University of Richmond
UR Scholarship Repository

Master's Theses | Student Research

8-1999

Glial cell alterations in the rat medial preoptic area and hippocampus as a function of reproductive state

Gordon Wentworth Gifford

Follow this and additional works at: http://scholarship.richmond.edu/masters-theses

Recommended Citation
Gifford, Gordon Wentworth, "Glial cell alterations in the rat medial preoptic area and hippocampus as a function of reproductive state" (1999). *Master's Theses.* Paper 625.

This Thesis is brought to you for free and open access by the Student Research at UR Scholarship Repository. It has been accepted for inclusion in Master's Theses by an authorized administrator of UR Scholarship Repository. For more information, please contact scholarshiprepository@richmond.edu.

Abstract

Glial Cell Alterations in the Rat Medial Preoptic Area and Hippocampus as a Function of Reproductive State

Gordon Wentworth Gifford III

Master of Arts in Psychology

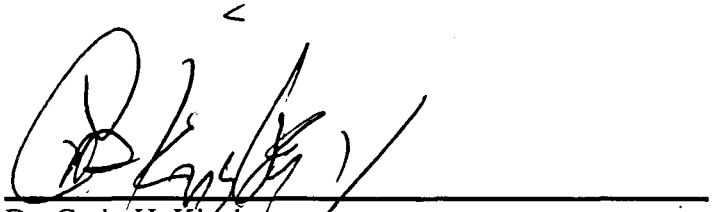
University of Richmond

1999

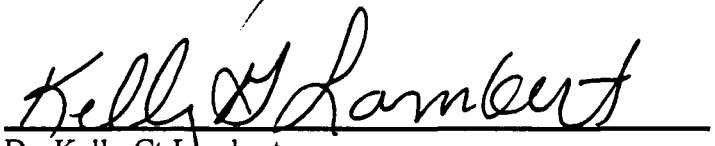
Dr. Craig Howard Kinsley

Changes in reproductive state are accompanied by fluctuating levels of female gonadal hormones at higher levels and for longer periods of time than the normal estrus cycle. These hormones have been noted, in cell culture and over the regular estrus cycle, to influence structural changes in neurons and glial cells in areas of the brain associated with the conduct of maternal behavior. The purpose of this project was to characterize changes in GFAP immunoreactivity and astrocyte morphology in the mPOA and hippocampus as a function of reproductive state and female steroid hormone treatment. Results of this investigation noted an increases of GFAP expression as a function of reproductive state associated with greater degrees of circulating hormones. Stereological cell counts reveled no increase in the number of cells expressing GFAP among these treatment conditions. Morphological analyses of process length and astrocyte classifications were identified as the source of increased levels of GFAP expression. Morphological analysis also revealed a greater representation of astrocytes with large numbers of processes and longer processes in those treatment conditions that received higher levels of circulating female steroid hormones.

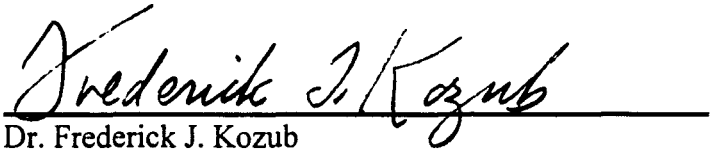
I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Arts.



Dr. Craig H. Kinsley



Dr. Kelly G. Lambert



Dr. Frederick J. Kozub

Glial Cell Alterations in the Rat Medial Preoptic Area and Hippocampus as a Function of Reproductive State

By

Gordon Wentworth Gifford III

B.A., Hampden-Sydney College, Hampden-Sydney, Virginia, 1997

A Thesis

Submitted to the Graduate Faculty

of the University of Richmond

in candidacy

for the degree of

Master of Arts and Sciences

in

Psychology

LIBRARY
UNIVERSITY OF RICHMOND
VIRGINIA 23173

August, 1999

Richmond, Virginia

Acknowledgements

Dr. Craig Kinsley for his strong mentor ship throughout this project. His insight fullness, patience, inspiration, support and sense of humor were all appreciated through out the completion of this work. I hope this and future work allows you see how much I have benefited from your efforts.

My wife Rebecca LaFleur for her sacrifice of long nights away from me while I was working in the lab and her endless ability to reenergize this effort during our time spent outside of Richmond Hall.

Dr. Aron Lichtman for providing me a job, understanding often when the pressures of graduate student life distracted me from our work together and introducing me to Dartmouth and the Medical College of Virginia.

Dr. Kelly Lambert for her help with my frustrated inquiries into Immunocytochemistry and glia morphology.

Dr. Fred Kozub for always providing an interesting insights and assisting me in approaching this project with a healthy level of skepticism.

Mom and Dad for helping me to reach self sufficiency and always being there when I needed your support.

Glial Cell Alterations in the Rat Medial Preoptic Area and Hippocampus as a Function of Reproductive State

Maternal responsiveness to the needs of offspring is necessary for the proper development and perpetuation of most species. Investigations into the expression of maternal behavior (MB) indicate that these activities are innate in origin and biologically dependent upon endocrine mechanisms. Bridges (1984) noted the role estradiol and progesterone play in the maintenance of pregnancy and MB by showing that the manipulation of these two hormones in rats has a direct effect on maternal responsiveness and the timing of its onset. These same mechanisms also play a role in human nurturing behavior, making new mothers more attracted to their newborn infants (Fleming et al., 1997a) and more responsive to the needs of their young (Fleming, et al., 1997b). Elicitation of MB by endocrine state occurs because of the effects of such hormones on the activity of specific areas of the brain. Placental prolactin secretion, and its stimulatory effects on neuronal activity within the medial preoptic area (mPOA) of the hypothalamus in rats, has been proven to play a critical role in priming the brain for MB at the end of gestation (Bridges, 1996). The trophic effect of estrogen in hippocampus (Trainer, et al., 1997) has also been found to coincide with improvements in the execution of spatial function as a result of reproductive experience (Madonia, et al., 1998). The effects of estrogen, progesterone, and prolactin, however, are not limited to a simple elevation of activity within the brain. These reproductive hormones also alter the morphology and physiology of neurons and supporting glial cells. The alteration of neurons by hormones is well established in the literature but , similar alterations within the neuroglia support are under represented. It is the goal of this

project to address this lack of data by quantifying changes in number and morphology of glial cells that occur as a function of the effects of reproductive hormones.

Maternal Behavior and Neuronal Plasticity

Until recently, the widely held view of neural plasticity stated that the brain is relatively unchanging after the completion of adolescent development (McEwen & Woolly, 1994). Naftolin, et al. (1993) have shown, however, that estrogen induces synaptic plasticity in neuron cultures collected from primates well into adulthood. A growing body of evidence demonstrates that brain sites responsible for the expression of MB have the same potential for adaptive change that continues well after adolescence. The medial preoptic area (mPOA) of the hypothalamus and the hippocampus (HI) are two such brain locations that have proven to be sensitive to the fluctuations in hormones that accompany changes in reproductive state (Keyser, et al, 1995; Trainer, et al., 1997). These areas are also critical for the correct execution of MB (Numan, 1994; Bridges & Freemark 1995).

The rat mPOA, analogous to the preoptic area in the human brain, plays an important role in the expression of MB. The necessity for focusing on the mPOA as a center for hormone activity was first suggested because it exhibited morphological sex differences in size (Gorski, et al., 1978), neurogenesis during development (Jacobson & Gorski, 1981) and the dendritic structures of its neurons (Ayoub, et al., 1983). Research into the behaviors governed by the mPOA have shown that axon sparing lesions of this area in female rats enhance mating receptivity while also decreasing behaviors that initiate such encounters (Hoshina, 1994). When exploring MB, electrolytic and chemical lesions of the mPOA cause disruptions in activities such as pup retrieval, nest building, crouching behavior and nursing (Numan, 1994). The mPOA also

appears to be one site at which reproductive hormones activate MB. Bridges and Freemark (1995) showed that the initiation of MB could be greatly influenced by the manipulation of prolactin levels in the mPOA. Similar modifications of MB have been noted with the manipulation of estrogen (Numan, Rosenblatt and Komisaruk, 1977) and progesterone (Bridges, et al. 1990) levels in the mPOA. Mechanisms explaining the influence of these hormones center on the binding of reproductive hormones on to nuclear receptors of neurons in the mPOA. This signal then influences protein and neurotransmitter production and the sensitivity of neurons to hormones and neurotransmitters as a result of receptor production (Numan, 1994).

The activational effects of reproductive hormones allow for a unique situation in which the mPOA could initiate a course of development well after adolescence. Garcia-Segura, et al. (1994b) noted that the effects of female gonadal hormones in hormonally sensitive areas of the brain include changes in the nuclear volume of neurons, dendritic morphology, innervation patterns and the number of synaptic inputs into neurons. Other research on the influence of reproductive hormones on morphological changes of neurons of the hypothalamus center on remodeling of synaptic connections (Naftolin, et al., 1996a). Naftolin, et al. (1996b) showed that high levels of circulating estrogen were responsible for a retraction of synaptic connections in an area of the brain posterior to the mPOA in the hypothalamus. This area of the hypothalamus named the arcuate nucleus, controls sex specific behaviors involved in mating. Duenas, et al. (1996) has also shown that estrogen, in conjunction with insulin-like growth factor-I, is one signaling pathway controlling the differentiation of hypothalamic neurons. Information on the direct effects of reproductive hormones on neurons of the mPOA is sparse. Keyser, et al. (1995) noted an increase in somal size of neurons of the mPOA during pregnancy and pregnancy

hormone treatment. These changes in neuronal and synaptic structure indicate that the hormonal fluctuations that accompany pregnancy and motherhood could give rise to changes in female brain morphology that ultimate influence MB. The precedent for morphological changes in the brain finds further support when looking at the HI, which has been noted to be involved in the expression of MB.

The role of the HI in spatial learning has been repeatedly proven in contexts ranging from the navigation of a Morris water by rats (Lalonde, 1997) to the recall of city layouts by taxi drivers (Maguire et al., 1997). This function in spatial activity also translates to the execution of MB. Numan (1994) noted that lesions of the HI in rats have a profound impact on the organization of MB toward pups. The behavior of these lesioned females is characterized by the making of many small nesting areas and a remarkable difficulty in finding and relocating all of their pups into one location (Kimble, et al., 1967). Often the disorganization of these activities results in the neglect of other maternal activities. In accordance with the hormonal regulation of MB, the HI also exhibits sensitivity to reproductive hormones. Woolley and McEwen (1992) have established that estrous cycle dependent estradiol concentrations are associated with fluctuations of synapse density in the HI as well as dendritic spine density (Woolley & McEwen, 1993). Hippocampal synapse density and dendritic spine density both appear to increase when the presence of estrogen is at its highest and decrease with the presence of progesterone (Woolley & McEwen, 1994). The hormonal regulation of pregnancy and MB causes a large increase in amount and duration of estrogen and progesterone exposure in the HI (Bridges, 1984). Trainer, et al. (1997) identified pronounced changes in dendritic spine concentrations, as a function of reproductive hormones that accompany reproductive state. This study also

provided evidence that increases in spine density may be permanent after pregnancy and the cessation of maternal care (Trainer, et al., 1997). The effect of these permanent changes have also been shown to affect how new mothers approach spatial learning tasks and to improve their ability to navigate a radial arm maze over virgin counterparts (Madonia, et al., 1998).

The above experimental findings support the view that fluctuating levels of reproductive hormones influence neurons in areas of the brain necessary in the proper expression of MB. The evidence that increased levels of reproductive hormones during pregnancy exaggerate these changes and make them more permanent is still limited but confirmatory of the idea that neurons make accommodations in accordance with the greater demands that nurturing of young requires. These increases in hormonal sensitivity and morphological changes in neurons indicate greater intercellular communication and are well established. Little investigation, however, has been given into the development of glial cells that support this increased neural activity during pregnancy and motherhood and could aid in the growth and guidance of neurons in the developing maternal brain.

Endocrine Sensitive Neuroglia

Kandel and Shwartz (1985) state that there are 10 to 50 times more glia than neurons in the central nervous system. Glial cells are divided into two categories macroglia, such as astrocytes and oligodendrocytes, and microglia. These cells support the function of neurons in the brain and have proven to be sensitive to female gonadal hormones in many aspects of their activity (Garcia-Segura et al, 1996b).

Microglia play a crucial role in immune response to brain injury. Estrogen and progesterone exposure have proven to both aid in this process of repair and removal of injured

neurons (Bucala, 1996) and to inhibit overactive non-injury induced immune response of microglia toward aging neurons in older animals (Ganter et al., 1992).

Oligodendrocytes, a form of glial cell that generate the myelin coating around axons, have proven to perform mylenization at an accelerated rate when exposed to progesterone (Chan et al., 1998).

Astrocytes, a type of glial cell found in the brain, have been shown to help maintain concentrations of hormones like progesterone (Jung-Testas et al., 1989a; Jung-Testas, et al., 1989b), use female gonadal steroids to modulate levels of the electrolyte potassium (Del Cerro et al., 1996) and to increase levels of various neural growth factors (Ma & Ojeda, 1994). Astrocytes also use female gonadal hormones to regulate estrous cycle activity in the hypothalamus (Ma et al., 1997) by covering or revealing neuronal surface area to control the number of synaptic connections between neurons (Kohama et al., 1995). The process through which radial glia, a specialized type of astrocyte, direct the growth and migration of neurons during brain development (Wang et al., 1994) has also been shown to be dependent upon hormonal levels in the brain (Hatten 1990; Ma & Ojeda, 1997). Other homeostatic functions of astrocytes include helping in the maintenance of cerebral temperature (Szelenyi, 1998), keeping levels of extracellular neurotransmitters constant during synaptic and gap-junctional communication (Giaume & McCarthy, 1996) and providing nutrition to the neurons throughout the brain (Kandel & Shwartz, 1985).

The effects that female gonadal hormones have on glia function indicate that these cells are most likely sensitive to similar reproductive hormones via a number of mechanisms. The work of Garcia-Segura and colleagues has well established both direct and indirect actions of

reproductive hormones on the functioning of glia in the hypothalamus and HI (Garcia-Segura et al, 1996a).

Jung-Testas & Baulieu (1998) have shown that glia, collected from the central nervous system of the rat, contain hormone receptors for progesterone and estrogen. When examining the effects of these hormones on glia proliferation progesterone was found to have an inhibitory effect while estrogen had the opposite effect (Jung-Testas, et al., 1994). Tranque, et al. (1987) used the antibody marker glial fibrillary acidic protein (GFAP), which stains type 1 and type 2 astrocytes as well as some forms of oligodendrocytes (Garcia-Segura et al., 1996a), to investigate the effects of estrogen exposure on hormone sensitive areas of the adult female rat brain. This study found that estrogen exposure had no effect on GFAP expression in the arcuate nucleus of the hypothalamus, but did significantly increase GFAP expression in the HI without having an effect on the number of astrocytes (Tranque et al., 1987). Research in culture exploring the specificity of hormone receptor expression in types of glia has established that estrogen receptors are present and equally represented in all types of glia stained by GFAP, specifically type 1 and type 2 astrocytes and oligodendrocytes (Santagati et al., 1994). Jung-Testas, et al. (1992) found similar evidence indicating progesterone receptors in these same types of glial cells and also discovered that progesterone receptors significantly increase when brought up in culture with estrogen (Jung-Testas et al., 1991). Evidence linking exposure of hormones to GFAP expression in culture is scarce, but Melcangi et al. (1996) have shown that progesterone exposure to type 1 astrocytes increases expression of GFAP.

Reproductive hormone dependent GFAP expression of astrocytes of the hypothalamus centers on a neuronal mediated mechanism (Garcia-Segura et al., 1995). This finding is one

explanation given for the sexually dimorphic expression of GFAP in this area of the brain (Chowen et al., 1995), as well as the fluctuating expression of GFAP over the estrous cycle in female rats (Torres-Aleman et al., 1992). Garcia-Segura (1994a) observed that increases in GFAP expression in the female rat arcuate nucleus of the hypothalamus during the afternoon of proestrus and the morning of estrous. These increases in GFAP were not due to increases in the number of astrocytes, but were related to the surface area of glial cells and the amount of neuronal surface area these cells covered. Torres-Aleman et al. (1992) also noted that exposure to estrogen caused arcuate nucleus astrocytes to change morphology from a flattened shape to more bipolar and stellate forms that had greater surface areas. Exposure of these astrocytes to progesterone, however, blocked the trophic effects of estrogen. This increase in astrocyte GFAP expression, however, only occurs in mixed cultures of hypothalamic neurons and astrocytes and does not occur in pure cultures of glial cells (Garcia-Segura et al., 1989). This finding indicates that the morphological effects of estrogen on hypothalamic astrocytes are regulated via a neuronal mechanism.

Little work investigating reproductive hormone responsive astrocytes has been focused on the mPOA. There is some evidence that a more direct mechanism may control GFAP expression in the mPOA. Langub & Watson (1992) have shown the presence of estrogen receptors on GFAP immunoreactive glia in the preoptic area of guinea pigs. There is no available literature that generalizes Langub's and Watson's (1992) findings to the female rat mPOA.

The pattern of glial cell reactivity to reproductive hormones in the HI follows a more direct hormone receptor mechanism. Day et al. (1993) have investigated the role of female

gonadal steroids in the development of glial cells in the HI. They have shown that manipulation of hormone levels in the brain can influence the expression of glial fibrillary acidic protein (GFAP) messenger RNA. The influence of estrogen and progesterone on GFAP expression in the dentate gyrus of the HI is similar to that found in the hypothalamus. This activity is characterized by a greater expression of GFAP that occurs without an increase in cell numbers during the afternoon of proestrus and the morning of estrous (Lunquin et al., 1992). Investigations of the mechanisms regulating the trophic effects of estrogen indicate a mechanism different than what is observed in the hypothalamus may be at work. Unlike hippocampal glia, pure cell cultures of hippocampal astrocytes express an elevation in GFAP (Lunquin et al., 1993). When progesterone is added to these cell cultures over the short term there is also no inhibition of the effect of estrogen on increased GFAP expression. These findings would suggest that estrogen influence over hippocampal astrocytes is a receptor mediated mechanism.

Astrocyte development within the maternal brain is worthy of research given the wide variety of ways glial cells interact with neurons and thus effect the expression of MB. In summary, glial cells perform a number of support functions within the brain that are also under the influenced by reproductive hormones. Glial cells found within the hypothalamus and the HI each have different response mechanisms to for the hormones estrogen and progesterone and both show fluctuations in the expression of GFAP over the normal estrous cycle. The changes in GFAP expression in both the HI and hypothalamus are attributed to changes in astrocyte morphology and not to changes in cell number. While these findings give a solid picture of adaptive glial cell changes to a normally cycling hormonal environment in the female rat, there is

little published work on the effects of increased and prolonged exposure to reproductive hormones that accompany pregnancy. There is also little information on the effects of reproductive hormones on the expression of GFAP in astrocytes of the mPOA. Recent preliminary findings from our own laboratory suggest that the number and morphology of astrocytes in the mPOA change as a function of reproductive state (Gifford & Kinsley, 1998). The above insights into the effects of reproductive hormones on astrocyte morphology lead to a number of interesting questions when attempting to understand the action of reproductive hormones on glial cells in areas of the brain responsible for the expression of MB.

Focus of Thesis

The goal of this project was to identify the effects of differing levels of reproductive and female steroid hormones on the morphology of glial cells.

The first priority in this study was the identification of a differential expression of GFAP in the mPOA and HI between females in a virgin, late pregnant and lactating state. After exposure to high levels of both estrogen and progesterone, astrocytes in pregnant females and lactating females should have a more robust expression of GFAP when compared to their virgin counterparts. Given the different mechanisms involved in the hormone guided changes in glia morphology within the HI and the mPOA, GFAP expression should also be distinct between these two structures.

The second inquiry centered on preliminary finding that increased expression of GFAP, in brains exposed to reproductive hormones, is not accompanied by the presence of more

neuroglia. The expression of maternal behavior coincides with a large increase in the activity of the mPOA and HI. The greater metabolic activity of neurons during pregnancy and maternal care should require a more efficient or more extensive neuroglia support structure. This disparity in expectations for glial cell proliferation necessitated an investigation into the number of glial cells present in the mPOA and HI during the virgin, pregnant and lactating states.

The third issue addressed in this study was the necessity to characterize any changes in the morphology of astrocytes that occur in the mPOA and HI after prolonged exposure to reproductive hormones. If the increased metabolic demands of the mPOA and HI during maternity are not served by an increase in the number of glia, then it is crucial to understand what modifications are being made in individual glial cells to address the more robust needs of neurons in these two areas of the brain. Estrogen has proven to have a trophic effect on astrocytes of the hypothalamus and HI. Progesterone has been shown to have a differential effect on the expression of GFAP in these two areas. If differences in GFAP expression were to be found between virgin, late pregnant and lactating females it would also be necessary to note if these changes are accounted for by morphological differences across these three differing hormonal states.

The final concern addressed by this study was to identify if any observed differences across groups were attributable to the differing levels of circulating hormones in the virgin, late pregnant and lactating states. The trophic effects of female gonadal hormones should result in a greater degree of GFAP-IR staining in ovariectomized females given a hormone regimen mimicking pregnancy over females that have had their ovaries removed. It also stands to reason

that any changes in glial cell number or morphology found across reproductive treatments could be generalized to a comparison of hormone deficient and hormone supplemented females.

Methods

General methods

Subjects. Forty (N=40) adult nulliparous female Sprague-Dawley rats were assigned to one of five age matched groups in accordance with a procedure by Bridges (1984): Intact diestrous virgins (n=8) (group Diestrous) who were sacrificed and had their brains removed on the day of diestrous; virgin, ovariectomized and implanted with blank Silastic capsules (n=8) (group OVX-); virgin, ovariectomized and implanted with Silastic capsules containing progesterone and estradiol (n=8) (group OVX+); late pregnant, day 21 (n=8) (group Late-pregnant); and day six postpartum lactating (n=8) (group Lactating). All animals were single housed in a light (12:12hr) and temperature (23° C) controlled room. These animals were left undisturbed except during routine cage cleaning and feeding.

Surgery and immunocytochemistry (ICC). All female subjects, at prescribed date of sacrifice, were overdosed on pentobarbital sodium. The animals were then profused for 2-3 minutes with cold phosphate buffered saline (PBS) and then 4% paraformaldehyde (PF) for 20-24 minutes. The brains were then removed and placed in 4% PF for 2 hours and then stored in 20% sucrose in PBS at 4° C for between 1 and 7 days until the day of brain sectioning.

On the day of sectioning, the brains were blocked and mounted for the area containing the mPOA and HI. In a Zeiss cryostat chamber brains were serial sectioned at 50 μ. Brain sections were then given a standard regimen of washes: 3 times in PBS for 10 minutes at room

temperature on a cell well shaker. After washing, brain sections were then exposed to a 1.25% nonfat dry milk in PBS solution for 1 hour at room temperature on shaker. The nonfat dry milk solution were then removed and replaced with 1:40,000 dilution of rabbit anti-GFAP (DiaSoren Inc., Stillwater, MN) in .3% Triton-X/PBS solution. Brain sections remained in primary antibody for 12-24 hours at 4° C.

After exposure to primary antibody was completed, sections were washed and then exposed to biotinylated secondary antibody at a 1:200 dilution in PBS (Vector Labs, Burlingame, CA) for 1 hour at room temperature on a shaker. Sections were then washed once again and then incubated in an avidin-biotin complex, (ABC) (Vector Labs) that has was prepared according to Vector Labs instructions, for one hour at room temperature. After incubation, sections were then washed and exposed for 3-5 minutes to a solution of DAB (.05%), NiSO4 (.008%), and 33 µl of H2O2 in 100ml of PBS. Sections were then washed in distilled water and stored at 4° C until mounted on slides. Brain sections were mounted on chrome-alum coated slides and set under a fume hood to dry. Slides were then hydrated for 1 minute with distilled water and then cleared with a series of solutions: 70% alcohol for 3 minutes; 90% alcohol for 3 minutes; 100% alcohol for 3 minutes and Xylene for 2 minutes. After clearing slides were then cover slipped using Permount and be set out to dry for 3 - 5 days.

Experiment 1: Natural reproductive state

Subjects. Animals assigned to the Virgin female group were killed during diestrous and had their brains removed.

Animals assigned to the Late-pregnant and Lactating groups were time mated. A morning vaginal lavage was used to determine if mating occurred the evening before. The

presence of sperm in the lavage fluid was used to indicate mating and the date of this observation was recorded as the first day of pregnancy.

Late-pregnant animals were killed on day 21 of pregnancy and their brains were collected. Lactating females had their litters culled to six pups, which then remained with the female. The Lactating group was killed on day six postpartum and their brains were collected.

Experiment 2: Female steroid hormone manipulation

Silastic capsule preparation. The Silastic implants were prepared using a procedure adapted from Bridges (1984). The implants were constructed from tubing (#602-305) purchased from Dow-Corning (Midland, MI). The implants were packed with crystalline steroid (progesterone or estradiol; Sigma, St. Louis, MO) and the ends sealed with Silastic cement. After drying, the capsules were washed in ethanol and incubated for 24 hours in physiological saline at room temperature to insure stable steroid infusion would commence immediately upon implantation.

Subjects. For the OVX+ group, females had their ovaries removed under Metophane inhalation anesthesia and were then implanted subcutaneously with steroid-containing Silastic capsules in a regimen that stimulates MB (Bridges, 1984): The females were implanted with three 30mm, Silastic capsules containing progesterone; the capsules remained for 11 days, at which time they were removed and replaced with a 2mm estradiol containing capsule which remained for ten days.

Females in the OVX- group had their ovaries removed under Metophane inhalation anesthesia and were implanted with blank capsules at the same times experienced by the OVX+

group. These groups were then killed and had their brains collected on day 21 after ovariectomy and primary capsule implantation.

Image analysis for mPOA

Thresholding analysis. BIOQUANT image analysis software was used to quantify the area of GFAP immunoreactive (GFAP-IR) staining for eight consecutive sections per animal of the mPOA. A box designating as containing the area of interest in mPOA was defined as a square 240 microns in width and 250 microns in height that created an area of 60,000 square microns at a magnification of 200x.

The view field box was located 800 microns, on the y-axis of the stage encoder, below the bottom of the anterior commissure and immediately right or left of the third ventricle wall as to exclude GFAP immunoreactive tissue on the boundary of the third ventricle and mPOA.

Identification of immunoreactive staining was accomplished by isolating a representative glial cell within the field of interest. The contrast of the bright field image was adjusted according to the observer's discretion to enhance the GFAP-IR astrocyte cell body and its processes. The threshold of determination between target staining and background was set to only label GFAP-IR within the field. Optimum threshold values varied from section to section and were adjusted according to the discretion of the observer. The data for each brain section were then recorded in both square microns and percentage of total area observed.

Stereological analysis. To determine differences in the number of astrocytes across each of the five treatment conditions, an unbiased count of GFAP-IR cells was conducted according to an adaptation of the well established point-counting method set forth by Weibel (1979).

This counting procedure was applied to the GFAP-IR stained profiles contained within the same field of interest used in the threshold analysis. A quantitative evaluation of surface density of GFAP-IR cells was estimated using the equation $S_d = I/L^2$, where S_d signifies the surface density of GFAP-IR astrocytes, I is the number of points a cell body crosses a stereological test grid and L is the length of lines contained within the grid. The stereological test grid used in this evaluation was based on the c16 grid of Weibel (1979). This grid was composed of 10 X 10 lines at 200μm for a total area of 40,000 μm^2 (L^2=40,000μm^2).

Morphological analyses. To determine differences in the morphology of GFAP-IR glia across groups two procedures set forth by Mong, et al. (1996) were applied to quantify process length and classify glia morphology.

Process length was quantified using the BIOQUANT length measurement tool. Three processes in three separate directions were measured per cell and the mean of the lengths was calculated. Four cells for each brain section were selected randomly for measurement. Four sections per brain were analyzed for a total of 16 cells per brain. Random selection consisted of four consecutive cells intersected by a horizontal stereological grid line. The grid line was chosen according to the output of a random number generator set for values between 1 and 10. In cases where fewer than three cells are found on a horizontal line the next line below was used.

The same four GFAP-IR cells selected for process measurement, were also selected for glia classification. Glia classification was determined using the three categories seen in Figure 1 and adapted from a procedure by Mong, et al. (1996). Class I cells were identified as having a bipolar appearance and only primary processes that are generally short and thick in appearance.

Class II cells were characterized as having a bipolar appearance and roughly 5 or more secondary processes. Class III cells were characterized as having a stellate appearance. Totals corrected for one square millimeter of brain area were then calculated for each of the four classification categories.

The experimenter was blind to the treatment condition for each set of brain sections while conducting the threshold analysis, stereological cell counts, and morphological analyses.

Image analysis for hippocampus

The region of interest for the HI, was identified by fully covering the medial most part of the HI with the view field. The view field was then moved laterally 1600 microns on the x-axis of the stage encoder and moved up to fully cover the peak of the CA1 region.

Threshold analysis, stereological cell counts, and morphological analyses for the HI were all conducted according to the same methods outlined for the mPOA. The experimenter was blind to the treatment condition for each set of brain sections while conducting these analyses.

Results

Experiment 1: Natural reproductive state

mPOA. The means and standard errors of each reproductive treatment condition for all analyses of the mPOA are contained in Table 1.

Figure 2 displays the mean expression of GFAP-IR in square microns that was recorded for the mPOA. One-way analysis of variance (ANOVA) revealed a significant difference among the treatment conditions, $F(2,381) = 42.326$, $p < .0001$. Post Hoc comparison in the form of a Fisher test showed significant differences between all groups. Late-pregnant females had the highest amount of GFAP-IR staining, followed by Lactating and Virgin females.

Figure 3 shows the mean number of glia estimated, per square millimeter of mPOA, by applying the point count method. One-way ANOVA failed to identify any significant difference among the reproductive treatment conditions, $F(2,93)= 0.183$, $p=$ n.s.

Figure 4 exhibits mean process lengths recorded for glia observed in the mPOA. One-way ANOVA discerned a significant difference among treatment conditions, $F(2,381)= 61.325$, $p<.0001$. A Fisher Post Hoc analysis noted a significant difference between all groups. The Lactating group had the longest process length, followed by the Late-pregnant and virgin groups.

Figure 5 presents the mean number of class I, II and III astrocytes observed out of sixteen samples taken for each brain. One-way ANOVA revealed significant differences among groups for class I, $F(2,21)= 28.916$, $p<.0001$, class II, $F(2,21)= 7.753$, $p<.01$, and class III cells, $F(2,21)= 21.181$, $p<.0001$.

A Fisher test revealed a number of significant mean differences for the above analyses. Diestrous virgin females had significantly more class I cells than their Lactating and Late-pregnant counterparts. There was no significant difference in the number of class I cells between the Lactating and Late-pregnant groups. Virgin females showed significantly less class II glia than the Late-pregnant and Lactating groups. The Late-pregnant and lactating groups showed no significant difference between each other. All three treatment conditions were found to be significantly different from each other for class III astrocytes. No class III astrocytes were found in the Virgin group and the Late-pregnant females had fewer cells than the Lactating females.

Hippocampus. The means and standard errors of each reproductive treatment condition for all analyses of the HI are contained in Table 2.

Figure 6 displays the mean expression of GFAP-IR in square microns that was recorded for the HI. One-way ANOVA revealed a significant difference among the treatment conditions, F (2,381)= 9.919, p<.0001. Post Hoc comparison, in the form of a Fisher test, showed significant differences between the Lactating and Virgin groups and the Lactating and Late-pregnant groups. No mean difference was found between the Virgin and Late-pregnant groups.

Figure 7 shows the mean number of glia estimated, per square millimeter of HI, by applying the point count method. One-way ANOVA identified a significant difference among the reproductive treatment conditions, F(2,93)= 0.183, p<.0001. The Fisher Post Hoc test indicated that only the Lactating group was significantly smaller than both the Late-pregnant and Diestrous Virgin groups.

Figure 8 exhibits mean process lengths recorded for glia observed in the HI. One-way ANOVA discerned a significant difference among treatment conditions, F(2,381)= 20.828, p<.0001. A Fisher Post Hoc analysis noted a significant difference between individual groups. The Lactating group had the longest process length, followed by the Virgin and Late-pregnant groups.

Figure 9 presents the mean number of class I, II and III astrocytes observed out of sixteen samples taken for each brain. One-way ANOVA revealed significant differences among groups for class I, F(2,21)= 3.664, p<.05, class II, F(2,21)= 17.902, p<.0001, and class III cells, F(2,21)= 26.077,p<.0001.

A Fisher test revealed a number of significant mean differences for the classification analyses. Late-pregnant females were found to have significantly more class I cells than their Lactating counterparts. There was no significant difference in the number of class I cells

between the Lactating and Virgin and Late-pregnant and Virgin groups. Both the Virgin and Late-pregnant groups showed significantly more class II glia than the Lactating groups. No significant difference existed in the number of class II astrocytes between the Late-pregnant and Diestrous Virgin females. Lactating females were found to have the highest number of class III glia. No mean difference was found between the Virgin and Late-pregnant groups.

Experiment II: Female steroid hormone manipulation

mPOA. The means and standard errors of the two hormone treatment conditions for all mPOA analyses are contained in Table 3.

Figure 10 exhibits the mean expression of GFAP-IR in square microns that was recorded for the two female steroid hormone treatments in the mPOA. One-way ANOVA indicated that the OVX+ hormone group had significantly more GFAP-IR staining than the OVX- group, $F(1,254)= 28.909$, $p<.0001$.

Figure 11 displays the mean number of glia per square millimeter of mPOA, estimated by the point count method. One-way ANOVA failed to identify any significant difference between the two hormone treatment conditions, $F(1,62)= 0.014$, $p=$ n.s.

Figure 12 presents mean process lengths recorded for glia observed in the mPOA. One-way ANOVA noted the OVX+ group had significantly longer process lengths than the OVX- group, $F(1,254)= 29.33$, $p<.0001$.

Figure 13 shows the mean number of class I, II and III astrocytes observed out of sixteen samples taken for each brain. One-way ANOVA indicated that the OVX+ group had significantly less class I astrocytes, $F(1,14)= 11.930$, $p<.01$, and more class III astrocytes,

$F(1,14)=74.667$, $p<.001$. No mean difference was found for the class II glia, $F(1,14)=3.431$, $p=$ n.s.

Hippocampus. The means and standard errors of the two hormone treatment conditions for all mPOA analyses are contained in Table 4.

Figure 14 exhibits the mean expression of GFAP-IR in square microns that was recorded for the two hormone treatments in the HI. One-way ANOVA indicated that the OVX+ hormone group had significantly more GFAP-IR staining than the OVX- group, $F(1,254)= 29.078$, $p<.0001$.

Figure 15 displays the mean number of glia per square millimeter of mPOA, estimated by the point count method. One-way ANOVA failed to identify any significant difference in the two hormone treatment conditions, $F(1,62)= 0.149$, $p=$ n.s.

Figure 16 presents mean process lengths recorded for glia observed in the mPOA. One-way ANOVA noted the OVX+ group had significantly longer process lengths than the OVX- group, $F(1,254)= 6.337$, $p<.0001$.

Figure 17 shows the mean number of class I, II and III astrocytes observed out of sixteen samples taken for each brain. One-way ANOVA indicated that the OVX+ group had significantly less class I astrocytes, $F(1,14)= 19.698$, $p<.001$. No effect was found for hormone treatment for the class II, $F(1,14)=0.187$, $p=$ n.s., or class III astrocytes, $F(1,14)=3.168$, $p=$ n.s.

Discussion

Medial Preoptic Area

The goal of this project was to identify any differential in the amount of GFAP immunoreactivity that may be caused by exposure to high levels of reproductive hormones and discover the cause of any noted changes. In accordance with the findings of Garcia-Segura, et al. (1996b) when exploring GFAP expression in the arcuate nucleus of the hypothalamus, it was hypothesized that there would be a significant increase in GFAP-IR in those groups that represented elevated levels of reproductive hormones. The results of each experiment focused on the mPOA fully supported this expectation.

The mPOA of those animals subjected to the reproductive treatments, the Late-pregnant and Lactating animals, had vastly larger amounts of GFAP staining compared to their Virgin counterpart. This effect appears to be largely due to the influence of female gonadal hormones when given the fact that ovariectomized females placed on a female steroid hormone replacement regimen mimicking pregnancy had similar increases in GFAP-IR expression over their ovariectomized counterparts.

In both the reproductive and female steroid hormone experiments, stereological assessment of the mPOA indicated that the hormone related increases in GFAP expression was not due to the increase in the number of glia serving the neurons of this area of the brain. This increase in GFAP-IR does appear to be a function of a change in the morphologies of glia represented when higher levels of hormones are present.

When looking at the classes of astrocytes present in the OVX+ hormones versus the OVX- groups, it is possible to see that the presence of hormones tend to coincide with a decrease in the number of class I astrocytes and an increase in the number of class III astrocytes. This effect is seen in an even greater degree when looking at the representation of class I and III

astrocytes across reproductive treatment conditions. The mPOA of Diestrous Virgin females is predominately inhabited by class I astrocytes while Lactating and Late-pregnant females show variation toward the more stellate appearances of the class II and III glia. Features of these stellate morphologies such as longer process length and greater number of processes appear to fully account for the differences in GFAP-IR staining of the mPOA. These results fit with the expected findings given the fact that Garcia-Segura, et al. (1996) noted the trophic effects of estrogen and progesterone on astrocytes in a mixed cell culture generated from the arcuate nucleus of the hypothalamus.

Hippocampus

A second goal of this experiment was to characterize how the direct action of female gonadal hormones affect the morphology of astrocytes located in the HI.

The effects of reproductive and female steroid hormones on glia morphology within the HI was expected to lead to a different pattern of GFAP expression than that witnessed in the mPOA. Del Cerro, et al. (1995) noted the trophic effects of estrogen in glia of the HI. Jung-Testas, et al. (1994) reported that progesterone has an opposite inhibitory effect on GFAP expression. When interpreting the results of hormonal state on GFAP expression in the HI the differential effects of these two hormones help explain this project's experimental findings.

It was hypothesized, despite the inhibitory effects of progesterone on GFAP–IR in the HI, that higher levels of reproductive and female steroid hormones should result in higher levels of GFAP expression. This hypothesis is supported by the greater amount of GFAP-IR staining found in the OVX+ hormone group when compared to the OVX- group.

Analysis of the reproductive treatments did not offer as clear a picture given the fact that no significant difference was found between the Late-pregnant and Diestrous Virgin groups. This lack of difference between the two groups could be attributable to the high levels of progesterone that circulate near the time of delivery. Increased levels of progesterone in the Late-pregnant group may have suppressed GFAP expression given the hormones atrophic effect on glia of the HI. This possibility does not equally explain why no suppression was found in the OVX + hormone group, especially when considering the hormone replacement regimen is designed to mimic the hormone levels of late pregnancy.

Another finding in the HI that did not fit well with the hypothesized results is the decrease in the number of cells counted in the lactating female. It is important to note that this decrease in cells counted is most likely due to a flaw in the counting procedure. The stellate appearance of these cells in largely represented in the Lactating treatment condition could have easily obscured smaller class I cells that normally would have been counted. The inability to count class I cells may have occurred because of the darker degree of staining and large number of processes of the stellate astrocytes. The true number of cells in this area of the brain for the Lactating female is most likely no different than it's Virgin and Late-pregnant counterparts.

When classifying the morphology of GFAP-IR glia of the HI across the stages of maternity and hormone treatments, the trophic effects of estrogen and, in some cases, progesterone to cells in culture (Melcangi et al., 1996) suggested that glia should assume a more robust stellate shape with extended exposure to reproductive hormones. This hypothesis was partially supported by the finding in the reproductive experiment that the lactating treatment had the largest number of class III astrocytes and the fewest number of class I glia. There was also

less class I cells in the OVX+ hormone group in the hormone treatment experiment. This confirmatory evidence, however, must also be considered in light of the fact that there was no clear pattern of the Late-pregnant group assuming a greater representation of stellate cells with exposure to hormones. In the case of the HI, the presence of receptors for reproductive hormones in hippocampal glia (Jung-Testas & Baulieu, 1998) are the most likely cause for the experimental findings not fitting well with the hypothesis. These cells are very sensitive to the hormonal environment, thus making it more difficult to predict their reaction to mixed hormone conditions. The findings of this experiment are not without precedent given the fact that the presence of progesterone alone has been noted to have an inhibitory effect on hippocampal GFAP expression, but also a trophic effect when paired with specific concentrations of estrogen (Jung-Testas, et al., 1994). The effects of progesterone could also be the cause of suppressed levels of GFAP activity witnessed in the HI of Late-pregnant females. If this were the case, however, one should expect suppression GFAP-IR in the similarly exposed OVX+ group. The limitations of this project made it difficult to identify all reproductive hormones that may be acting in this area of the brain due to experimental manipulation. These contradictory findings open the possibility to a mechanism beyond the influence of reproductive hormones used in this study that may have an effect upon GFAP activity in the HI.

In summary, this project did find support for the prediction that a differential in the expression of GFAP–IR staining is due to exposure to high levels of reproductive hormones. As predicted there was no significant increase in the number of GFAP-IR glia within the mPOA. Significant differences found among groups for hippocampal cell number were most likely due to observer error. The morphological classification data suggest that the changes in the

expression of GFAP in all experimental treatment conditions is due to the alteration in the number of projections exhibited by glia these hormone enriched groups. The decrease in the number of type I astrocytes, which express very little GFAP-IR, and the increase of the darker staining stellate astrocytes give rise to cells which have much longer projections. The processes of stellate and glia classified by the paper as class II bipolar astrocytes is much longer than the class I astrocyte. This increase in process length allows the cells to cover a larger area of influence. This suggests that cells within the mPOA glia support structure compensate for the greater level of neuronal activity by becoming more productive rather than simply dividing into more astrocytes.

The truly intriguing part of the presence of these stellate cells, during exposure to high levels of reproductive hormones, centers on one well know function that these types of glia perform during development (Wang et al., 1994). Specifically, stellate glia are involved in the support of neuronal migration and organization (Hatten, 1990). The emergence of these organizing support cells occur in conjunction with the enhancement of maternal behaviors that are in turn stimulated by the presence of reproductive hormones. This is a strong indication that these stellate cells may be involved in the organization of nuclei in the brain that is the substrate on which the development of maternal behaviors occurs.

Preliminary evidence supporting this possibility shows that these stellate glial cells may also be involved in the migration of newly generated neurons in the HI. During development astrocytes extend processes which serve as a tether on which neurons migrate to their final destination (Hatten, 1990). Kinsley, et al. (1999) reported that neurogenesis, which is observed to a much greater degree during brain development, occurs throughout the life span of female

rats. During reproduction the rate of neurogenesis drops off during late pregnancy and slightly recovers during lactation (Amory, et al., 1999). This decrease in neurogenesis during late pregnancy coincides with a decrease in process length in the HI observed in this project. This may serve to explain why an increases in process length was not observed in the Late-pregnant HI despite the fact that there was an increase in the number of class II astrocytes over their Virgin counterparts.

These findings come together to support the conjecture that areas of the brain that control maternal behavior, under the influence of reproductive hormones, flourish much like the brain during development. This possibility extends the period of brain development well past the time of adolescence and places it under the control of mechanisms which respond to internal changes of animals that endeavor the challenge of reproduction.

Further work into this topic must address the possibility that changes in glial cell morphology, due to greater exposure to female steroid hormones, may be as permanent as those changes observed for neurons in areas of the brain responsible for maternal behavior. An investigation into glial cell morphology of primiparous and multiparous females that have concluded lactation would give a clear insight into lasting effects of pregnancy on the support system of the maternal brain.

Works Cited

Amory, E.A., Wartella, J.E., Williams, A., Dillon, A., Ploszay, A., Griffin, G., Madonia, L., Graper, A., Babcock, S.G., Lambert, K.G. & Kinsley, C.H. (1999). Motherhood modifies neurogenesis in hippocampus. Paper presented at the annual meeting of the society for Neuroscience, Miami, FL.

Ayoub, D.M., Greenough, W.T., & Juraska, J.M. (1983). Sex differences in dendritic structures in the preoptic area of the juvenile macaque monkey brain. Science, 219, 197-198.

Bucala, R. (1996). MIF rediscovered: cytokine, pituitary hormone, and glucocorticoid-induced regulator of the immune response. FASEB Journal, 10(14), 1607-1613.

Bridges, R.S. (1984). A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. Endocrinology, 114, 930-940.

Bridges, R.S. & Freemark, M.S. (1990). Human placental lactogen infusions into the medial preoptic area stimulate maternal behavior in steroid-primed, nulliparous female rats. Hormones & Behavior, 29(2), 216-226.

Bridges, R.S., Robertson, M.C., Shiu, R.P.C., Friesen, H.G., Stuer, A.M., & Mann, P.E. (1996). Endocrine communication between conceptus and mother: A role for placental lactogens in the induction of maternal behavior. Neuroendocrinology, 64, 57-64.

Chan, J.R., Lornie, J., Phillips, I.I., & Glaser, M. (1998). Glucocorticoids and progestins signal the initiation and enhance the rate of myelin formation. Proceedings of the National Academy of Science, 95, 10459-10464.

Chowen, J.A., Busiguina, S., & Garcia-Segura, L.M. (1995). Sexual dimorphism and sex steroid modulation of glial fibrillary acidic protein messenger RNA and immunoreactivity levels in the rat hypothalamus. Neuroscience, 69, 519-532.

Chowen, J.A.,Torres-Aleman, I., & Garcia-Segura, L.M. (1992). Trophic effects of estradiol on fetal rat hypothalamic neurons. Neuroendocrinology, 56(6), 895-901.

Day, J.R., Laping, N.J., Lamper-Etchells, M., Brown, S.A., O'Callaghan, J.P., McNeill, T.H., & Finch, C.E. (1993). Gonadal steroids regulate the expression of glial fibrillary acidic protein in the adult male rat Hippocampus. Neuroscience, 55, 435-443.

Del Cerro, S., Garcia-Estrada, J., & Garcia-Segura, L.M. (1996). Neurosteroids modulate the reaction of astroglia to high extracellular potassium levels. GLIA, 18(4), 293-305.

Del Cerro, S., Garcia-Estrada, J., & Garcia-Segura, LM. (1995). Neuroactive steroids regulate astroglia morphology in hippocampal cultures from adult rats. GLIA, 14(1), 65-71.

Duenas, M., Torres-Aleman, I., Naftolin, F., & Garcia-Segura, LM. (1996). Interaction of insulin-like growth factor-I and estradiol signaling pathways on hypothalamic neuronal differentiation. Neuroscience, 74(2), 531-539.

Fleming, A.S., Steiner, M., & Corter, C. (1997a). Cortisol, hedonics, and maternal responsiveness in human mothers. Hormones & Behavior, 32(2), 85-98.

Fleming, A.S., Ruble, D., Krieger, H., & Wong, P.Y. (1997b). Hormonal and experimental correlates of maternal responsiveness during pregnancy and the puerperium in human mothers. Hormones & Behavior, 31(2), 145-158.

Ganter, S., Northoff, H., Mannell, D., & Gebicke-Harter, P.J. (1992). Growth control of cultured microglia. Journal of Neuroscience Research, 33, 218-230.

Garcia-Segura, L.M., Torres-Aleman, I., & Naftolin, F. (1989). Astrocytic shape and glial fibrillary acidic protein immunoreactivity are modified by estradiol in primary rat hypothalamic cultures. Developmental Brain Research, 47, 289-302.

Garcia-Segura, L.M., Chowen, J.A., Duenas, M., Torres-Aleman, I., & Naftolin, F. (1994a). Gonadal steroids as promoters of neuro-glial plasticity. Psychoneuroendocrinology, 19(5-7), 445-453.

Garcia-Segura, L.M., Chowen, J.A., Parducz, A., & Naftolin, F. (1994b). Gonadal hormones as promoters of structural synaptic plasticity: cellular mechanisms. Progress in Neurobiology, 44(3), 279-307.

Garcia-Segura, L.M., Duenas, M., Busiguina, S., Naftolin, F., & Chowen, J.A. (1995). Gonadal hormone regulation of neuronal-glial interactions in the developing neuroendocrine hypothalamus. Journal of Steroid Biochemistry & Molecular Biology, 53(1-6), 293-298.

Garcia-Segura, L.M., Chowen, J.A., Duenas, M., Parducz, A., & Naftolin, F. (1996a). Gonadal steroids and astroglial plasticity. Cellular & Molecular Neurobiology, 16(2), 225-237.

Garcia-Segura, L.M., Chowen, J.A., & Naftolin, F. (1996b). Endocrine glia: roles of glial cells in the brain actions of steroid and thyroid hormones and in the regulation of hormone secretion. Frontiers in Neuroendocrinology, 17(2), 180-211.

Giaume, C., & McCarthy, K.D. (1996). Control of gap-junctional communication in astrocytic networks. *T*rends in Neurosciences, 19(8), 319-325.

Gifford, G., Quadros, P., & Kinsley, C. (1998). Alterations in Astrocytes may

accompany changes in reproductive condition in the female rat. Paper presented at the annual meeting of the Virginia Academy of Sciences, May.

Gorski, R.A., Gordon, J.H., Shryne, J.E., & Southam, A.M. (1978). Evidence for a morphological sex difference within the medial preoptic area of the rat brain. Brain Research, 148, 333-346.

Hatten, M.E. (1990). Riding the glial monorail: a common mechanism for glial-guided neuronal migration in different regions of the developing mammalian brain. Trends in Neurosciences, 13(5), 179-184.

Hoshina, Y., Takeo, T., Nakano, K., Sato, T., & Sakuma, Y. (1994). Axon-sparing lesion of the preoptic area enhances receptivity and diminishes proceptivity among components of female rat sexual behavior. Behavioral Brain Research, 61(2), 197-204.

Jacobson, C.D., & Gorski, R.A. (1981). Neurogenesis of the sexually dimorphic nucleus of the preoptic area in the rat. Journal of Comparative Neurology, 196, 519-529.

Jung-Testas, I., & Baulieu, E.E. (1998). Steroid hormone receptors and steroid action in rat glial cells of the central and peripheral nervous system. Journal of Steroid Biochemistry & Molecular Biology, 65(1-6), 243-251.

Jung-Testas, I., Hu, Z.Y., Baulieu, E.E., & Robel, P. (1989a). Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. Endocrinology, 125(4), 2083-2091.

Jung-Testas, I., Hu, Z.Y., Baulieu, E.E., & Robel, P. (1989b). Steroid synthesis in rat brain dell cultures. Journal of steroid Biochemistry, 34, 511-519.

Jung-Testas, I., Renoir, J.M., Gasc, J.M., & Baulieu, E.E. (1991). Estrogen-inducible progesterone receptor in primary cultures of rat glial cells. Experimental Cell Research. 193(1), 12-19.

Jung-Testas, I., Renoir, M., Bugnard, H., Greene, G.L., & Baulieu, E.E. (1992). Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. Journal of Steroid Biochemistry & Molecular Biology, 41(3-8), 621-631.

Jung-Testas, I., Schumacher, M., Robel, P., & Baulieu, E.E. (1994). Actions of steroid hormones and growth factors on glial cells of the central and peripheral nervous system. Journal of Steroid Biochemistry & Molecular Biology, 48(1), 145-154.

Kandel, E.R., & Schwartz, J.H. (1985). Principles of Neuroscience: Second Edition. New York: Elsevier Science Publishing Co., Inc.

Keyser, L., Stafisso-Sandoz, G., Gerecke, K., Nightingale, L., Lambert, K. & Kinsley, C. H. (1995). Increase in somal size in medial preoptic area neurons following pregnancy and pregnancy-like steroidal treatment in the rat. Society of Neuroscience Abstract, 21, 464.

Kimble, D.P., Rogers, L., Hendrickson, C.W. (1967). Hippocampal lesions disrupt maternal, not sexual, behavior in the albino rat. Journal of Comparative Physiological Psychology, 63, 401-407.

Kinsley, C.H., Amory, E.A., Wartella, J., Gifford, G. & Lambert, K.G. (1999). Your mother (rat) should know: Pregnancy drives changes in neurogenesis and hippocampal neural and glial morphology and activity. Paper presented at the Maternal Brain Conference, Birstol, England.

Kohama, S.G., Goss, J.R., McNeill, T.H., & Finch, C.E. (1995). Glial fibrillary acidic protein mRNA increases at proestrus in the arcuate nucleus of mice. Neuroscience Letters, 183, 164-166.

Lalonde, R. (1997). Visiospatial abilities. International Review of Neurobiology, 41, 191-215.

Langub, M.C., & Watson, R.E. (1992). Estrogen receptor-immunoreactive glia, endothelia, and ependyma in guinea pig preoptic area and median eminence: electron microscopy. Endocrinology, 130(1), 364-372.

Luquin, S., Naftolin, F., & Garcia-Segura, L.M. (1993). Natural fluctuation and gonadal hormone regulation of astrocyte immunoreactivity in dentate gyrus. Journal of Neurobiology, 24(7), 913-924.

Ma, Y.J., Berg-von der Emde, K., Moholt-Siebert, M., Hill, D.F., & Ojeda, S.R. (1994). Region-specific regulation of transforming growth factor alpha (TGF alpha) gene expression in astrocytes of the neuroendocrine brain. Journal of Neuroscience, 14(9), 5644-5651.

Ma, Y.J., Berg-von der Emde, K., Rage, F., Wetsel, W.C., & Ojeda, S.R. (1997). Hypothalamic astrocytes respond to transforming growth factor-alpha with the secretion of neuroactive substances that stimulate the release of luteinizing hormone-releasing hormone. Endocrinology, 138(1), 19-25.

Ma, Y.J., & Ojeda, S.R. (1997). Neuroendocrine control of female puberty: glial and neuronal interactions. Journal of Investigative Dermatology Symposium Proceedings, 2(1), 19-22.

Madonia, L., Gifford, G., Tureski, K., & Kinsley, C. (1998). Parity and spatial learning. Poster session presented at the annual University of Richmond Research Symposium, April.

Maguire, E.A., Frackowiak, R.S.J., & Frith, C.D. (1997). Recalling routes around London: activation of the right Hippocampus in taxi drivers. Journal of Neuroscience, 17(18), 7103-7110.

McEwen, B.S., & Woolley, C.S. (1994). Estradiol and progesterone regulate neuronal structure and synaptic connectivity in adult as well as developing brain. Experimental Gerontology, 29(3-4), 431-436.

Melcangi, R.C., Riva, M.A., Fumagalli, F., Magnaghi, V., Racagni, G., & Martini, L. (1996). Effect of progesterone, testosterone and their 5 alpha-reduced metabolites on GFAP gene expression in type astrocytes. Brain Research, 711(1-2), 10-15.

Mong, J.A., Kurzweil, R.L., Davis, A.M., Rocca, M.S., & McCarthy, M.M. (1996). Evidence for Sexual Differentiation of Glia in Rat Brain. Hormones and Behavior, 30, 553-562.

Naftolin, F., Leranth, C., Perez, J., & Garcia-Segura, L.M. (1993). Estrogen induces synaptic plasticity in adult primate neurons. Neuroendocrinology, 57(5), 935-939.

Naftolin, F., Mor, G., Horvath, T.L., Luquin, S., Fajer, A.B., Kohen, F., & Garcia-Segura, L.M. (1996). Synaptic remodeling in the arcuate nucleus during the estrous cycle is induced by estrogen and precedes the preovulatory gonadotropin surge. Endocrinology, 137(12), 5576-5580.

Naftolin, F., Leranth, C., Horvath, T.L., & Garcia-Segura. L.M. (1996). Potential neuronal mechanisms of estrogen actions in synaptogenesis and synaptic plasticity. Cellular & Molecular Neurobiology, 16(2), 213-223.

Numan, M., Rosenblatt, J.S., & Komisaruk, B.R. (1977). Medial preoptic area and onset of MB in the rat. Journal of Comparative Physiological Psychology, 91, 146-164.

Numan, M. (1994). MB. In Knobil, E. & Neill, J.D. (Eds.), The physiology of reproduction. Second edition, New York: Raven Press, Ltd.

Santagati, S., Melcangi, R.C., Celotti, F., Martini, L., & Maggi, A. (1994). Estrogen receptor is expressed in different types of glial cells in culture. Journal of Neurochemistry, 63(6), 2058-2064.

Szelenyi Z. (1998). Neuroglia: possible role in thermogenesis and body temperature control. Medical Hypotheses, 50(3), 191-197.

Torres-Aleman, I., Rejas, M.T., Pons, S., & Garcia-Segura, L.M. (1992). Estradiol promotes cell shape changes and glial fibrillary acidic protein redistribution in hypothalamic astrocytes in vitro: a neuronal-mediated effect. GLIA, 6(3), 180-187.

Trainer, R., Quadros, P., Stafisso-Sandoz, G., & Kinsley, C. (1997). Differences in concentrations of dendritic spines on hippocampal neurons among virgin, pregnant and lactating rats. Poster session presented at the International Society for Developmental Psychobiology annual meeting, New Orleans, LA, October.

Tranque, P.A., Suarez, I., Olmos, G., Fernandez, B., & Garcia-Segura, L.M. (1987). Estradiol--induced redistribution of glial fibrillary acidic protein immunoreactivity in the rat brain. Brain Research, 406(1-2), 348-351.

Wang, L.C. Baird, D.H., Hatten, M.E., & Mason, C.A. (1994). Astroglial differentiation is required for support of neurite outgrowth. Journal of Neuroscience, 14(5 Pt 2), 3195-3207.

Weibel, E.R. (1979). Stereological methods / Ewald R. Weibel: Vol. 1 Practical methods for biological morphometry. New York: Academic Press.

Woolley, C.S., & McEwen, B.S. (1992). Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. Journal of Neuroscience, 12(7), 2549-2554.

Woolley, C.S., & McEwen, B.S. (1993). Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. Journal of Comparative Neurology, 336(2), 293-306.

Woolley, C.S., & McEwen, B.S. (1994). Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. Journal of Neuroscience, 14(12), 7680-7687.

Table 1.
Threshold, Stereology, Radii Length and Glia classification for mPOA

	Treatment Condition (Maternal)		
Measurement	Virgin Diestrous	Late-pregnant	Lactating
Threshold (square μ)	4202.12 ± 313.13	11012.58 ± 646.89	8579.61 ± 572.45
Mean # of glia	227.32 ± 16.98	211.39 ± 23.21	221.53 ± 15.50
Process Length (μ)	14.00 ± 0.53	19.58 ± 0.59	22.49 ± 0.53
Mean # of Class I	13.50 ± 1.24	6.25 ± 0.77	3.75 ± 0.73
Mean # of Class II	2.50 ± 1.24	6.75 ± 0.49	6.00 ± 0.46
Mean # of Class III	0.00 ± 0.00	3.00 ± 1.31	6.25 ± 1.08

Table 2.
Threshold, Stereology, Radii Length and Glia classification for hippocampus

	Treatment Condition (Maternal)		
Measurement	Virgin Diestrous	Late-pregnant	Lactating
Threshold (square μ)	2865.70 ± 174.80	3345.60 ± 189.99	4107.84 ± 228.08
Mean # of glia	224.92 ± 13.66	222.98 ± 15.46	147.69 ± 10.18
Process Length (μ)	17.34 ± 0.38	14.83 ± 0.37	18.65 ± 0.512
Mean # of Class I	5.875 ± 0.58	7.25 ± 0.65	4.75 ± 0.73
Mean # of Class II	7.88 ± 0.30	6.38 ± 0.57	3.25 ± 0.73
Mean # of Class III	2.25 ± 0.31	2.25 ± 0.16	8.00 ± 1.07

Table 3.
Threshold, Stereology, Radii Length and Glia classification for mPOA

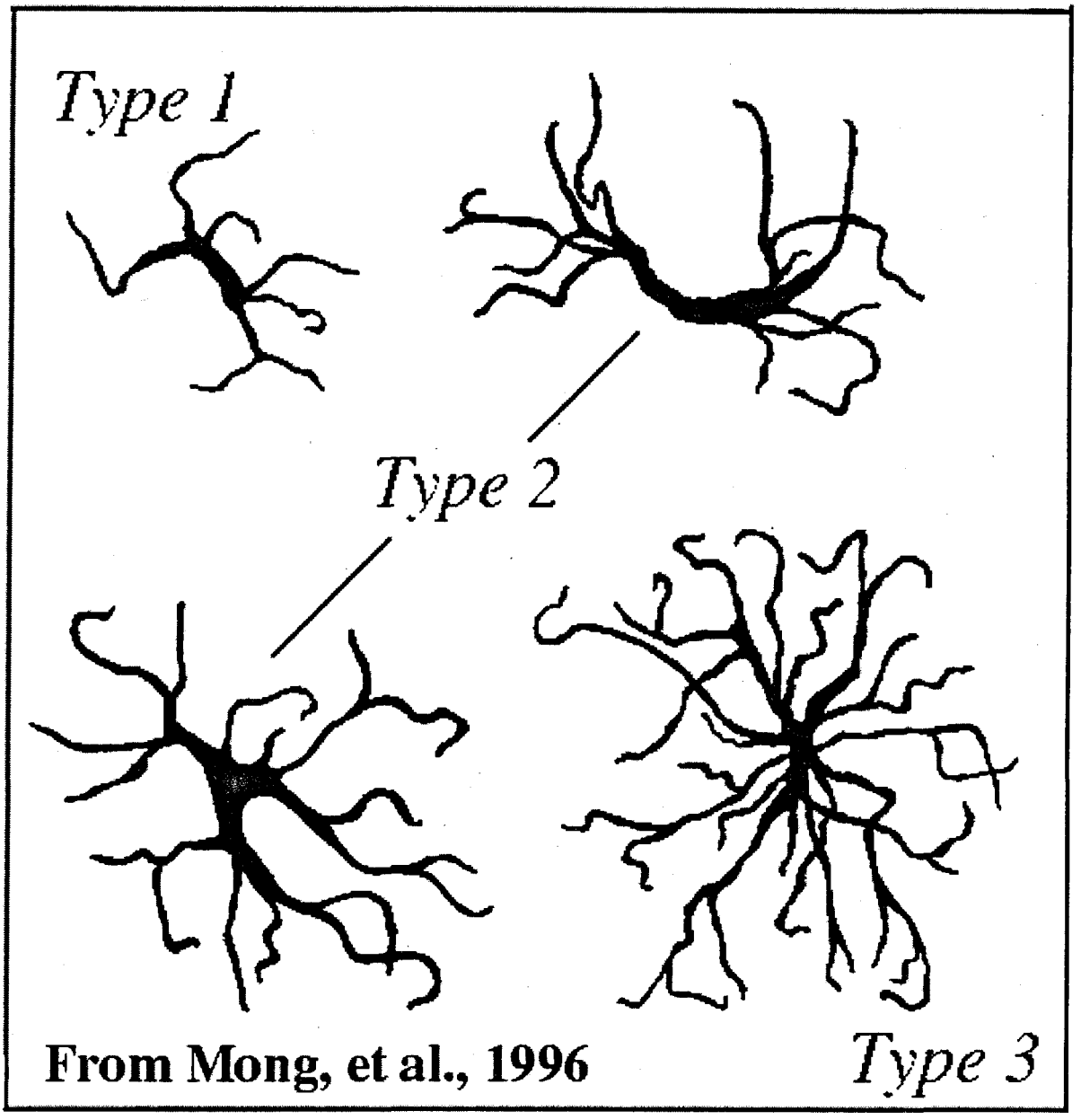
	Treatment Conditon (Hormone)	
Measurement	Ovex + Hormones	Ovex -
Threshold (square μ)	8194.63 ± 396.44	5563.39 ± 286.93
Mean # of glia	240.35 ± 16.89	237.46 ± 17.36
Process Length (μ)	22.568 ± 0.604	18.353 ± 0.49
Mean # of Class I	4.50 ± 0.57	7.25 ± 0.56
Mean # of Class II	7.00 ± 0.54	8.25 ± 0.412
Mean # of Class III	4.5 ± 0.42	0.50 ± 0.189

Table 4.
Threshold, Stereology, Radii Length and Glia classification for hippocampus

	Treatment Conditon (Hormone)	
Measurement	Ovex + Hormones	Ovex -
Threshold (square μ)	6481.34 ± 178.64	4954.87 ± 219.59
Mean # of glia	351.84 ± 10.83	344.60 ± 15.28
Process Length (μ)	24.92 ± 0.70	22.66 ± 0.56
Mean # of Class I	4.00 ± 0.38	1.25 ± 0.49
Mean # of Class II	5.75 ± 0.73	5.25 ± 0.90
Mean # of Class III	9.0 ± 0.92	6.75 ± 0.86

Figure Caption

Figure 1. Classification chart for glia morphology adapted from Mong, et al. 1999.



From Mong, et al., 1996

Figure Caption

Figure 2. Mean amount of GFAP-IR staining for mPOA measured in square microns using threshold analysis. Reproductive treatment elicited significantly different levels of staining among all groups.

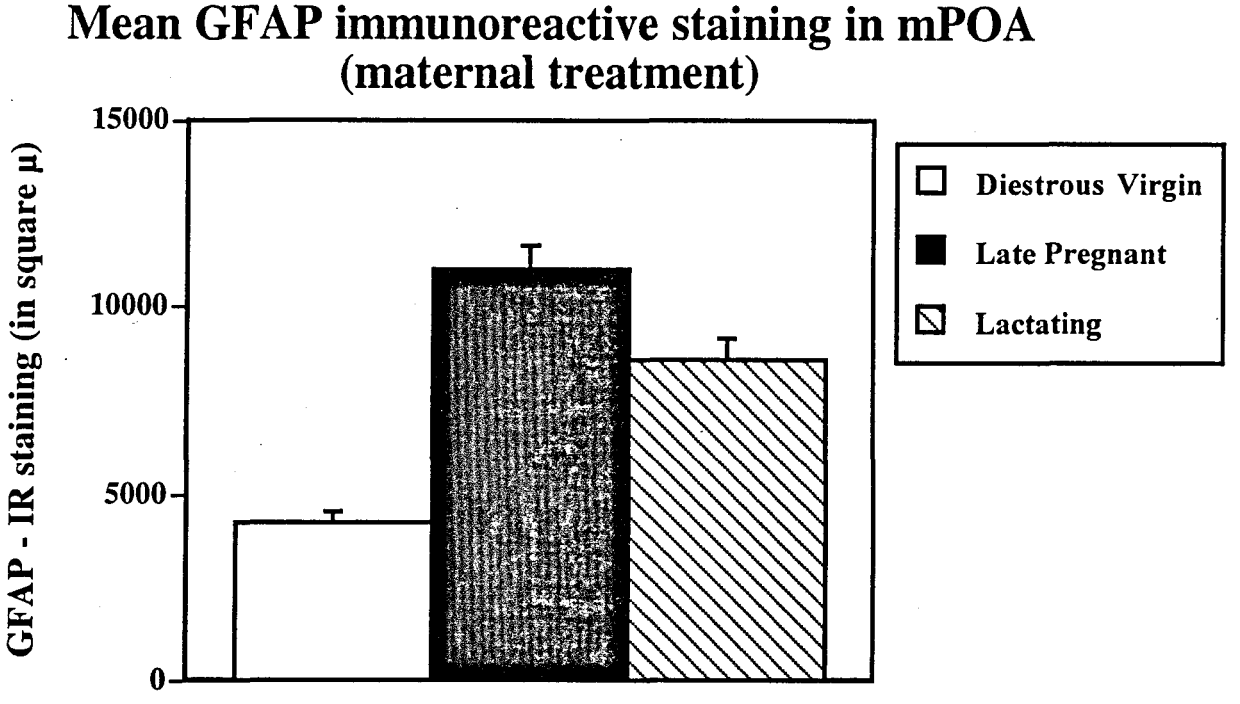

Mean GFAP immunoreactive staining in mPOA
(maternal treatment)
GFAP - IR staining (in square μ)
15000
10000
5000
0
Diestrous Virgin
Late Pregnant
Lactating

Figure Caption

Figure 3. Mean number of astrocytes per square millimeter of mPOA estimated using stereological analysis. No significant difference was identified among the reproductive treatment conditions.

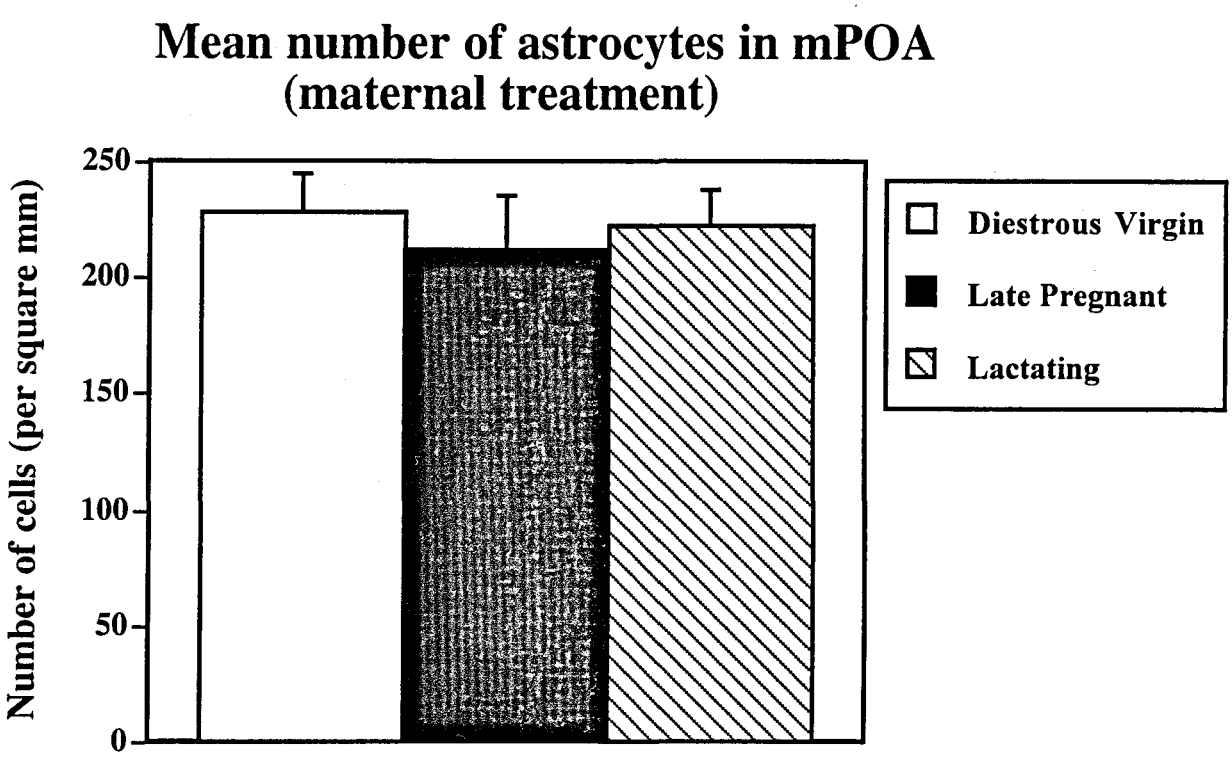

Mean number of astrocytes in mPOA
(maternal treatment)
Number of cells (per square mm)
250
200
150
100
50
0
Diestrous Virgin
Late Pregnant
Lactating

Figure Caption

Figure 4. Mean astrocyte process length in microns measured in the mPOA. Reproductive treatment conditions elicited a significant difference among all groups.

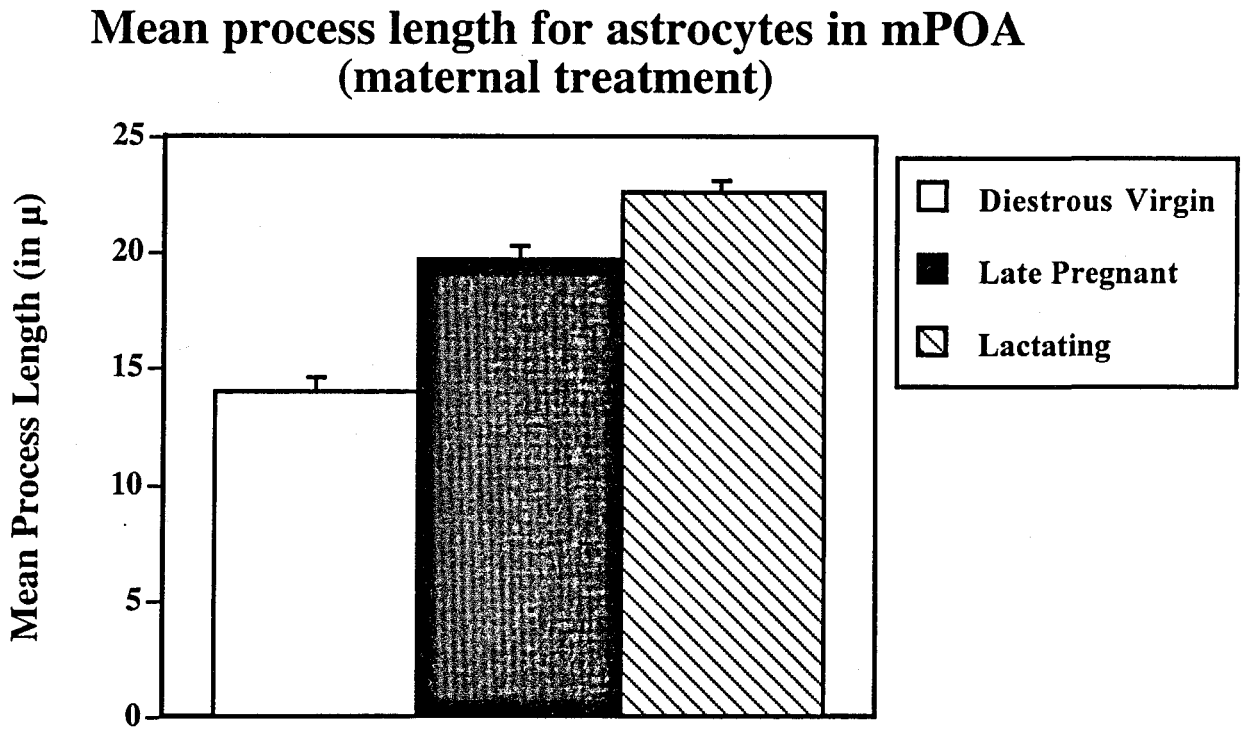

Mean process length for astrocytes in mPOA
(maternal treatment)
Mean Process Length (in μ)
25
20
15
10
5
0
Diestrous Virgin
Late Pregnant
Lactating

Figure Caption

Figure 5. Mean number of class I, class II and class III astrocytes counted out of a total of 16 cells sampled per brain in mPOA. Reproductive treatment conditions elicited significant differences among all groups for class I and class III glia. Lactating females had significantly lower numbers of class II astrocytes when compared to Diestrous Virgin and Late-pregnant counterparts. No significant difference was identified between Diestrous Virgin and Late-pregnant females for class II cells.

Mean representation of astrocyte classification in mPOA (maternal treatment)

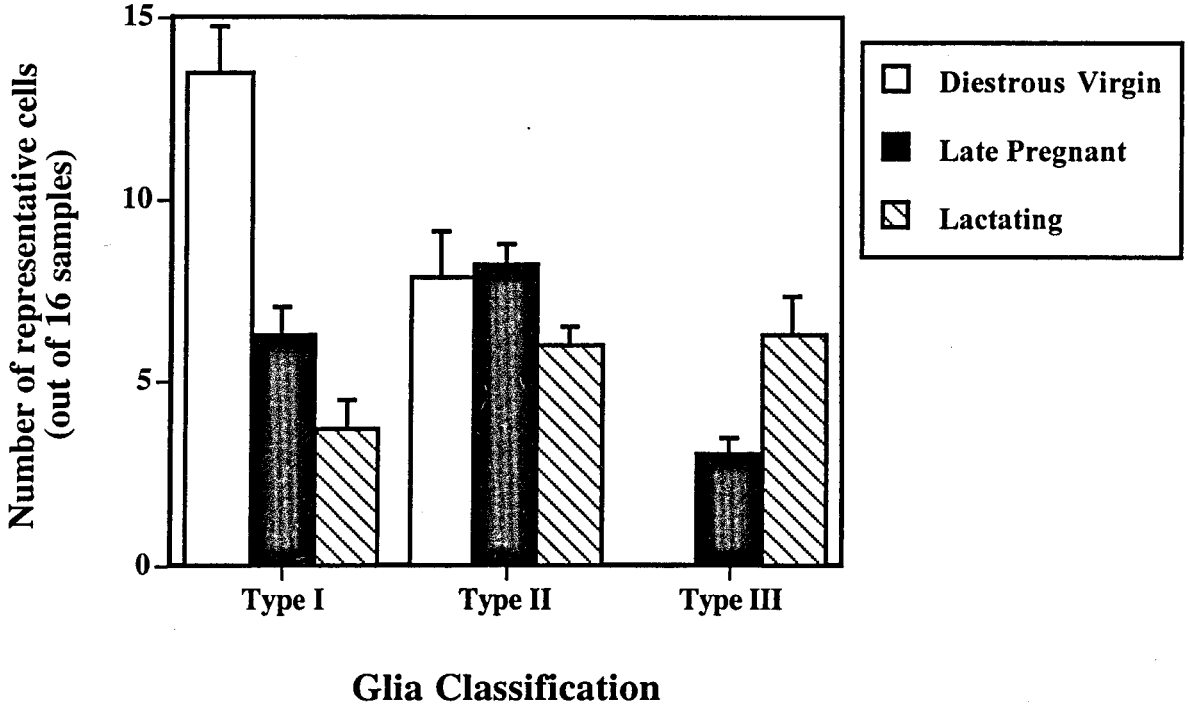


Figure Caption

Figure 6. Mean amount of GFAP-IR staining for HI measured in square microns using threshold analysis. Reproductive treatment elicited significantly greater levels of staining in the Lactating group over Virgin Diestrous group and Late-pregnant group. No significant difference was found between Virgin and Late-pregnant females.

Mean GFAP immunoreactive staining in hippocampus (maternal treatment)

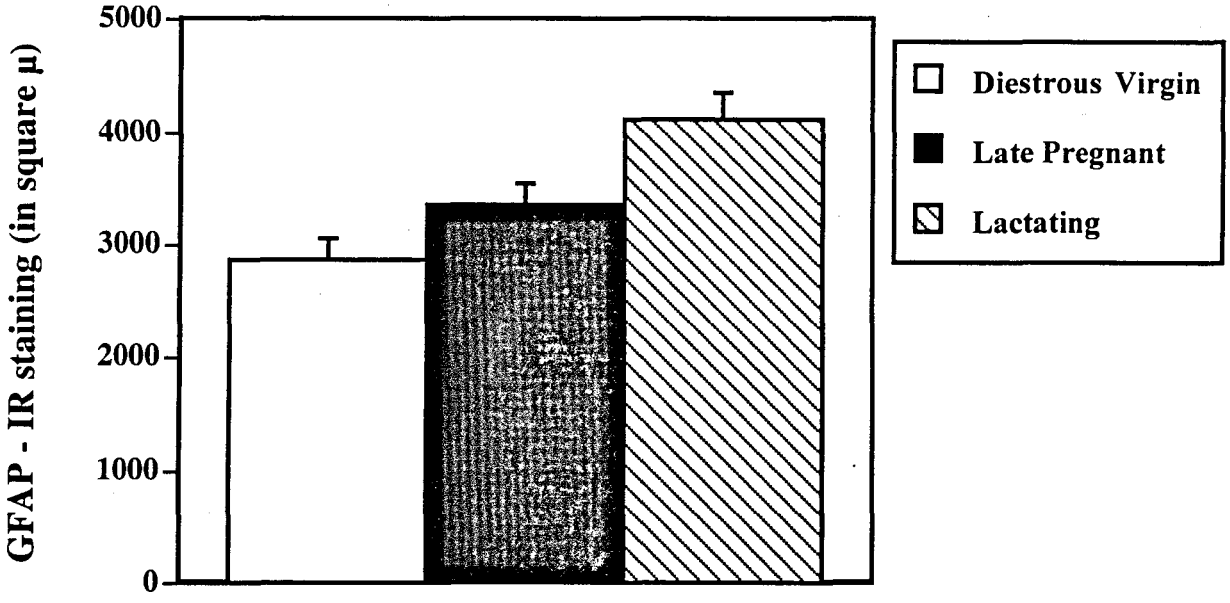


Figure Caption

Figure 7. Mean number of astrocytes per square millimeter of HI estimated using stereological analysis. Reproductive treatment conditions elicited a significantly lower amount of neurons in the lactating group when compared to the Late-pregnant and Virgin groups. These differences are most likely attributable to measurement error rather than the existence of a real difference between groups. No significant difference was identified between the Virgin and Late-Pregnant groups.

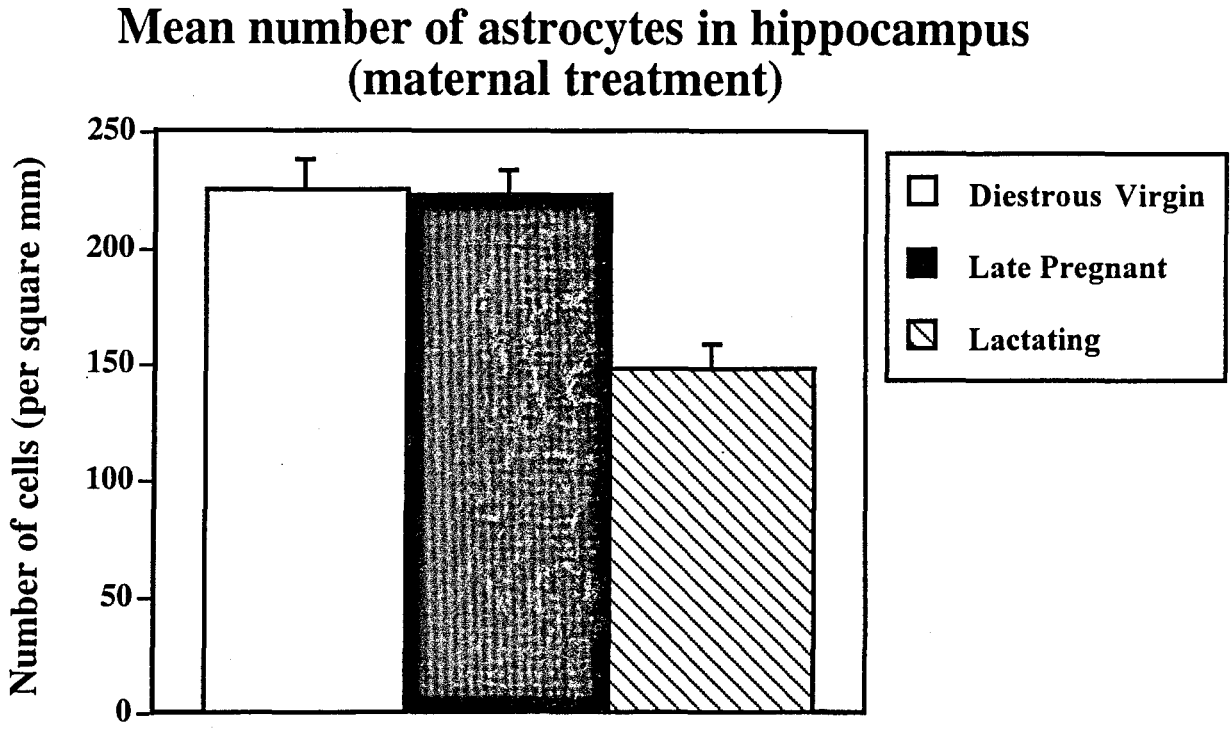

Mean number of astrocytes in hippocampus
(maternal treatment)
Number of cells (per square mm)
250
200
150
100
50
0
Diestrous Virgin
Late Pregnant
Lactating

Figure Caption

Figure 8. Mean astrocyte process length in microns measured in the HI. Reproductive treatment conditions elicited a significant difference among all groups.

Mean process length for astrocytes in Hippocampus (maternal treatments)

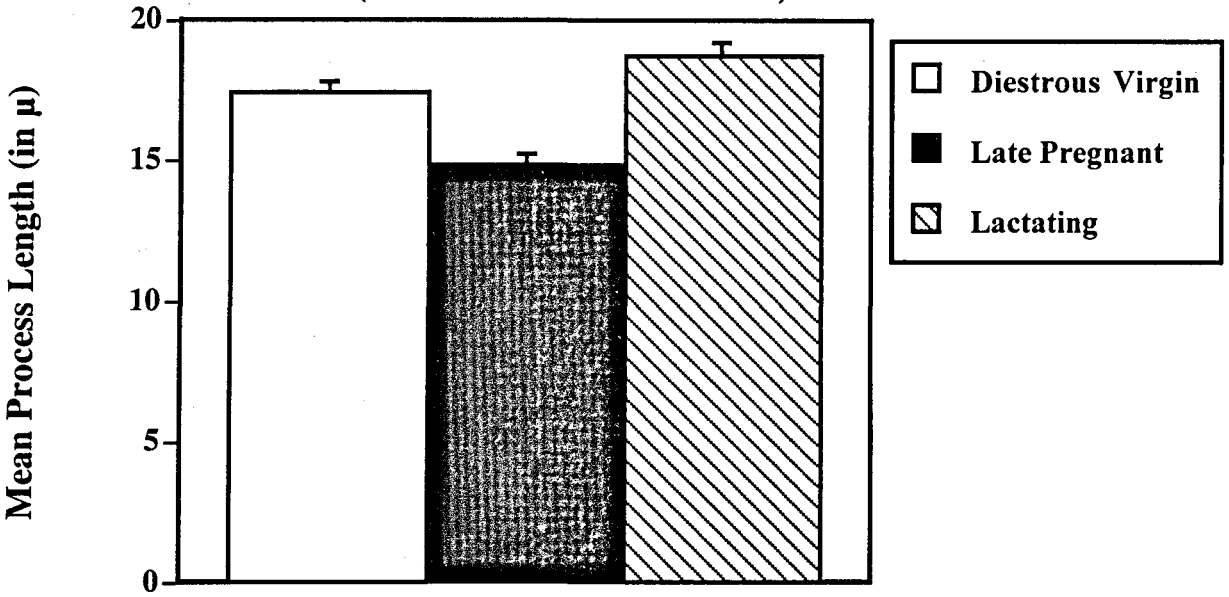


Figure Caption

Figure 9. Mean number of class I, class II and class III astrocytes counted out of a total of 16 cells sampled per brain in HI. For class I cells reproductive treatment conditions elicited only one significant differences between Lactating and Late-pregnant females. Significant differences in the number of class II glia were identified between Lactating and Late-Pregnant females as well as between Lactating and Diestrous Virgin females. Lactating females had significantly greater numbers of class III astrocytes when compared to Diestrous Virgin and Late-pregnant counterparts. No significant difference was identified between Diestrous Virgin and Late-pregnant females for class III cells.

Mean representation of astrocyte classification in hippocampus (maternal treatment)

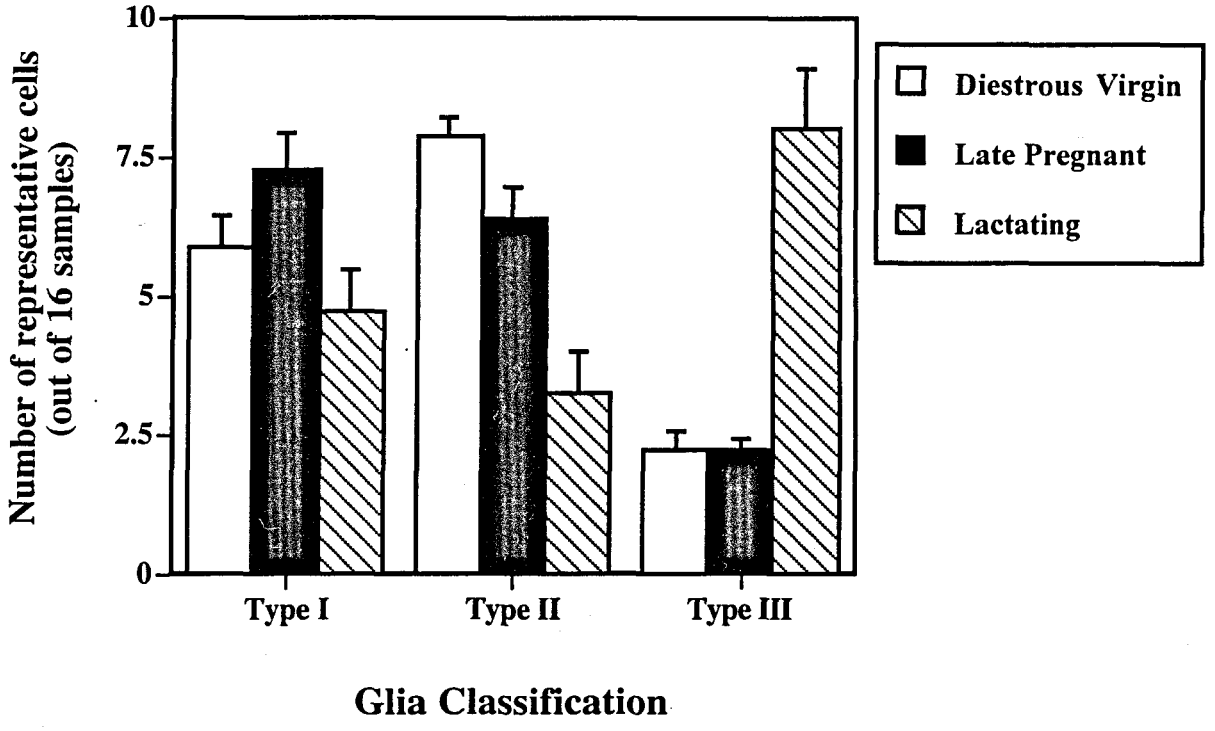


Figure Caption

Figure 10. Mean amount of GFAP-IR staining for HI measured in square microns using threshold analysis. Female steroid hormone treatment elicited significantly greater levels of staining in the OVX+ hormones group over OVX- group.

Mean GFAP Immunoreactive Staining in mPOA (hormone treatment)

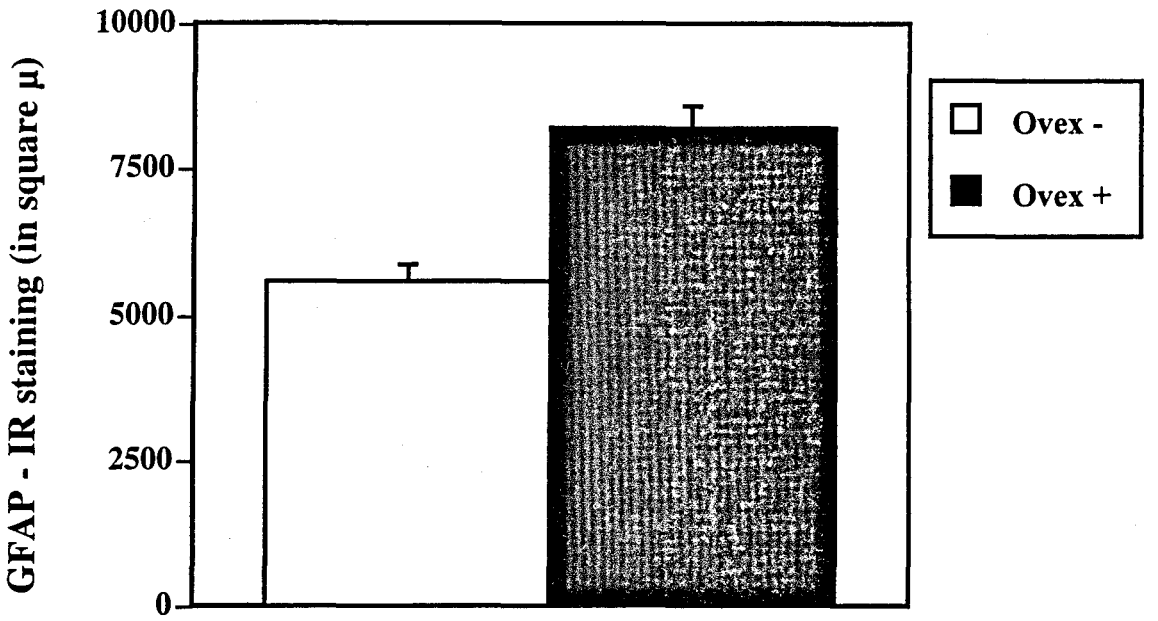


Figure Caption

Figure 11. Mean number of astrocytes per square millimeter of mPOA estimated using stereological analysis. No significant difference was identified between the female steroid hormone treatment conditions.

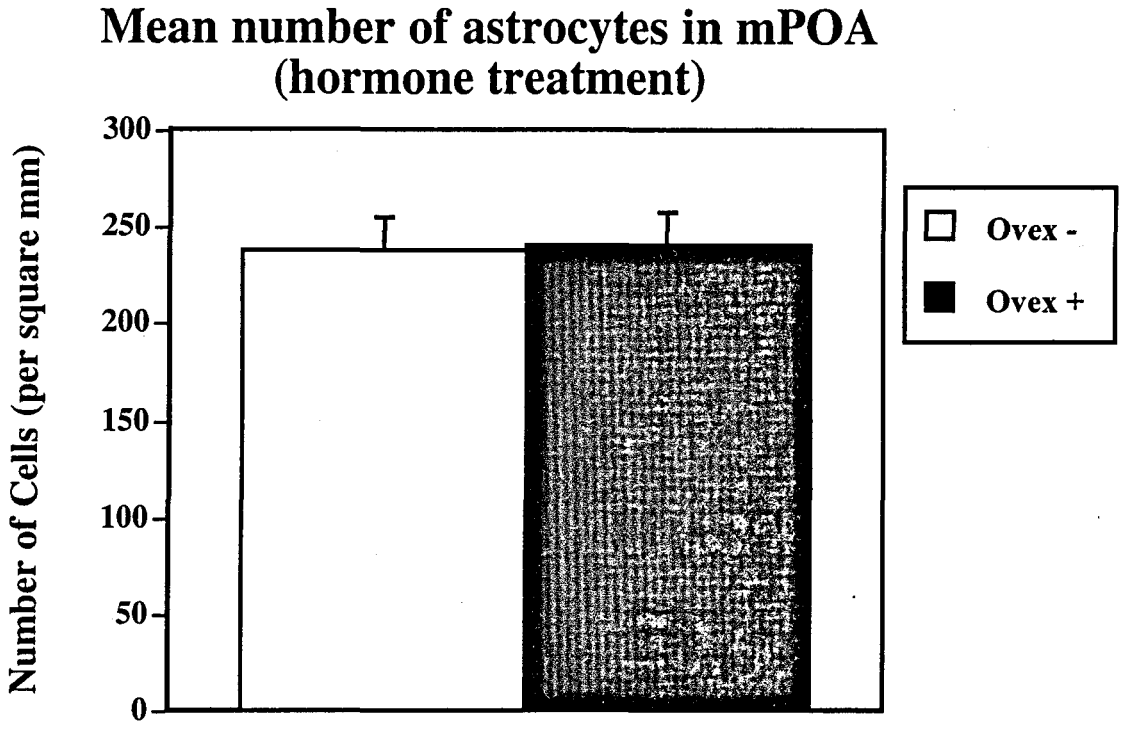

Mean number of astrocytes in mPOA
(hormone treatment)
Number of Cells (per square mm)
300
250
200
150
100
50
0
Ovex -
Ovex +

Figure Caption

Figure 12. Mean astrocyte process length in microns measured in the mPOA. Female steroid hormone treatment conditions elicited a significant difference between groups.

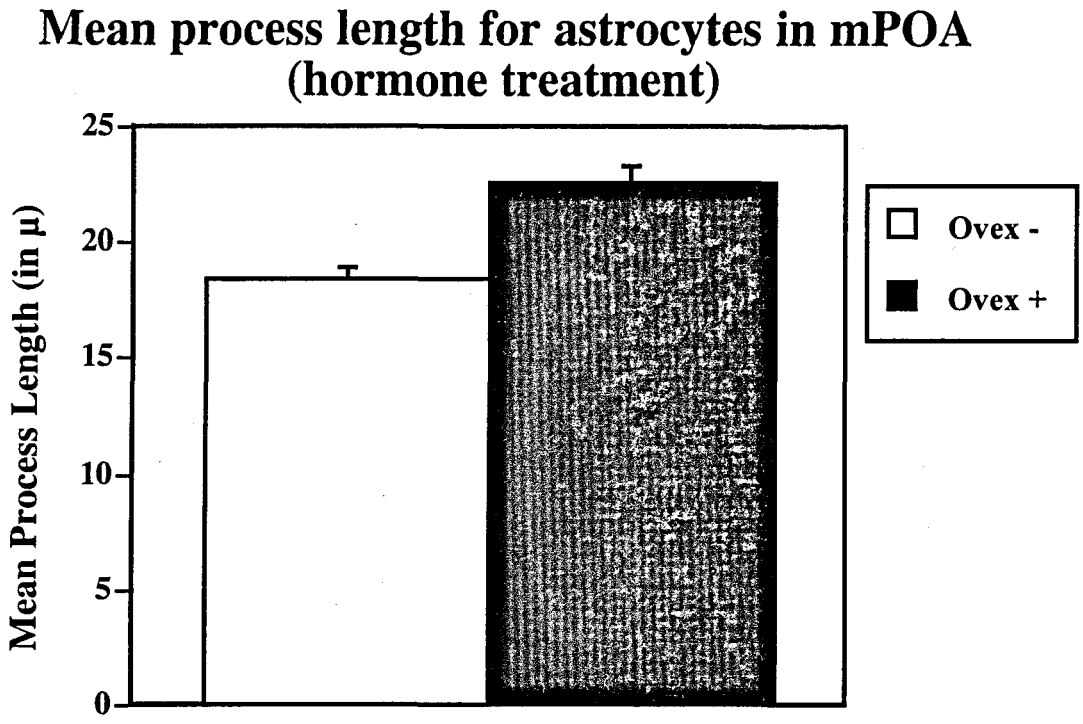

Mean process length for astrocytes in mPOA
(hormone treatment)
Mean Process Length (in μ)
25
20
15
10
5
0
Ovex -
Ovex +

Figure Caption

Figure 13. Mean number of class I, class II and class III astrocytes counted out of a total of 16 cells sampled per brain in mPOA. Female steroid hormone treatment conditions elicited significant differences between groups for all classes of cells.

Mean representation of astrocyte classification in mPOA (hormone treatment)

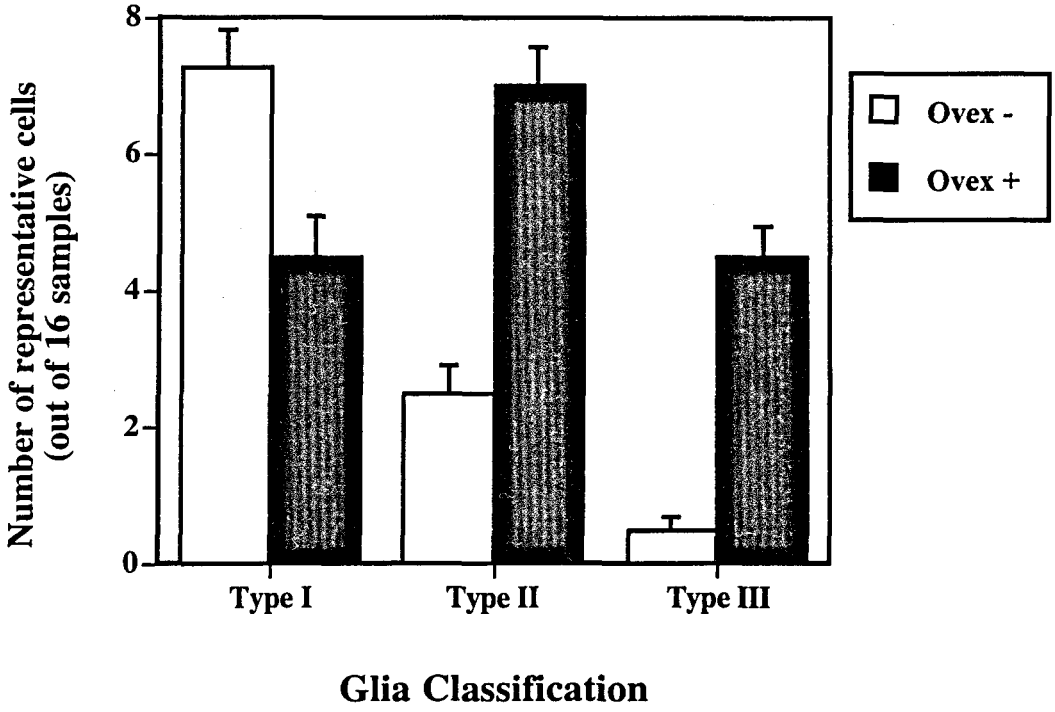


Figure Caption

Figure 14. Mean amount of GFAP-IR staining for HI measured in square microns using threshold analysis. Female steroid hormone treatment elicited significantly greater levels of staining in the OVX+ hormones group over OVX- group.

Mean GFAP immunoreactive staining in hippocampus (hormone treatment)

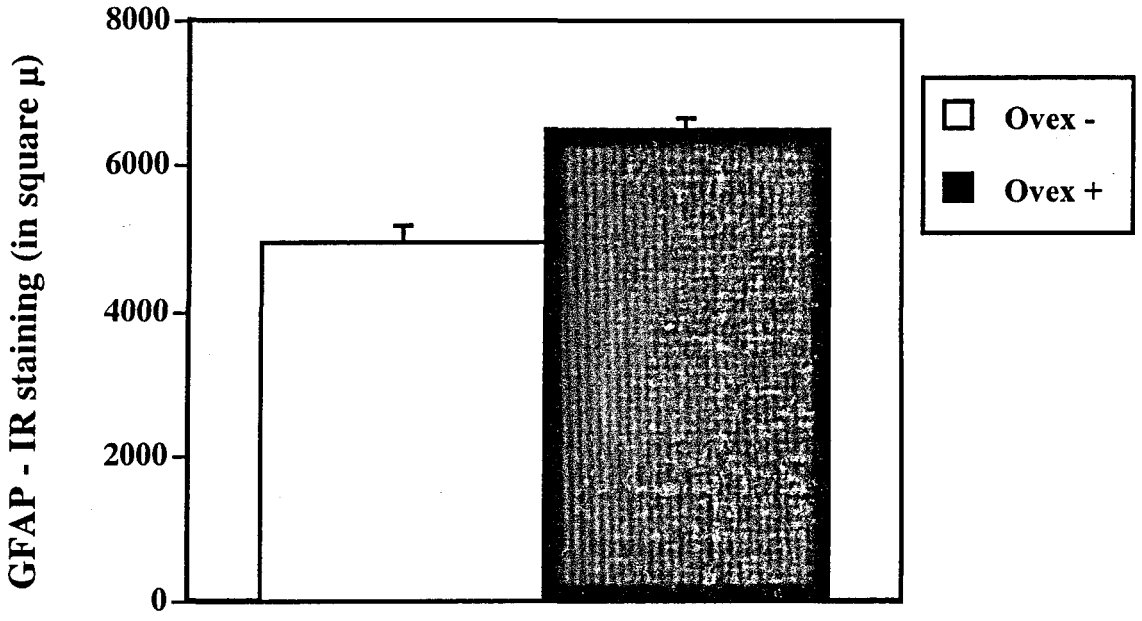


Figure Caption

Figure 15. Mean number of astrocytes per square millimeter of HI estimated using stereological analysis. No significant difference was identified among the female steroid hormone treatment conditions.

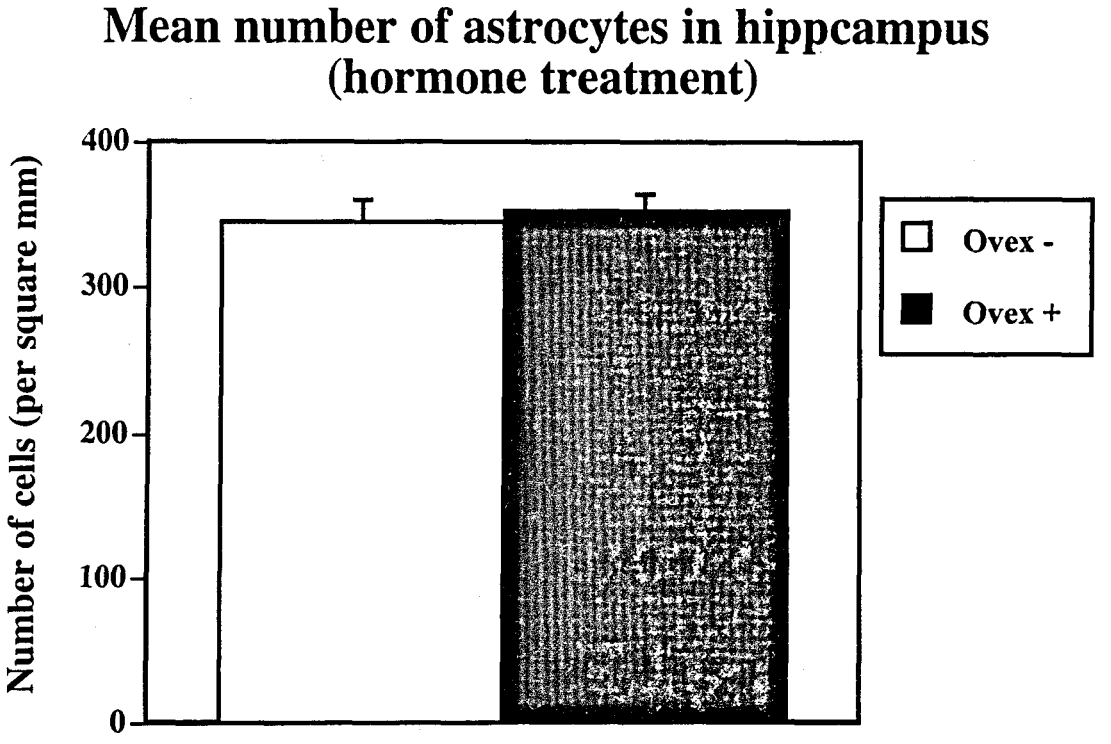

Mean number of astrocytes in hippcampus
(hormone treatment)
Number of cells (per square mm)
400
300
200
100
0
Ovex -
Ovex +

Figure Caption

Figure 16. Mean astrocyte process length in microns measured in the HI. Female steroid hormone treatment conditions elicited a significant difference between groups.

Mean process length for astrocytes in hippocampus (hormone treatment)

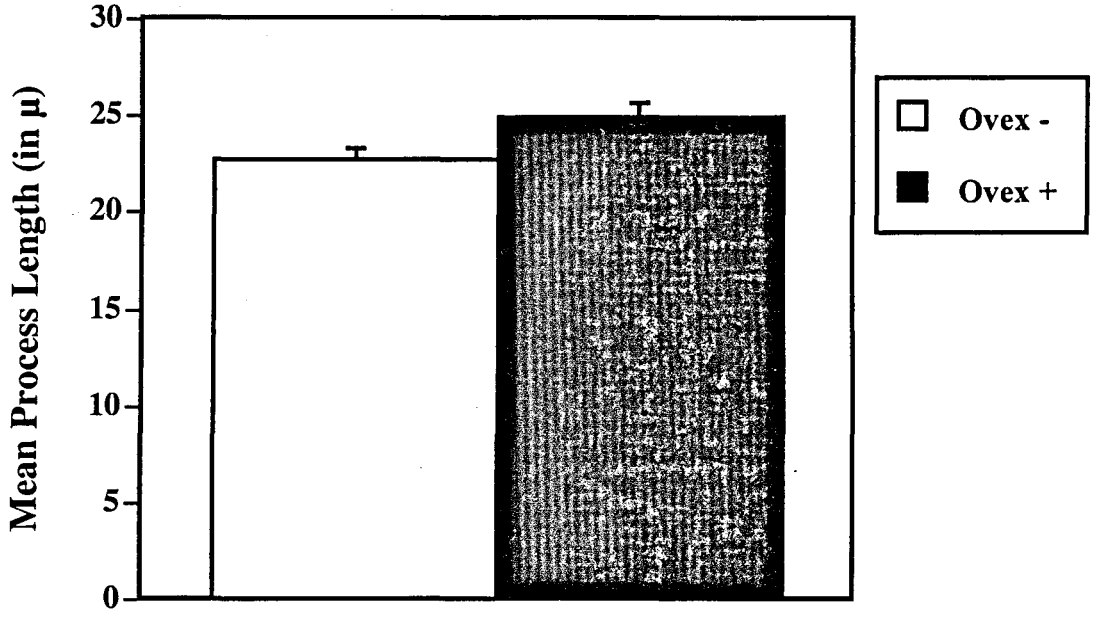


Figure Caption

Figure 17. Mean number of class I, class II and class III astrocytes counted out of a total of 16 cells sampled per brain in HI. Female steroid hormone treatment conditions resulted in significant differences between groups for class I and class III glia. No significant difference was identified between groups for class II cells.

Mean representation of astrocyte morphologies in hippocampus (hormone treatment)