CHANGES IN THE PARAVENTRICULAR NUCLEUS DURING DIFFERENT STAGES OF PREGNANCY AND LACTATION IN THE ALBINO RAT

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ABSTRACT

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By

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The magnocellular cells of the paraventricular and supraoptic nuclei are known to synthesize the hormones oxytocin and vasopressin. Although much is known about the stimuli that release these hormones (uterine stimulation, mammary stimulation, and osmotic stimuli) much less is known about how the hormones are produced. The fact that cell size and number of nucleoli in the cells of the supraoptic nucleus change during water deprivation and vasopressin production led to the realization that this phenomenon might also be true for cells in the paraventricular nucleus during oxytocin production. The present study is an analysis of the paraventricular neurones in intact male and female rats as well as during pre-parturient, lactating, and post-parturient non-lactating states in the female.

After histological preparation, the brains of four adult male rats, four adult female rats, four female rats one day prior to giving birth, four lactating mothers two days after giving birth, and four non-lactating females two days after giving birth were compared. Measurements were taken on percentage of cells containing two or more (multiple) nucleoli, percentage of cells whose nucleoli were bordering the nuclear membrane (marginated), and cell size. Both medial and lateral cells of the paraventricular nucleus were observed and data for these cell groups were treated separately.

Increases in the percentage of multiple nucleoli were seen for all three groups experiencing pregnancy conditions in comparison to the control females, and for the lactating females in comparison to males. For three of the groups studied, pre-parturient females, non-lactating females, and males, the lateral cells had a larger percentage of multiple nucleoli than the medial cells. Control hypothalamic cells showed no changes in percent of multiple nucleoli for any of the conditions studies. The ratio of percent multiples in the lateral cells to percent multiples in the medial cells was larger for males than females, and this was the only measure in which males differed from all the other females studied.

Margination proved to be a consistent variable to measure. No differences were found across groups or within

animals when either one or more than one nucleolus was found within the nucleus. However, when the cell contained multiple nucleoli an extremely high percentage of these (97%) had at least one nucleolus bordering the nuclear membrane.

Although cell sizes change during water deprivation and vasopressin production in the supraoptic nucleus, this phenomenon was not true for the paraventricular cells during increased oxytocin production. However, a medial-lateral cell size difference was found. In the sagittal planes both these cell groups are approximately the same size. But in the horizontal planes the lateral cells are larger than the medial cells, and significantly larger than lateral cells in the sagittal planes. This is further evidence that the medial and lateral cells are two different cell populations.

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To The Other Una

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INTRODUCTION

As early as 1906 it was discovered that posterior pituitary extracts had a stimulating effect on the uterus both in vivo and in vitro (Dale, 1906). Many years later it was suggested that milk ejection (ME) was a reflex which involved the activity of oxytocin (Ely and Peterson, 1941). Yet it was not until 1953 that the structure of oxytocin was analyzed by DuVingneaud and found to be a cyclic octapeptide linked by a disulfide bond.
(CyS-Tyr-ILEU-Glu(NH_2) - Asp(NH_2) - CyS-Pro-LEU-Gly(NH_2))
Since that time the work on this hormone has increased immensely with concentration on the areas of uterine motility, milk letdown, water regulation, and sperm transport, although the last is the least documented. Because of the well established effect of oxytocin on uterine contraction and milk letdown the female animal has been the primary subject of this research.

The origin of oxytocin in the hypothalamus has been the subject of much debate. Although it was originally thought that oxytocin was secreted from the paraventricular nucleus (PVN) and vasopressin from the supraoptic

nucleus (SON), the evidence now supports the hypothesis that both hormones are formed in both nuclei, but are produced in separate neurones. The theory of the specialization of the PVN for oxytocin production arose from early lesion studies. Electrolytic lesions in this nucleus cause a marked reduction of oxytocin activity in the neurohypophysis (Olivocrona, 1957) and similar lesions cause dystocia in pregnant cats (Nibbelink, 1961). Both oxytocin and vasopressin are found in the paraventricular nucleus and supraoptic nucleus (Lederis, 1961; Dyer et al, 1973). The more recent technique in which neurosecretory cells are identified by antidromic stimulation has given strong support for the production of oxytocin in both nuclei. A similar percentage of neurosecretory cells in both nuclei show a rapid acceleration in discharge rate about 15 seconds before a rise in intramammary pressure associated with ME (Wakerly and Lincoln, 1973a, 1973b). Other evidence for oxytocin production outside of the PVN is given by data showing that after bilateral electrolytic destruction of this nucleus in rats with diabetes insipidus, DI, rats lacking vasopressin, there are still substantial amounts of neurosecretory materials (probably oxytocin) stored in the pituitary one month later (Sokol, 1970).

Studies linking the PVN with oxytocin production include those of stimulation and recording. Electrical stimulation of the PVN (Cross, 1955a; Cross, 1958a) and of the median eminence and neurohypophysis in the rabbit

(Lincoln, 1971; Cross and Harris, 1950) resulted in ME and/or induced labor. Recording studies of antidromically identified units in the PVN at different times of the estrus cycle have also shown interesting results. For example, the mean firing rates are higher during pro-estrus and estrus than in metestrus and diestrus. Firing rates at the end of pregnancy are twice that at mid-pregnancy. The firing rates at 24 hours after parturition and during lactation were higher than those during metestrus, diestrus, and earlier pregnancy states (Negoro et al, 1973). An increase in discharge rates can be correlated with hormone secretion (Dyball and Dyer, 1971; Brooks et al, 1966). But interpretation of the increase in discharge rate for the level of the hormone in the blood is questionable, for it may reflect release into the pituitary, and storage, of oxytocin.

Swaab and Jonkind (1970) investigated hormone production in the PVN and SON by analyzing the neuro-secretory activity of these nuclei during each stage of the estrus cycle, pregnancy, parturition, and lactation, and in the adult male. The Golgi-specific enzyme, thiamine diphosphate phosphohydrolase (TPP-ase), was used for measuring neurosecretory activity. Their method was a semi-quantitive histochemical method which determines changes in enzyme distribution, not in enzyme activity. The SON and PVN reacted very similarly under these conditions. Enzymatic distribution was higher during both

parturition and lactation, lower during light induced persistent estrus, and even lower during day 21 of pregnancy. Enzyme distribution rates seen in males and in metestrus females were similar and the latter showed lower rates than did females in all the other conditions. The low enzymatic distribution during metestrus is similar to the low firing rates of PVN neurones seen at this time (Negoro et al, 1973). The similar reaction of both nuclei (the PVN and SON) at these times cannot be explained without knowing the level of each hormone, oxytocin and vasopressin, in these cells. During light induced persistent estrus the gonadotrophin hormone level changes and this may be influencing the high enzymatic distribution seen at this time. Because blood estrogen and prolactin concentrations are high on the day of parturition, one cannot overlook the possibility that this may have an excitatory effect on PVN neurones, which may be an explanation for the high TPP-ase distribution seen on this day. In ovariectomized rats, firing rates in the PVN increase when estrogen is administered (Negoro et al, 1973).

There are two cystine-rich proteins in the hypothalamus, known as neurophysin A and neurophysin B. Neurophysin A has been labelled as the carrier protein for vasopressin and neurophysin B as the carrier protein for oxytocin (Dean et al, 1968; Burford et al, 1971). To determine if the PVN synthesizes oxytocin and the SON synthesizes vasopressin, radioactive cystine was injected into the two

nuclei separately and the neurophysin A/B ratio calculated. The ratio was .76 ± .03 with PVN injections and 1.15 ± .16 with SON injections. Because this oxytocin neurophysin was labelled more in the PVN, one may conclude that more oxytocin is produced in the PVN, assuming that the association of neurophysin B with oxytocin is valid (Burford et al, 1972; Burford et al, 1974). Rats with bilateral lesions in the PVN show a significantly larger neurophysin A/B ratio in the posterior pituitary when killed 24 hours after the cystine injections, further evidence for the majority of oxytocin production in the PVN (Burford et al, 1973; Burford et al, 1974).

Although the manufacture of both oxytocin and vasopressin may occur in two different nuclei, it appears that the individual neurones are specialized for production of only one hormone. In rats with hereditary diabetes insipidus and presumably incapable of synthesizing vasopressin, the neurohypophysis shows areas of clustered granules and other areas lacking in stainable material. In normal rats deprived of water, and secreting oxytocin, the granules are not clustered but are spread evenly throughout. When these normal animals are rehydrated, there is a reaccumulation of secretory granules with no segregated clusters. These granules reflect the storage of oxytocin and vasopressin (Livingston, 1971). In the DI animals the non-staining areas may be the axon terminals of vasopressin producing cells (Sokol and

Valtin, 1967).

Howe (1962) has shown that only certain areas of the neurohypophysis are labelled with arginine, an amino acid present in vasopressin but not oxytocin. In a later study, when twenty-one antidromically identified cells in the PVN were recorded during reflex milk ejection, there were nine cells which did not change their firing rates. These might possibly be cells responsible for vasopressin production, or they may be inactive oxytocin producing cells. In our lab, with the light microscope, we have identified two different magnocellular cell groups in the PVN, the lateral and medial cell groups. It may be that one of these cell groups is responsible for oxytocin production, while the other is responsible for vasopressin production.

Moss, Urban, and Cross (1972) found that there are two sets of neurons in the PVN. Eighty-one percent of the antidromically identified cells were excited by acetylcholine and 83 percent were inhibited by norepinephrine. In the non- neurosecretory cells (those not identified by antidromic stimulation) 81% were excited by norepinephrine and 76% were inhibited by acetylcholine. However, this does not separate oxytocin producing cells from vasopressin producing cells. Further work on synaptic transmission is necessary for an understanding of how ADH and oxytocin are differentially released.

Stimuli for Oxytocin Release

There is a differential release of oxytocin and vasopressin to different stimuli. Some stimuli which are the most documented to cause release of oxytocin are vaginal and nipple manipulation, while hemorrhage induces ADH release (McNeilly et al., 1972) and .1 M $CaCl_2$ infusion releases both (Forsling et al., 1973). ADH does have some milk ejecting activity although it is only 1/6 as potent as oxytocin (Cross and VanDyke, 1953).

In many species, there is a rise in plasma levels of oxytocin associated with onset of suckling. Levels of 12-25 mU have been reported in the woman (Coch, 1968), 17-640 mU in the cow (Folley and Knaggs, 1965), and 31-375 mU in the rabbit (Bisset et al., 1970). With a normal milk yield in the rabbit, ADH was found in only one of eight animals (Bisset et al., 1970). Measures of ADH have been taken in other animals during ME and have been found to be below the threshold necessary for ME. These animals include the cow (Petters and Coussens, 1950) dog (Pickford, 1960) and woman (Theobald, 1959).

There is also favorable evidence for release of oxytocin during parturition, especially during the second stage of labor; i.e., during the expulsion of the fetus. This has been reported in the sheep and cow (Fitzpatrick and Walmsley, 1965) goat (Folley and Knaggs, 1965) and in the rabbit (Haldar, 1970). In the rabbit it is apparent that parturition is a stimulus for release of oxytocin

independent of vasopressin. The ratio of oxytocin to vasopressin was as high as 26/1 in one case and at least 5/1. In the goat, neurophysin is released simultaneously with oxytocin, and no rise in ADH is seen (McNeilly et al, 1972).

Wakerly and Lincoln (1971) recorded intramammary pressure in female rats with eight suckling young. The young were returned to their mother after 18 hours of separation. They found that there was a latency of 10-30 minutes after suckling began before there was a change in intramammary pressure. Thereafter there was an ME response every 10-20 minutes. This response is abolished by removal of the young, and is similar to that obtained by a rapid injection of .5 -1.5 mU of oxytocin. As was mentioned previously, there is a rapid acceleration in firing rate in some of the PVN cells 15 seconds before this ME response (Lincoln et al, 1973; Lincoln and Wakerly, 1971). It is interesting to note that suckling does not initiate release of oxytocin. Although during ME the pups are seen to arch their back and pull harder on the nipple, this causes no further change in PVN activity.

Because of the finding that bradykinin, 5-hydroxytryptamine, acetylcholine, and vasopressin will also cause ME, it had to be shown that oxytocin is the sole initiator of ME under normal conditions (Bisset et al, 1970). N-carbamyl-o-methyl, which competes for receptor sites with oxytocin, will block mammary contractions. But this

drug is also an antagonist of vasopressin. Yet with the hydrated animals within 1.5 hours after surgery there is a normal urine output, with an increase in urine flow following each ME. This was further evidence that oxytocin is released independently of ADH during ME (Wakerly et al, 1973).

Nucleoli

Although it is known that suckling of the nipple and stimulation of the uterus cause a rise in oxytocin secretion, the exact mechanism by which cells in the PVN respond is not known. It has been reported that cells in the SON increase their number of nucleoli within two hours when rats are water deprived (Hatton and Walters, 1973). This increase continues to rise up to 24 hours under deprivation conditions. With rehydration, the percent of multiples decreases although it does not return to the baseline level in 10 days of rehydration. Animals adapted to a desert habitat, where water conservation is necessary, are shown to have more multiple nucleoli in the supraoptic nucleus than animals adapted to a more moist environment (Hatton et al, 1972). The nucleolus is intimately concerned with the production of high molecular weight ribosomal RNA, and the production of proteins. Although there is a high concentration of protein within this nucleolus, it has not been determined if this organelle is a site for storage or synthesis of protein

(Busch and Smetana, 1970, p. 6). Yet the presence of multiple nucleoli may reveal one of the mechanisms at work in protein formation in these neurosecretory cells. The possibility that there is a nucleolar response of this kind during the increased production of the octapeptide, oxytocin, has now been analyzed.

Statement of purpose

The nucleoli of cells in the PVN have not been observed under conditions of lactation and parturition, except on day 8 of lactation where nucleolar size was measured. The presence of multiple nucleoli would be further evidence both of the role the PVN plays under these conditions, and of the underlying mechanisms at the cellular level for hormone secretion. The purpose of this experiment is to observe the number, position of nucleoli, and cell size in the PVN in the adult female rat, adult male rat, in the pre-parturient female, and in the post-parturient female under both lactating and non-lactating conditions. Although oxytocin is known to be produced in approximately equal amounts in both the normal male and female, the reason for the high presence of this hormone in the male remains unknown, and it will be interesting to compare the oxytocin producing cells of the two sexes.

Because some preliminary work (Hatton and Hutton, unpublished) has shown that there are two populations of PVN cells, the medial and lateral cells, which react as

heterogeneous bodies under water deprivation conditions, these two cell populations will be examined separately in this study. All data will be analyzed separately for the medial and lateral cells to determine if there are really two separate mechanisms at work in these differentiated cells.

All control adult male and female rats were kept on a constant light cycle, as opposed to the other groups which will be kept on a light-dark cycle (14 hours light, 10 hours dark). The purpose of this is to make the female acyclic, a light induced estrous condition, and to avoid oxytocic fluctuations which may occur in the cycling animal. It has been shown that firing rates in PVN neurones are higher during proestrous and estrous than during metestrous and diestrous (Negoro et al, 1973). Although it has not been shown that plasma levels of oxytocin vary during the different stages of the female's cycle, I feel that if firing rates do change there might be a multiple nucleolar difference as well. Thus I chose to make the control females acyclic, similar to the lactating and pregnant animals.

METHOD

Animals

The subjects in this study are adult male and female albino rats obtained from the Holtzman Company in Madison,

Wisconsin. These animals were housed individually with food and water present at all times. Ambient temperature ranged from 21-23 C. Five groups of rats were used in this study: four constant estrus females; four adult males; four females were sacrificed one day prior to expected parturition; eight females were sacrificed 48 hours after parturition, four of which were left with six lactating pups, and four of which had their pups removed at birth. Total number of pups and weight of each pup was recorded at birth for females sacrificed after parturition, or at time of perfusion for the pre-parturient (PP) group. All control adult males and females were kept under constant light conditions, whereas the remaining groups were maintained on light-dark cycles (14 hours light, 10 hours dark). The adult males and light-induced females were kept in our colony room for 30 days prior to perfusion.

Histological procedure

Animals were weighed and anesthetized with ether. A two-cc blood sample was taken from the heart of all animals and plasma hematocrit and specific gravity determined. Animals were then perfused transcardially with a 0.9% saline solution, followed by a formalin solution of one part 37% formalin and nine parts 0.9% saline. The brains were removed from the skull, dehydrated in alcohol solutions and embedded in celloidin.

From each group of four animals, two brains were sliced in horizontal sections and two were sliced in sagittal sections. The brains were sliced at 22 um and stained with thionin.

Cell characteristics

Magnocellular cells of the paraventricular nucleus (PVN) of the hypothalamus were analyzed. These cells are larger than the parvocellular cells and have a dark staining nucleolus and cytoplasm. The nucleus is round and clear. The cell area of both medial and lateral cells was measured for 60 medial and 60 lateral cells per animal. Samples of fifteen cells were taken from each horizontal or sagittal section containing the PVN, half of which came from each side of the brain. Only cells with observable nucleoli were taken for cell size measurements. The cells were magnified 2,170 times and the outlines of the perikarya were traced. Planimeter measures were used for the area determinations.

Nucleoli

Any dark staining, approximately round spot of the appropriate size (2-5um) within the nucleus was considered to be a nucleolus. One hundred and fifty cells from each side of the brain were sampled, making a total of 300 cells per animal. Of these half were lateral cells (cells in the more lateral, dorsal, and posterior regions) and half

were medial cells (cells in the more medial, ventral, and anterior regions of the nucleus). Both the position and number of nucleoli were observed. The two classifications of cells can be seen in Figure I.

Control cells

One hundred control cells from each animal were compared to PVN cells. These cells were taken from areas immediately surrounding the PVN.

Sampling procedure

Samples were taken on 25 cells from each section. Either odd or even thionin stained sections were listed. This was 7-8 sections in the horizontally sliced brain and 17-21 sections in the sagittally sliced brains. From these, six horizontal sections and twelve sagittal sections were chosen for counting. From these fifty cells were counted in the horizontal sections, 25 from each side of the brain, and 25 cells were counted from each parasagittally sliced section.

A Whipple-Hauser reticule was placed in one eyepiece of a Zeiss microscope. Under low power the PVN was brought into focus. Then under a higher power objective (40x) one part of the nuclear region was placed under the reticule and decisions about which rows or columns of the reticule the sampled cells were to come from were made before the microscope was focused under this power. The part of the

nucleus to be counted, while keeping medial and lateral sections separate, was varied from upper left, upper right, lower left, lower right, and center.

RESULTS

Physiological data

The physiological data are given in Table I for all animals with the exception of males on whom no blood data was recorded. Plasma protein, hematocrit measures as percent plasma, number of pups, and time of perfusion are reported. Two one-way analysis of variances were done to analyze both plasma protein and hematocrit counts on all the females in the study. It appears that the three groups of females that underwent pregnancy had higher hematocrits at the time of perfusion. However the AOV was non-significant (F=3.09, P .10, df=3/12). The differences for plasma protein counts were also non-significant (F=2.73, P .10, df=3/11). This may be due to the small sample size measured. The number of pups varied from 2-13 and there appears to be no relationship between this measure and the blood measures or the percentage of nucleoli in the paraventricular nucleus.

Medial and lateral cells

The paraventricular nucleus was photographed and these photomicrographs are seen in Figure I. Horizontally sliced

Table 1. Physiological Data

Animal	Plasma Protein	Number of Pups	Hematocrit % Plasma	Time of Perfusion
Normal Female				
1	6.45	-	54.0	10:00 AM
2	6.50	-	55.0	10:30 AM
3	7.00	-	56.0	11:00 AM
4	6.60	-	54.0	11:30 AM
Pre-Parturient				
10	6.00	8	59.0	11:15 AM
11	6.30	12	61.0	10:30 AM
12	6.50	11	61.0	9:45 AM
14	6.50	2	52.0	12:00 PM
Lactating Females				
1	6.40	6	58.0	11:15 AM
4	6.40	8	57.0	11:15 PM
7	5.90	9	64.0	8:30 AM
5	6.40	10	57.5	10:45 AM
Pups Removed				
2	6.30	6	60.0	11:45 AM
6	7.10	13	-	3:45 PM
8	6.70	5	60.0	2:00 PM
13	7.40	12	62.0	8:45 AM

Figure 1A. A photomicrograph of a horizontally sliced Thionin-stained section through the lateral cells of the paraventricular nucleus from a post-parturient non-lactating mother rat.

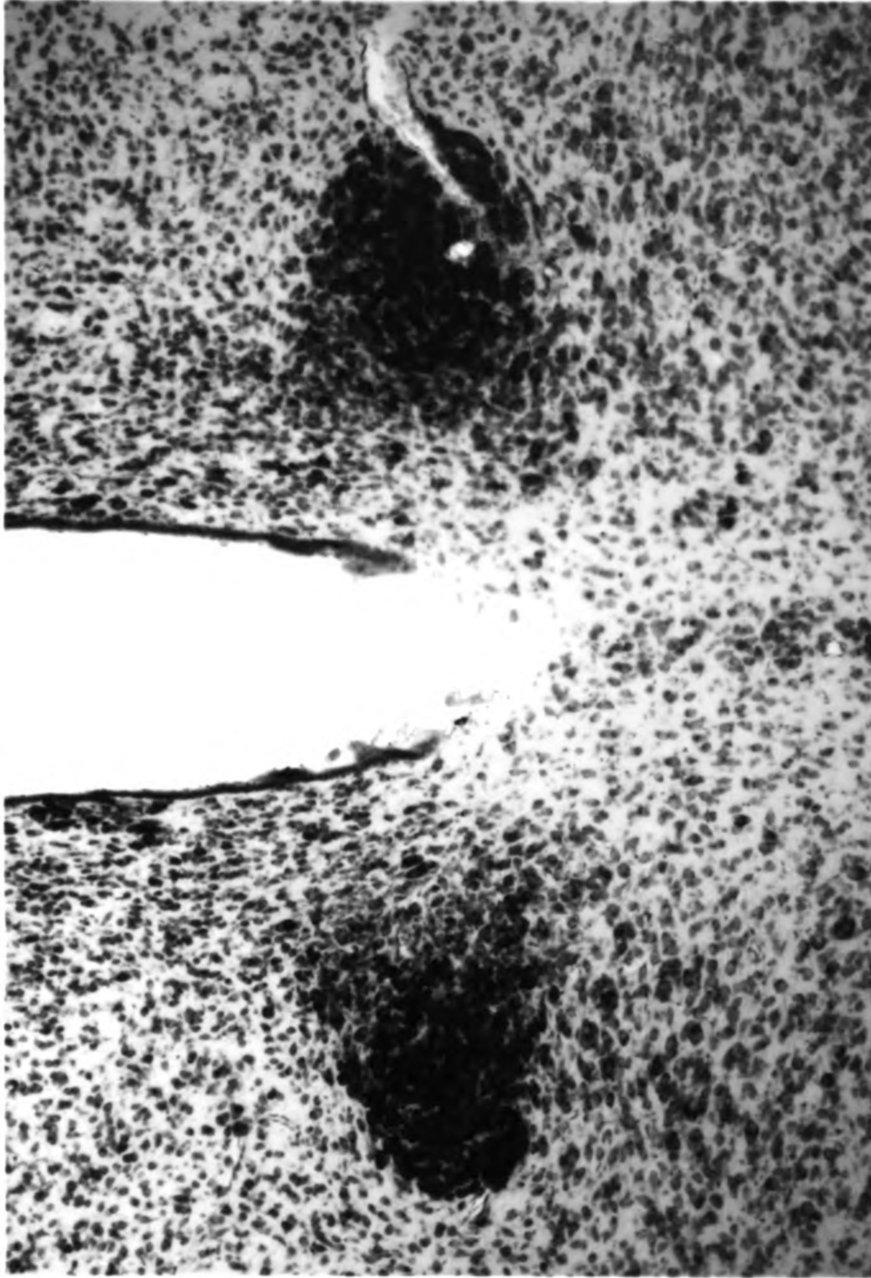

0.5 mm

Figure 1B. A photomicrograph of a horizontally sliced Thionin-stained section through the medial cells of the paraventricular nucleus from a post-parturient non-lactating mother rat.

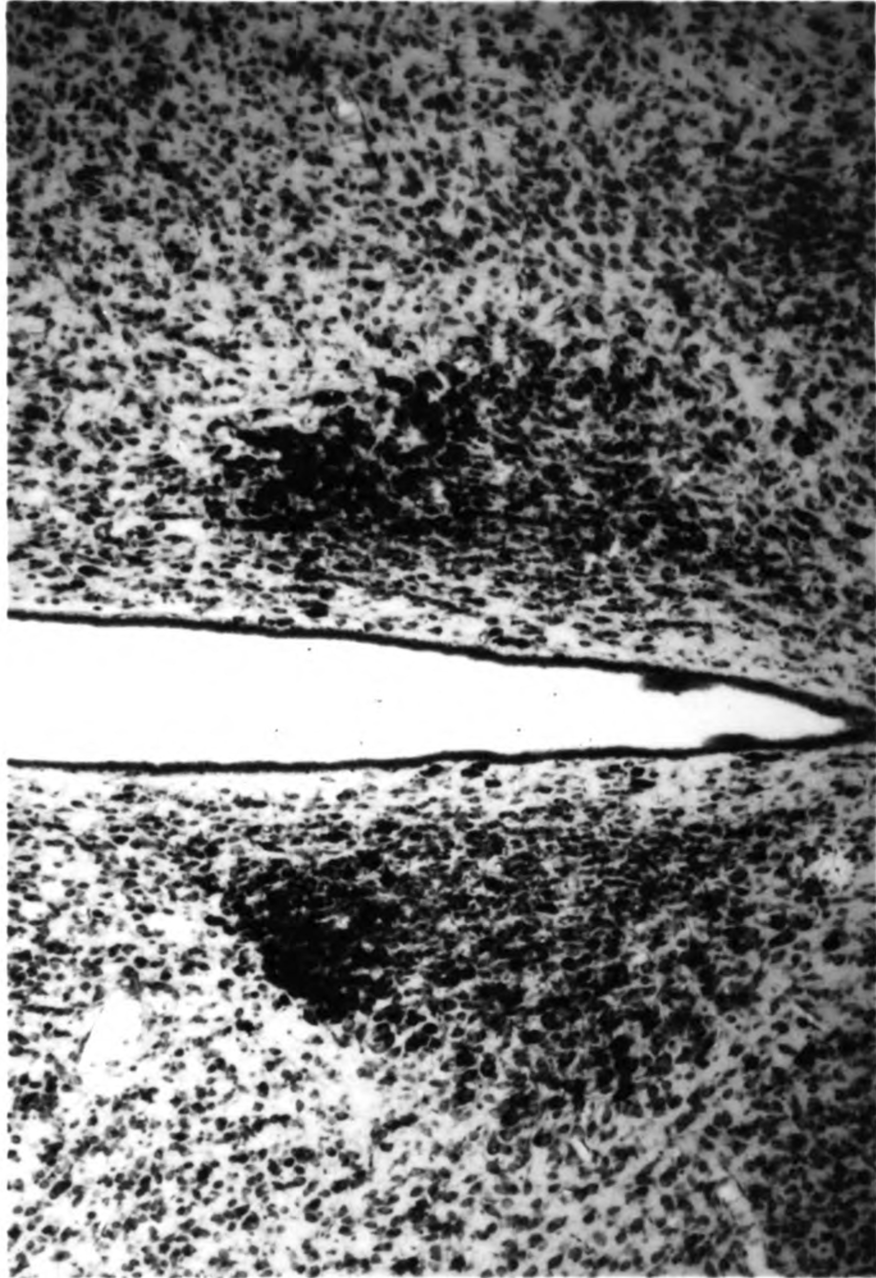

0.5 mm

Figure 1C. Photomicrograph of a horizontally sliced Thionin-stained section through the lateral cells of the paraventricular nucleus from a post-parturient non-lactating mother rat.

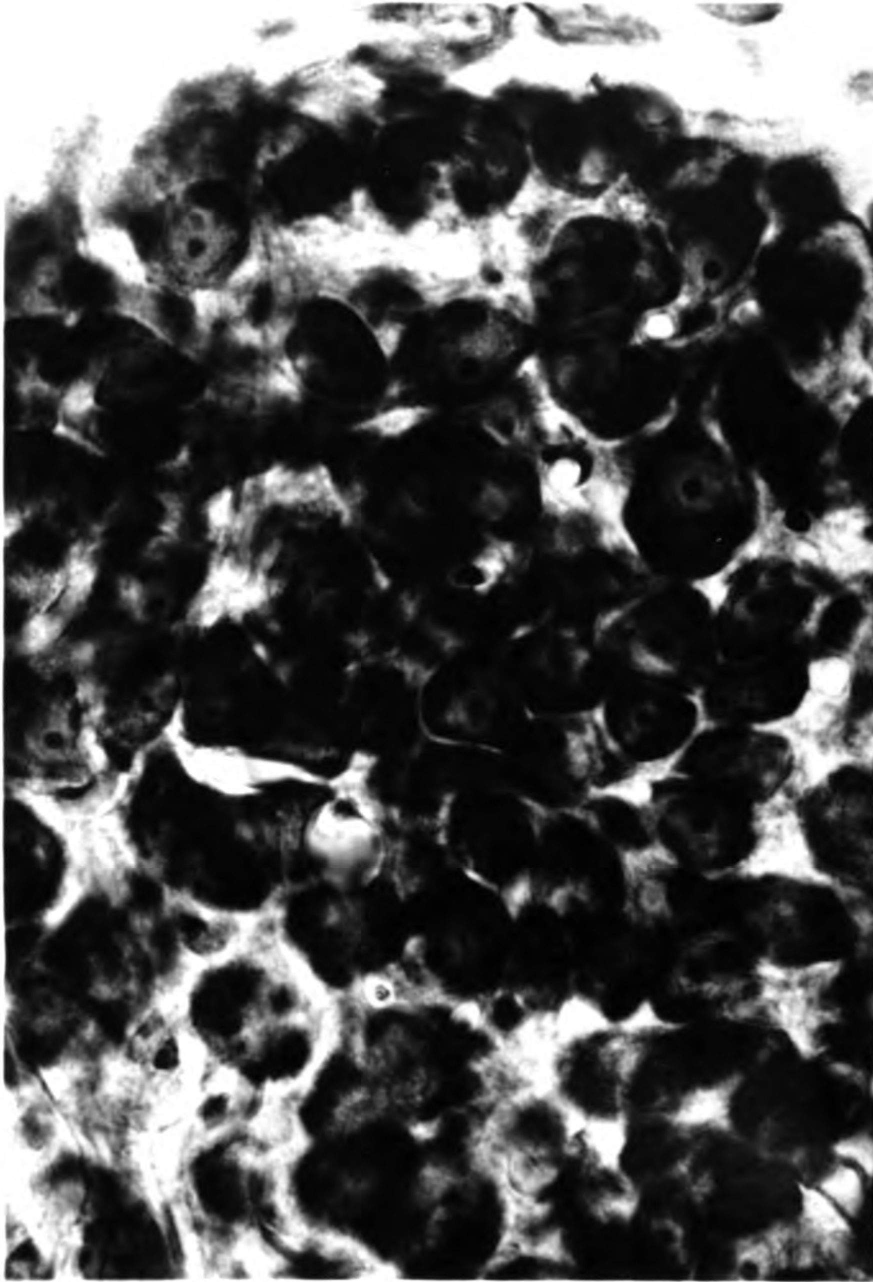
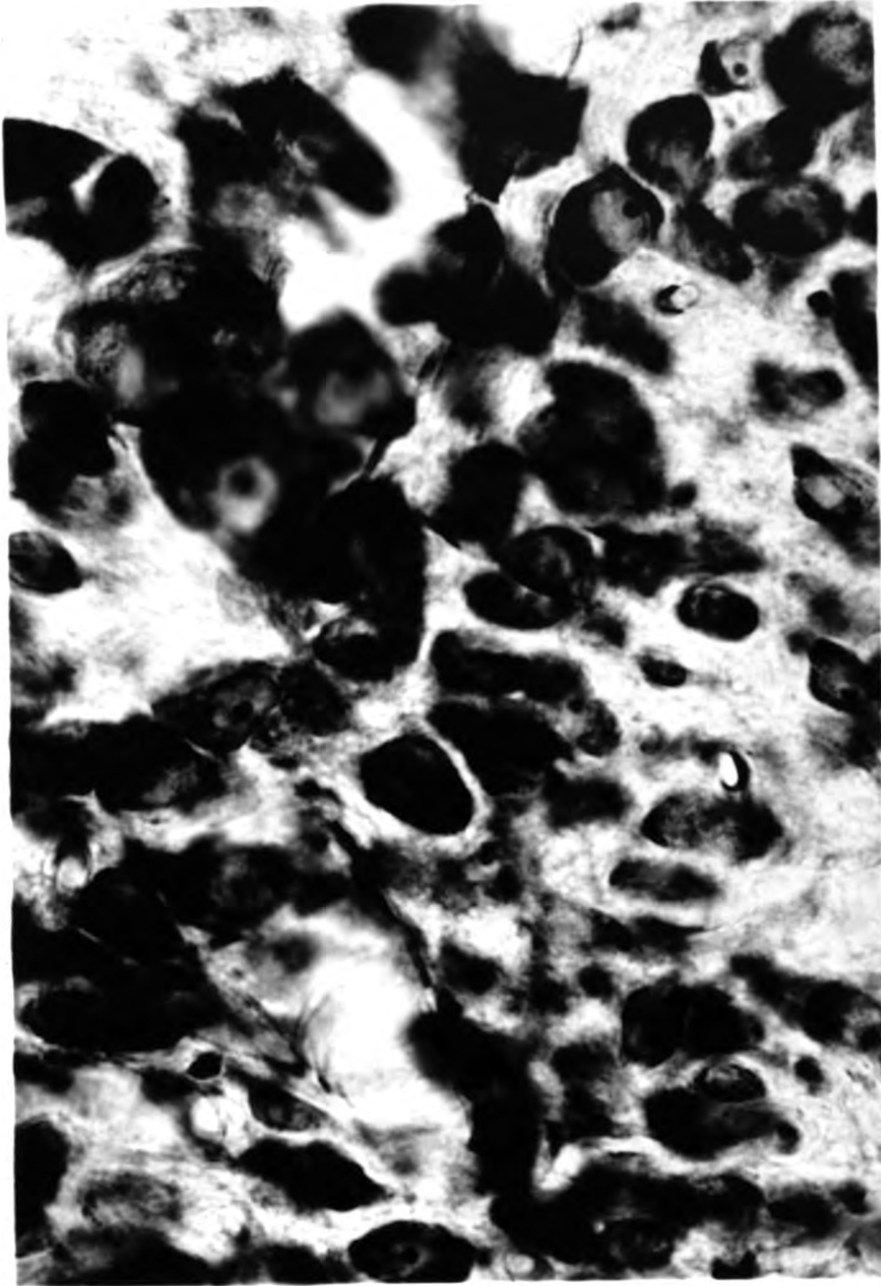

50 μm

Figure 1D. A photomicrograph of a horizontally sliced Thionin-stained section through the medial cells of the paraventricular nucleus from a post-parturient non-lactating mother rat.


50 μm

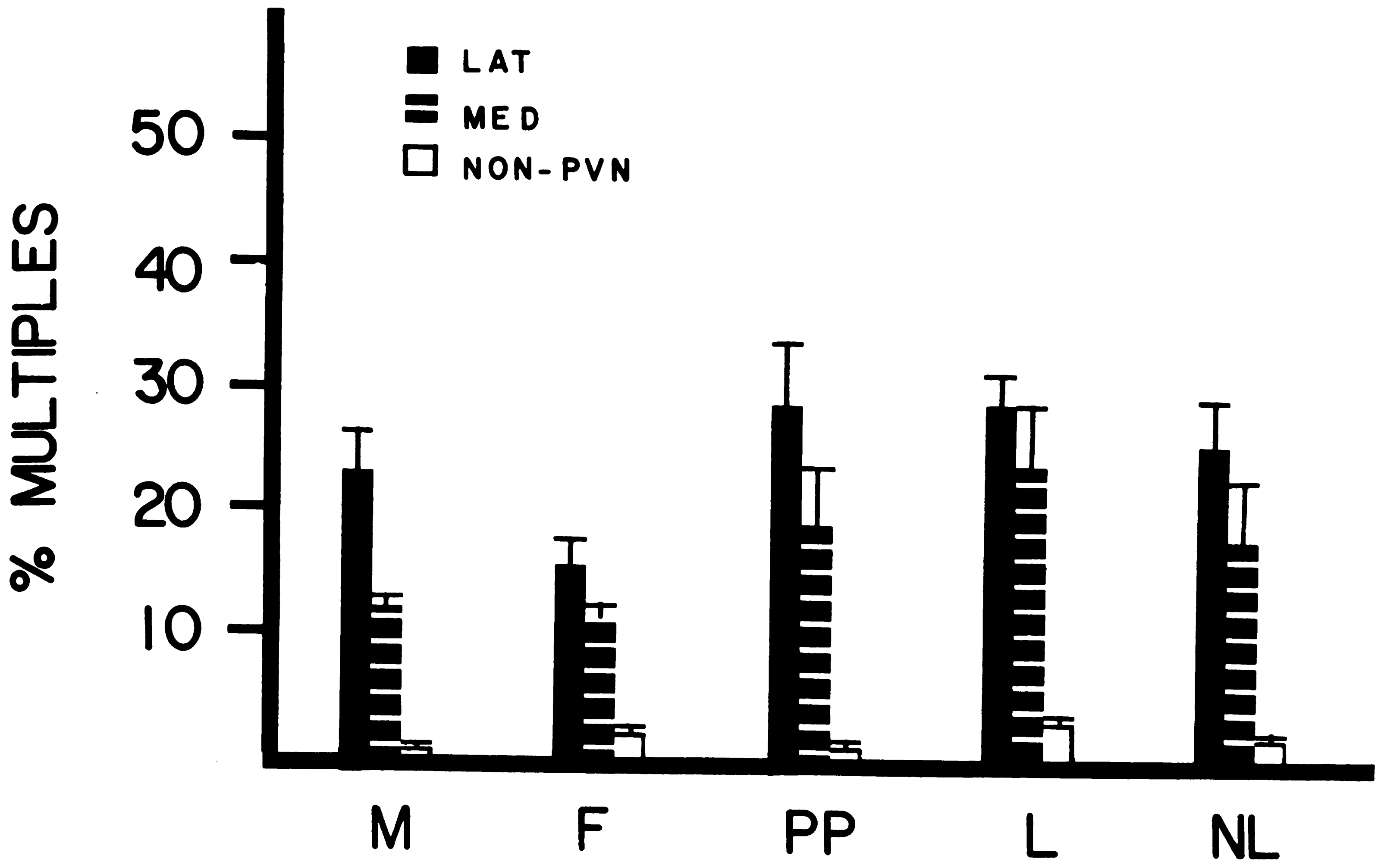
sections of a non-lactating animal were chosen for the photomicrographs. Both lateral and medial cells are shown under low and high power of the microscope. It can be seen that the lateral cells are generally larger than the medial cells.

Multiple nucleoli

The percentage of cells whose nucleus contained two or more nucleoli is plotted in Figure 2. Figure 2 shows the mean (+S.E.) for medial and lateral paraventricular cells and for non-paraventricular hypothalamic cells. The increase in multiple nucleoli which occurs in the PVN during and after parturition can be observed. Control cells showed no apparent increase or decrease in nucleoli throughout the experimental conditions and maintained an average of 1.5% multiples. Data for medial and lateral cells were analyzed separately because they are distinctly different cell populations and previous work (unpublished data) showed some difference in their responses to water deprivation.

An analysis was done (a two-factor mixed design, repeated measures on one factor) to analyze differences across groups and between medial and lateral cells. Differences were found both across groups (F=3.14, df = 4/15 $P < .05$) and within animals (medial vs. lateral) (F=49.07, df = 1/15 $P < .001$). No interaction was found. F-tests for simple effects were done to further analyze these results. Duncan's Range test was the statistical test used for

Figure 2. Mean percent (+ S.E.) of multiple nucleoli for lateral, medial, and non-paraventricular hypothalamic control cells for adult males (M), light-induced estrus females (F), pre-parturient females one day before parturition (PP), lactating females 48 hours after giving birth (L), and non-lactating females 48 hours after giving birth (NL).


% MULTIPLES
50
40
30
20
10
LAT
MED
NON-PVN
M
F
PP
L
NL

between group comparisons. There was a reliable increase in multiples ($P < .05$) from normal females to the three groups of females experiencing pregnancy conditions. The lactating (L) females showed a higher percentage of multiples than males ($P < .05$). There were no other significant differences between groups.

Medial vs. lateral cell differences within groups were significant at $P < .001$ for the non-lactating (NL) group, $P < .005$ for the pre-parturient (PP) group, and $P < .001$ for males. There were no significant medial-lateral differences in the other two groups.

Because the Duncan's range test between group comparisons combines both medial and lateral cells, a Wilcoxon rank sum was also done to compare the groups while keeping medial and lateral cell data separate. In the lateral cell group, differences were significant at $P < .05$ between normal females and lactating (L) females. In the medial cell group differences were significant ($P < .05$) between normal females and the L group, and between males and the L group.

By a one-way analysis of variance, a significant difference was seen in control cells (non-paraventricular hypothalamic cells) vs. experimental group cells on percent of multiple nucleoli ($\underline{F}=187.77$, $\underline{df} = 1/15$, $P < .001$).

A test for correlated measures was done across all animals, comparing the medial cell reaction to that of the lateral cells. $r = .7$.

An analysis, using the Mann-Whitney U test, was done to compare the ratio of percent multiples of lateral cells to medial cells between males and females. The ratio of lateral to medial cells is larger in males than in females ($P < .05$ one-tailed test).

Marginated nucleoli

When only one nucleolus was found within the nuclear membrane, and this nucleolus was touching the nuclear membrane, it was considered to be marginated. When two or more nucleoli were seen within the nucleus, and one or more of these were bordering the nuclear membrane, it was considered to be a marginated multiple cell. Table 2 gives the means for percent margination for all lateral and medial cells for each group. A mixed design analysis showed that there were no significant differences across groups, between medial and lateral cells, and no interaction for both single and multiple marginated cells.

These percents for singles were acquired by taking the total number of cells with one marginated nucleolus and dividing by the total number of cells with only one nucleolus in the nucleus. Similarly, the percents for multiples were acquired by taking the total number of cells with two or more nucleoli, one or more of which was marginated, and dividing by the total number of cells with two or more nucleoli.

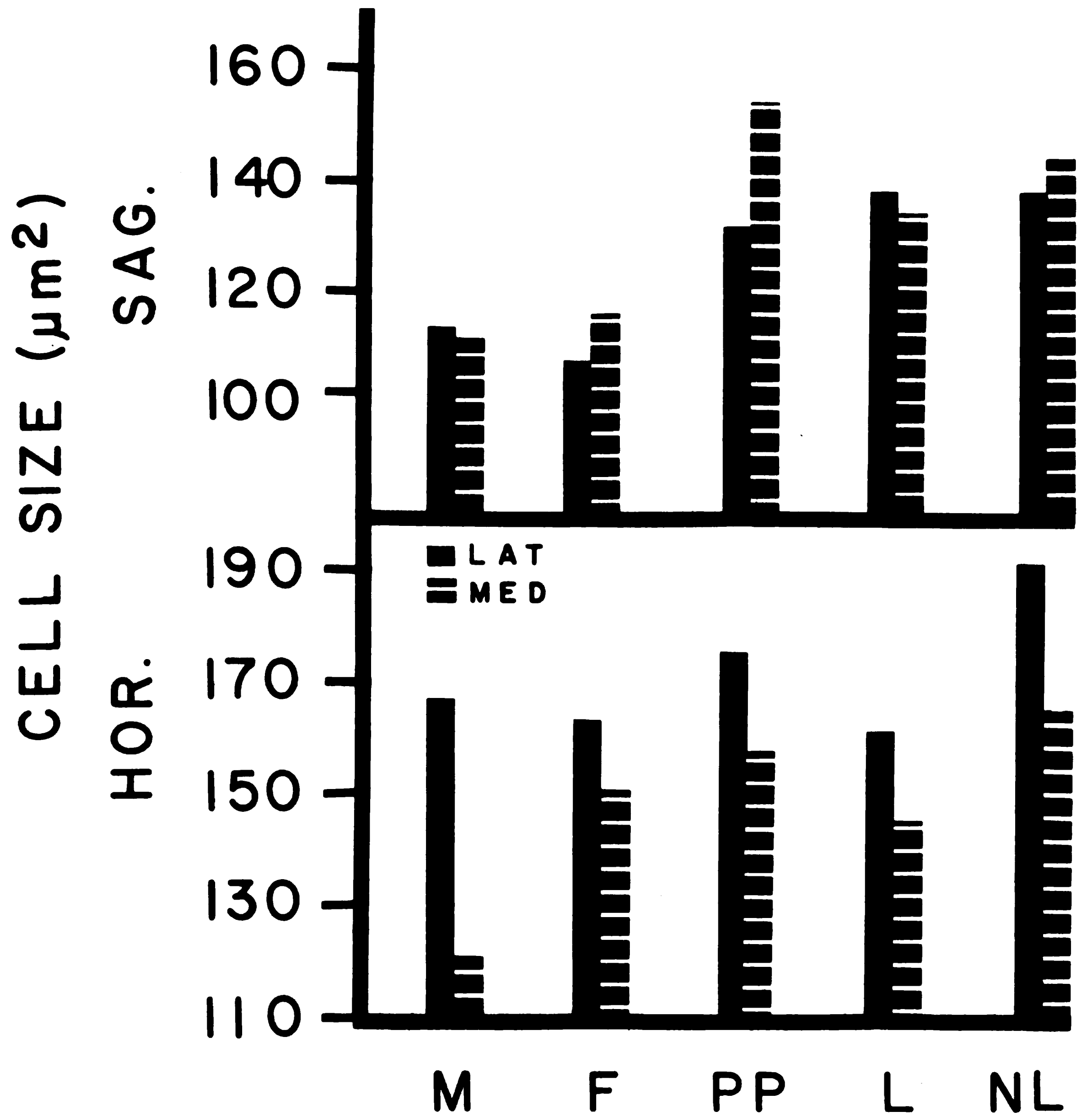
Table 2. Mean percent margination for medial and lateral cells.

Animal group	Single		Multiple	
	lateral	medial	lateral	medial
Normal females	35.4	34.6	94.1	100.0
Normal males	35.6	33.0	98.8	97.3
Pre-parturient females	41.7	37.9	94.9	99.3
Lactating females	39.9	37.9	97.3	97.1
Non-lactating females	38.1	35.2	94.5	94.1

Cell Size

Mean cell areas in square micro-meters and standard errors were calculated for each animal, with medial and lateral cell data kept separate. An analysis by a two factor mixed design comparing groups, and medial-lateral differences within groups, showed no significant differences. However, when an analysis using a 2 x 2 design was done comparing medial-lateral cell sizes across each plane of section, there were found to be reliable differences between horizontal and sagittal planes ($\underline{F}$=19.89, $P < .001$, $\underline{df}$ = 1/8), a medial-lateral cell size difference ($\underline{F}$=8.90, $P < .005$, $\underline{df}$ = 1/18), as well as an interaction ($\underline{F}$=24.7, $P < .001$, df = 1/18). When an analysis using $\underline{t}$-tests for related measures was done to compare cell sizes within each plane of section, it appeared that in the horizontal plane lateral cells are significantly larger than medial cells ($\underline{t}$=6.14, $P < .001$, $\underline{df}$ = 7, two-tailed), and in the sagittal planes there is no reliable difference between medial and lateral cell sizes. Figure 3 shows the means for medial and lateral cell sizes across groups in both the sagittal and horizontal planes. Table 3 gives the mean and standard error of cell areas for each animal in the study, maintaining a medial-lateral cell group separation. Also given here is the mean for all medial and lateral cells in each plane of section. An analysis using $\underline{t}$-tests for independent samples was done to compare cell size differences across planes for medial and lateral

Figure 3. Mean cell size in um^2 for lateral and medial cells in males (M), control females (F), pre-parturient females one day before parturition (PP), lactating females 48 hours after giving birth (L), and non-lactating females 48 hours after giving birth (NL). Mean cell areas are given for both horizontally and sagittally sliced brains.



CELL SIZE (μm²)
SAG.
HOR.
160
140
120
100
190
170
150
130
110
LAT
MED
M
F
PP
L
NL

Table 3. Cell areas of paraventricular neurones in um^2.

Animal	Medial Cells	Lateral Cells
Normal Female		
1 (Hor)	136.55 ± 4.67	152.90 ± 6.58
2 (Hor)	163.73 ± 6.16	174.35 ± 5.52
3 (Sag)	126.99 ± 4.25	107.03 ± 2.97
4 (Sag)	103.85 ± 3.40	104.06 ± 3.40
Normal Males		
1 (Hor)	122.46 ± 3.61	165.64 ± 4.88
2 (Hor)	117.65 ± 4.03	167.34 ± 5.31
3 (Sag)	117.01 ± 5.52	119.35 ± 3.82
4 (Sag)	105.12 ± 3.40	107.67 ± 3.82
Pre-parturient females		
10 (Hor)	167.56 ± 4.88	170.10 ± 5.95
11 (Hor)	145.47 ± 5.73	179.02 ± 6.80
12 (Sag)	152.90 ± 5.10	136.13 ± 4.25
14 (Sag)	153.75 ± 7.65	126.99 ± 4.88
Lactating Females		
5 (Hor)	154.18 ± 5.95	165.01 ± 6.80
1 (Hor)	136.55 ± 4.46	156.30 ± 6.16
4 (Sag)	139.10 ± 5.31	149.50 ± 6.58
7 (Sag)	126.99 ± 4.46	126.14 ± 4.46
Non-lactating females		
2 (Hor)	161.61 ± 5.95	191.98 ± 5.31
13 (Hor)	168.19 ± 6.37	190.70 ± 7.01
8 (Sag)	152.48 ± 5.52	154.39 ± 5.95
6 (Sag)	134.21 ± 5.95	121.47 ± 2.97
Mean Horizontal	147.44	171.33
Mean Sagittal	131.24	125.29

cell groups. For the medial cell group cell sizes in horizontal and sagittal planes did not differ. Yet, in the lateral cell population, cells sliced in the horizontal plane were reliably larger than those sliced in the parasagittal planes ($t=7.00$, $P<.001$, $df = 18$).

The standard errors were divided by the mean cell size for each individual animal to equate standard errors for each animal. This was done to determine if there was more variety in cell size for the medial cells compared to the lateral cells. The mean of all the S.E. divided by mean cell size for lateral cells was .035 and for medial cells was .037 and it was concluded that there was not more variation in cell size for the medial cells.

DISCUSSION

In viewing the overall results of this study, it becomes apparent that the most major differences are seen in the paraventricular nucleus of females under light induced estrus and in adult males in comparison to lactating females. There is an increase in multiple nucleoli in the PVN during lactation. This is not so surprising a result for the cell activity, measured by firing rates of the neurones (Negoro et al, 1973) and the TPP-ase distribution which is used to measure the neurosecretory activity of the cells (Swaab and Jonkind, 1970) are both higher in the nucleus of the lactating female than in the acyclic female

and adult male. The three groups of females that underwent pregnancy states were all killed within three days of each other, near parturition, and no reliable differences were found across groups in any of the measures analyzed. Some differences might be found between lactating females and females whose pups were removed at birth if the time of perfusion were extended beyond day two of lactation.

The female and male rats used in this study as controls were both submitted to constant light conditions. This renders the female acyclic, a condition of constant vaginal estrus. The normal pattern of gonadotrophin secretion changes, seen by the characteristic high levels of pituitary and plasma luteinizing hormone, decrease in ovarian weight, and increase in pituitary weight (Lawton and Schwartz, 1967; Maric et al, 1965). A steady secretion of estrogen is also characteristic of this persistent estrus condition. It is known that estrogen does raise the firing rates of neurones in the PVN of ovariectomized animals (Negoro et al, 1973) and this increased estrogen secretion at constant estrus may also have an effect of increasing the metabolic activity of cells in the PVN. During constant light, the TPP-ase distribution is increased (Swaab and Jonkind, 1970) over that of metestrus animals. In the SON the size of the nucleus and the nucleolus increases with constant light (Flament-Durand, 1967). Yet for this study constant light was required in order to avoid oxytocic fluctuations which

may occur in the cycling animal.

The lactating female rat

If increased nucleolar number can be used as a measure of increased RNA production, this study does not give direct support to the idea that the PVN is more active under constant light, for these animals do contain nuclei with a lower number of nucleoli than animals known to be releasing increased amounts of oxytocin, the lactating animals. It would be interesting to note if any differences do exist in the nucleolar structure and number during each of the four cyclic conditions: metestrous, pro-estrous, diestrous, and estrus.. The fact that no nucleolar differences were seen between the pre-parturient and post-parturient groups tends to suggest that the activity in the cells at these times is similar and more active than during constant estrus. During suckling, oxytocin is liberated in amounts greater than during non-suckling conditions (Bisset et al, 1970; Folley and Knaggs, 1966; Wakerly and Lincoln, 1971) and the cells must be able to replace this expended protein at a rapid rate. A reduction in Gomori-positive neuro-secretory material and a depletion of the osmiphilic core of the neurosecretory granules during suckling has been reported (in Norstrom et al, 1972). Within 15 minutes after the pups are removed from the mother there is a repletion of the dense core of the neurosecretory granules in the posterior pituitary (Monroe and Scott, 1966). These

elementary granules are considered to be the site of storage of posterior pituitary hormones, suggesting that with lactation oxytocin is being depleted from the pituitary (Livingston, 1971). On the third day of lactation, rats whose pups were removed for 200 minutes showed much less newly synthesized material in the neural lobe of the pituitary in comparison to the neural lobe of mothers who continued lactating. This was interpreted as being due to an increase in axonal transport due to suckling.

It is especially surprising that no differences were seen between the pre-parturient females and the lactating females. During lactation the neurosecretory activity in the PVN is significantly greater in relation to day 21 of pregnancy (Swaab and Jonkind, 1970) and the same authors report the work of Flament-Durand (1967) who found an increase in nuclear and nucleolar size as well as an increased cysteine-S^{35} incorporation in the PVN during lactation. There is a trend towards a larger amount of multiple nucleoli in the lactating female and with a larger sample size this trend might become more obvious. It is also possible that multiple nucleoli appear a day before neurosecretory activity increases and it would be necessary to examine the nuclei of animals during parturition to validate this possibility.

In the present study no measure was taken of the size of the nucleolus; what has been studied here is the <u>number</u>

of nucleoli and the cell size itself. The nucleolus is concerned with the production of high molecular weight RNA and thus plays a major role in protein synthesis. With an increased depletion of hormone and neurophysin during suckling, in comparison to the pre-parturient and post-parturient non-suckling states, one would expect an increased need of protein formation during lactation and a greater demand would be made upon the protein producing centers of the nucleus. As seen in Figure 2, there is a trend towards a higher percentage of multiple nucleoli during lactation, a trend which is especially apparent in the medial cells of this nucleus. This trend is supported in that normal females differ from the lactating females in both the medial and lateral cell groups, yet the number of nucleoli in the pre-parturient and non-lactating rat is not significantly increased over that of the control females. Measures made on male rats (percent multiples) also differ significantly from the lactating group in the medial cells, but not from any of the other conditions. In the SON the increase in number of nucleoli is seen within two hours of water deprivation and within 24 hours of water deprivation there is a rise in firing rates of cells in the SON which are significantly higher than the firing rates of animals given free access to water (Walters and Hatton, 1974). So there is indirect evidence, at least for the SON, that the number of nucleoli increase with an increased amount of vasopressin production and is correlated with an increase

in firing rates of the cells in the nucleus.

Firing rates in the paraventricular nucleus

Although there have been no studies reported for the rat which follow the amount of oxytocin either in the blood or in the PVN itself during pregnancy states, studies have been reported for the guinea pig (Burton et al, 1974) and for the cow, rabbit, and goat, (Fitzpatrick and Walmsley, 1962; Folley and Knaggs, 1965; Haldar, 1970) who showed an increase in oxytocin levels during the expulsive stages of labor. However, in the rat there are several studies analyzing firing rates of cells in the PVN during these different pregnancy and post-pregnancy states, yet to date no one has observed the firing rates of post-parturient non-lactating animals. At full term pregnancy firing rates were reported to be 2.8 ± 0.35 spikes/sec, within 24 hours after birth at 3.5 ± 0.35 spikes/sec. and during days 2-10 of lactation firing rates averaged 3.2 ± 0.24 spikes/sec. The rates continue to rise during parturition and through lactation and do not decrease until day 20 of lactation. In the non-pregnant rat rates vary throughout the cycle - a low of 1.5 ± 0.25 during metestrous and a high rate of 4.5 ± 0.33 during proestrous (Negoro et al, 1973). In the female lactating rat firing rates in the non-phasic discharging cells of the PVN increase from about 3/sec. to 50/sec., 14-26 seconds preceding milk ejection (Wakerly and Lincoln, 1973; Lincoln and Wakerly, 1971). Brooks et al,

1966) also showed that stimuli that elicit milk ejection in lactating cats (an oxytocin release) also cause an increase in neurone firing rates. The spontaneous firing rates of PVN neurones in male rats under normal conditions is slow - the majority less than 1/sec., although much variation can be seen (Dyball, 1971; Dyball and Koizumi, 1969).

It is probably an oversimplification to say that under all conditions there is a direct relationship between firing rates and hormone release (Dyball, 1971). Although blood oxytocin may be low in the day before birth, it appears by firing rates that the cells are more active than during half-term pregnancy and more active than in the male rat. If one can correlate firing rates with increases in multiple nucleoli as was done in the SON, these similar firing rates may help explain why only a trend was seen towards an increase in multiple nucleoli during lactation compared to the pre-parturient and non-lactating groups, and no real significant differences found. It also helps to explain why a significant increase was found in comparison to adult males. As was suggested by Dyball (1971) it is possible that these firing rates reflect the storage function of the cells, and not always hormone release.

<u>The post-parturient non-lactating female rat</u>

After birth a mother with no lactating pups should

have a decreasing need for oxytocin production and the neurosecretory activity of the cells producing oxytocin should subside. Within two days after rats which were without water for 24 hours were given free access to water the percentage of multiple nucleoli had decreased back towards that of control rats, although it did not return to a baseline condition by then (Hatton and Walters, 1973). Within two days after pups were removed from the mother only a trend towards a decrease in number of nucleoli can be seen. It is possible, although unlikely since rat pups continue to suckle constantly throughout the day, that two days without pups is not a long enough time for inhibition of the oxytocin producing neurones. If we accept the idea that the internal sensing system detects that lactation has been terminated, which appears to be the case since milk ejection and the increase in firing rates in the PVN preceding milk ejections never occurs in the absence of suckling pups, (Wakerly and Lincoln, 1971) the continued presence of multiple nucleoli seen at this time needs to be explained. In the PVN two days after birth may not be a long enough time for the nucleolar decline to become apparent. There may be no disadvantage for a cell to contain more than one nucleolus in the resting state, and after lactation has ceased, the nucleolus may be slowly disposed of. The exact effects of oxytocin on the uterus are uncertain, but it may be that oxytocin plays a

necessary role in the early retrograde changes that occur in this post-parturient uterus. Some astonishing results might be seen if these animals were compared to those animals on day 10 of lactation, both with and without lactating pups. By day 10 of lactation the early involutional changes of the uterus would have taken place, and one would be more certain that oxytocin secretion had decreased in the non-lactating mothers.

There is no way of excluding the possibility that some of the magnocellular cells sampled may be vasopressin producing or may be non-neurosecretory ordinary nerve cells associated with the neurosecretory cells. Numerous studies have shown that vasopressin to some extent is produced in the PVN although it is produced to a much greater extent in the SON (Dyball, 1971; Dyball and Koizumi, 1969; Burford et al, 1973; Burford et al, 1974; Koizumi and Yamashita, 1972). Vasopressin should be increasing towards birth in order that water concentration remain high for milk production. It must also be remembered that although oxytocin is six times more effective than vasopressin in stimulating milk ejection, the latter does also share this functional ability.

<u>The nucleolus</u>

The formation of the nucleolus is controlled by what has been termed the nucleolar organizer region (NOR), or the secondary constriction although in the rat these

constrictions are not observable (Busch and Smetana, p. 119). These regions are located only on the specific chromosomes concerned with nucleolar formation. They contain the DNA template involved in nucleolar and RNA synthesis (Busch and Smetana, p. 24, 116). The number of nucleoli varies from cell to cell, and in the PVN magnocellular cells as few as one and as many as three nucleoli were seen in individual cells. The nucleolus in nerve cells is known to react to different experimental manipulations by changing its number (Hatton and Walters, 1973), as well as changing its structure and size (Busch and Smetana, p. 425-430). It is also known that in non-neural tissue, hormones such as estrogens, androgens, and hydrocortisone cause increased RNA synthesis and probably an activation of the nucleolus (Busch and Smetana, p. 46, 499). A likely mechanism for an increase in number of nucleoli may be that there is a dis-inhibition of the loci on the genes coding for nucleoli, which under normal conditions are repressed allowing for only one nucleolus to form (Busch and Smetana, p. 138). What the active factors are that influence the nucleolus to produce an increased amount of RNA or cause the additional nucleolus are unknown at the present time. With two nucleoli the efficiency of RNA synthesis may be enhanced over that of one nucleolus. There is an increase in surface area with two nucleoli giving more area for transportation of RNA into the cytoplasm. It is also

possible that this second nucleolus is both biochemically of a different makeup than the original nucleolus and serving a somewhat different function for the cell.

The paraventricular nucleus in the male rat

No differences were found between normal males and females on measurements of multiple nucleoli or margination. But when a comparison of the ratio of percent multiples in the lateral cells to percent multiples in the medial cells is made between males and all females studied it appears that the males have a higher lateral to medial ratio. The lateral cells in the males are more active in relation to medial cells, if one may correlate increases in nucleolar number with increased protein synthesis and nuclear activity. The medial cells are not being inhibited for they contain nearly equal amounts of cells with multiple nucleoli as do the light-induced estrus females. The estimated amount of oxytocin secretion for the male rat in water balance is 18.7 mU /day and 28.9 mU /day for vasopressin. It may be that these lateral cells are more concerned with vasopressin production.

It is worthy to note that the baseline of percentage of multiple nucleoli in the lateral cells of the PVN in males is almost double that in the medial cells. And in the SON, also known to release both hormones, oxytocin and vasopressin, although predominately vasopressin, the baseline for multiple nucleoli is again half of what it is in

the medial cells. Yet the SON contains approximately 10,000-12,000 cells and 5% of these are multiples - about 500 cells. In the lateral cells of the PVN, a portion of the nucleus which contains about 1,250 cells, there are 23% of the cells with multiple nucleoli - about 287 cells. Thus it appears that there may be a direct relationship between the total percentage of multiple nucleoli and amount of hormone secreted. Only 12 of the medial cells (about 850 cells) in the males are multiples - about 100 cells, and since less oxytocin is produced in the male daily it may be that these are predominately responsible for oxytocin production.

Medial vs. lateral cells

Under two of the measures analyzed, percent multiple nucleoli and cell size, the medial and lateral cells appear to react differently. The lateral cells respond with a significantly higher percentage of multiple nucleoli for three of the conditions studied (the males, pre-parturient, and non-lactating) in comparison to the medial cells. With the exception of one animal found in the lactating group, all individual animals showed a higher percentage of multiples in the lateral cells. Thus we are really speaking of two different cell populations which are the makeup of what is typically thought of as one nucleus, the paraventricular nucleus. Under the conditions studied

here, as well as under water deprivation conditions, a medial-lateral cell difference is the case. From this study it is really impossible to determine which of these cells may be oxytocin or vasopressin producing without a measure of the hormone level in the nucleus, in the pituitary, and in the blood.

With only one nucleolus in the nucleus, there is an equal tendency for this nucleolus to border the nuclear membrane in both the medial and lateral cell groups. Approximately 37% of the nucleoli have this position. This is shown in Table 2. When more than one nucleolus is found in the nucleus, the lateral cells again contain nearly equal amounts of marginated nucleoli as do the medial cells - (97%-98%). Margination then leaves us with no appreciable differences to discuss.

The cell size of the two populations does differ. Lateral cells are larger than medial cells in the horizontal plane of section. And the lateral cells sliced in the horizontal plane are larger than those sliced in the parasagittal planes, yet this size difference is not seen in the medial cells. This could mean that the medial cells are spheres and randomly oriented where a slice in either plane through the center of the cell would give equal measurements. It could also mean that these cells are not spherical and are oriented consistently, and the mean of all cells sliced in each plane would be equal. By the size difference seen in the lateral cells it is obvious

that these cells are not spherical, but there exact shape cannot be determined from this data. So it becomes obvious that not only are the sizes of the two cell groups different, but they are reacting differently to the experimental manipulations. Recall here that the one male-female difference found was in the ratio of percent multiples in the lateral cells to percent multiples in the medial cells. It is interesting to note that cell size did not change due to the different conditions studied and this was true for both medial and lateral cells.

Different populations of cells in the PVN have been reported but there have been no reports made which have described differences between medial and lateral cell groups. Wakerly and Lincoln (1973) found two groups of cells on the PVN - one group whose unit activity changed in response to milk ejection and one group that was unresponsive to the suckling stimulus. Morris (1971) describes two types of neurosecretory neurones as seen under the light microscope. Also, within the anatomical boundary of the PVN in the rabbit are two cell populations which have diametrically opposite reactions to acetylcholine and noradrenaline (Moss et al, 1972). Yet it appears that both these cell types are scattered throughout the PVN and not segregated by any medial-lateral boundaries. Sundsten and his associates (1970) report neurosecretory cells which can be activated by neural lobe

stimulation spread throughout the nucleus. However they make no distinction as to medial and lateral cell boundaries, and no histology is shown. In the rabbit about half of these cells show no spontaneous activity at all (Sundsten et al, 1970).

From the studies reported and from the results of this study, one can conclude that the lateral cells are in general more active than the medial cells as seen by a larger percent of multiple nucleoli and also have a larger cell size. But towards a time of increased oxytocin output (during lactation) the number of nucleoli in the medial cells increases at a faster rate than in the lateral cells. Not only is the percent of multiple nucleoli in the medial cells of the lactating group elevated over that of the medial cells in all the other groups, but it has increased to the extent that the medial cells contain nearly equal percentages of multiples as do the lateral cells (23.8 and 28.8 respectively). It should be remembered that in this lactating group was found the one animal whose medial cells contained <u>more</u> multiple nucleoli than in the lateral cells. The medial-lateral difference in multiple nucleolar percentages was not significantly different in the normal females, but the percentage of cells with multiple nucleoli was low in both the medial and lateral cell populations (11.3 and 15.3 respectively). This may be some evidence that the medial cells are oxytocin producing. Further work is needed to give full support to the idea.

Margination

The reason for the nucleolus taking its position along the border of the nuclear membrane is not clearly understood. From this study it appears that in the nuclei of animals whose cells contain multiple nucleoli there is a great tendency for one or more of these nucleoli to be marginated. Approximately 97% of cells with multiple nucleoli have at least one marginated nucleolus, while only 37% of the cells with one nucleolus have this nucleolus bordering the nucleolar membrane. If one assumes that two nucleoli connotes more protein production it may be that more protein is being emitted into the cytoplasm and this being the reason for more nucleoli bordering the nuclear membrane. But further explanation for why the nucleolus exits to the side of the nucleus cannot be given at present.

Cell size

Nuclear size and nucleolar size of the magnocellular paraventricular cells have been measured and compared on day 8 after birth in both the lactating female and in the female whose pups were removed at birth. A significant enlargement in the sizes of both these structures for the lactating female was reported (Flament-Durand, 1967). The nuclear diameter of the PVN magnocellular cells is also known to increase under conditions of testosterone administrations, oestradiol treatment, and to decrease with

ovariectomy (Szentagothai et al, 1968, p. 288, 290). And although many researchers have commented on the large size of the magnocellular cells, no measurements of their size have been disclosed. Measurements for cell area in this study are given for two planes of section, the horizontal and sagittal plane. No correction for shrinkage was made.

Our results for cell size do not show any drastic changes under the conditions studied. However, looking at individual animals, one of the normal females and one of the normal males (both sagittal) showed unusually small cell areas, yet not small enough to make the difference significant. Again it may be that day two of lactation was not a long enough time after birth for differences to emerge between the lactating and non-lactating mothers, since it was on day 8 of lactation that nuclear and nucleolar sizes were enlarged. It may also be that the phenomena of cell enlargement, typical of the SON cells under water deprivation, occurs only when undue stress is put on the system to release unusually high quantities of hormone and protein. Although more oxytocin is released during lactation than any other time the neuroendocrine system has had 22 days to prepare for this event. Pregnancy and lactation are natural conditions for the female rat and it is known that about 1 m U of oxytocin occurs every ten to twenty minutes. Although this is about a fourfold increase in oxytocin output over the normal non-pregnant female it remains a non-stressful condition to

which the animal is predisposed. In contrast, cells in the SON are seen to increase in size after 24 hours of water deprivation and continue to increase in size with prolonged deprivation (Hatton and Walters, 1973). This condition of dehydration placed an immediate need upon vasopressin producing cells and could be considered an unusually stressful condition for animals who, for thirty days previous, had water and food present at all times.

Although no changes were seen across the experimental conditions, a quite interesting discovery was made when the medial and lateral cell sizes were compared. Not only do these cells contain differing amounts of multiple nucleoli, but they are of differing sizes.

Table 3 gives the average cell size for medial and lateral cells in each plane of section in um^2. The smallest cells are the sagittally sliced lateral cells - a mean of 125.29 um^2. The largest cells are also the lateral cells - found in the horizontally sliced brains, with an average area of 171.33 um^2. The lateral cells in the horizontal sections are larger than those in the sagittal sections and also larger than either horizontally or sagittally sliced medial cells. Since all cells used for size measurements had been sliced through their center, this could mean differences in sizes or orientation of the two groups. Regardless, this is further support for our notion that the medial and lateral cell groups should be looked at as two different cell populations.

Summary

The albino female rat, in the final day of gestation, and females who have given birth and remain with or without pups, have a more active paraventricular nucleus, as evidenced by the overall increase in multiple nucleoli, than adult females under constant light conditions. Although, at these times the paraventricular nucleus is known to be mainly responsible for oxytocin production, and the largest quantities of oxytocin are being emitted by the lactating female, there is only an indication that the PVN of this animal is more metabolically active than that of the non-lactating female which gave birth and the female immediately before birth. Overall the largest number of multiple nucleoli were found in the lactating mothers. Cell size did not change under the experimental conditions. Margination did not change under the conditions studied, but increased when more than one nucleolus was found within the nucleus. This was true for all groups.

Although the male albino rat is known to release about 18 mU of oxytocin daily, it is not yet understood what the purpose of this hormone is in the male. The only differences which we found between males and females was in the lateral-medial ratio of percent multiples. This emphasizes the need for further study of the nucleus and the actions of oxytocin in the male.

The paraventricular nucleus, which has always been considered a homogeneous body, is now known to consist of

two cell populations, the medial and lateral cells. We contend that these cells are functionally different as they show difference in both percent of multiple nucleoli and ell size. The data does not necessarily show one of these populations strictly responsible for oxytocin and the other responsible for vasopressin and the reason for their functional integrity remains a topic for study.

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APPENDIX A:

Materials

Appendix A

Materials and Equipment

Animals

Albino rats, Holtzman strain, from Madison, Wisconsin.

Sampling and analysis of blood

1. Refractometer, American Optical, $125.00
2. Centrifuge, International Equipment Co., $270.00
3. Capillary tubes
4. Micro-capillary Centrifuge, Model MB, International Equipment Co., $303.00
5. Test tubes, VWR, $4.25

Microscope Equipment

1. Zeiss Microscope
2. Photochanger
3. Extension Tube
4. Whipple-Hauser Disk

Photographic Equipment

1. Camera: 5" x 7" plate camera and optical bench arrangement
2. Film: Kodak Contrast, Process Ortho

Histology

1. Microtome, with knife; Arthur H. Thomas Co., $466.00
2. Thionin, Fischer Chemical, $9.00
3. Celloidin, Randolph Products, $10.00
4. Microscope slides, VWR, $7.60
5. Cover glasses, rectangles, 1 oz. box, Arthur H. Thomas Co., $4.10
6. Bottles, VWR, $16.40

APPENDIX B:

Procedure

Appendix B

Procedure

Celloidin Embedding

1. Perfuse brain of anesthetized animal with a solution of saline (.87% NaCl) followed by a solution of 10% formalin in 0.9% saline solution. Remove brain from the skull and let stand in formalin solution (10% formalin in 0.9% saline) for 5 days.

2. Place in an airtight container and cover the container with one layer of cheesecloth secured with a rubber band. Submerge the container in running water overnight.

3. The next morning begin the dehydration process. Remove the water from the jar and replace with the following solutions for the given amount of time:

80% alcohol (used)	1 day
fresh 80%	1 day
95% alcohol (used)	2 days
fresh 95%	3 days
used absolute alcohol	1 day
fresh absolute	1 day
ether-alcohol (50-50)	1/2 day
thin celloidin	5 days
medium celloidin	3 days
thick celloidin	3 days

While the brain is immersed in celloidin mixtures it is necessary to agitate, or the time must be lengthened.

thin celloidin = 5 grams celloidin: 100 cc ether alcohol

medium celloidin = 15 grams celloidin: 100 cc ether-alcohol

thick celloidin = 25 grams celloidin: 100 cc ether-alcohol
The ether-alcohol is made with two parts ether to one part absolute alcohol.

4. On the last day of thick celloidin make a small paper box for the brain. Fill the box with a small amount of thick celloidin and let stand in open air until celloidin in top hardens. Place brain in box. It should not sink to the bottom. Position the brain with probe or small wooden stick so that hypothalamus is on the top. Cover with thick celloidin.

5. Allow celloidin to harden until it does not adhere to your finger when touched. Place in a dessicator which has already been filled with several small vials of chloroform. Place the lid on the dessicator and secure tightly. Evacuate the air from the dessicator.

6. Check the blocks daily. When they have reached a firm consistency (like firm jello) remove them from dessicator and place in marked specimen jars filled with 70% alcohol. Leave for approximately 48 hours before attempting to remove the paper. Leave the block stored in 70% alcohol until ready to mount and slice.

<u>Mounting the block</u>

Mount the block one day before planning to section.

1. Pour ether-alcohol into a petrie dish and place the block in this dish with the surface that you are going to

cut up. Meanwhile place the stage in a container that holds it upright and dribble thick celloidin over it so as to cover the entire base of the stage. Set the block in the celloidin and dribble more celloidin around the edges of the block.

2. Alternate bathing the block with thick celloidin and 70% alcohol until block becomes firmly established on the stage.

3. Place block upside down in a wide mouthed jar making sure that it is covered with 70% alcohol. Leave overnight and begin sectioning in the morning or any time thereafter.

<u>Sectioning</u>

1. Mount the block and stage in holder and level by adjusting the necessary screws on the microtome. Stamp brain with inked stamp pad. Wet with 70% alcohol. Slice at desired thickness and remove the section from the knife and place in correct petrie dish (odd and even numbered sections are kept separate).

<u>Thionin Stain</u>

Thionin solution (1% aqueous) is made from the following:

200 ml of 1M acetic acid
36 ml of 1M sodium hydroxide
764 ml of distilled water
10 g of thionin powder

1. Mix above ingredients and heat at 90% for one hour. Filter and pour into a jar which is stored in the oven

at 55 degrees C.

2. Place sections in 1% aqueous thienin and leave in 54% oven for 15 minutes.

3. Rinse sections through distilled water until water remains fairly clear.

4. Rinse in 70% alcohol and repeat with new dish of 70% alcohol.

5. Transfer sections into a petrie dish half full of aniline-alcohol until differentiated.

6. When sections reach the desired color transfer them to fresh 95% alcohol, where they are stored until you are ready to mount them.

7. When ready to mount, send each section through two more rinses of 95% alcohol. Mount all the sections on a slide that will fit keeping them wet with 95% alcohol. Blot excess liquid from slide and place in dish of cajeput oil for 15 minutes.

8. Take through four changes of zylene to remove cajeput oil.

9. Cover slip the slides.

APPENDIX C:

Raw Data

NORMAL ADULT FEMALES

Normal adult Female #1

Born: 3-10-74
Arrived: 5-30-74
Perfused: 7-3-74 10:00 a.m.
Weight at time of perfusion: 251 g

Blood-plasma protein 6.45
hematocrit RBC - 45
WBC - 1
plasma - 54

Normal adult Female #2

Born: 3-10-74
Arrived: 5-30-74
Perfused: 7-3-74 10:30 a.m.
Weight at time of perfusion: 270 g

Blood-plasma protein 6.5
hematocrit RBC - 44
WBC - 1
plasma - 55

Normal adult Female #3

Born: 3-10-74
Arrived: 5-30-74
Perfused: 7-3-74 11:00 a.m.
Weight at time of perfusion: 262 g

Blood-plasma protein 7.0
hematocrit RBC - 43
WBC - 1
plasma - 56

Normal adult Female #4

Born: 3-10-74
Arrived: 5-30-74
Perfused: 7-3-74 11:30 a.m.
Weight at time of perfusion: 250 g

Blood-plasma protein 6.6
hematocrit RBC - 45
WBC - 1
plasma - 54

Pre-parturition female #10 (Hor)

sperm positive 4-29-74

Light-dark cycle 8-6

4-30 wght +190 g
5-19 wght +310 g

Perfused: 5-19 9:45 a.m.

#pups - 11 (5-6)
wt pups - in uterine born 64.2 gms.
ind. pups - 4.5, 4.6, 3.7, 4.3, 3.8, 4.1, 4.3,
4.6, 3.9, 4.2, 3.9

plasma protein - 6.0
hematocrit RBC - 38%
WBC - 1%
plasma - 61%

Pre-parturition female #11 (Hor)

sperm positive 4-29-74

Light-dark cycle 8-6

4-30 wght 222 g
5-19 wght 349 g

Perfused: 5-19 10:30 a.m.

pups - 12
wt pups - in uterine born 63.4 gms.
ind. pups - 3.6, 3.9, 3.3, 3.8, 3.5, 3.8, 3.3,
3.9, 3.5, 4.0, 3.8, 2.7

Blood - plasma protein 6.3
- hematocrit RBC 38%
WBC 1%
plasma 61%

Pre-parturition female #12 (Sag)

Sperm positive 4-29-74

Light-dark cycle 8-6
Birth due 5-20

4-30 wght 249 g
5-19 wght 363 g

Perfused: 5-19 11:15 a.m.

pups - 8(2-6)

wt pups in uterine born - 47.6
ind. pups - 4.1, 4.5, 4.1, 4.6, 3.8, 3.4, 3.0, 3.2

Blood - plasma protein 6.5
- hematocrit RBC 40%
WBC 1%
plasma 59%

Pre-parturition female #14 (Sag)

Sperm positive 4-29-74

Light-dark cycle 8-6

4-30 wght -185 g
5-19 wght -260 g

Perfused: 5-19 12:00 a.m.

#pups - 2
wt pups - in uterine born 13.4
ind. pups - 4.7, 4.5

Blood - plasma protein 6.5
- hematocrit RBC 47%
WBC 1%
plasma 52%

Rat #5 (Hor) 6 pups left w/mother

parturition: 6:00 a.m. 3-27
perfused : 10:45 a.m. 3-29

wt mother before birth 347 g
wt mother after birth 267.5 g
wt mother before death 266.0 g

pups 10 - 1 dead - 3 pups removed 2:00 p.m. 3-27
(at birth) wt pups - 6.4, 7.5, 6.3, 6.6, 6.2, 6.8, and remaining 4 = 25.5
(at death) wt pups - 8.9, 9.4, 9.0, 8.4, 9.1, 10.1

Blood - plasma protein 6.4
- hematocrit RBC 41.0%
WBC 1.5%
plasma 57.5%

Rat #1 (Hor) 6 pups left w/mother

parturition: 9:30 - 10:00 a.m. 3-27-74
perfusion : 11:15 a.m. 3-29-74

3-26 (4 pm) wt mother before parturition 351 g
wt mother after parturition 304 g
wt mother at death 298 g

(at birth) wt pups - 7.3, 7.0, 7.0, 7.2, 7.5, 6.9
(at death) wt pups - 9.5, 9.5, 9.3, 9.8, 8.9, 9.5

Blood - plasma protein 6.4
- hematocrit RBC 41%
WBC 1%
plasma 58%

Rat #4 (Sag) sp 3-5 6 pups left w/mother

parturition: 11:00 p.m. 3-26
perfusion : 11:15 p.m. 3-28

10:00 a.m. 3-26 wt before birth 337 g
wt after birth 268 g
wt before perfusion 274 g

pups born - 8 (9, 1 eaten) 2 pups removed 2:00 p.m. 3-26
(at birth) wt pups - all weigh 6.5 - 7.0 g
(at death) wt pups - 9.0, 8.5, 9.5, 8.6, 9.4

Blood - plasma protein 6.4
- hematocrit RBC 42%
WBC 1%
plasma 57%

Rat # 7 (Sag) sp 3-5 6 pups left w/mother

parturition: 3:00 a.m. 3-27
perfusion : 8:30 a.m. 3-29

3-26 wt mother before parturition 350.0 g
wt mother after parturition 277.0 g
wt mother at death 270.0 g

pups 9 (1 removed 8:00 a.m. 3-26)
(2 removed 2:00 p.m. 3-26)

(at birth) wt pups - 6.6, 6.5, 6.2 - removed
6.8, 7.4, 6.4, 6.8, 6.2, 7.1
(at death) wt pups - 9.6, 8.7, 8.5, 9.8, 8.4, 9.1

Blood - plasma protein 5.9
- hematocrit RBC 35%
WBC 1%
plasma 64%

Rat # 2 (Hor) sp 3-5-74 pups removed at birth

parturition: 9:30 - 10:00 a.m. 3-27
perfusion : 11:45 a.m. 3-29

wt mother before birth 365 g
wt mother after birth 306 g
wt mother at death 291 g

pups 6

(at birth) wt pups - 7.0, 6.8, 6.9, 6.9, 7.4, 6.7

Blood - plasma protein 6.3
- hematocrit RBC 39%
WBC 1%
plasma 60%

Rat #13 (Hor) sp 4-29-74 pups removed at birth

4-30 wt - 212 g
5-20 wt - 359 g birth (from 12-8 a.m. 5-21)

5-21 wt - 282 g (after parturition)
5-23 wt - 272 g (pups removed 9:15 a.m. 5-21)

pups - 12
wt pups - 6.5, 6.8, 7.0, 6.8, 7.0, 6.1, 6.5, 5.9, 6.2, 6.8, 6.5, 6.6

perfused: 8:45 a.m. 5-23
Blood - plasma protein 7.4
- hematocrit RBC 36%
WBC 2%
plasma 62%

Rat #8 (Sag) sp 3-5 pups removed at birth

parturition: 2:00 p.m. 3-27
perfusion : 2:00 p.m. 3-29

wt mother before birth 312 g
wt mother after birth 249 g
wt mother at death 238 g

pups - 5 (1 dead)
(at birth) wt pups - 6.6, 7.1, 6.7, 6.2, 6.7 (dead)

Blood - plasma protein 6.7
- hematocrit RBC 38.5%
WBC 1.5%
plasma 60%

Rat #6 (Sag) sp 3-5 pups removed at birth

parturition: 2:45 p.m. 3-26

wt mother after parturition 323 g
wt before perfusion (3-28) 291 g

pups - 13
wt pups at birth - 7.0, 5.0, 6.0, 6.5, 6.5, 6.0, 6.5, 6.5, 6.5, 7.0, 6.5, 6.0, 6.5

perfusion: 3:15 p.m. 3-28

Blood - plasma protein 7.1 plasma frozen

Raw Data Table 4. Nucleolar counts

Normal Females. Female 1. Horizontal

Section #		Right Side		Total	Section #	Left Side		Total
2	Lat	1-15	1_1-7		2	1-17	1_1-5	
		2- 0	2_1-3	25		2- 0	2_1-3	25
3	Lat	1-17	1_1-5		3	1-15	1_1-5	
		2- 0	2_1-3	25		2- 1	2_1-4	25
4	Lat	1-14	1_1-9		5	1-15	1_1-7	
		2- 0	2_1-2	25		2- 0	2_1-3	25
5	Med	1-14	1_1-4		6	1-18	1_1-6	
		2- 0	2_1-2	20		2- 0	2_1-1	25
6	Med	1-20	1_1-5		7	1-15	1_1-6	
		2- 0	2_1-5	30		2- 0	2_1-4	25
8	Med	1-16	1_1-8		8	1-16	1_1-6	
		2- 0	2_1-1	25		2- 0	2_1-3	25

Female 2. Horizontal

Section #		Right Side		Total	Section #		Left Side		Total
3	Lat	1-14	1_1-5		3	Lat	1-17	1_1-5	
		2- 0	2_1-6	25			2- 0	2_1-3	25
4	Lat	1-11	1_1-10		4	Lat	1-12	1_1-6	
		2- 0	2_1-4	25			2- 1	2_1-6	25
6	Lat	1-14	1_1-8		5	Lat	1-12	1_1-8	
		2- 0	2_1-3	25			2- 0	2_1-5	25
6	Med	1-13	1_1-9		7	Med	1-20	1_1-8	
		2- 0	2_1-3	25			2- 0	2_1-2	30
8	Med	1-10	1_1-14		8	Med	1-16	1_1-6	
		2- 0	2_1-1	25			2- 0	2_1-3	25
9	Med	1-12	1_1-9		9	Med	1-11	1_1-7	
		2- 0	2_1-4	25			2- 0	2_1-2	20

Table 4 cont.

Female 3. Sagittal

Section #	Right Side		Total	Section #	Left Side		Total
23 Med	1-15	1_1-8		6 Med	1-14	1_1-7	
	2- 0	2_1-2	25		2- 0	2_1-4	25
24 Med	1-14	1_1-8		7 Med	1-13	1_1-9	
	2- 0	2_1-3	25		2- 0	2_1-3	25
25 Med	1-10	1_1-11		9 Med	1-15	1_1-9	
	2- 0	2_1-4	25		2- 0	2_1-1	25
26 Lat	1-15	1_1-8		2 Lat	1-15	1_1-4	
	2- 0	2_1-2	25		2- 0	2_1-1	20
28 Lat	1-17	1_1-8		4 Lat	1-15	1_1-9	
	2- 0	2_1-0	25		2- 1	2_1-5	30
29 Lat	1-15	1_1-5		5 Lat	1- 9	1_1-12	
	2- 0	2_1-5	25		2- 1	2_1-3	25

Female 4. Sagittal

Section #	Right Side		Total	Section #	Left Side		Total
19 Med	1-15	1_1-6		7 Med	1-13	1_1-8	
	2- 0	2_1-4	25		2- 0	2_1-4	25
20 Med	1-16	1_1-7		8 Med	1-13	1_1-10	
	2- 0	2_1-2	25		2- 0	2_1-2	25
22 Med	1-17	1_1-3		9 Med	1-12	1_1-10	
	2- 0	2_1-5	25		2- 0	2_1-3	25
24 Lat	1-13	1_1-7		2 Lat	1- 9	1_1-9	
	2- 0	2_1-5	25		2- 0	2_1-7	25
25 Lat	1-10	1_1-11		4 Lat	1-14	1_1-10	
	2- 0	2_1-4	25		2- 0	2_1- 7	25
26 Lat	1-12	1_1-10		5 Lat	1-11	1_1-6	
	2 -0	2_1-3	25		2- 1	2_1-7	25

Male 1. Horizontal.

Section #		Right Side		Total	Section #		Left Side		Total
154	Lat	1-15	1_1-5		154	Lat	1-14	1_1-8	
		2- 0	2_1-5	25			2- 0	2_1-3	25
156	Lat	1-28	1_1-15		156	Lat	1-28	1_1-16	
		2- 0	2_1-7	50			2- 0	2_1-6	50
160	Med	1-16	1_1-6		160	Med	1-18	1_1-5	
		2- 0	2_1-3	25			2- 0	2_1-2	25
162	Med	1-11	1_1-12		162	Med	1-20	1_1-5	
		2- 0	2_1-2	25			2- 1	2_1-3	29
164	Med	1-16	1_1-6		164	Med	1- 8	1_1-11	
		2- 0	2_1-3	25			2- 0	2_1-2	21

Male 2. Horizontal.

Section #		Right Side		Total	Section #		Left Side		Total
138	Lat	1- 8	1_1-5		136	Lat	1-12	1_1-6	
		2- 0	2_1-2	15			2- 0	2_1-2	20
140	Lat	1-13	1_1-5		138	Lat	1- 7	1_1-13	
		2- 0	2_1-12	30			2- 0	2_1-10	30
144	Lat	1-14	1_1-9		140	Lat	1- 6	1_1-11	
		2- 1	2_1-6	30			2- 0	2_1-8	25
144	Med	1-16	1_1-14		144	Med	1-14	1_1-14	
		2- 0	2_1-1	31			2- 0	2_1-9	37
146	Med	1-18	1_1-8		146	Med	1-10	1_1-13	
		2- 0	2_1-4	30			2- 0	2_1-2	25
148	Med	1- 6	1_1-8		148	Med	1- 6	1_1-6	
		2- 0	2_1-0	14			2- 0	2_1-1	13

Raw Data cont.

Male 3. Sagittal.

Section #		Right Side		Total	Section #		Left Side		Total
244	Lat	1-12	1_1-5		204	Lat	1-16	1_1-3	
		2- 0	2_1-8	25			2- 0	2_1-6	25
242	Lat	1-12	1_1-8		206	Lat	1-12	1_1-4	
		2- 0	2_1-5	25			2- 1	2_1-8	25
240	Lat	1- 9	1_1-9		208	Lat	1-15	1_1-5	
		2- 0	2_1-7	25			2- 0	2_1-5	25
234	Med	1-13	1_1-6		216	Med	1-15	1_1-7	
		2- 0	2_1-6	25			2- 0	2_1-3	25
236	Med	1-18	1_1-4		214	Med	1-17	1_1-5	
		2- 0	2_1-3	25			2- 0	2_1-3	25
238	Med	1-15	1_1-6		212	Med	1-14	1_1-8	
		2- 0	2_1-4	25			2- 1	2_1-2	25

Male 4. Sagittal.

Section #		Right Side		Total	Section #		Left Side		Total
226	Lat	1-13	1_1-7		198	Lat	1-12	1_1-6	
		2- 0	2_1-5	25			2- 0	2_1-7	25
228	Lat	1-16	1_1-2		200	Lat	1-11	1_1-7	
		2- 0	2_1-7	25			2- 0	2_1-7	25
230	Lat	1-10	1_1-8		202	Lat	1-15	1_1-7	
		2- 0	2_1-7	25			2- 0	2_1-3	25
222	Med	1-17	1_1-5		206	Med	1-17	1_1-4	
		2- 0	2_1-3	25			2- 0	2_1-4	25
222	Med	1-16	1_1-6		208	Med	1-19	1_1-3	
		2- 0	2_1-3	25			2- 0	2_1-3	25
224	Med	1-20	1_1-5		210	Med	1-12	1_1-9	
		2- 0	2_1-0	25			2- 0	2_1-4	25

Raw Data cont. Pre-parturition Females.

Pre-parturition 10. Horizontal

Section #		Right Side		Total	Section #		Left Side		Total
7	Lat	1-11	1_1-8		6	Lat	1-14	1_1-8	
		2- 0	2_1-6	25			2- 0	2_1-3	25
8	Lat	1- 7	1_1-9		7	Lat	1- 9	1_1-11	
		2- 1	2_1-8	25			2- 1	2_1-4	25
9	Lat	1-11	1_1-8		8	Lat	1- 7	1_1-11	
		2- 0	2_1-6	25			2- 0	2_1-7	25
8	Med	1- 5	1_1-7		9	Med	1-12	1_1-12	
		2- 0	2_1-3	15			2- 0	2_1-1	25
10	Med	1-13	1_1-7		10	Med	1-17	1_1-3	
		2- 0	2_1-5	25			2- 0	2_1-5	25
11	Med	1-13	1_1-7		12	Med	1-10	1_1-11	
		2- 0	2_1-5	25			2- 0	2_1-4	25
12	Med	1- 7	1_1-2						
		2- 0	2_1-1	10					

Pre-parturition female 11. Horizontal

Section #		Right Side		Total	Section #		Left Side		Total
7	Lat	1-12	1_1-11		7	Lat	1-15	1_1-4	
		2- 0	2_1-2	25			2- 1	2_1-5	25
8	Lat	1-15	1_1-7		8	Lat	1-11	1_1-9	
		2- 0	2_1-3	25			2- 1	2_1-4	25
9	Lat	1-12	1_1-8		10	Lat	1-12	1_1-9	
		2- 0	2_1-5	25			2- 0	2_1-4	25
11	Med	1-16	1_1-8		11	Med	1-10	1_1-12	
		2- 0	2_1-1	25			2- 0	2_1-3	25
13	Med	1-15	1_1-8		12	Med	1-17	1_1-7	
		2- 0	2_1-2	25			2- 0	2_1-1	25
14	Med	1-14	1_1-9		14	Med	1-15	1_1-6	
		2- 0	2_1-2	25			2- 0	2_1-4	25

Raw Data cont.

Pre-parturient female 12.
Sagittal

Section #	Right Side		Total	Section #	Left Side		Total
14 Med	1-15	1_1-4		7 Med	1-10	1_1-9	
	2- 0	2_1-6	25		2- 0	2_1-6	25
15 Med	1- 9	1_1-5		9 Med	1- 9	1_1-11	
	2- 0	2_1-11	25		2- 0	2_1-5	25
16 Med	1-10	1_1-7		10 Med	1-12	1_1-6	
	2- 0	2_1-8	25		2- 0	2_1-7	25
17 Lat	1- 8	1_1-5		2 Lat	1-10	1_1-3	
	2- 1	2_1-11	25		2- 0	2_1-12	25
18 Lat	1-10	1_1-6		4 Lat	1-10	1_1-5	
	2- 1	2_1-8	25		2- 1	2_1-9	25
19 Lat	1- 9	1_1-8		5 Lat	1-11	1_1-5	
	2- 0	2_1-8	25		2- 0	2_1-6	25

Pre-parturient Female 14. Sagittal

Section #	Right Side		Total	Section #	Left Side		Total
6 Med	1-14	1_1-7		7 Med	1-10	1_1-8	
	2- 0	2_1-4	25		2- 0	2_1-7	25
7 Med	1-16	1_1-5		8 Med	1-14	1_1-5	
	2- 0	2_1-4	25		2- 0	2_1-6	25
8 Med	1-11	1_1-8		9 Med	1- 9	1_1-10	
	2- 0	2_1-6	25		2- 1	2_1-5	25
12 Lat	1- 9	1_1-8		1 Lat	1-12	1_1-9	
	2- 0	2_1-8	25		2- 0	2_1-4	25
14 Lat	1- 7	1_1-10		2 Lat	1- 9	1_1-5	
	2- 1	2_1-7	25		2- 0	2_1-11	25
15 Lat	1-11	1_1-5		4 Lat	1- 8	1_1-8	
	2- 0	2_1-9	25		2- 0	2_1-9	25

Raw Data. Table 4 cont.

Lactating Females.

Lactating Female 5. Horizontal

Section #	Right Side		Total	Section #	Left Side		Total
5 Lat	1-19	1_1-4		5 Lat	1- 9	1_1-5	
	2- 0	2_1-2	25		2- 1	2_1-10	25
7 Lat	1-13	1_1-3		6 Lat	1-10	1_1-6	
	2- 0	2_1-9	25		2- 1	2_1-8	25
8 Lat	1-16	1_1-4		7 Lat	1- 6	1_1-10	
	2- 0	2_1-5	25		2- 0	2_1-9	25
11 Med	1-14	1_1-6		9 Med	1- 5	1_1-3	
	2- 0	2_1-5	25		2- 0	2_1-2	10
12 Med	1- 9	1_1-8		10 Med	1-13	1_1-9	
	2- 0	2_1-8	25		2- 0	2_1-3	25
13 Med	1-12	1_1-7		12 Med	1-11	1_1-10	
	2- 1	2_1-5	25		2- 0	2_1-4	25

Lactating Female 1. Horizontal

Section #	Right Side		Total	Section #	Left Side		Total
8 Lat	1- 8	1_1-9		7 Lat	1-16	1_1-7	
	2- 0	2_1-8	25		2- 0	2_1-2	25
9 Lat	1-15	1_1-5		8 Lat	1-12	1_1-6	
	2- 0	2_1-5	25		2- 0	2_1-7	25
10 Lat	1-11	1_1-5		9 Lat	1- 7	1_1-13	
	2- 0	2_1-9	25		2- 0	2_1-5	25
10 Med	1-10	1_1-5		10 Med	1-15	1_1-6	
	2- 1	2_1-9	25		2- 0	2_1-4	25
12 Med	1- 4	1_1-4		11 Med	1- 8	1_1-6	
	2- 0	2_1-2	10		2- 0	2_1-4	15
13 Med	1- 8	1_1-5		12 Med	1- 6	1_1-5	
	2- 0	2_1-12	25		2- 0	2_1-9	20
14 Med	1- 7	1_1-4		14 Med	1- 9	1_1-2	
	2- 0	2_1-4	15		2- 0	2_1-4	15

Raw Data cont.

Lactating Female 4. Sagittal.

Section #		Right Side		Total	Section #		Left Side		Total
16	Med	1-21	1_1-13		9	Med	1-11	1_1-2	
		2- 2	2_1-14	50			2- 0	2_1-12	25
19	Med	1-13	1_1-3		11	Med	1-11	1_1-5	
		2- 1	2_1-8	25			2- 0	2_1-9	25
22	Lat	1-10	1_1-6		12	Med	1-14	1_1-3	
		2- 0	2_1-9	25			2- 0	2_1-8	25
23	Lat	1-10	1_1-6		3	Lat	1- 4	1_1-5	
		2- 1	2_1-8	25			2- 0	2_1-6	15
25	Lat	1-15	1_1-4		4	Lat	1- 8	1_1-4	
		2- 1	2_1-5	25			2- 0	2_1-5	17
					5	Lat	1-10	1_1-9	
							2- 0	2_1-14	33
					7	Lat	1- 6	1_1-2	
							2- 0	2_1-2	10

Lactating Female 7. Sagittal.

Section #		Right Side		Total	Section #		Left Side		Total
3	Med	1- 9	1_1-12		19	Med	1-15	1_1-6	
		2- 0	2_1-4	25			2- 0	2_1-4	25
4	Med	1-10	1_1-8		20	Med	1-13	1_1-8	
		2- 0	2_1-7	25			2- 0	2_1-4	25
6	Med	1-10	1_1-12		21	Med	1-13	1_1-9	
		2- 0	2_1-3	25			2- 0	2_1-3	25
7	Lat	1-10	1_1-7		14	Lat	1-12	1_1-6	
		2- 0	2_1-8	25			2- 0	2_1-7	25
9	Lat	1- 8	1_1-10		15	Lat	1- 8	1_1-9	
		2- 0	2_1-7	25			2- 1	2_1-7	25
10	Lat	1- 8	1_1-12		16	Lat	1- 5	1_1-14	
		2- 0	2_1-5	25			2- 0	2_1-6	25

Raw Data cont.

Pups removed. Female with pups removed 2. Horizontal.

Section #		Right Side		Total	Section #		Left Side		Total
1	Lat	1-11	1_1-10		2	Lat	1-10	1_1-5	
		2- 0	2_1-4	25			2- 1	2_1-9	25
2	Lat	1-14	1_1-5		3	Lat	1-17	1_1-4	
		2- 0	2_1-6	25			2- 0	2_1-4	25
4	Lat	1-11	1_1-5		4	Lat	1-11	1_1-9	
		2- 0	2_1-9	25			2- 1	2_1-4	25
4	Med	1-13	1_1-8		5	Med	1-17	1_1-4	
		2- 0	2_1-4	25			2- 0	2_1-4	25
6	Med	1-12	1_1-11		6	Med	1-13	1_1-6	
		2- 1	2_1-1	25			2- 0	2_1-6	25
7	Med	1-14	1_1-9		8	Med	1-16	1_1-7	
		2- 0	2_1-2	25			2- 0	2_1-2	25

Pups removed 13. Horizontal.

Section #		Right Side		Total	Section #		Left Side		Total
1	Lat	1-11	1_1-3		1	Lat	1-16	1_1-3	
		2-3	2_1-8	25			2- 0	2_1-6	25
3	Lat	1-10	1_1-5		3	Lat	1-13	1_1-8	
		2- 0	2_1-10	25			2- 1	2_1-3	25
4	Lat	1-13	1_1-2		4	Lat	1-13	1_1-5	
		2- 1	2_1-9	25			2- 2	2_1-5	25
4	Med	1 -3	1_1-1		5	Med	1-14	1_1-3	
		2- 0	2_1-1	5			2- 1	2_1-7	25
5	Med	1-16	1_1-4		6	Med	1-13	1_1-8	
		2- 1	2_1-4	25			2- 0	2_1-4	25
7	Med	1- 8	1_1-4		7	Med	1 -9	1_1-5	
		2- 1	2_1-12	25			2- 2	2_1-9	25
8	Med	1-13	1_1-3						
		2- 0	2_1-4	20					

Raw Data cont.

Pups removed 8. Sagittal.

Section #		Right Side		Total	Section #		Left Side		Total
16	Med	1-10	1_1-11		6	Med	1-14	1_1-11	
		2- 0	2_1-4	25			2- 0	2_1-0	25
18	Med	1-14	1_1-7		7	Med	1-14	1_1-9	
		2- 0	2_1-4	25			2- 0	2_1-2	25
19	Med	1-13	1_1-11		10	Med	1-11	1_1-10	
		2- 0	2_1-1	25			2- 0	2_1-4	25
20	Lat	1-15	1_1-8		1	Lat	1-16	1_1-9	
		2- 0	2_1-2	25			2- 0	2_1-0	25
21	Lat	1-15	1_1-6		2	Lat	1- 8	1_1-11	
		2- 0	2_1-4	25			2- 0	2_1-6	25
23	Lat	1-11	1_1-9		3	Lat	1- 7	1_1-12	
		2- 0	2_1-5	25			2- 0	2_1-6	25

Pups removed 6. Sagittal.

Section #		Right Side		Total	Section #		Left Side		Total
18	Med	1- 9	1_1-8		7	Med	1-12	1_1-8	
		2- 0	2_1-8	25			2- 0	2_1-5	25
19	Med	1-12	1_1-13		8	Med	1-15	1_1-6	
		2- 0	2_1-0	25			2- 0	2_1-4	25
20	Med	1-15	1_1-4		9	Med	1-17	1_1-5	
		2- 1	2_1-5	25			2- 1	2_1-2	25
23	Lat	1- 9	1_1-10		1	Lat	1-16	1_1-6	
		2- 1	2_1-5	25			2- 0	2_1-3	25
25	Lat	1- 6	1_1-7		2	Lat	1- 6	1_1-9	
		2- 0	2_1-12	25			2- 0	2_1-10	25
26	Lat	1-10	1_1-8		4	Lat	1- 7	1_1-13	
		2- 0	2_1-7	25			2- 0	2_1-5	25

Table 5. Total nucleolar counts. Percentages of multiple nucleoli for 300 cells in the paraventricular nucleus of individual animals.

animal	lateral cells	medial cells	total
females			
1 (Hor)	12.0	10.7	11.3
2 (Hor)	18.7	10.0	14.3
3 (Sag)	12.0	11.3	11.7
4 (Sag)	18.7	13.3	16.0
males			
1 (Hor)	14.0	10.7	12.3
2 (Hor)	27.3	11.3	19.3
3 (Sag)	26.7	14.7	20.7
4 (Sag)	24.0	11.3	17.7
Pre-parturient females			
10 (Hor)	24.0	16.0	20.0
11 (Hor)	16.7	8.7	12.7
12 (Sag)	40.0	28.7	34.3
14 (Sag)	32.7	22.0	27.3
Lactating females			
5 (Hor)	30.0	20.0	25.0
1 (Hor)	24.0	22.7	23.4
4 (Sag)	34.0	36.0	35.0
7 (Sag)	27.3	16.7	22.0
Females with pups removed			
2 (Hor)	24.7	13.3	19.0
13 (Hor)	32.0	30.7	31.3
8 (Sag)	15.3	10.0	12.7
6 (Sag)	28.7	17.3	23.0

Table 6. The mean and standard deviation of percentage of multiple nucleoli for lateral and medial cells for each animal group studies.

Animal	Lateral Cells	Medial cells
Normal Females	15.3 ± 2.0	11.3 ± .8
Normal Males	25.1 ± 3.8	17.8 ± 4.6
Pre-parturient Females	28.3 ± 5.2	18.9 ± 4.3
Lactating Females	28.8 ± 2.2	23.8 ± 4.3
Females with Pups removed	25.1 ± 3.8	17.8 ± 4.6

Table 7. Percentage of multiple nucleoli in 120 control cells surrounding the paraventricular nucleus for individual animals.

Animal	Percent Multiples
Females	
1 (Hor)	3.0
2 (Hor)	2.0
3 (Sag)	2.0
4 (Sag)	0.0
Males	
1 (Hor)	1.0
2 (Hor)	0.0
3 (Sag)	1.0
4 (Sag)	0.0
Pre-parturient Females	
10 (Hor)	1.0
11 (Hor)	0.0
12 (Sag)	2.0
14 (Sag)	0.0
Lactating Females	
5 (Hor)	4.0
1 (Hor)	4.0
4 (Sag)	2.0
7 (Sag)	1.0
Females with pups removed	
2 (Hor)	2.0
13 (Hor)	0.0
8 (Sag)	2.0
6 (Sag)	2.0

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