it is possible that 20 or fewer CAG repeats are associated with reduced circulating androgen levels and therefore reduced rather than enhanced AR function.

Sex, apoE4 and salivary testosterone levels in nondemented elderly

Sex-dependent effects of apoE4 on testosterone levels might further contribute to the increased susceptibility of women to age-related cognitive decline. Recently, we reported the effects of apoE4 on cognitive performance and salivary testosterone levels in 116 nondemented elderly women and men, ranging in age from 62 to 92 (mean age ± SEM, 81.60 ± 0.57 years) and recruited from two neighboring retirement communities in Portland, Oregon. For salivary testosterone levels, there was a sex x ε4 interaction, with higher salivary testosterone levels in ε4 carrying than non-ε4 carrying men and lower salivary testosterone levels in ε4 carrying than non-ε4 carrying women (Berteau-Pavy, Park, and Raber, 2007). These data suggest that in ε4 carrying men increased salivary testosterone levels might function as compensatory change to prevent more severe cognitive impairments, as in older men testosterone levels are positively associated with cognitive function (Barrett-Connor, Goodman-Gruen, and Patay, 1999; Cherrier, Asthana, Plymate, Baker, Matsumoto, Peskind, Raskind, Brodkin, Bremner, Petrova, LaTendresse, and Craft, 2001; Cherrier, Matsumoto, Amory, Asthana, Bremner, Peskind, Raskind, and Craft, 2005; Janowsky, Chavez, and Orwoll, 2000; Janowsky et al., 1994; Kenny, Bellantonio, Gruman, Acosta, and Prestwood, 2002; Tan and Pu, 2003; Vermeulen, 2001; Yaffe, Lui, Zmuda, Ferrell, and Cauley, 2002). In men, the difference between immediate and delayed novel image recognition correlated with salivary testosterone levels ($r = 0.473$, $p = 0.015$). When the data were grouped by performance level, ε4-carrying men had the highest salivary testosterone levels at each performance level. Similarly, during the hidden session of a virtual reality spatial navigation test (Memory Island), salivary testosterone levels in male ε4 carriers correlated with time to reach the target location (latency) ($r = -0.92$, $p = 0.03$), cumulative distance to the target location ($r = 0.87$, $p = 0.06$), % trials in which the target was located within 2 min ($r = 0.85$, $p = 0.07$), and % time spent in the quadrant of the virtual island previously containing the target ($r = 0.89$, $p = 0.04$).

Summary

Increasing evidence supports a role for androgens and AR in cognitive function. As apoE4 reduces androgen binding to AR, this effect might contribute to increased susceptibility to cognitive impairments in apoE4 female mice and apoE4-carrying women. As androgens can antagonize detrimental effects of apoE4 effects on cognitive function and AR binding and might also protect against other AD-related factors, increased efforts are warranted to further explore the role of AR in health and disease.

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References

Acevedo S, Gardell LR, Bradley SR, Piu F, Raber J. Selective androgen receptor modulators antagonize apolipoprotein E4-induced cognitive impairments. Lett Drug Design Discovery. 2008in press

Alexander G, Packard M, Hines M. Testosterone has rewarding affective properties in male rats. Behav Neurosci 1994;108:424–428. [PubMed: 8037886]

Allan G, Lai M, Sbriscia T, Linton O, Haynes-Johnson D, Bhattacharjee S, Dodds R, Fiordeliso J, Lanter J, Sui Z, Lundeen S. A selective androgen receptor modulator that reduces prostate tumor size and prevents orchidectomy-induced bone loss in rats. J Steroid Biochem Mol Biol 2007;103:76–83. [PubMed: 17049844]

Apostolinas S, Rajendren G, Dobrjansky A, Gibson M. Androgen receptor immunoreactivity in specific neural regions in normal and hypogonadal mice: effect of androgens. Brain Res 1999;817:19–24. [PubMed: 9889303]

Arendt T, Bruckner MK, Gertz HJ, Marcova L. Cortical distribution of neurofibrillary tangles in Alzheimer's Disease matches the pattern of neurons that retain their capacity of plastic remodeling in the adult brain. Neurosci 1998;83(4):991–1002.

Arendt T, Schindler C, Brückner MK, Eschrich K, Bigl V, Zedlick D, Marcova L. Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein ε4 allele. J Neurosci 1997;17:516–529. [PubMed: 8987775]

Barley J, Ginsberg M, Greenstein B, MacLusky N, Thomas P. An androgen receptor in rat brain and pituitary. Brain Res 1975;100:383–393. [PubMed: 172193]

Barrett-Connor E, Goodman-Gruen D, Patay B. Endogenous sex hormones and cognitive function in older men. J Clin Endocrinol Metab 1999;84:3681–3685. [PubMed: 10523014]

Beatty WW. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. Horm Behav 1979;12:112–163. [PubMed: 573741]

Bellosta S, Mahley RW, Sanan DA, Murata J, Newland DL, Taylor JM, Pitas RE. Macrophage-specific expression of human apolipoprotein E reduces atherosclerosis in hypercholesterolemic apolipoprotein E–null mice. J Clin Invest 1995;96:2170–2179. [PubMed: 7593602]

Berger-Sweeney J, Arnold A, Gabeau D, Mills J. Sex differences in learning and memory in mice: Effects of sequence of testing and cholinergic blockade. Behav Neurosci 1995;109:859–873. [PubMed: 8554711]

Berteau-Pavy F, Park B, Raber J. Effects of sex and APOE e4 on object recognition and spatial navigation in the elderly. Neuroscience 2007;147:6–17. [PubMed: 17509769]

Boyles JK, Zoellner CD, Anderson LJ, Kosik LM, Pitas RE, Weisgraber KH, Hui DY, Mahley RW, Gebicke-Haerter PJ, Ignatius MJ, Shooter EM. A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. J Clin Invest 1989;83:1015–1031. [PubMed: 2493483]

Brown, C.; Colton, C.; Vitek, M. Anti-inflammatory properties of testosterone are mediated by apolipoprotein E genotype. Developmental Origins of Aging in the Brain and Blood Vessels; The 4th Neurobiology of Aging Conference; 2003. p. P37

Brown C, Wright E, Colton C, Sullivan PM, Laskowitz DT, Vitek M. Apolipoprotein E isoform mediated regulation of nitric oxide release. Free Radic Biol Med 2002;32:1071–1075. [PubMed: 12031891]

Brown C, Xu Q, Okhubo N, Vitek M, Colton C. Androgen-mediated immune function is altered by the apolipoprotein E gene. Endocrinology 2007;148:3383–3390. [PubMed: 17395708]

Bucci DJ, Chiba AA, Gallagher M. Spatial learning in male and female Long-Evans rats. Behav Neurosci 1995;109:180–183. [PubMed: 7734074]

Burgess L, Handa R. Hormonal regulation of androgen receptor mRNA in the rat brain and anterior pituitary gland of the male rat. Molec Brain Res 1993;19:31–38. [PubMed: 8361343]

Cherrier MM, Asthana S, Plymate S, Baker L, Matsumoto AM, Peskind E, Raskind MA, Brodkin K, Bremner W, Petrova A, LaTendresse S, Craft S. Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology 2001;57:80–88. [PubMed: 11445632]

Cherrier MM, Matsumoto AM, Amory J, Asthana S, Bremner W, Peskind E, Raskind MA, Craft S. Testosterone improves spatial memory in men with Alzheimer disease and mild cognitive impairment. Neurology 2005;64:2063–2068. [PubMed: 15985573]

Choong C, Kemppaines J, Zhou ZX, Wilson E. Reduced androgen receptor gene expression with first exon CAG repeat expansion. Mol Endocrinol 1996;10:1527–1592. [PubMed: 8961263]

Corder EH, Robertson K, Lannfelt L, Bogdanovic N, Eggertsen G, Wilkins J, Hall C. HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. Nat Med 1998;4(10):1182–4. [PubMed: 9771753]

Corder EH, Saunders AM, Pericak-Vance MA, Roses AD. There is a pathologic relationship between ApoE-ε4 and Alzheimer's disease. Arch Neurol 1995;52:650. [PubMed: 7619017]

Couse JF, Korach KS. Exploring the role of sex steroids through studies of receptor deficient mice [see comments]. J Mol Med 1998;76(7):497–511. [PubMed: 9660168]

Del Bo R, Comi GP, Bresolin N, Castelli E, Conti E, Degiuli A, Ausenda CD, Scarlato G. The apolipoprotein E ε4 allele causes a faster decline of cognitive performances in Down's syndrome subjects. J Neurol Sci 1997;145:87–91. [PubMed: 9073034]

Einon D. Spatial memory and response strategies in rats: Age, sex, and rearing differences in performance. J Exp Psychol 1980;32:473–489.

Engelhart M, Geerlings M, Meijer J, Kiliaan A, Ruitenberg A, van Swieten J, Stijnen T, Hofman A, Witteman J, Breteler M. Inflammatory proteins in plasma and risk of dementia: The Rotterdam Study. Archives of Neurology 2004;61:668–672. [PubMed: 15148142]

Enzinger C, Ropele S, Strasser-Fuchs S, Kapeller P, Schmidt H, Poltrum B, Schmidt R, Hartung H, Fazekas F. Lower levels of N-acetylaspartate in multiple sclerosis patients with the apolipoprotein E epsilon 4 allele. Arch Neurol 2003;60:65–70. [PubMed: 12533090]

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. J Am Med Assoc 1997;278:1349–1356.

Fazekas F, Strasser-Fuchs S, Kolleger H, Berger T, Kristoferitsch Schmidt H, Enzinger C, Schiefermeier M, Schwarz C, Kornek B, Reindl M, Huber K, Grass R, Wimmer G, Vass K, Pfeiffer K, Hartung H, Schmidt R. Apolipoprotein E epsilon 4 is associated with rapid progression of multiple sclerosis. Neurology 2001;57:853–857. [PubMed: 11552016]

Flood J, Roberts E. Dehydroepiandrosterone sulfate improves memory in aging mice. Brain Res 1988;118:178–181. [PubMed: 2968829]

Fratiglioni L, Viitanen M, von Strauss E, Tontodonati V, Herlitz A, Winblad B. Very old women at highest risk of dementia and Alzheimer's disease: incidence data from the Kungsholmen Project, Stockholm. Neurology 1997;48(1):132–8. [PubMed: 9008508]

Frye CA. Estrus-associated decrements in a water maze task are limited to acquisition. Physiol Behav 1995;57:5–14. [PubMed: 7878125]

Frye CA, Lacey EH. Posttraining androgens' enhancement of cognitive perfromance is temporally distinct from androgens' increases in affective behavior. Cognitive Affect Behav Neurosci 2001;1:172–182.

Galea LAM, Kavaliers M, Ossenkopp KP, Hampson E. Gonodal hormone levels and spatial learning performance in the Morris water maze in male and female meadow voles, Microtus pennsylvanicus. Horm Behav 1995;29:106–125. [PubMed: 7782059]

Gao S, Hendrie HC, Hall KS, Hui S. The relationships between age, sex, and the incidence of dementia and Alzheimer disease: A meta-analysis. Arch Gen Psychiatry 1998;55:809–815. [PubMed: 9736007]

Gao W, Reiser P, Coss C, Phelps M, Kearbey J, Miller D, Dalton J. Selective androgen receptor modulator treatment improves muscle strength and body composition and prevents bone loss in orchidectomized rats. Endocrinology 2005;146:4887–4897. [PubMed: 16099859]

Garcia-Ovejero D, Veiga S, Garcia-Segura L, Doncarlos L. Glial expression of estrogen and androgen receptors after brain injury. J Comp Neurol 2002;450:256–271. [PubMed: 12209854]

Geinisman Y, Detoledo-Morrell L, Morrell F, Heller RE. Hippocampal markers of age-related memory dysfunction: behavioral, elctrophysiological and morphological perspectives. Prog Neurobiol 1995;45:223–252. [PubMed: 7777673]

Gouchie C, Kimura D. The relationship between testosterone levels and cognitive ability patterns. Psychoneuroendocrinology 1991;16(4):323–34. [PubMed: 1745699]

Goudsmit E, Van De Poll NE, Swaab DF. Testosterone fails to reverse spatial memory decline in aged rats and impairs retention in young and middle-aged animals. Behav Neural Biol 1990;53:6–20. [PubMed: 2302142]

Guangda X, Bangshun X, Xiujian L, Yangzhong H. Apoepsilon(4) allele increases the risk for exercise-induced silent myocardial ischemia in non-insulin-dependent diabetes mellitus. Atherosclerosis 1999;147(2):293–6. [PubMed: 10559515]

Hampson E. Spatial cognition in humans: possible modulation by androgens and estrogens. J Psychol Neurosci 1995;20:397–404.

Handa R, Reid D, Resko J. Androgen receptors in brain and pituitary of female rats: Cyclic changes and comparisons with the male. Biology Reprod 1986;34:293–303.

Ho Y, Deckelbaum R, Chen Y, Vogel T, Talmage D. Apolipoprotein E inhibits serum-stimulated proliferation and enhances serum-independent cell proliferation. J Biol Chem 2001;276:43455–43462. [PubMed: 11551921]

Hogervorst E, Williams J, Budge M, Barnetson L, Combrinck M, Smith AD. Serum total testosterone is lower in men with Alzheimer's disease. Neuroendocrinology Letters 2001;22:163–168. [PubMed: 11449190]

Holtzman DM, Pitas RE, Kilbridge J, Nathan B, Mahley RW, Bu G, Schwartz AL. Low density lipoprotein receptor-related protein mediates apolipoprotein E-dependent neurite outgrowth in a central nervous system-derived neuronal cell line. Proc Natl Acad Sci USA 1995;92:9480–9484. [PubMed: 7568158]

Hörtnagl H, Hansen L, Kindel G, Schneider B, Tamer AE, Hanin I. Sex differences and estrous cycle-variations in the AF64A-induced cholinergic deficit in the rat hippocampus. Brain Res Bull 1993;31:129–134. [PubMed: 8095840]

Howieson D, Camicioli R, Quinn J, Silbert L, Care B, Moore M, Dame A, Sexton G, Kaye J. Natural history of cognitive decline in the old old. Neurology 2003;60:1489–1494. [PubMed: 12743237]

Isgor C, Sengelaub D. Prenatal gonodal steroids affect adult spatial behavior, CA1 and CA3 pyramidal cell morphology in rats. Horm Behav 1998;34:183–198. [PubMed: 9799628]

Ishunina T, Fisser B, Swaab D. Sex differences in androgen receptor immunoreactivity in basal forebrain nuclei of elderly and Alzheimer patients. Exp Neurol 2002;176:122–132. [PubMed: 12093089]

Janowsky JS, Chavez B, Orwoll E. Sex steroids modify working memory. J Cogn Neurosci 2000;12:407–414. [PubMed: 10931767]

Janowsky JS, Oviatt SK, Orwoll ES. Testosterone influences spatial cognition in older men. Behavioral Neuroscience 1994;108(2):325–32. [PubMed: 8037876]

Ji ZS, Pitas RE, Mahley RW. Differential cellular accumulation/retention of apolipoprotein E mediated by cell surface heparan sulfate proteoglycans. J Biol Chem 1998;273:13452–13460. [PubMed: 9593678]

Jones R, Pugh P, Hall J, Channer K, Jones T. Altered circulating hormone levels, endothelial function and vascular reactivity in the testicualr feminised mouse. Eur J Endocrinol 2003;148:111–120. [PubMed: 12534364]

Joseph R, Gallagher RE. Gender and early environmental influences on activity, overresponsiveness, and exploration. Dev Psychol 1980;13:527–544.

Joseph R, Hess S, Birecree E. Effects of hormone manipulations and exploration on sex differences in maze learning. Behav Biol 1978;24:364–377. [PubMed: 743070]

Kenny A, Bellantonio S, Gruman C, Acosta R, Prestwood K. Effects of transdermal testosterone on cognitive function and health perception in older men with low bioavailable testosterone levels. J Gerontol A Biol Sci Med Sci 2002;57:M321–M325. [PubMed: 11983727]

Kerr J, Beck S, Handa R. Androgens selectively modulate c-fos messenger RNA induction in the rat hippocampus following novelty. Neuroscience 1996;74:757–766. [PubMed: 8884771]

Kerr JE, Allore JE, Beck SG, Handa RJ. Distribution and hormonal regulation of androgen receptor (AR) and AR mesenger mRNA in the rat hippocampus. Endocrinol 1995;136:2113–3221.

Kesner RP, Bolland BL, Dakis M. Memory for spatial locations, motor responses and objects. Exp Brain Res 1993;93:462–470. [PubMed: 8519335]

Kolb B, Cioe J. Sex-related differences in cortical function after medial frontal lesions in rats. Behav Neurosci 1996;110:1271–1281. [PubMed: 8986331]

Kus L, Handa R, Hautman J, Beitz A. Castration increases [125I]MK801 binding in the hippocampus of male rats. Brain Res 1995;683:270–274. [PubMed: 7552365]

Laskowitz DT, Horsburgh K, Roses AD. Apolipoprotein E and the CNS response to injury. J Cereb Blood Flow Metab 1998;18:465–471. [PubMed: 9591838]

Lehmann D, Butler H, Warden D. Association of the androgen receptor CAG repeat polymorphism with Alzheimer's disease in men. Neurosci Lett 2003;340:87–90. [PubMed: 12668243]

Leranth C, Hajszan T, MacLusky N. Androgens increase spine synapse density in the CA1 hippocampal subfield of ovariectomized female rats. J Neurosci 2004;24:495–499. [PubMed: 14724248]

Lu SF, McKenna SE, Cologer-Clifford A, Nau EA, Simon NG. Androgen receptor in mouse brain: Sex differences and similarities in autoregulation. Endr 1998;139:1594–1601.

Luine VN, Renner KJ, McEwen BS. Sex-dependent differences in estrogen regulation of choline acetyltransferase are altered by neonatal treatments. Endocrinology 1986;119:874–878. [PubMed: 3732148]

Mahley R, Huang Y. Apolipoprotein E: From atherosclerosis to Alzheimer's disease and beyond. Curr Opin Lipidol 1999;10:207–217. [PubMed: 10431657]

Mahley RW. Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. Science 1988;240:622–630. [PubMed: 3283935]

Malgor LA, Valsecia M, Verges E, De Markowsky EE. Blockade of the in vitro effects of testosterone and erythropoietin on Cfu-E and Bfu-E proliferation by pretreatment of the donor rats with cyproterone and flutamide. Acta Physiol Pharmacol Ther Latinoam 1998;48:99–105. [PubMed: 9695882]

Masterman T, Zhang Z, Hellgren D, Salter H, Anvret M, Lilius L, Lannfelt L, Hillert J. APOE genotypes and disease severity in multiple sclerosis. Multiple Sclerosis 2002;8:98–103. [PubMed: 11990879]

McEwen BS. Actions of sex hormones on the brain: Organization and activation in relation to functional teratology. Prog Brain Res 1988;73:121–134. [PubMed: 3047792]

McEwen BS, Alves SE, Bulloch K, Weiland NG. Ovarian steroids and the brain: Implications for cognition and aging. Neurology 1997;48:S8–S15. [PubMed: 9153161]

McGarvey T, Nguyen T, Malkowicz S. An interaction between apolipoprotein E and TERE1 with a possible association with bladder tumor formation. J Cell Biochem 2005;95(419):419–428. [PubMed: 15782423]

McKenna N, Lanz R, O'Malley B. Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 1999;20:321–344. [PubMed: 10368774]

McNemar Q, Stone CP. Sex difference in rats on three learning tasks. J Compar Psychol 1932;14:171–180.

Means LW, Dent MF. The effects of number of trials per day, retention interval, gender and time of day on acquisition of a two-choice, win-stay, water-escape working memory task in the rat. J Neurosci Meth 1991;39:77–87.

Mroz K, Carrel L, Hunt P. Germ cell development in the XXY mouse: evidence that X chromosome reactivation is independent of sexual differentiation. Dev Biol 1999;207:229–238. [PubMed: 10049577]

Naghdi N, Nafisy N, Majlesi N. The effects of intrahippocampal testosterone and flutamide on spatial locallization in the Morris water maze. Brain Res 2001;897:44–51. [PubMed: 11282357]

Nathan BP, Bellosta S, Sanan DA, Weisgraber KH, Mahley RW, Pitas RE. Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. Science 1994;264:850–852. [PubMed: 8171342]

Nathan BP, Chang K-C, Bellosta S, Brisch E, Ge N, Mahley RW, Pitas RE. The inhibitory effect of apolipoprotein E4 on neurite outgrowth is associated with microtubule depolymerization. J Biol Chem 1995;270:19791–19799. [PubMed: 7649988]

Nathoo N, Chetty R, van Dellen J, Barnett G. Genetic vulnerability following traumatic brain injury: the role of apolipoprotein E. Mol Pathol 2003;56:132–136. [PubMed: 12782758]

Olton DS, Branch PJ, Best PJ. Spatial correlates of hippocampal unit activity. Exp Neurol 1978;58:387–409. [PubMed: 620700]

Pfankuch T, Rizk A, Olsen R, Poage C, Raber J. Role of circulating androgen levels in effects of apoE4 on cognitive function. Cognitive Brain Res 2005;1053:88–96.

Poirier J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. Trends Neurosci 1994;17:525–530. [PubMed: 7532337]

Poirier J, Baccichet A, Dea D, Gauthier S. Cholesterol synthesis and lipoprotein reuptake during synaptic remodelling in hippocampus in adult rats. Neuroscience 1993;55(1):81–90. [PubMed: 8350994]

Pouliot W, Handa R, Beck S. Androgen modulates NMDA mediated depolarization in CA1 hippocampal pyramidal cells. Synapse 1996;23:10–19. [PubMed: 8723131]

Qin L, Wu X, Block M, Liu Y, Breese GR, Hong JS, Knapp DJ, Crews F. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 2007;55:453–462. [PubMed: 17203472]

Raber J, Bongers G, LeFevour A, Buttini M, Mucke L. Androgens protect against apolipoprotein E4-induced cognitive deficits. J Neurosci 2002;22:5204–5209. [PubMed: 12077215]

Raber J, Huang Y, Ashford J. ApoE genotype accounts for the vast majority of AD risk and AD pathology. Neurobiol Aging 2004;25:641–650. [PubMed: 15172743]

Raber J, Wong D, Buttini M, Orth M, Bellosta S, Pitas RE, Mahley RW, Mucke L. Isoform-specific effects of human apolipoprotein E on brain function revealed in *ApoE* knockout mice: Increased susceptibility of females. Proc Natl Acad Sci USA 1998;95:10914–10919. [PubMed: 9724804]

Raber J, Wong D, Yu GQ, Buttini M, Mahley RW, Pitas RE, Mucke L. Alzheimer's disease: Apolipoprotein E and cognitive performance. Nature 2000;404:352–354. [PubMed: 10746713]

Reinisch JM, Ziemba-Davis M, Sanders SA. Hormonal contributions to sexually dimorphic behavioral development in humans. Psychoneuroendocrinology 1991;16:213–278. [PubMed: 1961841]

Rizk-Jackson A, Robertson J, Raber J. Tfm-AR modulates the effects of ApoE4 on cognition. J Neurochem. 2007in press

Robyr D, Wolffe A, Wahli W. Nuclear hormone receptor coregulators in action: diversity for shared tasks. Mol Endocrinol 2000;14:329–347. [PubMed: 10707952]

Roof RL. Neonatal exogenous testosterone modifies sex difference in radial arm and Morris water maze performance in prepubescent and adult rats. Behav Brain Res 1993;53:1–10. [PubMed: 8466654]

Roof RL, Havens MD. Testosterone improves maze performance and induces development of a male hippocampus in females. Brain Res 1992;572:310–313. [PubMed: 1611529]

Roses, AD.; Saunders, AM. ApoE, Alzheimer's disease, and recovery from brain stress. In: de la Torre, JC.; Hachinski, V., editors. Ann NY Acad Sci. 826. New York: 1997. p. 200-212.

Schmidt S, Barcellos L, DeSombre K, Rimmler J, Lincoln R, Bucher P, Saunders A, Lai E, Martin E, Vance J, Oksenberg J, Hauser S, Pericak-Vance M, Haines J, Group MSG. Association of polymorphisms in the apolipoprotein E region with susceptibility to and progression of multiple sclerosis. Am J Hum Gen 2002;70:708–717.

Simental J, Sar M, Wilson E. Domain functions of the androgen receptor. J Steroid Biochem Mol Biol 1992;43:37–41. [PubMed: 1525065]

Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. J Comp Neurol 1990;294:76–95. [PubMed: 2324335]

Sing R, Pervin S, Shryne J, Gorski R, Chaudhuri G. Castration increases and androgens decrease nitric oxide synthase activity in the brain: physiologic implications. Proc Natl Acad Sci USA 2000;97:3672–3677. [PubMed: 10725371]

Srivastava R, Srivastava N, Averna M. Estrogen up-regulates apolipoprotein E (apoE) gene expression by increasing apoE mRNA in the translating pool via the estrogen receptor a-mediated pathway. J Biol Chem 1997;272:33360–33366. [PubMed: 9407129]

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Engchild J, Salvesen GS, Roses AD. Apolipoprotein E: High-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer's disease. Proc Natl Acad Sci USA 1993;90:1977–1981. [PubMed: 8446617]

Sun C, Robl J, Wang T, Huang Y, Kuhns J, Lupisella J, Beehler B, Golla R, Sleph P, Seethala R, Fura A, Krystek SJ, An Y, Malley M, Sack J, Salvati M, Grover G, Ostrowski J, Hamann L. Discovery of potent, orally-active, and muscle-selective androgen receptor modulators based on an N-aryl-hydroxybicyclohydantoin scaffold. J Med Chem 2006;49:7596–7599. [PubMed: 17181141]

Tabibnia G, Cooke BM, Breedlove SM. Sex difference and laterality in the volume of mouse dentate gyrus granule cell layer. Brain Res 1999;827(1–2):41–45. [PubMed: 10320691]

Tan R, Pu S. A pilot study on the effects of testosterone in hypogonadal aging male patients with Alzheimer's disease. The Aging Male 2003;6:13–17. [PubMed: 12809076]

Tang G, Xie H, Xu L, Lin D, Ren D. Genetic study of apolipoprotein E gene, alpha-1 antichymotrypsin gene in sporadic Parkinson disease. Am J Med Genet 2002;114:446–449. [PubMed: 11992569]

Tardiff B, Newman M, Saunders A, Strittmatter W, Blumenthal J, White W, Croughwell N, Davis R, Roses A, Reves J. Preliminary report of a genetic basis for cognitive decline after cardiac operations. Ann Thorac Surg 1997;64:715–720. [PubMed: 9307463]

Teter B, Xu PT, Gilbert JR, Roses AD, Galasko D, Cole GM. Human Apolipoprotein E isoform-specific differences in neuronal sprouting in organotypic hippocampal culture. J Neurochem 1999;73:2613–2616. [PubMed: 10582625]

Tsuboi Y, Joseph K, Cookson N, Dickson D. APOE E4 is a determinant for Alzheimer pathology in progressive supranuclear palsy. Neurology 2003;60:240–245. [PubMed: 12552038]

Tut T, Ghadessi F, Trefiro M, Pinsky L, Yong E. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 1997;82:3777–3782. [PubMed: 9360540]

Ueda T, Mawki N, Bruchovsky N, Sadar M. Ligand-independent activation of the androgen receptor by Interleukin 6 and the role of steroid receptor coactivator-1 in prostate cancer cells. J Biol Chem 2002;277:38078–38094.

Umesono K, Evans R. Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell 1989;57:1139–1146. [PubMed: 2500251]

van Haaren F, van Hest A, Heinsbroek RPW. Behavioral differences between male and female rats: Effects of gonadal hormones on learning and memory. Neurosci Biobehav Rev 1990;14:23–33. [PubMed: 2183097]

Van Meer P, Acevedo S, Raber J. Impairments in spatial memory retention of GFAP-apoE4 female mice. Beh Brain Res 2007;176:372–375.

Vazquez-Pereyra F, Rivas-Arancibia S, Castillo ALD. Modulation of short-term and long-term memory by steroid sexual hormones. Life Sci 1995;56:255–260.

Vermeulen A. Androgen replacement therapy in the aging male" a critical evaluation. J Clin Endocrinol Metab 2001;86:2380–2390. [PubMed: 11397827]

Weisgraber KH, Pitas RE, Mahley RW. Lipoproteins, neurobiology, and Alzheimers disease: Structure and function of apolipoprotein E. Curr Opin Struct Biol 1994;4:507–515.

Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J Neurosci 1990;10:4035–4039. [PubMed: 2269895]

Wozniak M, Itzhaki R, Faragher E. Apolipoprotein E-epsilon 4 protects against severe liver disease caused by hepatitis C virus. Hepatology 2002;36:456–463. [PubMed: 12143056]

Yaffe K, Haan M, Byers A, Tangen C, Kuller L. Estrogen use, APOE, and cognitive decline: Evidence of gene-environment interaction. Neurology 2000;54(10):1949–1954. [PubMed: 10822435]

Yaffe K, Lui L, Zmuda J, Ferrell R, Cauley J. Sex hormones and cognitive function in older men. J Am Ger Soc 2002;50:707–712.

Zareparsi S, Camicioli R, Sexton G, Bird T, Swanson P, Kaye J, Nutt J, Payami H. Age at onset of Parkinson disease and apolipoprotein E genotypes. Am J Med Genet 2002;107:156–161. [PubMed: 11807891]

Table 1

Object recognition of sham and castrated *Apoe*$^{-/-}$, apoE3, and apoE4 male mice[1]

	Old Location	Novel Location	Novel Object
Sham *Apoe*$^{-/-}$	32 ± 5	49 ± 4*	74 ± 4
Castrated *Apoe*$^{-/-}$	32 ± 7	58 ± 5**	45 ± 10
Sham apoE3	35 ± 2	47 ± 3*	69 ± 5
Castrated apoE3	28 ± 8	46 ± 7*	55 ± 11
Sham apoE4	28 ± 5	47 ± 4**	58 ± 7
Castrated apoE4	35 ± 3	39 ± 3	61 ± 5

* $p < 0.05$ versus old location.

** $p < 0.01$ versus old location.

[1] Pfankuch, Rizk, Olsen, Poage, and Raber, 2005

Table 2

Spatial memory retention of sham and castrated *Apoe*$^{-/-}$, apoE3, and apoE4 male mice in the water maze probe trial[1]

	Left	Target	Right	Opposite
Sham *Apoe*$^{-/-}$	19 ± 5	49 ± 6**	16 ± 5	17 ± 5
Castrated *Apoe*$^{-/-}$	20 ± 6	28 ± 5	34 ± 5	18 ± 4
Sham apoE3	16 ± 3	48 ± 8*	22 ± 5	14 ± 4
Castrated apoE3	16 ± 4	48 ± 9*	20 ± 8	16 ± 6
Sham apoE4	30 ± 7	42 ± 7	17 ± 5	12 ± 3
Castrated apoE4	21 ± 6	49 ± 7**	16 ± 6	14 ± 3

* $p < 0.05$ versus any other quadrant.

** $p < 0.01$ versus nay other quadrant.

[1] Pfankuch, Rizk, Olsen, Poage, and Raber, 2005

Table 3

Passive avoidance learning and memory of sham and castrated *Apoe*$^{-/-}$, apoE3, and apoE4 male mice[1]

	Trials to Criterion	Memory Retention Day 2
Sham *Apoe*$^{-/-}$	3.0 ± 0.5	261 ± 20
Castrated *Apoe*$^{-/-}$	2.6 ± 0.4	247 ± 28
Sham apoE3	2.8 ± 0.2	288 ± 8
Castrated apoE3	2.8 ± 0.3	244 ± 30
Sham apoE4	3.8 ± 0.4	289 ± 8
Castrated apoE4	3.9 ± 0.4	300 ± 0

[1] Pfankuch, Rizk, Olsen, Poage, and Raber, 2005