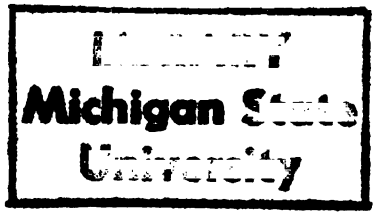

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THE MATERNAL EXPERIENCE IN THE RAT: AN INVESTIGATION INTO CELLULAR CHANGES ACCOMPANYING PREGNANCY, PARTURITION, MOTHERHOOD (REAL OR IMAGINED), AND BEYOND

presented by

ADRIENNE KAY SALM

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of the requirements for

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Glenn I. Hatton
Major professor

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THE MATERNAL EXPERIENCE IN THE RAT: AN INVESTIGATION INTO CELLULAR CHANGES ACCOMPANYING PREGNANCY, PARTURITION, MOTHERHOOD (REAL OR IMAGINED), AND BEYOND.

By

Adrienne Kay Salm

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ABSTRACT

THE MATERNAL EXPERIENCE IN THE RAT: AN INVESTIGATION INTO CELLULAR CHANGES ACCOMPANYING PREGNANCY, PARTURITION, MOTHERHOOD (REAL OR IMAGINED), AND BEYOND.

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Supraoptic nucleus (SON) neurons produce, and release from their axons in the neurohypophysis, the hormones oxytocin (OX) and vasopressin (VP) during parturition, lactation and, perhaps, concurrently with the expression of maternal behaviors. At these times they exhibit morphological plasticity, i.e.: 1) increases in cell-cell apposition (two neurons with no intervening glial process), 2) dendritic bundling (two or more dendritic profiles in apposition), and 3) formation of double synapses (one presynaptic axon terminal contacting two dendrites or somata). Simultaneously, many of the axons, normally enveloped in the cytoplasm of pituicytes, lose their pituicyte ensheathment.

Six groups of female Sprague-Dawley rats- virgins (controls), virgins in which maternal behavior had been induced by exposure to rat pups (MBs), immediately prepartum, immediately postpartum, 14-days lactating, and 10-days postweaning, were studied. The questions under investigation were: 1) Does the SON change with maternal

behavior 2) What is the peptidergic content of neurons which form cell-cell appositions during the "Motherhood experience" and 3) Do the morphological traces of Motherhood persist to 10-days postweaning?

Electron microscopy (EM) of control and MB SONs showed no differences in double synapse formation or soma-somatic apposition. However, MBs displayed more dendrites, large (9-12 dendrites) dendritic bundles, dendritic profiles, and dendritic profile area in bundles. The dendritic zone area was larger in MBs. In the neurohypophysis, MBs had fewer enclosed axons per animal.

Immunoelectron microscopy of prepartum, postpartum and 14-day lactating subjects showed that OX-Neurophysin positive cells in the postpartum group had more soma-somatic apposition than did the controls or the prepartum group.

Conventional EM of postweaning and control SONs showed no differences between groups in cell-cell apposition, number of double synapses, or dendritic bundling. The areas of single dendritic profiles and of the dendritic zone were larger in the postweaning group, as was the number of dendrites.

These data indicate a functional role for oxytocinergic somatic appositions during parturition, the rapid reversal of some traces of the "Motherhood experience", and that the SON changes with maternal behavior.

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LIST OF ABBREVIATIONS

CSF - cerebrospinal fluid

dcvs - dense cored vesicles

EM - electron microscopy

ICC - immunocytochemistry

MB - maternal behaver

MNCs - magnocellular neuroendocrine cells

OX - oxytocin

PVN - paraventricular nucleus

PW - postweaning

SON - supraoptic nucleus

VP - vasopressin

INTRODUCTION

Part IA: Morphological correlates of maternal behavior in the SON.

Maternal behaviors are a constellation of activities which have been described in many species (see Fahrbach and Pfaff, 1982, for review) and which are comprised of various care-giving activities which parental animals engage in with their offspring, presumably to promote the offspring's, as well as the species, well-being and longevity. It has recently been shown quite dramatically (Pedersen et al., 1982; 1984a; Fahrbach et al., 1984) that an intracerebroventricular injection of the nonapeptide oxytocin (OX), a hormone which promotes the milk-ejection reflex during lactation and uterine contractions during parturition, into ovariectomized estrogen-primed virgin female Sprague-Dawley rats results in full maternal behavior for that species (FMB; grouping and regrouping of pups, licking of pups, crouching over grouped pups, nest building, and retrieval of pups to a central location (Figure 1). Further evidence for a role of OX in FMB is that the onset of FMB can be blocked by the administration of an antibody against OX to ovariectomized estrogen-primed virgin rats (Pedersen et al., 1984b). Two major regions of the brain which produce oxytocin are the hypothalamic paraventricular nucleus (PVN) and the supraoptic nucleus (SON). PVN, with

Figure 1. Cartoon depicting the four activities which were considered to be maternal behaviors.

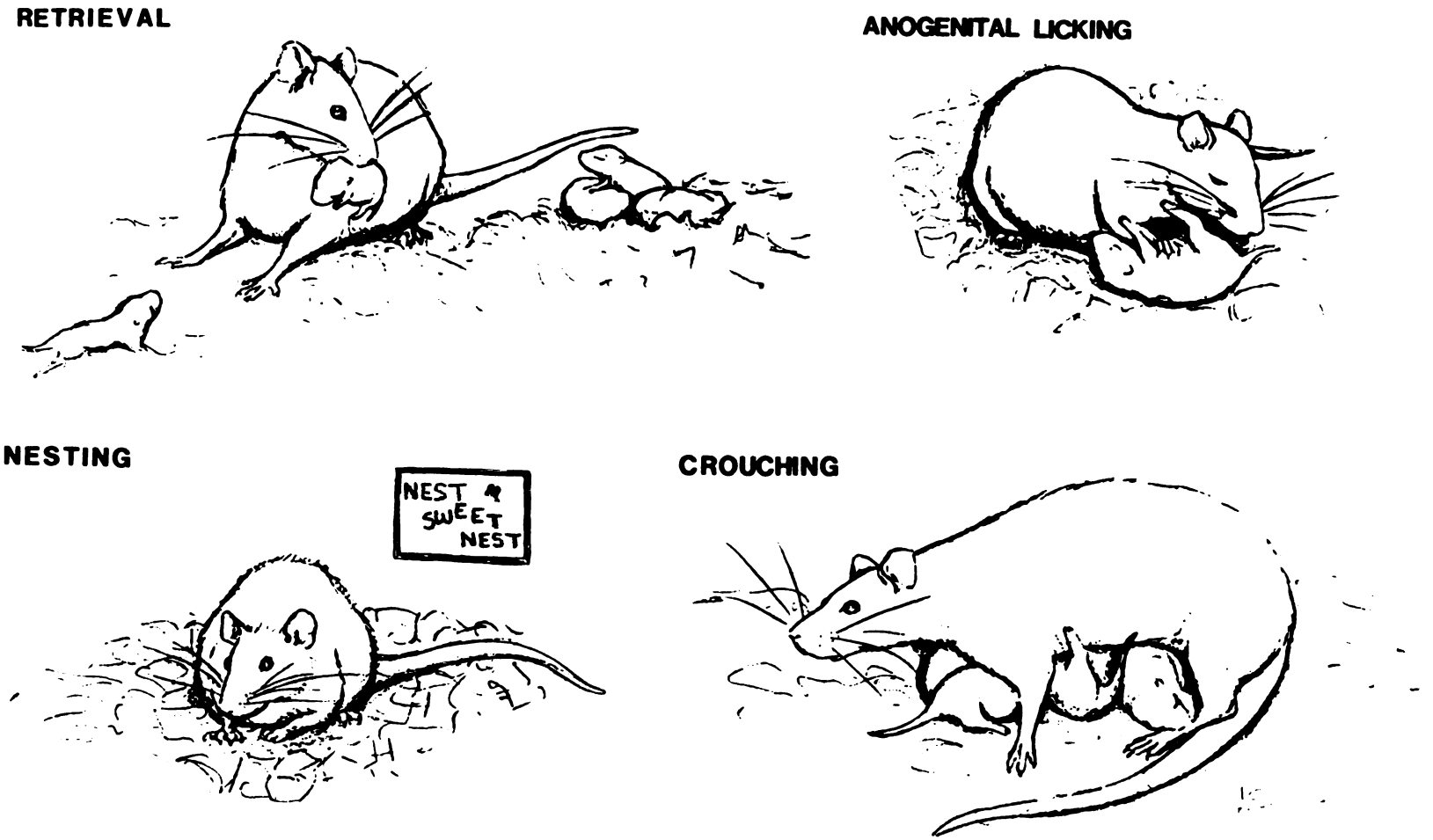

RETRIEVAL
ANOGENITAL LICKING
NESTING
NEST
SWEET
NEST
CROUCHING

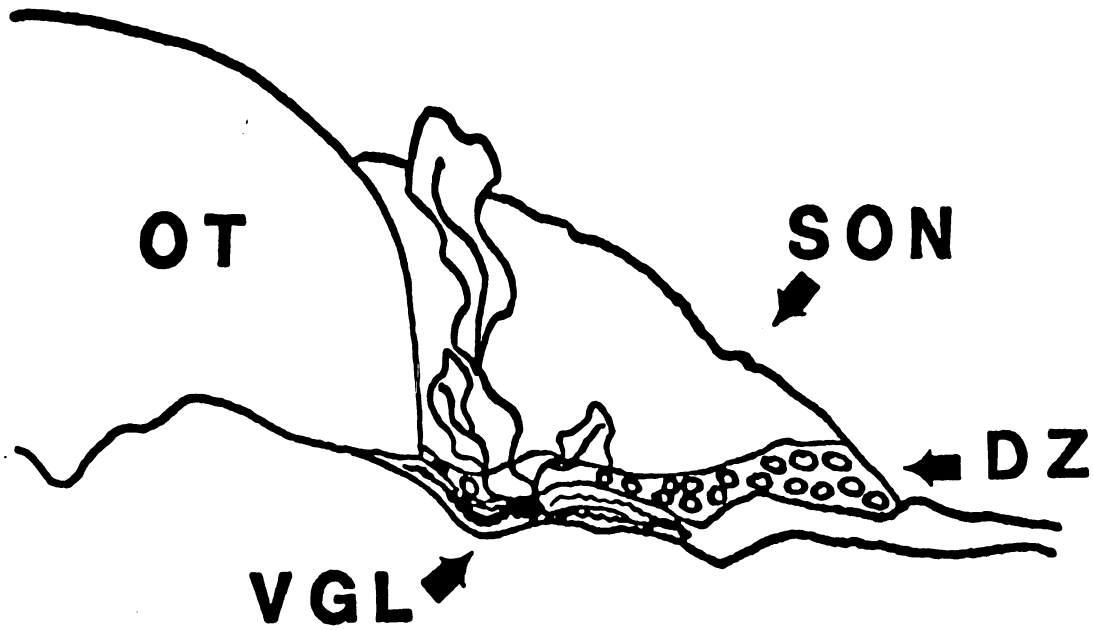
Figure 1.

its extrahypothalamic projections (Swanson and Sawchenko, 1983) might be considered to be a likely way station in an oxytocin-dependent pathway mediating FMB. However, when the PVN is bilaterally lesioned or its lateral connections severed, there is no disruption of FMB in already maternally behaving rats (Numan, 1984). Eliminating PVN, other anatomical data allow the SON to be somehow linked to the occurrence of maternal behavior in primiparous lactating rats as well as in ovariectomized, estrogen-primed, nulliparous rats in which maternal behavior has been induced by exposure to rat pups. It is fairly well established (Fahrbach and Pfaff, 1982) that of the many brain areas suspected of mediating maternal behaviors in the past (not including SON) only the medial preoptic area (MPOA) appears to be absolutely necessary for the induction and maintenance of FMB. Because the MPOA sends projections to the SON (Conrad and Pfaff, 1976; Swanson, 1976), the SON becomes a strong candidate for promoting oxytocin-induced FMB. This is particularly true in light of the recent demonstration that some SON neurons have axon collaterals which terminate in the nearby lateral hypothalamus (Mason, Ho and Hatton, 1984), a region known to contain MPOA efferents (Conrad and Pfaff, 1976). Thus, the influence of SON neurons now appears to include the CNS itself.

If the SON somehow participates in the onset of maternal behavior, might morphological changes occur in the SON that are concurrent with this behavior? If so, what

form might they take? Since 1976 a growing body of work from Hatton, Tweedle, and associates (see Hatton et al.,1984, for review, as well as Theodosis, Poulain and Vincent (1981) and Theodosis and Poulain (1984a & b) has established that during conditions of stimulation (dehydration, parturition and lactation) neurons of the SON, which produce and release vasopressin (VP) in addition to oxytocin, display significant morphological plasticity. This plasticity is in the form of changes in the interrelationships of neurons and astrocytes and includes: 1) increases in cell-cell apposition (two neurons with no intervening glial process between them), 2) dendritic bundling (two or more dendritic profiles in direct apposition), and 3) the formation of double synapses (one presynaptic axon terminal simultaneously contacting two dendrites or two somata), apparently brought about by astroglial process retraction (Figures 2-5). Additional complexity is indicated by the finding that of the stimuli studied so far only the chronic stimulation associated with gestation and lactation produce double synapse formation in the dendritic zone (Perlmutter et al., 1984a). Ten days of 2% saline treatment produces double synapse formation in the somatic zone (Tweedle and Hatton, 1984a), but not in the dendritic zone (Perlmutter et al., 1984c). Indeed, evidence for two populations of double synapses (one dendritic and one somatic) has been presented (Tweedle and Hatton, 1984b).

Based on the available circumstantial evidence it



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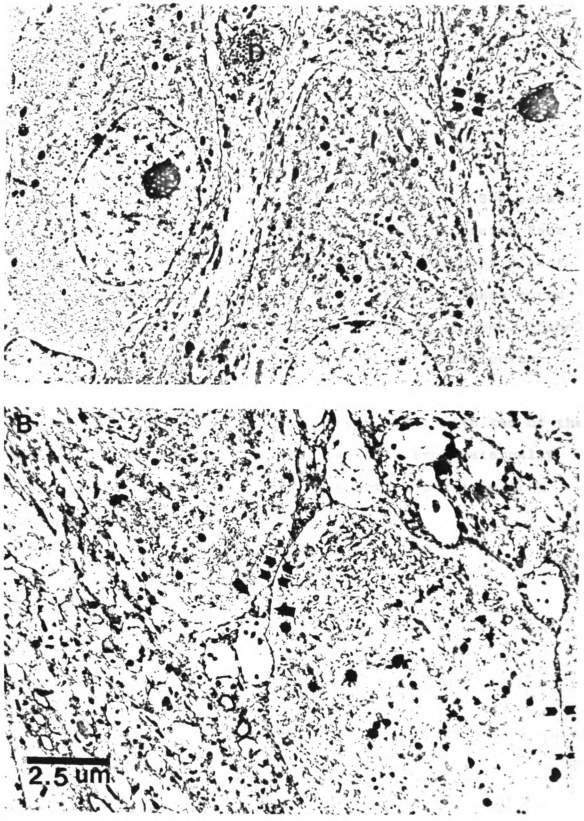

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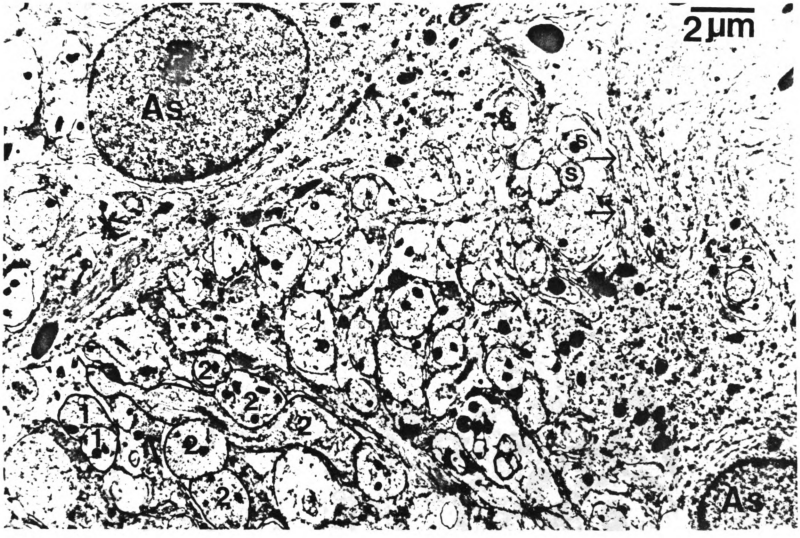

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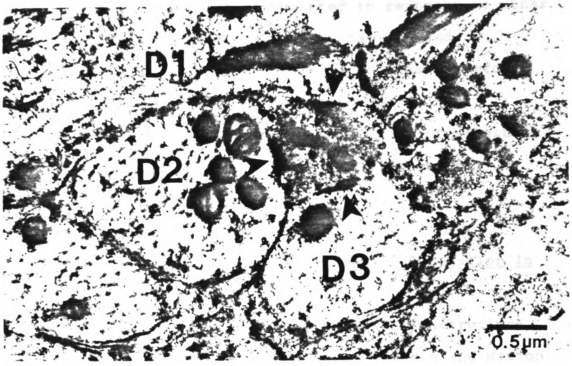

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therefore seems reasonable to ask whether maternal behavior is accompanied by ultrastructural manifestations of hormone production and/or release in SON neurons which are perhaps similar to those previously documented in response to other stimuli associated with gestation, lactation, and dehydration. Part IA of the present study was therefore undertaken to determine if changes occurred in SON ultrastructure concurrent with the exhibition of maternal behavior, without the animal experiencing pregnancy, parturition, and lactation.

Part IB: Morphological correlates of maternal behavior in the posterior pituitary.

Together with the examination of SON ultrastructure under varying physiological conditions, Tweedle and Hatton and associates have studied ultrastructural changes in the posterior pituitary under those same conditions. They have found that this structure, which contains axonal endings of PVN and SON cells which release their hormones into the fenestrated capillaries found there, also exhibits a striking degree of plasticity when the neurohypophysial system is stimulated. In particular, the axons of the MNCs, many of which are normally enveloped by the cytoplasm of pituitary neuroglia (pituicytes) have been seen to lose their glial ensheathment under conditions of stimulation. So far it has been shown that immediately after parturition and during dehydration, and in response to a high osmolality

medium in vitro, the number of neurosecretory axons which are totally enclosed by pituicyte cytoplasm decreases significantly (Tweedle and Hatton, 1980a, 1982; Perlmutter et. al., 1984d). In Part IB of the present study the possibility of similar changes occurring with the stimuli associated with the induction of maternal behavior was investigated.

Part II: Immunoelectron microscopy of SON neurons at late gestation, parturition, and lactation.

Despite energetic investigation and the substantial inroads made into understanding this complex peptidergic system there is much yet to be learned. Many of the questions to be resolved involve the formation of somatic direct appositions, double synapses and dendritic bundling over conditions of the pregnancy cycle in relation to the peptide content of SON neurons and their dendrites. In particular, the previous work (Hatton and Tweedle, 1982; Perlmutter et al., 1984a) employing tissue from virgin rats, prepartum, postpartum, and lactating rats has revealed a different time course of events in the dendritic zone versus at the cell body level. On the measures of percentage of cell bodies or of dendrites directly apposed, percentage of somatic or dendritic membrane in direct apposition, and percentage of cell bodies or dendrites receiving double synapses, the largest increases in the dendritic zone were found immediately postpartum. The changes at the cell body

level did not reach their maximum until fourteen days of lactation. This suggests that differences exist in the functional demands placed on different levels of the SON cells at parturition and lactation. Since there is evidence (Fuchs and Saito, 1971) that both OX and VP are released in large quantities during parturition, whereas only oxytocin is thought to show enhanced release during lactation (Wakerley et al., 1973), the maximal dendritic effect at parturition may reflect the activation of both peptidergic cell types at this time. The same line of speculation holds for those differences seen in the dendritic area between pre- and postpartum groups. The peptide content of the dendrites during parturition thus becomes of special interest. Also, assuming that the morphological changes seen at the level of the cell bodies reflect functional demands, such changes during lactation would be expected exclusively to involve OX-containing somata. Whether this is the case remains to be elucidated. Although Theodosis and Poulain (1984b) have reported OX- immunostained profiles apposed to non-immunostained profiles, in tissue from lactating subjects, methodological problems prevent easy interpretation of these data. In Part II, the hormone content of apposed and nonapposed neurons and, where possible, the hormone content of dendrites participating in bundles, was assessed by means of immunoelectron microscopy, in tissue from control, prepartum, postpartum and lactating rats.

Part III: Morphology of SON neurons after a 10 day postweaning period.

The time course of post-weaning changes is still something of a mystery. In the SON dendritic zone area, Perlmutter et al., (1984a) found that the lactating group showed a trend toward a decrease from postpartum values on the three measures of dendritic bundling, dendritic membrane apposition, and the occurrence of double synapses. Whether these decreases reflected an eventual return to base line is not clear. Theodosis and Poulain (1984a) found that at one month post-weaning, the extent of dendro-dendritic and soma-somatic appositions was not significantly different from that seen in virgins. Similarly, the number of double synapses was not significantly different from virgins by one month post-weaning. Knowledge of the time course of the return to normal values, particularly of the interval between 14-days of lactation and one month post-weaning, would tell something of the lability of the system. Does reinsertion of the glial processes occur rapidly after weaning? How long does the system, once engaged, retain the characteristics which might enable it to respond to the stimulus of suckling? Further, Theodosis and Poulain (1984a) did not distinguish between double synapses found at the dendritic versus somatic levels, so it is unknown whether the return to normal values differs on this measure for the two regions. In Part III of this dissertation, the

morphology of neurons and dendrites in the SONs of rats which had undergone pregnancy, parturition, 14 days of lactation and ten days of a post-weaning interval was studied to determine if traces of their maternal experience were still evident at this time.

This dissertation therefore examines ultrastructural and hormonal events which occur in the supraoptic nucleus (SON) concurrently with the maternal experience in the Sprague-Dawley rat. The data presented herein were gathered to answer three basic questions: 1) Does the participation of the SON in the events surrounding maternity include even the promotion of the maternal behaviors which this species engages in with its offspring?, 2) What is the peptidergic content of the neurons which undergo morphologic rearrangement over the course of the "Motherhood experience" and 3) What is the lability of the system, i.e., how long do the morphological traces of Motherhood persist?

For the sake of simplicity, these questions are addressed here as though they are separate experiments addressing separate issues. It should be remembered that such a division is merely experimentally convenient. It may be illuminating to consider the phenomena under study as representing different facets of an underlying continuum of events.

METHODS

Part I. Morphological correlates of maternal behavior.

Induction of maternal behavior in virgin rats.

Fourteen virgin Sprague-Dawley female rats were equally divided into control and experimental groups. They were housed in a 12:12 hr light/ dark cycle with food and water ad libitum. Induction of maternal behavior was accomplished with the protocol of Yogev et al. (1980): after 2 days of habituation to 30.48 cm by 35.56 cm by 16.51 cm plastic cages with wire tops, and to the standard light cycle, subjects were constantly exposed to two foster young, 3-15 days old. The foster young were exchanged daily and placed in opposite corners of the cage as far from the experimental subject as possible. For a fifteen minute observation period after introduction of the new pups each day, subjects were scored on the incidence of three behaviors: 1) retrieval of both pups to the same place in the cage, 2) crouching over pups in a nursing posture and 3) pup-licking, especially of the anogenital region. Subjects were also provided with shredded paper towels and/or tissue paper and evaluated for nest-building behavior. The behaviors were independently scored by two observers until a concensus as to what constituted occurrence of the behaviors was reached. Subjects were considered to be maternal from the first of two consecutive days that the first three behaviors were observed and they were sacrificed on day three of FMB after 5 minutes of observation. Control subjects were housed in

plastic cages in the same nursery as were the MBs, for 2 days prior to sacrifice. Control subjects were sacrificed at age 120 days, while the experimental group ages varied: 101 days (2 animals), 104 days (2 animals), 112 days (2 animals), 138 days (1 animal). Time of sacrifice was 2 - 4 hrs after lights on. Vaginal lavage was performed to determine the phase of the reproductive cycle at which sacrifice occurred for both groups.

Electron microscopy.

Rats were deeply anesthetized with ether and then transcardially perfused with physiological saline followed by 150-200 ml of a solution of 3.5% glutaraldehyde and 1% paraformaldehyde in a 0.1M cacodylate buffer. Brains were removed from the skulls and placed in fixative for an additional 2-3 hr prior to being cut into into 500 µm slices with a tissue chopper. The left and right SON were then cut from each slice with a single edged razor, dehydrated with a graded series of alcohols, and embedded in a water soluble resin (L.R. White, E.F. Fullam, Inc., Latham, N.Y.). Platinum and gold colored sections (60-90 nm) were cut with an ultramicrotome from blocks of resin containing the middle third of the SON, as partitioned into anterior, middle and posterior portions, and collected on nickel grids coated with 0.25% formvar. In rare instances it was necessary to use tissue from the anterior or posterior SON. Thin sections were also cut from blocks containing the posterior pituitaries of control and experimental subjects.

Representative grids containing tissue from the SON or posterior pituitary were counterstained with saturated uranyl acetate for 30 minutes followed by lead citrate for 6 min. Grids were examined with a Philips 201C electron microscope.

Data collection- SON.

Electron micrographs were obtained from one thin section each from both the left and right SON of each subject. The ventral dendritic zone of the SON was photographed in its entirety, the criteria for inclusion being that the dendrites were ventral to magnocellular neurons and/ or had dendrites containing dense cored vesicles (dcvs; presumably containing hormone) amongst them. Grid bars were used as a guide to insure that all of the area of the dendritic zone was photographed without overlap. Dendrites were identified as having microtubules, rough endoplasmic reticulum and/or free ribosomes, electron lucent cytoplasm, at times the presence of 100-200 nm dcvs, and often, being postsynaptic to presynaptic profiles. Care was taken to include as many dendrites as possible in each negative. Astrocyte cell bodies and processes were often included in the photographs since they exist in this region. However, the ventral glial lamina (VGL), consisting almost exclusively of astrocyte cell bodies and processes was not photographed except when the presence of numerous dendritic profiles warranted its sampling. Sampling of the somatic zone was accomplished by locating, at a magnification too

low to discern direct appositions (1612X, actual calibration), 2 or more cell bodies in close proximity to one another and then increasing the magnification for photography. MNCs were identified primarily by their location in the tightly packed SON adjacent to the optic track and, at higher magnification, the presence of 100-200 nm dense cored vesicles in their cytoplasm. Pictures were taken at a magnification of 3059X and enlarged 2.5 times (print magnification 7590X). During the printing process, random code numbers were assigned to the micrographs by an associate in order to guard against observational bias during scoring. Micrographs were then scored for 1) the presence of direct appositions between cell bodies, 2) the incidence and size of dendritic bundles containing two or more dendrites, 3) length of apposed and non-apposed membranes between dendrites or somata, and 4) the presence of double synapses. Membrane lengths and dendritic areas were determined by tracing membrane lengths on micrographs which were placed on a Houston Instruments (Houston, Texas) digitizing tablet 114 which was interfaced with a Zenith 150 personal computer (software by R. Luzenski). Data analysis was aided, when appropriate, by the use of an Apple IIe personal computer and statistical programs (Human Systems Dynamics, Northridge, CA) for t-tests, 2 sample correlations, and analysis of variance (ANOVA). Nonparametric tests were employed when appropriate.

Data collection- posterior pituitary.

Without being aware of experimental group membership, fifteen pituicytes from each neurohypophysis were randomly sampled by a confederate (B. Kohn) at 4740X. A pituicyte was first located on the electron microscope at low magnification (1612X). The magnification was then increased to 3059X and the perimeter of the pituicyte membrane followed around its entire extent. The total number of axonal processes completely enclosed by the pituicyte was counted.

Part II- Immunoelectron microscopic correlation of morphology and peptide content of SON neurons in control, prepartum, postpartum and lactating animals.

Experimental subjects.

Twenty-one 18 day pregnant Sprague-Dawley rats were received from Holtzman Co. (Kenosha, WI) and divided equally into 3 groups. Animals from two of the groups, designated prepartum and postpartum, were each housed in separate hanging wire cages in which flooring and nesting material were provided. The third group, designated lactating, were each housed separately in 30.48 cm by 35.56 cm by 16.51 cm plastic cages with wire tops. All were exposed to a 12:12 light cycle with food and water ad libitum.

On day 21 of gestation subjects in the prepartum group were deeply anesthetized with ether and transcardially perfused as described for the maternal behavers and the control group. All were confirmed as being in the late

stages of pregnancy based on two observations: 1) the fetal pups were well developed, to the point of being viable when removed surgically and placed with foster mothers, and 2) other rats determined to be sperm positive on the same date began labor within 3 hours of the prepartum group's sacrifice. Rats in the postpartum group were sacrificed within 12 hours of delivering full litters of pups. Litters of the group designated as lactating were culled or added to in order that each subject was nursing 9-10 pups. On the fourteenth day of lactation rats were sacrificed as described for the other groups. The age at which the various groups were sacrificed were as follows: prepartum, 112 days; postpartum, 112 days; and lactators, 128 days. Time of sacrifice was 2 - 4 hr after lights off. Brain tissue from all three groups, in addition to tissue from the control group described in Part IA, was prepared for electron microscopy as described for maternal behavers and controls in Part 1A, except that no counterstains were applied.

Immunocytochemistry.

Prologue. The goal of the EM immunocytochemist is to produce tissue which has good morphology, specific unequivocal labelling, and a paucity of excess reaction product. For this task, he or she should have the constitution of an Indiana Jones.

Three mouse monoclonal antibodies (MABS) were employed

as primary antisera. Two of these were the generous gift of Dr. H. Gainer and were raised in his laboratory. Their specificities have been thoroughly determined (Ben-Barak et al., 1985). Monoclonal antibody (MAB) #38 has been characterized as specifically binding to an as yet undetermined portion of the oxytocin-associated neurophysin peptide. MAB #41 is somewhat less specific, being predominantly directed against an unknown part of the vasopressin-associated neurophysin peptide (Russell et al., 1980). Some cross-reactivity of this MAB with (a presumably) oxytocin-associated peptide has been noted when used at high concentrations (>= 1:50) in postembedding ICC. The third MAB used, designated MAB "Day one" was the generous gift of Dr. W. L. Smith and was raised against bovine platelets. To date, it has only been found to bind with certain parenchymal elements of bovine liver. This third MAB was used to insure that the mouse MABs were not non-specifically binding to the cells of the SON.

The ICC protocol employed in this study was a post-embedding technique, that is, the reagents were applied to the tissue after it had been embedded, thin sectioned, and collected on grids. The formvar step was necessary to prevent the rather unstable resin from disintegrating when exposed to the electron beam in the microscope. After sectioning, the grids were carefully inserted into squares of Silgard (Dow Corning), a pliable, rubbery substance, into which slits had been made with a single edged razor. Grids

were inserted such that each thin section would be exposed to reagents. Reagents were applied onto the Silgard squares where surface tension maintained their covering of all of the grids. With this method it was thus possible to immunocytochemically stain a great many grids at one time. The protocol followed was:

1. phosphate buffered saline (PBS), pH 7.4- 8 minutes.
2. 1% sodium meta-periodate in PBS- 10 minutes.
3. 4 rinses with PBS @ 1 minute.
4. 10% normal goat serum (NGS) in PBS, 10 minutes.
5 Blot NGS from grids with filter paper.
6. Primary MABs #38 and #41 in PBS (1:100), MAB Day 1 in PBS (1:50), 1 hour.
7. Reapply fresh MABs, 1 hour.
8. 6 rinses with PBS, @ 1 minute.
9. Goat anti-mouse peroxidase conjugate (Boeringer-Mannheim) in PBS (1:20), 2 hours.
10. 6 rinses with PBS, @ 1 minute.
11. 10 mg/ 20 ml PBS 3,3'-diaminobenzidene, filtered with a 1.2 um pore size, Triton-X 100 free Millipore filter, 30 minutes.
12. 6 rinses with PBS, @ 1 minute.
13. 0.5% osmium tetroxide in PBS, 30 sec.
14. Each Silgard square was then dipped 4-8 times each in three successive beakers of distilled water and blotted dry with filter paper.

All incubations in sera were done on a lighted

histology table. Grids were variously stained on three separate Silgard squares, one per primary MAB employed. The Silgard squares were placed on separate pieces of filter paper which had been dampened with PBS. They were then covered with the lids of glass petri dishes and placed approximately 1.5 feet underneath a 100 watt incandescent light bulb. All PBS was filtered prior to rinses and making solutions with a .22 µm pore size, Triton-X 100 free Millipore filter. Step 13 was done under a safety hood. No counterstains such as lead citrate or uranyl acetate were applied to the tissue.

All the grids ultimately examined in this study were stained in one session, in an attempt to reduce the variability inherent in this technique. ICC was performed on a rainy Tuesday afternoon in mid-April.

Examination of grids.

Data from a total of 48 grids, representing tissue from 28 animals (eight grids were lost in processing) was collected on the electron microscope. Because of a lack of contrast due to the absence of counterstain, the .25% Formvar coating on the grid, and an additional low-contrastiness contributed by the L.R. White resin, grids were examined with the emission control of the Philips 201c set at 3. Setting the kv control at 40 was also employed in an effort to improve contrast. Under these conditions it was possible to quantitatively examine an entire SON (1 grid) in thin section in 30 - 60 min. For grids stained

with each MAB, either #38 (OX-NP) or #41 (VP-NP), counts were made of the number of MNCs in close apposition, the number unapposed, and whether they were judged to be immunopositive or immunonegative. Efforts were also made to identify double synapses and determine the peptide content of the somatic or dendritic profiles they contacted.

So- which of you cells are stained?

Unlike ICC of pituitary tissue which yields unequivocally stained or unstained axonal endings (Figure 6), ICC of MNCs, even between grids stained on the same Silgard square, yields somewhat variable staining density of dcvs. Therefore, it was necessary to develop, and consistently apply, decision rules for judging dcvs to be either stained or unstained.

Preliminary tests with various combinations of counterstains such as lead citrate, uranyl acetate, and osmium, applied to immunostained and control grids (primary antisera omitted) revealed that the use of lead citrate and/or uranyl acetate, often resulted in dark, dense cored vesicles within MNC cytoplasm - in both control and ICC-treated grids. Only osmium was found to leave the dcvs in a condition which did not falsely appear "stained". The tip-off for this phenomena was the observation that the production of falsely -positive dcvs was accompanied by the production of dense staining of the lysosomes within the cytoplasm also; both apparently having a similar affinity for the counterstains. In the absence of lead citrate and

Figure 6. Example of the interrelationships of axons and pituicytes (P) and in the neurohypophysis. The tissue has been immunocytochemically stained with an antiserum raised against the vasopressin-associated neurophysin. Specifically stained endings (arrows) are interspersed with unstained endings (*). Some "bleeding" of reaction product onto the underlying formvar film can be seen near intensely stained endings. One ending (**), judged to be unstained, is entirely enclosed by pituicyte cytoplasm. Inset: neurohypophysial tissue which was exposed to "Day-1" antiserum, raised against bovine platlets, exhibits no immunocytochemical reaction product. The darker granules seen in "unstained" endings are likely due to the brief exposure to osmium. Tissue from maternal behaver #2.

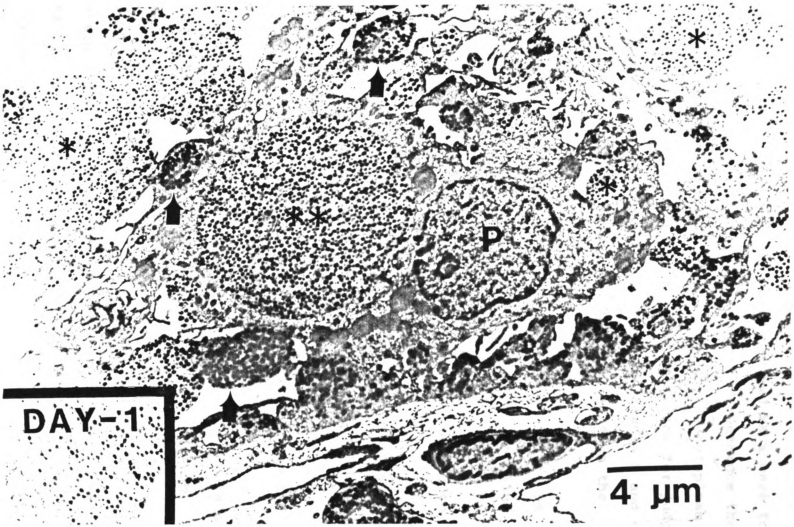


Figure 6.

uranyl acetate, but with the application of the .5% osmium used in this procedure, well immunostained MNCs were found to exhibit dcvs which were far darker in appearance than nearby lysosomes, as well as the rest of the cytoplasm and neuropil. This then, became a major criterion for judging whether a cell was truly immunostained (Figures 7 and 8). Other decision rules which evolved were based on the following features:

1. Size. Well stained dcvs were generally seen to be somewhat larger than normal (100-200 nm) due to being covered by reaction product (Figure 8).

2. Numbers. Well stained cells generally appeared to contain a wealth of dcvs because they were more visible (Figure 7). This alone, however, was insufficient in order to declare a cell stained, as some unequivocally stained cells seemed to be depleted of their dcvs. Because the VP-NP MAB #41 had been reported to somewhat cross-react with OX-NP when used at high concentrations (1:50), on grids stained with that MAB, cells with only one or two "stained" vesicles were judged to be unstained in the absence of other information (Figure 7).

3. Staining of nearby cells. When there were very well stained cells in close proximity to a cell with questionable "staining" the former would be judged to be unstained (Figure 7).

4. Absence of high background. Excess reaction product was seen to distribute itself as small round, often dcv-sized

Figure 7. Example of immunoreactive and non-immunoreactive cell bodies in the SON. This tissue was stained using an antibody to the vasopressin-associated neurophysin. The cell in the center (boundaries outlined with arrows) was judged to be non-immunoreactive despite the presence of a few densely-appearing dense cored vesicles (DCVS), based on the prevalence of "unstained" DCVS (right-hooked arrows) and the robust staining seen in nearby cells. Well-stained DCVS are marked by left-hooked arrows. The long arrow points to non-specific glitch. Tissue from control animal #1.

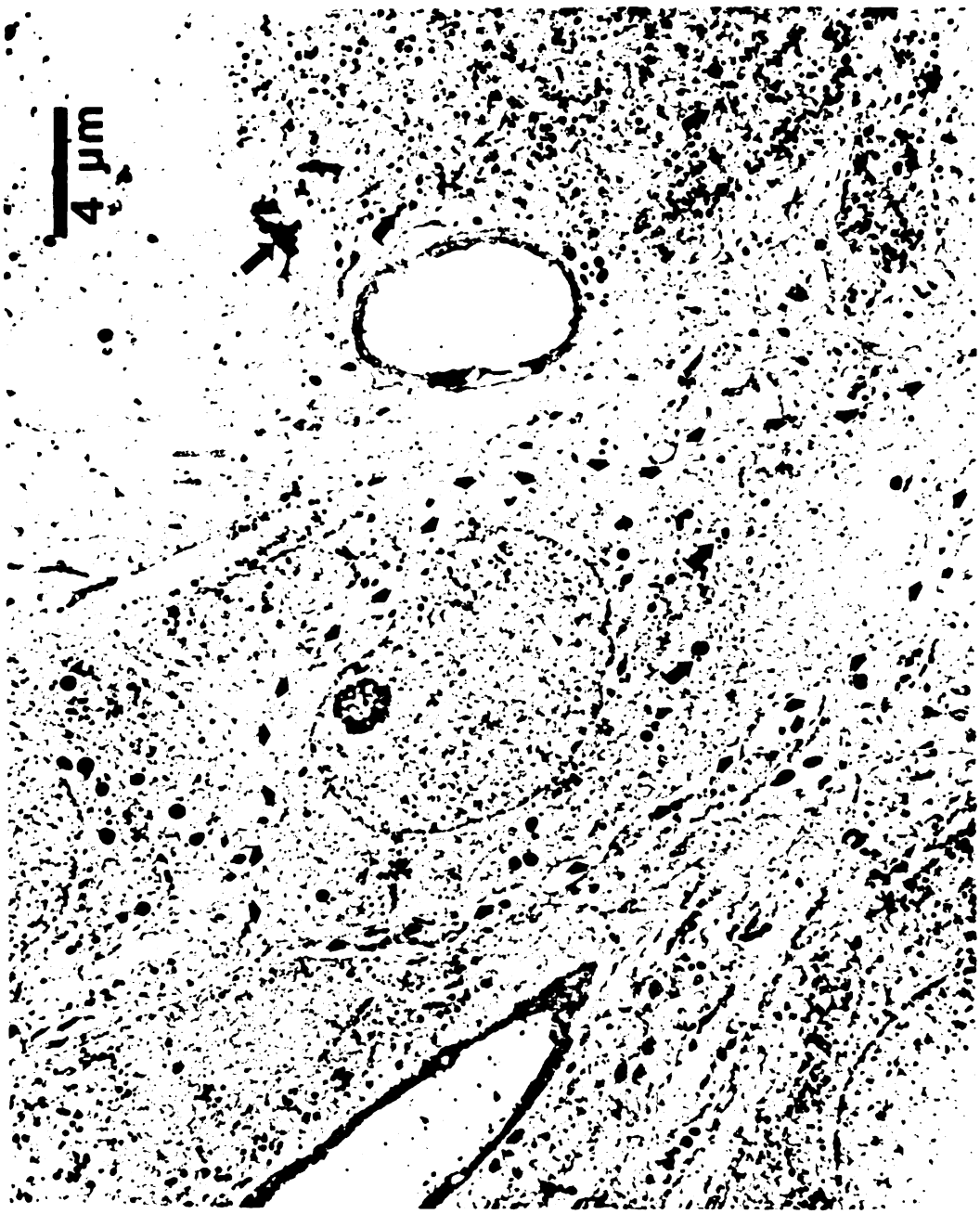


Figure 7.

Figure 8. Two electron micrographs taken at high magnification show the appearance of immunocytochemically stained verses unstained dense cored vesicles (DCVS). The top panel is of tissue which was not exposed to a primary antibody whereas the bottom panel is of tissue immunostained for the vasopressin-associated neurophysin. Compare the density of the DCVS to the density of nearby lysosomes (LY). Notice that the immunostained DCVS are larger than their unstained counterparts, denser than nearby lysosomes and, in some cases, encrusted with pentagonal peroxidase-antiperoxidase molecules. Nuc = nucleus.

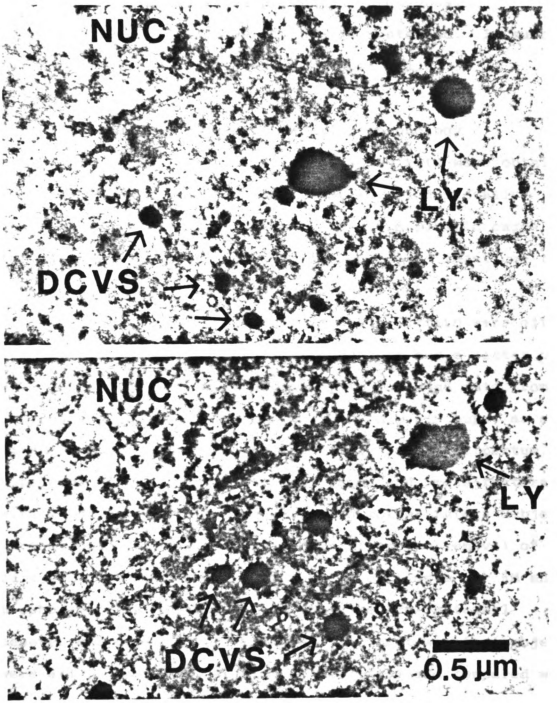


Figure 8.

droplets. On occasion, it prevented unequivocal identification of cells as stained or unstained. In such cases data was not collected from those cells.

Part III- Analysis of SON postweaning morphology

Seven 18-day pregnant Sprague-Dawley rats were obtained from Holtzman Co. Upon arrival they were individually housed in 30.48 cm by 35.56 cm by 16.51 cm plastic cages in the laboratory animal "nursery" in conditions identical to those described for the induced-maternal behavior experiment. At one day postpartum their litters were either culled or added to, such that each subject had a litter of 9-10 pups. (It was later necessary to included data from an alternate postweaning subject who had only six pups, as some tissue was damaged in processing). At day 14 postpartum, pups were removed from their home cages and cross-fostered with other non-experimental lactating mother rats. Subjects were allowed to survive for 10 days after the removal of the pups prior to sacrifice. Preparation of tissue for EM and morphological analysis were as described for maternal behaver and control tissue in Part I. Post-weaning subjects were all 112 days old at time of sacrifice and compared with control animals 120 days of age. Time of sacrifice was 2 - 4 hr after lights off.

RESULTS

PART IA- Morphometry of the SON of maternally behaving virgin rats.

Behavioral observations (Figure 1).

Experimental subjects displayed the maternal behaviors of retrieval of pups to a central location, licking of pups, including anogenital licking, and crouching over pups in a nursing posture after 5 to 16 days of being housed with rat pups. Six out of the seven rats also used the materials provided to build distinct nests, i.e., digging depressions in the bedding and arranging the paper towel strips around the perimeter in a methodical fashion, where they would deposit the rat pups after retrieval, although these did not appear as deep or appear to employ as many paper towel strips as those built by actual nursing mother rats with litters of 8 or more offspring.

Morphometry.

Somatic appositions. (Figure 3).

A total of 298 ($\bar{X}$=42) and 246 ($\bar{X}$=35) magnocellular neurons were examined from the maternally behaving and control groups respectively. The percentage of cells seen to be in apposition was 28.9 $\pm$ 3.99% vs 19.5 $\pm$ 1.78%, controls and maternal behavers (MBs) respectively, a non-significant difference (Mann Whitney-U test, $p<.08$). Likewise, the percentage of membrane in direct apposition

(5.0 ± .58% versus 3.9 ± .63%, controls and MBs respectively), was not significantly different.

Double synapses-somata. (Figure 3).

Two populations of double synapses were seen to occur in the somatic regions of MBs and controls; both of which were rare events. The first consisted of one presynaptic axon simultaneously contacting two postsynaptic somata. An average of only 2.88 ± 1.22% and 2.54 ± 1.52% of the somata in the MB and control groups were seen to receive such synapses, a non-significant difference. The second kind of double synapse observed was one presynaptic axon contacting one cell body and one adjacent dendrite simultaneously. No significant differences were found between MBs ($\bar{X}$=2.35 ± 1.54%) and controls ($\bar{X}$=5.00 ± 1.29%) on this measure. Neither were differences detected when the two measures were combined for analysis.

Dendritic bundling. (Figure 4).

Because previous work established that dendritic bundles of up to four dendrites were often seen in the dendritic zone of normal animals (Perlmutter et al., 1984a), data were equally categorized into bundles of 1-4, 5-8 and 9-12 dendrites. A two-factor analysis of variance (ANOVA) was employed to assess differences between control and maternally behaving groups in the average number of dendrites per bundle in bundles falling into each of these categories. An overall difference ($p<.025$, $F= 7.06$, $df= 1, 12$) was found between the two groups for the average number

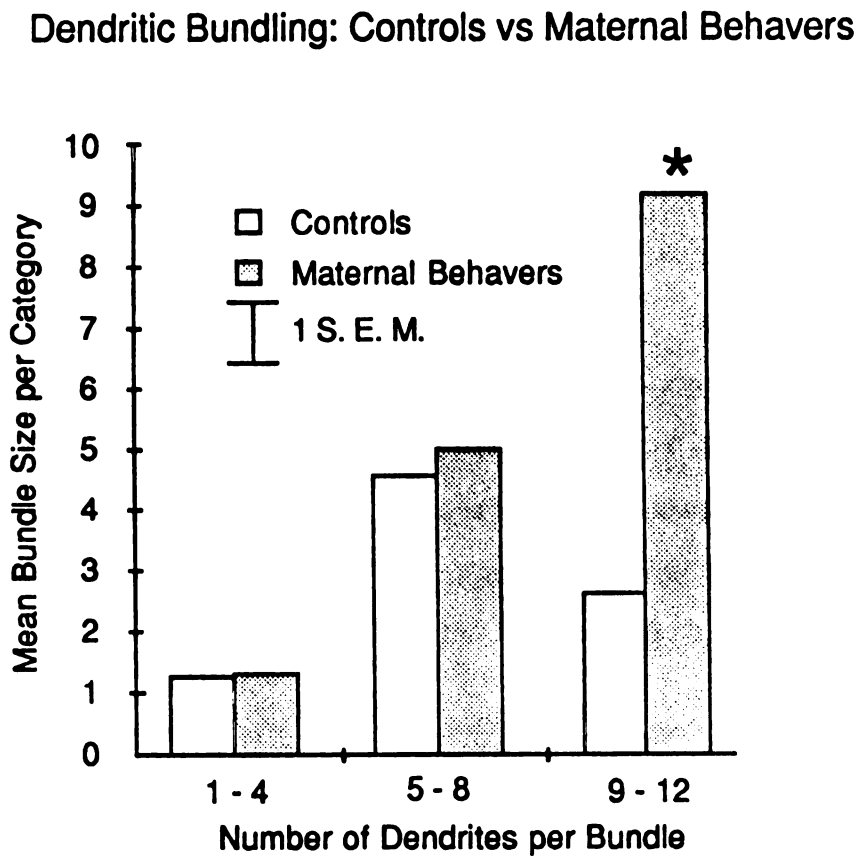


Figure 9. Dendritic bundling in control and maternally behaving animals. * $p<.02$; Tukey's test. S.E.M.= 1.07.

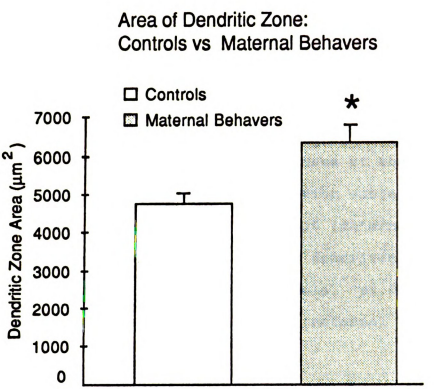
of dendrites per bundle. Post-hoc comparisons (Tukey's test; Gill, 1978) revealed that whereas no differences existed between groups in the average number of dendrites per bundle in bundles of 1-4 and 5-8, the two groups differed significantly ($p<.025$, $q= 6.08$, $df= 2,12$) in the occurrence of bundles containing 9-12 dendrites ($\bar{X}s$ = 9.26 ± 1.07 and 2.71 ± 1.07; MBs vs controls; Figure 9).

Percentage of dendritic membrane in direct apposition.

Analysis of data gathered with the aid of the digitizing tablet failed to detect any differences between groups in either the absolute length of dendritic membrane in direct apposition ($\bar{X}s$ ± S.E.M. = 232 ± 31 µm, controls; 269 ± 36 µm, MBs) or when the percentage of membrane in direct apposition was computed as a percentage of total membrane (apposed membrane/ apposed membrane + unapposed membrane). The means and standard errors for the latter measures were 13.64 ± 1.04% (controls) vs 12.48 ± 1.08% (MBs).

Number of dendrites observed. (Figure 10).

An average of 622 ± 42.53 and 511 ± 39.64 dendrites were observed per 2 thin sections per animal in the maternally behaving and control groups respectively. By Student's t-test, these differences were found to be significant ($p < .05$, $t= 1.77$, $df=12$; since no pyknotic profiles or other visible signs of degeneration were observed in the material, the possibility of dendritic loss in the MB group did not seem realistic, thus a one-tailed critical value was accepted). The correlation (Pearson's product-moment


Area of Dendritic Zone:
Controls vs Maternal Behavers
Controls
Maternal Behavers
*
Dendritic Zone Area (μm²)
7000
6000
5000
4000
3000
2000
1000
0

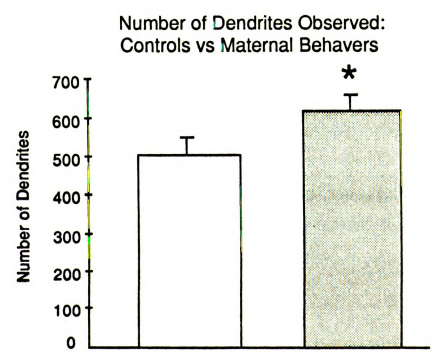

Number of Dendrites Observed:
Controls vs Maternal Behavers
*
Number of Dendrites
700
600
500
400
300
200
100
0

Figure 10.

Figure 10. Top: Differences in the area of the dendritic zones of control and maternally behaving subjects. *p<.01; t-test. S.E.M.= 221 (controls) and 409 (maternal behavers). Bottom: Differences in the number of dendrites counted in control and maternally behaving animals. *p<.05; t-test. S.E.M.s= 39.64 (controls) and 42.53 (maternal behavers).

correlation coefficient) between the total number of dendrites observed per animal and the mean number of dendrites in bundles of 9-12 per animal was not significantly different from zero ($r = .32$, $p > .05$).

Area of dendritic zone. (Figure 10).

Due to heterogeneous variance between groups a median test (Siegel, 1957) was used to determine that significantly ($p<.0004$) more micrographs of the ventral dendritic zone were obtained from tissue from maternally behaving animals relative to controls. An average of 7.71 vs 5.71 micrographs were taken per animal, respectively. When considered in terms of area, each negative, taken at the magnification of 3,059X, encompassed an area of approximately 835 μm^2. The mean area occupied by the dendritic zone per two thin sections per animal was 4774 $\pm$ 221 μm^2 versus 6326 $\pm$ 409 μm^2 for controls and MBs respectively, a statistically significant ($p<.01, t=3.092$, $df=12$) difference. A partial correlation analysis was performed to determine to what extent the greater number of micrographs obtained from the MB group contributed to the more frequent observation of large bundles in that group. With the amount of variance contributed by the number of micrographs factored out, no significant correlation ($r=.08$, $p>.05$) was found between the number of dendrites observed in dendritic bundles of 9 or more dendrites and the total number of dendrites observed.

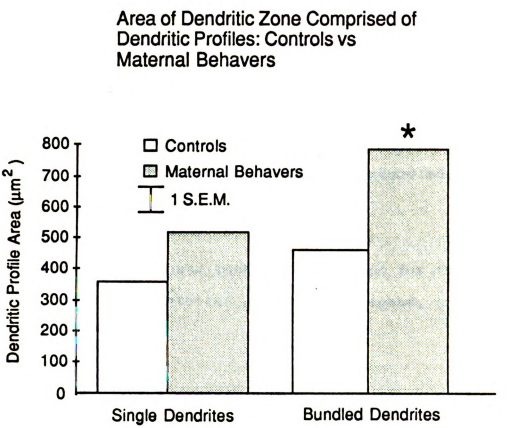


Figure 11. Differences in the area of dendritic profiles in the dendritic zones of controls and maternal behavers. * $p<.05$; Tukey's test. S.E.M.= 87.

Area of dendrites. (Figure 11).

Analysis of the total area of dendritic profiles in bundles and in single dendrites (ANOVA) revealed an overall difference ($F= 7.59$, $df = 1, 12$, $p< .02$) between controls and MBs in dendritic area. Tukey's test for individual comparisons showed a significantly larger dendritic area for those dendrites participating in bundles in the MB group ($q= 3.68$, $df = 2, 12$, $p < .05$; $\bar{X} \pm$ S.E.M. = $462 \pm 87\ \mu m^2$., controls and $785 \pm 87\ \mu m^2$, MBs). No differences were found between the two groups in the area of single, unbundled, dendrites.

Dendritic double synapses.

No differences were found between the groups for the percentage of dendrites contacted by double synapses, (1.21% vs 0.92%; controls vs MBs).

Vaginal cytology.

Inspection of vaginal smears showed that animals from both groups were at various points in the estrous cycle at the time of sacrifice. As determined by this method (Martin, 1976) MBs were distributed amongst the points of the cycle as follows: metestrus (1), proestrus (3), diestrus (2) and estrus (1). Four of the control animals were observed to be in metestrus while the others exhibited vaginal cytology associated with proestrus (1), estrus (1), and diestrus (1).

Miscellaneous observations.

No significant correlations were found between the number of days the MBs were exposed to rat pups and: the

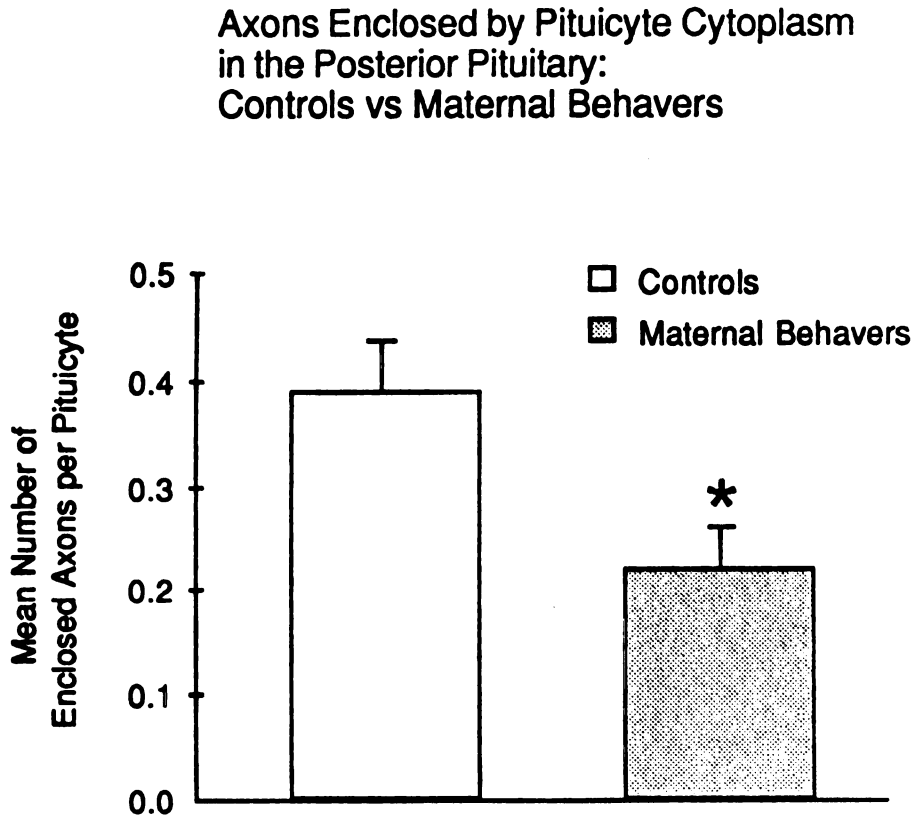


Figure 12. Differences between maternal behavers and controls in the number of axons/ pituicyte totally enclosed by pituicyte cytoplasm. * $p < .035$. S.E.M.= .05 (controls) and .04 (maternal behavers).

average number of dendrites in bundles of 9 - 12 ($r = -.28$, $df = 5$); the number of dendrites ($r = -.12$, $df = 5$); or the dendritic zone area ($r = -.10$, $df = 5$).

In a small amount of material from two maternally behaving animals, profiles of processes containing dense cored vesicles similar to those observed in SON dendrites and somata, i.e., 100-200 nm in size with an electron lucent "shell" were observed in regions approximately 600-1000 µm lateral to SON, well outside the boundaries of that nucleus. A very few cells were seen to display other indices of cellular activation. Nine cells displayed swollen endoplasmic reticulum (Figure 3). Two of these were from 1 MB animal, the other eight were from 3 control animals. One cell each from 1 control and 3 MBs displayed double nucleoli.

PART IB-Morphological correlates of maternal behavior-posterior pituitary.

Pituicyte enclosure of axons in the posterior pituitary (Figures 6 & 12).

A tally of the number of axons enclosed by pituicyte cytoplasm revealed that the group means ± standard errors for the number of axons enclosed per pituicyte were 0.22 ± 0.04 for MBs and 0.39 ± 0.05 for controls, a significant ($p<.035$, $t=2.377$, $df=12$) decrease of 43% for the MBs.

Part II- Electron microscopic immunocytochemistry of SON neurons.

Control procedures.

Examination of grids which had been exposed to MAB Day-one as the primary antiserum, or those which had buffer substituted for primary antiserum showed a lack of reaction product over dcvs of SON neurons and pituitary axons. Nor was reaction product observed over any part of the cytoplasm or surrounding neuropil in the SON and neural lobe. Due to the high amount of antigens concentrated in a small area, staining of posterior pituitary tissue with anti-OX-NP or anti-VP-NP might be expected to produce cross-reactivity (Ben-Barak et. al., 1985; Whitnall et. al., 1985). However, a selective staining of axonal profiles was obtained in this region (Figure 6). Serial sections which had been alternately stained with OX-NP and VP-NP MABs exhibited dendritic and somatic profiles which appeared to be specifically immunostained, i.e., immunopositive (OX-NP+ or VP-NP+) in one section and immunonegative in the other. (Figures 13 & 14). A total of 527 ($\bar{X} \pm$ S.E.M./ group = 131 $\pm$ 6.38) and 705 (151 $\pm$ 28.14) cells were examined in anti-OX-NP and anti-VP-NP stained tissue, respectively. Based on the data of Swaab et al., 1975a, it might be expected that, at the level of the SON which was sampled, 50% of the cells observed on each grid should have been immunopositive for either anti-OX-NP or anti-VP-NP. A tally of the percentage of cells sampled which were immunopositive for either antigen showed that an average of 87.53 $\pm$ 3.38 %

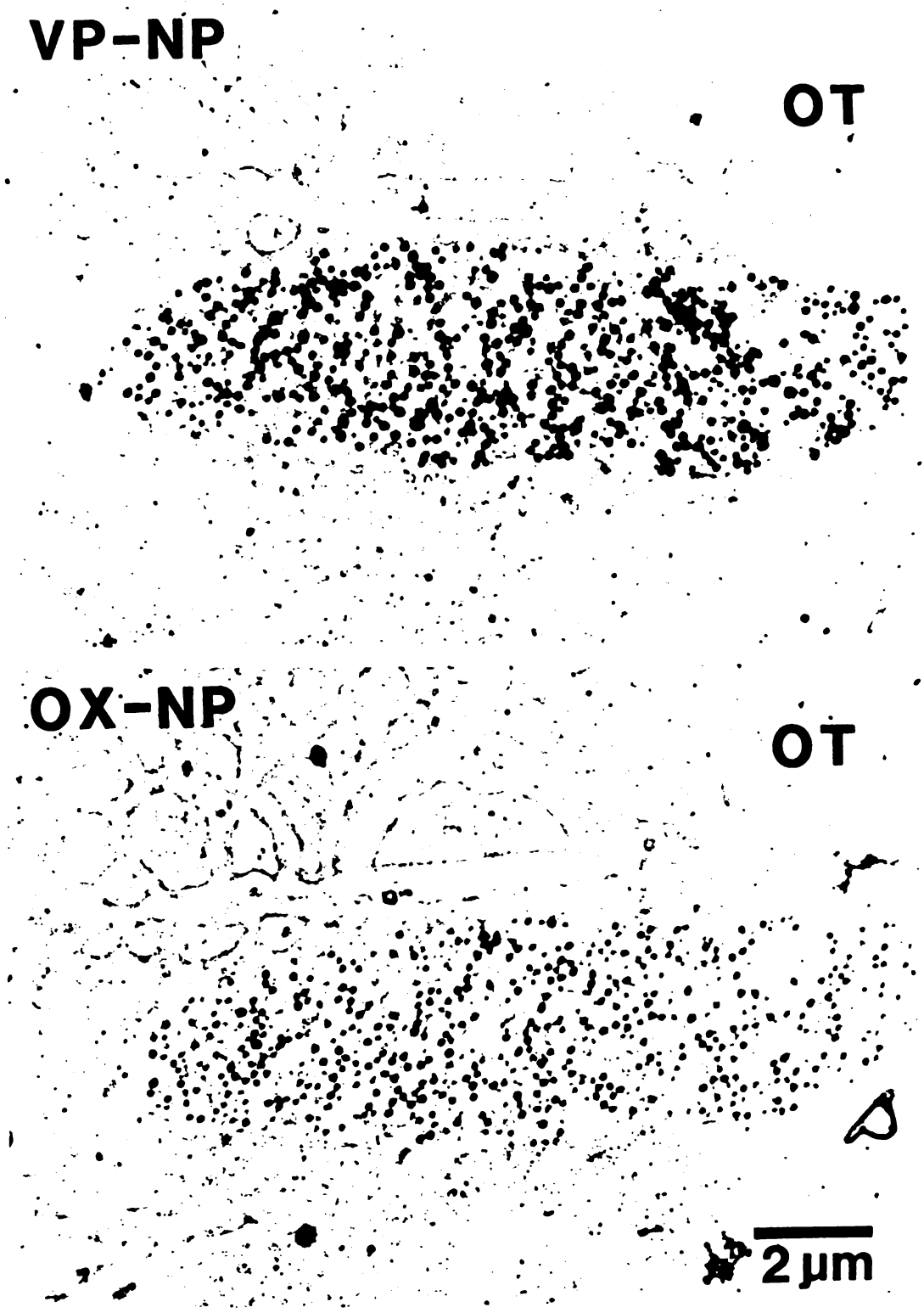


Figure 13.

Figure 13. Serial sections of tissue from post-partum animal #4 pass through a dendrite adjacent to the optic tract (OT) which is immunoreactive to a vasopressin-associated neurophysin (VP-NP) antibody but not to an oxytocin-associated neurophysin antibody (OX-NP).

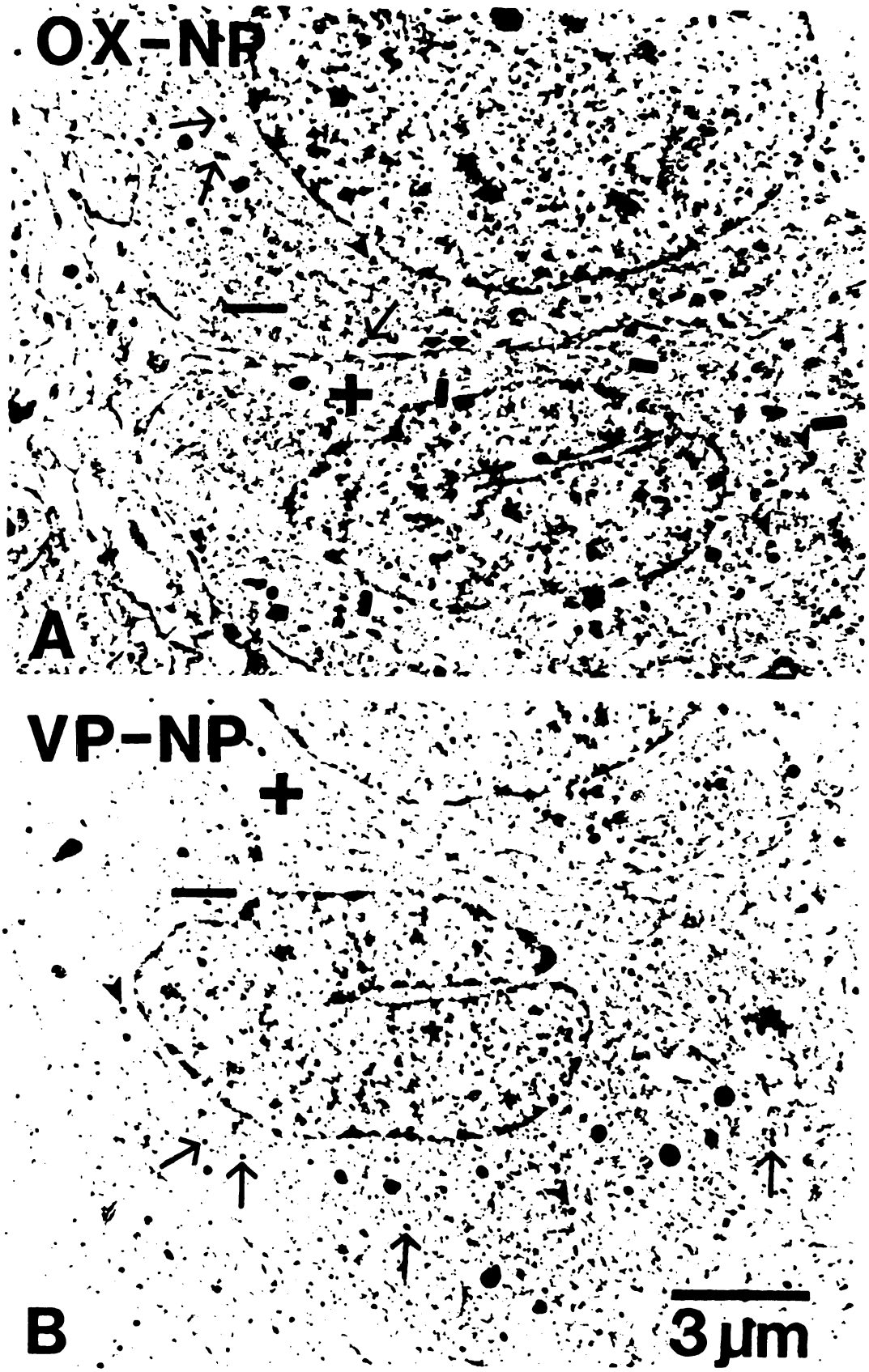


Figure 14.

Figure 14. Serial sections from lactating animal #6 through two cell bodies which have been stained with alternate antisera show antibody specificity and heterotypic cell-cell apposition. The + and - indicate cells judged to be immunoreactive and non-immunoreactive respectively. The small, winged arrows point to stained dense cored vesicles and the long, straight arrows point to unstained dense cored vesicles. Darts point to non-specific "glitch" as determined at a higher magnification. Panel A shows tissue stained with an antibody to the oxytocin-associated neurophysin (OX-NP). The short, fat arrows point to a region of direct membrane apposition. The crystalline structures are of unknown origin. Panel B shows tissue which has been stained with an antibody to the vasopressin-associated neurophysin (VP-NP).

per animal of the cells sampled were deemed immunopositive for one antigen or the other. The percentage of immunopositive cells/ antiserum for all groups was 49 ± 2.2% (OX-NP) and 45 ± 8.4% (VP-NP).

Peptidergic identity of apposed SON somata as a function of reproductive state. (Table 1).

A two-factor ANOVA was employed to assess whether OX-NP+ or VP-NP+ cells preferentially formed close appositions during late gestation, parturition, and lactation. The mean percentages of apposed control group neurons immunopositive for OX-NP and VP-NP were 9.87% and 10.22% respectively. To control for possible differences in antibody affinity, data from the experimental groups were expressed as a percentage of the mean number of apposed control group neurons immunopositive for the same antigen. The analysis confirmed previous observations that the number of cells in direct apposition varies in relation to reproductive state ($p<.02$, $F = 6.87$, $df= 3, 40$). As for the interaction between cellular peptide content and the likelihood of forming direct appositions, no significant interaction was found ($p>.05$, $F= 2.99$, $df= 3, 40$), indicating that one peptidergic cell type was participating to a greater extent in cell-cell appositions. Individual comparisons (Tukey's test) reveal that whereas VP-NP+ cells did not differ significantly from controls in percentage of apposition across reproductive states, the percentage of apposed OX-NP+ cells in the postpartum group was significantly greater ($p<.05$; $q=5.15$, prepartum vs

postpartum; df = 4, 16) than that seen in both control and prepartum groups.

Table 1. Peptide content of directly apposed cells.

	Mean % Cells in Apposition (% of Controls)	
	OX-NP +	VP-NP +
Controls	100 ± 37	100 ± 37
Prepartum	* 93 ± 37	116 ± 37
Postpartum	** 281 ± 37	182 ± 37
Lactating	123 ± 37	225 ± 37

** = different from controls ($p < .05$).

* = different from postpartums ($p < .05$).

Homotypic versus heterotypic direct appositions (Table 2).

A second analysis (two-way ANOVA) was performed to determine if the apposition between cells of like peptide content (homotypic) or unlike peptide content (heterotypic) changed with reproductive state, and whether one of these was more prevalent than the other. No changes were detected in the type of apposition across reproductive state ($F = 0.598$, $df = 3, 88$). However, a highly significant difference was found in the occurrence of heterotypic versus homotypic cell-cell appositions ($P < .003$, $F = 9.40$, $df = 1, 88$). Individual comparisons (Tukey's test) revealed that there were significantly ($p < .05$, $q = 3.49$, $df = 2, 24$) more homotypic appositions in the prepartum group than

heterotypic appositions.

Table 2. Homotypic vs heterotypic direct appositions

	Mean % Appositions	
	Homotypic	Heterotypic
Control	11 ± 3.0	6 ± 3.0
Prepartum	* 22 ± 3.0	12 ± 3.0
Postpartum	24 ± 3.0	17 ± 3.0
Lactating	22 ± 3.0	19 ± 3.0

*= different from corresponding % heterotypic appositions ($p<.05$).

Double synapses and bundled dendrites.

Collecting data on these items proved to be, if not impossible, then foolhardy. Whether due to the absence of counterstain, hence contrast, or other processes employed for ICC, only six double synapses were seen in the immunostained tissue- one of these was contacting one cell body and one unstained dendrite. Two double synapses were between cells both immunostained with anti-OX-NP, two were between two cells stained with anti-VP-NP, and one was between two cells which were negative for anti-VP-NP. Similarly, dendritic peptide content was only discernIble in those dendrites containing DCVs. Further, without counterstain it too often became an intuitive exercise determining if dendritic membranes were indeed apposed.

Part III- Morphometry of postweaning group (Lead citrate - uranyl acetate stained tissue).

Somatic appositions (Figure 3).

A total of 246 ($\bar{X}$ ± S.E.M./animal =35.1 ± 2.56) and 332 ($\bar{X}$=47.4 ± 3.14) cell bodies were examined for direct apposition in control (N = 7; same as used in MB comparison) and postweaning (N = 7) animals each. No significant differences were found on this measure between the postweaning group and controls. The mean ± S.E.M. percentages of cell bodies in soma-somatic apposition were 29.7 ± 4.5% (postweaning) and 28.9 ± 4.0% (controls).

Percentage of somata contacted by double synapses (Figure 3).

No statistically significant differences were found in the percentage of SON somata contacted by: 1) double synapses which contacted two somata ($\bar{X}$ ± S.E.M., postweaning vs control, = 6.54 ± 2.12%; 2.54 ± 1.52%) or 2) double synapses which contact one soma and one dendrite (5.54 ± .99%; 5.00 ± 1.29%, or 3) when values for the two kinds of double synapses were combined.

Dendritic bundling (Figure 4).

Dendritic bundles containing up to 14 dendrites were observed in tissue from the postweaning group. Analysis of overall differences between groups was performed as for the maternal behavior experiment. The average number of dendrites in bundles of 1-4 dendrites, 5-8 dendrites, and in bundles containing 9 or more dendrites was compared for the

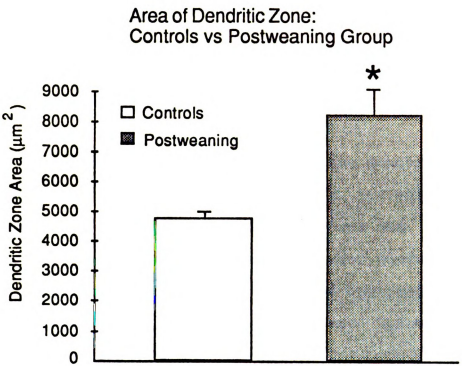
two groups with a two-factor ANOVA. The average number of dendrites in the three categories of bundle size was not found to be significantly greater than controls in the postweaning group ($F=4.10$, $df= 1, 12$, $p>.05$). Neither were differences found on the measure of percentage of dendritic membrane in apposition ($t=.965$, $df=12$, $p>.05$). Means and standard errors for controls and postweaning animals were 13.63 ± 1.0 and 12.11 ± 1.0 respectively.

Number of dendrites observed (Figure 15).

An average ($\pm$ S.E.M.) of 849 ± 107 versus 511 ± 39.6 dendrites were counted per two thin sections per animal in the dendritic zones of postweaning and control animals, respectively. A statistical comparison for data with heterogeneous variance revealed this difference to be significant (Mann-Whitney U, $U = 6$, $p<.02$).

Area of dendritic zone (Figure 15).

Due to heterogeneous variance a median test was used to determine that significantly more micrographs of the dendritic zone were obtained from tissue from postweaning rats ($p<.0003$). An average of 5.71 versus 9.85 micrographs were obtained from controls versus the postweaning group. When interpreted in terms of area sampled, a mean area of 4774 ± 220 μm^2 versus 8235.15 ± 642.53 μm^2 was occupied by the dendritic zones per two thin sections per animal in control and postweaning animals respectively. This difference was found to be significant ($U = 0$, $p<.001$) when tested with a Mann-Whitney U test. Data gathered with the aid of the digitizing tablet revealed that the area of


Area of Dendritic Zone:
Controls vs Postweaning Group
Dendritic Zone Area (μm²)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Controls
Postweaning
*

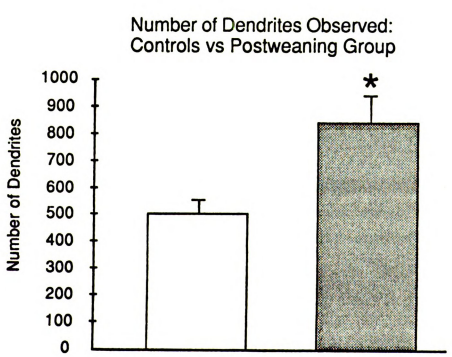

Number of Dendrites Observed:
Controls vs Postweaning Group
Number of Dendrites
1000
900
800
700
600
500
400
300
200
100
0
*

Figure 15.

Figure 15. Top: Differences in the area of the dendritic zone of postweaning and control group animals. *p<.001, Mann-Whitney U test. S.E.M.s= 220 (controls) and 642 (postweaning group). Bottom: Differences between postweaning and control groups in the number of dendrites observed in the dendritic zone. *p<.02; Mann-Whitney U test. S.E.M.s= 39.6 (controls) and 107 (postweaning group).

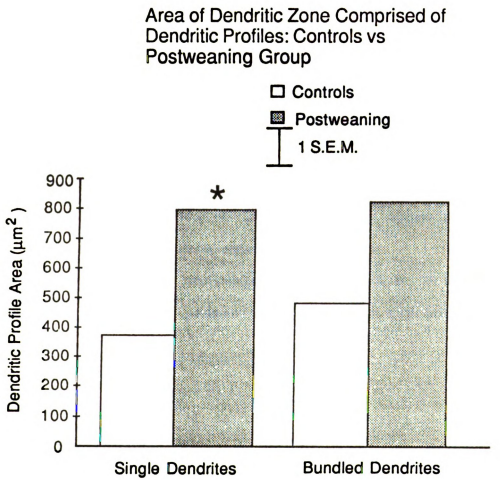


Figure 16. Differences between postweaning and control groups in the area of dendritic profiles. $p<.05$, Tukey's test. S.E.M.= 122.

dendritic profiles was significantly greater than controls in the postweaning group. ($F=15.78$, $df=1, 12$, $p<.002$; Figure 16). Post-hoc comparisons (Tukey's test) showed that a significantly larger area was comprised of single dendrites, whereas no differences were found in the area covered by bundled dendrites ($q= 4.37$, $df= 2, 12$, $p<.05$). The mean area ($\pm$ S.E.M., μm^2) of the dendritic zone devoted to dendritic profiles was 357 $\pm$ 122 (single controls), 462 $\pm$ 122 (bundled controls), 793 $\pm$ 122 (single postweaning), and 818 $\pm$ 122 (bundled dendrites).

Double synapses: dendritic zone (Figures 4 & 5).

The percentage of dendrites in postweaning animals which were contacted by double synapses was not significantly greater than controls (Means $\pm$ S.E.M. = 2.50 $\pm$ 0.48% versus 1.21 $\pm$ 0.30 respectively; $p>.05$, $t=2.081$, $df= 12$).

Discussion

Part I. Morphological correlates of induced maternal behavior.

Once again the SON has displayed a dissociation between events at the cell body level versus the dendritic level (Hatton and Tweedle, 1982; Perlmutter et al., 1984a). While no changes were found in the extent of direct apposition in the somatic region concurrent with the induction of maternal behavior, the stimulus or stimulus complex which elicits such behavior appears to have promoted substantial changes in the dendritic zone. The data presented here which demonstrate that changes occur in SON in virgin animals in which maternal behavior has been induced, suggest a role for the cells of the SON which goes beyond contributing to the well established "reflexive" hormonal events surrounding motherhood in the rat such as the release of hormones at parturition (Fuchs and Saito, 1971) or the milk ejection reflex (Wakerley et al., 1973). The correlation of ultrastructural changes in the SON with a complex behavior in this species suggests that a hormonal mechanism may underlie the expression of such behavior as well as constituting a novel demonstration of neural plasticity in adult animals.

The question arises: what stimulus or stimulus complex was responsible, either directly or indirectly, for the observed changes in the dendritic regions of the SON? The

necessary conditions for the establishment of maternal (or paternal) behavior in rodents has been well studied (Beach, 1956 a & b; Benuck and Rowe, 1975). It is known that virgin female rats, (usually ovariectomized and receiving estrogen replacement therapy) can become maternal in the absence of olfactory and visual cues (Herrenkohl and Rosenberg, 1972; Rosenblatt, 1979). It appears that the only absolutely necessary condition for the induction of maternal behavior is that the "mother" have actual physical contact with the pups. Because of this, it has been thought that some substance is transferred from the pups to the induced-behaver upon contact, perhaps pheromones in the urine. In this study it was noticed that quite often anogenital licking preceded the occurrence of full-blown maternal behavior by several days. At this time, the actual stimuli for induction of maternal behavior remains a mystery. Also, it should not be assumed that the stimuli which lead to maternal behavior are exactly the same as those leading to morphological changes. In this study control animals were housed predominantly (except for two days prior to sacrifice) in a separate room away from the nursery, i.e., away from non-contact kinds of pup stimuli such as ultrasounds for example. The possibility exists therefore, that merely being housed in the same room as pups could lead to dendritic bundling. There is some evidence that this is not the case, however. Most notable are the data from the postweaning group (which will be discussed at greater length in Part III of this discussion) which

indicated that the extent of bundling was not different from controls. This group, obviously, had been extensively exposed to both contact and non-contact pup stimuli in addition to the morphometric analysis for dendritic bundling for this group being identical to that performed for the MB group. Nonetheless, in light of these issues, it is hoped that further studies will be undertaken to more precisely delineate those factors responsible for the morphological changes observed in the MB group in this study. One interesting manipulation would be to examine the SON ultrastructure of virgin rats who had been exposed to pups but who had failed to display maternal behavior.

Assuming that the observed changes in SON are indeed related to the expression of maternal behavior, it is also unknown whether the SON is a primary mediator of maternal behavior. Given that this study represents the first time that SON has been associated with maternal behavior the more conservative interpretation is that it receives afferent input from another region which is firmly established as promoting maternal behavior, the medial preoptic nucleus (Fahrbach and Pfaff, 1982; Conrad and Pfaff, 1976). This notion gains credence when the necessity for estrogen in the production of maternal behavior is considered. Estrogen-priming is essential for eliciting of maternal behavior in ovariectomized virgin female rats and greatly reduces the latency to onset of the behavior in intact females. The number of receptors for this steroid is far greater in the medial preoptic area as compared to the SON

(Sar and Stumpf, 1975)

Comparison of SON bundles versus other CNS bundles.

The phenomenon of dendritic bundling (see Roney, Sheibel, and Shaw, 1979, for review) has been reported in the literature since 1970. Bundles have been variously noted in the spinal cord (Anderson et al., 1976), brainstem (Felten, 1977), cerebral cortex (Peters and Walsh, 1972), nucleus reticularis thalami (Scheibel and Scheibel, 1972), habenular nucleus (Iwahori, 1977), and olfactory bulb (Scheibel and Scheibel, 1975). Some investigators have included in bundles dendrites which are as much as 1 μm apart although that literature seems to be based primarily on light microscopic work. In the present study only dendrites whose membranes appeared to be in direct apposition were counted as being a part of any one bundle. The number of dendrites in a bundle in the CNS varies quite widely, from 2-3 dendrites/ bundle in layer five of the visual cortex of the cat (Roney et al., 1979) to as many as 1200-1600 in motorneurons in the ventral horn of the rat (Anderson et al., 1976). The extent of the bundling seen amongst the dendrites of SON neurons (2-14 dendrites/ bundle in this dissertation) is similar to that reported for many regions of somatosensory cortex in a wide range of species (Roney et al., 1979). However, functional comparisons of the two regions based on extent of bundling would be undoubtedly a foolhardy exercise at this juncture. Another feature of bundling elsewhere in the CNS is an apparently regular occurrence of bundles within any one region

(Scheibel et al., 1975). This certainly did not seem to be the case in SON where bundling seemed to occur randomly in the total population of dendrites. Finally, one feature of SON bundling which, so far, appears to be indisputably unique (to my knowledge) in the literature is that the extent of the bundling is plastic, varying over physiological (Perlmutter et al., 1984) and now behavioral states.

Changes in the size of the dendritic field.

The finding of significantly more dendrites and a larger dendritic profile area in the dendritic fields of maternal behavers was unexpected. At this juncture it is unknown whether the increased number of profiles represent the formation of new primary dendrites or the branching of already existing dendrites. Given the distance of the dendritic zone from the somatic zone, the latter explanation seems more likely. On first inspection it might appear that the finding of more dendrites was an artifact created by simply taking more micrographs. This is unlikely since the total area of the dendritic zone was sampled in each group and the criteria for identifying dendrites in this region held the same for both groups. The source of these apparently new dendrites could be one of the following: 1) sprouting of new dendritic branches from already existing dendrites; 2) an ingrowth of dendrites from a population of dendrites existing outside of the SON; or 3) an ingrowth of dendrites from proliferating neurons in SON. The third possibility can be rejected on the grounds that there are no

data which indicate that neurons in SON proliferate in adult animals, even with the most robust stimulation, prolonged dehydration for example. The second possibility may be a bit more reasonable than the third: however Golgi studies of this region indicate that all the dendrites present ventral to SON emanate from SON neurons (Armstrong et al., 1982). A development of new dendritic branches seems the most reasonable explanation for the increased number of dendrites in the SON of maternal behavers. Such a phenomenon is not without precedence in other parts of the nervous system. Buell and Coleman (1981) using a Van der Loos modification of the Golgi method have shown that in the parahippocampal gyrus, normal aged humans had longer and more branched terminal apical dendrites than did younger adults or adults with senile dementia. More related to the issues of plasticity, Rutledge et al., (1974) found that long term electrical stimulation of cortex in cats (thought to represent increased neuronal activity), led to a greater branching of the apical dendrites of contralateral cortical pyramidal cells. With respect to the maternal behavers examined in this study, it does not seem too radical to say that something in these animals' environments ultimately influenced their brain anatomy. One is reminded of the early "enriched environment" work of Bennett, Diamond, Krech, and Rosenzweig (1964; to cite but one of their published reports) who found that placing toys in the cages of rats resulted in morphological and biochemical changes in cortex, but not in "rest of brain" (they had their

priorities). This line of research was extended to include effects of an enriched environment on dendritic morphology. Uylings et al., (1978) found that an enriched environment consisting of toys being placed in rats' cages for thirty days resulted in increased branching of basal terminal dendrites of visual cortex pyramidal neurons. The addition of pups into the cages of the maternal behavers could conceivably be viewed as enriching the environments of these rats. In the case of normal mother rats, it would be particularly efficient if the appearance of pups into their environments led to an enhanced capacity of SON neurons to respond to incoming signals from both the environment as well as other brain regions.

It should be noted that the increased size of the dendritic region, as determined from the area photographed, is likely not entirely due to a greater number of dendrites. While an effort was made during sampling to include as many dendrites as possible in each micrograph, astrocytes and endothelial cells were also present in the micrographs and it is possible that the number and/or area of these cells increased also. Murray (1968) and Paterson and Le Blond (1977) have both made observations suggesting that dehydration is accompanied by astrocytic and endothelial cell proliferation in SON, at least in young animals. Although the validity of these results has been disputed (G. I. Hatton, personal communication) such a proliferation might account for part of the increase in size of the dendritic zone observed in this study.

If, in fact, an increase in the extent of SON dendritic arborization does occur in response to activation of cells in that nucleus, what biological purpose could it serve? One obvious possibility is that it serves to expand the area of the neuron which is receptive to afferent input, thus perhaps rendering the cell more responsive during times of increased hormone demand. A second possibility is that the new dendrites participate in bundles with dendrites of other neurons, thereby coordinating the activity of many cells. This notion receives some support from the finding that the area of the dendritic zone covered by single dendrites and the average area/dendritic profile did not differ for controls and MBs, the "extra" dendrites apparently being destined for bundles. If the addition of new dendrites, or dendritic branches, were purely a random event, one would expect to find a generalized increase in area for both single and bundled dendrites. For the present, the purpose of more dendrites in SON during activated states remains an enigma.

Recent work defining the nature of the stimuli which may produce morphological changes in SON has dichotomized experimental manipulations into chronic (lactation or 10 days of 2% saline substitution for drinking water) and acute (4-24 hrs water deprivation) categories. Apparently the formation of double synapses results from chronic stimulation, whereas dendritic bundling and somatic appositions occur rapidly in response to short term dehydration (Hatton et al., 1984; Perlmutter et al., 1984b)

as well as chronic stimulation conditions (Perlmutter et al., 1984a & c). Based on these findings the stimulus associated with the induction of maternal behavior might be considered to fall into the acute category, given that there were no changes found in the number of double synapses either in the dendritic or the somatic region. Since the animals used in this study were sacrificed after 3 days of exhibiting maternal behavior it might be interesting to see if the number of double synapses would have increased had the animals been behaving maternally for a longer period. Such results also once again raise the question of what exactly is the stimulus which promotes maternal behavior. Simple exposure to pups might be rejected as an explanation because the experimental subjects were exposed to pups for up to 19 days prior to sacrifice - hardly an "acute" situation. It may be that the "acute" verses "chronic" stimulus dichotomy is not always sufficient to predict the morphological changes which will occur in an "activated" SON.

Morphological changes in the posterior pituitary.

The finding of morphological changes in the posterior pituitaries of maternal behavers was rather suprising. Previous reports of reduced axonal enclosure by pituicytes have been of dehydrated (Tweedle and Hatton, 1980) post-partum (Tweedle and Hatton, 1982) or osmotically stimulated pituicytes in vitro (Perlmutter et al., 1984d). These conditions are, or mimic, states when neurohypophysial hormones are released into the general circulation. A

reduction in axonal enclosure by pituicytes at these times is thought to allow release of hormone from the axon into the fenestrated capillaries of the pituitary. The biological significance of similar changes in the pituitaries of maternal behavers is hard to fathom, as it was supposed that any influence of SON neurons on the production of maternal behavior would likely be exerted centrally, via recently described collateral projections to the nearby lateral hypothalamus (Mason et al., 1984). Interesting data relating to this issue comes from Jakubowski and Terkel (1980) who claimed that maternal virgin rats were seen to lactate after 14-18 days of exhibiting maternal behaviors. In this study, subjects were sacrificed on the third day of exhibiting maternal behavior, too short of an interval to induce lactation, if such is indeed possible. One could entertain the notion that the changes seen in the posterior pituitary in this study were harbingers of more profound changes to come. It is hoped that further studies will examine such phenomena. Finally, as is the case with those morphological changes seen in the SON, these data do not address the question of which peptidergic cell types undergo changes under the conditions of this study. Preliminary examination of ICC-treated material have yielded equivocal results, largely due to a failure to produce enough adequately stained tissue for sampling. While the data of Pedersen et al., (1982) indicate that oxytocin is a far more potent elicitor of maternal behavior than vasopressin, the latter peptide was

not without some effect. Therefore, the possibility remains that both peptidergic cell types participate in the changes seen in the SON dendritic zone and pituitaries of maternal behavers.

Part II. Immunoelectron microscopic correlation of morphology and peptide content of SON neurons in virgin, prepartum, postpartum and lactating animals.

Almost 50% of the cells examined in this study were found to be immunopositive for OX-NP while a somewhat more conservative (45%) estimation of NP-VP staining was obtained. Based on previous work which indicates that there are fairly even numbers of oxytocinergic and vasopressinergic cells (Swaab et al., 1975a) at the middle third of the SON which was sampled, and the specific staining produced on serial sections, it appears that a consistent and accurate estimation of peptide content was obtained.

The results of the analysis of the peptide content of apposed neurons as a function of reproductive state point to a selective formation of direct appositions of oxytocinergic cells immediately after parturition (Table 1.). Assuming that such direct appositions reflect functional demands made upon the cells and in regards to oxytocin at least, these data coincide well with those of Fuchs and Saito, (1971; rats) and Fuchs and Dawood (1980; rabbits) who reported a significant release of oxytocin at parturition, presumably to promote smooth muscle contractions of the uterus during

the birth process. The question arises as to what purpose direct appositions between oxytocinergic cells could serve at this time. One hypothesis is that the resulting reduction in intercellular space could promote ephaptic interactions between these neurons and/or result in an increased extracellular K+ concentration, thereby increasing overall cellular excitability. Another possibility is that close appositions would allow the formation of gap junctions between these neurons (Andrew et. al., 1981) again promoting a coordinated activity of many neurons which could result in a massive release of oxytocin at parturition (Poulain and Wakerley, 1982).

The finding that homotypic appositions were significantly higher than heterotypic appositions in the prepartum group, in one respect, is not surprising given that like-hormone containing cells tend to be grouped together in the SON. However, because no differences were found for this group, relative to controls, in the percentage of cells in direct apposition the biological significance of such events is doubtful.

The percentage of cells seen in apposition in ICC treated control sections (20%) was lower than that seen in controls under the conditions employed in Part I (29%), i.e., examination of micrographs of tissue stained with lead citrate and uranyl acetate. One likely explanation for this is that in the absence of counterstain in the ICC treated material, that "spot" (or tiny) appositions, commonly observed in the counterstained material, were less likely to

be identified. The estimation of apposition in the control group in ICC treated tissue (20%) was considerably higher however, than that found by Hatton and Tweedle (1982; 3.5%) and somewhat higher than that reported by Theodosis et al., 1981; 12%) for virgin female rats when somatic dendritic appositions were included in the sample. The reproductive state producing maximal somatic appositions in this study (postpartum) was also different from that found by Hatton and Tweedle, (1982) who found maximal apposition with 14 days of lactation. Both results are at variance with the work of Theodosis and Poulain (1984b) who found maximal apposition in prepartum animals- which was equivalent to that seen in lactating animals (they did not examine an immediately postpartum group. These differences are hard to reconcile, but several factors should be considered. First, the area sampled in this study was from the mid-portion of the SON, where the population of oxytocinergic and vasopressinergic cells is roughly split 50-50 (Swaab et al., 1975; 1976) whereas the sections sampled in the other three studies were apparently obtained more randomly from throughout the SON. Given the somewhat segregated distribution of the two cell types in the SON where oxytocinergic cells are found dorsally and anteriorly and vasopressinergic cells are found more ventrally and posteriorly, it is conceivable that different populations of cells were sampled in the four studies. A second factor worth considering is the time of sacrifice of the animals. In the study of Hatton and Tweedle (1982) animals were

sacrificed ~2-4 hours after lights-off whereas in this study subjects were sacrificed 2-4 hours after lights-on. It is possible that the interaction of diurnal fluctuations in hormone release, particularly in the case of vasopressin, could interact with factors controlling the release of hormones during lactation to result in the overall higher values for neuronal apposition during lactation seen by Hatton and Tweedle (1982).

The failure to obtain data regarding either dendritic bundling or double synapse formation across the reproductive cycle is unfortunate. In the case of dendritic bundles, although many stained profiles were seen, only those containing dcvs could be identified as such and their numbers were small in comparison to those not containing dcvs. It is possible that the best hope for identifying the peptide content of bundled dendrites is the far-from-perfect and intensively laborious preembedding technique. Such an attempt would require large amounts of processed tissue, but would result in reaction product being deposited in dendritic profiles whether they had dcvs or not. Theodosis and Poulain (1984a) used this technique to show that oxytocinergic dendrites and somata form direct appositions with other immunopositive, as well as immunonegative, dendrites and somata. Further, they identified a double synapse contacting an immunoreactive dendrite and somata. Judging by the brevity of their report however, a quantitative analysis of such tissue would require much time and effort. Alternatively, the post-embedding technique,

coupled with colchicine injections which might prevent the transport of dcvs out of cell bodies and dendrites, could possibly solve the mystery of who is bundling with whom. With respect to double synapses, none of those unequivocally identified were found contacting one stained and one unstained cell body, suggesting at least that such synapses do not contact different hormone containing cells. While not constituting an adequate sample, these findings are consistent with the data of Perlmutter (1984d) who managed to find 19 double synapses in her immunoelectron microscopic study of the SON of dehydrated rats.

Part III. Postweaning group morphometry.

The portrait of the SON which was obtained by morphological measures of postweaning group tissue is, with one major exception, one of a nucleus not significantly different from controls. For all of the originally proposed measures, i.e., somatic direct apposition, dendritic bundling, percentage of somatic or dendritic membrane in direct apposition, and the number of somata or dendrites contacted by double synapses, there were no differences detected relative to controls. One could almost make the argument that nothing much in the way of morphological changes seemed to have occurred here were it not for one observation: an average of roughly 300 more dendrites/ animal existed in the samples of the dendritic zones of the postweaning animals examined. As these numbers were gathered by counting the number of dendrites on two thin

sections in the mid-portion of SON, it is unknown whether they merely hint at a far greater change which occurred throughout the nucleus or if such changes were specific to that part of the nucleus. Further, the size of the entire dendritic area, including dendrites, astrocytes and endothelial cells, was seen to be an average of 3500 um^2 larger then the comparable area in controls, for the two thin sections sampled in the postweaning group. Like the dendritic zones of the maternal behavers discussed earlier, the total area of dendritic profiles was significantly greater in this group also. However, as might be expected, since no differences were found in the extent of dendritic bundling, the difference in dendritic profile area was localized to single dendrites.

Comparisons of data for the postweaning group obtained in this study with that found by Theodosis and Poulain (1984b) show that, while the absolute values of measures differed, they too found no significant differences from controls on any of the variables measured. Combined postweaning group values for double synapses contacting both somata and dendrites was some 4.5% in this study vs 2.8% in theirs. On the measure of percentage of somatic membrane in apposition Theodosis and Poulain found that at one month post-weaning, this measure was 0.4% vs 0.7% (controls and postweaning respectively) whereas in this study at ten days postweaning the values were 5% and 4%. Finally, they found values of 3% vs 2%, (control vs postweaning groups) on the measure of dendritic membrane in apposition as compared to

values of 13.6% vs 12.1% for the same variables measured in this study.

The data presented here confirm the reversibility of the somatic, dendritic, and synaptic changes that have been shown previously to occur in the SON with gestation, parturition and lactation. Further, the data indicate that morphological traces of the maternal experience disappear in a fairly short time, i.e., within 10 days postweaning. This is in contrast to the results of Perlmutter et al., (1984c) who investigated the reversibility of dendritic bundling in response to dehydration. They found that even 14 days of rehydration was insufficient to decrease dendritic bundling to control levels. It thus appears that, in the dendritic zone but not the somatic zone that the stimulus of dehydration produces more long-lasting effects than does gestation and lactation. Changes which have not been previously noted to occur with maternity are the rather profound differences seen in this study in the dendritic zone: the formation of new dendrites or increased branching of already existing dendrites as well as an overall increase in the size of the dendritic zone which may include astrocytic and endothelial cell changes. Some of the biological implications of these phenomena have already been discussed in relation to similar changes in the dendritic zone of maternal behavers and will not be reiterated here. In relation to the pregnancy "cycle" however, it cannot be automatically assumed that these dendritic changes are evident during gestation, parturition or lactation. It

would be interesting therefore to examine the SON during these states using light microscopic techniques such as Golgi staining or Golgi-like immunostaining (Sofroniew and Glassmann (1981) to determine whether such changes occur. The latter technique, of course, would have the advantage of being able to identify the peptide content of any changing dendrites.

General Discussion.

The values obtained for the control group used in this dissertation were consistently elevated when compared to those data reported by Theodosis et. al.(1981), Hatton and Tweedle (1982) and Theodosis and Poulain (1984b). One factor which could be responsible for this is the stage of the estrous cycle at which the animals were sacrificed, as steroid hormone levels have been shown to influence vasopressin release (Skowsky et al., 1979). However, when data for maternal behavers and control animals were combined (N= 14) no significant correlation could be found between the stage of the estrous cycle and either somatic appositions or dendritic bundling.

A more plausible reason for the seemingly elevated control values might be that the time of sacrifice of the group used in this study was ~ 12 hours out of synchrony with the time of sacrifice of the control group of Hatton and Tweedle (1982). Unfortunately this information is unavailable for the work of Theodosis and colleagues. While animals in this study were sacrificed 2-4 hours after the onset of the "lights-on" portion of the 12:12 hr light

cycle, Hatton and Tweedle sacrificed their control group 2-4 hours after "lights-off" (they had their animals on a reversed light cycle). The data of Schwartz et al., (1983) and Reppert et al.,(1983) showed that a diurnal fluctuation of vasopressin exists in the cerebrospinal fluid (CSF) of rats and that peak concentrations of the hormone occur just prior to "lights-on" and persist to about hour 6 of "lights-on". This is in contrast to the baseline levels observed during almost the entire "lights-off" period. While the source of neurohypophysial hormones in the CSF is still a mystery, it does suggest that magnocellular neurons are influenced by the light cycle. Some evidence also exists for diurnal fluctuations of plasma vasopressin with high values found four hours after "lights-on" (Mohring and Mohring, 1975). Armstrong and Hatton (1978) also found that multiple nucleoli were most numerous at the end of the daylight hours, an indication of increased metabolic activity. If diurnal fluctuation is responsible for the elevated control values then one more stimulus complex can be added to the growing list of stimuli which influence SON morphology. It may be that merely a few hours difference in the course of a diurnal cycle are necessary to effect morphological changes in SON. (What a difference a day makes!). That morphological changes, at least in the SON somatic region, can occur with such rapidity has been demonstrated by Tweedle and Hatton (1977). It would therefore be interesting to determine if the morphological changes described here and by others can be produced by

manipulations of the light-dark cycle.

LIST OF REFERENCES

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Andrew, R.D., MacVicar, B., Dudek, F.E. & Hatton, G.I. (1981) Dye transfer through gap junctions between neuroendocrine cells of the rat hypothalamus. Science 211, 1187-1189.

Armstrong, W.E. & Hatton, G.I. (1978) Morphological changes in the rat supraoptic and paraventricular nuclei during the diurnal cycle. Brain Res. 157, 407-413.

Armstrong, W.E., Scholer, J. & McNeill, T.H. (1982) Immunocytochemical, Golgi and electron microscopic characterization of putative dendrites in the ventral glial lamina of the rat supraoptic nucleus. Neuroscience 7, 679-694.

Anderson, W.J., Stromberg, M.W. and Hinsman, E. J. (1976) Morphological characteristics of dendritic bundles in the lumbar spinal cord of the rat. Brain Res. 110: 215-227.

Beach, F. and Jaynes, J. (1956) Studies on maternal retrieving in rats, I. Recognition of young. J. Mammology. 37:177-180.

Beach, F. and Jaynes, J. (1956) Studies of maternal retrieving in rats, III: Sensory cues involved in the lactating female's response to her young. Behavior. 10: 104-125.

Bennett, E.L., Diamond, M.C., Krech, D. and Rosenzweig, M.R. (1964) Chemical and anatomical plasticity of brain. Science. 146:610-619.

Ben-Barak, Y., Russell, J.T., Whitnall, M.H., Ozato, K. & Gainer, H. (1985) Neurophysin in the hypothalamo-neurohypophysial system I. Production and characterization of monoclonal antibodies. J. Neurosci. 5: 81-97.

Benuck, I. and Rowe, F.A. (1975) Centrally and peripherally induced anosmia: Influences on maternal behavior in lactating female rats. Physiology and Behavior. 14: 439-447.

Buell, S.J. and Coleman, P.D. (1981) Quantitative evidence for selective dendritic growth in normal human aging but not in senile dementia. Brain Res. 214: 23-41.

Brimble, M.J. and Dyball, R.E.J. (1977) Characterization of the responses of oxytocin- and vasopressin-secreting neurons in the supraoptic nucleus to osmotic stimulation. J. Physiol. 271:253-271.

Conrad L.C.A., and Pfaff, D.W. (1976) Efferents from the medial basal forebrain and hypothalamus. I. An autoradiographic study of the medial preoptic area. J. Comp. Neurol. 169: 185-220.

Fahrbach, S.E., and Pfaff, D.W. (1982) Hormonal and neural mechanisms underlying maternal behavior in the rat. In: The Physiological Mechanisms of Motivation, D. W. Pfaff, Ed. Springer-Verlag, New York.

Fahrbach, S.E., Morrell, J.I., and Pfaff, D.W. (1984) Oxytocin induction of short-latency maternal behavior in nulliparous, estrogen-primed female rats. Hormones and Behav. 18: 267-286.

Felten D.L. (1977) Dendritic monoamines in the squirrel monkey brain. Brain Res. 120: 553-558.

Fuchs, A.-R. and Dawood, M.Y. (1980) Oxytocin release and uterine activity during parturition in rabbits.

Fuchs, A.-R. and Saito, S. (1971) Pituitary oxytocin and vasopressin content of pregnant rats before during and after parturition. Endocrinol. 88: 574- 578.

Gill, J. (1978) Design and analysis of experiments in the animal and medical sciences. Vol. 1. The Iowa State University Press. Ames, 180-182.

Hatton, G.I., Perlmutter, L.S., Salm, A.K. & Tweedle, C.D. (1984) Dynamic neuronal glial interactions in hypothalamus and pituitary: implications for control of hormone synthesis and release. Peptides 5 (Suppl 1): 121-138.

Hatton, G.I. & Tweedle, C.D. (1982) Magnocellular neuropeptidergic neurons in hypothalamus: Increases in membrane apposition and number of specialized synapses from pregnancy to lactation. Brain Res. Bull. 8: 197-204.

Herrenkohl, R.L. and Rosenberg, P.A. (1972) Exteroceptive stimulation of maternal behavior in the naive rat. Physiology and Behavior. 8:595-598.

Iwahori, N. (1977) A Golgi study on the habenular nucleus of the cat. J. Comp. Neurol. 171:319-344.

Jakubowski, M. and Terkel, J. (1980) Induction by young of

prolonged dioestrus in virgin rats behaving maternally. J. Reprod. Fert. 58: 55-60.

Martin, C. (1979) Endocrine Physiology. Oxford University Press. New York. p294.

Mason, W.T., Ho, Y.W. and Hatton, G.I. (1984) Axon collaterals of supraoptic neurons: anatomical and electrophysiological evidence for their existence in the lateral hypothalamus. Neuroscience, 11: 169-182.

Mohring, B. and Mohring, J. (1975) Plasma ADH in normal Long-Evans rats and in Long-Evans rats heterozygous and homozygous for hypothalamic diabetes insipidus. Life Sci. 17:1307-1314.

Murray, M. (1968) Effects of dehydration on the rate of proliferation of hypothalamic neuroglial cells. Exp. Neurol. 20:460-468.

Numan, M. (1984) Preoptic projections to the paraventricular hypothalamic nucleus are not essential for the normal display of maternal behavior in post-partum lactating rats. Conference on Reproductive Behavior Absts. pg. 77.

Paterson, J.A., and C.P. LeBlond (1977) Increased proliferation of neuroglia and endothelia cells in the supraoptic nucleus and hypophysial neural lobe of young rats drinking hypertonic sodium chloride solution. J. Comp. Neurol. 175:373-390.

Pedersen, C.A., Ascher, J.A., Monroe, Y.L., and Prange, A.J. Jr. (1982) Oxytocin induces maternal behavior in virgin female rats. Science, 216: 648-650.

Pedersen, C.A., Caldwell, J.D., and Prange, A.J.Jr. (1984a) Oxytocin reverses novelty inhibition of onset of ovarian steroid-induced maternal behavior. Conference on Reproductive Behavior Absts. pg. 61.

Pedersen, C.A., Caldwell, J.D., and Prange, A.J.Jr. (1984b) Oxytocin antiserum blocks onset of ovarian steroid-induced maternal behavior: intracerebroventricular oxytocin promotes lordosis behavior. Conference on Reproductive Behavior Absts. pg. 16.

Perlmutter, L.S., Tweedle, C.D., & Hatton, G.I. (1984a) Neuronal/glial plasticity in the supraoptic dendritic zone: dendritic bundling and double synapse formation at parturition. Neuroscience, 13: 769 - 779.

Perlmutter, L.S., Tweedle, C. D., and Hatton, G. I. (1984b) Plasticity in supraoptic neurons: dendritic bundle

formation in response to short-term dehydration and rehydration. Anat. Rec. Vol.208: 136A-137A.

Perlmutter, L.S., Tweedle, C. D., and Hatton, G. I. (1984c) Plasticity in the supraoptic dendritic zone: dendritic bundling (but not double synapse) formation varies with chronic dehydration. Soc. Neurosci. Absts. Vol. 10: 976.

Perlmutter, L.S., Hatton, G. I., and Tweedle, C. D. (1984d) Plasticity in the in vitro neurohypophysis: effects of osmotic changes on pituicytes. Neuroscience. 12:503-511.

Perlmutter, L. S. (1984e) Plasticity and the supraoptic neuron: dynamic neuronal/glial interactions. Dissertation. Michigan State University.

Peters, A. and Walsh, T. M. (1972) A study of the organization of apical dendrites in the somatic sensory cortex of the rat. J. Comp. Neurol. 144: 2563-268.

Poulain, D.A. and Wakerley, J.B. (1982) Electrophysiology of hypothalamic magnocellular neurons secreting oxytocin and vasopressin. Neuroscience. &:773-808.

Reppert, S.M., Schwartz, W.J., Artman, H.G., and D.A. Fisher (1983) Comparison of the temporal profiles of vasopressin and oxytocin in the cerebrospinal fluid of the cat, monkey and rat. Brain Res. 261: 341-345.

Roney, K. J., Scheibel, A. and Shaw, G.L. (1979) Dendritic bundles: survey of anatomical experiments and physiological theories. Brain Res. Rev. 1: 225-271.

Rosenblatt, J.S., Siegel, H.I. and Mayer, A.D. (1979) Progress in the study of maternal behavior in the rat: Hormonal, nonhormonal, sensory, and developmental aspects. In J.S. Rosenblatt, R.A. Hinde, E. Shaw, and C. Beer (Eds.) Advances in the study of behavior (vol. 10). New York, Academic Press, 225-311.

Russell, J.T, Brownstein, M.J., and Gainer, H. Biosynthesis of vasopressin, oxytocin, and neurophysins: Isolation and characterization of two common precursors (propressophysin and prooxyphysin). Endocrinology. 107: 1880-1891.

Rutledge, L.T., Wright, C., and Duncan, J (1974) Morphological changes in pyramidal cells associated with increased use. Exp. Neurol. 44:209-228.

Sar, M. and Stumpf, W.E. (1975) Atlas of estrogen target cells in rat brain. In Anatomical Neuroendocrinology. Int Conf. Neurobiology of CNS-Hormone Interactions.

Karger-Basel, pp 104-119.

Scheibel, M.E., Lindsay, R.D., Tomiyasu, U. and Scheibel, A.B. (1975) Progressive dendritic changes in aging human cortex. Exp. Neurol. 47: 392-403.

Scheibel, M.E. and Scheibel, A.B. (1972) Specialized organizational patterns within the nucleus reticularis thalami of the cat. Exp. Neurol., 34:316-322.

Scheibel, M.E. and Scheibel, A.B. (1975) Dendrite bundles, central programs and the olfactory bulb. Brain Res. 95:407-421.

Schwartz, W.J., Coleman, R.J. and Reppert, S.M. (1983) A daily vasopressin rhythm in rat cerebrospinal fluid. Brain Res. 263: 105-112.

Siegel, S. (1956) Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York, pp 111 - 116.

Skowsky, R.W., Swan, L., and Smith, P. (1979) Effects of sex steroid hormones on arginine vasopressin in intact and castrated male and female rats. Endocrinol. 104:105.

Sofroniew, M.V. and Glassmann, W. (1981) Golgi-like immunoperoxidase staining of hypothalamic magnocellular neurons that contain vasopressin, oxytocin or neurophysin in the rat. Neuroscience. 6:619-643.

Swaab, D.F. Nijveldt, F., and Pool, C. W. (1975a). Distribution of oxytocin and vasopressin in the rat supraoptic and paraventricular nucleus. J. Endocrinol. 67: 461-462.

Swaab, D.F., C.W. Pool, and Nijveldt F. (1975b) Immunofluorescence of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system. J. Neural Trans., 36: 195-215.

Swanson, L.W. (1976) An autoradiographic study of the efferent connections of the preoptic region in the rat. J. Comp. Neurol. 167: 227-256.

Swanson, L.W. and Sawchenko, P.E. (1983) Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Ann. Rev. Neurosci., 6: 269-324.

Theodosis, D.T., Poulain, D.A. & Vincent, J.-D. (1981) Possible morphological bases for synchronisation of neuronal firing in the rat supraoptic nucleus during lactation. Neuroscience 6: 919-929.

Theodosis, D.T. & Poulain, D.A. (1984a) Evidence that

oxytocin-secreting neurones are involved in the ultrastructural reorganisation of the rat supraoptic nucleus apparent at lactation. Cell Tiss. Res. 235: 217-219.

Theodosis, D.T. & Poulain, D.A. (1984b) Evidence for structural plasticity in the supraoptic nucleus of the rat hypothalamus in relation to gestation and lactation. Neuroscience 11:183-194.

Tweedle, C.D. & Hatton, G.I. (1976) Ultrastructural comparisons of neurons of supraoptic and circularis nuclei in normal and dehydrated rats. Brain Res. Bull. 1: 103-121.

Tweedle, C.D. and Hatton, G.I. (1977) Ultrastructural changes in rat hypothalamic neurosecretory cells and their associated glia during minimal dehydration and rehydration. Cell Tiss. Res. 181:59-72.

Tweedle, C.D. and Hatton, G.I.(1980a) Evidence for dynamic interactions between pituicytes and neurosecretory axons in the rat. Neuroscience 5:661-667.

Tweedle, C.D. and Hatton, G.I. (1980b) Glial cell enclosure of neurosecretory endings in the neurohypophysis of the rat. Brain Res. 192: 555-559.

Tweedle, C.D. and Hatton, G.I. (1982) Magnocellular neuropeptidergic terminals in neurohypophysis: Rapid glial release of enclosed axons during parturition. Brain Res. Bull. 8:205-209.

Tweedle, C.D. & Hatton, G.I. (1984a) Synapse formation and disappearance in adult rat supraoptic nucleus during different hydration states. Brain Res. 309: 373-376.

Tweedle, C.D. & Hatton, G.I. (1984b) Ultrastructure of catecholaminergic synapses in the rat supraoptic nucleus. Soc. Neurosci. Abst. 10: 209.

Uylings, H.B.M., Kuypers, K., Diamond, M.C., and Veltman, W.A.M. (1978) Effects of differential environments on plasticity of dendrites of cortical pyramidal neurons in adult rats. Exp. Neurol. 62:658-677.

Wakerley, J.B., Dyball, R.E.J., and Lincoln, D.W. (1973) Milk ejection in the rat: the result of selective release of oxytocin. J. Endocrinol. 57: 557-558.

Whitnall, M.H., Ben-Barak, Y., Ozato, K. and Gainer, H. (1985) Neurophysin in the hypothalamo-neurohypophysial system. II. Immunocytochemical studies of the ontogeny of oxytocinergic and vasopressinergic neurons. J. Neurosci.

5:98-109

Yogev, L., Jakubowski, M. and Terkel, J. (1980) Two modes of prolactin secretion induced by young in maternally behaving maternal rats. Endocrinology, 107: 1808-1812.

APPENDIX A

KEY TO APPENDIX A

C = Control animal

MB = Maternal Behaver

PW = Postweaning animal

Pre = Prepartum animal

Pos = Postpartum animal

Lac = Lactating animal

Apps = appositions

App'd = apposed

Dbls = double synapses

Dend = dendro-

ICC = immunocytochemistry

MAB+ = immunostained cell

MAB- = nonimmunostained cell

APPENDIX A

Table 3. RAW DATA-SOMATA

CODE	# Cells Observed	% Cells App'd	% Membrane App'd
C1	42	21.42	6.20
C2	37	32.43	4.98
C3	44	52.27	7.94
C4	30	26.66	2.89
C5	38	28.94	4.93
C6	32	18.75	3.48
C8	23	21.73	4.69
MB1A	58	22.41	4.67
MB1	50	16.00	2.65
MB2	41	26.82	6.28
MB4	18	11.11	1.46
MB5	44	20.45	2.98
MB6	20	20.00	3.17
MB7	67	19.40	6.02
PW1	54	44.44	10.89
PW2	37	27.02	2.90
PW4	49	36.73	5.59
PW5	44	36.36	2.93
PW6	39	25.64	2.35
PW7	63	33.33	2.98
PW8	46	4.34	0.11

APPENDIX A

Table 4. RAW DATA- % SOMATA WITH DOUBLE SYNAPSES

CODE	Soma-somatic	Dend-Somatic	Combined Dbls.
C1	0.00	2.38	2.39
C2	0.00	10.81	10.81
C3	9.09	4.54	13.63
C4	0.00	3.33	3.33
C5	0.00	5.26	5.26
C6	0.00	0.00	0.00
C8	8.69	8.69	17.39
MB1A	3.44	12.06	15.51
MB1	4.00	2.00	6.00
MB2	9.75	2.43	12.19
MB4	0.00	0.00	0.00
MB5	0.00	0.00	0.00
MB6	0.00	0.00	0.00
MB7	2.98	0.00	2.98
PW1	11.11	3.70	14.81
PW2	0.00	8.10	8.10
PW4	16.32	2.04	18.36
PW5	4.54	2.27	6.82
PW6	0.00	7.69	7.69
PW7	9.52	6.34	15.87
PW8	4.34	8.69	13.04

APPENDIX A

Table 5. RAW DATA- DENDRITES

CODE	# DENDRITES	# MICROGRAPHS	% MEMBRANE APP
C1	536	4	11.96
C2	694	6	16.36
C3	604	6	11.64
C4	383	6	9.75
C5	500	6	12.04
C6	384	6	16.94
C8	475	6	16.83
MB1A	685	9	11.17
MB1	543	8	14.22
MB2	622	8	12.52
MB4	446	7	8.85
MB5	719	7	16.80
MB6	540	5	8.77
MB7	799	9	15.05
PW1	1264	14	9.87
PW2	635	11	8.94
PW4	537	8	15.82
PW5	507	8	10.16
PW6	977	10	13.20
PW7	838	8	10.64
PW8	1186	10	16.18

APPENDIX A

Table 6. RAW DATA- % DENDRITES RECEIVING DOUBLE SYNAPSES/ AREA OF DENDRITES IN BUNDLES AND SINGLES (μM^2).

CODE	% DBL SYNAPSES	BUNDLE AREA	SINGLES
C1	1.86	182.22	217.85
C2	1.72	516.90	562.63
C3	1.98	556.30	514.21
C4	0.00	337.07	334.11
C5	2.00	690.46	419.25
C6	.52	533.42	198.74
C8	.42	423.58	256.48
MB1A	.87	728.81	460.10
MB1	.73	1061.56	389.50
MB2	.90	770.11	569.79
MB4	.44	611.45	641.84
MB5	1.39	524.93	157.54
MB6	1.11	517.94	733.42
MB7	1.00	1280.58	667.14
PW1	2.37	1044.84	1377.17
PW2	1.25	859.44	925.91
PW4	4.84	735.93	478.65
PW5	.98	415.16	496.34
PW6	3.68	1549.65	614.17
PW7	1.67	927.52	745.48
PW8	2.69	1999.47	915.56

APPENDIX A

Table 7. RAW DATA-MEAN NUMBER OF DENDRITES IN BUNDLES OF 1-4, 5-8 AND >= 9

BUNDLE SIZE: CODE	1-4	5-8	>=9
C1	1.35	0.00	0.00
C2	1.34	5.50	0.00
C3	1.41	6.33	0.00
C4	1.32	5.00	0.00
C5	1.34	5.16	10.00
C6	1.41	5.33	0.00
C8	1.38	5.20	9.00
MB1A	1.36	5.83	12.00
MB1	1.45	6.33	9.00
MB2	1.33	6.00	12.00
MB4	1.33	5.50	0.00
MB5	1.39	5.75	11.00
MB6	1.38	0.00	12.00
MB7	1.41	6.15	9.00
PW1	1.37	5.35	0.00
PW2	1.32	6.00	0.00
PW4	1.38	5.75	12.00
PW5	1.40	5.50	9.00
PW6	1.56	5.76	10.83
PW7	1.33	5.50	9.50
PW8	1.51	5.63	10.66

APPENDIX A

Table 8. RAW DATA-NEUROHYPOPHYSIS: MEAN # ENCLOSED AXONS PER ANIMAL/ PER PITUICYTE.

CODE	AXONS PER PITUICYTE	AXONS PER ANIMAL
C1	.40	6
C2	.20	3
C3	.33	5
C4	.27	4
C5	.60	9
C6	.60	9
C7	.33	5
MB1A	.20	3
MB1	.20	3
MB2	.13	2
MB4	.07	1
MB5	.27	4
MB6	.27	4
MB7	.40	6

APPENDIX A

Table 9. RAW DATA-VAGINAL CYTOLOGY
STAGE OF ESTROUS CYCLE

CODE	
C1	METESTRUS
C2	METESTRUS
C3	ESTRUS
C4	PROESTRUS
C5	METESTRUS
C6	METESTRUS
C8	DIESTRUS
MB1A	METESTRUS
MB1	PROESTRUS
MB2	DIESTRUS
MB4	DIESTRUS
MB5	ESTRUS
MB6	PROESTRUS
MB7	PROESTRUS

APPENDIX A

Table 10. RAW DATA- ICC

	% of MAB+ Apposed		% of Control	
CODE	OX-NP	VP-NP	OX-NP	VP-NP
C1		13.15		128.66
C2		12.50		122.30
C4	10.52	0.00	106.58	0.00
C5	3.84	18.18	38.90	177.88
C6	17.39	0.00	176.19	0.00
C7	14.28	11.11	144.68	108.70
C8	3.33	16.66	33.73	163.01
Pre1		24.13		236.10
Pre2		25.00		244.61
Pre3	15.38	0.00	155.82	0.00
Pre4	6.25	0.00	63.33	0.00
Pre5	10.86	5.80	110.00	56.75
Pre6	0.00	9.09	0.00	88.94
Pre7	13.33	19.23	135.00	188.16
Pos1		25.00		244.61
Pos2		22.72		222.30
Pos3	20.00	8.33	202.63	81.50
Pos4	35.48	20.58	359.47	201.36
Pos5	23.33	3.20	236.37	31.31
Pos6	34.78	21.73	352.38	212.62
Pos7	25.00	28.57	253.29	279.54
Lac1		32.00		313.11
Lac2		23.50		229.94
Lac3	11.11	6.60	112.56	64.57
Lac4	12.50	35.00	126.64	342.46
Lac5	15.38	30.00	155.82	293.54
Lac6	16.27	16.66	164.84	163.01
Lac8	5.66	17.24	57.34	168.68

APPENDIX A

Table 11. RAW DATA- ICC APPOSITIONS

% MAB+ CODE	Heterotypic OX-NP	Apps. VP-NP	% MAB-	Homotypic. OX-NP	Apps. VP-NP
C1		13.15			13.15
C2		6.25			9.37
C4	0.00	0.00		0.00	0.00
C5	3.84	27.27		3.84	0.00
C6	0.00	0.00		0.00	16.66
C7	0.00	0.00		0.00	11.11
C8	6.66	16.66		6.66	16.66
Pre1		15.51			24.13
Pre2		25.00			12.50
Pre3	30.76	0.00		0.00	28.57
Pre4	0.00	0.00		6.25	57.14
Pre5	13.04	14.70		13.04	23.52
Pre6	0.00	18.18		20.00	27.27
Pre7	0.00	23.07		30.00	26.92
Pos1		31.25			12.50
Pos2		13.63			27.27
Pos3	13.33	0.00		13.33	33.33
Pos4	19.35	26.47		25.80	23.52
Pos5	20.00	6.40		23.33	19.35
Pos6	0.00	26.08		43.47	26.08
Pos7	14.58	26.57		31.25	14.28
Lac1		6.45			35.48
Lac2		20.58			29.41
Lac3	0.00	13.33		22.20	40.00
Lac4	25.00	30.00		0.00	30.00
Lac5	15.38	13.33		15.38	30.00
Lac6	32.55	33.33		9.30	12.50
Lac7	11.76	24.13		29.41	6.89

APPENDIX A

Table 12. RAW DATA- Maternal Behavior

Animal ID:MB-1

Day	Behaviors: Crouching	Anogenital Licking	Retrieving
1	0	0	0
2	0	0	0
3	1	0	0
4	1	0	0
5	15	0	0
6	12	1	1
7	16	3	1
8	5	3	1

Animal ID:MB-1A

Day	Behaviors: Crouching	Anogenital Licking	Retrieving
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	1	0	0
9	0	0	0
10	1	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0
16	-	-	-
17	1	1	1
18	1	0	0
19	1	1	1
20	-	-	-
21	1	1	1

APPENDIX A

Table 13. RAW DATA- Maternal Behavior

Animal ID:MB-2

Day	Behaviors: Crouching	Anogenital Licking	Retrieving
1	0	0	0
2	0	0	0
3	1	0	0
4	0	0	0
5	14	0	0
6	7	0	0
7	15	0	0
8	2	2	0
9	0	0	0
10	9	1	0
11	12	2	0
12	12	6	0
13	9	4	0
14	11	1	0
15	9	1	0
16	6	5	0
17	14	3	1
18	14	9	1
19	1	3	1

Animal ID:MB-5

Day	Behaviors: Crouching	Anogenital Licking	Retrieving
1	0	2	0
2	0	0	0
3	1	0	0
4	10	1	0
5	15	0	1
6	14	5	2
7	14	2	1
8	14	2	0
9	13	9	1
10	13	8	1
11	1	4	1

APPENDIX A

Table 14. RAW DATA- Maternal Behavior

Animal ID:MB-4

Day	Behaviors: Crouching	Anogenital Licking	Retrieving
1	0	0	0
2	0	0	0
3	1	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	11	2	0
9	1	0	0
10	1	1	0
11	1	0	0
12	6	2	0
13	1	0	0
14	0	0	0
15	1	2	0
16	3	0	1
17	7	5	1
18	12	4	1
19	2	4	3

Animal ID:MB-6

Day	Behaviors: Crouching	Anogenital Licking	Retrieving
1	0	0	0
2	0	1	0
3	1	0	0
4	3	2	0
5	15	2	0
6	13	5	1
7	15	4	1
8	1	2	2

APPENDIX A

Table 15. RAW DATA- Maternal Behavior

Animal ID:MB-7

	Behaviors		
	Crouching	Anogenital Licking	Retrieving
Day			
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	15	9	1
6	12	4	1
7	15	2	1
8	13	6	0
9	2	9	2
10	9	7	1
11	3	3	1

APPENDIX B

APPENDIX B

Procedure for L.R.White embedding

1. Chop tissue with tissue chopper.
2. "Core" tissue with a single edged razor blade. Expose tissue to the following solutions:
3. 1.0% osmium tetroxide in 0.15M cacodylate buffer- 1 hr
4. 30 % ETOH, 0.5 hr
5. 50 % ETOH, 0.5 hr
6. 70 % ETOH, 0.5 hr
7. 2:1, L.R.White : 70% ETOH solution, 0.5 hr
8. 100% L.R.White, 0.5 hr
9. 100% L.R.White, 1 hr.
10. Place tissue in bottom of gelatin capsules (size #00) and fill capsule with 100% L.R. White.
11. Polymerize in a 50 degree oven, 48 hrs.

APPENDIX C

APPENDIX C

Supplies

Electron microscopy

Electron microscopy film # 4489, 3.25" by 4" - Kodak (MSU Stores)

Ektamatic SII activator - Kodak (MSU Stores)

Ektamatic S30 stabilizer - Kodak (MSU Stores)

Forceps, #7, curved - Ted Pella, Inc.

Formvar, 0.25% in ethylene dichloride - Ted Pella, Inc

Gelatin capsules, size #00 - Lilly

Grid Boxes, Pelco - Ted Pella, Inc.

Grids, nickel, ultra-high transmission, thin bar, 200 mesh - Ladd Research Industries

Lead Citrate - Ladd

L.R. White Resin (acrylic) medium grade - Fullam

Osmium tetroxide, crystalline - Polysciences

Photographic paper, 8" by 10" AGFA-Gevart Rapidoprint - VWR Scientific

Photographic paper, 8" by 10" Polycontrast Rapid II medium weight - Kodak (MSU Stores)

Photographic paper, 8" by 10" Kodabrome II RC medium weight - Kodak (MSU Stores)

Silgard 184 elastomer with 184 curing agent, # 1.1 kit. -

APPENDIX C

Supplies

Prehler Co.

Uranyl acetate - Electron Microscopy Sciences

Fixation

EM grade 20% paraformaldehyde - Polysciences

Glutaraldehyde, 25% - Electron Microscopy Sciences

Sodium cacodylate - Polysciences

Immunocytochemistry

DAB (3,3' -diaminobenzidine 4HCL) - Sigma

Filter equipment - see Master's Thesis

Goat anti-mouse IgG $F(ab')^2$ fragment conjugated to peroxidase - Boehringer-Mannheim

Monoclonal Antibodies - Drs. H. Gainer (NIH) and W. Smith (Mich. St. Univ.)

Normal goat serum - Antibodies Inc.

Sodium m-periodate - Sigma