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Reproductive experience and aging : possible neuroprotective effects of motherhood

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Abstract

Hormonal fluctuations associated with pregnancy and post partum periods create significant changes in the brain and behavior in female rats. Animals were tested in a land version of the Morris Water maze for three days at 6, 12, 18 and 24 months. At the ages of 12, 18, and 24 months animals were also tested in the same maze using a reversal task. At the conclusion of the study brains were analyzed for Amyloid Precursor Protein (APP) to determine the amount of neurodegeneration among the groups. Multiparous animals showed significantly superior performance , followed by primiparous animals, and nulliparous animals across all ages of testing . Brain analysis indicated multiparous animals had significantly fewer APP positive cells than virgin and primiparous animals. These findings suggest that reproductive experience is beneficial to females, providing possible advantages that follow the mother well past her reproductive prime, through the remainder of her life.

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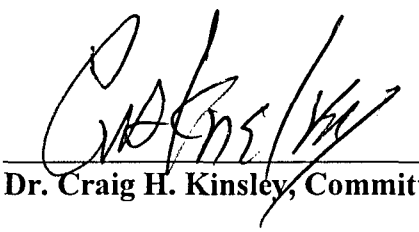

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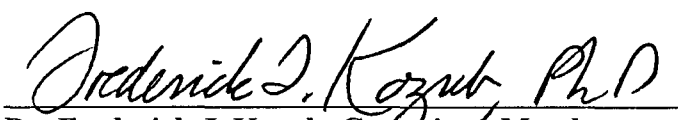

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REPRODUCTIVE EXPERIENCE AND AGING: POSSIBLE NEUROPROTECTIVE EFFECTS OF MOTHERHOOD

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Reproductive Experience and Aging: Possible Neuroprotective Effects of Motherhood

The brain is a malleable organ, easily influenced by the different experiences an organism encounters throughout its existence. The resulting neural changes affect behavior, in most situations lasting for life. Whereas much of the interests of science and research have focused on the reproductive act, very little focus is placed on the reproductive experience and the maternal state. Maternal behavior is the most crucial and natural behavior to study, and may lead to many insights into mammalian and human behavior. One could posit that pregnancy, birth, and the postpartum period are not one time experiences; rather the effects of the experience remain throughout life, and produce beneficial changes in the mother. It then seems obvious that long term effects of motherhood may extend into old age, carrying with it both behavioral and physiological effects. Much like pregnancy, aging is most evident in marked changes in body and brain physiology and function. Clear biochemical and structural changes characterize physiological aging of the brain. This process is an imbalance of sorts that is seen most apparently in neuromodulators and neurotransmitters (Ferari , Flavia,Dino,Guiseppe & Solerte 1995).Current aging and neurodegenerative disorder research focus primarily on two areas related to maternal experience and the female reproductive cycle: the potential neuroprotective effects of estrogen and the positive influence of mental and physical enrichment in prevention and reduction in the amount of neural degeneration and time of onset. Both of these phenomena are characteristic of pregnancy and its aftermath.

During pregnancy, the female brain undergoes a significant amount of morphological change, with greater complexity seen in neurons and glial cells, which provide a sort of scaffolding to the neurons. Further, there appear to be effects upon the brain's production of new neurons, neurogenesis (Amory, 2000;Young et al., 1999)., Our research on neurogenesis has indicated that while during pregnancy neurogenesis decreases in the female rat, the levels of neurogenesis increase greatly following contact and stimulation of the pups. These findings suggest that the pups act as an enriched environment for the mother, providing physical and cognitive stimulation associated with her day to day physiological functioning and survival. Young and colleagues have demonstrated that enriched environments in both mice and rats not only enhance neurogenesis, but also promote long term survival of neurons (Young, Lawlor & Leone, 1999). Research conducted by Amory (2000) and Madonia (2001) has demonstrated that reproductive experience modifies neurogenesis, and that the offspring play a major role in the stimulation and possible maintenance of neurogenesis in their mothers.

A number of our behavioral studies have shown that reproductive experience influences learning and memory. In particular, our findings indicate mothers can learn more quickly and retain information in memory longer than those without reproductive experience (Kinsley et al., 1999). With that in mind, the current research explored a very intriguing set of questions that are of major concern in aging. First, does past reproductive experience produce neuroprotective effects during aging? That is, does the experience of motherhood prevent and or reduce the neural degeneration and cell death that accompanies aging? If this is the case, then what neural changes occurring during the reproductive experience produce these neuroprotective effects? The goal of this research

is to begin to elucidate the long term benefits of reproductive experience through a careful study of neural degeneration, and the associated behavioral consequences, such as memory impairment.

Background and Significance

Maternal Behavior: Pregnancy, Lactation and the postpartum period

The mammalian reproductive cycle consists of three distinct phases: pregnancy, parturition and lactation, These distinct phases throughout the life cycle make it essential that a continuum of adaptive changes occur in order that the maternal physiology satisfies both the needs of the mother and the changing needs of the fetus or neonate (Russell, Douglas & Ingram, 2001). Numerous changes in the brain are necessary for the expression of neuroendocrine functions and behaviors that are most conducive to the health and maintenance of the offspring. The reproductive experience is undoubtedly one of the most dramatic changes a female will ever experience, both physiologically and behaviorally. These physiological changes begin at the establishment and associated maintenance of the pregnancy. Soon afterwards hormonal signals from the embryo enter the mother's circulatory system, this starts a series of endocrine changes in the mother in order to maintain and prepare her, mentally and physically for the pregnancy.

During the first pregnancy two dramatic changes occur in the endocrine experience. First there is a dramatic increase in levels of progesterone in her body that far exceeds any concentration in her blood stream, and thus her brain and tissues, previously experienced. There is also a large increase in concentration of chorionic gonadotrophin , which is new to a former nulliparous body .During this period remarkable physiological

changes occur as a direct consequence of the actions of the pregnancy hormones. The first to occur is the suppression of fertility during the pregnancy and following into the postpartum lactation period. Next, marked changes in metabolism occur, with an increase in hunger and body weight. There is also a massive increase in body fluids, with a 40% increase in blood volume during pregnancy in order to provide for the hemo-dynamic needs of the developing fetus. (Russell et al., 2001). Changes in the hypothalamic-pituitary- adrenal (HPA) axis also occur, which affect everything from metabolism to gene transcription, and most notably, stress and fear responses. A striking reduction in neural excitation has been found in limbic brain regions that process stressors and regulate responses on neurons in pregnancy and lactation. Da Costa, Wood Ingram and Lightman (1996) indicated there is a reduction in HPA response (release of corticosteroids etc) to stress during pregnancy and lactation. It has also been noted that this decrease manifests itself in behavior as well, as pregnant and lactating mothers exhibit a decrease in anxiety related behaviors in stressful/ novel environments. This continues throughout the life of the mother (Wartella, 2000). There are a number of possible functions for reduced stress in a mother, with a large factor being protection of the fetus and offspring from corticosteroids. The mother has lower levels of these stress hormones and consequently has a more successful pregnancy and parturition. This also is beneficial for the development of mentally and physically healthy offspring, as prenatal stress seems to affect offspring throughout life, with decreased cognitive and immune function (Vallee, Maccari, Dellu, Simon, & Mayo, 1999). The reduced stress hormone levels also prove advantageous to a female providing the ability to successfully function during dangerous and frightening (novel) situations in order to provide food, shelter and

protection from predators for both the offspring and herself. This ability could prove long lasting for the mother. The seat of maternal behaviors lies in specific areas of the brain, such as the bed nucleus of the stria terminalis, the basal forebrain and the hypothalamus. The MPOA (medial preoptic area of the hypothalamus) plays a major role in the expression of maternal behavior.

Through pregnancy and the secretion of sex hormones the adaptive changes in the mother's brain occur rapidly, and thus lead to the development of maternal behavior. In the rat, changes occurring before parturition prepare the mother for the offspring, the most obvious being nest building, in which the female digs and clears out a protected area for holding and protecting the pups. This nest provides protection and warmth to the pups. This behavior appears immediately at the time of parturition. After giving birth the new mother rat will immediately begin her maternal duties with stereotypical patterns of behavior. These include retrieving, grouping, and crouching (Numan, 1994). Retrieving and grouping of the pups are the means by which the mother groups the litter together in the nest site and carries displaced pups back to the nest. Nursing is the behavior characterized by the mother arching herself over the nested litter for suckling. Crouching occurs when the mother covers her pups with a tent- like posture over the nest in order to provide them with protection and warmth. Finally licking and grooming the pups provides the stimulation of the anogenital region, thereby allowing the neonate the ability to deficate and urinate (Numan, 1994).

The adaptive changes of pregnancy are a consequence of signals occurring after conception, in large part to hormonal influence, while postpartum the signals are a result of the interactions between mother and offspring and the associated neuroendocrine

mechanisms (Russell et al., 2001). During pregnancy and peri partum, levels of estrogen, progesterone and placental lactogens prepare the female for lactation. During the postpartum period however, the suckling stimulation of the offspring maintains milk production and inhibits ovulation and the hormonal profile is changed significantly.The hormonal profiles of these females thereby maintain the maternal behaviors and decrease stress levels. While it is well established that the hormones of pregnancy affect the maternal areas of the brain, recent work suggests that estrogen has affects on other brain structures, such as the area associated with learning and memory, the hippocampus (McEwen, 1997).

Estrogen

The female brain displays a plasticity that is truly exceptional. Much of this plasticity is regulated by the cyclic hormonal alterations characteristic of the female, particularly, those associated with pregnancy and the maternal state (Numan et al.,1994). For years research has indicated the powerful influence of these hormones on the bodies of women and the potential protective effects of these hormones, such as the ability to improve natural memory decline seen in aging women, as well as potential protection against Alzheimer's Disease (Janowsky,Chavez,Orwell, 2000; McEwen,1994; Shaywitz , Shaywitz and Pugh , 1999)

Until recently there were only two main functions of sex steroids commonly studied (for both males and females) in the central nervous system. One was participation in brain organization during neural development and activation of sexual behaviors during maturity (Janowsky et al., 2000). While the hormonal influence of estrogen on

brain anatomy and functionality affects the areas of the brain associated with maternal behavior, other areas are affected as well (McEwen, 1994).

Current studies have shown that gonadal steroids influence neuronal structure, plasticity and survival across numerous regions, in particular the hippocampus. Research conducted by Yankova and Woolley (2001) indicates the presence of estrogen in female rats increases the density and divergence of dendritic spines of previously connected cells in the hippocampus. These connections suggest an immense increase in neural transmission efficiency, with a 25% increase in speed of transmission in estrogen treated animals compared to control animals (Yankova et al., 2001). There also seems to be formation of new excitatory synapses with estrogen treatment and an increase in hippocampal dendritic spines (McEwen, 1997). Estrogen receptors appear to be found in the areas associated with spatial learning and memory: the CA1, CA3 and dentate gyrus of the hippocampus (Wooley, Gould, Frankfurt, McEwen, 1990).

Other research indicates estrogen can influence the expression of behaviors not associated directly with reproduction. Behaviorally, estrogen is associated with enhanced learning and memory. Most studies indicate estrogen improves performance of rats on measures of learning and memory, in particular tasks dependent on spatial reference memory. In a study conducted by Daniels (1997), prior exposure to estrogen enhanced learning and memory performance, but it did not have to be in the female system during the period of testing. This study indicated that estrogen could induce changes in neuronal function that persisted well beyond the period of exposure. It becomes clear that this has a direct relevance on the aging female brain, in particular the aging mother's brain.

Other behavioral and endocrinological studies suggest that sex steroids can modify and improve memory in aging (Janowsky et al., 2000; Bunk , 2000; Tanila, Sipila, Shapiro, Eichenbaum, 1996). Also research undertaken by Roberts, Glardick, Lalsley and Rapp of University of California Davis (1997), have followed female rhesus monkeys throughout their reproductive cycles and used Delayed Response Tasks sensitive to aging. Roberts and colleagues found that peri – and post menopausal females exhibit significant impairments compared to those still in their reproductive prime. This suggests that menopause accelerates the effects of normal aging on learning and memory, rather than this decline being explained by the socio-cultural influences of aging, as has previously suggested (Roberts et al., 1997). Clinical studies have also illustrated that the lack of estrogen in menopausal women may be a relevant factor in Alzheimer Diseases (AD) pathogenesis. The hippocampus, which shrinks in Alzheimer disease patients, is found to be larger in postmenopausal women with estrogen supplements than in women without hormone therapy (Jagust, 2001). Estrogen may protect against metabolic insults of glucodeprivation and beta amyloid, and acts as a growth factor, or as protection against neural cell death (Zhang, Rubinow, Xiang, Li, 2001).

Complementary to these studies is research in endocrinology, which indicates parity, (reproductive experience) in women affects endocrine profiles and menstrual cycles for years following the birth of their offspring. It was also proposed by Musey (1987) that parity influences the reproductive aging of a female, in a sense protecting her system. Ginsberg et al (1991) indicated that the protective influence of parity can be seen to increase the length of time until menopause by 3- 5 years, while nulliparous women, that is women with no reproductive experience, have an early menopause. This

phenomenon is also seen in non-human primates and rats. For example, in female Sprague Dawley rats, parous rats are known to exhibit normal estrous cycling and remain fertile longer than nulliparous rats.

Aging and Cognition

Advanced age in rats is associated with a decline in spatial learning and memory. The effects are seen in the hippocampus, the main area of spatial learning and memory. Neurons and glia within the hippocampus of aged, spatial - learning impaired rats exhibit uniquely altered gene expression profiles, and isolated mitochondria in these areas are found to be severely damaged (Barone, Tandon, McGinty & Tilson, 1991). Age related effects associated with hippocampus include declines in the number of neurons and synapses in the dentate gyrus, the CA1 and CA3 regions of the hippocampus and exhibited in decreased performance in the Morris Water maze (Poe et al., 2001). Over twenty years ago Richard Morris introduced the Morris Water maze and since it has become the traditional measure of cognitive decline in aged rats, as it tests the ability of the rat to use spatial cues and spatial ability and memory. As these are abilities traditionally associated with the hippocampus, the Morris water maze can reflect a decline in hippocampal integrity through poor performance (Dudchenko, 2001). Gallagher, Burwell and Burchinal (1993) found the water maze is sensitive to age differences in performance with the most marked differences seen in the early stages of acquisition, suggesting it just takes old rats longer to learn the maze, rather than differences in speed or motivation. It was also found that Sprague Dawley rats have age related impairment in cue learning. These aged rats become less proficient at learning the information required for efficient navigation to a specific location. It has also been

seen in Sprague Dawleys that aging fundamentally alters the nature of hippocampal info processing of novel cues (Tanila et al., 1997). Also closely related to aging and cognition are the processes of apoptosis, neurodegeneration and the development of amyloid precursor protein (APP) and beta amyloid plaques, associated with Alzheimer's Disease.

Alzheimer's Disease, Beta Amyloid and APP

Alzheimer's Disease (AD) is a progressive degenerative disease of specific neurons. The cell death leading to cognitive decline and eventual dementia in the elderly. Beta amyloid proteins cause fibrillogenic peptides that cause the characteristic tangles in brains of Alzheimer's patients that lead to the malfunction of connections between the neurons. The protein is produced in neurons for regulating cell survival, synaptic plasticity and growth. Metabolic changes that occur with aging lower the level of neuroprotective forms of beta amyloid, thus causing neural degeneration. Development of plaques and tangles during the progression of Alzheimer's Disease are the identifying characteristics of its pathology. Although there is no consistent pattern of plaque and tangle development, as there are numerous spatial and temporal patterns, the resulting loss of neurons and synaptic connections are ultimately the same.

The senile plaques are an early and critical event in the pathogenesis of AD and form predominately in the region of the hippocampus, the crucial area of learning and memory (Selkoe, 1997). The plaques are also found in moderate or large numbers in limbic structures and association neocortex (Selkoe, 1999). Senile plaques consist of extracellular Beta Amyloid filaments, which are associated with dystrophic dendrites and

axons, and inflamed and over reactive microglia and astrocytes. These senile plaques are generated by a deposition in the brain of fibrils associated with beta amyloid.

Beta amyloid is a peptide of 40-43 amino acids, harbored within a larger transmembranal component known as (beta) amyloid precursor protein or APP (Stephan, Laroche & Davis, 2001). This amyloid precursor protein (APP) plays a pivotal role in the early stages of the neurodegeneration associated with Alzheimers. APP is a large membrane glyco- protein whose N- terminus projects into extracellular space.
It is widely expressed in the CNS, found in synapses, axons, and dendrites at both the surface and within the cells (Phinney ,Calhoun, Wolfer, Lipp & Zheng, 1999). However, increased formation of fibrillar AB peptides has been associated with genetic mutations in amyloid precursor protein (Selznick, 2000). It seems this related to an alteration in the processing pattern of the protein resulting in the generation of beta amyloid. This occurs by an alteration in the glycosylation state by the generation of ogliosacchrides. This causes a decrease the secretion of the neuroprotective soluble form of the protein and a parallel increase in the deposition in the cellular protein, where it interferes with cell functioning (Georgeopoulou, Mclaughlin, McFarlane & Breen, 2001) (see also Figure 1).

It is thought that APP and its metabolic byproduct AP are toxic and are processed in the Golgi complex and Endoplasmic Reticulum resulting in intracellular accumulations of protein that impair the neighboring neurons and lead to cell death (Koo et al.,, 1990; Lafena, Hall, Ngo, 1996; van Leeuwen, 1998). Hardy (1997) also suggested aged neurons could possibly secrete APP through their dendrites. APP is also seen in neurons, axons and dendrites (Koo et al., 1990). Hass & Selkoe (1993) propose that neuronal APP undergoes fast axonal transport to the synaptic terminal where APP fragments are then

released. At the synaptic terminals APP can be internalized along with recycled synaptic vesicles. APP is then sorted away from the vesicles and directed to the somatodendritic compartment and sorted at the cell surface, which interferes with proper function, and results in cell death.

It is essential to understand the connection between APP and B- Amyloid when conducting animal research. Whereas the pathology of AD is not known to develop in rodents as it does in humans, recent research indicates that APP is found in aged rats. APP has also been found in the injured CNS of young rats, indicating the involvement of APP in the injury and degeneration of neurons (Xie, Yao, Wu, 2000). Closely related, and some believe intimately connected, is the relationship between APP, Beta Amyloid and apoptosis. The precise mechanism is not completely understood, yet there are a number of factors possible. Theories range from dietary deficiencies that leave older brains more vulnerable to the toxic affects of aging (Kruman, Kummanuel, Lohani, Pederson & Mattson, 2002) to post - translational modification of APP by glycosylation acting as a key event in the processing of APP. In most cases however it is believed that altered processing of APP in AD may promote AB deposition, which renders neurons vulnerable to apoptosis (Masliah, Mallory, Tanaka & Hansen, 1998). (See below for description of apoptosis). Another related view is that neurodegeneration in AD might result from gain of toxicity or loss of trophic activity related to abnormal processing of APP (Masliah, 1997).

Beta amyloid , Apoptosis and the Caspases

Apoptosis, also known as programmed cell death, is the mechanism by which cells die through an activation of a naturally occurring cellular "suicide program" (Martinou & Sadoul, 1996). This programmed cell death is essential to the brain during development, but it is also related to neuron vulnerability as seen in old age and neurodegenerative diseases (Hara, Yoshinobu, Aujin & Hideki, 1996). While AB is known to induce cell death, the pathways that lead to cell death are still relatively unknown. Some evidence suggests roles for beta amyloid and biochemical cascades associated with apoptosis could be to blame for cell loss in neurodegeneration, including Alzheimer's Disease. The possibility that apoptotic cascades are activated in neurons in Alzheimer's Disease has gained support in a number of varied studies (Chan, Griffin, Mattson, 1999). For example studies in post-mortem AD patients show an increase in number of neurons with damaged DNA and mitochondria (Mococci, MacGarvey & Beal, 1994), and an increase in expression in genes linked to apoptosis (Tortosa, Lopez, Ferrer, 1998).

Cysteine proteases known as caspases are generally thought to be the essential mediators that execute the pathways and steps involved in the biochemical cascade of apoptosis (Allen, 2001). These caspases are divided into 3 categories: Initiator caspases, proinflammatory caspases and executor caspases. Executor caspases include caspases 3, 6, and 7, and are thought to directly cause the execution of the apoptotic program. Recent studies of Caspases have indicated that Caspase 3 acts on multiple substrates to culminate in the classic morphological features of apoptosis (Selznick, 2000) (Janicke, 1998). Caspase - 3 immunoreactivity is also increased in brain tissue from AD patients (Masliah

et al., 1998), as well as in cultured rat hippocampal cells undergoing apoptosis after exposure to beta amyloid (Chan, Griffin & Mattson, 1999). Allen (2001) also found in rat cortical neurons, caspase 3 was involved in beta amyloid induced neuronal apoptosis, with significant cleavage of caspase 3 in cortical neurons infused with beta amyloid. Apoptosis in these neurons is inhibited when the beta amyloid infused cells are also infused with anti-caspase 3, a caspase inhibitor, however these cells are weakened due to mitochondrial damage that has occurred , including the loss of mitochondrial membrane potential (Allen, 2001; Green,1998). These findings along with similar studies conducted by Selznick et al (2000) support an important role for caspase 3 in beta amyloid toxicity. Also indicative of the role of Caspase -3 is an in depth study, conducted by Chan et al(1999), exploring the role of caspases and the mechanisms by which they cause cell death and degradation. The study suggests a scenario in which AB induces apoptotic pathways in neurons that, during the early stages, receptors are targets of caspase -3. Chan et al provide two possible mechanisms by which this could occur, one being that the receptors are directly cleaved by caspases. The other supports the possibility that caspase 3 induces activation of proteases that degrade receptors.

B-Amyloid the Hippocampus and Memory

The beta amyloid precursor proteins lead to neural damage that impairs behavioral performance on learning and memory tasks (Mattson, 1997). Cognitive deficits in AD have been linked to several types of neurodegeneration, including neuron and synapse loss (Phinney et al., 1999). Other findings indicate that a common feature of neurodegeneration includes hippocampal atrophy as well as severe B- amyloid deposits in the cerebral cortex and hippocampus of humans (Fox, Warrington, Freeborough,

Hartikatzen & Kennedy, 1996); (Stephan & Laroche, 2001); (Fukuta, Nitta, Itoh, & Funikawa, 2001). In rat models of AD, using APP, there seems to be an increase in neuron damage in the hippocampus , indicating that hippocampus damage in rats may be comparable to the damage seen in AD humans. Other data have shown that rats with amyloid damage to the dentate gyrus are impaired in reference memory, particularly spatial navigation in the Morris water maze (Barone, 1991); (Conrad & Roy, 1993). The dentate gyrus is also one of the first areas in the brain to show Alzheimer's plaques in humans (Duyckaerts, 1997). In rodent models investigating the presence of APP, behaviorally tested APP positive rats show deficits in working memory and spatial navigation, found in a region of the hippocampus known as the CA1 region. (McNaughton, 1991). In light of these studies we found it necessary to explore closely the role of pregnancy, motherhood and its potential protective effects on the brain throughout the life of a mother.

First, pregnancy is known to increase brain complexity. Also related to this is the theory that the act of motherhood itself and the multi-sensory experience of caring for pups, may create an enriched environment that substantially increases the neural complexity and provides neuroprotection to these previously developed neurons, glia and synaptic connections. This leads one to believe that a mother not only leaves her young with her experience, but the young also provide her with a more complex brain, a benefit that will follow her throughout life. Also fascinating are the neuroendocrine influences of reproductive hormones, in particular estrogen. Estrogen increases brain connectivity, efficiency and then provides protection of these structures in the hippocampus, an area crucial to learning and memory. We must also note the fact that hormones are prolonged

in the female system by the number of reproductive experiences she experiences. It is possible mothers would begin aging with more neurons and more complex brains than those without reproductive experience, and have more protection from the prolonged presence of reproductive hormones. Therefore females with more reproductive experiences would exhibit a less serious decline in neurons and associated behavioral and cognitive deficits from the inevitable loss of neurons through the process known as apoptosis.

Observations of diverse behavioral / cognitive performance, neuroprotection and apoptosis (cell death) in the brain, between those with different reproductive experiences, have lead to some fascinating speculation that we have begun to test empirically. The current project examines the possibility that parity may modify the neurodegeneration and associated cognitive decline that occurs with aging. If, as we have shown, reproductive experience alters glia, neuron morphology and neurogenesis, in adult females, it is worthwhile examining to what extent APP and therefore, neurodegeneration in aged females might be affected. Finally, a behavioral measure should indicate, on learning and memory tasks that animals with more reproductive experiences will show superior performance compared to those animals with fewer reproductive experiences or no reproductive experiences. We also expect to find the most complex neural connections and the least amount of APP and neurodegeneration in the multiparous animals, and the largest amounts of APP and neurodegeneration to be exhibited in the nulliparous animals.

Project Design and Methods

In order to determine the effects of reproductive experience on neural changes associated with aging we used both behavioral and anatomical measures. Behavioral measures were part of a longitudinal study that following the female animals across the life-span, with the same measures taken every six months for two years.

The anatomical measures were taken after the two-year period of behavioral testing to determine if the amount of neural degeneration is different between animals of different reproductive experiences (nulliparous, primiparous and multiparous).

Subjects

Thirty (n=30), six - month old aged matched Sprague Dawley Rats, (birth date: January 2000) consisting of three groups of 10 animals based on reproductive experience. (Nulliparous, Primiparous, Multiparous), were ordered as classified from Harlan - Tekland . Nulliparous / Virgin animals were classified as animals with no prior reproductive experience and no prior contact with pups. Primiparous animals were described as having one reproductive experience (one pregnancy) with a mean litter size of 12 pups. (No litters are culled). Females were allowed constant contact/ care of pups for 21 days after which point pups were weaned. Multiparous animals were described as having two reproductive experiences (two pregnancies) with a mean litter size of 12 pups. (No litters are culled). Females were allowed constant contact/ care of pups for 21 days after which point pups were weaned.

Multiparous animals were then given a "resting period" of a two to four weeks without contact with pups or other animals. After this period the female was bred again and underwent a subsequent pregnancy and 21 days post partum constant care of pups,

until weaning. Animals were then given a rest period and sent to the University of Richmond neuroscience lab on July 3, 2000. All animals were double- housed and cared for according to federal guidelines, and the University IACUC, for the length of the study.

Maze Testing

Original Dry Land Maze Test

Animals were behaviorally tested in a dry land maze version on the Morris Water Tank with eight baited food wells (see figure 10 for layouts). All animals were food deprived five days prior to testing in order to place weight at 85-90% of original weight.

To do this animals were placed on a restrictive diet and weighed at 0900 each morning to ensure healthy weight loss. Each day during this time, animals were habituated to a .25 gram piece of "Froot Loops" cereal, which was placed into the cage after weight was determined in the morning. After 85-90% of the animals original weight was reached, the animals were given three days of ten-minute habituation training in which the number of baited wells were gradually decreased.

On day one of habituation animals were placed one at a time in the maze with all eight wells baited with a piece of "Froot Loops", and given ten minutes to explore the maze and find as many baited wells as possible. The second day animals were again given ten minutes to explore, but the baited wells were decreased to four, with one baited well in each corner. On day three animals had ten minutes to find the food in two wells. After the third day of habituation, testing began, the object of this testing was for the animal to find the one baited well, within a short period of time, using general spatial cues within the maze.

Tests consisted of three trials, limited in time to 180 second each, ending when the animal found the baited well or the time limit is met. The animal was then removed and the next trial began, with the animal being placed in at a new starting position each trial (northeast, southeast and southwest) of the baited well. The timed testing occurred over a three-day period. At the conclusion of the study, latency to find the well was recorded and a mean for each day was determined and compared between the different groups of animals. The first group of testing for all groups (multiparous, primiparous and virgin) was completed at six months of age. The data collected is now the baseline data for the continuation of the longitudinal component of the research study.

These testing procedures were repeated every six months until animals reached the age of 24 months that is, at six, twelve eighteen and twenty four months. The age of twenty-four months was chosen because it is considered to be old age(~ 85 years in humans) in Sprague Dawley Rats (Zivic - Miller and Harlan Tekland- phone correspondence). The same measures were taken and means were determined within and between subjects across the four different testing sessions.

Reversal Task in Dry Land Maze

Beginning at twelve months of age we tested animals in a reversal task in the same dry land maze apparatus as listed above. At the end of the three day testing period of the original task in the dry land maze, a new task was introduced, rotating the food reward from the initially learned well to an adjacent well. Animals were given a 180 second acquisition period before testing.

The tests consisted of three trials, limited in time to 180 second each, and ending when the animal finds the baited well or the time limit is met. The animal was then

removed and the next trial began, with the animal being placed in at a different starting position each trial. (northeast, southeast, etc). The timed testing occurred over a three-day period. At the conclusion of the study, latency to find the well was recorded and a mean for each day was determined and compared among the different groups based on reproductive experience.

These testing procedures were repeated every six months until animals reached the age of 24 months that is, at twelve, eighteen and twenty four months. The same measures were taken and means were determined within and between subjects across the four different testing sessions.

At the conclusion of testing animals were returned to the animal rooms for a period of two weeks. Animals were returned to their normal diet and housing procedures, according to protocol 99-1. This allowed sufficient time in order to prevent the testing stressors (i.e. food deprivation, stress from novel environment etc.) from confounding the neural analysis. After two weeks neural analysis procedures began.

Neural Analysis

The neural analysis measures used were measures of precursors of neurodegeneration, known as Amyloid Precursor Protein (APP). The measures were obtained through the use of immunohistochemistry. The procedure began with the perfusion protocol in which first, animals were euthanized using sodium pentobarbitol. The animals were then perfused with cold PBS for 2-3 minutes, followed by cold 4% paraformaldehyde (PF) for 20 minutes to stiffen the brain and eliminate blood and blood-related products from it. The brains were removed and post fixed for 24 hours in 4 % PF, and then switched to 20% sucrose/PBS solution overnight at 4C. Next, sections of brain

were cut at ~40 microns on a cryostat and every 6th section was placed into wells containing PBS. Ten sections were collected from each brain for each measure taken, with a total of 60 sections were cut from each brain. (We saved alternate sections for other analyses including: Caspase-3 , Insulin receptors, Mitochondrial factors and Nissl staining for accompanying research).The next day sections were washed for 10 minutes with 3% H_2O_2/ PBS, followed by three 10 minute washes of PBS. The sections were then incubated in 10% normal goat serum (NGS) on a shaker at room temperature. Finally, slices were incubated overnight on a refrigerated vortex in the APP primary antibody diluted 1:200 in PBST (-Chemicon antibody #Mab343).

During day two, the sections were washed three times for ten minutes with PBS. Next, the tissues were exposed to the secondary antibody (goat -Anti - rabbit) for one hour on a room temperature shaker, followed by three ten minute washes in PBS. Sections were then incubated in Avidin Biotin Complex (ABC) for one hour on shaker, followed by two ten minute washes in PBS. The sections were then visualized with DAB/NiCl diluted in distilled H_2O_2 for five minutes, washed in PBS and mounted on subbed slides.

The mounted tissue was then viewed under the microscope and the cells with the Alzheimer precursor protein present on the membrane were labeled and counted by a blind observer through the use of Bioquant computer technology. To ensure correct labeling of APP, cells to be counted were assessed and verified by Chemicon technical staff. We chose to count cells that contained 50% or greater of the reaction product on the cell surface. An assistant blind to the conditions counted cells from a specified section taken in both the dentate gyrus and the CA1 regions. From the sections counted one

"punch". A "punch" is defined as the area visible on one Bioquant computer screen at magnification of 40X (equivalent to 153.72um X 114.42um of the tissue) was taken from the highest point of the dentate gyrus.. The area counted for the CA1 region was taken from the area of the CA1 region directly above the area counted for the dentate gyrus.

Statistics

A 3 (reproductive experience: nulliparous, primiparous, multiparous) by 4 (age: 6 months, 12 months, 18 months, 24 months) by 3 (day of testing: day 1, day 2, day 3) mixed analysis of variance (ANOVA) was performed on the data for both the original and reversal tasks. For measurements in APP, a one way analysis of variance (ANOVA) :mean number of APP positive cells by reproductive experience (Multiparous, Primiparous and Nulliparous /virgin)was performed. Post hoc analysis used was Fishers LSD test at the.05 level. Finally, a Pearson correlation was run between the mean latency of the animals in the maze at 24 months and the number of APP positive cells. This measure was taken for both the original and reversal tasks and in both the CA1 region and the Dentate Gyrus of the hippocampus.

Results

Behavioral Data

Original land maze results

Main Effects

Three significant main effects were revealed by the ANOVA. First, there was a significant main effect for reproductive experience, $F(2, 1008) = 344.13$, $p < .01$ reach criterion. Second, a significant main effect was also found for age, $F(3, 1008) = 236.71$, $p < .01$. Third, a significant main effect was revealed for day of testing,

$F(2, 1008) = 58.11, p < .01$ (see Table 1 for means and standard deviations, and Figure 2 for graphical representations).

2-Way Interaction Effects

Three significant two-way interaction effects were revealed by the ANOVA. First, reproductive experience significantly interacted with age, $F(6, 1008) = 20.29$, $p < .01$.. Second, there was a significant interaction between reproductive experience and day of testing, $F(4, 1008) = 15.40, p < .01$. Finally, a significant interaction was seen between age and day of testing, $F(6, 1008) = 2.98, p < .01$.

Fisher's LSD Post Hoc tests $p<.05$ revealed in general, multiparous rats took less time through the maze at all ages than either the nulliparous group or the primiparous group. All three groups had the lowest times at six months than at any other age; however, primiparous rats took longest. Nulliparous rats performed similarly on days 1 and 3; best performance was seen on day 2. Primiparous rats performed significantly worse on day 1 than any other day; no difference was seen between scores for days 2 and 3. Multiparous rats performed significantly better on each day, improving from day 1 to day 3.

Performance was better for multiparous rats on all days than either nulliparous or primiparous rats. For all age groups, significantly worse performance is seen on day 1. For ages 12, 18 and 24 the best performance was seen on day 3, even though there is no significant difference between day 2 and day 3 for ages 18 and 24.

3-Way Interaction Effects

There was a significant interaction among all three variables: reproductive experience, age and day, $F(12, 1008) = 6.09, p < .01$. Fisher's LSD Post Hoc revealed within the nulliparous group, a significant effect of day was only seen at age 24 months; performance on day 3 was significantly best. Within the primiparous group, a significant effect of day was seen at both age 18 and 24 months. At both ages, performance on days 2 and 3 was significantly better than day 1.Within the multiparous group, a significant effect of day was seen for ages 6, 12 and 24 months. At six months, best performance was seen on day 3 but no differences were seen between days 1 and 2. At 12 months, all days were significantly different from one another; performance improved each day with lowest times on day 3. At 24 months, no differences were seen between days 2 and 3; however, these days were significantly better than day 1. No differences were seen between days at age 18 months for multiparous rats.

Reversal task results

Results

A 3 (reproductive experience: nulliparous, primiparous, multiparous) by 4 (age:12 months, 18 months, 24 months) by 3 (day of testing: day 1, day 2, day 3) mixed analysis of variance (ANOVA) was performed on the reversal data.

Main Effects

Two significant main effects were revealed by the ANOVA. First, there was a significant main effect for reproductive experience, $F(2,269) = 73.31, p < .01$ (see Figure 3 for graphical representation). A significant main effect was revealed for day of testing,

$F(2,269) = 11.74, p < .01$. That is multiparous animals performed better than nulliparous and primparous animals, and primiparous animals performed better than nulliparous animals. We also found significant improvement for all groups of animals between days one two and three, thereby indicating that all animals learned the memory task in the allotted testing period.

2-Way Interaction Effects

There was no significant two-way interaction effects revealed by the ANOVA. Reproductive experience did not significantly interact with age. There was no significant interaction between reproductive experience and day of testing and no significant interaction was seen between age and day of testing

3-Way Interaction Effects

There was no significant three- way interaction effect revealed by the ANOVA.

APP Analysis

We chose to measure Amyloid Precursor Protein (APP) in two regions: CA1 and Dentate Gyrus. The area measures are regions of the hippocampus sensitive to aging and are areas considered to be implicated in solving spatial memory tasks. (Poe, Linville, Riddle, Sorintag, Brunso-Beechtold, 2001). A one way analysis of variance (ANOVA) mean number of APP positive cells by reproductive experience (Multiparous, Primiparous and Nulliparous/ virgin) revealed significant differences.

Dentate Gyrus

In the Dentate Gyrus we found significant differences $F(2,19)= 11.36, p<.03$. The LSD post hoc indicated differences between the Multiparous and Nulliparous animals, $p<.05$. The multiparous animals had significantly fewer APP positive cells (M= 81.29)

than nulliparous animals (M=100.57), while there was a non-significant difference between primiparous (92.67) animals and both nulliparous and multiparous animals (see Figure 4).

CA1 Region

In the CA1 region of the hippocampus we did not find significant differences $F(2,19)=14.93.$, $p>.05$. However we noticed a trend that supported our hypothesis, and therefore ran post hoc tests. Post hoc tests revealed a significant difference between the multiparous animals and primiparous animals, $p,<.05$ and significant differences between the multiparous animals and the nulliparous animals.

There was no significant difference in number of APP positive cells between primiparous and nulliparous animals. Multiparous animals had significantly fewer APP positive cells (M=78.71) than both primiparous (M=94.33), and nulliparous animals (94.43) (see Figure 5).

Correlation

A Pearson correlation was performed between the mean latency of the animals in the maze at 24 months and the number of APP positive cells. This measure was taken for both the original and reversal tasks and in both the CA1 region and the Dentate Gyrus of the hippocampus

Original Task

There was a significant negative correlation between the number of APP positive cells in the CA1 region $r = .45$, $p<.03$ ($r^2= .204$) and the dentate gyrus $r = .51$, $p<.02$ ($r^2 = .26$) and the mean latency to find the well in the original task .

That is the more APP positive cells found in the brain, the slower the latency to find the food well in animals at 24 months of age (see Figures 6 and 7 for graphical representation).

Reversal task

There was a significant negative correlation between the number of APP positive cells in the CA1 region $r = .49$, $p<.05$ ($r^2=.24$) and the dentate gyrus $r= .53$, $p<.04$ ($r^2=.28$) and mean latency in the reversal task in the land maze. Again, we see the more APP cells found in the hippocampus, the poorer the performance (see Figures 8 and 9).

Discussion

The behavioral data illustrate that multiparous animals possess superior performance in comparison to nulliparous animals, and that this performance seems to continue across the life span of the females. This is consistent with the findings of Kinsley, Lambert, Gifford, Trainer & Madonia, (1999). Also it was found that while primiparous animals show enhanced performance relative to nulliparous rats, the group does not perform as well as multiparous animals. These findings suggest that although reproductive experience results in an enhancement of performance, a critical change occur in females between the first and subsequent pregnancies.

Whereas there was a consistent significant decline in performance across the groups as age increased, the performance of multiparous animals surpasses that of primiparous and nulliparous animals, and primiparous performance exceeds that of nulliparous animals throughout the life span. Based on the results of our longitudinal behavioral study, the superior spatial skills appear to remain with the mother for life.

It should also be noted that the performance was highly variable among all groups, as seen by a large standard deviation, memory decline in aging has been shown to be highly variable (Gallagher, Burwell,& Burchinal ,1993). Extensive research also indicates that normal aging is accompanied by a decline in the ability to acquire new information, this effect is most likely caused by a change in the way information is encoded as a result of fewer neurons. That is, the neurons lost may very well be a break in the inner connections of neurons that form memories. (Tanila, 1997; Tsai, 2002).

Researchers have ruled out the possibility of impaired performance due to changes in motivation, motor speed or vision, rather the problem seems to stem from an inability to gather or code the new information correctly and quickly (Gallagher, Burwell& Burchinal, 1993). Most likely this inflexibility in learning new spatial information is associated with fewer neural connections due to changes in the aging hippocampus. (Jeltsh et al., 2001). The results indicate while there is a decline in performance with age in primiparous and multiparous females, the decline is not as severe as typical aging in animals without reproductive experience. This raises a question as to what causes the differences we see in these reproductively experienced animals that leaves them more fit during old age.

A number of possibilities exist to explain these findings, with estrogen as a primary factor. One mechanism by which estrogen influences memory is through restructuring dendrites and synapses in the hippocampus, an area of learning and memory (Okeefe & Nadel, 1978). It also increases dendritic branching and synaptic connectivity (Yankova et al., 2001). Other research suggests estrogen improves memory in maze performance (Daniels, 1997), and prevents memory and performance decline in aged

females. Estrogen seems to have neuroprotective effects for females. (Janowsky, Chavez & Orwell, 2000). Based on research indicating that multiple pregnancies lengthen the reproductive period and the amount of time ovarian hormones circulate in the female, the prolonged period of estrogen exposure could prove to be a major factor in the superior performance seen in multiparous animals in comparison to animals with fewer reproductive experiences.

Another factor we cannot rule out is the possibility that the mother's experience with her offspring created an enriched environment. Classic enriched environment studies have shown that environmental factors can influence aspects of the central nervous system associated with behavior (Rosenzweig, Bennet , Herbert & Morimoto, 1978). These changes have been seen in both neurochemical relationships between neurons and in neuroanatomy of certain brain regions. (Rosenzweig, et al., 1978; Myhrer, Utsikt, Fjelland, Iverson & Evy et al., 1992).

Basic maternal behaviors provide a vast amount of stimulation to the mother: auditory (ultra sonic calls), ventral tactile stimulation (suckling) and facial tactile stimulation of the mouth and whiskers from licking and retrieving pups. Unpublished observations of Madonia (2001) noted large amounts of neurogenesis not only in the area of learning and memory (dentate gyrus), but also the barrel cortex, an area associated with cues received from the whiskers. These findings support not only the idea that there is an enriched environment created by caring for the pups, but also that this creates neurogenesis and possibly promotes the new cell survival throughout life.

Research has indicated that people's capacities during aging seem to depend on their particular environment. While much of this research has been conducted in rats,

such as that conducted by Young et al which demonstrated enriched environments not only enhance neurogenesis, but also promote cell survival in the long term (Young, Lawlor & Leone, 1999). Related to this research is work conducted by Dean (1997) in which interviews conducted in old human participants who described their daily activities. The activities were then compared to rat studies in enriched environments. Findings suggested in both humans and animals environmental elements such as nutrition, exercise, complex cognitive activities and social and physical contact with others can prove to provide enriched environments and continued development and maintenance of mental ability.

If raising offspring does create an enriched environment, and enriched environments enhance neurogenesis and maintain these cells throughout life, this suggests mothers have more neurons and, therefore, more connections. Additionally, these connections remain protected in the mother throughout her life.

Another possible advantage reproductively experienced animals may possess is a decreased stress response. Wartella (2000) suggested the stress responses seen in pregnant and lactating animals remain long after the lactation period ends. It is quite possible that the decreased stress responsiveness in multiparous and primiparous animals enhances performance in the initially stressful environment of a maze. It is well noted in aging research that old rats characteristically tend to explore new environments, such as mazes, with hesitation and then fail to explore them sufficiently, even when motivated by hunger (Tanila, Sipila, & Eichenbaum, 1997). This suggests that older animals demonstrate more pronounced fear responsiveness than young animals. If reproductive

experience does reduce stress responsiveness, then it is possible that this reduced stress is seen even in old age and therefore could partially account for our findings.

To explore the neural substrates of our behavioral findings we chose to look at the CA1 region of the hippocampus as well as the dentate gyrus of the hippocampus. Numerous studies have shown that the hippocampus is the key brain location of learning and memory and more specifically the CA1 region and the dentate gyrus are the center of spatial learning and memory. (Amenta, Mignini, Ricci, Sabatoni & Tayebati, 2001; Jeltsh, Bertrand, Lazarus & Cassel, 2001; Gallagher, Burwell, & Burchinal, 1993). Several studies also have shown CA1 and dentate gyrus processing is altered naturally in aging (Poe, Linville, riddle, Sorintag & Brunso, 2001; McEchron, Weible, & Disterhoft, 2001). Age related effects associated with the hippocampus include a decrease in the number of neurons and synapses in the dentate gyrus and theCA1 regions, with a decrease in cognitive ability as assessed by the Morris water maze (Poe et al., 2001).

Another factor related to brain aging, albeit atypical brain aging, is amyloid precursor protein deposits. APP leads to neural damage that impairs behavioral performance on learning and memory tasks (Mattson, 1997). Other data have shown that rats with amyloid damage to the dentate gyrus are impaired in reference memory, particularly spatial navigation in the Morris water maze. (Barone, 199; Conrad & Roy, 1993). The dentate gyrus is also one of the first areas in the brain to show Alzheimer's plaques in humans. (Duyckaerts, 1997). In rodent models investigating the presence of APP, behaviorally tested APP positive rats show deficits in working memory and spatial navigation, found in the CA1 region of the hippocampus. (McNaughton(1991). Our findings indicated that not only did reproductively experienced animals outperform

nulliparous animals in a land version of the Morris water tank, but also showed fewer amounts of APP positive cells.

Why should mothers have a lifelong benefit from their maternal experiences much earlier in life. One theory possibly supporting these findings is the grandmother hypothesis. That is, aging women gain an inclusive fitness advantage from being around to influence and invest in their grandchildren/offspring (Scott-Peccei, 2001).The theory states that aged females past the reproductive prime can direct their remaining reproductive effort more profitability toward enhancing the reproductive success of existing progeny. This behavior clearly exists in humans. In other mammals, specifically female elephants characteristically care for the "grandchildren". Female elephants live in extended family units and protected by a matriarch, females assist their daughters delivering the first calves and behave in a parental manner to these offspring (Douglas – Hamilton & Douglas – Hamilton, 1975). In rats studies of maternal behavior actually find that the capacity to respond to pups is significantly enhanced over young or "middle-aged" rats. The response to donor pups is almost immediate in aged females (Gonzalez & Diez, 1990).

In addition, old mothers have the added benefit of prolonged exposure to estrogens and an extended period of reproductive fitness prior to her stint as a grandmother. Add the benefit of the stimulation and enriched environment created by caring for her offspring and grand offspring, and this female not only has possible neural and cardiovascular protection from the estrogen, but also has a neuroprotective, enriched environment throughout her life. This protection and enrichment very well could prove to

be a factor in the maintenance of cognitive abilities, and hence additional care directed toward her kin.

We are aware that our experiment has limitations. For example, the life experience and reproductive experiences are somewhat artificial, as it is typically thought to be impossible to find naturally occurring virgin females in the wild. On the other hand, behavioral observations of mice colonies indicate there are numerous instances of nulliparous and primiparous females in colonies with dominant multiparous animals (Crowcroft, 1973). Also in any reproductive experience there are a number of variables that we are unable to control. This includes inter-individual differences in litter size of those who have delivered, the type of mating experiences encountered, length of gestation period, as well as individual differences in hormone levels.

We also are mindful that it is premature to draw any definite conclusions about specific relationships between maternal experience and neuroprotection throughout the life cycle. However we feel that these data, as well as past data from our lab, indicate that animals with reproductive experience have a distinct advantage over animals with out reproductive experience in artificial as well as natural environments. Females with reproductive experience show enhanced brain connections (Amory, 2000; Madonia, 2001) and enhanced performance on spatial tasks (Kinsley et al., 1999).

The superior ability of these females to learn and remember how to maneuver the maze in order to find food directly relates to maintenance of both the mother and her offspring. In the wild this ability can be greatly beneficial. These animals are subject to the caprice of an unpredictable environment. As food supplies wane, reproductively experienced animals have an advantage in finding limited food. This advantage enables

the reproductively experienced female and her offspring to survive. Studies of wild rats support this theory. Klein and Glass, have found that in wild rat traps in Baltimore, the preponderance of animals caught are lactating females, and this suggests that these females are able to navigate their environment more quickly and efficiently to find food for survival than males or females without reproductive experience. This would indicate in the game of survival of the fittest mothers are by far the "most fit". This fitness seems to follow the mother well past her reproductive prime, and throughout her life, perhaps taking advantage of her abilities to nurture grand offspring.

In addition to our findings that females with reproductive experience have exceptional performance in spatial maze throughout the life cycle and brains with less degeneration at the end of life, other research in the lab may indicate that these differences originate from genetic changes that occur during pregnancy and lactation. DNA microanalysis conducted on virgin and lactating animals indicates a significant increase in the production in both the synuclein gene, a gene involved in neurodegeneration associated with Parkinson's disease, and in the gene for Huntington's disease (Griffin et al, 2001). This indicates that there are differences in genetic expression between reproductively experienced animals and animals without reproductive experiences. Changes on the genetic level could indeed persist for life and prove to be a major factor in the differences we have found in the levels of neurodegeneration of these animals during old age.

Also related is a recent study examining the interaction between dopamine and synuclein in Parkinson's disease, in which it was found that the brain protein synuclein combines with dopamine (DA) in nerve cells producing reactive oxygen molecules that,

can kill the nerves. Looking at the genes of the indiviuals who develop Parkinson's it has been found that they express a mutated form of the alpha synuclein gene (Xu, Kao, Lee, Jin, Yankner, 2002).The possibility that animals with reproductive experience express the synuclein gene differently suggests that the genes could relate to differences in the synuclein's action with DA, and therefore less neurodegeneration in aging. These genetic findings could also prove to be another factor that related the reduced neural degeneration in reproductively experienced animal.

Conclusion

Related to previous studies in aging, hormones, and cognition these data suggest definite changes occur in the brains of females who undergo the experiences of pregnancy and lactation. The data also suggests these changes, once made, remain with the female past her reproductive prime and act as a benefit for the remainder of her life. Even more interesting the data suggest the changes are more pronounced in animals with multiple reproductive experiences.

We feel that our findings will prove significant to an area of behavioral neuroscience that has received little attention. Still we understand that we can not make grandiose affirmations, such as maternal behavior makes women smarter, nor that it is a fountain of youth or a cure for senility or Alzheimer's Disease. Future research will determine the significance of this study and its generalizability to humans.

It is our goal to determine the true effect of maternal experience on neuron and brain morphology, as reflected in learning and behavior throughout the life cycle. The data suggest that pregnancy and the postpartum period may produce a neuroprotective

effect, as seen through levels of APP. Further, we are very interested in the role of the pups and their ability to provide an enriched environment to the mothers. Therefore, the influence of the pups also represents a very important variable to be studied.

The brains of the animals tested were well distributed and are currently being analyzed to determine if there are differences between groups based on reproductive experiences in hippocampal density (Nissl stains) other measures of cell death (caspase 3) as well as mitochondrial functioning. Together, these data will provide a significant piece of the puzzle about the dynamic maternal brain.

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Table 1. Latency for animals to find baited food well in dry land maze (original task)at 6,12,18,and 24 months of age.

		Latency to baited well		
Reproductive Experience	Age	Day 1	Day 2	Day 3
Nulliparous	6 Months	85.10	62.43	89.23
	(Std.dev)	(38.88)	(40.07)	(55.38)**
	12 Months	179.76	177.73	174.77
	(Std.dev)	(0.71)	(5.36)	(13.08)
	18 Months	170.50	167.71	172.67
	(Std.dev)	(22.87)	(19.57)	(11.48)
	24 Months	180.00	172.42	160.08
	(Std.dev)	(0.00)	(11.95)	(24.48)
Primiparous	6 Months	130.57	107.97	101.63
	(Std.dev)	(49.04)	(46.95)	(50.90)
	12 Months	173.23	174.50	169.53
	(Std.dev)	(14.18)	(10.40)	(19.06)
	18 Months	154.00	126.04	125.50
	(Std.dev)	(31.89)	(20.11)	(30.03)
	24 Months	166.29	142.92	129.83
	(Std.dev)	(15.95)	(25.46)	(45.77)
Multiparous	6 Months	50.48	65.22	26.26
	(Std.dev)	(39.38)	(40.49)	(31.28)
	12 Months	163.19	114.15	60.70
	(Std.dev)	(42.37)	(51.28)	(30.60)
	18 Months	97.56	85.81	66.89
	(Std.dev)	(57.31)	(51.20)	(30.60)
	24 Months	138.84	85.97	83.37
		(36.24)	(35.37)	(36.32)

Figure 1. Diagram illustrating APP development. Follows the development of the amyloid precursor protein from altered glycosylation, to intraceelular and extracellular deposits. The diagram then follows the path of the altered protein through the development of plaques and tangles and concludes with cell death.

Figure 2. Mean latency to find baited food well in land version of the Morris water maze (in seconds) by reproductive group * denotes significant difference between multiparous and nulliparous groups *# denotes significance between all groups

Figure 3. Mean latency to find baited food well in reversal task(in seconds) by reproductive group.* denotes significant difference between multiparous and nulliparous groups *# denotes significance between all groups

Figure 4 . Mean number of APP positive cells counted in the Dentate Gyrus (hippocampus) of aged (24 month –old) multiparous, primiparous and nulliparous females. * denotes significant difference between multiparous and nulliparous groups

Figure 5. Mean number of APP positive cells counted in the CA1 (hippocampus) of aged (24 month –old) multiparous, primiparous and nulliparous females. * denotes significant difference between multiparous and primiparous groups and multiparous and nulliparous groups.

Figure 6. Correlation between latency to find baited food well on original task and mean number of APP positive cells in the CA1 region for aged (24 month –old) multiparous, primiparous and nulliparous females.

Figure 7. Correlation between latency to find baited food well on original task and mean number of APP positive cells in the Dentate Gyrus for aged (24 month –old) multiparous, primiparous and nulliparous females.

Figure 8. Correlation between latency to find baited food well on reversal task and mean number of APP positive cells in the CA1 for aged (24 month –old) multiparous, primiparous and nulliparous females.

Figure 9. Correlation between latency to find baited food well on reversal task and mean number of APP positive cells in the Dentate Gyrus for aged (24 month –old) multiparous, primiparous and nulliparous females.

Figure 10. Apparatus Diagram. Maze Organization across days and trials.

Figure 1

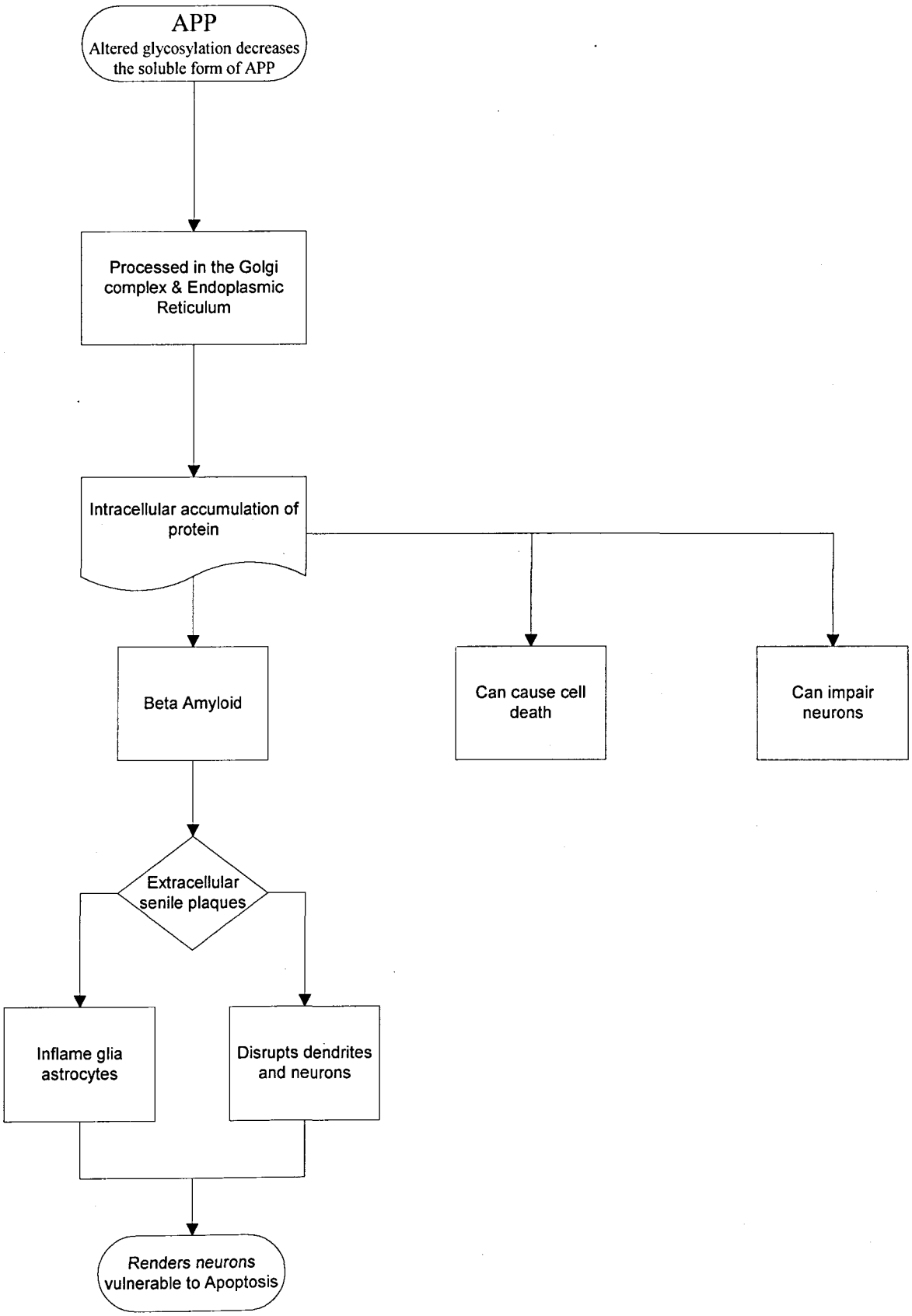

APP
Altered glycosylation decreases the soluble form of APP
Processed in the Golgi complex & Endoplasmic Reticulum
Intracellular accumulation of protein
Beta Amyloid
Can cause cell death
Can impair neurons
Extracellular senile plaques
Inflame glia astrocytes
Disrupts dendrites and neurons
Renders neurons vulnerable to Apoptosis

Figure 2.

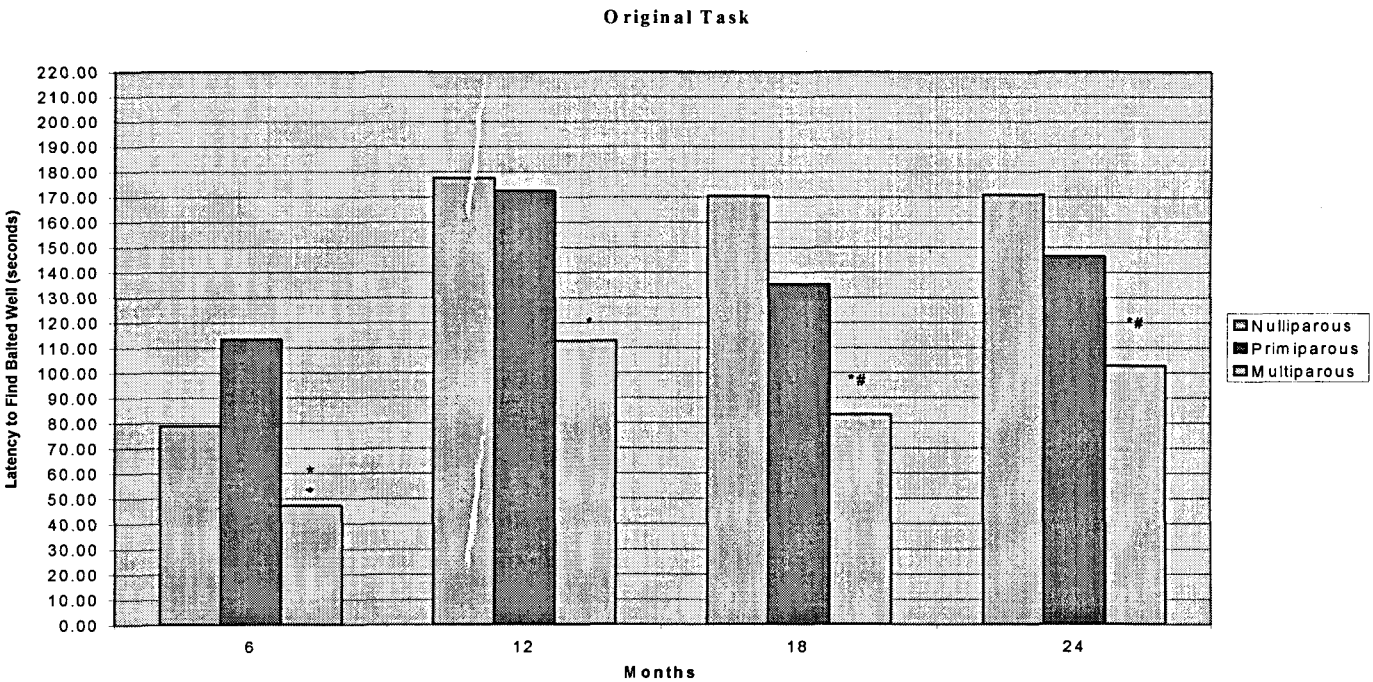

Original Task
Latency to Find Baited Well (seconds)
220.00
210.00
200.00
190.00
180.00
170.00
160.00
150.00
140.00
130.00
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
6
12
18
24
Months
Nulliparous
Primiparous
Multiparous

Figure 3.

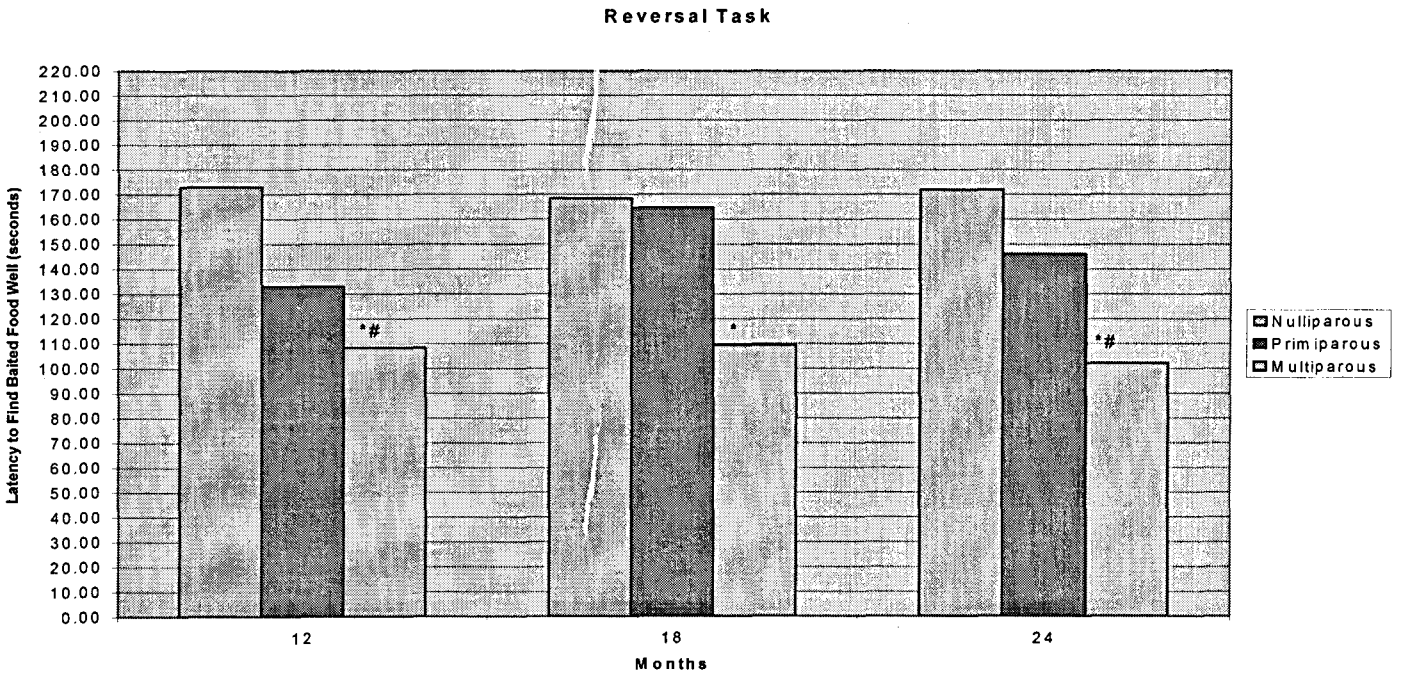

Reversal Task
Latency to Find Baited Food Well (seconds)
220.00
210.00
200.00
190.00
180.00
170.00
160.00
150.00
140.00
130.00
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
*#
*
*#
12
18
24
Months
Nulliparous
Primiparous
Multiparous

Figure 4.

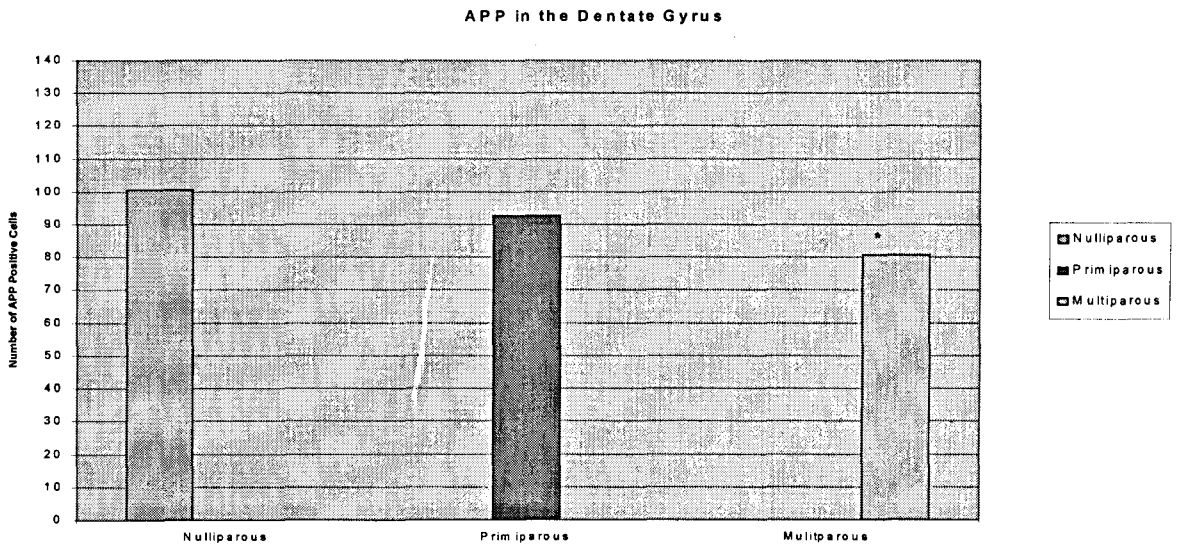

APP in the Dentate Gyrus
Number of APP Positive Cells
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Nulliparous
Primiparous
Mulitparous
*
Nulliparous
Primiparous
Multiparous

Figure 5.

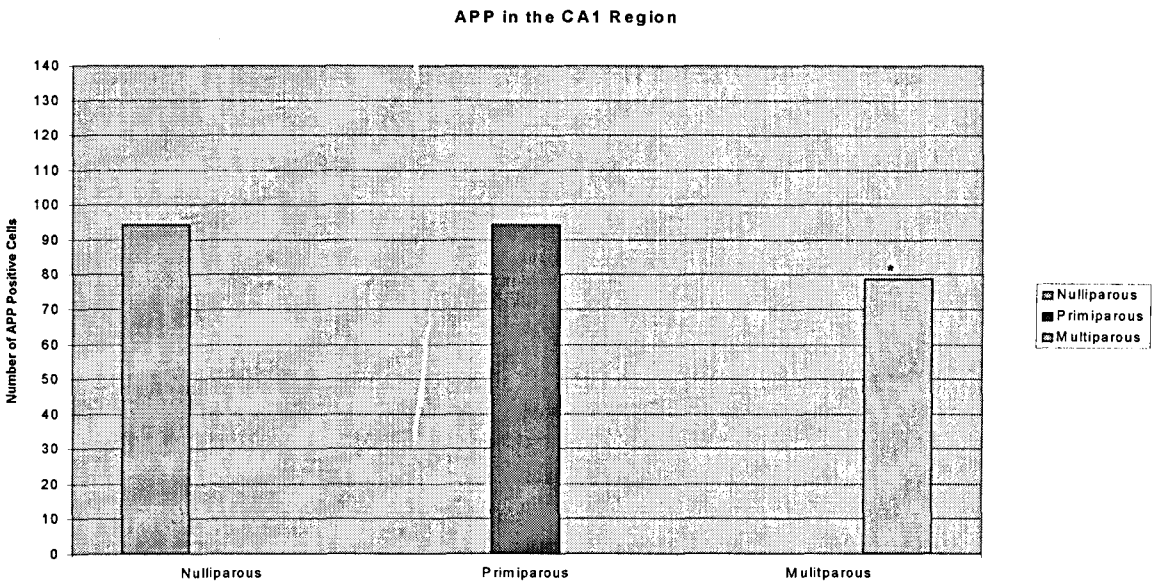

APP in the CA1 Region
Number of APP Positive Cells
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Nulliparous
Primiparous
Mulitparous
*
Nulliparous
Primiparous
Multiparous

Figure 6

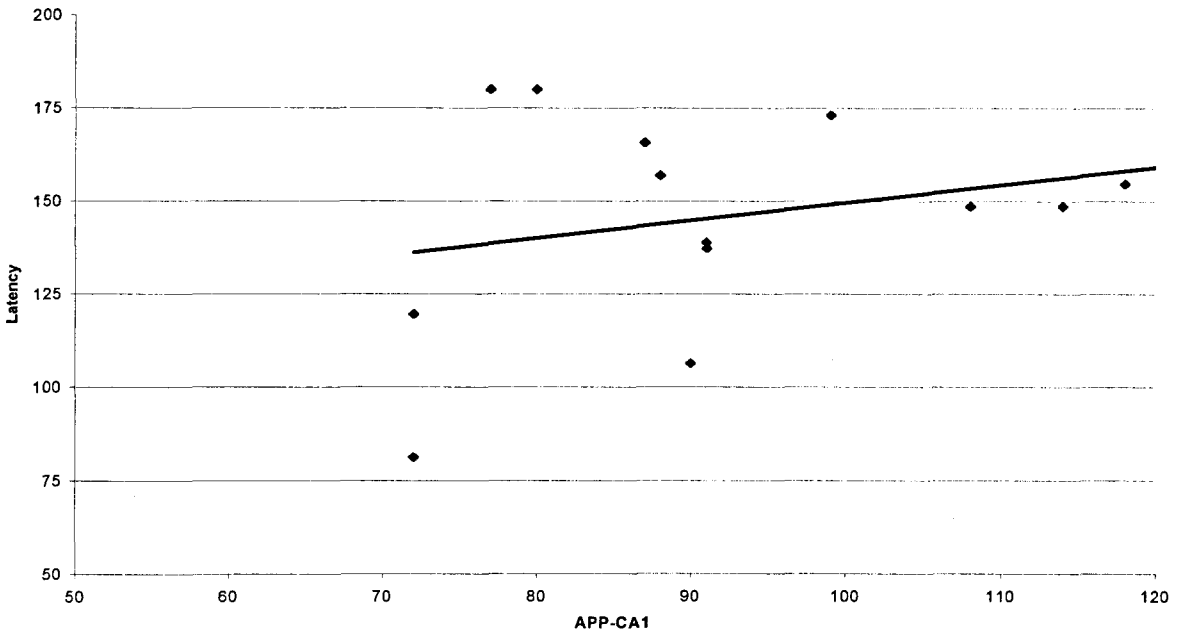

200
175
150
125
100
75
50
Latency
50
60
70
80
90
100
110
120
APP-CA1

Figure 7.

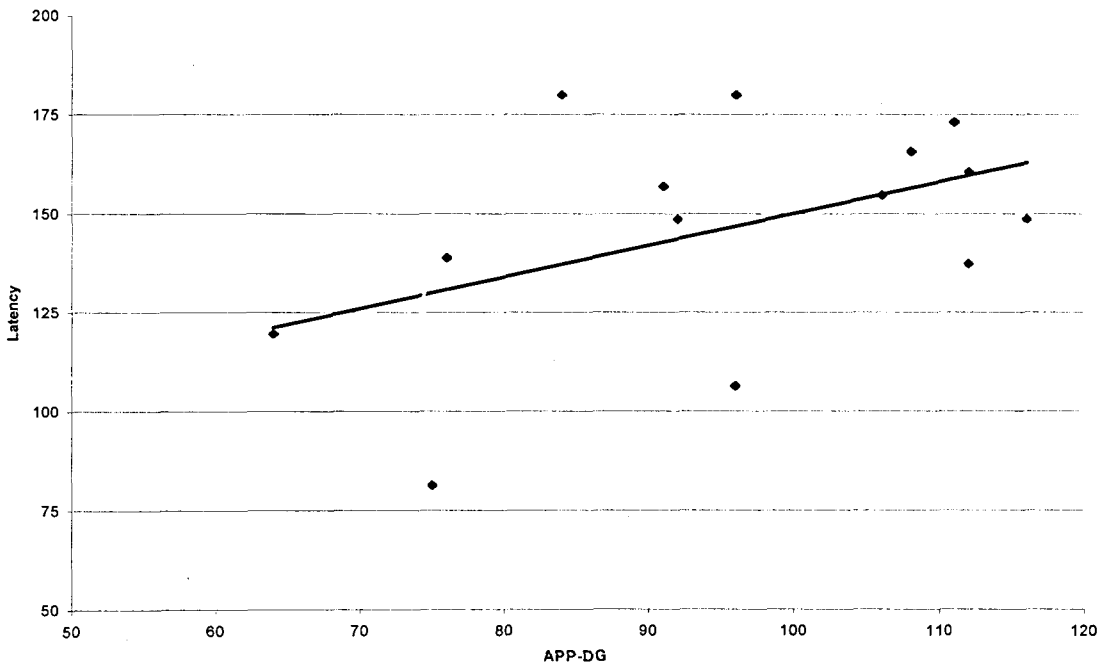

200
175
150
125
100
75
50
Latency
50
60
70
80
90
100
110
120
APP-DG

Figure 8

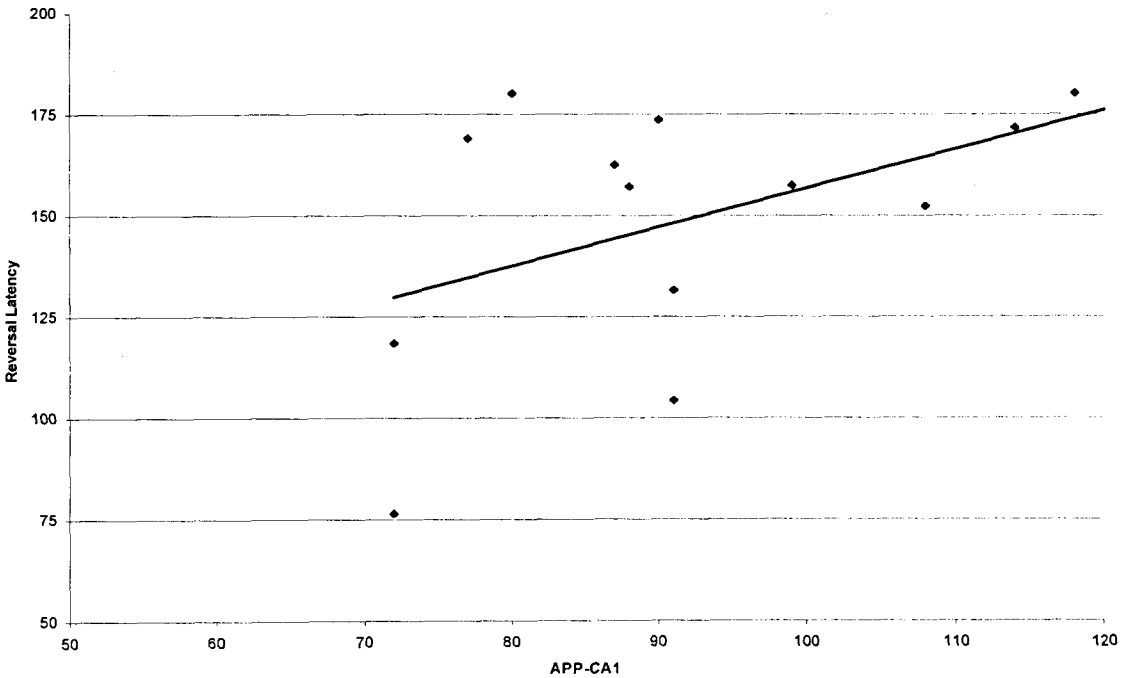

200
175
150
125
100
75
50
Reversal Latency
50
60
70
80
90
100
110
120
APP-CA1

Figure 9

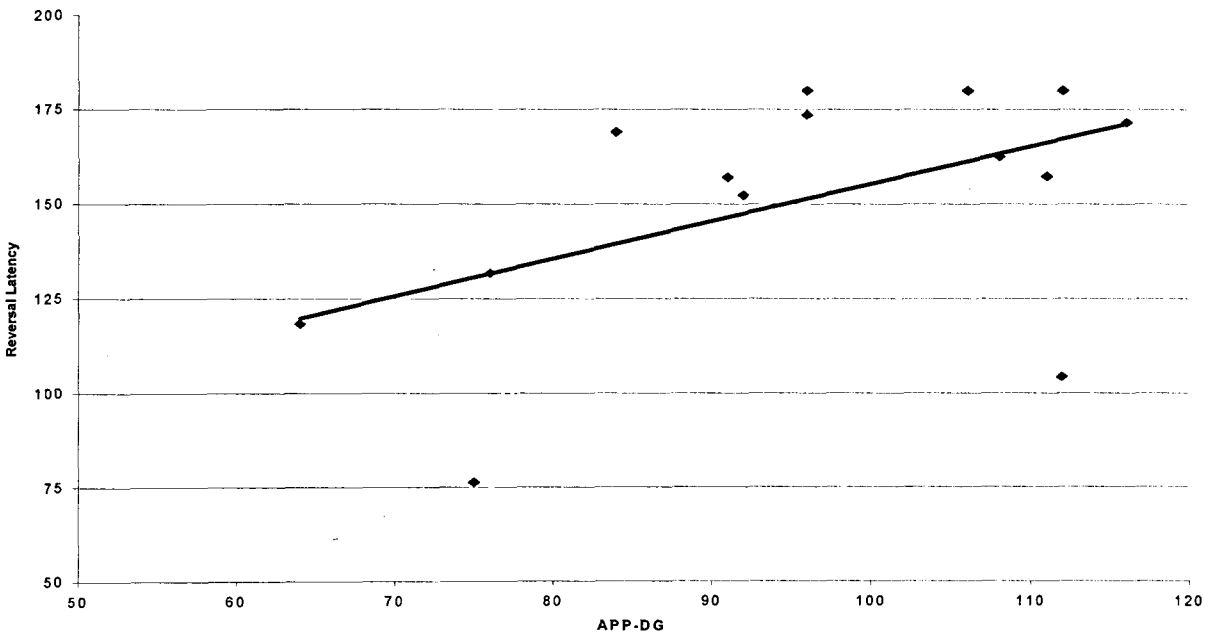
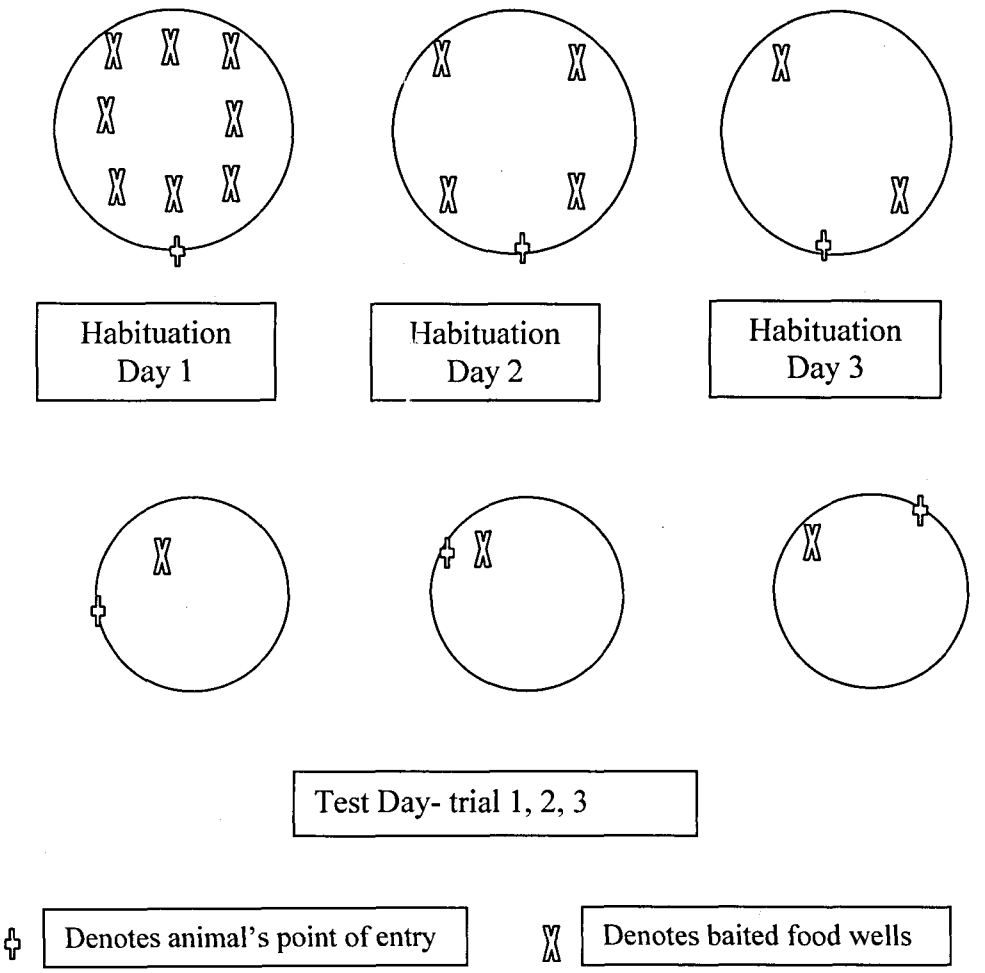

200
175
150
125
100
75
50
Reversal Latency
50
60
70
80
90
100
110
120
APP-DG

Figure 10: Maze Organization across days and trials



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Publications:

- Keyser,L., Stafisso-Sandoz,G., Gerecke, K., Jasnow, A., Gatewood, J., Kinsley, C. Alterations of medial preoptic area neurons following pregnancy and pregnancy- like steroidal treatment in the rat. Brain Research Bulletin. 55(6).737-745.
- Kinsley,C., Trainer, R., Stafisso-Sandoz,G., Quadros, P., Gatewood, J., Lambert, K. Motherhood modifies concentrations of hippocampal neuronal dendritic spines. (*In preparation*)
- Gatewood, J., Eaton,M., Madonia,L., Lambert,K., Kinsley,CH. Reproduction-Facilitated Aging: Parity and Pup Stimulation May Forestall some Aspects of Senescent Memory Loss. *(In preparation)*

Abstracts and Meeting Presentations:

- Gatewood,J., Madonia,L., Lambert,K., Kinsley,C. Reproduction-Facilitated Aging: Parity and Pup Stimulation May Forestall some Aspects of Senescent Memory Loss. Poster Presentation :Society for Neuroscience (November 2001).
- Lambert.K., Fischer-Stenger,K., Devries,AC., Glasper,E., Lin,S., Gatewood,J., Kinsley,CH. Social contact during chronic stress modulates stress responsivity and immunological functioning in Peromyscus californicus. Poster presentation :Society for Neuroscience (November 2001)
- Glasper,E., Lambert,K., Gatewood,J., Kinsley,CH. Behavioral and Neurobiological Correlates of the addiction syndrome: Effects of maternal experience on withdrawl – induced changes in mesolimbic activity. Presented at the annual meeting of the International Society for Behavioral Neuroscience, Cancun Mexico. (April 2001).
- Madonia,LF., Amory,EA., Wartella,J., Lambert,K, , Gatewood,J., Kinsley,C. Neurogenesis and neural behavioral stress responsiveness are altered by motherhood and or pup exposure. Poster presentation: Society for Neuroscience,(November 2000).