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The Hormones of Pregnancy Alter Somal Size in the Medial Preoptic Area of the Rat Brain

Lori Anne Keyser

Thesis Advisor- Dr. Craig Howard Kinsley

Formerly non-responsive females will display maternal behavior (MB) following pregnancy and parturition. The behavioral alterations are believed to occur in response to hormonal changes that accompany pregnancy. The medial preoptic area (MPOA) regulates hormone-induced MB. The current study examined neuronal changes which might account for the modified behavior. Twenty adult female Sprague-Dawley rats were assigned a hormone condition: ovariectomized (OVX), ovariectomized/hormone-treated (P+E$_2$), intact diestrus (DI), or pregnant (PREG). Animals were killed, and their brains fixed in Golgi-Cox solution. Somata of the MPOA and related cortex were measured in each group using a Bioquant imaging system. Pregnant females had significantly larger somal areas in the MPOA than all other groups. Cortical soma size remained relatively unchanged between groups. These data suggest that the hormonal changes characteristic of pregnancy are capable of modifying neurons in the adult central nervous system. Modifications in the MPOA neuron, therefore, may play a role in the onset, maintenance, and retention of MB.
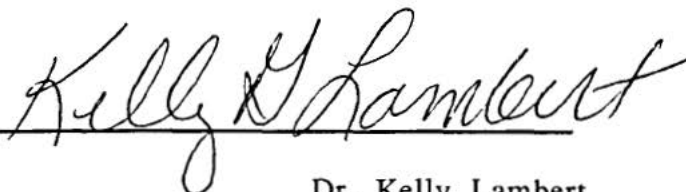
I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Arts.

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Dr. Craig H. Kinsley, Thesis Advisor

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THE HORMONES OF PREGNANCY ALTER SOMAL SIZE

IN THE MEDIAL PREOPTIC AREA OF THE RAT BRAIN

By

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The Hormones of Pregnancy Alter Somal Size in the Medial
Preoptic Area of the Rat Brain

It is well established that the central nervous system
undergoes early sexual differentiation. Investigators have
found sex differences in the size of the sexually dimorphic
nucleus of the preoptic area (SDN-POA), as well as synaptic
connectivity in the MPOA (Gordon, Shryne, & Southam, 1978;
Raisman & Field, 1971). Bleier, Byne, & Siggelkow (1982)
detected dimorphisms in patterns of cell distributions and
density in the medial preoptic area of the rat, guinea pig,
hamster, and mouse. Alterations have also been reported in
the fine structural organization of the central nervous
system. Ayoub & Greenough (1983) uncovered differences
between males and females in number of dendritic branches in
medial preoptic area (MPOA) neurons. The many changes in the
CNS are due primarily to exposure to gonadal hormones
(Gorski, 1990).

Historically, sexual differentiation was hypothesized to
occur in response to gonadal steroids present in circulation
during "critical periods" of development (Goy & McEwen, 1980;
Toran-Allerand, 1976). Investigators believed that adult
brains were resistant to hormonal influences. Evidence has
emerged, however, which shows that hormones influence
morphological plasticity in the mature, adult brain

(Nottebohm, 1980; Gomez & Newman, 1991). For example, recent
studies have demonstrated that certain hormonal conditions
modify adult brain structures. The songbird telencephalon is
responsible for the production of complex song in males
(Nottebohm, & Arnold, 1976; Gurney, 1981). Nottebohm (1980)
observed that administration of the male steroid,
testosterone, to female canaries in levels sufficient to
induce song, resulted in growth in the size of the vocal
control nuclei of the telencephalon comparable to that of
males. Further, Bloch and Gorski (1988) observed reductions
in the SDN-POA of adult male rats who were gonadectomized and
injected with the female steroids, estrogen and progesterone.

Variations in hormone levels are also sufficient to
produce morphological changes in the adult CNS. Seasonal
fluctuations in testicular activity are known to alter the
size of nuclei in adult canaries and mice (Forger &
Breedlove, 1987; Nottebohm, 1980). Furthermore, increased
amounts of estrogen (E_2) have been shown to expand the size
and change the shape of neurons (e.g., E_2-treated neurons
became more rounded) in females (Cohen & Pfaff, 1981; Carrer
& Aoiki, 1982; Jones, Pfaff, McEwen, & 1985; Miller &
Erskine, in press). The withdrawal of hormones can also
produce alterations. Gomez and Newman (1991) showed that
neurons in the posterior medial nucleus of the amygdala lose

volume following castration in males.

Behavioral sex differences are due to hormonal influences. In the adult animal, it is thought that gonadal steroids enter neurons and bind to stereospecific receptors, which bind to nuclear chromatin, alter genomic function, and result in modified protein synthesis and function; the hormonal modification of neuronal circuitry is then translated to complex behaviors (Gorski, 1990). Steroid sex hormones control the discharge of gonadotropin releasing hormones from the pituitary to regulate certain behavioral responses. One behavior considered to be hormonally influenced is maternal behavior in females. Researchers have been able to stimulate maternal behavior in virgin females through hormone regimens which mimic pregnancy (Mayer, Monroy, & Rosenblatt, 1990; Bridges, 1984). It has been postulated that the development of parental responsiveness through hormonal modifications may result in permanent neuronal changes (Kinsley, 1994; Kinsley & Bridges, 1988).

Previous investigations have demonstrated that the MPOA regulates maternal behavior (MB) in females (Fahrbach & Pfaff, 1986; Numan, 1974; Numan, Rosenblatt, & Komisaruk; 1977). Knife cuts to the MPOA impair performance of maternal behaviors by lactating animals (Numan, 1974; Jacobson, Terkel, Gorski, & Sawyer, 1980; Numan & Callahan, 1980).

Pfaff & Keiner (1973) suggested that the large number of estrogen receptors in the MPOA make it a major site of action in the facilitation of MB. Fahrbach & Pfaff (1986) treated ovariectomized, virgin female rats with estradiol implants directly in the MPOA and found that the rats responded rapidly to pups. Gubernick, Sengelaub, and Kurz (1993) found an increase in somal size of MPOA neurons when female mice became mothers. Therefore, reproductive status also appears to be an important variable underlying CNS structural changes.

Maternal behavior includes crouching, nursing, licking, nest building, and retrieving pups. Although these behaviors can be exhibited by nulliparous as well as parous females, rapid onset is thought to be induced by the hormones of pregnancy (Seigel & Rosenblatt, 1975; Moltz, Lubin, Leon, & Numan, 1970; Bridges, 1984). Previous studies have demonstrated significantly reduced latencies to respond maternally in ovariectomized nulliparous animals from 6-7 days to 35-40 hours following hormone administration (Moltz, Lubin, Leon, & Numan, 1970; Siegel & Rosenblatt, 1975). In addition, the duration of steroid exposure also appears to be important to the latency of onset of MB. Bridges (1984) found that as the length of E_2 and progesterone exposure increased, latencies to exhibit MB declined.

Several studies pointed to estrogen as the primary hormone in maternal behavior. Siegel and Rosenblatt (1975) found that hysterectomized/ovariectomized rats treated with E_2 displayed short latencies to respond maternally. When comparisons were made between male, virgin female, and pregnant female rats, Koch and Ehret (1989) found that pregnant females have the highest number of nuclei with estrogen-receptor labeling and the highest number of E_2 target cells. Their results indicate that estrogen production increases significantly during pregnancy. Fahrbach, Morrell, and Pfaff (1986) traced the estrogen-concentrating neurons in the MPOA, showing that estrogen neurons project from the MPOA to the ventromedial midbrain. Subsequently, others have postulated that maternal behavior could be initiated by the activation of this pathway (Numan, 1990).

The role of progesterone in MB is evidently a facilitatory one. It has been suggested that progesterone exposure might increase the sensitivity of E_2 receptors, by "priming" the neurons for the subsequent increase in E_2 (Bridges, 1984). Progesterone levels remain high during the first part of pregnancy and then dramatically decline just before parturition. This phenomenon has been aptly termed "progesterone withdrawal". Consequently, E_2 receptors become

hypersensitive to estrogen, causing females to respond maternally (Doerr, Siegel, & Rosenblatt, 1981). Although progesterone production increases during pregnancy, its presence is not crucial for MB.

Lactogenic hormones, specifically prolactin (PRL), and placental lactogens, have also been implicated in maternal responsiveness. PRL, a protein hormone, is released into the bloodstream from the pituitary, whereas placental hormones are released from the placenta (Numan, 1994). The findings of a recent study (Bridges, 1994, p.37) indicated that lactogens reach the cerebrospinal fluid through "receptor-mediated transport across the blood-CSF barrier". Once in the CSF, the lactogenic molecules are then free to bind to receptors, especially in the MPOA. Moreover, the role of E_2 and P in promoting the synthesis of PRL receptors which bind to circulating PRL thereby stimulating MB was considered (Bridges, Numan, Ronsheim, Mann, & Lupini,1990). Prolactin, in fact, may be essential for onset of maternal responsivity. Bridges, DiBiase, Loundes, & Doherty (1985) found that sequential treatment with progesterone and estradiol stimulated rapid onset of full maternal behavior in rats with intact pituitary glands, but failed to stimulate maternal behavior in hypophysectomized rats. Although PRL has been shown to induce MB, investigators have determined that

circulating PRL levels are elevated only during the first half of pregnancy (Amenomori, Chen, & Meites, 1970). Placental lactogens, in turn, are elevated from days 10 to 22 of pregnancy, providing continually high levels of lactogens in the bloodstream throughout pregnancy (Numan, 1994). The effect of placental lactogens on MB has also been demonstrated. Bridges (1994) stimulated rapid onset of MB through hPL (a placental lactogen) infusions directly into the MPOA of ovariectomized nulliparous females.

These data suggest that hormones, in combination with the "enriched" environment of motherhood (pup stimulation) may produce structural changes in the MPOA. Thus, hormone-brain structural changes may produce marked facilitation of maternal behavior. Once established, maternal behavior is preserved independent of hormonal regulation. That is, once neuronal networks which elicit maternal behavior are hormonally activated, the behavior is retained or easily activated for a long period of time (Bridges, 1975; Bridges, 1978, Kinsley & Bridges, 1988a). Organizational changes that take place in the brains of primiparous and multiparous females resulting from the experience of motherhood are hypothesized to account for this phenomenon. For example, Modney and Hatton (1990) found that parous females possessed extensively reorganized cell-cell interactions with new

specialized synapses forming on the dendrites of magnocellular neurons. Such alterations may account for the behavioral changes that transpire during parturition and beyond.

Preliminary results from our laboratory suggest a relationship between pregnancy levels of progesterone and estrogen and the size of soma in the MPOA and cortex. Comparisons made between ovariectomized control females and ovariectomized females implanted with hormone capsules suggested that hormones could influence the size of MPOA neurons while leaving cortical neurons unchanged (Keyser, Stafisso-Sandoz, & Kinsley, 1994).

The goal of the current investigation was to gather additional data in order to support prior conclusions, as well as explore the influence of further hormonal environments on somal size. The two previous groups will be retained and increased (Keyser et.al., 1994) and two additional groups will be added: one comprised of intact, normally cycling females, and one group of pregnant females. The additional groups will complement earlier findings as well as provide further information. The intact control group will furnish data on typical somal size in virgin females during a period of normally low P and E_2, whereas the pregnant group will allow us to determine if other factors

expressed/released during pregnancy influence somata in the MPOA.

Method

Subjects

Twenty-one virgin female Sprague-Dawley rats bred in our laboratory served as subjects. All rats were mature adults, approximately 90 days of age, weighing 205-300g at the start of the study. Females were randomly assigned to one of three treatment groups (see below). (One animal was not used in the study due to difficulties that occurred with staining). The animals were housed from weaning in 20 x 45 x 25 cm. polypropylene cages filled with loosely packed wood shavings for bedding. Animals were maintained on a 12h light/dark schedule, in rooms kept at approximately 20° C. Purina rat chow and water were available ad lib. All animals used in this experiment were maintained according to the standards set forth by the University of Richmond Institutional Animal Care and Use Committee and the National Institutes of Health.

Treatment Groups

To eliminate the effects of endogenous hormones, on experiment day one, animals in the first two groups were bilaterally-ovariectomized using ether anesthesia. Rats were then treated with subcutaneously-implanted Silastic capsules (3 by 30mm) prepared according to Bridges (1985) protocol.

Group one (OVX) rats received blank implants. Group two (E2+P) females were implanted with capsules containing progesterone (Sigma, St. Louis, MO). Post-surgery, animals were housed individually to allow wounds to heal. On day eleven, progesterone-filled capsules were removed and replaced by (2mm) capsules containing estradiol (E2) (Sigma, St. Louis, MO). Blank capsules were replaced with new blanks of corresponding size to the E2 capsules. On day 21 all rats were killed with an overdose of sodium pentobarbital (administered intraperitoneally).

Females in group three (DI) (n=3) were checked daily via vaginal smear to detect when they were in diestrus. Diestrus was chosen as the point of investigation during the estrous cycle because of the low levels of circulating endogenous hormones (Freeman, 1994). Diestrus was determined by comparisons with cytology slides. At that time, rats were killed with an overdose of sodium pentobarbital (administered intraperitoneally).

Females in group four (PREG)(n=6) were housed with a sexually experienced male and checked daily via vaginal smear to determine whether sperm was present in the vagina. The day that sperm was detected was considered day one of pregnancy and the female was removed and housed individually until day 21 of pregnancy, at which time they were killed

with an overdose of sodium pentobarbital (administered
intraperitoneally).

Histology

At the time of death, brains were removed and blocked
into three sections by making two frontal cuts, one anterior
to the cerebellum and another anterior to the optic chiasm.
Sections were stored in Golgi-Cox solution (Ramon-Moliner,
1970) prepared in accordance with the Ruscio-Kinsley
variation (unpublished protocol) for ten days.

Brains were sectioned in the coronal plane into 150µm
thick slices, in serial frontocaudal progression, using a
vibratome (Ted Pella, Burlingame, CA) filled with a
physiological saline (.9%) bath. Slices were serially
mounted directly onto gelatin-coated slides, air-dried, and
exposed to alkalyzing (lithium hydroxide) solution,
dehydrated, cleaned in xylene, and coverslipped with
Permount.

Data Quantification

The MPOA and corresponding cortex were located on the
prepared sections with the aid of a stereotaxic atlas
(Paxinos & Watson, 1986) and the anterior commissure as a
prominent landmark. The stained sections were examined under
a Zeiss Axioplan microscope at x400. An investigator,
without prior knowledge of the group to which the animal

belonged, selected and measured the largest cortical and MPOA neurons with completely stained perikarya. A stage encoder was utilized to record regions already quantified. Somal areas were calculated using Bioquant image analysis software (R & M Biometrics, Nashville, TN).A total of 50 neurons (25 MPOA and 25 cortex) per animal were collected. Because the cerebral cortex is not considered a region with high estrogen receptor concentrations (Pfaff & Keiner, 1973), cortical somal areas (from the same slices as MPOA sites) were collected to determine if the hormonal effects were site-specific.

Statistical Analysis

Somal areas from the MPOA and cortex of each animal were averaged, and the average of each animal was used in subsequent analyses. Mean scores (dependent variable) was used in place of individual observations to calculate group means. Averaging neurons per animal was used to keep the number of data points within reasonable limits (Gubernick, et.al., 1993).

Statistical analyses were performed using a 2 x 4 between-subjects ANOVA with hormone exposure (OVX control, OI control, E2+P implanted, or PPEG) and brain region (MPOA or cortex) as the independent variables. Tukey post-hoc comparisons were then performed to determine where significant

effects occurred. SPSS-X MANOVA and CLR ANOVA were utilized for the analyses. Significance levels were set at p<.05.

Results

As expected, hormone condition was found to produce a significant effect on somal size ($F[3,30]=6.48$, $\underline{MSE}=$ 2847.21,$\underline{p}<.01$). Figure 1 indicates that MPOA somata grew larger as animals were exposed to more complex hormone conditions. In contrast to the MPOA differences, cortical soma size remained relatively unchanged by the animals' hormonal state.

Insert Figure 1 about here

Somal size differences were also expressed between brain regions ($F[1,30]=43.61$, $\underline{MSE}=$ 2847.21,$\underline{p}<.01$). MPOA somata were consistently larger than cortical somata across all four treatment conditions. In addition, an interaction effect between hormone condition and brain region ($F[3,30]=5.15$, $\underline{MSE}=$ 2847.21, $\underline{p}<.01$) was noted.

Closer examination of the data utilizing Tukey post-hoc comparisons revealed differences between the hormone conditions. Post-hoc tests indicated that MPOA somal size in PREG rats was significantly larger than OVX and DI females, $\underline{p}<01$. Although no significant differences were found between

PREG and E$_2$+P females, visual inspection of the MPOA means
(Table 1) reveals a trend of expanding somal size. With
regard to size in cortical neurons, no significant difference
was found among hormone conditions.

Insert Table 1 about here

Post-hoc procedures examining regional differences
determined that cortical somas were significantly smaller
(p<.01) than MPOA somata in E$_2$+P females as well as PREG
animals. It is interesting to note that although regional
differences were not significant in either OVX or DI females,
MPOA somata were larger in both instances.

Discussion

The current investigation provides further evidence to
corroborate the influence of gonadal hormones on the adult
mammalian central nervous system. More specifically, these
data illustrate that a relatively brief hormonal aberration,
such as pregnancy, is capable of altering somal structure.
These neuronal mutations would account for the behavioral
changes manifested by parturient rats (i.e., the onset of
MB).

With regard to regional (MPOA and cortex) differences found in the effects of hormones on somal size, the presence of steroid receptor sites appears to be responsible. Previous studies have demonstrated the abundance of estrogen receptors in the MPOA, and a paucity of receptors sites in the cortex (Koch & Ehret, 1989; Pfaff & Keiner, 1973). Therefore, one could attribute the site-specific effects to receptor concentrations. Because the MPOA contains an abundance of receptors to which circulating estrogens bind, the surge in gene activation and protein synthesis might modify neural activity in MPOA cells, thereby producing an increase in somal size, as well as behavior changes following pregnancy. Although significant differences in MPOA somal size were found only between the PREG animals and OVX and DI females, somata of E_2+P animals were over 62% larger than those of each control group. These data provide support for the theory that although elevated levels of E_2 and P are important in the induction of maternal behavior, other agents involved in pregnancy are also responsible for neuronal change. Placental lactogens are one possible source to consider. It has been suggested that PRL may be responsible for the increase in E_2 receptors (Bridges, 1990; Muldoon, 1981) detected during pregnancy. Kinsley and Bridges (1988b) found that juvenile males had higher levels of

circulating PRL than females. They concluded that the high PRL levels could account for males rapid onset of MB when exposed to stimulus pups (as cited by Kinsley, 1994).

It is also interesting to note that although elevated amounts of gonadal hormones and lactogens elicit an increase in somal area, lack of hormones do not appear to cause a decrease in somal size. Our findings contradict those found by Gomez & Newman (1991). In their study, neurons in the posterior medial nucleus of the amygdala of castrated male hamsters experienced a decrease in somal size compared to intact males. The discrepancy among findings could have resulted from differences in time elapsed between gonadectomy and data collection (12 weeks, Gomez & Newman vs. 3 weeks for the current study), inter-species differences, sex differences, or regional differences.

It is unknown whether the somal size changes which originate during pregnancy are only transient. For example, Gould, Woolley, Frankfurt, & McEwen (1990) demonstrated differences in dendritic spine density between control and hormone-treated rats. The transformation was found to be short acting and implied that spine density may fluctuate during the rat estrous cycle. They also found that ovariectomized rats not given hormone supplements experienced an actual decrease in dendritic spines.

In contrast, Bridges (1975) found that once maternal behavior is initiated, animals exhibit shortened latencies to respond maternally. Kinsley (1994) went on to suggest the constancy of these behavioral changes over time. Future research is needed to determine whether somal alterations that accompany pregnancy and parturition are permanent.

Although it is hypothesized that the somal size differences discovered in the current study were due to hormonal interactions and fluctuations, maternal behavior is a complex system comprised of a myriad of components. Therefore, one must consider what other variables are operating. In a seminal investigation, Rosenblatt (1967) noted hormonal stimulation as a contributing factor to maternal responsiveness. Rosenblatt determined that exposure to pup stimuli elicited MB from males and ovariectomized virgin female rats. Since, researchers have studied various hormonal-linked components of MB, analyzing sensory stimulation, opioid activity, etc.(Kinsley, Bauer, Beverly, Turco, Wellman, & Graham, 1991; Rosenblatt & Fleming, 1975).

In summary, the present investigation has demonstrated that the event of pregnancy alters somal size in female rats. Such alterations might account for the behavioral changes that occur in lactating females. That the adult female brain exhibits such dramatic cellular plasticity suggests a

lifelong role for gonadal steroid hormones in shaping physiology and behavior. Examinations of MB, therefore, provide us with a valuable model for understanding the interaction among hormones, brain, and behavior.

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Figure Caption

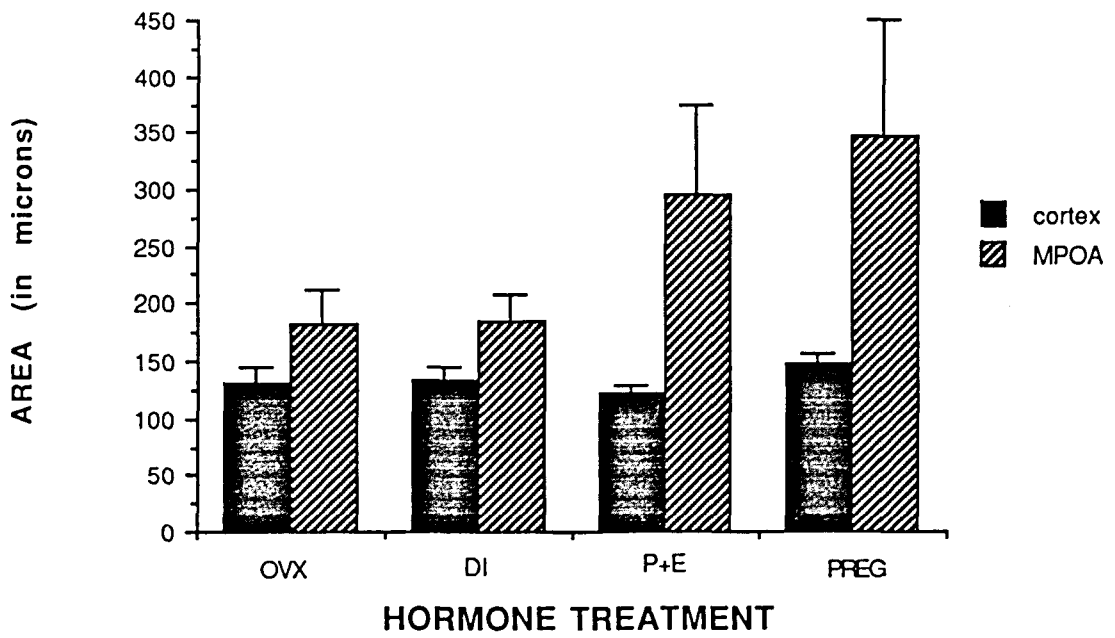Figure 1. Mean somal area (in square microns) by condition and region.

Table 1.

Mean (+ SD) Somal Area (in microns) of Cortical and MPOA Neurons

Group	n	Mean (sd) Cortical Somal Area	Mean (sd) MPOA Somal Area
Ovariectomized Control	6	131.42 (13.30)	181.62 (31.64)
Diestrus	3	133.75 (11.77)	184.84 (23.21)
Hormone Implant	5	122.08 (6.32)	294.54 (78.84)
Pregnant	6	146.98 (9.31)	345.91 (103.82)

Biography

Lori Anne Keyser was born on February 4, 1969, in Morgantown, WV. She received her Bachelor of Arts in Psychology from West Virginia University in 1991. Lori received a pre-doctoral fellowship from the Medical College of Virginia in August, 1994, and intends to continue working under the fellowship until the fall of 1996, when she plans to return to school to pursue a Ph.D. in clinical psychology.