[Frontiers in Bioscience 5387-5405, May 1, 2008]

# Apolipoprotein E may be a critical factor in hormone therapy neuroprotection

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## 1. ABSTRACT

In this review we examine the evidence for ovarian hormone neuroprotection in chronic neurological diseases, including stroke. We propose that neuroprotection may involve the ability of estrogens to modulate apolipoprotein E (apoE) and its receptor, the low density lipoprotein receptor related protein (LRP). Results from numerous studies have demonstrated that (1) nerve regeneration is severely delayed in apoE-gene knockout (KO) mice as compared to wild-type (WT) littermates; (2) 17beta estradiol replacement in ovariectomized mice resulted in a significant increase in levels of apoE and LRP, in the olfactory bulb (OB) and other brain areas; (3) estradiol treatment increased both apoE and neurite outgrowth in cortical and olfactory neuronal cultures; and (4) estradiol treatment had no effect on neurite outgrowth in cultures deprived of apoE or in the presence of apoE4. In essence these studies suggest that apoE is a critical intermediary for the beneficial effects of 17beta estradiol on nerve repair, which can lead to functional reorganization (plasticity). Future studies of HT should evaluate the effects of apoE genotype and production estradiol on neuroprotection.

## 2. INTRODUCTION

For years hormone replacement therapy (HRT-shortened to HT) and currently occasionally referred to as menopausal hormone replacement (mHT), was touted as a neuroprotective agent. Numerous epidemiology studies reported neuroprotective effects showing a decreased prevalence of numerous chronic neurological diseases. However, as discussed below, findings from the Women's Health Initiative (WHI), a prospective study, did not find any evidence of neuroprotection (1). In fact, the risks for medical problems appeared to be greater in the treatment groups than the placebo-treated groups. These findings, disseminated by popular press, resulted in a precipitous decline in the use of HT. The pendulum of common knowledge had clearly swung to a position contraindicating HT other than for a few selected menopausal symptoms. Five years later, we are finding that the results of the WHI, although statistically correct as reported, may have been entirely too negative. Studies now being published are suggesting a window of opportunity for neuroprotection by HT. Furthermore, studies suggest that a common protein, apolipoprotein E (apoE), may be an integral modulator of HT neuroprotection. Appropriate hormone replacement schedules and attention to apoE genotype may vastly improve the efficacy of neuroprotection by HT while avoiding some of the contraindications.

## 3. THE CURRENT STATUS OF HORMONE REPLACEMENT THERAPY (HT) AND NEUROPROTECTON

Previous epidemiological/observational studies of hormone therapy (HT) reported neuroprotective effects on numerous chronic neurological diseases (discussed below). These observations were prospectively addressed in the design and execution of the Women's Health Initiative (WHI) trial that evaluated a mixture of equine estrogens Premarin (Prem) and medroxyprogesterone (Pro) on women's health. Results from the WHI clearly identified contraindications of continuous PremPro (1). This study found increased incidence of deep vein thrombosis and breast cancer over five years. To our knowledge, no reports of increased incidence of these complications were reported in the Premarin-only group and several studies suggested that the medroxyprogesterone may be a negative factor in, at least, breast cancer (2,3). Similar untoward findings were made in the Wisdom Study (4). Consequently, the current approved indications for HT use are to relieve symptoms of the menopause (vasomotor symptoms and vaginal atrophy) and to prevent postmenopausal osteoporosis. In light of lay press reports of the WHI data, use of HT has declined by approximately 50% (5,6).

If HT is neuroprotective, then decline in use presents a demographic time bomb for our aging population. US Census estimates predict that approximately 46 million women will be over the age of 65 in 2050. About 7 million of these women will be demented based on 15% overall prevalence rates. Hence, if only 30% of women started HT at menopause and HT decreased risk by 30%, we could likely decrease the number of demented women by over 600,000. Clearly, we need to determine the reason for the discrepancy between epidemiological and prospective studies. Our goal in this review is to identify some critical features of HT in neuroprotection that might have been overlooked in previous studies. In particular, we emphasize the possibility that modulation of apoE may be a core mechanism of HT neuroprotection.

## 4. DEFINITION OF NEUROPROTECTION

Defining clinical neuroprotection is key in identifying possible mechanisms. Neuroprotection can occur when the disease process is present but clinical evidence of progression is delayed. This is most evident in Alzheimer's disease (AD) which is associated with advanced age. Recent studies, and our personal experiences with neuropathology samples, strongly suggest that individuals may be cognitively intact at the time of death while harboring substantial Alzheimer-like pathology (7). In the absence of clinical information, we may have incorrectly concluded that these patients had clinical dementia.

We propose that neuroprotection can act in two basic modes; 1) directly affecting the disease mechanism, and 2) affecting the clinical progression. These two modes are not identical. Direct effects on the disease etiology are usually inferred when neuroprotective effects of a compound are identified and are usually the goal of "neuroprotection" trials. However, slowing of clinical disease progression, in the absence of directly affecting the disease process, may be just as important for decreasing the prevalence of a disease.

We hypothesize that clinical expression of a progressive neurological disease occurs when a threshold of tissue damage is reached. We propose that many treatments that affect clinical expression do so by slowing the progression to that threshold. Particularly, repair of disease-caused damage could delay the progression of clinical expression of the disease.

Delay of clinical expression can occur by numerous mechanisms including protection of cells from lethal injuries, perhaps production of new cells from pluripotent stem cells, or repair and reorganization of remaining cells and connections (plasticity). Protection could physically reduce the amount of compromised tissue, hence reducing the total damage. Repair may generate new connections to replace functions previously performed by the irreversibly damaged tissue. Hence, repair could compensate for neuron death and synaptic loss by forming new synapses and this repair will delay clinical expression (e.g., dementia).

Importantly, the neuroprotective intervention may have relatively minor effects on the basic mechanisms underlying the etiology of the disease. For example, relapsing-remitting multiple sclerosis is generally thought to represent damage by an immune cell-mediated

mechanism interspersed with periods of repair. If the magnitude of recovery is augmented during the remission phase, then disability progression is slower, independent of the primary cause. A drug that could improve recovery would delay the course of disease although have little or no effect on the mechanism (s) underlying demyelination. We propose that understanding the full range of neuroprotection by HT involves modulation of the disease course.

Why natural selection would assign estrogens (and perhaps progesterone) a neuroprotective role probably relates to maternal physiology during pregnancy. During pregnancy the mother is at risk for stroke, exhibits peripheral resistance to insulin, and shows changes in osmoregulation, increased cardiac output, and alteration of vascular resistance. Circulating estrogens increase during pregnancy, increase uterine blood flow and are key regulators of progesterone synthesis (8). A role for estrogen in neuroprotection may be an evolutionary strategy to protect the mother to insure the success of her offspring. Hence, to us, it is not surprising that estrogen would have a generalized neuroprotective effect on the brain. The obvious question is then, what is the mechanism underlying the neuroprotective role of estrogens?

## 5. THE MENSTRUAL CYCLE and MENOPAUSE

### 5.1. Menstrual cycle

Possible mechanisms of neuroprotection of HT need to be understood in the context of the normal menstrual cycle (or estrus cycle in rodents and other species) and menopause, when menstrual cycles become more irregular and subsequently cease. During the normal menstrual cycle of about 28 days, the ovarian hormones, 17beta estradiol and progesterone, show striking cyclic variation. Circulating levels of estradiol vary from 20 to 600 pg/mL during the menstrual cycle, with highest levels just prior to the pituitary gonadotropin surge and ovulation. Circulating progesterone varies from approximately 0.1 ng/mL to 19.4 ng/mL with peak levels occurring about 1 week after ovulation. Levels of both hormones are lowest during menstruation. (9,10).

The estrus cycle in the rodent compresses into 4-5 days the endocrine and ovarian events that occur over the typical 28-day human menstrual cycle. Growth of small antral ovarian follicles occurs during diestrus and proestrus with concomitant increase in circulating 17β estradiol that is produced by these growing follicles. Peak circulating levels of estradiol occur by mid-proestrus and decline after the preovulatory gonadotropin surge. Preovulatory LH and FSH peak late on proestrus and ovulation occurs in the early hours of estrus. LH levels rapidly decline after the preovulatory surge. FSH declines then exhibits a secondary rise that peaks during the early morning of estrus. Progesterone begins to rise after the preovulatory gonadotropin surge (11). Hence, the female brain, during the reproductive years, is exposed to continuously varying levels of ovarian hormones and pituitary gonadotropins.

### 5.2. Menopause

Menopause is characterized by the cessation of menstrual cyclicity, decline in ovarian hormone secretion and rise in circulating FSH. Ovarian changes that precipitate the decline in ovarian hormone production occur several years prior to the cessation of menstruation. As a woman ages the number of ovarian follicles decreases and by the onset of menopause few, if any, follicles remain. During the years just prior to menopause, the perimenopause transition, gonadal inhibin production decreases. In response to the declining levels of inhibin, FSH rises. Menstrual cycles become irregular in response to the alterations in gonadal and hypophyseal hormones and menstruation eventually ceases. Menopause is clinically defined as elevated FSH (> 23-40 IU/L) and the absence of menstrual periods for 12 months. The permanent cessation of menstruation is caused by failure of ovarian follicular development and estradiol production in the presence of elevated gonadotrophin levels (8-11).

### 5.3. HT types

Current prescribing indications for HT are for the relief of menopausal symptoms including hot flashes, mood swings, vaginal atrophy and osteoporosis. It is not currently indicated for neuroprotection. The most advertised type of HT is the combination of Premarin (estrogens derived from pregnant mare's serum) combined with medroxyprogesterone (Pro) having the trade name PremPro. This is the combination used in many trials including the HERS study and the Woman's Health Initiative (WHI). This drug is taken orally on a daily regimen. In women without a uterus, only Premarin is given in a daily dose. These approaches to HT result in relatively constant levels of estrogens and progestins in contrast to normal fluctuation in serum levels and, as noted below, may be suboptimal for neuroprotection.

Another orally supplied regimen is typically composed of 21 days of estrogens followed by several days of a progesterone analogue. The addition of a progesterone analogue in both of the orally available protocols is included because previous studies show that estradiol-replacement alone can result in an increased risk for endometrial cancer. Hence the carcinogenic effect is opposed with a progesterone analogue.

Several alternative methods utilize a depot approach either administered subcutaneously or by a patch applied to the skin. The important point to be made about these regimens is that the alternating estradiol-progesterone and the depot approach are significantly different than the PremPro daily dosing in that they do not result in unchanging levels of ovarian hormone in the system. The depot approach probably results in higher levels immediately after application than at the end of the pharmaceutical life of the depot; often about 30 days. Levels again rise at replacement. Hence levels of estradiol would fluctuate which we suspect is critical in neuroprotective effects (see below).

**Table 1.** Estrogen replacement studies

| Subjects [1] | Study Type [2] | Intervention [3] | Dependent Variable [4] | Outcomes[5] | References[6] |
|---|---|---|---|---|---|
| Normal | Retrospective | Mixed | Repeated testing on MMSE | HT associated with less cognitive decline when not having apoE4 | 74 |
| Normal | Prospective | Mixed | Short Term Memory Performance | HT improved | 75 |
| Normal | Prospective (WHI) | PremPro | Cognitive tests | Greater decline in the treated than placebo group | 24, 25 |
| Normal | Retrospective | Mixed | Clinical Data | No Protective Effects | 14 |
| Normal | Retrospective | Mixed | Chart Review, Death Certificate | Decreased Risk for developing dementia | 13 |
| Normal | Prospective | Mixed | Clinical Data | Decreased risk for developing dementia | 12, 15-17 |
| | | | | Decreased risk for those starting early | 18 |
| Demented | Prospective | PremPro | Multiple Cognitive Tests | No improvement in cognitive function | 20-23 |
| Demented | Prospective | Estradiol Patch | Multiple cognitive Tests | Short term improvement | 24 |
| Parkinson Disease | Retrospective | Mixed | Clinical Evaluation | Less severe progression or later onset | 32,33 |
| Stroke | Retrospective | Mixed | Questionnaire, death certificates | Decreased Likelihood | 27,29 |
| Multiple Sclerosis (MS) | Retrospective | Mixed | Questionnaire | Decreased Symptoms | 35,38,39 |
| | | | | No effects | 36 |
| MS-relapsing remitting | Retrospective | Estriol | Laboratory Testing | Decreased Size and number of lesions | 37 |

This table summarizes the results of multiple studies with various estrogen replacement strategies. 1) Subjects: Putative condition at the start of the study. Normal-Normal cognitive performance; Demented-Evidence of cognitive decline. Prospective studies applied clinical evaluations; Parkinson Disease-defined clinically; Stroke-by chart review, death records or questionnaire; Multiple Sclerosis-by clinical diagnosis. 2) STUDY TYPE: Prospective-patients were followed after an initial evaluation for varying time periods. Retrospective-patient's condition and HT intervention was determined by chart review. 3) INTERVENTION: Mixed- The type of estrogens used was not or could not be specified and may have included opposed estrogens. Estrogens-estrogens only but the mixture was not reported; Prempro-a combination of Premarin and a progesterone (medroxyprogesterone); Estradiol-presumably 17 beta estradiol. 4) DEPENDENT VARIABLE: Conclusions based on this variable 5 OUTCOMES: Outcome description. REFERENCE: Citation number.

## 6. HT AND NEUROPROTECTION

### 6.1. HT and dementia

A review of the literature (Table 1) shows that a history of HT decreased the risk for dementia by about 30% (12-18). We can assume that most of these cases were probably Alzheimer disease, although most studies were unable to thoroughly evaluate the cause of the dementia (see Table 1). A history of early oophorectomy has been associated with an increased risk for dementia (19). A logical extension of these observations is the intervention with HT in women with AD. However, these studies have, by and large, not been successful.

Intervention studies with HT in demented women have not generally shown positive results (20-24). To our knowledge only one study has shown an improvement with HT and this intervention used high doses with a transdermal patch (24). Intervention studies in cognitive intact women have also shown rather negative results. The mental health aspect of the Women's Health Initiative Mental Status study (WHIMS) found no protective effects of PremPro on cognitive function, and, in fact, a slight decline (25,26). These discrepancies are important to address. Non hormonal explanations for contrast between the results of the WHIMS and previous observational studies can lie in publication bias (negative findings are unlikely to be published) or biases of sample selection (women who use HT might be better educated or live a healthier life-style). Nonetheless, failure of neuroprotection in the WHIMS and in interventions in women with dementia contrasts with epidemiology studies of HT and with neuroprotective effects of estradiol replacement reported in animal studies. As discussed below, we suspect that apolipoprotein might be a critical factor in these differences.

### 6.2. HT and other chronic diseases

Possible neuroprotection by HT is not unique to dementia (Table 1). A history of using HT has been associated with protection against both stroke and hypertensive brain damage (27-30), and silent strokes have been suggested to be a factor in the clinical expression of dementia in the presence of AD pathology (31). A history of HT also decreased the risk for Parkinson disease dementia (32-33.) and early oophorectomy was associated with an increased risk for PD (34). HT was also associated with less severe multiple sclerosis (35-38) and MS symptoms may become less severe during pregnancy when ovarian hormone levels are elevated (39). In sum multiple studies suggested an effect of ovarian hormone replacement on disease progression. An Occam's razor approach to explaining neuroprotection by HT suggests that, rather than affecting disease-specific processes, HT may result in generalized neuroprotection. Neuroprotection by estradiol appears likely, but the mechanism (s) require clarification for optimal application.

## 7. APOLIPOPROTEIN E ISOFORMS AND NEUROLOGICAL DISEASE

### 7.1. ApoE isoforms and receptors

ApoE is a 35kD lipid associated protein (40,41). In brief, the C-terminus of apoE binds to the lipoprotein

complex while the N-terminus extends from the complex to bind with receptors. In humans, three major isoforms of apoE are found and referred to as apoE2, apoE3 and apoE4, and coded by three alleles, ε2, ε3 and ε4 (42-45). These three isoforms vary by one amino acid at two sites. This variability is proposed to modify the tertiary structure of apoE to modify its ability to bind to both intracellular and extracellular receptors (44).

The apoE receptors comprise a family of receptors that range in weight from 130 to 600 kD and include the very low density lipoprotein receptors (VLDLr), the low density lipoprotein receptor (LDLr), the apoE receptor (apoE2r), the lipoprotein receptor related protein (LRP), megalin and GP330. Most of these are found in the brain. Of particular importance is the c-terminus of some of these receptors, including the LRP, has an NPxY motif that modulates numerous intracellular processes in addition to lipid internalization. More information about these receptors can be found in excellent reviews (46-48).

However, a key observation in these studies for relating HT to apoE is that estradiol replacement can increase protein levels of both apoE (49-52) and its receptor, LRP, while not affecting levels of the LDLr (53). Hence, HT could increase apoE and LRP and thereby underlay HT effects. Effects of these proteins on cell function are complex and are briefly discussed below.

### 7.2. ApoE and chronic neurological diseases

ApoE isoform clearly has a critical role in developing dementia. Epidemiology studies revealed that the apoE4 allele showed a dose-response effect on developing the dementia associated with Alzheimer disease. In fact, those homozygous for apoE4 had roughly a 90% risk for dementia by the age of 90 while those homozygous for apoE3 had roughly a 30% risk (54). Some studies suggested that apoE2 may be neuroprotective and lessen the risk. However, the low frequency of this isoform made epidemiological risk estimates unreliable. Of note is that the risk for dementia was also modified by polymorphisms of LDL-r family members including LDLr (55) and LRP (56). ApoE binds strongly to the putative cause of Alzheimer disease, beta amyloid. This observation has led to a general hypothesis that apoE affects the course of dementia by modulating the transport of beta amyloid (57,58)

What is less appreciated is that having the apoE4 isoform of the gene also increases the risk or progression rate for multiple other chronic neurological diseases. The apoE alleles ε2 and ε4 increased the likelihood of developing Parkinson disease (59,60) and this effect was greater in women than in men (61). The severity of pathology appeared to be exacerbated by having apoE4 (62). In addition, the risk for dementia in PD appeared greater in the presence of apoE4 (63), although this clinical presentation could have been co-occurrence of AD pathology and Parkinson pathology referred to, generically, as diffuse Lewy body disease.

Other chronic conditions were also affected by apoE isoform. ApoE genotype was associated, in a complex manner, with the clinical progression of fronto-temporal dementia (64). The apoE ε4 genotype was a major risk factor for dementia following brain trauma (65,66) and the progression of relapsing-remitting multiple sclerosis appeared to be exacerbated by the presence of the ε4 allele, although the risk was not increased (67,68). The progression of amyotrophic lateral sclerosis (ALS) appeared to be more rapid when apoE4 was present (69,70). Finally, the progression of TLE epilepsy (71), a refractory response to anti-epileptic drugs (72) and the likelihood of post-traumatic seizures (73) were increased by having an apoE4 allele.

As with HT, the range of diseases affected by the risk factor of apoE isoform suggests that a disease-specific mechanism is not likely to underlay disease progression. We hypothesize that a relevant interpretation of the role of apoE is facilitation of protection and repair. Specifically, disease progression is more rapid (and it becomes clinically evident earlier) in individuals with the apoE4 gene because, in general, damage may be increased and repair attempts by the brain are less effective.

### 7.3. Interaction between apoE isoforms and HT

Only a few studies have evaluated the interaction between HT and apoE. To our knowledge, two epidemiology studies have examined the role of HT and apoE isoform on dementia and cognitive function. Women treated with HT who had the apoE4 allele did not show the same degree of neuroprotection by HT from cognitive decline as those with other genotypes (74). HT appeared to improve cognitive function only in post-menopausal women not having the ε4 allele (75). Estradiol may not be neuroprotective in patients that have a history of stroke and apoE4 (76). An estrogen receptor (ER) alpha polymorphism interacted with apoE4 genotype to increase the risk for developing of the Alzheimer type dementia greater than that of apoE4 alone (77).

What becomes clear in these studies is that both HT and apoE modify the course of chronic neurological diseases but may not necessarily modify the underlying etiology of the disease. Studies in experimental animals show that HT, principally 17beta estradiol, can be neuroprotective. The presence of apoE is generally neuroprotective when compared to the absence of apoE. However, an interaction between hormone replacement and apoE expression has not been systematically studied. A review of animal studies follows.

## 8. ANIMAL STUDIES OF NEUROPROTECTION BY ESTRADIOL REPLACEMENT

### 8.1. Stroke studies

Estradiol replacement after ovariectomy has shown mixed effects on neuroprotection in animal models of ischemia (stroke). We suspect that the mixed effects largely reflect the variability in the interval between estradiol treatment and induction of the stroke. Effective post-stroke estradiol replacement had to occur within hours

after the stroke (30,78-80). If estradiol replacement in ovariectomized (OVX) animals was started about one week or less prior to stroke, about 85% of published studies show a protective effect (81-103). Conversely, two weeks or more of estradiol treatment prior to stroke induced a protective effect in only about 25% of the studies (104-113). Of note is that neuroprotection from stroke by estradiol did not occur in the absence of apoE (114) bringing the focus to apoE

We found that continuous estradiol following OVX increased apoE and LRP, but only for about a week after replacement (52). Acute replacement of estradiol could increase apoE which could, in turn, limit stroke damage by upregulating lipid availability. Modulating LRP could also modulate $Ca2^{+}$ entrance into neurons through the n-methyl d-aspartate (NMDA) receptor slowing an apoptotic cascade (115). LRP also modulates tissue plasminogen activator that is involved in tissue destruction and phagocytosis (116-118). Identifying mechanisms of protection from the acute effects of strokes requires more study.

### 8.2. Synaptogenesis

Synaptogenesis associated with the estrous cycle and estradiol replacement following OVX is a widely known animal model. Numerous studies in rats showed that post-synaptic dendritic structures (spines) varied with the estrous cycle in female rats with a peak on proestrus in intact rats. Six days following OVX, two days of estradiol replacement increased spine and synaptic density by 30% in hippocampal CA1 pyramidal neurons peaking at 2-3 days (119,120). Progesterone replacement facilitated the decline in spine number following OVX. A general conclusion of these studies was that the mechanism of spine production involved increased activation of the NMDA receptor caused by inhibition of the gamma amino butyric acid (GABA) receptor. Evaluation of synaptophysin, an integral membrane protein of synaptic vesicles (see 121), showed a similar response to estradiol replacement (122,123).

### 8.3. Cholinergic neurotransmission

Enzymes associated with acetylcholine transmission are modulated by estradiol. The highest levels of mRNA for the rate limiting enzyme for acetylcholine, choline acetyltransferase (ChAT), were found on diestrus day 1 in the medial septum (which projects to hippocampus) and on diestrus day 2 in striatum and in nucleus basalis of Meynert which projects to neocortex (124,125). Estradiol replacement increased ChAT in multiple brain regions (124,126,127). However, continuous exposure to estradiol for two weeks or more had variable effects on ChAT; some brain regions increased, some decreased and some did not change (128-132). Moreover, studies disclosed "a window of opportunity" for estradiol replacement following OVX (131,132). If estradiol replacement occurred after this window, no effects were observed.

### 8.4. Regeneration and repair

*In vivo* studies of estradiol-facilitated growth and repair are relatively rare. However, they generally showed that estradiol replacement facilitated axonal regeneration. Islamov (133) reported that estradiol replacement could improve sciatic nerve regeneration. Studies also showed that sprouting of axons in denervated hippocampus was more rapid in the presence of estradiol (123,134,135). Finally, estradiol could modulate genesis and survival of neuronal precursor cells (136-138).

Studies of neuronal cultures have shown that including estradiol in a culture media increased the rate of neurite growth. An early study (139) used explanted cultures of hypothalamic tissue. Other studies, using cell culture methods of dissociated embryonic tissue also found facilitation of process growth (140-142).

## 9. ANIMAL MODELS OF NEUROPROTECTION BY APOLIPOPROTEIN E

The precise mode (s) by which apoE is neuroprotective is not clear. Decreased densities of hippocampal and neocortical synaptophysin (a marker for presynaptic terminals) and microtubule-associated protein 2 (a dendritic marker) were reported in old apoE deficient mice (143). However, another study did not observe frank neuropathological changes in either young (approximately 6 months) or old (more than 20 months) apoE deficient mice (144). A clear explanation for these differences requires study of possible founder effects of KO mice.

### 9.1. Stroke studies

ApoE clearly affects the severity of damage from experimental strokes. The severity of damage subsequent to ischemia was substantially increased in mice lacking the apoE gene (apoE knockouts or KO) compared to WT mice (145). Adding the apoE3 gene in apoE KO mice appeared to decrease the damage while the apoE4 gene appeared to increase damage (146,147). Key studies to understanding how apoE might work reported delayed damage following stroke in the presence of apoE4 (148) and faster clearance of debris in mice with the apoE gene compared to KO mice (149,150). Hence, the total damage from an acute event (stroke) might be modulated by repair processes, the rate of which are modulated by apoE. Slow clearance of debris may result in a prolonged period of glial activation and increased damage from glial-produced toxins.

### 9.2. Repair in apoE KO mice

ApoE may facilitate sprouting by recycling cholesterol and other lipids via lipoprotein receptors on target cells (40,151-154). These lipoprotein particles have been proposed to scavenge cholesterol from the degenerating myelin and provide it to growth cones of sprouting axons via low-density lipoprotein receptor-mediated endocytosis (40,155,156).

ApoE protein increased by 250- to 350-fold 3-weeks following injury of peripheral nerve in rats (41,157). These data suggested that apoE was important for peripheral nerve regeneration, however, regenerating nerves in both apoE deficient and control mice were morphologically indistinguishable at two and four weeks following sciatic nerve crush (155,158). We speculate that

other apolipoproteins in the PNS may have been capable of functionally substituting for apoE.

Following a brain injury, apoE mRNA expression in the hippocampal dentate gyrus increased six days following injury, and several days after reactive gliosis is identified (156,159). Olfactory nerve lesion resulted in a two fold increase in whole olfactory bulb apoE and a roughly 10 fold increase in both astroglia and microglia containing detectable apoE (160). Anderson et al, (144) reported little difference between the apoE deficient mice and wild-type controls on plasticity and repair in contrast to another that reported poorer repair when apoE4 was expressed by neurons (161). The presence of apoE4, rather than apoE3, may have delayed repair processes. Delayed repair may prolong reactive astrogliosis. Prolonged reactive gliosis may have inhibited axonal growth (162) and apoE4 may have been deficient in facilitating axonal re-growth resulting in a more prolonged period of reactive gliosis. Some critical function in neuroplasticity and axonal process growth was suggested by studies that showed impaired long-term potentiation in hippocampus of apoE deficient mice (163,164).

Of note for regulation of apoE was that cortical spreading depression, which was associated with oxidative stress, increased apoE message (165) and reactive gliosis (166). In this paradigm, KCl bathed the cortex causing the neurons to initially depolarize and then become quiescent. Hence, in response to declining neuronal activity, apoE might be upregulated. Regulation of apoE following damage or stress in the CNS may indicate an important role in brain protection and repair.

**9.3. Olfactory nerve repair in apoE KO mice**

The rodent olfactory bulb may present an ideal model for understanding the role of apoE in regeneration and repair. In the normal mouse and human, we showed weak but consistent immunoreactivity for apoE surrounding the terminal field of olfactory receptor neurons, the glomerulus (167). In brief, weak but detectible immunoreactivity for apoE surrounded the glomeruli, and was present in the olfactory nerve. Subsequent studies showed that apoE occurred in sustentacular cells in the olfactory receptor nerve layer, in the glia surrounding the olfactory nerve and in the olfactory bulb proper (168). Hence, apoE was present along the whole length of the olfactory nerve and could function to support neurite growth for the length of the olfactory nerve. These observations suggested to us that apoE was associated with the continuous olfactory receptor axonal extension that occurs in the olfactory bulb. Therefore, we have focused on the olfactory nerve and bulb as a valuable model of regeneration and repair.

The major source of axonal input to the olfactory bulb is the olfactory nerve. The olfactory nerve can be reversibly injured by irrigating the olfactory epithelium, where the cell bodies reside, with zinc sulfate or Triton X100. This procedure does not directly injure the olfactory bulb. Hence, this model provides a simplified system for studying the role of apoE in olfactory nerve regeneration and repair in the absence of direct damage to the measured structure.

Following irrigation of the olfactory epithelium with Triton X-100, receptor neurons that project into the olfactory bulb died, but the remaining precursor (basal) cells were able to reconstitute the olfactory epithelium and after several weeks re-innervated the olfactory bulb (169). Hence we could perform a peripheral lesion of the olfactory nerve and monitor recovery in the olfactory bulb which is part of the central nervous system.

Three days following olfactory epithelium irrigation, apoE increased two-fold by semi-quantitative Western blotting of whole olfactory bulb. Immunocytochemical localization showed intense apoE immunoreactivity covered the degenerating olfactory nerve and surrounded the glomeruli (160). Both astroglia and microglia cell bodies contained clearly visible apolipoprotein E throughout the bulb, but neurons contained no detectable apoE immunoreactivity. In both normal and apoE-KO mice, Western blot levels of olfactory marker protein (OMP-which is restricted to adult olfactory nerves) and area measurements of the terminal fields of olfactory nerves (glomeruli) showed a rapid decline after olfactory epithelium irrigation that reach a nadir within seven days. By 14 days levels of OMP and area were increasing in normal mice. ApoE was still significantly elevated two weeks following nasal irrigation, well into the period of olfactory nerve regeneration. Statistically normal levels of OMP were reached by 21 days, although further increase of OMP was observed at 42 days post lesion.

Markedly delayed regeneration of the olfactory nerve occurred in apoE-KO mice (169). We found no significant recovery at 14 or 21 days post-lesion with either OMP or areal measures of glomeruli. Levels of OMP started to increase at 42 days but only reached statistical comparability with the WT mice at 52 days post lesion. Of note was that the end point of regeneration was not different in the two genotypes suggesting that apoE affected the rate, but not the endpoint, of recovery. The clear implication from these studies was that apoE was intimately involved in both degeneration and regeneration.

We hypothesize that the delay in olfactory nerve regeneration in KO mice in the absence of apoE represents inefficient lipid utilization. During degeneration and regeneration apoE was upregulated and glia internalized free lipids from degenerating processes. During regeneration these lipids were available to support membrane synthesis by regenerating axons. In the absence of this exogenous supply of lipid derived from degenerated processes carried by apoE, regeneration required either *de novo* lipid synthesis by neurons and transport to the growth cone, lipid supply by less efficient methods, and/or possibly excessive glial activation that could cause excessive damage. We suspect that a primary role of apoE in neuroplasticity is to process and supply lipids to neurons for regeneration.

### 9.4. Culture models of apolipoprotein E and neuroplasticity

Several studies showed that apoE3, but not apoE4, combined with cholesterol, increased neurite growth *in vitro*. When combined with a source of lipid, apoE3 stimulated neurite extension from dorsal root ganglion neurons, whereas apoE4 inhibited it (170,171). Other studies using adult culture techniques have replicated this finding (see 172). Importantly, the effects of apoE on facilitating neurite outgrowth appear to be locally mediated (173). In a compartmented cell culture model, in which the media surrounding the cell body is mechanically isolated from the media surrounding the axon, apoE supplied to the growing axon facilitates axonal growth. If added to the cell body media, apoE has no significant effect on axonal growth. This suggests that apoE may act locally on a growing axon, perhaps to supply lipids for the extending cell membrane.

An enduring question, that has major clinical importance, is whether apoE4 is merely ineffective in supporting neurite growth or is actually neurotoxic and inhibits neurite growth. Disruption of the neuronal cytoskeleton by apoE4 was suggested as a possible etiology of growth delay in culture (171). In fact, we have performed studies of apoE growth facilitation finding in one study that apoE4 was ineffective, but in another, with "identical" conditions, that apoE4 inhibited growth compared to no apoE (174,175). Identifying causes for this discrepancy is important from a clinical point of view in that interventions that increase apoE production in individuals that are heterozygous for the apoE4 isoform could be injurious.

### 9.5. Microglial activation, estradiol and apoE

Another consideration for a role in apoE in neuroprotection is a role in suppressing microglial activation and inflammation and concomitant neuronal injury. ApoE replacement decreased microglial activation and secretion of proinflammatory factors including TNFα, IL-6, IL-1β and NO (176-189). In general, apoE4 was less effective than apoE3 in inhibiting microglial activation and NO production. Of note is a recent paper (190) that indicates that in an experimental model of MS, EAE, estrogen receptor (ER) alpha ligands delay disease onset and suppress inflammation. Conversely, ERbeta ligands improve the recovery during the chronic phase of the disease. Hence a biphasic effect of estrogens may be indicated, with an ERalpha mediated response protecting from damage and ERbeta aiding regeneration and repair.

### 9.6. Neurogenesis and ApoE

It is not clear if apoE modulates neurogenesis from stem cells although a suggestion of that possibility is present (191-193). The possible role of apoE and neuronal precursor cell function requires examination.

## 10. INTERACTION BETWEEN ESTRADIOL AND APOE ON AXONAL REGENERATION

### 10.1. *In vivo* studies of interactions between estradiol and apoE

In a key *in vivo* study, estradiol was found to increase the rate of hippocampal regeneration, but only in the presence of apoE (123). Mice that did not produce apoE did not show facilitation of regeneration. Experimentally induced stroke studies have shown the neuroprotective effect of estradiol replacement is not seen in the absence of apoE (114). Culture studies (discussed below) have shown the same dependency of estradiol on apoE.

### 10.2. *In vitro* studies of interactions between estradiol and apoE

We performed an i*n vitro* study to examine the combined effects of estradiol replacement and apoE isoforms (175). Our cultures were derived from adult mice and contained both neurons and glia. During preparation, the cells were stripped of processes. We found a dose response curve for estradiol increasing apoE in the medium that matched the dose-response curve for neurite growth, leading to the logical conclusion that increasing apoE increased neurite growth. This study also examined the role of estradiol combined with apoE isoforms. In the absence of apoE or combined with apoE4, physiological doses of estradiol had no effect on neurite growth. In contrast, apoE2 or apoE3 both increased neurite growth. Combined with estradiol, growth was significantly greater than apoE alone emphasizing the synergistic relationship. The same effects were seen in mice that were transgenic for either apoE3 or apoE4. This study showed that estradiol could increase process growth in cultured neurons but that the effect required the presence of apoE2 or apoE3. ApoE isoform apparently had a permissive effect on neurite growth stimulated by estradiol replacement. Of interest was that lipid transport into neurons by apoE3 was better than that by apoE4 which, in turn, was better than that of no apoE. Therefore, lipid transport alone did not account for the effects of estradiol on process growth.

This leads to the likelihood that estradiol probably had other effects on neuronal growth besides apoE increase. *In vivo* studies have shown that estradiol is able to increase neuronal activity. Estradiol may do this through modulation of the NMDA receptor (194). Our recent data, discussed below, show that estradiol can also modulate the expression of a multifunctional receptor, LRP (53). Studies have suggested that ligand binding by LRP may modulate the Ca2+ permeability of NMDA receptors (115). Hence, this system presents a simple model whereby estradiol could increase both the supply of lipids to neurons (by upregulating apoE) and simultaneously change the excitability of neurons by indirectly modulating the NMDA receptor and calcium influx. Moreover, apoE and the LRP could be neuroprotective by suppressing Ca2+ influx. This possibility of complex interactions between apoE, other ligands and LRP requires further study.

## 11. OVARIAN HOMONE CONTROL OF APOE and LRP

### 11.1. Normal variation in apoE expression in the brain

An obvious question this review raises is how does estradiol modulate apoE production? Previous studies suggested that estradiol acted through an ERalpha-like receptor (50,195). However, our data suggest that

estradiol likely controls apoE in an indirect manner. ApoE levels showed regionally-specific variation with the estrous cycle in females (51). ApoE protein in males tended to be slightly less than peak levels in females. The highest brain levels of apoE protein in females occurred on diestrus and proestrus in hippocampus, cingulate and frontal cortex with lowest levels on estrus (51). Hippocampal levels tended to correspond to apoE mRNA levels which peaked on diestrus in CA3 and proestrus in CA1 of hippocampus (196). Comparison of the mRNA data and our protein data suggest even more regional variation within the hippocampus that was masked in homogenate studies. In striking contrast to cortex was that highest levels of apoE in the olfactory bulb and cerebellum were on estrus with lower levels on di- and proestrus (51). Clearly a major difference between brain regions exists.

**11.2. Short and long-term effects of estradiol replacement**

We found that physiological doses of estradiol increased apoE in adult cell culture media four hours after addition. ApoE remained elevated for four days in these mixed neuron-glial cultures from adult mice (175). However, the *in vivo* response to estradiol replacement was more complex than a simple receptor mediated process would suggest.

Estradiol increased whole brain apoE mRNA and protein at five days after replacement in rodents (49-52). In two studies we found that five days of estradiol pellet replacement had a small effect on increasing neocortical apoE (ca. 20%), a stronger effect in hippocampus (ca. 40%) and a substantial effect in olfactory bulb and cerebellum (100%) (51,52). This magnitude of hippocampal change corresponds to a more recent report (195).

In subsequent studies we examined the effects of continuous estradiol replacement for up to 49 days (52). We used estradiol pellet replacement at the time of OVX and followed apoE, glial fibrillary acidic protein (GFAP-an indicator of reactive astrocytes), synaptophysin (a presynaptic protein), LDLr and LRP. This study presented three interesting results. The first was that apoE and synaptophysin increased seven days after estradiol replacement in the four brain regions examined (olfactory bulb, cerebellum, somatosensory cortex, and hippocampus) compared to the OVX-non-replaced mice. The second observation was that at 14 days after continuous estradiol, levels of apoE and synaptophysin declined to levels comparable to the vehicle-treated mice in all regions and stayed low over the 49 days. We found no changes in the LDLr in any area or at any time following estradiol replacement. LRP increased in hippocampus, somatosensory cortex and olfactory bulb at seven days but did not increase in the cerebellum (53). The third observation involved GFAP. GFAP, in the estradiol-replaced mice, was not different from the non-replaced mice at seven or 14 days. However, GFAP in the non-replaced mice began to increase and was significantly higher than the estradiol replaced group by 49 days. Long-term estradiol replacement appeared to suppress glial activation, a conclusion made in previous studies (197).

The explanation for this pattern of results is not obvious at this point, but these data raise interesting questions about protocols commonly used for HT. We found that acute increases of apoE, LRP and synaptophysin by estradiol replacement occurred in the mouse brain. However, levels of these proteins declined to OVX levels during continuous estrogen replacement. Hence, in the mouse, neuroprotective effects of estradiol may decline with continuous exposure longer than seven days. This decline may explain the general failure of more than two weeks of continuous estradiol to be neuroprotective in stroke studies in rodents as discussed above. In the clinical arena, continuous PremPro or Premarin replacement may lose neuroprotective effects. Perhaps intervals of interruption of HT are necessary to maximize its neuroprotective effect.

**11.3. Estrogen receptors in the mouse brain**

We also evaluated the distribution of estrogen receptors (ER) in these brain areas with immunocytochemical techniques. In the cortical areas we identified both ERalpha and ERbeta-like immunoreactivity (51,198). We found weak ERalpha-like nuclear immunoreactivity in lamina II of, primarily, somatosensory-parietal cortex neurons. ERbeta-like immunoreactivity (with a Zymed antisera) was present in some neurons in deeper layers of the neocortex and in small processes and cells that were probably glia (198). In the olfactory bulb we found ERbeta in olfactory bulb glia in the glomerular layer and ERbeta exclusively in Bergmann glia in the cerebellum (51). In contrast, we found little or no ERalpha-like immunoreactivity which replicates previously published studies (199,200). Although negative results with immunocytochemistry must always be considered with suspicion, the absence of ERalpha in glia, the primary source of apoE (201,202) suggest more complex control of apoE by estrogens. Of note, in two searches of independent genome data bases we found only a three base pair consensus sequence for an estrogen response element in the promoter region of mouse apoE. This suggests that apoE is not regulated by estradiol acting through a classical estrogen receptor. The precise mode of estradiol control of apoE expression is not clear.

**11.4. Acute responses to estradiol replacement**

In unpublished studies we used acute (2-24 hours) estradiol replacement after OVX to address questions of aging, vs. duration of OVX raised by the previous study. Mice were OVX at two ages, 16 or 23 weeks and received a single subcutaneous dose of estradiol, which was expected to increase serum estradiol to proestrus levels. A third group of mice was OVX at 16 weeks but not replaced until 23 weeks. This design permitted comparison of aging and delay of replacement. We found that a single dose of estradiol in the immediate (five days) replacement groups did not increase apoE within 24 hours. However, estradiol replacement seven weeks following OVX increased apoE protein levels within twelve hours in all brain regions. In no condition was synaptophysin increased at these short intervals of estrogen replacement as would have been expected from previous studies (119,120).

LRP showed a more complex response to estradiol replacement compared to that of apoE. In the hippocampus of 16wk mice, replacing estradiol suppressed the levels of LRP protein two hours after administration. A comparable, but less robust suppression was observed in the somatosensory cortex. In contrast to the young (16wk) mice, estradiol replacement had no effect on the mice that were OVX at 23 weeks and replaced five days later. Elevation of LRP at twelve hours in hippocampus occurred in the long-term OVX mice. These mice expressed the lowest baseline levels of LRP. LDLr showed no effects of estradiol replacement. Our data suggest that levels of LRP are not directly modulated by apoE levels (115). This study also showed that OVX and estradiol replacement had an age-specific effect on LRP and that studies of "long-term" OVX effects are age-sensitive. Specifically, a 16 week old mouse showed a very different response to OVX and immediate estradiol replacement than a 23 week old mouse.

Clearly we are dealing with a moving target in interpreting the "effects" of hormone replacement on apoE. A "unique" response to estradiol replacement does not exist. Effects of five to seven days of estradiol replacement are not identical to an acute response. Both apoE and GFAP are strongly entrained to the estrus cycle, (51,198). But the effects of acute estradiol replacement on both apoE and indicators of glial activation are complex. Estradiol must be interacting with other ovarian steroids or neurosteroids to modulate apoE expression. For example, it is possible that high levels of progesterone that occur on estrus and metestrus prime glia to respond to slight increases in estradiol that occur late in diestrus. In addition, reproductive history (i.e., perhaps the number of estrous cycles), duration between ovariectomy and estrogen replacement, and duration of treatment all act to change the response to estradiol replacement. The outcome of these studies raises multiple questions about aging and the timing of estradiol replacement that may have profound implications in the clinical application of HT.

## 12. INTERACTION BETWEEN ASTROGLIA AND NEURONS ON APOE PRODUCTION

We propose that to properly interpret the results of estradiol replacement on apoE, LRP and probably other proteins, we must consider the inter-relationship between neurons and glia. A complex neuron-glia interaction has been emphasized in several reviews (203-206). Estradiol stimulation of apoE production probably requires, in a broadly defined sense, glial activation as shown by temporally GFAP preceding apoE production (156). The rat promoter region for GFAP has a functional estrogen response element and estrogen has been shown to increase GFAP mRNA message in pure glial cultures. However, inclusion of neurons in a culture eliminates this response (205).

Neuronal activity/inactivity may modulate the glial response to a specific stimulus. Conversely, glia may modulate a neuronal response to stimuli. Astroglia produce lactic acid that may be a primary energy source for neurons (207,208). Glia also serve as a sink for glutamate released by neurons. Glutamate is processed to glutamine, which is transferred back to neurons (209-211). Glial derived protein (s) have been indicated as critical for the formation of neuronal synapses (212-214). Glutamate and other neurotransmitters can increase glial intracellular Ca2+ which may, in turn, act to favor depolymerized GFAP (215,216). Depolymerization of GFAP could result in retraction of astroglial processes permitting expansion of neuronal processes and new synaptogenesis. Conversely, neuronal inhibition by γ-amino butyric acid (GABA) agonists increased polymerized GFAP (217,218). We have shown that estradiol replacement increased LRP, which has been proposed to suppress NMDA channel mediated Ca+2 influx (115). Hence what happens in glia affects neurons, and vice versa. Although this statement appears fatuous, it should be remembered for interpretation of experimental interventions in a monotypic cell culture.

Given the glia-neuron interaction, the net effect of in vivo estradiol replacement, identified by some dependent variable (e.g., apoE), represents the combined response of these two cell types. This process could easily occur over hours or days. Several reports suggest that estradiol replacement can directly activate neurons (219-223). Stimulation could directly affect neuron-neuron activation via an NMDA receptor as has been proposed for estradiol effects on synaptogenesis (223). Initially, neuronal stimulation by estradiol may suppress glial activity and function. However, neuronal stimulation may be self limiting resulting in a release from neuronal inhibition of glial. Release from inhibition may result in supply of energy (lactic acid) to neurons and the release of apoE. ApoE would then be able to supply lipids for repair or remodeling of the neuronal membrane. Of note is that spreading cortical depression, which eventually results in quiescent neurons, is associated with upregulation of apoE message (165). Hence, the ultimate "outcome" of estradiol replacement may represent a constellation of sequential cellular responses.

## 13. PERSPECTIVE

A close inter-relationship between estradiol replacement and apoE is strongly suggested by a review of the diseases affected by both. Both apoE and estradiol appear to decrease the severity of stroke and facilitate neuronal repair. Figure 1 presents a possible model for multifaceted neuroprotective effects of estradiol mediated by apoE. We suspect that apoE may be able both to limit the acute damage done from an injurious event and improve repair and plasticity. Hence, estrogen replacement may be able to delay the progression of numerous chronic neurological diseases. Moreover, estradiol can improve neurogenesis from neuronal precursor cells and neurogenesis may further slow disease progression. However, no data, to our knowledge, indicates a possible role of apoE in neurogenesis from progenitor cells, but the effect may have been overlooked. If apoE is able to improve neuronal protection and repair, then one obvious goal is to find approaches to increase brain levels of apoE following injury.

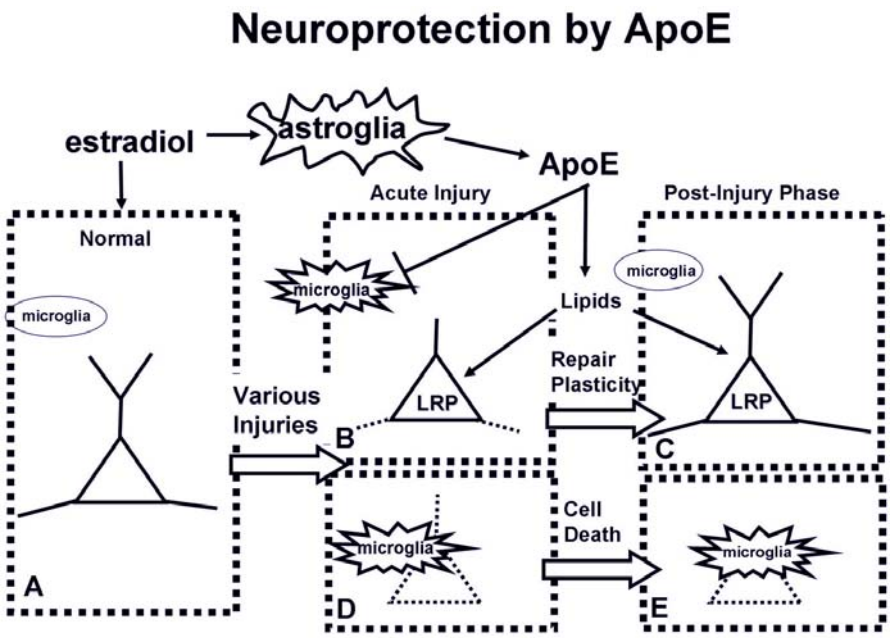


**Figure 1.** Possible interactions between estradiol and apoE. This figure presents our hypotheses about possible interactions between estradiol and apoE. Addition of estradiol has direct effects on neurons (A) including increasing levels of the LRP, a multifunctional apoE receptor[46-48,53]. Simultaneously to the neuronal effect, or within a brief time period, estradiol also stimulates astroglia (and perhaps microglia) to release or increase apoE[49-52]. Increased apoE, concurrent with injury (B), may have two neuroprotective actions in this acute phase. ApoE may sequester and transport lipids for uptake by injured neurons to replace oxidized membrane lipids and limit damage. ApoE may also suppress microglial activation and the release of cytotoxic agents[177-181]. Binding with LRP by apoE, and perhaps by other ligands, can modify numerous cellular processes that may also be neuroprotective[116]. During the Post-Injury phase, (C) apoE supplies lipids to neurons to facilitate process extension and synaptogenesis[124,170] allowing more rapid recovery following injury. In the absence of apoE or in the presence of apoE4 (D) neither of these protective actions occurs. Extra lipids are not available and continued upregulation of cytokines and NO from activated microglia induce extensive cell damage to neurons. Moreover, in the absence of apoE, (E) regeneration may be slowed due to lack of available lipids and microglial activation persists, exacerbating their toxicity at the injury site.

Judiciously applied estradiol replacement may be one approach to increase neuroprotection. Patient history and menopausal status are important considerations in selecting the most appropriate intervention. As demonstrated by the WHI study, HT initiated decades after the menopause may not be neuroprotective (25,26) and perhaps injurious. In all likelihood, HT should be initiated during the perimenopausal interval (18). HT may not be indicated if apoE4 does not promote repair or is actually injurious and the patient carries one or two apoE ε4 genes. Interventions that increase apoE4 might result in more rapid deterioration. For example, approximately 40-60% of demented women carry at least one apoE4 allele. This overabundance of the apoE4 isoform might explain the apparent more rapid rate of decline in one study of PremPro replacement than the placebo controls in women with dementia (20), as well as the general failure of HT studies in demented patients.

Confirmation of a critical interaction between estrogen and apoE might lead to more rational approaches to pharmacological interventions. Intervention could be tailored for direct stimulation of apoE by selective estrogen response modulators while avoiding the negative effects of stimulation of all estrogen receptors. For example, if apoE is primarily produced by stimulation of an ERbeta like receptor, then selective ERbeta agonists might be more efficacious than estradiol alone. Finally, the role of progesterone has not been explored, and as our data on the estrous cycle suggest, estradiol may strongly interact with other hormones. The likelihood that apoE is a critical variable in the neuroprotective effects of HT requires focusing on possible approaches to increase the appropriate isoform of apoE in a timely and functional manner.

## 14. ACKNOWLEDGMENTS

These studies were supported by the Illinois Department of Public Health Alzheimer Disease Fund and the Southern Illinois University School of Medicine Central Research Committee Research Fund. None of the authors has any financial interests related to this work. We wish to sincerely thank: Shari Beckman-Randall and Jennifer (Miao) Li for expert technical assistance; Xiang Xing Cheng for her work on the LRP; and countless medical and graduate students for their participation in the studies presented herein

## 15. REFERENCES


1. Rossouw J. E, G. L. Anderson, R. L. Prentice, A. Z. LaCroix, C. Kooperberg, M. L. Stefanick, R. D. Jackson, S. A. Beresford, B. V. Howard, K. C. Johnson, J. M. Kotchen, J. Ockene: Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* 288, 321-333 (2002)

2. Saitoh M,, M. Ohmichi, K. Takahashi, J. Kawagoe, T. Ohta, M. Doshida, T. Takahashi, H. Igarashi, A. Mori-Abe, B. Du, S. Tsutsumi, H. Kurachi: Medroxyprogesterone acetate induces cell proliferation through up-regulation of cyclin D1 expression via phosphatidylinositol 3-kinase/Akt/nuclear factor-kappaB cascade in human breast cancer cells. *Endocrinology* 146, 4917-4925 (2005)

3. Seeger H, D. Wallwiener, A. O. Mueck: The effect of progesterone and synthetic progestins on serum- and estradiol-stimulated proliferation of human breast cancer cells. *Horm Metab Res* 35, 76-80 (2003)

4. Vickers M. R, A. H. MacLennan, B. Lawton, D. Ford, J. Martin, S. K. Meredith, B. L. DeStavola, S. Rose, A. Dowell, H. C. Wilkes, J. H. Darbyshire, T. W. Meade, WISDOM group: Main morbidities recorded in the women's international study of long duration oestrogen after menopause (WISDOM): a randomised controlled trial of hormone replacement therapy in postmenopausal women. *BMJ* 335, 239-251 (2007)

5. Haas J. S, C. P. Kaplan, E. P. Gerstenberger, K. Kerlikowske: Changes in the use of postmenopausal hormone therapy after the publication of clinical trial results. *Ann Intern Med* 140, 184-188 (2004)

6. Hersh A. L, M. L. Stefanick, R. S. Stafford: National use of postmenopausal hormone therapy: annual trends and response to recent evidence. *JAMA* 291, 47-53 (2004)

7. Bennett D. A, J. A. Schneider, Z. Arvanitakis, J. F. Kelly N. T. Aggarwal, R. C. Shah, R. S. Wilson: Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology* 66, 1837-1844 (2006)

8. S. G. Gabbe, J. R. Niebyl, J. L. Simpson, (eds) Obstetrics: normal and problem pregnancies. 5th ed, Churchill Livingstone/Elsevier, Philadelphia, PA, 2007.

9. M. A. Stenchever. Comprehensive gynecology. 4th ed., Mosby, St. Louis, MO, 2001.

10. P. Reed H. Larsen, M. Kronenbery, S. Melmed, K. S. Polonsky (eds.) Williams textbook of endocrinology, 10th ed., Saunders, Philadelphia, PA. 2002.

11. J. D. Neill. (ed.) Knobil and Neill's Physiology of Reproduction, 3rd ed., Elsevier, Boston, MA, 2006.

12. Kawas C, S. Resnick, A. Morrison, R. Brookmeyer, M. Corrada, A. Zonderman, C. Bacal, D. D. Lingle, E. Metter: A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. *Neurology* 48, 1517-1521 (1997)

13. Paganini-Hill A, V. Henderson: Estrogen deficiency and risk of Alzheimer's disease in women. *Am J Epidemiol* 140, 256-261 (1994)

14. Brenner D. E, W. A. Kukull, A. Stergachis, G. van Belle, J. D. Bowen, W. C. McCormick, L. Teri, E. B. Larson: Postmenopausal estrogen replacement therapy and the risk of Alzheimer's disease: a population-based case-control study. *Am J Epidemiol* 140, 262-267 (1994)

15. Tang M. X, D. Jacobs, Y. Stern, K. Marder, P. Schofield, B. Gurland, H. Andrews, R. Mayeux: Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 348, 429-432 (1996)

16. LeBlanc E. S, J. Janowsky, B. K. Chan, H. D. Nelson: Hormone replacement therapy and cognition: systematic review and meta-analysis. *JAMA* 285, 1489-1499 (2001)

17. Zandi P. P, M. C. Carlson, B. L. Plassman, K. A. Welsh-Bohmer, L. S. Mayer, D. C. Steffens, J. C. Breitner: Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. JAMA 288, 2123-2129 (2002)

18. Henderson V. W, K. S. Benke, R. C. Green, L. A. Cupples, L. A. Farrer, MIRAGE Study Group: Postmenopausal hormone therapy and Alzheimer's disease risk: interaction with age. *J Neurol Neurosurg Psychiat* 76, 103-105 (2005)

19. Rocca W. A, J. H. Bower, D. M. Maraganore, J. E. Ahlskog, B. R. Grossardt, M. de Andrade, and L. J. Melton III: Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause *Neurology* 69, 1074-1083 (2007)

20. Mulnard R. A, C. W. Cotman, C. Kawas, C. H. van Dyck, M. Sano, R. Doody, E. Koss, E. Pfeiffer, S. Jin, A. Gamst, M. Grundman, R. Thomas, L. J. Thal: Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. Alzheimer's Disease Cooperative Study. *JAMA* 283, 1007-1015 (2000)

21. Wang P. N, S. Q. Liao, R. S. Liu, C. Y. Liu, H. T. Chao, S. R. Lu, H. Y. Yu, S. J. Wang, H. C. Liu: Effects of estrogen on cognition, mood, and cerebral blood flow in AD: a controlled study. *Neurology* 54, 2061-2066 (2000)

22. Henderson V. W, A. Paganini-Hill, B. L. Miller, R. J. Elble, P. F. Reyes, D. Shoupe, C. A. McCleary, R. A. Klein, A. M. Hake, M. R. Farlow: Estrogen for Alzheimer's disease in women: randomized, double-blind, placebo-controlled trial. *Neurology* 54, 295-301 (2000)

23. Thal L. J, R. G. Thomas, R. Mulnard, M. Sano, M. Grundman, L. Schneider. Estrogen levels do not correlate with improvement in cognition. *Arch Neurol* 60, 209-212 (2003)

24. Asthana S, L. D. Baker, S. Craft, F. Z. Stanczyk, R. C. Veith, M. A. Rasking, S. R. Plymate. High-dose estradiol improves cognition for women with AD: results of a randomized study. *Neurology* 57, 605-612 (2001)

25. Espeland M. A, S. R. Rapp, S. A. Shumaker, R. Brunner, J. E. Manson, B. B. Sherwin, J. Hsia, K. L. Margolis, P. E. Hogan, R. Wallace, M. Dailey, R. Freeman, J. Hays: Women's Initiative Memory Study. Conjugated equine estrogens and global cognitive function in postmenopausal women: Women's Health Initiative Memory Study. *JAMA* 291, 2959-2968 (2004)

26. Shumaker S, A, C. Legault, L. Kuller, S. R. Rapp, L. Thal, D. S. Lane, H. Fillit, M. L. Stefanick, S. L. Hendrix, C. E. Lewis, K. Masaki, L. H. Coker: Women's Health Initiative Memory Study. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women:

Women's Health Initiative Memory Study. *JAMA* 291, 2947-2958 (2004).
27. Paganini-Hill A: Estrogen replacement therapy and stroke. *Prog Cardiovasc Dis* 38, 223-242 (1995)
28. Stein D. G.: Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? *Trends Neurosci* 24, 386-91 (2001)
29. Paganini-Hill A, M. Perez Barreto: Stroke risk in older men and women: aspirin, estrogen, exercise, vitamins, and other factors. *J Gend Specif Med* 4, 18-28 (2001)
30. Simpkins J. W, S. H. Yang, Y. Wen, M. Singh: Estrogens, progestins, menopause and neurodegeneration: basic and clinical studies. *Cell Mol Life Sci* 62, 271-280 (2005)
31. Snowdon D. A, L. H. Greiner, J. A. Mortimer, K. P. Riley, P. A. Greiner, W. R. Markesbery: Brain infarction and the clinical expression of Alzheimer disease. The Nun Study *JAMA* 277, 813-817 (1997)
32. Saunders-Pullman R, J. Gordon-Elliott, M. Parides, S. Fahn, H. R. Saunders, S. Bressman: The effect of estrogen replacement on early Parkinson's disease. *Neurology* 52, 1417-1421 (1999)
33. Currie L. J, M. B. Harrison, J. M. Trugman, J. P. Bennett, G. F. Wooten. Postmenopausal estrogen use affects risk for Parkinson disease. *Arch Neurol* 61, 886-888 (2004)
34. Rocca W. A, J. H. Bower, D. M. Maraganore, J. E. Ahlskog, B. R. Grossardt, M. de Andrade, and L. J. Melton III: Increased risk of parkinsonism in women who underwent oophorectomy before menopause. Neurology 64, 1458-1464. (2007)
35. Smith R, J. W. Studd: A pilot study of the effect upon multiple sclerosis of the menopause, hormone replacement therapy and the menstrual cycle. *J R Soc Med* 85, 612-613 (1992)
36 Holmqvist P,, M. Wallberg, M. Hammar, A. M. Landtblom, J. Brynhildsen: Symptoms of multiple sclerosis in women in relation to sex steroid exposure. *Maturitas* 54, 149-153 (2006)
37. Voskuhl R. R: Hormone-based therapies in MS. *Int MS J* 10, 60-66 (2003)
38. Sicotte N. L, S. M. Liva, R. Klutch, P. Pfeiffer, S. Bouvier, S. Odesa, T. C. Wu, R. R. Voskuhl: Treatment of multiple sclerosis with the pregnancy hormone estriol. *Ann Neurol* 52, 421-428. (2002)
39. Houtchens M. K,: Pregnancy and multiple sclerosis. *Semin Neurol* 27, 434-441. (2007)
40. Mahley R. W: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240, 622-630 (1988)
41. Boyles J. K, C. D. Zoellner, L. J. Anderson, L. M. Kosik, R. E. Pitas, K. H. Weisgraber, D. Y. Hui, R. W. Mahley, P. J. Gebicke-Haerter, M, J. Ignatius: A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J Clin Invest* 83, 1015-1031 (1989)
42. Menzel H. J, G. Assmann, S. C. Rall Jr., K. H. Weisgraber ¸ R. W. Mahley: Human apolipoprotein A-I polymorphism. Identification of amino acid substitutions in three electrophoretic variants of the Munster-3 type, *J Biol Chem* 259, 3070-3076 (1984)
43. Hallman D. M, E. Boerwinkle, N. Saha, C. Sandholzer, H. J. Menzel, A. Csazar, G. Utermann: The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations, *Am J Hum Genet* 49, 338-349 (1991)
44. Weisgraber K. H: Apolipoprotein E: structure-function relationships. *Adv Protein Chem* 45, 249-302 (1994)
45. Davignon J, R. E. Gregg, C. F. Sing: Apolipoprotein E polymorphism and atherosclerosis, *Arteriosclerosis* 8, 1-21 (1988)
46. Herz J, D. K. Strickland: LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest* 108, 779-784 (2001)
47. Herz J, H. H. Bock: Lipoprotein receptors in the nervous system. *Ann Rev Biochem* 71, 405-434 (2002)
48. Strickland D. K, S. Ranganathan: Diverse role of LDL receptor-related protein in the clearance of proteases and in signaling. *J Thromb Haemost* 1, 1663-1670 (2003)
49. Srivastava R. A, N. Bhasin, N. Srivastava: Apolipoprotein E gene expression in various tissues of mouse and regulation by estrogen *Biochem Mol Biol Int* 38, 91-101 (1996)
50. Levin-Allerhand J, B. S. McEwen, C. E. Lominska, D. B. Lubahn, K. S. Korach, J. D. Smith: Brain region-specific up-regulation of mouse apolipoprotein E by pharmacological estrogen treatments. *J Neurochem* 79, 796-803 (2001)
51. Struble R. G, E. R. Rosario, M. L. Kircher, S. M. Ludwig, P. J. McAdamis, K. Watabe, M. E. McAsey, C. Cady, B. P. Nathan: Regionally specific modulation of brain apolipoprotein E in the mouse during the estrous cycle and by exogenous 17β estradiol. *Exp Neurol* 183, 638-44 (2003)
52. McAsey M. E, C. Cady, L. M. Jackson, M. Li, S. Randall, B. P. Nathan, R. G. Struble: Time course of response to estradiol replacement in ovariectomized mice: brain apolipoprotein E and synaptophysin transiently increase and glial fibrillary acidic protein is suppressed. *Exp Neurol* 197, 197-205 (2006)
53. Cheng X, M. E. McAsey, M. Li, S. Randall, C. Cady, B. P. Nathan, R. G. Struble: Estradiol replacement increases the low-density lipoprotein receptor related protein (LRP) in the mouse brain. *Neurosci Lett* 417, 50-54 (2007)
54. Saunders A. M, K. Schmader, J. C. Breitner, M. D. Benson, W. T. Brown, L. Goldfarb, D. Goldgaber, M. G. Manwaring, M. H. Szymanski, N. McCown: Apolipoprotein E epsilon 4 allele distributions in late-onset Alzheimer's disease and in other amyloid-forming diseases. *Lancet* 342, 710-711 (1993)
55. Gopalraj R. K, H. Zhu, J. F. Kelly, M. Mendiondo, J. F. Pulliam, D. A. Bennett, S. Estus: Genetic association of low density lipoprotein receptor and Alzheimer's disease. *Neurobiol Aging* 26, 1-7 (2005)
56. Kolsch H, U. Ptok, I. Mohamed, S. Schmitz, M. L. Rao, W. Maier, R. Heun: Association of the C766T polymorphism of the low-density lipoprotein receptor-related protein gene with Alzheimer's disease. *Am J Med Genet B Neuropsychiatr Genet* 121, 128-130 (2003)
57. Holtzman D. M: In vivo effects of ApoE and clusterin on amyloid-beta metabolism and neuropathology. *J Mol Neurosci* 23, 247-254 (2004)

58. Bu G, J. Cam, C. Zerbinatti: LRP in amyloid-beta production and metabolism. Ann N Y Acad Sci. 1086, 35-53 (2006)
59. Tang G, H. Xie, L. Xu, Y. Hao, D. Lin, D. Ren: Genetic study of apolipoprotein E gene, alpha-1 antichymotrypsin gene in sporadic Parkinson disease. *Am J Med Genet* 114, 446-449 (2002)
60. Zareparsi S, R. Camicioli, G. Sexton, T. Bird, P. Swanson, J. Kaye, J. Nutt, H. Payami: Age at onset of Parkinson disease and apolipoprotein E genotypes. *Am J Med Genet* 107, 156-161 (2002)
61. Buchanan D. D, P. A. Silburn, J. A. Prince, G. D. Mellick: Association of APOE with Parkinson disease age-at-onset in women. *Neurosci Lett* 411, 185-188 (2007)
62. Ghebremedhin E, K. Del Tredici, M. Vuksic, U. Rub, D.R. Thal, G. J. Burbach, A. Rosenberger, H. Bickeboller, T. Deller, R. A. de Vos, E. N. Jansen Steur, H. Braak: Relationship of apolipoprotein E and age at onset to Parkinson disease neuropathology. J Neuropathol *Exp Neurol* 65, 116-123 (2006)
63. Huang X, P. Chen, D. I. Kaufer, A. I. Troster, C. Poole: Apolipoprotein E and dementia in Parkinson disease: a meta-analysis. *Arch Neurol* 63, 189-193 (2006)
64. Short R. A, N. R. Graff-Radford, J. Adamson, M. Baker, M. Hutton: Differences in tau and apolipoprotein E polymorphism frequencies in sporadic frontotemporal lobar degeneration syndromes. *Arch Neurol* 59, 611-615 (2002)
65. Jordan B. D, N. R. Relkin, L. D. Ravdin, A. R. Jacobs, , A. Bennett, S. Gandy: Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. *JAMA* 278, 136-140 (1997)
66. Friedman G, P. Froom, L. Sazbon, I. Grinblatt, M. Shochina, J. Tsenter, S. Babaey, Z. Yehuda Groswasser: Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology* 52, 244-248 (1999)
67. Masterman T, Z. Zhang, D. Hellgren, H. Salter, M. Anvret, L. Lilius, L. Lannfelt, J. Hillert: APOE genotypes and disease severity in multiple sclerosis. *Mult Scler* 8, 98-103 (2002)
68. Fazekas F, S. Strasser-Fuchs, H. Kollegger, T. Berger, W. Kristoferitsch, C. Schmidt, H. Enzinger, M. Schiefermeier, C. Schwarz, B. Kornek, M. Reindl, K. Huber, R. Grass, G. Wimmer, K. Vass, K. H. Pfeiffer, H. P. Hartung, R. Schmidt: Apolipoprotein E epsilon 4 is associated with rapid progression of multiple sclerosis. *Neurology* 57, 853-857 (2001)
69. Li Y. J, M. A. Pericak-Vance, J. L. Haines, N. Siddique, D. McKenna-Yasek, W. Y. Hung, P. Sapp, C. I. Allen, W. Chen, B. Hosler, A, M. Saunders, L. M. Dellefave, R. H. Brown, T. Siddique: Apolipoprotein E is associated with age at onset of amyotrophic lateral sclerosis. Neurogenetics 5, 209-213 (2004)
70. Drory V. E, M. Birnbaum, A. D. Korczyn, J. Chapman: Association of APOE epsilon4 allele with survival in amyotrophic lateral sclerosis. J Neurol Sci 190, 17-20 (2001)
71. Briellmann R. S, Y. Torn-Broers, B. E. Busuttil, B. J. Major, R. M. Kalnins, M. Olsen, G. D. Jackson, A. G. Frauman, S. F. Berkovic: APOE epsilon4 genotype is associated with an earlier onset of chronic temporal lobe epilepsy. *Neurology* 55, 435-437 (2000)
72. Sporis D, J. Sertic, N. Henigsberg, D. Mahovic, N. Bogdanovic, T. Babic. Association of refractory complex partial seizures with a polymorphism of ApoE genotype. *J Cell Mol Med* 9, 698-703 (2005)
73. Diaz-Arrastia R, Y. Gong, S. Fair, K. D. Scott, M. C. Garcia, M. C. Carlile, M. A. Agostini, P. C. Van Ness: Increased risk of late posttraumatic seizures associated with inheritance of APOE epsilon4 allele *Arch Neurol* 60, 818-22 (2003)
74. Yaffe K, M. Haan, A. Byers, C. Tangen, L. Kuller: Estrogen use, APOE, and cognitive decline: evidence of gene-environment interaction. *Neurology* 54, 1949-1954 (2000)
75. Burkhardt M. S, J. K. Foster, S. M. Laws, L. D. Baker, S. Craft, S. E. Gandy, B. G. Stuckey, R. Clarnette, D. Nolan, B. Hewson-Bower, R. N. Martins: Oestrogen replacement therapy may improve memory functioning in the absence of APOE epsilon4. *J Alzheimers Dis* 6, 221-228 (2004)
76. Rippon G. A, M. X. Tang, J. H. Lee, R. Lantigua, M. Medrano, R. Mayeux: Familial Alzheimer disease in Latinos: interaction between APOE, stroke, and estrogen replacement. *Neurology* 66, 35-40 (2006)
77. Mattila K. M, K. Axelman, J. O. Rinne, M. Blomberg, T. Lehtimaki, P. Laippala, M. Roytta, M. Viitanen, L. Wahlund, B. Winblad, L. Lannfelt: Interaction between estrogen receptor 1 and the epsilon 4 allele of apolipoprotein E increases the risk of familial Alzheimer's disease in women. Neurosci Lett 282, 45-8 (2000)
78. Liu R, X. Wang, Q. Liu, S. H. Yang, J. W. Simpkins: Dose dependence and therapeutic window for the neuroprotective effects of 17beta-estradiol when administered after cerebral ischemia. Neurosci Lett 415, 237-241 (2007)
79. Simpkins J. W, G. Rajakumar, Y. Q. Zhang, C. E. Simpkins, D. Greenwald, C. J. Yu, N. Bodor, A. L. Day: Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. *J Neurosurg* 87, 724-730 (1997)
80. Zhang Y. Q, J. Shi, G. Rajakumar, A. L. Day, J. W. Simpkins: Effects of gender and estradiol treatment on focal brain ischemia. *Brain Res* 784, 321-324 (1998)
81. Hall E. D, K. E. Pazara, K. L. Linseman: Sex differences in postischemic neuronal necrosis in gerbils. *J Cereb Blood Flow Metab* 11, 292-298 (1991)
82. Shi J, Y. Q. Zhang, J. W. Simpkins: Effects of 17beta-estradiol on glucose transporter 1 expression and endothelial cell survival following focal ischemia in the rats. *Exp Brain Res* 117, 200-206 (1997)
83. Dubal D. B, M. L. Kashon, L. C. Pettigrew, J. M. Ren, S. P. Finklestein, S. W. Rau, P. M. Wise: Estradiol protects against ischemic injury. *J Cereb Blood Flow Metab* 18, 1253-1258 (1998)
84. Toung T. J, R. J. Traystman, P. D. Hurn: Estrogen-mediated neuroprotection after experimental stroke in male rats. *Stroke* 29, 1666-1670 (1998)
85. Rusa R, N. J. Alkayed, B. J. Crain, R. J. Traystman, A. S. Kimes, E. D. London, J. A. Klaus, P. D. Hurn: 17beta-estradiol reduces stroke injury in estrogen-deficient female animals. *Stroke* 30, 1665-1670 (1999)
86. Alkayed N. J, S. J. Murphy, R. J. Traystman, P. D. Hurn, V. M. Miller: Neuroprotective effects of female

gonadal steroids in reproductively senescent female rats. *Stroke* 31, 161-168 (2000)
87. Fukuda K, H. Yao, S. Ibayashi, T. Nakahara, H. Uchimura, M. Fujishima, E. D. Hall: Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats. *Stroke* 31, 155-160 (2000)
88. Veliskova J, L. Velisek, A. S. Galanopoulou, E. F. Sperber: Neuroprotective effects of estrogens on hippocampal cells in adult female rats after status epilepticus. *Epilepsia* 41, Suppl 6:S30-35 (2000)
89. Dubal D. B, P. M. Wise: Neuroprotective effects of estradiol in middle-aged female rats. *Endocrinology* 142, 43-48 (2001)
90. Harukuni I, P. D. Hurn, B. J. Crain: Deleterious effect of beta-estradiol in a rat model of transient forebrain ischemia. *Brain Res* 900, 137-142 (2001)
91. Watanabe Y, M. T. Littleton-Kearney, R. J. Traystman, P. D. Hurn: Estrogen restores postischemic pial microvascular dilation. *Am J Physiol Heart Circ Physiol* 281, H155-160 (2001)
92. Santizo R. A, H. L. Xu, S. Ye, V. L. Baughman, D. A. Pelligrino: Loss of benefit from estrogen replacement therapy in diabetic ovariectomized female rats subjected to transient forebrain ischemia. *Brain Res* 956, 86-95 (2002)
93. He Z, Y. J. He, A. L. Day, J. W. Simpkins: Proestrus levels of estradiol during transient global cerebral ischemia improves the histological outcome of the hippocampal CA1 region: perfusion-dependent and-independent mechanisms. *J Neurol Sci* 193, 79-87 (2002)
94. Mehta S. H, K. M. Dhandapani, L. M. De Sevilla, R. C. Webb, V. B. Mahesh, D. W. Brann: Tamoxifen, a selective estrogen receptor modulator, reduces ischemic damage caused by middle cerebral artery occlusion in the ovariectomized female rat. *Neuroendocrinology* 77, 44-50 (2003)
95. Fan T, S.H. Yang, E. Johnson, B. Osteen, R. Hayes, A. L. Day, J. W. Simpkins: 17beta-Estradiol extends ischemic thresholds and exerts neuroprotective effects in cerebral subcortex against transient focal cerebral ischemia in rats. *Brain Res* 993, 10-17 (2003)
96. Shughrue P. J, I. Merchenthaler: Estrogen prevents the loss of CA1 hippocampal neurons in gerbils after ischemic injury. *Neuroscience* 116, 851-861 (2003)
97. Wen Y, S. Yang, R. Liu, E. Perez, K. D. Yi, P. Koulen, J. W. Simpkins: Estrogen attenuates nuclear factor-kappa B activation induced by transient cerebral ischemia. *Brain Res* 1008, 147-154 (2004)
98. Xu H. L, V. L. Baughman, D. A. Pelligrino: Estrogen replacement treatment in diabetic ovariectomized female rats potentiates postischemic leukocyte adhesion in cerebral venules. *Stroke* 35, 1974-1978 (2004)
99. Carswell H. V, D. Bingham, K. Wallace, M. Nilsen, D. I. Graham, A. F. Dominiczak, I. M. Macrae: Differential effects of 17beta-estradiol upon stroke damage in stroke prone and normotensive rats. *J Cereb Blood Flow Metab* 24, 298-304 (2004)
100. Chaovipoch P, K. A. Jelks, L. M. Gerhold, E. J. West, S. Chongthammakun, C. L. Floyd: 17beta-estradiol is protective in spinal cord injury in post- and pre-menopausal rats. *J Neurotrauma* 23, 830-852 (2006)
101. Wise P. M.: Estrogen therapy: does it help or hurt the adult and aging brain? Insights derived from animal models. *Neuroscience* 138, 831-835 (2006)
102. Sandstrom N. J, M. H. Rowan: Acute pretreatment with estradiol protects against CA1 cell loss and spatial learning impairments resulting from transient global ischemia. Horm Behav. 51, 335-345 (2007)
103. Wise P. M, D.B. Dubal: Estradiol protects against ischemic brain injury in middle-aged rats. *Biol Reprod* 63, 982-985 (2000)
104. Wang Q, R. Santizo, V. L. Baughman, D. A. Pelligrino, C. Iadecola: Estrogen provides neuroprotection in transient forebrain ischemia through perfusion-independent mechanisms in rats. *Stroke* 30, 630-637 (1999)
105. Chi O. Z, X. Liu, H. R. Weiss: Effects of 17beta-estradiol on blood-brain barrier disruption during focal ischemia in rats. *Horm Metab Res* 34, 530-534. (2002)
106. Bingham D, I. M. Macrae, H. V. Carswell. Detrimental effects of 17beta-oestradiol after permanent middle cerebral artery occlusion. *J Cereb Blood Flow Metab* 25, 414-420 (2005)
107. Gordon K. B, I. M. Macrae, V. Carswell: Effects of 17beta-oestradiol on cerebral ischaemic damage and lipid peroxidation. *Brain Res* 1036, 155-162 (2005)
108. Theodorsson A, E .Theodorsson: Estradiol increases brain lesions in the cortex and lateral striatum after transient occlusion of the middle cerebral artery in rats: no effect of ischemia on galanin in the stroke area but decreased levels in the hippocampus. *Peptides* 26, 2257-2264 (2005)
109. Yong Y, H. J. Xie, Y. F. Zhang, Q. D. Yang, D. F. Liao, H. L. Yang, P. K. Yan, Z. J. Liu: 17beta-estradiol potentiates ischemia-reperfusion injury in diabetic ovariectomized female rats. *Brain Res* 1054, 192-199 (2005)
110. Gulinello M, D. Lebesgue, T. Jover-Mengual, R. S. Zukin, A. M. Etgen: Acute and chronic estradiol treatments reduce memory deficits induced by transient global ischemia in female rats. *Horm Behav* 49, 246-260 (2006)
111. Suzuki S, C. M. Brown, C. D. De la Cruz, E. Yang, D. A. Bridwell, P. M. Wise: Timing of estrogen therapy after ovariectomy dictates the efficacy of its neuroprotective and anti-inflammatory actions. *Proc Natl Acad Sci U S A* 104, 6013-6018 (2007)
112. Won C. K, H. H. Ji, P. O. Koh: Estradiol prevents the focal cerebral ischemic injury-induced decrease of forkhead transcription factors phosphorylation. *Neurosci Lett* 398, 39-43 (2006)
113. Chi O. Z, C. Hunter, X. Liu, H.R. Weiss: Effects of 17beta-estradiol on blood-brain barrier disruption during focal cerebral ischemia in younger and older rats. *Horm Metab Res* 38, 377-381 (2006)
114. Horsburgh K, I. M. Macrae, H. Carswell: Estrogen is neuroprotective via an apolipoprotein E-dependent mechanism in a mouse model of global ischemia. *J Cereb Blood Flow Metab* 22, 1189-1195 (2002)
115. Qiu Z, K. A. Crutcher, B. T. Hyman, G. W. Rebeck: ApoE isoforms affect neuronal N-methyl-D-aspartate calcium responses and toxicity via receptor-mediated processes. *Neuroscience* 122, 291-303 (2003)

116. Yepes M, D. A. Lawrence: Neuroserpin: a selective inhibitor of tissue-type plasminogen activator in the central nervous system. *Thromb Haemost* 91, 457-464 (2004)
117. Biehle S. J, J. Carrozzella, R. Shukla, J. Popplewell, M. Swann, N. Freeman, J. F. Clark: Apolipoprotein E isoprotein-specific interactions with tissue plasminogen activator. *Biochim Biophys Acta* 1689, 244-251 (2004)
118. Benchenane K, V. Berezowski, C. Ali, M. Fernandez-Monreal, J. P. Lopez-Atalaya, J. Brillault, J. Chuquet, A. Nouvelot, E. T. MacKenzie, G. Bu, R. Cecchelli, O. Touzani, D. Vivien: Tissue-type plasminogen activator crosses the intact blood-brain barrier by low-density lipoprotein receptor-related protein-mediated transcytosis. *Circulation* 111, 2241-2249 (2005)
119. Gould E, C. S. Woolley, M. Frankfurt, B. S. McEwen: Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci* 10, 1286-1291 (1990)
120. Woolley C. S, B. S. McEwen: Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12, 2549-2554 (1992)
121. Thiele C, M. J. Hannah, F. Fahrenholz, W. B. Huttner: Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat Cell Biol* 2, 42-49. (2000)
122. Brake W. G. S. E. Alves, J. C. Dunlop, S. J. Lee, K. Bulloch, P. B. Allen, P. Greengard, B. S. McEwen: Novel target sites for estrogen action in the dorsal hippocampus: an examination of synaptic proteins. *Endocrinology* 142, 1284-1289 (2001)
123. Stone D. J, I. Rozovsky, T. E. Morgan, C. P. Anderson, C. E. Finch: Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease. *J Neurosci* 18, 3180-3185 (1998)
124. Gibbs R. B, D. Wu, L. B. Hersh, D. W. Pfaff: Effects of estrogen replacement on the relative levels of choline acetyltransferase, trkA, and nerve growth factor messenger RNAs in the basal forebrain and hippocampal formation of adult rats. *Exp Neurol* 129, 70-80 (1994)
125. Gibbs R. B: Fluctuations in relative levels of choline acetyltransferase mRNA in different regions of the rat basal forebrain across the estrous cycle: effects of estrogen and progesterone. *J Neurosci* 16,1049-1055 (1996)
126. Luine V. N: Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. Exp Neurol 89, 484-490 (1985)
127. Kaufman H, C. Vadasz, A. Lajtha: Effects of estradiol and dexamethasone on choline acetyltransferase activity in various rat brain regions. *Brain Res* 453, 389-392 (1988)
128. Lapchak P. A, D. M. Araujo, R. Quirion, A. Beaudet: Chronic estradiol treatment alters central cholinergic function in the female rat: effect on choline acetyltransferase activity, acetylcholine content, and nicotinic autoreceptor function. *Brain Res* 525, 249-255 (1990)
129. Singh M, E. M. Meyer, W. J. Millard, J. W. Simpkins: Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. *Brain Res* 644, 305-312 (1994)
130. Singh M, E. M. Meyer, J. W. Simpkins: The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. *Endocrinology* 136, 2320-2324 (1995)
131. Gibbs R. B: Effects of estrogen on basal forebrain cholinergic neurons vary as a function of dose and duration of treatment. *Brain Res* 757, 10-16 (1997)
132. Gibbs R. B, R. Gabor: Estrogen and cognition: applying preclinical findings to clinical perspectives. *J Neurosci Res* 74, 637-643 (2003)
133. Islamov R. R, W. A. Hendricks, R. J. Jones, G. J. Lyall, N. S. Spanier, A. K. Murashov: 17Beta-estradiol stimulates regeneration of sciatic nerve in female mice. *Brain Res* 943, 283-286. (2002)
134. Kadish I, T. J. Van Groen: Low levels of estrogen significantly diminish axonal sprouting after entorhinal cortex lesions in the mouse. *Neurosci* 22, 4095-4102 (2002)
135. Morse J. K, S. W. Scheff, S. T. DeKosky: Gonadal steroids influence axon sprouting in the hippocampal dentate gyrus: a sexually dimorphic response. *Exp Neurol* 94, 649-658 (1986)
136. Tanapat P, N. B. Hastings, E. Gould: Ovarian steroids influence cell proliferation in the dentate gyrus of the adult female rat in a dose- and time-dependent manner. *J Comp Neurol* 481, 252-265 (2005)
137. Gould E, P. Tanapat, T. Rydel, N. Hastings: Regulation of hippocampal neurogenesis in adulthood. *Biol Psychiatry* 48, 715-20 (2000)
138. Leuner B, E. Gould, T. J. Shors: Is there a link between adult neurogenesis and learning? *Hippocampus* 16, 216-224 (2006)
139. Toran-Allerand C. D, L. Ellis, K. H. Pfenninger: Estrogen and insulin synergism in neurite growth enhancement in vitro: mediation of steroid effects by interactions with growth factors? *Brain Res* 469, 87-100 (1988)
140. Ferreira A, A. Caceres: Estrogen-enhanced neurite growth: evidence for a selective induction of Tau and stable microtubules. *J Neurosci* 11, 392-400 (1991)
141. Diaz H. A. Lorenzo, H. F.Carrer, A. Caceres: Time lapse study of neurite growth in hypothalamic dissociated neurons in culture: sex differences and estrogen. *J Neurosci. Res* 33, 266-281 (1992)
142. Brinton R. D, J. Tran, P. Proffitt, M. Montoya. 17 beta-Estradiol enhances the outgrowth and survival of neocortical neurons in culture. Neurochem Res 22, 1339-1351 (1997)
143. Masliah E, M. Mallory, N. Ge, M. Alford, I. Veinbergs, A. D. Roses: Neurodegeneration in the central nervous system of apoE-deficient mice. Exp Neurol 136, 107-122. 1995)
144. Anderson R, J. C. Barnes, T. V. Bliss, D. P. Cain, K. Cambon, H. A. Davies, M. L. Errington, L. A. Fellows, R. A. Gray, T. Hoh, M. Stewart, C. H. Large, G. A. Higgins: Behavioural, physiological and morphological analysis of a line of apolipoprotein E knockout mouse. Neuroscience 85, 93-110 (1998)

145. Laskowitz D. T, K. Horsburgh, A. D. Roses: Apolipoprotein E and the CNS response to injury. J Cereb Blood Flow Metab 18, 465-471 (1998)
146. Sheng J. G, R. A. Jones, X. Q. Zhou, J. M. McGinness, L. J. Van Eldik, R. E. Mrak, W. S. Griffin: Interleukin-1 promotion of MAPK-p38 overexpression in experimental animals and in Alzheimer's disease: potential significance for tau protein phosphorylation. *Neurochem Int* 39, 341-348 (2001)
147. McColl B. W, A. L. McGregor, A. Wong, J. D. Harris, A. Amalfitano, S. Magnoni, A. H. Baker, G. Dickson, K. Horsburgh: APOE epsilon3 gene transfer attenuates brain damage after experimental stroke. *J Cereb Blood Flow Metab* 27, 477-487 (2007)
148. Mori T, T. Town, M. Kobayashi, J. Tan, S. C. Fujita, T. Asano: Augmented delayed infarct expansion and reactive astrocytosis after permanent focal ischemia in apolipoprotein E4 knock-in mice. *J Cereb Blood Flow Metab* 24, 646-656 (2004)
149. Fagan A. M, B. A. Murphy, S. N. Patel, J. F. Kilbridge, W. C., Mobley, G Bu, D. M. Holtzman:. Evidence for normal aging of the septo-hippocampal cholinergic system in apoE (-/-) mice but impaired clearance of axonal degeneration products following injury. *Exp Neurol* 151, 314-325 (1998)
150. Kitagawa K, M. Matsumoto, K. Kuwabara, T. Ohtsuki, M. Hori: Delayed, but marked, expression of apolipoprotein E is involved in tissue clearance after cerebral infarction. J Cereb Blood Flow Metab 21, 1199-1207 (2001)
151. Herz J, U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl, K. K. Stanley: Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. EMBO J. 7, 4119-4127 (1988)
152. Kowal R. C, J. Herz, K. H. Weisgraber, R. W. Mahley, M. S. Brown, J. L. Goldstein: Opposing effects of apolipoproteins E and C on lipoprotein binding to low density lipoprotein receptor-related protein. J Biol Chem 265, 10771-10779 (1990)
153. Takahashi S. Y. Kawarabayasi, T. Nakai, J. Sakai, T. Yamamoto: Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. Proc Natl Acad Sci USA 89, 9252-9256 (1992)
154. Willnow T. E , J. L. Goldstein, K. Orth, M. S. Brown, J. Herz: Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance J Biol Chem 267, 26172-26180 (1992)
155. Goodrum J. F, J. E. Weaver, N. D. Goines, T. W. Bouldin: Fatty acids from degenerating myelin lipids are conserved and reutilized for myelin synthesis during regeneration in peripheral nerve. J Neurochem 65, 1752-1759 (1995)
156. Poirier J, A. Baccichet, D. Dea, S. Gauthier: Cholesterol synthesis and lipoprotein reuptake during synaptic remodelling in hippocampus in adult rats. Neuroscience 55, 81-90 (1993)
157. Snipes G. J, C. B. McGuire, J. J. Norden, J. A. Freeman: Nerve injury stimulates the secretion of apolipoprotein E by nonneuronal cells. *Proc Natl Acad Sci USA* 83, 1130-1134 (1986)
158. Popko B, J. F. Goodrum, T. W. Bouldin, S. H. Zhang, N. Maeda: Nerve regeneration occurs in the absence of apolipoprotein E in mice. *J Neurochem* 60, 1155-1158 (1993)
159. Poirier J, M. Hess, P. C. May, C. E. Finch: Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. *Brain Res Mol Brain Res* 11, 97-106 (1991)
160. Nathan B. P, R. Nisar, S. Randall, J. Short, M. Sherrow, G. K. Wong, R. G. Struble: Apolipoprotein E is upregulated in olfactory bulb glia following peripheral receptor lesion in mice. *Exp Neurol* 172, 128-136 (2001)
161. Buttini M, H. Akeefe, C. Lin, R. W. Mahley, R. E. Pitas, T. Wyss-Coray, L. Mucke: Dominant negative effects of apolipoprotein E4 revealed in transgenic models of neurodegenerative disease. *Neuroscience* 97, 207-210 (2000)
162. Blain J. F, P. M. Sullivan, J. Poirier: A deficit in astroglial organization causes the impaired reactive sprouting in human apolipoprotein E4 targeted replacement mice. *Neurobiol Dis* 21, 505-514 (2006)
163. Krugers H. J, M. Mulder, J. Korf, L. Havekes, E. R. de Kloet, M. Joels: Altered synaptic plasticity in hippocampal CA1 area of apolipoprotein E deficient mice. *Neuroreport* 8, 2505-2510 (1997)
164. Veinbergs I, M. W. Jung, S. J. Young, E. Van Uden, P. M. Groves, E. Masliah: Altered long-term potentiation in the hippocampus of apolipoprotein E-deficient mice. *Neurosci Lett* 249, 71-77 (1998)
165. Choudhuri R, L. Cui, C. Yong, S. Bowyer, R. M. Klein, K. M. Welch, N. E. Berman: Cortical spreading depression and gene regulation: relevance to migraine. *Ann Neurol* 51, 499-506 (2002)
166. Kraig R. P, L. M. Dong, R. Thisted, C. B. Jaeger: Spreading depression increases immunohistochemical staining of glial fibrillary acidic protein. *J Neurosci* 11, 2187-2198 (1991)
167. Struble R. G, J. Short, M. Ghobrial, B. P. Nathan: Apolipoprotein E immunoreactivity in human and mouse olfactory bulb. *Neurosci Lett*. 267, 137-140 (1999)
168. Nathan B. P, S. Nannapaneni, S. Gairhe, I. Nwosu, R. G. Struble: The distribution of apolipoprotein E in mouse olfactory epithelium. *Brain Res* 1137, 78-83 (2007)
169. Nathan B. P, R. Nisar, J. Short, S. Randall, E. Grissom, G. Griffin, P. V. Switzer, R. G. Struble: Delayed olfactory nerve regeneration in ApoE-deficient mice. *Brain Res* 1041, 87-94 (2005)
170. Nathan B. P, S. Bellosta, D. A. Sanan, K. H. Weisgraber, R. W. Mahley, R. E. Pitas: Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. *Science* 264, 850-852 (1994)
171. Nathan B. P, K. C. Chang, S. Bellosta, E. Brisch, N. Ge, R. W. Mahley, R. E. Pitas: The inhibitory effect of apolipoprotein E4 on neurite outgrowth is associated with microtubule depolymerization. *J Biol Chem* 270, 19791-19799 (1995)
172. Teter B, P. T. Xu, J. R. Gilbert, A. D. Roses, D. Galasko, G. M. Cole: Apolipoprotein E isoform-specific differences in neuronal sprouting in organotypic hippocampal culture. *J Neurochem* 73, 2613-2616 (1999)

173 . Hayashi H, R. B. Campenot, D. E. Vance, J. E. Vance: Glial lipoproteins stimulate axon growth of central nervous system neurons in compartmented cultures. *J Biol Chem* 279, 14009-14015 (2004)
174. Nathan B. P, Y. Jiang, G. K. Wong, F. Shen, G. J. Brewer, R. G. Struble: Apolipoprotein E4 inhibits, and apolipoprotein E3 promotes neurite outgrowth in cultured adult mouse cortical neurons through the low-density lipoprotein receptor-related protein. *Brain Res* 928, 96-105 (2002)
175. Nathan B. P, A. G. Barsukova, F. Shen, M. McAsey, R. G. Struble: Estrogen facilitates neurite extension via apolipoprotein E in cultured adult mouse cortical neurons. *Endocrinology* 145, 3065-3073, (2004)
176. Colton C. A, D. L. Gilbert: Production of superoxide anions by a CNS macrophage, the microglia. *FEBS Lett* 223, 284-288 (1987)
177. Barger S. W, A. D. Harmon: Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature* 388, 878-881 (1997)
178. Laskowitz D. T, A. D.Thekdi, S. D.Thekdi, S. K. Han, J. K. Myers, S. V.Pizzo, E. R Bennett: Downregulation of microglial activation by apolipoprotein E and apoE- mimetic peptides. *Exp Neurol* 167, 74-85 (2001)
179. Laskowitz D. T, W. D. Matthew, E. R. Bennett, D. Schmechel, M. H. Herbstreith, S. Goel, , M. K. McMillian: Endogenous apolipoprotein E suppresses LPS-stimulated microglial nitric oxide production. *Neuroreport* 9, 615-618 (1998)
180. Colton C. A, M. Czapiga, J. Snell-Callanan, O. N. Chernyshev, M. P. Vitek: Apolipoprotein E acts to increase nitric oxide production in macrophages by stimulating arginine transport. *Biochim Biophys Acta* 1535, 134-144 (2001)
181. Lynch J. R, J. A. Pineda, D. Morgan, L. Zhang, D. S. Warner, H Benveniste, D. T. Laskowitz: Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. *Ann Neurol* 51, 113-117 (2002)
182. Colton C. A, C. M. Brown, M. Czapiga, M. P. Vitek: Apolipoprotein-E allele-specific regulation of nitric oxide production. *Ann N Y Acad Sci* 962, 212-225 (2002)
183. Lynch J. R, D. Morgan, J. Mance, W. D. Matthew, D. T. Laskowitz: Apolipoprotein E modulates glial activation and the endogenous central nervous system inflammatory response. *J Neuroimmunol* 114, 107-113 (2001)
184. Brown C. M, E. Wright, C. A. Colton, P. M.Sullivan, , D. T.Laskowitz, , M. P. Vitek: Apolipoprotein E isoform mediated regulation of nitric oxide release. *Free Radic Biol Med* 32, 1071-1075 (2002)
185. Brown C. M., E. Wright, C. A. Colton, P. M.Sullivan, D. T. Laskowitz, M. P. Vitek: Apolipoprotein E isoform mediated regulation of nitric oxide release. *Free Radic Biol Med* 32, 1071-1075 (2002)
186. Czapiga M, C. A. Colton: Microglial function in human APOE3 and APOE4 transgenic mice: altered arginine transport. *J Neuroimmunol* 134, 44-51 (2003)
187. Colton C. A, C. M. Brown, D. Cook, L. K. Needham, Q. Xu, M. Czapiga, A. M. Saunders, D. E. Schmechel, K. Rasheed, M. P. Vitek: APOE and the regulation of microglial nitric oxide production: a link between genetic risk and oxidative stress. *Neurobiol Aging* 23, 777-785 (2002)
188. Colton C. A, C. M. Brown, M. P. Vitek: Sex steroids, APOE genotype and the innate immune system. *Neurobiol Aging* 26, 363-372. (2005)
189. Colton C. A, L. K. Needham, C. Brown, D. Cook K. Rasheed J. R. Burke, W. J. Strittmatter, D. E. Schmechel, M. P. Vitek: APOE genotype-specific differences in human and mouse macrophage nitric oxide production. *J Neuroimmunol* 147, 62-67 (2004)
190. Tiwari-Woodruff S, L. B. Morales, R. Lee, R. R. Voskuhl: Differential neuroprotective and antiinflammatory effects of estrogen receptor (ER)alpha and ERbeta ligand treatment. *Proc Natl Acad Sci U S A* 104, 14813-14818. (2007)
191. Krathwohl M. D, J. L. Kaiser. Chemokines promote quiescence and survival of human neural progenitor cells. *Stem Cells* 22, 109-118. (2004)
192. Caruso A, M. Motolese, L. Iacovelli, F. Caraci, A. Copani, F. Nicoletti, G. C. Terstappen, G. Gaviraghi, A. Caricasole: Inhibition of the canonical Wnt signaling pathway by apolipoprotein E4 in PC12 cells. *J Neurochem* 98, 364-371 (2006)
193. Levi O, D. M. Michaelson: Environmental enrichment stimulates neurogenesis in apolipoprotein E3 and neuronal apoptosis in apolipoprotein E4 transgenic mice. *J Neurochem* 100, 202-210 (2007)
194. Nilsen J, R. D. Brinton: Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. *Proc Natl Acad Sci U S A* 100, 10506-10511 (2003)
195. Wang J. M, R. W. Irwin, R. D. Brinton: Activation of estrogen receptor alpha increases and estrogen receptor beta decreases apolipoprotein E expression in hippocampus in vitro and in vivo. Proc Natl Acad Sci U S A 103, 16983-16988 (2006)
196. Stone D. J, I. Rozovsky, T. E. Morgan, C. P. Anderson, H. Hajian, C. E. Finch: Astrocytes and microglia respond to estrogen with increased apoE mRNA in vivo and in vitro. Exp Neurol 143, 313-318 (1997)
197. Garcia-Estrada J, J. A. Del Rio, S. Luquin, E. Soriano, L. M. Garcia-Segura: Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. Brain Res 628, 271-278 (1993)
198. Struble R. G, S. Afridi, S. Beckman-Randall, M. Li, C. Cady, B. Nathan, M. E. McAsey: Neocortical and hippocampal glial fibrillary acidic protein immunoreactivity shows region-specific variation during the mouse estrous cycle. *Neuroendocrinology* 83, 325-335 (2006)
199. Shughrue P. J, M. V. Lane, I. Merchenthaler: Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J Comp Neurol* 388, 507-25 (1997)
200. Shughrue P. J, I. Merchenthaler: Evidence for novel estrogen binding sites in the rat hippocampus. *Neuroscience* 99, 605-612. 2000)
201. Pitas R. E, J. K. Boyles, S. H. Lee, D. Foss, R. W. Mahley: Astrocytes synthesize apolipoprotein E and

metabolize apolipoprotein E-containing lipoproteins. *Biochim Biophys Acta* 917, 48-61 (1987)
202. Boyles J. K, R. E .Pitas, E. Wilson, R. W. Mahley, J. M. Taylor: Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J Clin. Invest* 76, 1501-1513 (1985)
203. Stone D. J, Y. Song, C. P. Anderson, K. K. Krohn, C. E. Finch, I. Rozovsky: Bidirectional transcription regulation of glial fibrillary acidic protein by estradiol in vivo and in vitro. *Endocrinology* 139, 3202-3209 (1998)
204. Araque A, G. Carmignoto, P. G. Haydon. Dynamic signaling between astrocytes and neurons. *Ann Rev Physiol* 63, 795-813 (2001)
205. Garcia-Segura L. M, M. M. McCarthy. Role of glia in neuroendocrine function. *Endocrinology* 145, 1082-1086 (2004)
206. Struble R. G, B. P. Nathan, C. Cady, X. Cheng, M. McAsey: Estradiol regulation of astroglia and apolipoprotein E: An important role in neuronal regeneration. *Exp Gerontol* 42, 54-63 (2007)
207. Schurr A, J. J. Miller, R. S. Payne, B. M. Rigor: An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. *J Neurosci* 19, 34-39 (1999)
208. Kasischke K. A, H. D. Vishwasrao, P. J. Fisher, W. R. Zipfel, W. W. Webb: Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science* 305, 99-103 (2004)
209. Danbolt N. C: The high affinity uptake system for excitatory amino acids in the brain. *Prog Neurobiol* 44, 377-396. 1994)
210. Hertz L, R. Dringen, A. Schousboe, S. R. Robinson: Astrocytes: glutamate producers for neurons. *J Neurosci Res* 57, 417-428 (1999)
211. Schousboe A, N. Westergaard, L. Hertz: Neuronal-astrocytic interactions in glutamate metabolism. *Biochem Soc Trans* 21, 49-53 (1993)
212. Pfrieger F. W, B. A. Barres: Synaptic efficacy enhanced by glial cells in vitro. *Science* 277, 1684-1687 (1997)
213. Mauch D H., K. Nagler, S. Schumacher, C. Goritz, E. C. Muller, A. Otto, F. W. Pfrieger: CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294, 1354-1357 (2001)
214. Ullian E. M, S. K. Sapperstein,. K. S.Christopherson, B. A Barres:. Control of synapse number by glia. *Science* 291, 657-661 (2001)
215. Inagaki M, Y. Gonda, K. Nishizawa, .S Kitamura, C. Sato, S. Ando, K. Tanabe, K. Kikuchi, S. Tsuiki, Y. Nishi: Phosphorylation sites linked to glial filament disassembly in vitro locate in a non-alpha-helical head domain. *J Biol Chem* 265, 4722-4729 (1990)
216. Kommers T, L. Vinade, C. Pereira, C. A. Goncalves, S. Wofchuk, R. Rodnight: Regulation of the phosphorylation of glial fibrillary acidic protein (GFAP) by glutamate and calcium ions in slices of immature rat spinal cord: comparison with immature hippocampus. *Neurosci Lett* 248, 141-143 (1998)
217. Matsutani S, N. Yamamoto: Neuronal regulation of astrocyte morphology in vitro is mediated by GABAergic signaling. *Glia* 20, 1-9 (1997)
218. Runquist M, G. Alonso: Gabaergic signaling mediates the morphological organization of astrocytes in the adult rat forebrain. *Glia* 41, 137-151 (2003)
219. Joels M: Steroid hormones and excitability in the mammalian brain. *Front Neuroendocrinol* 18, 2-48 (1997)
220. Kis Z, D. Budai, G. Imre, T. Farkas, S. Horvath, J. Toldi: The modulatory effect of estrogen on the neuronal activity in the barrel cortex of the rat. An electrophysiological study. *Neuroreport* 12, 2509-2512 (2001)
221. McEwen B: Estrogen actions throughout the brain. *Recent Prog Horm Res* 57, 357-384 (2002)
222. Rudick C. N, C. S. Woolley: Estrogen regulates functional inhibition of hippocampal CA1 pyramidal cells in the adult female rat. *J Neurosci* 21, 6532-6543 (2001)
223. Rudick C. N, R. B. Gibbs, C. S. Woolley: A role for the basal forebrain cholinergic system in estrogen-induced disinhibition of hippocampal pyramidal cells. *J Neurosci* 23, 4479-4490 (2003)

**Key Words:** Apolipoprotein E, menopause, Neuroprotection, Hormone Replacement, Review

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