

Effects of Age on Gene Expression during Estrogen-Induced Synaptic Sprouting in the Female Rat

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Age and estrogen treatment influenced fiber outgrowth and compensatory neuronal sprouting after unilateral entorhinal cortex lesions (ECL) which model Alzheimer disease-like deafferentation in the dentate gyrus of the hippocampus. In young F344 rats (3 months old), ovariectomy (OVX) decreased reactive fiber outgrowth by 60%. Sprouting in middle-aged rats (18 months old) was reduced in intact females; no further reduction was caused by OVX. Several astrocyte mRNAs were measured in the dentate gyrus of young and middle-aged female rats in three different estrogen states (sham OVX, OVX, or OVX + estradiol) 1 week after ECL. Glial fibrillary acidic protein (GFAP) mRNA was twofold greater in middle-aged rats than young, although both ages showed threefold increases in response to ECL. In prior studies GFAP was found to be decreased by estradiol treatment 3–4 days after ECL; in this study GFAP mRNA had returned to sham OVX levels in young rats by 7 days post-ECL. Surprisingly, estradiol treatment increased GFAP mRNA levels by 25% above OVX in middle-aged rats. Apolipoprotein E (apoE) mRNA was decreased 20% by age in the dentate, although both age groups showed a 25% increase in apoE mRNA in response to ECL. Apolipoprotein J (apoJ) mRNA was increased 20% in the dentate gyrus of middle-aged rats, and both age groups responded to ECL with a 65% increase in apoJ mRNA. The estrogen state did not alter levels of either apolipoprotein mRNA in the deafferented dentate. The data suggest that the estrogen-induced decrease of GFAP in response to lesions does not persist at 7 days post-ECL during sprouting. Overall effects of age on the dentate gyrus include elevated GFAP mRNA and decreased apoE mRNA. The cortical wound site showed consistent enhancement of GFAP mRNA in both age

groups by estradiol above sham OVX and greater responses in middle-aged rats. © 2000 Academic Press

Key Words: aging; apoE; apoJ; brain lesion; estrogen; GFAP; sprouting; reafferentation; synaptogenesis; hippocampus.

INTRODUCTION

Synaptic density and functions are sensitive to 17 β -estradiol in the adult rodent brain. In the unlesioned brain, dendritic spine number is increased by estradiol in hippocampal CA1 neurons (81) and granule neurons of the dentate gyrus (40). Synapse number also varies during the estrous cycle in the arcuate nucleus of the hypothalamus (48, 51). Moreover, estradiol interacts with response to lesioning. In response to entorhinal cortex lesions (ECL)² which model the deafferentation of the dentate gyrus of the hippocampus seen in Alzheimer's disease (AD), treatment with estradiol increases reactive fiber sprouting (44, 45, 77) and synaptogenesis (73). In both AD and after experimental ECL in rodents, the molecular layer of the dentate gyrus is similarly reinnervated through sprouting (16); the main sources of afferent sprouting are fibers from the commissural/associational (C/A) neurons to the inner molecular layer, whereas the outer molecular layer receives cholinergic fibers from the septohippocampal pathways and from local interneurons. Because of the strong association of AD with aging, it is pertinent that compensatory sprouting in response to ECL is diminished in 24-month-old (senescent) male rats in the inner molecular layer of the dentate gyrus (61, 62) as is cholinergic sprouting to the outer molecular layer (62). Moreover, aging female rats show progressively greater susceptibility to neurotoxicity from MK-801, a

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² Abbreviations used: apoE, apolipoprotein E; apoJ, apolipoprotein J; C/A, commissural/associational; ECL, entorhinal cortex lesion; GFAP, glial fibrillary acidic protein; AD, Alzheimer's disease; ERT, estrogen replacement therapy; OVX, ovariectomy; BBB, blood-brain barrier; ADX, adrenalectomy.

glutamatergic NMDA receptor antagonist, whereas male rats showed no age effect (3). In contrast, estradiol-induced sprouting in the dentate gyrus showed no effects of age in female rats (40). These findings in rodents are pertinent to the ongoing discussion on association of estrogen replacement therapy (ERT) with AD risk (20, 47, 52, 68, 76).

This study examines the general relationship between age, ovarian steroids, and glial mRNAs in sprouting. Three proteins regulated by sex steroids in astrocytes which also show age-related changes in expression are of particular interest: glial fibrillary acidic protein (GFAP), apolipoprotein E (apoE), and apolipoprotein J (apoJ), which is also known as clusterin. GFAP is an intermediate filament protein that is upregulated in reactive astrocytes (28). GFAP increases twofold per cell in the aging brain (49, 53, 83) and is also increased in response to ECL (56, 62). The role of astrocyte reactivity in synaptogenesis is incompletely understood; while reactive astrocytes can enhance neuronal sprouting through the production of neurotrophic factors, treatment of cultured astrocytes with antisense GFAP mRNA enhances neurite outgrowth (29).

ApoE is a mediator of cholesterol transport in the CNS (21, 34, 55) and peripheral circulation (24), which has a major role in compensatory synaptogenesis (35, 73, 77) and maintenance of dendritic complexes with age (36). Aging rats show trends for decreased apoE that would not support synaptogenesis. *In vivo* and *in vitro* apoE mRNA is detected in microglia, though astrocytes are the predominant location (56, 74). The increase in risk of AD with apoE4 allele dose (6) makes control of this protein of special interest to mechanisms in aging (11, 39, 57).

ApoJ is also implicated in cholesterol transport and is increased in response to brain lesions and in AD (13, 37). Cholesterol transport in the rat brain appears to function via an apoE–apoJ lipoprotein particle secreted by astrocytes (26, 66) although mouse astrocytes *in vitro* produce distinct apoE and apoJ lipoprotein particles (10). ApoJ mRNA shows alterations with age in the 24-month-old male rat brain that vary by region in both magnitude and direction of effect (43).

Because gonadal steroids regulate levels of GFAP (9, 15, 32, 75), apoE (69, 70, 74), and apoJ (9), we hypothesized that estradiol treatment may increase synaptic sprouting through an alteration of glial phenotype. For example, estrogen decreases GFAP immunoreactivity up to 72 h after a brain stab wound (15) and 96 h after a deafferenting lesion (58). This change is opposite to in direction to the increased GFAP levels seen during aging, which suggests that estrogen may shift the glial phenotype away from that in aging to one that is more supportive of synaptogenesis.

METHODS

Surgery and estrogen replacement. Young (3 months old) and middle-aged (18 months old) female F344 rats were obtained from Charles River Laboratories (Wilmington, MA) and maintained in a controlled light and temperature environment with food and water ad libitum. Surgery for ovariectomy (OVX) or sham OVX was done with ketamine:xylazine anesthesia (46:4.6 mg/kg). In sham OVX, skin and peritoneal wall incisions were made. After 1 week, anesthetized rats were given unilateral stereotaxic entorhinal cortex lesions which severed the perforant path, using a retractable wire knife (16) (Scouten wire knife; Kopf, Tujunga, CA). The knife is inserted into the entorhinal cortex (2.2 mm anterior, 5 mm lateral from lambda and 1 mm ventral from dura). The extended blade was then lowered 5 mm ventrally twice at angles to avoid the hippocampus. Immediately after surgery rats were given subcutaneous Silastic implants containing 150 mg/ml 17 β -estradiol in sesame oil or oil only sham implants. One week after surgery rats were decapitated under anesthesia, and brains (36; $n = 6$ /group) were immediately frozen in isopentane (-18°C) and stored (at -70°C) until sectioning.

A standard method for examining C/A fiber outgrowth in response to ECL involves a second lesion of the contralateral entorhinal cortex 2 days before sacrifice (60, 62), which removes sprouting of fibers originating from the contralateral entorhinal cortex. However the entorhinal cortex, which responds to ECL with reactive sprouting and increased SYN production (4), also shows transient changes in certain presynaptic protein mRNAs during the estrous cycle (7). This suggests that reactive synaptogenesis from the contralateral entorhinal cortex is also under estrogenic control, and for this reason we chose not to lesion on the contralateral side. While this paradigm obscured the boundary of the C/A plexus, we were able to estimate the increase in the fiber plexus by measuring the percentage of area covered by Holmes stain-positive fibers in the inner one-third of the molecular layer (Fig. 1). A paradigm of short-term ovariectomy and estradiol replacement was used to ensure that changes seen were the result of the effects of estradiol on compensatory synaptogenesis; long-term ovariectomy and estradiol replacement have been shown to have effects on synapse number in the absence of CNS lesions (40).

In the sprouting studies, OVX and ECL were performed as above. At 2 weeks post-ECL, rats were anesthetized and perfusion fixed in phosphate buffer (pH 7.4), containing 4% paraformaldehyde. Brains ($n = 3$ –5/group) were immersion fixed for 1 day at 4°C in buffered paraformaldehyde, immersed in 30% sucrose for 3 days (4°C), and sectioned by cryostat (16 mm). Uteri were weighed after blotting to estimate ovarian function.

Fiber staining. Holmes fiber staining followed the method of Sheehan and Hrapchak (67). After hydration, sections were incubated in 1% silver nitrate in dark for 2 h. Sections were impregnated overnight (37°C) in 9% boric acid buffer, 8% borax buffer, 1% silver nitrate, and 10% pyridine. Sections were then reduced in 10% sodium sulfite with 1% hydroquinone. Sections were then incubated in 0.2% gold chloride/6 min, 2% oxalic acid/8 min, and 5% sodium thiosulfate/5 min.

In situ hybridization. Sections were sliced at 16 μm and mounted on Superfrost Plus slides (Fisher, Pittsburgh, PA). Slides were washed in PBS and dehydrated in an ethyl alcohol series. Sections were prehybridized for 1 h at 55°C (prehybridization buffer 0.75 M NaCl, 50% formamide, 10% dextran sulfate, 0.05 phosphate, pH 7.4) and hybridized with an ^{35}S -labeled cRNA probe. Sections were hybridized for 3 h/55°C. Sense cRNA probes were controls for background.

Image analysis and statistics. All slides were coded and measured under blind conditions. *In situ* signals were measured with IPLab Spectrum image analysis software (Signal Analytics Corporation) in the molecular layer of the deafferented and contralateral dentate gyrus from X-ray film images. Holmes stain-positive fiber density (percentage of area covered by fibers) was measured under high power. Data were analyzed by two-way ANOVA.

RESULTS

All rats in this study received unilateral lesions of the entorhinal cortex which interrupts the perforant path to the dentate gyrus and induces compensatory sprouting (16). The effects of ovarian steroid manipulations on the responses to injury were evaluated relative to the unlesioned side (see Methods). The 18-month-old F344 rats are considered middle-aged (i.e., not senescent) because of general good health and low mortality rates (30). At 18 months, fertility is very low due to imminent exhaustion of ovarian follicles (79).

Experiment 1: Ovariectomy and Sprouting at 14 Days Postlesion

ECL-induced sprouting in the C/A fiber plexus was estimated as the percentage of area covered by Holmes stain-positive fibers in the inner one-third of the molecular layer of the dentate gyrus, measured 14 days post-ECL (Fig. 1). In young ECL rats, OVX decreased by 60% the area covered by fibers (from 9 to 4%, $P < 0.02$). The contralateral dentate gyrus showed a similar trend, with OVX decreasing area covered by fibers (from 8 to 3%; Fig. 2B; $P < 0.01$). In middle-aged rats given ECL with sham OVX, the percentage of area covered by fibers was half of that in young rats, in both the lesioned and the contralateral hippocampus (Figs.

2A and 2B). OVX did not cause a decrease in area covered by fibers in either dentate gyrus. The Treatment \times Age interaction effect was significant (two-way ANOVA, $P < 0.05$) for area covered by fibers in the ipsilateral, but not in the contralateral (unlesioned) dentate gyrus.

One factor in the loss of the OVX effect in middle-aged rats could be prior age-related loss of ovarian function. By 18 months, a subgroup of rats will have very low estradiol levels due to the depletion of steroid-producing follicles (79). For individuals with low estradiol production, OVX should have a smaller effect on fiber density in the dentate molecular layer. We used uterine weight as a direct measure of ovarian function and estrogen production in young and middle-aged rats. Both young and middle-aged rats responded to OVX with a significant decrease in uterine weight (Fig. 2C). Because this peripheral estrogen target organ retains a full response to OVX or estradiol replacement in middle-aged rats, we infer that the loss of response to OVX in fiber density is a result of aging changes within the brain.

Experiment 2: Effect of Ovariectomy and Estrogen on Glial Gene Expression at 7 Days Postlesion

In the Dentate Gyrus

To determine which genes may be involved in estrogen-dependent synaptic sprouting in young rats (and in impairments at middle-age) we examined mRNA levels in the molecular layer of the dentate gyrus at 7 days post-ECL, during the peak of sprouting (71). In this experiment, half of the OVX rats (both young and middle-aged) received Silastic implants of 17β -estradiol to determine if effects of OVX could be reversed with estradiol alone.

GFAP. At middle-age, GFAP mRNA levels were twofold higher than in young rats in both the ipsilateral and the contralateral dentate gyrus (Figs. 3A and B; $P < 0.0001$). Both ages responded to ECL with threefold increases in GFAP mRNA in the ipsilateral dentate gyrus relative to the contralateral dentate gyrus (Fig. 3B; $P < 0.0001$). This response suggests that the GFAP mRNA response to ECL in middle-aged rats is not limited by a ceiling effect due to the prior increase with age. Middle-aged OVX rats treated with estradiol had 25% higher GFAP mRNA than OVX rats without estradiol (Fig. 3A; $P < 0.05$); young rats showed a similar (nonsignificant) trend. These results differ from changes in GFAP expression during the estrous cycle, in which GFAP mRNA and transcription levels in the dentate gyrus are lowest when circulating estradiol levels are highest at proestrus (75). Thus the GFAP mRNA response in the deafferented dentate gyrus is the opposite of that expected based upon estrous cycle data.

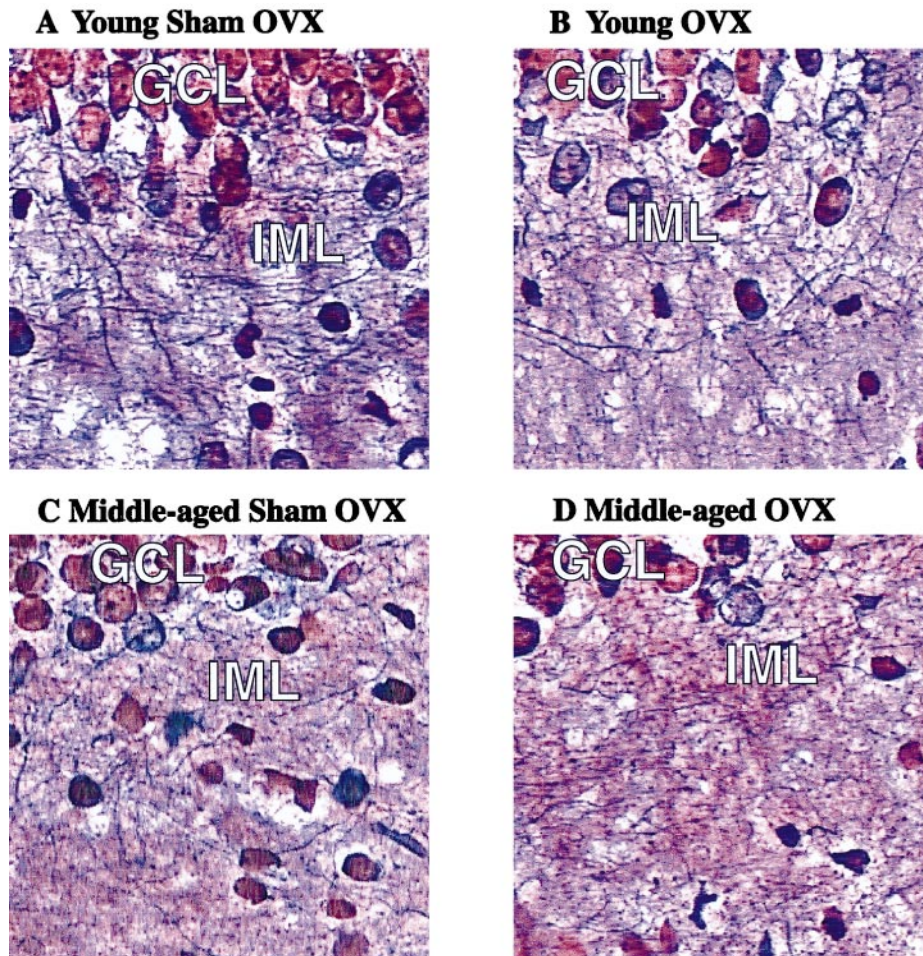


FIG. 1. Area covered by Holmes stain-positive fibers in the inner molecular layer of the deafferented dentate gyrus. (A) Young, sham OVX rats show much of the inner molecular layer covered by fibers 2 weeks after ECL. In young, OVX rats (B) percentage of area covered by Holmes stain-positive fibers is reduced to below one-half that seen in control rats. (C) In middle-aged, sham OVX rats, area covered by fibers was at approximately the level seen in OVX young rats; no further decrease was detected (D) in response to OVX in middle-aged rats. GCL, granule cell layer; IML, inner molecular layer of dentate gyrus.

ApoE. After ECL, apoE mRNA was 27% higher in the deafferented molecular layer than in the contralateral side of both young ($P < 0.01$) and middle-aged ($P < 0.002$) rats. ApoE mRNA in middle-aged rats was 20% lower than in young rats in both the ipsilateral and the contralateral dentate gyrus ($P < 0.025$) (Fig. 4). No effect of estradiol replacement was detected in either age group in either dentate gyrus. As found for GFAP, this result departed from the direction of changes during the estrous cycle as well as in cultured glia, which suggests that apoE mRNA levels may differ between hippocampal regions, e.g., upregulated in the CA1 neuron layer or downregulated in CA3, in response to estradiol (74).

ApoJ. After ECL in the deafferented dentate gyrus, apoJ mRNA levels were increased above those in the contralateral dentate in both young (60% increase) and middle-aged (70% increase) rats (Figs. 5A and 5B). Across all treatment groups, aging increased apoJ

mRNA levels in the lesioned dentate gyrus by 22%. Estradiol replacement did not affect apoJ mRNA in either age group. In young rats, however, OVX increased apoJ mRNA ($P < 0.025$). Because this effect of OVX was not reversed by estradiol replacement, apoJ expression may be inhibited by another ovarian steroid, possibly progesterone.

At the Wound Site

These astrocyte mRNAs were also measured at the wound site in the entorhinal cortex made by the knife insertion, 7 days post-ECL. We caution that ECL by knife cut transiently disrupts the local blood-brain barrier, allowing an influx of blood-borne factors which would normally be excluded, including circulating monocyte/macrophages and other cells (41).

GFAP. OVX decreased GFAP mRNA at the wound site (Fig. 6A) in both age groups: young, 15% decrease

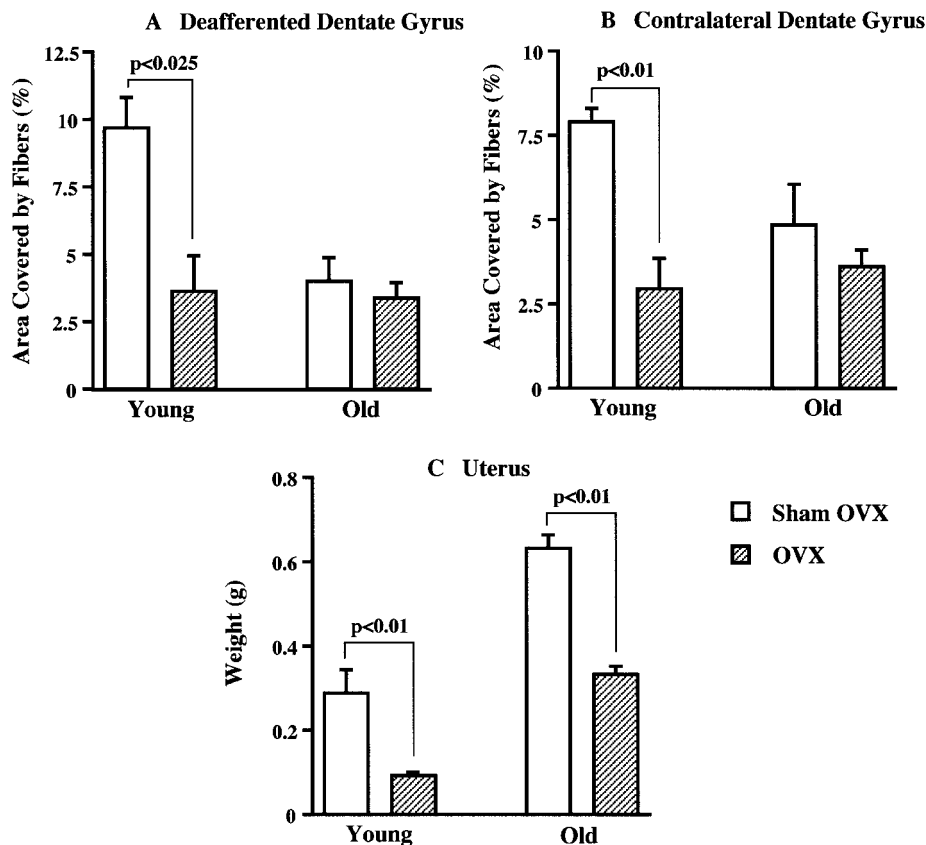


FIG. 2. Effects of ovariectomy on commissural/associational (C/A) fibers and uterine weight in young and middle-aged rats. (A) Two weeks after ECL, the density of the C/A fiber plexus in the deafferented inner molecular layer of the dentate gyrus in OVX young rats is 60% lower than in intact controls. In the same region in middle-aged rats, the fiber plexus appears to be at a minimal level and shows no further decrease with OVX. (B) The contralateral dentate gyrus shows a similar trend, with OVX decreasing percentage of area covered by fibers in young rats, and middle-aged rats appearing to be at a minimal level with no further reduction possible. The loss of an ovarian steroid effect in middle-aged animals does not appear to be the result of a loss of ovarian function; uterine weight (C) was decreased in both young and middle-aged rats in response to OVX. Thus while peripheral tissue responds similarly to OVX in both young and middle-aged animals, the dentate gyrus shows a loss of effect in middle-aged animals.

($P < 0.05$); and middle-aged, 25% decrease ($P < 0.05$). Furthermore, estradiol of OVX rats treatment increased GFAP mRNA to levels above those in sham OVX animals: young, 20% increase ($P < 0.05$); and middle-aged, 15% increase ($P < 0.05$). Thus the effect of estrogen on GFAP levels in response to a penetrating brain injury at 7 days post lesion is opposite that observed 3 days postlesion (15), which suggests a biphasic response of GFAP expression to wounding.

ApoE. OVX increased apoE mRNA at the wound site in both age groups: young, +100% ($P < 0.01$); and middle-aged, +50% ($P < 0.05$) relative to controls. In young rats, estradiol replacement returned apoE mRNA to control levels, whereas in middle-aged rats apoE levels remained elevated even with estradiol replacement (Fig. 6B).

ApoJ. OVX decreased apoJ mRNA levels at the wound site in young rats ($P < 0.05$; Fig. 6C). This decrease was not reversed by estradiol replacement, suggesting a possible progesterone effect. As observed

for the other mRNAs, apoJ mRNA responses at the wound site differed from that in the deafferented dentate gyrus where OVX increased apoJ mRNA levels. As in the dentate gyrus, no effect was detected in the middle-aged rats.

DISCUSSION

These results confirm that compensatory synaptic sprouting in the hippocampus is dependent on ovarian steroids, as judged by Holmes fiber histochemistry (44, 45, 73). Estrogen-dependent sprouting was also observed during physiological variations of blood estradiol on synaptic density in the hippocampal pyramidal CA1 neurons (81, 82), on dentate gyrus granule neurons (40), and in hippocampal slice cultures (77). Previous studies showed impaired compensatory synaptogenesis in aged male rats (60, 62), which we extend here to female rats. Although the sensitivity of ECL-induced sprouting to OVX is impaired by middle age as

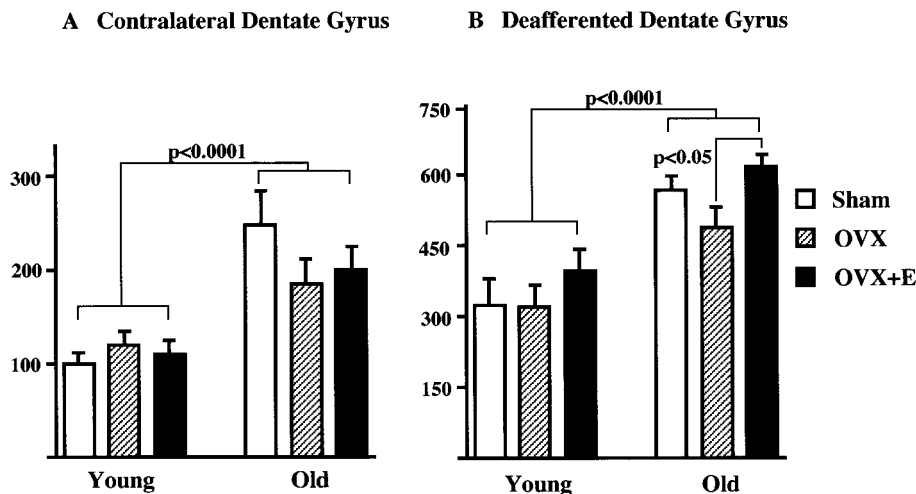


FIG. 3. GFAP mRNA in the deafferented and contralateral dentate gyrus at 7 days postlesion. (A) In the contralateral molecular layer of the dentate gyrus, no significant estrogen effect was observed in either young or middle-aged rats. Middle-aged rats showed a twofold increase in GFAP mRNA when compared to young rats ($P < 0.0001$). (B) In middle-aged rats, GFAP levels were increased twofold over levels in young rats ($P < 0.0001$). While young rats did not show a significant estrogen effect, estradiol-replaced middle-aged rats had a 25% increase in GFAP mRNA over OVX rats ($P < 0.05$). Data are displayed as percentages of young control level (ECL, Sham OVX, Contralateral).

judged by the Holmes fiber stain, synapse density was not directly measured here. In an elegant study, Miranda *et al.* (40) showed that short-term replacement of estradiol in middle-aged rats rapidly induced dendritic spines in the dentate gyrus (the postsynaptic contacts of the fibers in question here) back to the level observed in young rats. These rats were in an estrogen-deficient state for more than 1 year (without neurological lesions). This observation gives rise to two possible interpretations: a return to the young phenotype or a gain of function in middle-aged rats. The observed deficit in sprouting after deafferenting ECL lesions could be due to age changes in estrogen responsiveness in the afferent projections, however, independent of any in-

trinsic capacity of dentate gyrus neurons to form additional dendritic spines.

GFAP and Glial Reactivity

In Experiment 2, we examined the mRNAs expressed in astrocytes that are involved in estrogen-induced compensatory synaptic sprouting. We also examined effects of aging to identify if genes which might be factors in the age-related impairment of compensatory synaptogenesis and OVX response. Astrocyte reactivity, as defined by an increase in GFAP, is a hallmark of brain injury observed in virtually all neurodegenerative conditions (50, 72). Moreover, GFAP

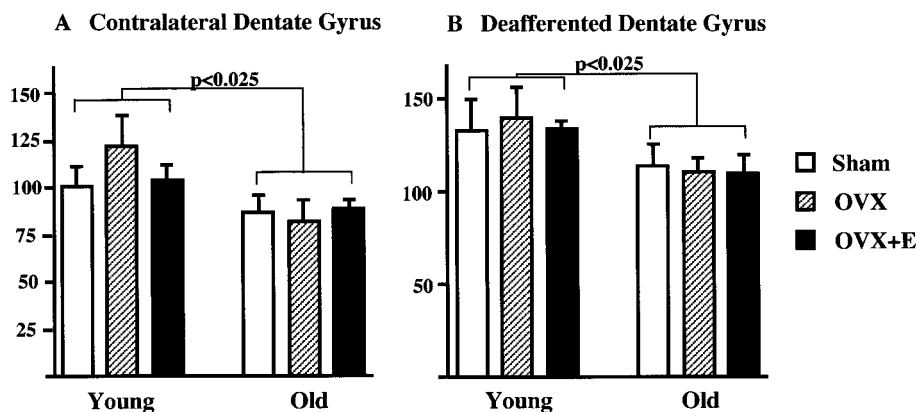


FIG. 4. ApoE mRNA in the deafferented and contralateral dentate gyrus at 7 days postlesion. (A) In the contralateral molecular layer of the dentate gyrus, no significant estrogen effect was observed in either young or middle-aged rats. Middle-aged rats showed a 20% decrease in apoE mRNA when compared to young rats ($P < 0.025$). (B) In the deafferented dentate gyrus of middle-aged rats, apoE levels were again decreased 20% below levels in young rats ($P < 0.025$). Neither young nor middle-aged rats displayed a significant estrogen effect. Data are displayed as percentages of young control level (ECL, Sham OVX, Contralateral).

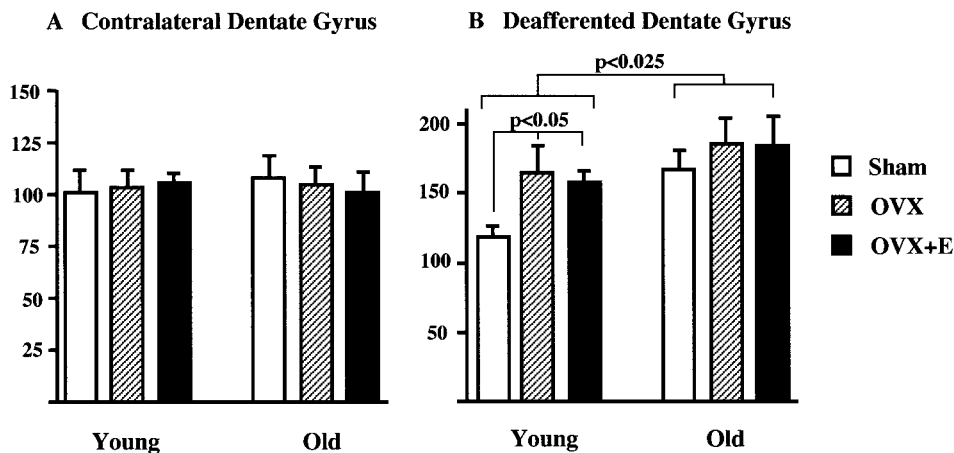


FIG. 5. ApoJ mRNA in the deafferented and contralateral dentate gyrus at 7 days postlesion. (A) In the contralateral dentate gyrus, no significant age or estrogen effects were detected. (B) In the deafferented dentate gyrus, while both young and middle-aged rats responded to ECL with an increase in apoJ mRNA, levels were 22% higher in middle-aged than in young rats ($P < 0.025$). Additionally, young rats showed a further increase in apoJ mRNA with OVX ($P < 0.025$) which was not reversed by estradiol replacement. This suggests an inhibitory effect on apoJ levels by progesterone. This effect was not detected in middle-aged animals. Data are displayed as percentages of young control level (ECL, Sham OVX, Contralateral).

expression per astrocyte increases progressively during aging in the absence of definable neuropathology in rodent and human brains (18, 19, 27, 43, 49, 83).

Abundant evidence indicates that increased GFAP can be detrimental to reactive synaptogenesis through glial scar formation. Dorsal root ganglion neurons transplanted in the area of a CNS injury have the ability to grow both toward and away from the wound site; however, contact with the glial scar stops neurite outgrowth (8). Similarly, the retina, which retains the ability to reestablish connection in response to lesions, is notably lacking in scar formation in a rat model (33). This raises the question of the role of glial scar formation in the CNS. Removal of GFAP-positive scar-forming astrocytes after injury (by treatment with ganciclovir in mice expressing HSV-TK from the GFAP promoter) results not only in increased neurite outgrowth, but also increased neuronal degeneration, monocyte infiltration, and failure to repair the blood-brain barrier (BBB) (5). Thus glial scar formation may be a "necessary evil" or cost of adaptation in response to brain injury, where neurite outgrowth must be sacrificed to prevent further neuronal degeneration. Although they do not form a "classic" glial scar, the highly reactive astrocytes in the dentate gyrus during reafferentation may play a similar paradoxical role by both inhibiting neurite outgrowth and enhancing synaptogenesis. Because aging rats (which were 4–12 months older than in this study) have enhanced GFAP response to brain lesions concurrent with the decrease in compensatory synaptogenesis (18, 63), the increase of GFAP during aging could contribute to the loss of sprouting with age.

The role of estrogen in neurite outgrowth and sprouting may also involve glial reactivity. Estradiol supports

synaptogenesis in some hippocampal neurons, while decreasing the local GFAP response to a penetrating brain injury (15). Inflammatory mechanisms have been implicated in glial scar formation (14). This observation may explain the biphasic effect of estrogen on astrocyte reactivity we have observed in response to injury. For example, in the deafferented hippocampus at 3 days postlesion, GFAP mRNA was reduced by estradiol, followed by decrease GFAP immunoreactivity at 7 days postlesion in estradiol-treated rats (58). This mRNA decrease is reversed to become an increase at 7 days postlesion. In particular we hypothesize that estradiol may have an anti-inflammatory role in the initial phase of the wound response, such that the resulting decrease in astrocyte reactivity observed may be due to a reduction in the stimulus (inflammation). Direct anti-inflammatory actions of estradiol such as the suppression of TNF- α production have been demonstrated in inflammatory cells (1, 59). This would allow for increased neurite outgrowth without the increased neuronal degeneration resulting from total removal of the astrocyte reactivity response. During the second phase of the wound response (following neurite outgrowth), estrogen may be acting directly or indirectly on astrocytes to increase reactivity and their role in synapse formation.

The hypothesis of estradiol as an anti-inflammatory agent does not, however, explain all experimental data. For example, the increase in GFAP in the aging brain may be a response to inflammation or oxidative stress (42). If this were the case, the contralateral hippocampus in middle-aged rats in this study would have been expected to show decreased GFAP in response to estradiol treatment (which was not observed). Furthermore, the twofold increase in GFAP mRNA in the aged rat

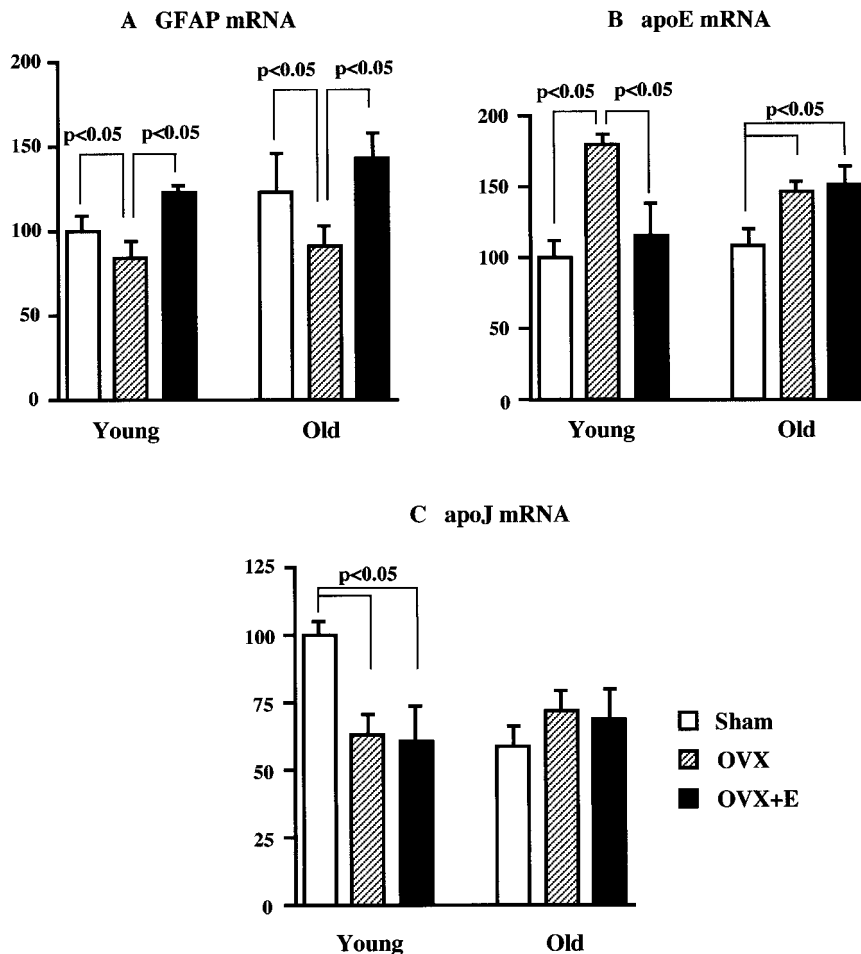


FIG. 6. The mRNA response at the wound site in young and middle-aged animals. (A) Both young and middle-aged rats showed a decrease in GFAP mRNA levels with OVX that was reversed by estradiol replacement. (B) Both young and middle-aged rats showed an increase in apoE mRNA in response to OVX. This was reversed by estradiol replacement in young, but not middle-aged rats. (C) Young rats showed a decrease in apoJ mRNA levels in response to OVX, which was not reversed by estradiol replacement, again suggesting a progesterone effect in young rats. Middle-aged rats did not show any OVX or estradiol effect.

brain is not maximal, as the middle-aged rat brain is still capable of mounting a threefold increase in GFAP mRNA in response to brain lesioning. This same phenomenon has also been observed in response to nigrostriatal lesioning (18). It is also important to point out that we are not suggesting a role for GFAP itself directly in the process of synaptogenesis, but rather are using it as a marker of glial reactivity. Transgenic mice lacking the GFAP gene still respond normally to CNS injury (54, 80), probably because they can still produce intermediate filaments. GFAP/vimentin double knockout mice (which cannot produce intermediate filaments) show impaired glial scar formation accompanied by bleeding (54).

Apolipoprotein Responses to Lesioning and Steroids

During reafferentation following ECL, the brain responds with an increase in both apoE (55, 56) and apoJ (22, 37) mRNA production, which may mediate trans-

port of cholesterol and other hydrophobic membrane components for synaptogenesis (55). As observed for GFAP, the middle-aged rats show decreased apoE mRNA in the lesioned and contralateral dentate gyrus which would be consistent with decreased synaptic sprouting. In both areas apoE mRNA is significantly decreased with age, consistent with other studies on male rats in the unlesioned hippocampus as well (43). It is of potential interest that the apoJ mRNA response to ECL is about 30% larger in sham OVX middle-aged rats than in young, a response that would be expected to coincide with increased synaptic sprouting. This may be a lesion-specific phenomenon, as apoJ mRNA levels do not increase in the unlesioned hippocampus with age.

Some evidence implied that apoE mRNA would be increased by estrogen treatment in the lesioned hippocampus. In the mouse brain, apoE mRNA is increased by estrogen treatment (69); likewise, apoE

knock-out mice have do not show estradiol-dependent synaptic sprouting after ECL (73, 77). Similarly, rat mixed glial cultures respond to estrogen treatment with increased apoE, and estrous cycle data suggest bidirectional control of apoE mRNA in various regions of the rat brain (75). OVX and estradiol replacement did not affect apoE mRNA levels in either the deafferented or contralateral dentate gyrus at 7 days postlesion.

ApoE-, apoJ-, and GFAP mRNAs at the Wound Site

In each case, astrocyte mRNA responses at the wound site in the entorhinal cortex differed from that in the deafferented dentate gyrus in response to OVX and estradiol replacement. While apoE mRNA was not sensitive to estradiol in the dentate gyrus, estradiol replacement of OVX-lesioned rats decreased apoE mRNA levels at the wound site. Likewise, apoJ showed no OVX response in the dentate gyrus, whereas at the lesion site OVX cause a substantial decrease in apoJ mRNA which was not reversed by estradiol replacement. One explanation for this discrepancy may be local opening of the BBB at the wound site. Cell-cell interactions are critical for the astrocytic mRNA response to estrogen for both apoE (74) and GFAP (75). With the BBB compromised, white blood cells and macrophages invade the wound site, which may alter the responses of local astrocytes. Estradiol treatment decreases circulating apoE levels in humans (2, 46) and baboons (25). The effects in the periphery of rodents are not as clear and differ between various strains of mice (70). Thus infiltrating macrophages and other blood cells could alter the mRNA response in the brain in two ways: indirectly, by interacting with astrocytes in a different manner than resident microglia thereby altering the mRNA response, or directly through production of apoE and apoJ mRNA, which would be increased in OVX rats.

Hormonal Influences on Aging in the Brain

In addition to phenotypic changes in neurons and glia, changes in peripheral hormones can influence reafferentation. Age changes in cholinergic projections could be a factor in the decreased sprouting: although no effect of aging in female rats was observed in the dorsal hippocampus, the globus pallidus showed a 30% decrease in ChAT activity (31). Estrogen treatment increases ChAT mRNA in the rat basal forebrain (17, 38), suggesting that age and ovariectomy may interact in cholinergic neurons.

Adrenal glucocorticoids influence sprouting both individually and in conjunction with estradiol, and an increase with age in circulating levels is well established in rodents (12). In female rats, adrenalectomy (ADX) increases fiber outgrowth to the lesioned hippocampus (64), suggesting that decreased sprouting

with age may be a result of increased CORT levels. This effect, however, is highly dependent upon the presence of circulating estrogen. In ADX/OVX female rats, CORT treatment *decreases* fiber outgrowth (44), whereas in intact female rats CORT treatment *increases* outgrowth (65). In this study intact, middle-aged female rats showed a decrease in the density of Holmes fibers (Fig. 1). This effect is present without sex steroid manipulation and could be influenced by increased circulating CORT levels; however, it is doubtful that this is the exclusive cause of the age-related decrease. Male rats also show decreased sprouting with age, and their response to CORT may be opposite that of females. ADX alone has no effect on sprouting in males (64), and CORT treatment in the intact male rat decreases reafferentation (65). While the effects of CORT on fiber outgrowth are sexually dimorphic and cannot explain a phenomenon as consistent as the loss of fiber density/outgrowth with age, they may influence the estrogen response throughout the life cycle. Importantly, the middle-aged rats in this study represent a select subgroup that has survived both to middle age and through two surgeries. If survival at this age is tied to hormonal response it would confound these experiments; this is impossible to determine at this point.

Estrogen and Alzheimer's Disease

These results are of interest in the ongoing discussion of the effect of ERT on the risk of developing AD. Many post hoc studies indicate a decreased risk of AD in women undergoing ERT (23, 52, 76, 78). However, two recent randomized, placebo-controlled trials have failed to show any benefit to ERT in women in the early stages of AD (20, 47). If estrogen is determined to delay and/or prevent the onset of AD, postmortem studies of women who had undergone ERT will be of great interest. Our results suggest that studies of the AD hippocampus must control for estrogen use, as estrogen-induced synaptogenesis could drastically affect counts of synaptic markers.

CONCLUSIONS

In conclusion, our results suggest that mRNA production in the glia of aged rats is altered in a manner consistent with decreased neurite outgrowth and compensatory synaptogenesis. Middle-aged rats show increased GFAP levels, which has been frequently (though not exclusively) shown to be associated with decreased synaptogenesis. Similarly, aged rats show decreases in mRNA for apoE, a protein that is necessary for normal synaptogenesis. These specific changes in glial phenotype, however, do not appear to be causal in the loss of the OVX effect on synaptic sprouting with age.

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REFERENCES

- An, J., R. C. Ribeiro, P. Webb, J. A. Gustafsson, P. J. Kushner, J. D. Baxter, and D. C. Leitman. 1999. Estradiol repression of tumor necrosis factor- α transcription requires estrogen receptor activation function-2 and is enhanced by coactivators. *Proc. Natl. Acad. Sci. USA* **96**: 15161–15166.
- Applebaum-Bowden, D., P. McLean, A. Steinmetz, D. Fontana, C. Matthys, G. R. Warnick, M. Cheung, J. J. Albers, and W. R. Hazzard. 1989. Lipoprotein, apolipoprotein, and lipolytic enzyme changes following estrogen administration in postmenopausal women. *J. Lipid Res.* **30**: 1895–1906.
- Auer, R. N. 1996. Effect of age and sex on *N*-methyl-D-aspartate antagonist-induced neuronal necrosis in rats. *Stroke* **27**: 743–746.
- Bergmann, M., A. Post, I. Rittel, I. Bechmann, and R. Nitsch. 1997. Expression of synaptophysin in sprouting neurons after entorhinal lesion in the rat. *Exp. Brain Res.* **117**: 80–86.
- Bush, T. G., N. Puvanachandra, C. H. Horner, A. Polito, T. Ostefeld, C. N. Svendsen, L. Mucke, M. H. Johnson, and M. V. Sofroniew. 1999. Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron* **23**: 297–308.
- Corder, E. H., A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines, and M. A. Pericak-Vance. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families [see comments]. *Science* **261**: 921–923.
- Crispino, M., D. J. Stone, M. Wei, C. P. Anderson, G. Tocco, C. E. Finch, and M. Baudry. 1999. Variations of synaptotagmin I, synaptotagmin IV, and synaptophysin mRNA levels in rat hippocampus during the estrous cycle. *Exp. Neurol.* **159**: 574–583.
- Davies, S. J., D. R. Goucher, C. Doller, and J. Silver. 1999. Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. *J. Neurosci.* **19**: 5810–5822.
- Day, J. R., N. J. Laping, T. H. McNeill, S. S. Schreiber, G. Pasinetti, and C. E. Finch. 1990. Castration enhances expression of glial fibrillary acidic protein and sulfated glycoprotein-2 in the intact and lesion-altered hippocampus of the adult male rat. *Mol. Endocrinol.* **4**: 1995–2002.
- Fagan, A. M., D. M. Holtzman, G. Munson, T. Mathur, D. Schneider, L. K. Chang, G. S. Getz, C. A. Reardon, J. Lukens, J. A. Shah, and M. J. LaDu. 1999. Unique lipoproteins secreted by primary astrocytes from wild type, apoE (–/–), and human apoE transgenic mice. *J. Biol. Chem.* **274**: 30001–30007.
- Farlow, M. R., D. K. Lahiri, J. Poirier, J. Davignon, L. Schneider, and S. L. Hui. 1998. Treatment outcome of tacrine therapy depends on apolipoprotein genotype and gender of the subjects with Alzheimer's disease. *Neurology* **50**: 669–677.
- Finch, C. E. 1990. *Longevity, Senescence, and the Genome*. Univ. of Chicago Press, Chicago, IL.
- Finch, C. E. 1999. *Clusterin in Normal Brain Functions and during Development*. Landes, Austin, TX.
- Fitch, M. T., C. Doller, C. K. Combs, G. E. Landreth, and J. Silver. 1999. Cellular and molecular mechanisms of glial scarring and progressive cavitation: In vivo and in vitro analysis of inflammation-induced secondary injury after CNS trauma. *J. Neurosci.* **19**: 8182–8198.
- Garcia-Estrada, J., J. A. Del Rio, S. Luquin, E. Soriano, and L. M. Garcia-Segura. 1993. Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. *Brain Res.* **628**: 271–278.
- Geddes, J. W., D. T. Monaghan, C. W. Cotman, I. T. Lott, R. C. Kim, and H. C. Chui. 1985. Plasticity of hippocampal circuitry in Alzheimer's disease. *Science* **230**: 1179–1181.
- Gibbs, R. B., D. Wu, L. B. Hersh, and D. W. Pfaff. 1994. 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.
- Gordon, M. N., W. A. Schreier, X. Ou, L. A. Holcomb, and D. G. Morgan. 1997. Exaggerated astrocyte reactivity after nigrostriatal deafferentation in the aged rat. *J. Comp. Neurol.* **388**: 106–119.
- Hansen, L. A., D. M. Armstrong, and R. D. Terry. 1987. An immunohistochemical quantification of fibrous astrocytes in the aging human cerebral cortex. *Neurobiol. Aging* **8**: 1–6.
- 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, and M. R. Farlow. 2000. Estrogen for Alzheimer's disease in women: Randomized, double-blind, placebo-controlled trial. *Neurology* **54**: 295–301.
- Ignatius, M. J., P. J. Gebicke-Harter, J. H. Skene, J. W. Schilling, K. H. Weisgraber, R. W. Mahley, and E. M. Shooter. 1986. Expression of apolipoprotein E during nerve degeneration and regeneration. *Proc. Natl. Acad. Sci. USA* **83**: 1125–1129.
- Johnson, S., C. S. Young-Chan, N. J. Laping, and C. E. Finch. 1996. Perforant path transection induces complement C9 deposition in hippocampus. *Exp. Neurol.* **138**: 198–205.
- Kawas, C., S. Resnick, A. Morrison, R. Brookmeyer, M. Corrada, A. Zonderman, C. Bacal, D. D. Lingle, and E. Metter. 1997. A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: The Baltimore Longitudinal Study of Aging [published erratum appears in *Neurology*, 1998, Aug; **51**(2):654]. *Neurology* **48**: 1517–1521.
- Koo, C., T. L. Innerarity, and R. W. Mahley. 1985. Obligatory role of cholesterol and apolipoprotein E in the formation of large cholesterol-enriched and receptor-active high density lipoproteins. *J. Biol. Chem.* **260**: 11934–11943.
- Kushwaha, R. S., D. M. Foster, P. H. Barrett, K. D. Carey, and M. G. Bernard. 1991. Metabolic regulation of plasma apolipoprotein E by estrogen and progesterone in the baboon (*Papio* sp). *Metabolism* **40**: 93–100.
- LaDu, M. J., S. M. Gilligan, J. R. Lukens, V. G. Cabana, C. A. Reardon, L. J. Van Eldik, and D. M. Holtzman. 1998. Nascent astrocyte particles differ from lipoproteins in CSF. *J. Neurochem.* **70**: 2070–2081.
- Landfield, P. W., G. Rose, L. Sandles, T. C. Wohlstatter, and G. Lynch. 1977. Patterns of glial hypertrophy and neuronal degeneration in the hippocampus of aged memory-deficient rats. *J. Gerontol.* **32**: 3–12.
- Laping, N. J., B. Teter, N. R. Nichols, I. Rozovsky, and C. E. Finch. 1994. Glial fibrillary acidic protein: Regulation by hormones, cytokines, and growth factors. *Brain Pathol.* **4**: 259–275.
- Lefrancois, T., C. Fages, M. Peschanski, and M. Tardy. 1997. Neuritic outgrowth associated with astroglial phenotypic changes induced by antisense glial fibrillary acidic protein (GFAP) mRNA in injured neuron-astrocyte cocultures. *J. Neurosci.* **17**: 4121–4128.

30. Lipman, R. D., G. E. Dallal, and R. T. Bronson. 1999. Effects of genotype and diet on age-related lesions in ad libitum fed and calorie-restricted F344, BN, and BNF3F1 rats. *J. Gerontol. A Biol. Sci. Med. Sci.* **54**: B478–B491.
31. Luine, V. N., K. J. Renner, S. Heady, and K. J. Jones. 1986. Age and sex-dependent decreases in ChAT in basal forebrain nuclei. *Neurobiol. Aging* **7**: 193–198.
32. Luquin, S., F. Naftolin, and L. M. Garcia-Segura. 1993. Natural fluctuation and gonadal hormone regulation of astrocyte immunoreactivity in dentate gyrus. *J. Neurobiol.* **24**: 913–924.
33. MacLaren, R. E. 1996. Development and role of retinal glia in regeneration of ganglion cells following retinal injury. *Br. J. Ophthalmol.* **80**: 458–464.
34. Mahley, R. W. 1988. Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. *Science* **240**: 622–630.
35. Masliah, E., M. Mallory, N. Ge, M. Alford, I. Veinbergs, and A. D. Roses. 1995. Abnormal synaptic regulation in hAPP695 transgenic and apoE knockout mice. In *Research Advances in Alzheimer's Disease and Related Disorders* (I. Iqbal, J. A. Morimer, B. Winblad, and H. M. Wisniewski, Ed.), pp. 405–413. Wiley, New York.
36. Masliah, E., M. Mallory, N. Ge, M. Alford, I. Veinbergs, and A. D. Roses. 1995. Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp. Neurol.* **136**: 107–122.
37. May, P. C., M. Lampert-Etchells, S. A. Johnson, J. Poirier, J. N. Masters, and C. E. Finch. 1990. Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. *Neuron* **5**: 831–839.
38. McMillan, P. J., C. A. Singer, and D. M. Dorsa. 1996. The effects of ovariectomy and estrogen replacement on trkA and choline acetyltransferase mRNA expression in the basal forebrain of the adult female Sprague–Dawley rat. *J. Neurosci.* **16**: 1860–1865.
39. Meyer, M. R., J. T. Tschanz, M. C. Norton, K. A. Welsh-Bohmer, D. C. Steffens, B. W. Wyse, and J. C. Breitner. 1998. APOE genotype predicts when—not whether—one is predisposed to develop Alzheimer disease [letter] [see comments]. *Nature Genet.* **19**: 321–322.
40. Miranda, P., C. L. Williams, and G. Einstein. 1999. Granule cells in aging rats are sexually dimorphic in their response to estradiol. *J. Neurosci.* **19**: 3316–3325.
41. Morgan, T. E., N. R. Nichols, G. M. Pasinetti, and C. E. Finch. 1993. TGF-beta 1 mRNA increases in macrophage/microglial cells of the hippocampus in response to deafferentation and kainic acid-induced neurodegeneration. *Exp. Neurol.* **120**: 291–301.
42. Morgan, T. E., I. Rozovsky, S. K. Goldsmith, D. J. Stone, T. Yoshida, and C. E. Finch. 1997. Increased transcription of the astrocyte gene GFAP during middle-age is attenuated by food restriction: Implications for the role of oxidative stress. *Free Rad. Biol. Med.* **23**: 524–528.
43. Morgan, T. E., Z. Xie, S. Goldsmith, T. Yoshida, A. S. Lanzrein, D. Stone, I. Rozovsky, G. Perry, M. A. Smith, and C. E. Finch. 1999. The mosaic of brain glial hyperactivity during normal ageing and its attenuation by food restriction. *Neuroscience* **89**: 687–699.
44. Morse, J. K., S. T. DeKosky, and S. W. Scheff. 1992. Neurotrophic effects of steroids on lesion-induced growth in the hippocampus. II. Hormone replacement. *Exp. Neurol.* **118**: 47–52.
45. Morse, J. K., S. W. Scheff, and S. T. DeKosky. 1986. Gonadal steroids influence axon sprouting in the hippocampal dentate gyrus: A sexually dimorphic response. *Exp. Neurol.* **94**: 649–658.
46. Muesing, R. A., V. T. Miller, J. C. LaRosa, D. B. Stoy, and E. A. Phillips. 1992. Effects of unopposed conjugated equine estrogen on lipoprotein composition and apolipoprotein-E distribution. *J. Clin. Endocrinol. Metab.* **75**: 1250–1254.
47. 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, and L. J. Thal. 2000. Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: A randomized controlled trial—Alzheimer's Disease Cooperative Study [see comments]. *JAMA* **283**: 1007–1015.
48. Naftolin, F., G. Mor, T. L. Horvath, S. Luquin, A. B. Fajer, F. Kohen, and L. M. Garcia-Segura. 1996. Synaptic remodeling in the arcuate nucleus during the estrous cycle is induced by estrogen and precedes the preovulatory gonadotropin surge. *Endocrinology* **137**: 5576–5580.
49. Nichols, N. R., J. R. Day, N. J. Laping, S. A. Johnson, and C. E. Finch. 1993. GFAP mRNA increases with age in rat and human brain. *Neurobiol. Aging* **14**: 421–429.
50. Norton, W. T., D. A. Aquino, I. Hozumi, F. C. Chiu, and C. F. Brosnan. 1992. Quantitative aspects of reactive gliosis: A review. *Neurochem. Res.* **17**: 877–885.
51. Olmos, G., F. Naftolin, J. Perez, P. A. Tranque, and L. M. Garcia-Segura. 1989. Synaptic remodeling in the rat arcuate nucleus during the estrous cycle. *Neuroscience* **32**: 663–667.
52. Paganini-Hill, A., and V. W. Henderson. 1996. Estrogen replacement therapy and risk of Alzheimer disease. *Arch. Intern. Med.* **156**: 2213–2217.
53. Pasinetti, G. M., M. Hassler, D. Stone, and C. E. Finch. 1999. Glial gene expression during aging in rat striatum and in long-term responses to 6-OHDA lesions. *Synapse* **31**: 278–284.
54. Pekny, M., C. B. Johansson, C. Eliasson, J. Stakeberg, A. Wallen, T. Perlmann, U. Lendahl, C. Betsholtz, C. H. Berthold, and J. Frisen. 1999. Abnormal reaction to central nervous system injury in mice lacking glial fibrillary acidic protein and vimentin. *J. Cell Biol.* **145**: 503–514.
55. Poirier, J., A. Baccichet, D. Dea, and S. Gauthier. 1993. Cholesterol synthesis and lipoprotein reuptake during synaptic remodeling in hippocampus in adult rats. *Neuroscience* **55**: 81–90.
56. Poirier, J., M. Hess, P. C. May, and C. E. Finch. 1991. Astrocytic apolipoprotein E mRNA and GFAP mRNA in hippocampus after entorhinal cortex lesioning. *Brain Res. Mol. Brain Res.* **11**: 97–106.
57. Roses, A. D. 1997. Apolipoprotein E, a gene with complex biological interactions in the aging brain. *Neurobiol. Dis.* **4**: 170–185.
58. Rozovsky, I., M. Wei, C. P. Andersen, and C. E. Finch. 1999. Estrogen–injury interactions in astrocyte–neuron cocultures: Decreased GFAP expression and enhanced neurite outgrowth. In *Society for Neuroscience Annual Meeting, Miami Beach, FL*, 712.5.
59. Salem, M. L., M. S. Hossain, and K. Nomoto. 2000. Mediation of the immunomodulatory effect of beta-estradiol on inflammatory responses by inhibition of recruitment and activation of inflammatory cells and their gene expression of TNF-alpha and IFN-gamma. *Int. Arch. Allergy Immunol.* **121**: 235–245.
60. Schauwecker, P. E., H. W. Cheng, R. M. Serquinia, N. Mori, and T. H. McNeill. 1995. Lesion-induced sprouting of commissural/associational axons and induction of GAP-43 mRNA in hilar and CA3 pyramidal neurons in the hippocampus are diminished in aged rats. *J. Neurosci.* **15**: 2462–2470.
61. Schauwecker, P. E., and T. H. McNeill. 1995. Enhanced but delayed axonal sprouting of the commissural/associational pathway following a combined entorhinal cortex/fimbria fornix lesion. *J. Comp. Neurol.* **351**: 453–464.

62. Scheff, S. W., L. S. Benardo, and C. W. Cotman. 1980. Decline in reactive fiber growth in the dentate gyrus of aged rats compared to young adult rats following entorhinal cortex removal. *Brain Res.* **199**: 21–38.
63. Scheff, S. W., and S. T. DeKosky. 1983. Steroid suppression of axon sprouting in the hippocampal dentate gyrus of the adult rat: Dose–response relationship. *Exp. Neurol.* **82**: 183–191.
64. Scheff, S. W., J. K. Morse, and S. T. DeKosky. 1988. Hydrocortisone differentially alters lesion-induced axon sprouting in male and female rats. *Exp. Neurol.* **100**: 237–241.
65. Scheff, S. W., J. K. Morse, and S. T. DeKosky. 1988. Neurotropic effects of steroids on lesion-induced growth in the hippocampus. I. The asteroidal condition. *Brain Res.* **457**: 246–250.
66. Shanmugaratnam, J., E. Berg, L. Kimerer, R. J. Johnson, A. Amaratunga, B. M. Schreiber, and R. E. Fine. 1997. Retinal Muller glia secrete apolipoproteins E and J which are efficiently assembled into lipoprotein particles. *Brain Res. Mol. Brain Res.* **50**: 113–120.
67. Sheehan, D. C., and B. B. Hrapchak. 1980. The Holmes stain. In *Theory and Practice of Histotechnology* (D. C. Sheenan and B. B. Hrapchak, Eds.), pp. 256–257. Mosby, St. Louis.
68. Simkins, J., M. Singh, and J. Bishop. 1994. The potential role for estrogen therapy in treatment of cognitive decline and neurodegeneration associated with Alzheimer's disease. *Neurobiol. Aging* **15**: S195–S197.
69. Srivastava, R. A., N. Bhasin, and N. Srivastava. 1996. Apolipoprotein E gene expression in various tissues of mouse and regulation by estrogen. *Biochem. Mol. Biol. Int.* **38**: 91–101.
70. Srivastava, R. A., N. Srivastava, M. Aversa, R. C. Lin, K. S. Korach, D. B. Lubahn, and G. Schonfeld. 1997. Estrogen up-regulates apolipoprotein E (ApoE) gene expression by increasing ApoE mRNA in the translating pool via the estrogen receptor alpha-mediated pathway. *J. Biol. Chem.* **272**: 33360–33366.
71. Steward, O., and J. Loesche. 1977. Quantitative autoradiographic analysis of the time course of proliferation of contralateral entorhinal efferents in the dentate gyrus denervated by ipsilateral entorhinal lesions. *Brain Res.* **125**: 11–21.
72. Steward, O., E. R. Torre, L. L. Phillips, and P. A. Trimmer. 1990. The process of reinnervation in the dentate gyrus of adult rats: Time course of increases in mRNA for glial fibrillary acidic protein. *J. Neurosci.* **10**: 2373–2384.
73. Stone, D. J., I. Rozovsky, T. E. Morgan, C. P. Anderson, and C. E. Finch. 1998. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: Implications for Alzheimer's disease. *J. Neurosci.* **18**: 3180–3185.
74. Stone, D. J., I. Rozovsky, T. E. Morgan, C. P. Anderson, H. Hajian, and C. E. Finch. 1997. Astrocytes and microglia respond to estrogen with increased apoE mRNA in vivo and in vitro. *Exp. Neurol.* **143**: 313–318.
75. Stone, D. J., Y. Song, C. P. Anderson, K. K. Krohn, C. E. Finch, and I. Rozovsky. 1998. Bidirectional transcription regulation of glial fibrillary acidic protein by estradiol in vivo and in vitro. *Endocrinology* **139**: 3202–3209.
76. Tang, M. X., D. Jacobs, Y. Stern, K. Marder, P. Schofield, B. Gurland, H. Andrews, and R. Mayeux. 1996. Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease [see comments]. *Lancet* **348**: 429–432.
77. Teter, B., M. E. Harris-White, S. A. Frautschy, and G. M. Cole. 1999. Role of apolipoprotein E and estrogen in mossy fiber sprouting in hippocampal slice cultures. *Neuroscience* **91**: 1009–1016.
78. van Duijn, C. M., J. S. Meijer, and J. M. C. Witteman. 1996. Estrogen, apolipoprotein E and the risk of Alzheimer's disease. *Neurobiol. Aging* **17**: S79–S80.
79. vom Saal, F. S., C. E. Finch, and J. F. Nelson. 1994. The natural history of reproductive aging in humans, laboratory rodents, and selected other vertebrates. In *Physiology of Reproduction* (E. Knobil, Ed.), pp. 1213–1314. Raven Press, New York.
80. Wang, X., A. Messing, and S. David. 1997. Axonal and nonneuronal cell responses to spinal cord injury in mice lacking glial fibrillary acidic protein. *Exp. Neurol.* **148**: 568–576.
81. Woolley, C. S., and B. S. McEwen. 1992. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat [published erratum appears in *J. Neurosci.*, 1992, Oct; **12**(10):following table of contents]. *J. Neurosci.* **12**: 2549–2554.
82. Woolley, C. S., and B. S. McEwen. 1993. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *J. Comp. Neurol.* **336**: 293–306.
83. Yoshida, T., S. K. Goldsmith, T. E. Morgan, D. J. Stone, and C. E. Finch. 1996. Transcription supports age-related increases of GFAP gene expression in the male rat brain. *Neurosci. Lett.* **215**: 107–110.