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Maternal experience and Alzheimer's disease : degenerative differences in the female rat

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Maternal Experience and Alzheimer's Disease:
Degenerative Differences in the Female Rat

by

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Abstract

Alzheimer's disease is a degenerative disease found in many aging adults. The presence of amyloid precursor protein (APP) is an early indicator of the onset of Alzheimer's, primarily in memory-related brain regions like the hippocampus. Hormones accompanying pregnancy, such as estrogen, may provide the female brain with protection against neurodegeneration and deposits of APP. The present study will compare concentrations of APP in the brains of parous and nulliparous animals and examine the interaction of APP with estrogen receptor beta (ERβ). Young and aged animals will also be compared to determine any early effects of APP or ERβ. It is proposed that because of the neuroprotective effects of preganacy, the parous animals will have lower concentrations of APP and higher concentrations of ERβ.

Maternal Experience and Alzheimer's Disease: Degenerative Differences in the Female Rat

Alzheimer's disease is quickly becoming one of the most prevalent and devastating afflictions of today's aging population. Alzheimer's disease is the fourth leading cause of death in Western nations, and it is estimated that approximately 24 million people worldwide suffer from Alzheimer's-related dementia (Miller, McLoughlin, Kwok-Fai, Tennant, & Rogelj, 2006). There is no cure or effective treatment currently available for Alzheimer's disease, but given its frequent occurrence in older adults, the need for treatment is becoming more and more pronounced. In addition to seeking treatment for Alzheimer's, scientists are still working to understand the intricate details of the complex pathology of this disease (Kar, Slowikowski, Westaway, & Mount, 2004).

Alzheimer's disease is most distinctly characterized by neural plaques and neurofibrillary tangles, which both lead to eventual lesions in the brain, as well as a decrease in number of neurons and functional synaptic connections. Therefore, neurons are unable to efficiently pass on messages, and cognitive functioning begins to decline (Stephan, Laroche, & Davis, 2001). The development of these degenerative features is linked to deposits of amyloid precursor protein (APP) in the brain, especially in regions associated with memory such as the hippocampus. High concentrations of APP deposits are often found extremely early in the pathogenesis of the disease, even before specific Alzheimer's symptoms emerge (Stephan, Laroche, & Davis, 2001). Most notably, β-amyloid peptide (Aβ) is derived from the APP deposits in the brain. The Aβ acts as the core for the formation of neural plaques (Kar et al., 2004).

The role of Aβ and APP in the pathogenesis of Alzheimer's disease has been evaluated in many studies of animal models. Stephan, Laroche, and Davis (2001) found that rats injected

with small amounts of Aβ experienced a significantly accelerated development of neural plaques. The injected animals also performed poorly on cognitive tasks, made more behavioral mistakes in navigation, and made a greater number of errors on a working memory task than the non-injected control group. These decreases in memory performance indicate that the presence of Alzheimer's-related proteins in the brain lead to both negative behavioral modifications and disruptions in underlying neuroanatomy. APP can have detrimental effects on the strength and speed of neuronal firing. In a mouse model of Alzheimer's, results indicated that hippocampal neurons lacking in APP had a higher frequency and strength of synaptic transmissions, and there were more functional neurons in the areas of the brain without APP (Priller, Bauer, Mitteregger, Krebs, Kretzschmar, & Herms, 2006). Once APP and Aβ effectively deposit the neural tangles and plaques, the target regions of the brain start to lose neurons and functional synapses, resulting in the beginnings of the slow cognitive degeneration associated with Alzheimer's disease (Kar et al., 2004).

Sex Differences and Hormones

Alzheimer's disease is more prevalent in women than in men. This sex difference can be attributed to an overall longer female lifespan and differences in the duration of the disease; women with Alzheimer's tend to live longer with the disease than men (Baum, 2005). The body may also provide some natural defenses against the effects of Alzheimer's. Specifically, hormones may play a mediating role in the development and progression of the disease. It has been suggested that testosterone may protect men from Alzheimer's disease, while estrogen may have similar protective effects for women (Baum, 2005).

Studies on hormone replacement therapy have confirmed the beneficial effects of female hormones like estrogen on the pathogenesis of Alzheimer's disease. Henderson, Benke, and Greene (2005) found that a hormone therapy regimen including estrogen might reduce the risk of

developing early onset Alzheimer's. It was also concluded that the hormone therapy must be administered at a certain critical time period, during the early stages of postmenopausal symptoms, for protective effects against Alzheimer's to be executed. Beginning a hormone therapy regimen years after the onset of menopause and the loss of endogenous estrogen is too late, and the beneficial effects that result from earlier hormone administration will not occur (Baum, 2005). Generally, hormone replacement therapy exhibits a 'healthy cell bias,' in which neurons that are healthy during administration will experience the benefits of therapy, while hormones will cause further damage in unhealthy neurons (Brann, Dhandapani, Wakade, Mahesh, & Khan, 2007). It is also important to consider dosage when administering hormone replacement therapy. Baum (2005) reported an inverted U-shaped pattern in the effectiveness of hormone dosages; a small dosage did not show any effects and a large dosage could have toxic and harmful effects. An excessive administration of exogenous estrogen can disrupt the body's natural fluctuations of hormones, which can actually have detrimental effects on the functioning of the brain (Marriott & Wenk, 2004). Moderate amounts of hormone replacement therapy that more closely mimic the body's natural cycle may provide the best protection against the degeneration of Alzheimer's disease (Marriott & Wenk, 2004).

The Role of Estrogen Receptors

Estrogen is a female reproductive hormone, traditionally associated with gender differentiation during the developmental period and increased synaptic plasticity during puberty and the ovarian cycle. More recently, estrogen has been implicated in playing a role in vital actions of the brain, including the release of neurotransmitters, electrical excitability, and synaptic function (Genazzani, Pluchino, Luisi, & Luisi, 2007). The positive role of estrogen in neuroaction has made it a focus of recent research on the prevention of neurodegenerative diseases. Estrogen depletion in the aging brain may be an early risk factor for Alzheimer's

disease, and a continued presence of estrogen in the brain may preserve specific areas in the brain targeted by disease pathology (Brann et al., 2007). Although estrogen-based hormone replacement therapy has been linked to reduced risk of Alzheimer's onset, the side effects have made the therapy controversial. The actions of the therapy are beneficial in the brain, but can have detrimental impacts on peripheral tissues, including an increased risk of breast and uterine cancer (Carroll & Pike, in press). Because of these harmful effects, research on estrogen therapies has focused on making treatments more specific to the areas of the brain associated with neurodegeneration.

One target-specific focus of research has involved the differentiation between two different estrogen receptors: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Both receptors have been linked to neuroprotection in the brain, especially in learning and memory-related areas (Genazzani et al., 2007). ERα and ERβ are both present in the hippocampus, and deposits of Alzheimer's-related proteins are regulated through each of these receptors (Brann et al., 2007). However, the different mechanisms of ERα and ERβ are not fully understood. Overall, the actions of estraidol, a clinical form of estrogen, are found to be predominantly dependent on ERα, indicating that it is the primary estrogen receptor (Carroll & Pike, in press).

Research has shown that ERβ, a more recently discovered receptor, might also play a primary role in estrogen regulation in the brain, especially in association with Alzheimer's pathology. ERβ is closely associated with learning and memory because it is the predominant type of estrogen receptor found in the hippocampus (Genazzani et al., 2007). Because of its increased presence in the hippocampus, hormone therapy that is ERβ specific might have especially beneficial effects in the brain (Wang, Irwin, & Brinton, 2006). Research by Forsell,

Enmark, Axelman, Blomberg, Wahlund, Gustafsson, et al. (2001) further linked ERβ and Alzheimer's by confirming that a mutation in the ERβ gene might increase susceptibility to Alzheimer's disease. Given this recent research on its presence in the hippocampus and association with Alzheimer's disease, the present study will focus on the expression of ERβ in the brain.

Maternal Experience

The benefits of the natural hormonal changes associated with the female reproductive experience are well established. Motherhood leads to improvements in learning and memory, both of which are functions of the hippocampus. Oxytocin, another hormone associated with reproduction, also enhances neural functioning and potentiation of neural messages (Pawluski, Walker, & Glaea, 2006). Pregnant female rats demonstrated better performance on tests of spatial memory than virgin rats, which can be attributed to the elevated and relatively stable levels of estrogen present in the body during pregnancy (Galea, Ormerod, Sampath, Kostaras, Wilkie, & Phelps, 2000). Galea et al. (2000) also established that pregnant rats spent more time in the target quadrant of the water maze and traveled shorter distances to the reward than nonpregnant animals, demonstrating the positive effects of pregnancy on learning and memory.

In addition to hormonal changes, maternal animals must respond to a large number of novel stimuli as a result of exposure to pups. Suddenly, new mothers find that they are responsible for the survival of their offspring (Love, Torrey, Glasper, DeVries, Lambert, & Kinsley, 2005). They must quickly acquire new skills, such as improving their hunting abilities, so that they can obtain food for themselves as well as their new pups. New mothers must learn to forage and recall the spatial location of food in an effort to spend less time away from their offspring (Love et al., 2005). The first pregnancy is therefore critical, as the female brain begins to change in response to new hormone levels and a range of sensory stimuli from the pups. The

maternal rat must make her own modifications and obtain new skills to ensure the survival of her offspring, without any previous experience or knowledge in this area (Pawluski et al., 2006). Subsequent pregnancies can increase the benefits of initial hormone and pup exposure. While primiparous (single reproduction) animals outperformed nulliparous (no reproduction) animals on tests of learning and memory, multiparous (more than one reproduction) animals often performed better than both groups (Gatewood, Morgan, Eaton, McNamara, Stevens, & Kinsley, 2005). Therefore, repeated exposure to reproductive hormones and offspring can lead to a further enhancement in neural functioning and memory.

The present study looks to extend the findings of Gatewood et al. (2005) that the paired exposure to both pups and the natural hormonal changes of the female reproductive experience preserves the aging brain from degeneration and provides neuroprotection against memory declines. Parous animals have lower levels of amyloid precursor protein in their brains, and multiparous animals have significantly less of the protein deposited in their brains than primiparous animals (Gatewood, 2005). These results suggest that the protection provided by maternal experience can extend specifically to defense against the risks of Alzheimer's disease. Given that the results of hormone replacement studies have supported the benefits of moderate dosages that mimic the actual female hormone cycle, it is possible that the natural exposure to female reproductive hormones provides an effective barrier against the onset of Alzheimer's, even at a relatively young age (Marriott & Wenk, 2004). It is also possible that moderate dosages of hormone replacement therapy and natural reproductive hormone levels have equally beneficial effects (Love et al., 2005).

A preliminary study in female rats did indicate higher concentrations of APP in nulliparous animals than primiparous animals, which were aligned with the findings of previous research (Gatewood et al., 2005). An immunofluorescent investigation of the expression of APP

in the female hippocampus showed that primiparous animals had a lower concentration of APP in the hippocampal region of their brains than age-matched nulliparous animals. While it is expected that maternal experience will offer some protection against the onset of neurodegeneration, the present study looks to specifically examine the expression of ERβ in the female rat brain. An understanding of how the deposits of ERβ and APP interact and are expressed together can give some further indication to the specific protective mechanisms of pregnancy hormones in the brain.

The current study will expand upon past findings investigating the early signs of neurodegeneration in younger rats. While past research has focused on the presence of Alzheimer's-related declines in aged animals, more research in younger animal models will indicate the effects of APP and memory decline early in life. Priller et al. (2006) found that in animals lacking APP, the enhancements of neuron and synapse functioning were more pronounced in young mice, aged three weeks, than older mice, aged 11 months. Studies involving the administration of degenerative protein have also supported their quick and early action. In animals injected with Aβ, collections of amyloid protein and neuron loss were seen around injection sites in as little as seven weeks (Stephan et al., 2001). Given the support of early administration of therapeutic remedies like hormone replacement therapy, it is important to identify the early progression of the disease to effectively stop the first signs of Alzheimer's pathology.

The present study will investigate the effects of maternal experience, its accompanying hormones, and its novel stimuli on the emergence of neuroanatomical changes related to Alzheimer's disease in young and old female rats. It is expected that our findings will replicate past research that deposits of APP increase with age, while the presence of estrogen receptors decrease with age (Priller et al., 2006; Brann et al., 2007). It is expected that these neural

differences will be seen predominantly in the hippocampus, a region of the brain essential for memory. Specifically, the current study will focus its investigation on the CA1 region of the hippocampus, an area of neural density and a high concentration of ERβ receptors (Carroll & Pike, in press).

Hypotheses

1. It is hypothesized that primiparous animals will have less APP than nulliparous animals, as measured by the area of APP expression.

2. It is hypothesized that younger animals will have less APP than older animals, as measured by the area of APP expression.

2a. It is hypothesized that old primiparous animals will have less APP than old nulliparous animals.

2b. It is hypothesized that old primiparous animals will have more APP than young primiparous and young nulliparous animals.

2c. It is hypothesized that old nulliparous animals will have more APP than young primiparous and young nulliparous animals.

2d. It is hypothesized that young primiparous animals will have less APP than young nulliparous animals.

3. It is hypothesized that primiparous animals will have more ERβ than nulliparous animals, as measured by the area of ERβ expression.

4. It is hypothesized that younger animals will have more ERβ than older animals, as measured by the area of ERβ expression.

4a. It is hypothesized that old primiparous animals will have more ERβ than old nulliparous animals.

4b. It is hypothesized that old primiparous animals will have less ERβ than young primiparous and young nulliparous animals.

4c. It is hypothesized that old nulliparous animals will have less ERβ than young primiparous and young nulliparous animals.

4c. It is hypothesized that young primiparous animals will have more ERβ than young nulliparous animals.

Method

Animals

The animals used in the present study were six female rats, and they were ordered from Zivic-Miller Laboratories, Inc. The animals were housed in the University of Richmond Animal Facility. Each animal was housed individually in a 25 cm × 40 cm × 25 cm cage with corncob bedding, and food and water was available ad libitum in the home cage. The animals differed only in their ages and reproductive experience. Two of the rats were 12 months of age at the time of perfusion, and four of the animals were four months of age at the time of perfusion. The older animals were perfused at the age of approximately 12 months because it is a time when potentially degenerative neuroanatomy is expected to be observed, yet the animals have not fully reached old age (Gatewood et al., 2005). The younger animals were perfused at the age of four months because it is an early time in the developmental period of the rat when early signs of neurodegeneration are expected to emerge (Priller et al., 2006). Of the older rats, one was a primiparous animal (having one pregnancy) and the other was a nulliparous animal (having no pregnancies). Two of the younger rats were primiparous, while the other two were nulliparous. The older parous animal was given a 21-day postpartum experience with her offspring before the pups were weaned, and the younger parous animals were given a 14-day postpartum experience. The parous and nulliparous animals were exposed to identical living conditions, and the home

cages of all of the animals were housed in the same room so as to be exposed to the same light and dark schedule.

Tissue Preparation

The animals were exposed to carbon dioxide gas and sacrificed by an overdose of sodium pentobarbital. The animals were then perfused with both PBS and 4% paraformaldehyde (PF), and their brains were surgically removed. The dissected brains were immersed in PF for approximately five hours, and then switched to a solution of 30% PBS sucrose for two days. The sucrose was switched after the first day.

The brains were sectioned for the area of interest, the anterior region of the hippocampus, with the aid of an atlas (Paxinos & Watson, 1998). That section of the brain was then sliced by a cryostat set at -18 °C into sections that were 40 μ in thickness. Approximately 48 slices were cut for each animal, and placed in groups of four into 12-well culture dishes filled with TBS. After several initial TBS washes, the sections were immersed in a primary antibody cocktail overnight at 4 °C. The primary antibody cocktail was composed of a rabbit anti-amyloid precursor protein polyclonal antibody (Invitrogen 51-2700) and a goat anti-ERβ polyclonal antibody (Santa Cruz Biotechnology sc-6821). The anti-APP primary antibody was used at a dilution of 1:500 (2 μg/ml), and the anti-ERβ primary antibody was used at a dilution of 1:200 (5 μg/ml). As a negative control measure to check for nonspecific staining, two wells per tray were not exposed to the overnight primary antibody incubation, although all of the other steps of the protocol were followed for these tissue sections. The second day, after more TBS washes, the sections were immersed in the secondary antibody cocktail for two hours at 4 °C. The secondary cocktail was compiled of a goat anti-rabbit Alexa Fluor 488 secondary antibody (Invitrogen A11070) and a rabbit anti-goat Alexa Fluor 594 secondary antibody (Invitrogen A21223). Both secondary antibodies were used at a dilution of 1:400 (2.5 μg/ml). During the secondary antibody wash, the

culture trays were covered in aluminum foil to prevent exposure of the sensitive fluorescent antibodies to light. (see Appendix A for immunofluorescent protocol).

After the completion of staining, the tissue sections were mounted onto gelatin subbed slides and allowed to dry overnight. The slides were covered in foil to minimize light exposure. Once they were dry, the sections were coverslipped with Prolong Gold antifade reagent with DAPI (Invitrogen P36935). Prolong Gold preserves the tissue and fluorescent signal, while the DAPI is used as a marker of cell bodies because it binds to nuclear chromosomes and emits a blue fluorescent stain of DNA (Invitrogen, 2008).

Tissue Analysis

The tissue sections were analyzed with a Zeiss Imager.Z1 microscope. The program AxioVision (release 4.6.1) was also used to analyze images and quantify protein expression in the tissue sections. The CA1 of the hippocampus was initially identified at 5× magnification, and an image of the CA1 for each tissue section was captured at 40× magnification. Each image captured a 220.4 μm × 170 μm portion of the CA1. A merged image of multiple fluorescent channels was captured to show the combined presence of the green APP fluorescence, the red ERβ fluorescence, and the blue DAPI fluorescence (see Figures B1-F1).

As part of the AxioVision software, Program Wizard was used to quantify the area of APP and ERβ fluorescent expression in each tissue section. First, the merged images were separated, and the green APP stain and red ERβ stain were quantified separately. The program selected areas of cell bodies expressing an optimal amount of fluorescent stain and calculated the total summed area of the amount of stain per image. The areas of expression were calculated in μm^2, and the means of APP and ERβ expression were calculated for each animal.

Results

Amyloid Precursor Protein Analysis

Primiparous animals had less APP expression (M = 2069.76 μm^2, SD = 1623.86) than nulliparous animals (M = 4236.30 μm^2, SD = 1229.70). An one-way ANOVA was run and did not indicate a significant difference, $F(1,4) = 3.39$, $p = 0.14$ (see Table H1).

Young animals had less APP expression (M = 2372.65 μm^2, SD = 1510.64) than older animals (M = 4713.79 μm^2, SD = 1088.58). An one-way ANOVA was run and did not indicate a significant difference $F(1,4) = 3.64$, $p = 0.13$ (see Table I1).

To further determine differences in APP expression, a 2 × 2 between-subjects ANOVA was run. The old primiparous animal had more APP expression (M = 3944.04 μm^2, SD = 0.00) than the young primiparous animals (M = 1132.62 μm^2, SD = 66.77). The old nulliparous animal also had more APP expression (M = 5483.53 μm^2, SD = 0.00) than the young nulliparous animal (M = 3612.68 μm^2, SD = 831.22). A significant main effect of age was found, $F(1,2) = 21.02$, $p < 0.05$, and a marginally significant main effect of maternal experience was found, $F(1, 2) = 15.49$, $p = 0.06$. There was not a significant interaction of age and maternal experience, $F(1,2) = 0.85$, $p = 0.45$ (see Table J1).

Estrogen Receptor Beta Analysis

Primiparous animals had more ERβ expression (M = 1200.47 μm^2, SD = 656.96) than nulliparous animals (M = 331.80 μm^2, SD = 215.19). An one-way ANOVA was run and did indicate a significant difference, $F(1,4) = 10.10$, $p < 0.05$ (see Table K1).

Young animals had less ERβ expression (M = 719.90 μm^2, SD = 584.06) than older animals (M = 1458.61 μm^2, SD = 1272.43). An one-way ANOVA was run and did not indicate a significant difference $F(1,4) = 1.10$, $p = 0.35$ (see Table L1).

To further determine differences in ERβ expression, a 2 × 2 between-subjects ANOVA was run. The old primiparous animal had more ERβ expression (M = 2358.35 μm^2, SD = 0.00) than the young primiparous animals (M = 1221.53 μm^2, SD = 40.04). The old nulliparous animal also had more ERβ expression (M = 558.86 μm^2, SD = 0.00) than the young nulliparous animal (M = 218.28 μm^2, SD = 123.61). A significant main effect of age was found, $F(1,2) = 86.19$, $p < 0.05$, and a significant main effect of maternal experience was found, $F(1, 2) = 310.19$, $p < 0.05$. There was also a significant interaction of age and maternal experience, $F(1,2) = 25.04$, $p < 0.05$ (see Table M1).

Discussion

The present study supported the hypothesis that primiparous animals would have significantly higher levels of ERβ expression than nulliparous animals. The hypothesis that primiparous animals would have less APP than nulliparous animals was not supported. Also, the hypotheses that younger animals would have less APP than older animals and that younger animals would have more ERβ than older animals were not supported. However, there was a main effect of age on APP expression and main effects of age and maternal experience on ERβ expression. Finally, an interaction of age and maternal experience on ERβ expression was also established. These trends in the data might have interesting implications for the study of Alzheimer's disease and potential therapeutic targets.

One of the most interesting trends in the data was that primiparous animals did have lower mean levels of APP expression in the CA1 of the hippocampus than nulliparous animals. These findings indicate that maternal experience might offer some protection against the depositing of APP in the brain. Since the present study investigated only naturally-occurring, baseline levels of APP and ERβ, these results support past findings that moderate dosages of

hormone replacement therapy that mimic the body's natural levels might have the best neuroprotective effects (Marriott & Wenk, 2004). However, it is important to note that there was not an overall significant difference in APP expression between parous and nulliparous animals. While pregnancy hormones might offer some neurprotection, the results of the present study indicate that there are other factors influencing the pathogenesis of Alzheimer's. Hormone replacement therapy might not be the best treatment option in all cases. Potential detrimental effects of hormone replacement therapy have been established and were laid forth in the Women's Health Initiative Study, including an increased risk of dementia and cancer in peripheral tissues (Baum, 2005).

The present study did find a significant difference in the expression of ERβ. Primiparous animals had more ERβ than nulliparous animals. The combined presence of ERβ and APP in the tissue sections examined indicates that ERβ might have a role in offering neuroprotection. However, more research needs to be completed on the specific mechanisms of ERβ, especially in contrast to what is known about the mechanisms of ERα. It is possible that one type of receptor offers a higher degree of neuroprotection than the other type. Therapies that specifically target the most beneficial receptor type could be particularly effective in refining the actions of hormone replacement therapy and combating the onset of Alzheimer's disease (Carroll & Pike, in press).

Another interesting trend in the present study was the age differences found in measured area of APP expression. Higher mean areas of APP were found in older animals. There was a significant main effect of age and APP expression, but the overall age difference was not found to be statistically significant, indicating that there was also a presence of APP in the hippocampal regions of the younger animals. This finding supports past research that the onset of Alzheimer's

disease symptoms can begin relatively early in the lifespan, and that the effects of the changes in neuroanatomy can begin to take their toll at a young age (Stephan et al., 2001).

The trend seen in age differences in ERβ expression was also found, although this trend opposed the proposed hypothesis. Overall, younger animals had lower means of ERβ expression than older animals, and a significant main effect of maternal experience and ERβ expression was established. These results do not support past findings of a decline in estrogen receptor expression with age (Brann et al., 2007). In contrast, the present results suggest that ERβ levels might remain relatively stable or even increase across the lifespan. These findings have implications in considering dosage levels for hormone replacement administration, especially in older women. In order to maintain an effective moderate dose, the changes in baseline hormone levels that come with age need to be further understood.

It is important to note that the trend of more expression of ERβ may be attributed to the fact that the older and younger primiparous animals had differing lengths of postpartum periods with their offspring. Post-pregnancy events, including sensory exposure to pups and lactation, have been found to contribute to the increased neural plasticity accompanying motherhood, above and beyond the effects of the pregnancy hormones alone (Love et al., 2005). Since the older animals had a longer postpartum period with their pups, it is possible that the additional week of offspring sensory exposure led to further neuroanatomical changes and allowed them to maintain higher levels of ERβ expression.

A primary limitation of the present study is the small sample size. Only six animals were investigated, and the older age group only consisted of two animals. It would be important to replicate the current findings with a larger sample of animals. Another limitation in the present study was the limited area of the brain examined. While the CA1 of the hippocampus has been

found to be essential for memory related-processes, in addition to being a main target of Alzheimer's pathogenesis, it is possible that other areas of the brain play an equally important role in the progression of the disease. It would be interesting to study other areas of the brain linked to learning and memory, such as the amygdala, or even other regions within the hippocampus. For example, the dentate gyrus has also been found to be a site of high neural density and protein expression that might also be a target of Alzheimer's pathogenesis (Gatewood et al., 2005).

Future research could expand upon the present study by including a behavioral task, in addition to brain analysis. Studies have indicated that overall, parous animals outperform nulliparous animals on learning and memory-related tasks (Love et al., 2005; Galea et al., 2000; Pawluski et al., 2006). Additionally, animal models injected with APP have demonstrated decreased performances in working-memory tasks (Stephan et al., 2001). It would be interesting to include a behavioral component to investigate the combined effects of maternal experience as well as Alzheimer's-related protein deposits in the brain on behavioral performance. This research would result in a further connection between behavioral performance and underlying neuroanatomical structure.

The findings of the present study have implications for the further study of Alzheimer's disease pathology and potential sites of therapeutic action. The present findings indicate a complex and dynamic interaction between deposits of APP and the presence of ERβ. This relationship that is seen in even natural, baseline levels indicates the further complications that can be brought on by additional hormone administration. Future research needs to be continued to find sites of action and optimal doses for exposure to exogenous estrogen. An effective administration of hormones would result in neuroprotection against the onset of degenerative

proteins, without making the body susceptible to potentially the potentially detrimental effects of too much hormone exposure.

Finally, the present study has implications for the elucidation of the complex aspects of Alzheimer's pathology. Specifically, the results indicate that the disease might have a unique and specific progression in the female brain because of the differential effects of sex hormones on neuroanatomy. Since Alzheimer's disease is more prevalent in women, it is important to further understand the particular ways in which the pathogenesis of the disease attacks the female brain. Further understanding of the actions of Alzheimer's in the brain and the protective impact of female hormones might introduce novel sites of therapeutic action that could moderate the devastation of this neurodegenerative disease.

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Appendix A

Immunofluorescent Staining Protocol

TBS (1L)
900 ml dH_20
6.05 g Tris base
9 g NaCl

TBS ++ (500 ml)
15 ml 3% normal goat serum
1.25 ml 10% Triton
500 ml TBS

Staining: Day One
5 min TBS
5 min TBS
5 min TBS

60 min TBS ++

Overnight in 1º Ab diluted in TBS++ (keep at 4 ºC)

Staining: Day Two
15 min TBS
15 min TBS

15 min TBS++

****The rest of the steps should be done with absolute minimal exposure to light*

120 min 2º Ab diluted in TBS ++ (keep at 4 ºC)

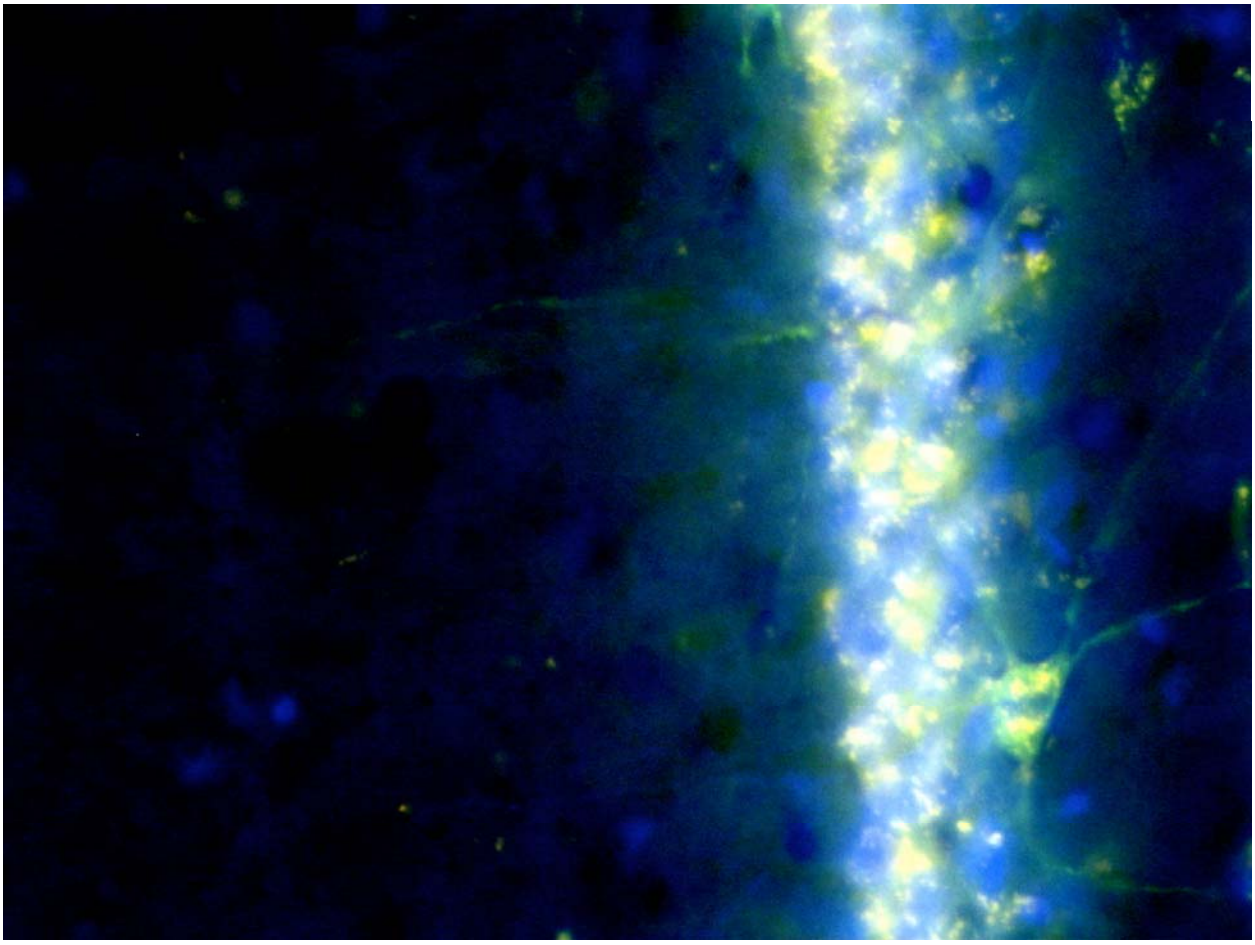
5 min TBS
5 min TBS

Tissue Preparation: Day Three
Allow mounted slides to dry overnight, then coverslip with Prolong Gold with DAPI

****Allow coverslipped slides to dry for three-five days before placing in a slide box*

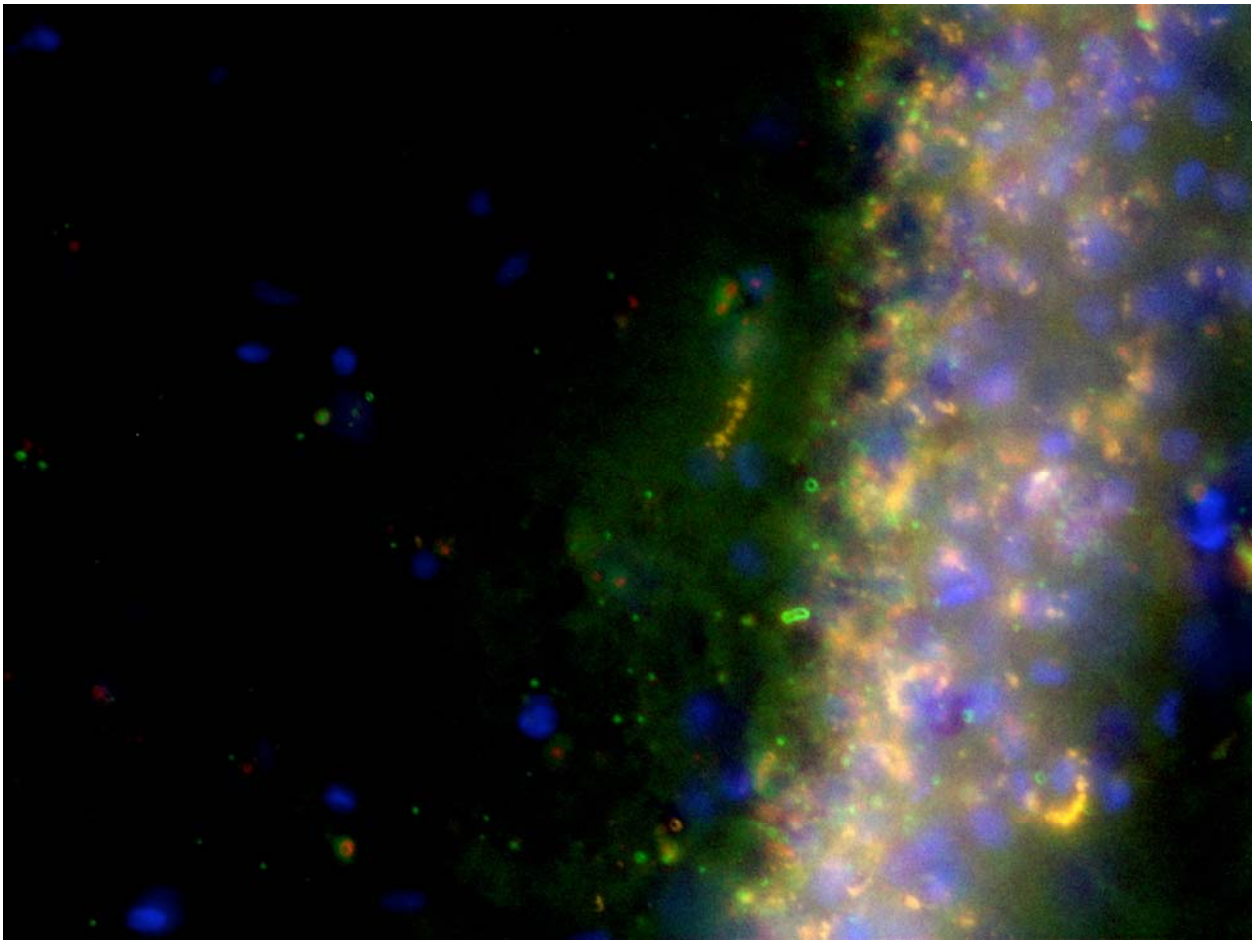
Appendix B

Figure B1



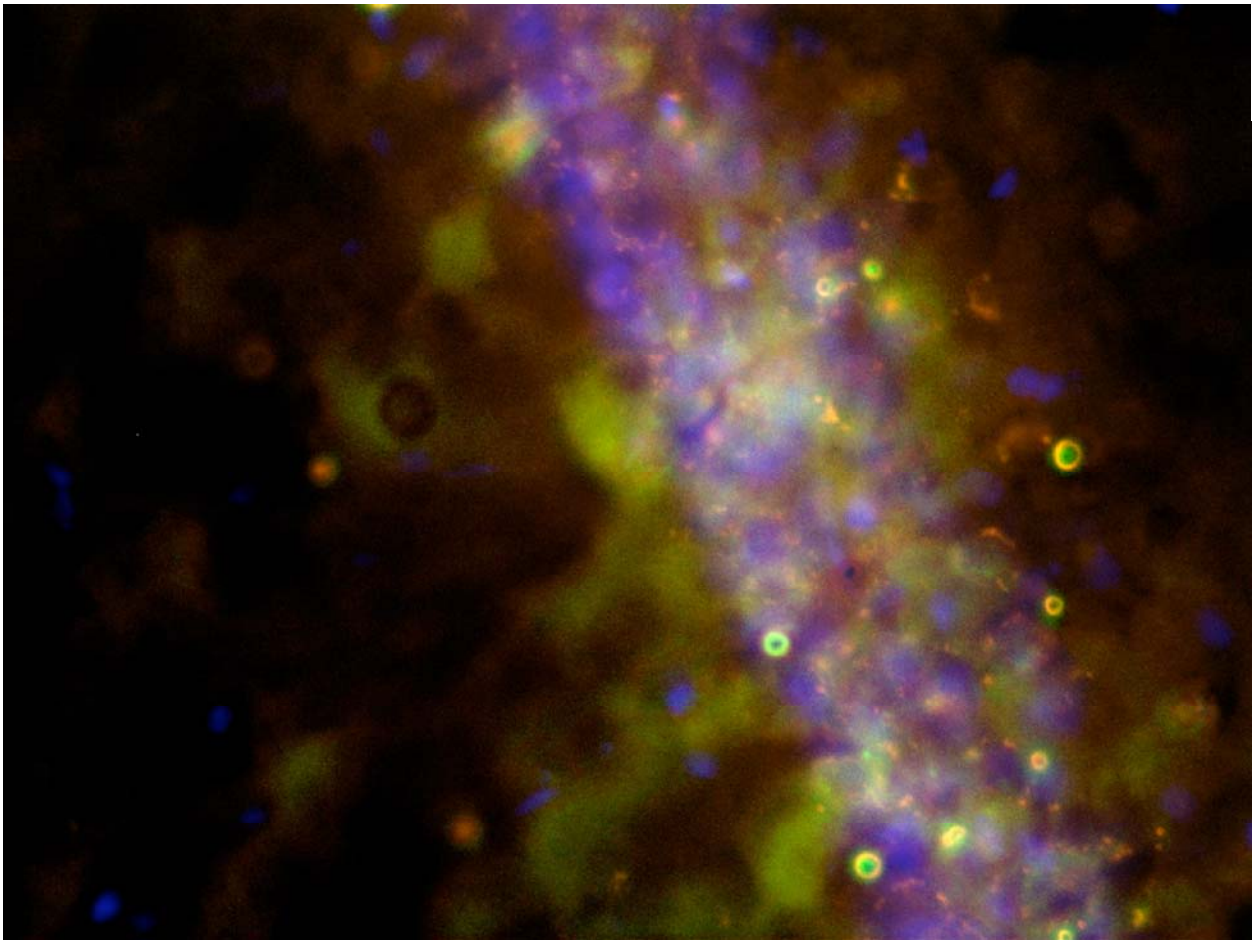
Appendix C

Figure C1



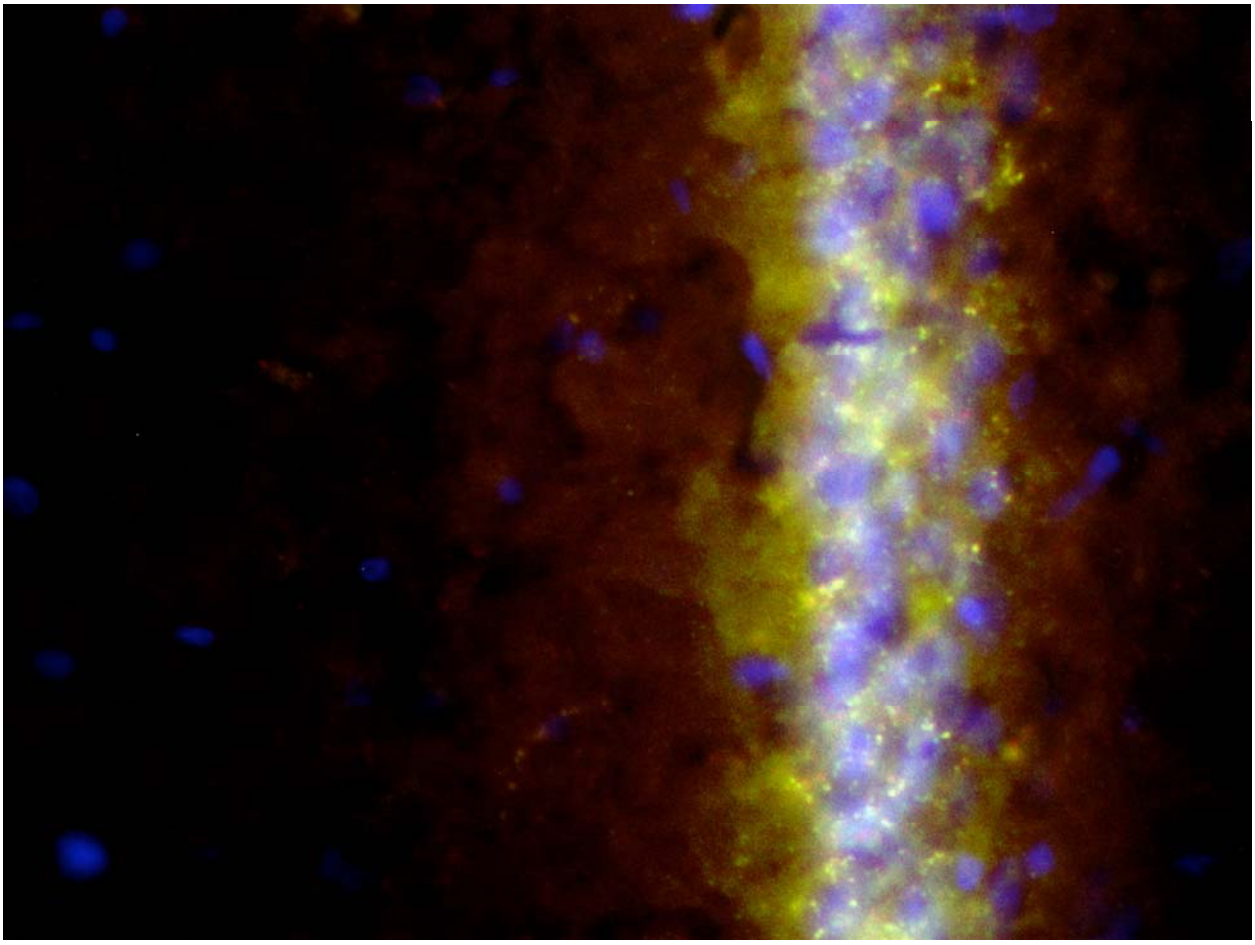
Appendix D

Figure D1



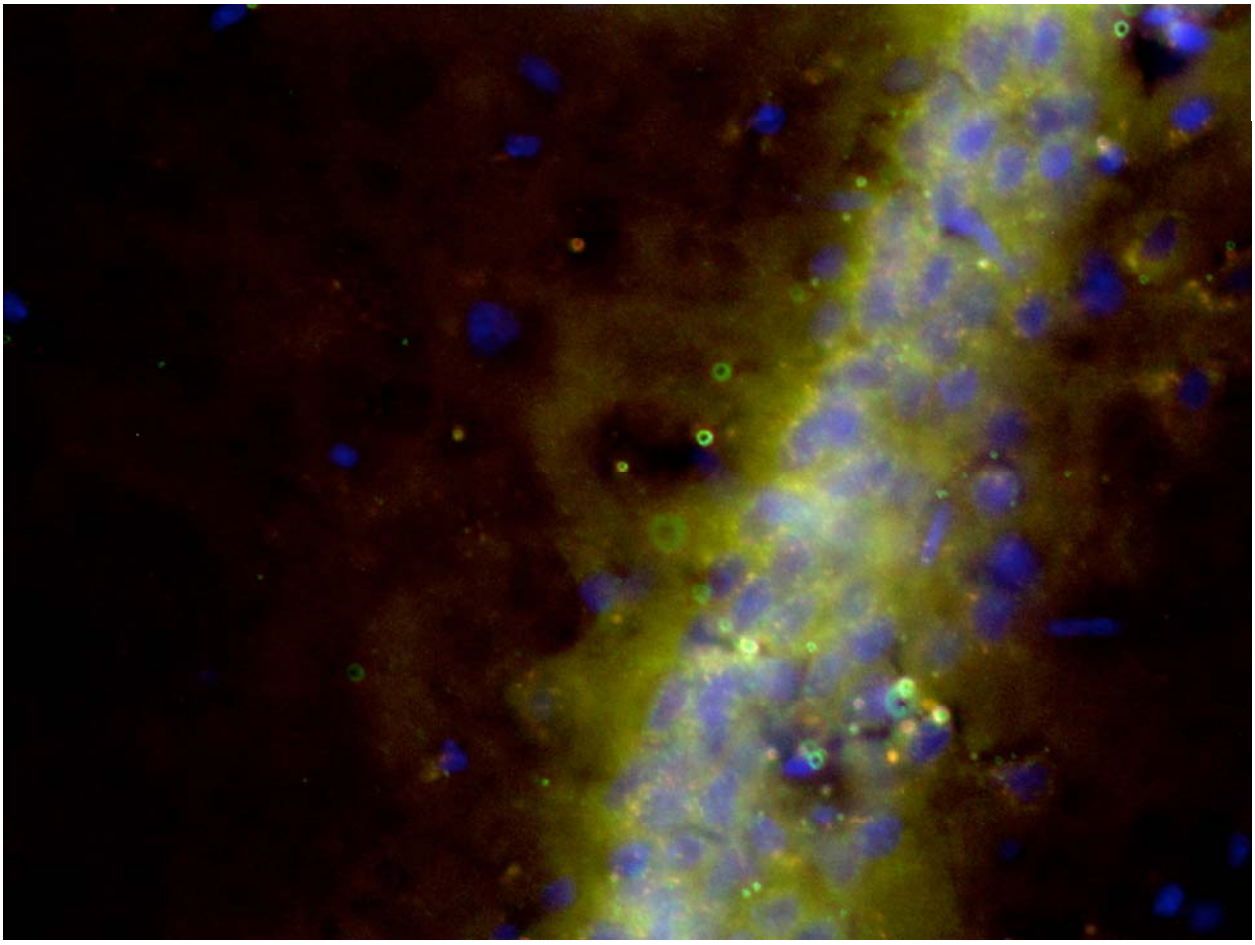
Appendix E

Figure E1



Appendix F

Figure F1



Appendix G

Figure Captions

Figure B1. Preliminary immunofluorescent stain for amyloid precursor protein (green) and DAPI (blue) in CA1 of an old primiparous animal, 40× magnification.

Figure C1. Immunofluorescent stain for amyloid precursor protein (green), estrogen receptor-beta (red), and DAPI (blue) in CA1 of an old primiparous animal, 40× magnification.

Figure D1. Immunofluorescent stain for amyloid precursor protein (green), estrogen receptor-beta (red), and DAPI (blue) in CA1 of an old nulliparous animal, 40× magnification.

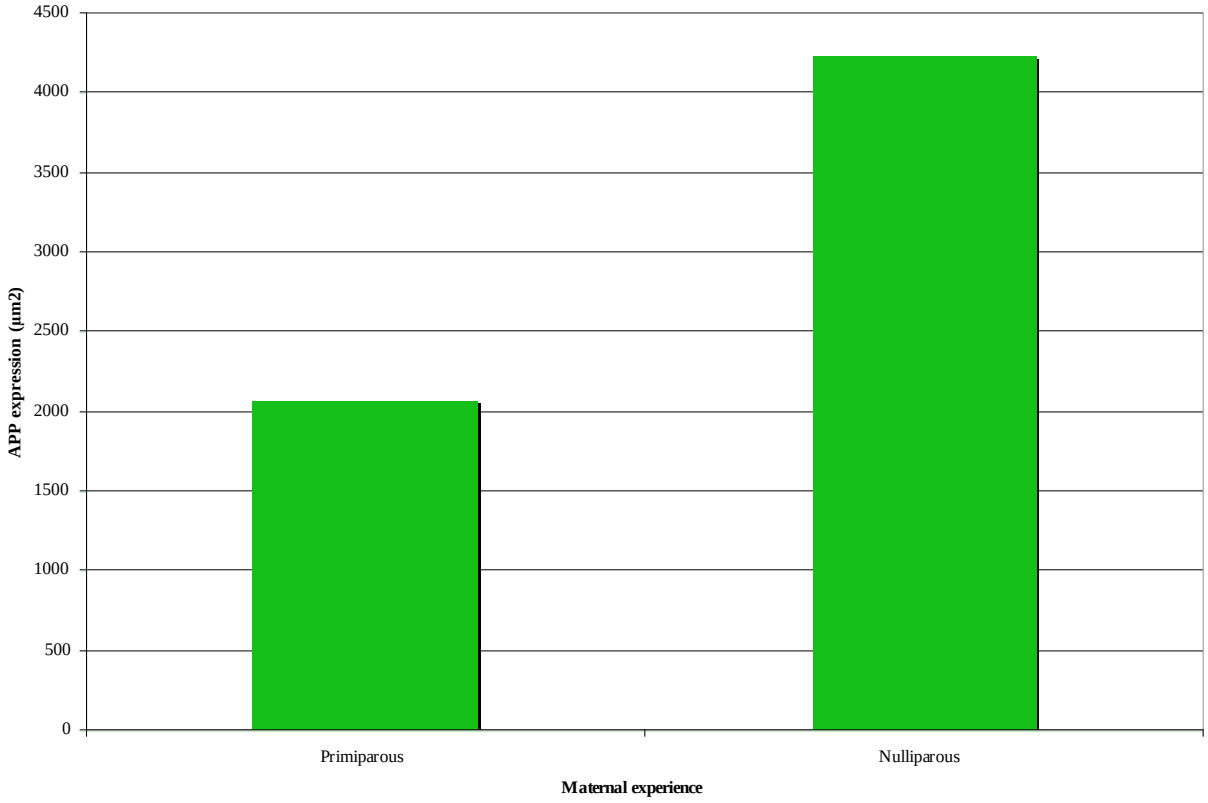
Figure E1. Immunofluorescent stain for amyloid precursor protein (green), estrogen receptor-beta (red), and DAPI (blue) in CA1 of a young primiparous animal, 40× magnification.

Figure F1. Immunofluorescent stain for amyloid precursor protein (green), estrogen receptor-beta (red), and DAPI (blue) in CA1 of a young nulliparous animal, 40× magnification.

Appendix H

Table H1

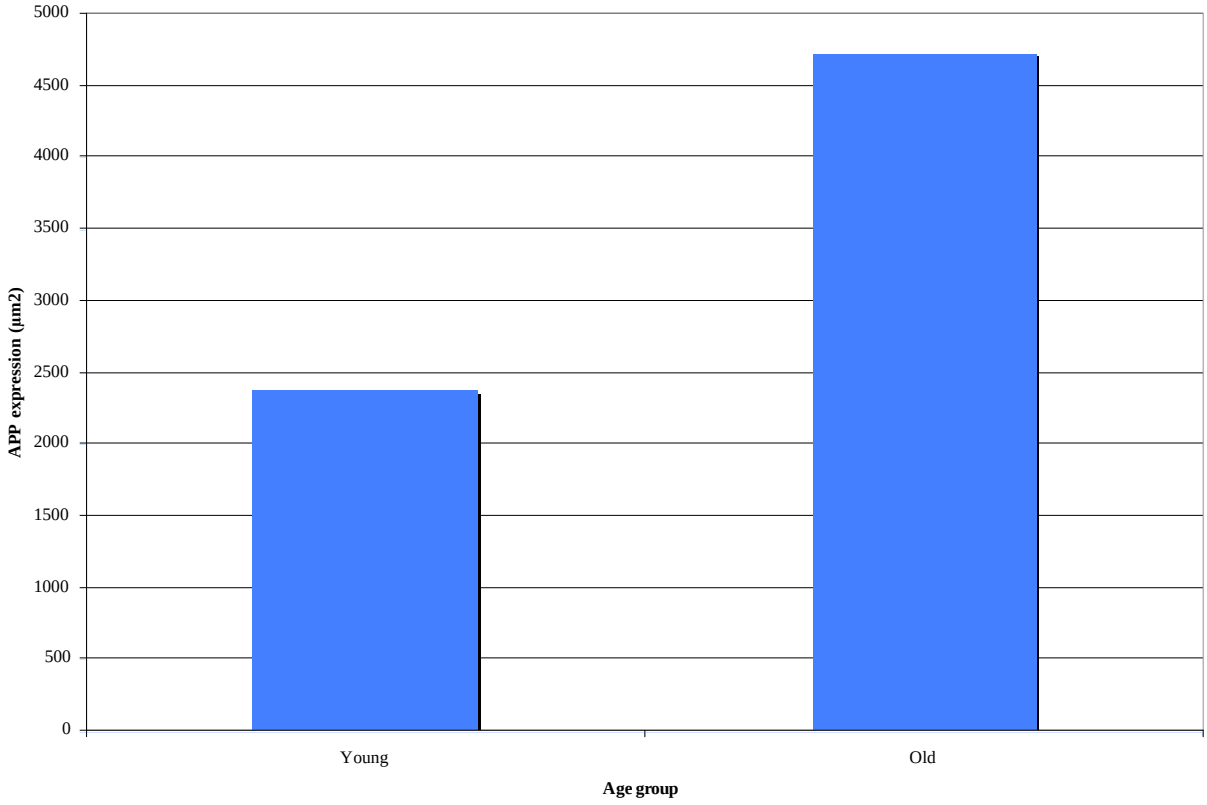
Mean Levels of Amyloid Precursor Protein Expression in Animals with Different Maternal Experience



Appendix I

Table I1

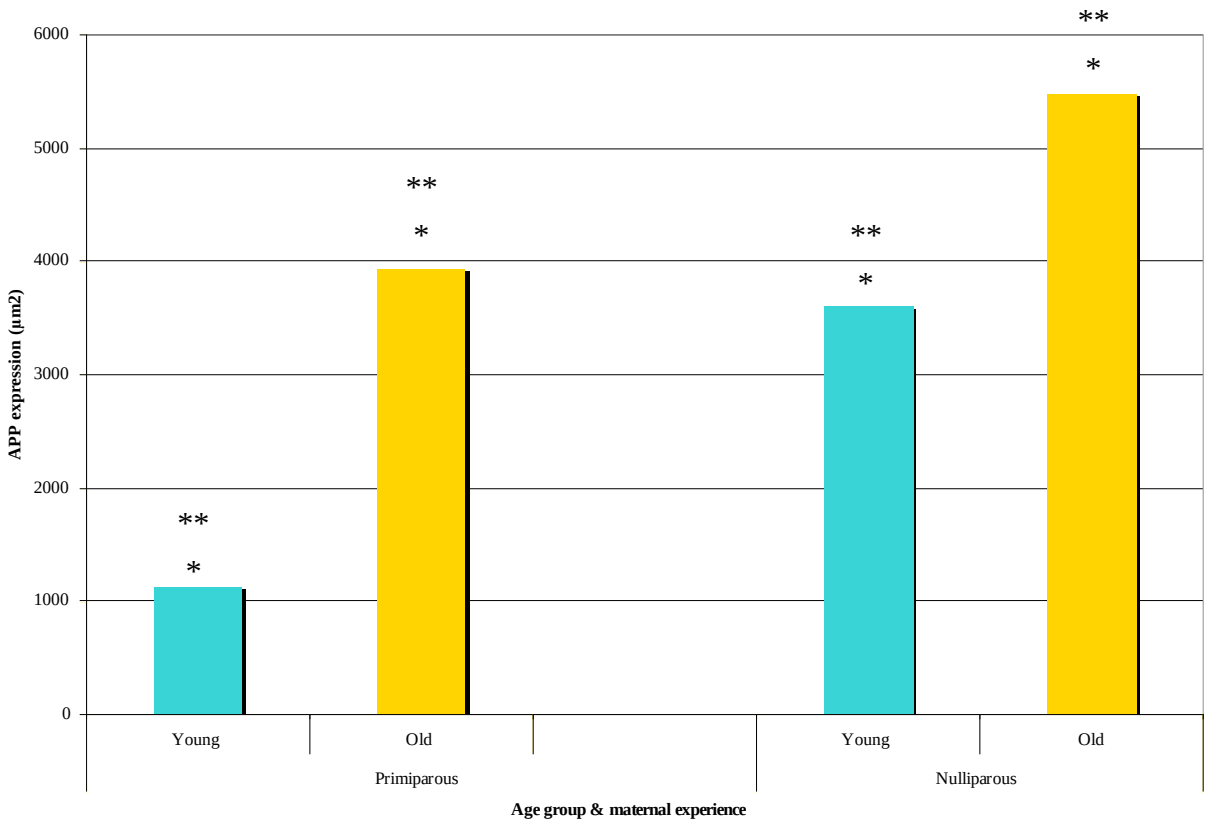
Mean Levels of Amyloid Precursor Protein Expression in Animals from Different Age Groups



Appendix J

Table J1

Mean Levels of Amyloid Precursor Protein Expression in Animals from Different Age Groups with Different Maternal Experience

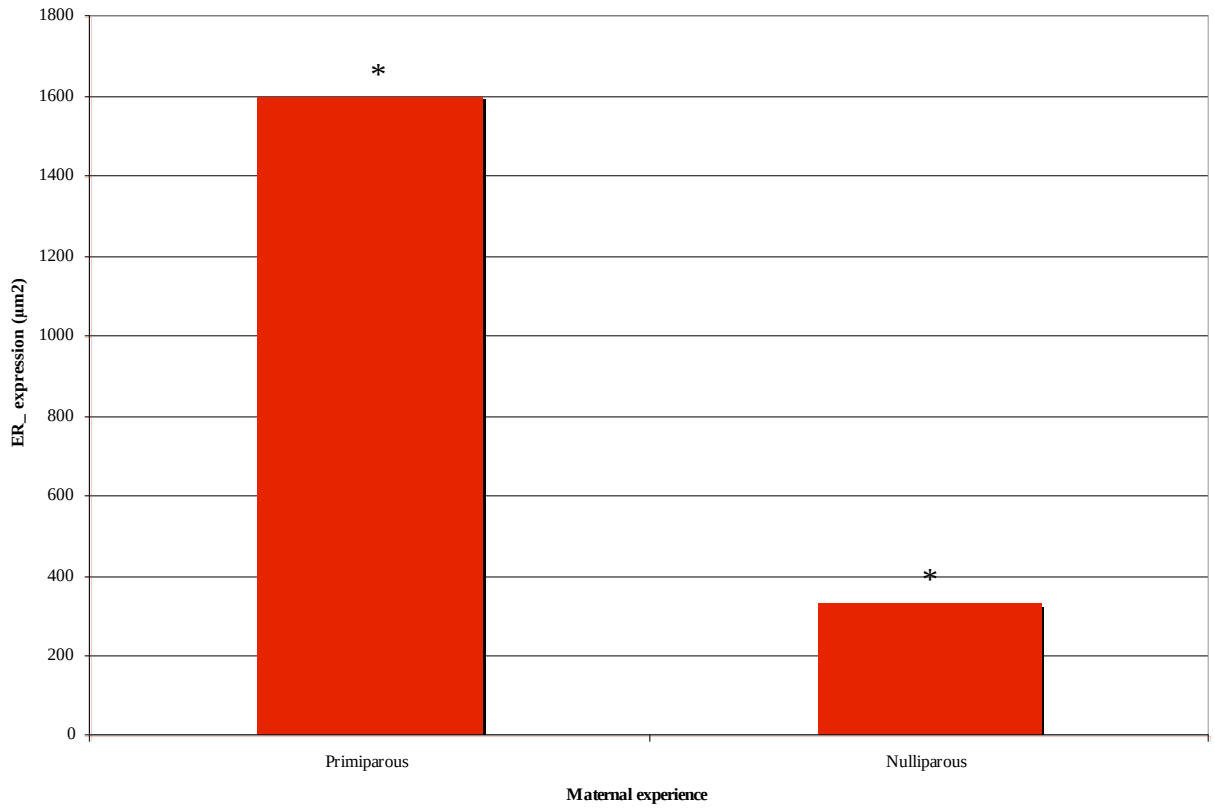


* indicates a significant main effect of age at the 0.05 level
** indicates a marginally significant effect of maternal experience

Appendix K

Table K1

Mean Levels of Estrogen Receptor Beta Expression in Animals with Different Maternal Experience

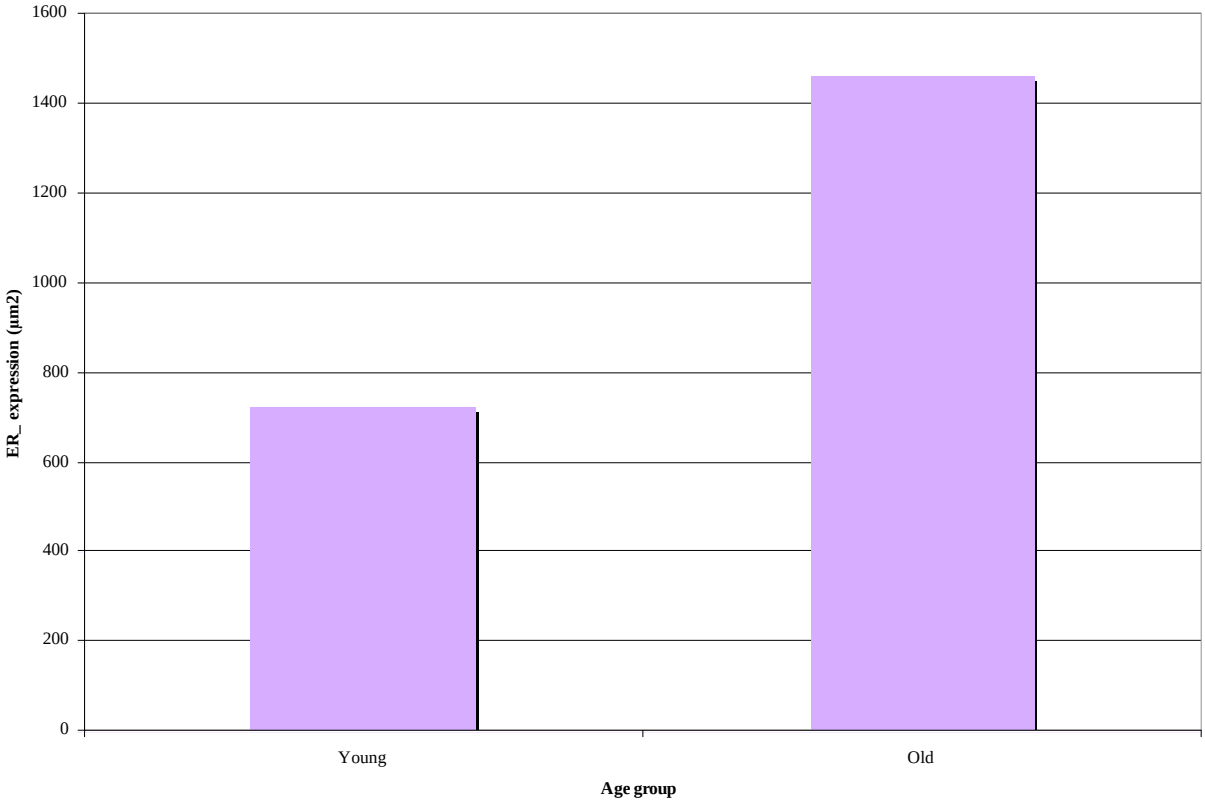


*indicates a significant difference at the 0.05 level

Appendix L

Table L1

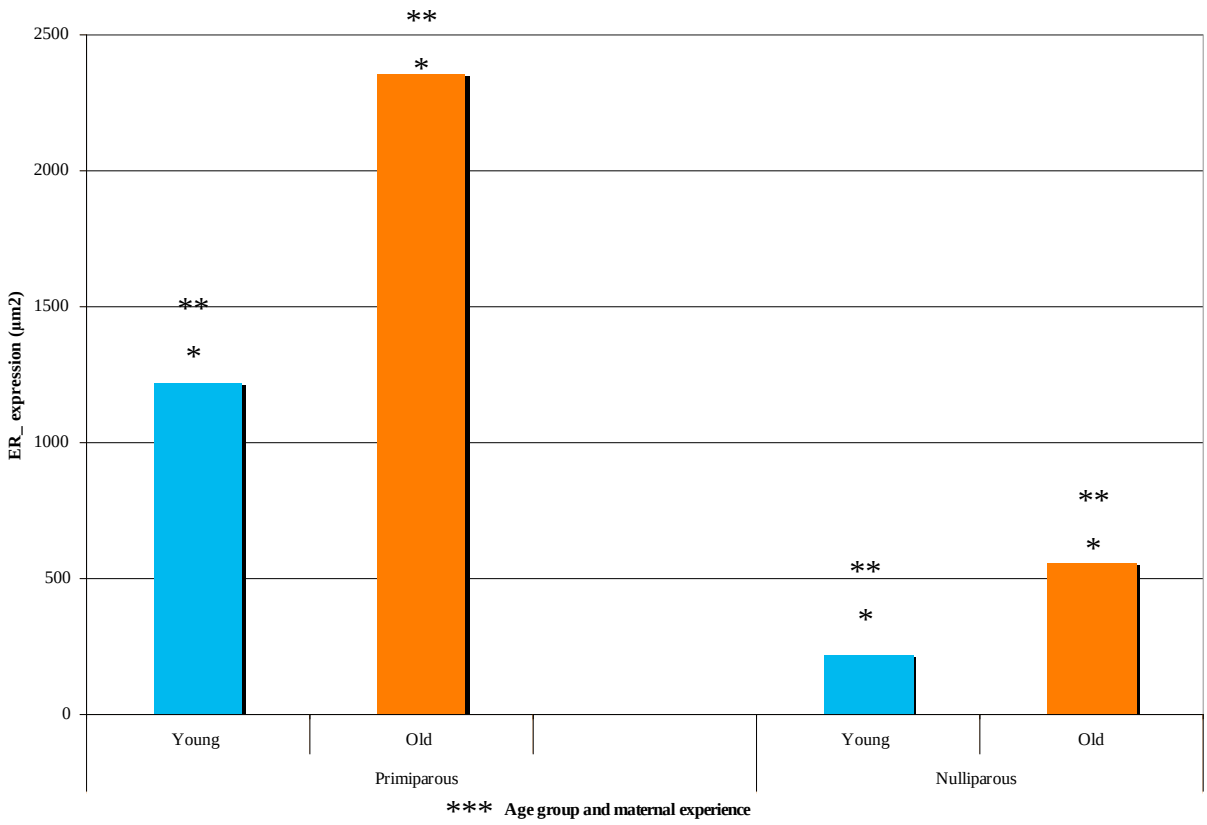
Mean Levels of Estrogen Receptor Beta Expression in Animals from Different Age Groups



Appendix M

Table M1

Mean Levels of Estrogen Receptor Beta Expression in Animals from Different Age Groups with Different Maternal Experience



* indicates a significant main effect of age at the 0.05 level
** indicates a significant effect of maternal experience at the 0.05
***indicates a significant interaction of age and maternal experience at the 0.05 level