RESPONSIVENESS OF SUPRAOPTIC NEUROENDOCRINE CELLS TO SEQUENTIAL OXYTOCIN- AND VASOPRESSIN-EVOKING STIMULI

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CHAPTER I

INTRODUCTION

Speculation on the function of the posterior pituitary may be traced back to Galen in the first century B.C. who found that fish, amphibians, reptiles, birds, and mammals possess a pituitary gland. Several hypotheses were put forth, but our current understanding of the function of the neurohypophysis began in the late 19th and early 20th century when vasopressic (Oliver and Schafer, 1895), oxytocic (Dale, 1906), milk-ejecting (Ott and Scott, 1910), and antidiuretic (Farini, 1913; Von den Velden, 1913) properties were found in extracts of the posterior pituitary. Subsequent research has established the presence, synthesis, and release of oxytocin and vasopressin from the hypothalamo-neurohypophyseal system (HNS) (Sloper, 1966). The HNS consists of the paired supraoptic nuclei (SON), paraventricular nuclei (PVN), internuclear zone (INZ), and the axons and telodendria of neurons originating in these areas which terminate for the most part, in the neurohypophysis (Knigge and Silverman, 1974).

It has also been determined that release of the neurohypophyseal hormones requires propagation of action potentials in the neurons of the HNS (magnocellular neuroendocrine cells) and that this release may be evoked by a variety of stimuli (Douglas, 1974). Stimuli known to evoke oxytocin release (oxytocinogogues) include distension of the vagina, cervix, and uterus, suckling, milking, massage of the genitalia

of both sexes, and odors of conspecifics (Tindal, 1974). Stimuli known to evoke vasopressin release (vasopressinogogues) include decreased extracellular volume, increased plasma osmolarity, pain, emotional stress, and emesis (Hayward, 1977). These stimuli are not necessarily entirely specific, however, in causing release of only oxytocin or only vasopressin (Weitzman, 1977).

Spontaneous and evoked activity of single antidromically (AD) identified magnocellular neuroendocrine cells has been studied. Four spontaneous firing patterns have been identified among AD identified cells: continuously active slow, continuously active fast, low frequency bursting, and silent (Haskins, 1976; Jennings, et al., 1978). Various degrees of excitation have been observed in response to vaginal distension, suckling, hemorrhage, increased plasma osmolarity, and pain, but rarely have experiments utilized more than one stimulus or been performed on unanesthetized animals (Hayward, 1977).

Of particular interest is the sequential effect of stimuli known to release oxytocin and those which are known to release vasopressin. It has been demonstrated in lactating, urethane-anesthetized rats that all AD identified cells responded with increased firing rates to dehydration or intraperitoneal injection of hypertonic saline while 50% of these were also responsive to suckling (Poulain and Wakerely, 1977; Brimble and Dyball, 1977). No such study has been performed on unanesthetized animals.

The estradiol implanted, unanesthetized ewe serves as an excellent model for studies of effects of sequentially applied vasopressin- and oxytocin-associated stimuli on AD identified SON neuroendocrine cells. An abundance of information is available regarding evoked release of

oxytocin and vasopressin in unanesthetized sheep and goats measured by bioassay (Moses and Miller, 1974; Share, 1974; Tindal, 1974) and radioimmunoassay (Weitzman, 1977). The use of estradiol implants is well established to enhance oxytocin release in these species (Roberts, 1973). An excellent stereotaxic atlas is available for the Southdown sheep diencephalon (Rogers, 1976). The model has been made even more attractive by publication of information on spontaneous activity of AD identified SON neurons and of the activity evoked by fast and slow hypertonic intrajugular infusions (Haskins, 1976; Jennings, et al., 1978).

This investigation uses the estradiol-implanted unanesthetized ewe as a model to examine the activity of AD identified magnocellular neuroendocrine cells of the SON evoked by a variety of oxytocin- or vasopressin-associated stimuli. Stimuli were applied to be within a range of intensities expected to be encountered by sheep in natural situations. Those stimuli applied which have been demonstrated to evoke oxytocin release are vaginal distension, vulvar massage, and urine and fecal odor of sexually mature rams. In a short series of experiments, the release of oxytocin and vasopressin evoked by vaginal distension, hemorrhage, and intrajugular injection of hypertonic saline were measured by radioimmunoassay.

The major goal of this investigation was to determine the degree of convergence and specificity of various sensory stimuli on magnocellular neuroendocrine cells of the SON in the unanesthetized sheep.

Those hypotheses addressed in the experiments measuring plasma oxytocin and vasopressin include: 1) vulvar massage, vaginal distension, and ram odors will be associated with a transient release of oxytocin;

2) vulvar massage, vaginal distension, and ram odors will cause no release of vasopressin in the estradiol-implanted ewe; 3) hypertonic sodium chloride and 15% hemorrhage will cause release of vasopressin in the estradiol-implanted ewe; and 4) hypertonic sodium chloride infusion and 15% hemorrhage will cause no release of oxytocin in the estradiol-implanted ewe.

Those hypotheses addressed in the experiments monitoring magnocellular neuroendocrine cell activity include: 1) the firing pattern of a portion of AD identified SON neuroendocrine cells will be altered by vaginal distension, vulvar massage, or ram odors; 2) the firing pattern of that same portion of SON neuroendocrine cells will not be altered by 15% hemorrhage or hypertonic sodium chloride; 3) the firing pattern of a portion of AD identified SON neuroendocrine cells will be altered by 15% hemorrhage or hypertonic sodium chloride infusion; 4) the firing pattern of that portion of AD identified SON neuroendocrine cells will not be altered by vulvar massage, vaginal distension, or ram odors; and 5) prior exposure to ram odors will increase the responsiveness of some SON neuroendocrine cells to vulvar massage.

CHAPTER II

REVIEW OF SELECTED LITERATURE

Although not an exhaustive review of the literature available relating to the hypothalamo-neurohypophyseal system, this section does inform by reviewing selected literature most pertinent to the questions addressed in this investigation. The body of literature drawn from is large, encompassing almost a century of research.

Posterior pituitary extracts were found to contain a "vasopressic principle" (Oliver and Schafer, 1895), an "oxytocic principle" (Dale, 1906), a galactagogue (Ott and Scott, 1910), and antidiuretic properties (Farini, 1913; Velden, 1913), and, although the connection of the supraoptic nucleus and the posterior pituitary was first described by Cajal in 1894 (Harris and Donavan, 1966), the functional significance of this connection was not understood.

An early hypothesis favored the idea of synthesis of these "principles" by cells of the intermediate lobe, migration into the posterior lobe, forming hyaline bodies which would fall apart into particles released into the blood (Herring, 1915). This hypothesis remained in vogue until the histochemical studies of Sharrer and Sharrer (1940) and Bargmann (1949) and, later, of Bargmann and Sharrer (1951), showed clear evidence of neurosecretory material in the hypothalamo-neurohypophyseal system (HNS), both in the magnocellular nuclei and in the axons of the neurohypophysis.

Synthesis of the neurosecretory material was investigated by autoradiographic study of incorporation of S^{35}-labelled DL-cysteine by Sloper in 1958 (Sloper, 1966) which indicated rapid and preferential uptake by the supraoptic nucleus (SON) and paraventricular nucleus (PVN). Sachs and Takabatke (1964) observed transport of radioactive vasopressin by autoradiography down the HNS of dogs whose fourth ventricles were infused with S^{35}-labelled cystine. Isotopically labelled oxytocin and vasopressin were recovered from the posterior pituitary of rats whose cerebrospinal fluid had been injected with labelled tyrosine (Pickering and Jones, 1971). Thus, presence, synthesis, and transport of oxytocin and vasopressin within the HNS were confirmed.

Several years before Bargmann's work was published in 1949, uterine contractions in the rabbit and cat were found to be elicited by electrical stimulation of the hypophyseal stalk (Haterius and Ferguson, 1938; Harris, 1947), thus initiating the electrophysiological investigation of the HNS. Harris (1947) also observed an antidiuresis following electrical stimulation of the hypophyseal stalk giving evidence for the release of both oxytocin and vasopressin in response to electrical activity in the HNS. Similar results were obtained by electrical stimulation of the cell bodies (Anderson, 1951; Cross, 1955). In vivo studies in the lactating rabbit revealed an electrical stimulus frequency dependent response of milk-ejection, requiring a stimulus frequency no less than 25-30 Hz (Harris, et al., 1969), although in vivo investigation of lactating rats demonstrated elevated plasma oxytocin, uterine contractions, and milk-ejection in response to thirty minutes of 6 Hz electrical stimulation of the hypophyseal stalk

(Boer, et al., 1980). Dreifuss, et al. (1971), confirmed that the action potential was a requirement for neurohypophyseal hormone release by using tetrodotoxin to block action potentials and failing to cause electrically evoked hormone release.

The establishment of association of neurohypophyseal hormone release with electrical stimulation was followed by recording of the electrical activity of the HNS. Use of large electrodes, recording direct current in the region of the SON, led Von Euler (1953) to describe DC "osmopotentials" in response to intravenous injections of hypertonic solutions. Further research paralleled the development of technology. Single anterior hypothalamic neurons of urethane-anesthetized rabbits were found to respond to intracarotid hypertonic injection while monitored by extracellular electrodes (Cross and Green, 1959). A number of researchers followed by recording from unidentified neurons in the SON and PVN (Brooks, et al., 1962; Brooks, et al., 1966a; Brooks, et al., 1966b; Ishikawa, et al., 1966; Koizumi, et al., 1964; Suda, et al., 1963). Utilizing Prussian blue staining to confirm recording location, these investigations demonstrated that neurons in the region of the magnocellular nuclei spontaneously discharge irregularly or continuously (Hayward, 1977).

Kandel (1964) developed the technique of antidromic identification of magnocellular neuroendocrine cells during intracellular recording in the preoptic nucleus of anesthetized goldfish. This technique proved valuable both for identification of magnocellular neuroendocrine cells and also for discovering evidence of recurrent inhibition and facilitation. Recurrent inhibition has been demonstrated by recording inhibitory post synaptic potentials intracellularly following

antidromic pituitary stimulation (Kandel, 1964; Koizumi and Yamashita, 1972) and by apparent inhibition of spontaneous firing of identified magnocellular neuroendocrine cells recorded extracellularly following antidromic pituitary stimulation (Dreifuss and Kelly, 1972; Hayward and Jennings, 1973a; Kelly and Dreifuss, 1970; Negoro and Holland, 1972; Negoro, et al., 1973). Further, Koizumi and Yamashita (1972) found AD-identified SON cells firing at high frequency (500-800 Hz) which may be neuroendocrine "Renshaw" cells. Existence of facilitory collaterals was suggested by intracellular recording of magnocellular neuroendocrine cells of isolated bullfrog HNS which exhibited decreased latency of response to close-spaced (5 msec) subthreshold antidromic stimuli and increased latency of response to further spaced (11 msec) stimuli (Koizumi, et al., 1973).

A number of studies were subsequently performed on antidromically identified neurons of the supraoptic and paraventricular nuclei. In studies of both anesthetized and unanesthetized animals, three types of spontaneous firing patterns were found prior to 1975: silent (3-10%); continuously active (65-77%); and phasic or burster (20-25%) (Hayward, 1977). Later works expanded the classification of spontaneous firing patterns of neurons in the SON: 1) 6% silent; 2) 21% continuously active fast (CAF); 3) 33% continuously active slow (CAS); and 4) 39% low frequency bursting (LFB) (Haskins, 1976; Jennings, et al., 1978). These same studies identified three additional classifications of spontaneous firing patterns recorded from neurons in the SON region but not found in antidromically identified neurons: 1) high frequency bursting; 2) continuously active bursting; and 3) continuously active regular (Haskins, 1976; Jennings, et al., 1978). The investigators

suggested these may represent firing patterns typical of interneurons, pacemakers, or perhaps osmoreceptor cells.

Other investigations, fundamental to the developing concepts of the HNS, determined many of the stimuli evoking the release of oxytocin and vasopressin. Verney, in his work with unanesthetized dogs, demonstrated antidiuresis in response to intrajugular hypertonic saline injection, intracarotid hypertonic saline injection, intracarotid dextrose and sodium sulphate, emotional stress, and hemmorhage (Verney, 1947). Saito, et al. (1969) showed an increase in plasma vasopressin in dogs following hypertonic saline injections, as did Ahmed (Ahmed, et al., 1967) in similar work with humans. Injection of hypertonic solutions into the third ventricle of unanesthetized goats also elevates plasma vasopressin (Andersson, et al., 1967).

Reduction of extracellular fluid volume, also, seems a major stimulus for vasopressin release. Research in the early 1950's and 1960's showed hemorrhage decreased urine flow (Henry and Ganer, 1951; Ginsburg and Heller, 1963; Weinstein, et al., 1960; Baratz and Ingram, 1960). Research in the late 1960's and early 1970's revealed an equivalent of 8% to 10% decrease in blood volume increased bioassayable plasma ADH in anesthetized and unanesthetized animals (Henry, et al., 1968; Share, 1968; Johnson, et al., 1970; Szcepanska-Sadowska, 1972; Dunn, et al., 1973; Goetz, et al., 1974). Investigators using separate and concurrent osmotic and volume stimuli applied to unanesthetized sheep discovered both increased plasma osmolarity and decreased left atrial pressure to increase plasma vasopressin independently and together, suggesting that neither input dominated the other in terms of vasopressin release (Zehr, et al., 1969; Johnson, et al., 1970).

Development of sensitive radioimmunoassays for vasopressin and oxytocin confirmed elevation of plasma vasopressin in response to hypertonic saline injection, hemorrhage, and dehydration (Dogterom, et al., 1977; Weitzman, et al., 1978), but also indicated release of oxytocin.

Pain and emotional disturbance are stimuli for vasopressin release (Verney, 1947; Rothballer, 1966). Further research, stimulating midbrain and limbic areas suspected of nociceptory involvement, demonstrated release of vasopressin (Hayward and Smith, 1963; Hayward, 1972).

Several other stimuli cause release of vasopressin, including a number of chemicals. Intraventricular injections of angiotension II and carbachol have caused release of vasopressin in Sprague-Dawley rats (Hoffman, et al., 1977). Intraventricular injections of angiotensin II in the unanesthetized goat have been demonstrated to potentiate the release of vasopressin in response to intracarotid injections of hypertonic sodium choloride (Andersson and Olsson, 1977). Alpha adrenergic blockade causes elevated oxytocin and vasopressin levels in the rat (Guzek and Janus, 1980) and intravenous substance P has been shown to elevate plasma vasopressin levels in anesthetized dogs (Gullner, et al., 1979). Intravenous nicotine is effective in releasing large amounts of vasopressin in the unanesthetized monkey (Hayward and Pavasuthipaisit, 1976). Recent work has confirmed that a nictonic-cholinergic system mediates vasopressin release by demonstrating an inhibition of osmotic stimulation of vasopressin release by nicotinic blocking agents (Sladek and Joynt, 1979).

Nausea, retching, and vomiting also cause release of vasopressin (Andersson and Larson, 1954; Hayward, 1974; Baylis and Robertson,

1977). Not all stimuli held to induce release of vasopressin are without conflicting data; ether and acceleration stress caused a rapid decrease in plasma vasopressin measured by radioimmunoassay, contradicting other literature indicating increased plasma vasopressin levels in response to noxious stimuli (Keil and Severs, 1977). However, the literature leads the reader to the conclusion that release of vasopressin is governed by plasma and cerebrospinal osmolarity, blood and extracellular fluid volume, angiotensin II, and by emotional disturbance. No doubt, other stimuli contributing to vasopressin control will be elucidated.

The liberation of oxytocin has been determined to be associated with a variety of stimuli. In 1941, both the Ferguson reflex and the milk ejection reflex were described, whereby oxytocin is released by distension of the hollow organs of the female genital tract and by suckling, respectively (Ferguson, 1941; Ely and Petersen, 1941). Ferguson observed rabbits, chloralose and urethane anesthetized, 9-48 hours post partem. By dilation of one uterine horn, the cervixes, or the vagina, contractions were elicited in the unstretched horn or in both horns. The effect of vaginal distension was judged by Ferguson to be the weakest (Ferguson, 1941).

Both reflexes were studied subsequently by a number of investigators. Debackere, et al. (1961) studied the Ferguson reflex and the effect of massage of seminal vesicles and ampullae of female and male sheep, respectively, by an ingenious cross-circulation technique. Stimulation in both cases caused an increase in pressure in the udder cisterns of the cross-connected ewe, not only indicating release of oxytocin, but elegantly demonstrating independence from neural effectors in the effects of the hormone.

Additional work, determining optimum and minimum conditions for release of oxytocin ensued. Measurement of oxytocin by bioassay in lactating, cycling, and pregnant ewes in response to vaginal distension revealed an increase of oxytocin in lactating and cycling ewes, but an actual decrease in oxytocin levels in pregnant ewes (Roberts and Share, 1968). Concluding that a factor peculiar to pregnancy interfered with the release of oxytocin, Roberts and Share (1969) injected normally cycling ewes with either progesterone or estradiol for two weeks and observed no significant increase in plasma oxytocin in those ewes treated with progesterone but a 2660% increase in those treated with estradiol. Reaching the conclusion that the Ferguson reflex depended on estrogen exposure, Roberts (1973) tested the responsiveness of cycling, ovariectomized, and ovariectomized-estradiol-treated goats to vaginal distension. Results of this study showed increased plasma oxytocin levels in cycling and ovariectomized-estradiol-treated subjects and decreasing responsiveness in ovariectomized subjects. Yet, only after three months of estradiol treatment were all ovariectomized subjects responsive, and this responsiveness declined in summer, suggesting a further seasonal influence on the responsiveness of the system to estradiol treatment. Release of oxytocin in response to vaginal distension was confirmed by radioimmunoassay and, in addition, showed dexamethasone treatment to attenuate the release of oxytocin (Blank and DeBias, 1977).

Stimuli associated with coitus have been shown to elevate plasma oxytocin levels in cows indicated by increased uterine motility (Van Demark and Hays, 1952), in women (Fox and Knagges, 1969), and in goats (McNeilly and Ducker, 1972). Their work earlier in 1952 led Van Demark

and Hays (Hays and Van Demark, 1952) to show that oxytocin injection increased uterine motility which was inhibited by pretreatment with epinephrine, and, finally, showed that manual massage of the cervix, cervical os, and the vulva each caused release of oxytocin in the cow (Hays and Van Demark, 1953). McNeilly and Ducker (1972) showed bio-assayed oxytocin to be released throughout coitus in 8 of 36 experiments with estrus goats, but in seven of these, oxytocin release had begun prior to actual coitus, and in eleven others oxytocin release began and ended prior to coitus. In 23 experiments, oxytocin release continued for 3-15 minutes after the male had left the room. This provided evidence that stimuli other than mechanical were affecting oxytocin release. In several further experiments sound of the male and sight of the male were ineffective in release of oxytocin, while smell of the male caused release of oxytocin, as did presence of a teaser female goat.

Other stimuli known to cause oxytocin release are suckling and milking (Tindal, 1974). Milk ejection, caused by adequate plasma oxytocin levels, has been shown to be inhibited by epinephrine (Sibaja and Schmidt, 1975) and prostaglandin $F_{2\alpha}$ (Prilusky and Deis, 1976). Norepinephrine, dopamine, and apomorphine are known to cause increase in intramammary pressure (Clarke, et al., 1979). Anesthetics may not affect oxytocin release, as in the case of tribromoethanol, inhibit oxytocin release, as in the case of pentobarbitone, or potentiate oxytocin release, as in the case of urethane (Dyball, 1975). A pair of investigations have revealed that synchronized electroencephalographs (EEG) are prerequisites for milk injection in the rat, documenting a complicating factor in studies of the oxytocin neuroendocrine system

(Voloschin and Tramezzani, 1979; Lincoln, et al., 1980). A synchronized EEG occurs during slow wave sleep, under the influence of most anesthetics, and in the female rat during suckling, if not aroused (Lincoln, et al., 1980).

The literature has thus far shown that distension of the genital tract of the female, massage of the genital tract of the male and female, suckling, milking, presence of an estrus female, smell of a male, apomorphine, dopamine, norepinephrine, and several anesthetics cause oxytocin release in a number of species. Furthermore, it has been demonstrated that a synchronized EEG is a prerequisite for milk ejection in the rat, and that oxytocin release is enhanced by estrogen and inhibited by progesterone, epinephrine, and prostaglandin $F_{2\alpha}$. It has also been shown that hemorrhage and hypertonic saline cause release of both vasopressin and oxytocin (Weitzman, et al., 1978; Dyball, 1968).

The nuclear hypothesis which was held forth for some years stated that oxytocin was located mainly in the paraventricular nuclei and vasopressin was located mainly in the supraoptic nuclei (Lederis, 1961). The use of immunohistochemical techniques, however, showed both oxytocin and vasopressin to be present in both pairs of magnocellular nuclei in the rat (Swaab, et al., 1975a; Swaab, et al., 1975b; Vandesande and Dierickx, 1975; George, et al., 1975). Further work indicated that oxytocin-containing and vasopressin-containing neurons have a characteristic distribution within the nuclei of the rat, monkey, and man. Oxytocin-containing cells are located about the periphery of the PVN and form a dorsal cap on the SON, while vasopressin-containing neurons are located centrally in the PVN and

ventrally in the SON (Zimmerman and Defendini, 1977).

Having established the existence and hormone content of the HNS, a variety of stimuli known to cause release of oxytocin and vasopressin, and the requirement for the action potential of the magnocellular neuroendocrine cells to cause release of oxytocin and vasopressin, a number of investigators studied the correlation of stimuli known to cause release of the hormones with the electrical activity of antidromically identified magnocellular neuroendocrine cells.

Prominent among workers in the field was a group who worked primarily with urethane-anesthetized rats. It should be noted, however, that urethane is known to release both oxytocin and vasopressin in the rat (Dyball, 1975). The effects of anesthetics on antidromically identified neurons in the paraventricular and supraoptic nuclei have been examined. Firing rate of the neurons was not significantly different under the influence of urethane, known to cause release of vasopressin from the rate seen with chloral hydrate or from the rate seen with tribromoethanol, which is not known to release vasopressin (Dyball and McPhail, 1974).

Dyball and Koizumi (1969) showed cells in the SON and PVN to be excited or depressed by vagal stimulations or rapid intracarotid injection of acetylcholine, carbachol, 0.85% saline, $CaCl_2$, or 5% saline. The definition of excitation or inhibition in this study was a 20% increase or decrease in mean firing rate twenty to thirty seconds post stimulus. Five percent sodium chloride injection caused excitation in about 30% of the cells and caused inhibition in about 15% of the cells. Wakerely and Lincoln (1973) observed 58% of PVN cells to respond with a 2-4 second firing of 28-84 Hz to suckling which was

associated with milk ejection. Further study of SON neurons during suckling showed a characteristic response of a 30-fold increase (median) of mean firing rate for 0.9-4.7 seconds, 10.5 to 17.4 seconds prior to milk-ejection in 50% of the cells studied (Lincoln and Wakerely, 1974). Dyball (1971) recording from antidromically identified neurons in the PVN and SON, showed bioassayable oxytocin and vasopressin to increase during intracarotid, hypertonic saline injections, and that 70% of the SON neurons were excited as evidenced by a 30% increase in mean firing rate.

It was also observed that the relationship between activity of single neurons and hormone release was not a simple one. Lincoln and Wakerely (1975), however, determined that peak firing rate of suckling-sensitive neuroendocrine cells was determined by intensity of the stimulus and that a peak rate of 30 Hz was associated with milk-ejection consistently in the urethane-anesthetized rat.

Dutton and Dyball (1979) supported the idea of phasic firing increasing vasopressin release in the isolated rat HNS by using recorded, excited "vasopressinergic" neurons as stimulus triggers, and also showed a direct relationship between elevated frequency and increased vasopressin release.

It was demonstrated that those cells responding to suckling (50%), "oxytocinergic" cells, responded to dehydration or by intraperitoneal injection of hypertonic saline as the other half of the cells, but with a different firing pattern; "vasopressinergic" cells responded with higher firing rates and tended to fire in bursts at high plasma osmolarity, while "oxytocinergic" cells rarely fired in this phasic pattern and their firing rate was generally lower (Poulain and

Wakerely, 1977; Brimble and Dyball, 1977).

The Ferguson reflex was also investigated in the urethane-anesthetized rat. Lincoln, et al. (1977) showed the Ferguson reflex only under deep anesthesia (urethane plus sodium pentobarbitane) and vaginal distension to activate antidromically identified SON cells with a smaller magnitude, more varied response than with a suckling stimulus. Vaginal distension interrupted milk ejection and the two stimuli seemed not to interact in a positive fashion. In another investigation, it was found that only 15% of the antidromically identified PVN cells responded to vaginal distension and that the typical response was less than the thirty-fold increase observed in response to suckling shown in other studies (Freund-Mercier and Richard, 1977). In yet another investigation, 69.2% of the identified PVN neurons exhibited an elevation of firing rate in estrogen-treated rats in response to vaginal distension, a significantly greater percentage than in ovariectomized rats (Negoro, et al., 1973).

Although odors of a ram have been shown to release oxytocin in ewes (McNeilly and Ducker, 1972), only a single study has been performed on the activity of antidromically identified neuroendocrine activity of antidromically identified neuroendocrine activity cells in response to sexual odors. Urine of estrus female rats was found to cause an elevation of AD identified PVN unit activity of long duration in male rats (Young, 1976).

With a broad base of research with the urethane-anesthetized rat model, attempts were made to record from antidromically identified neuroendocrine cells in the conscious rat. This was found to be difficult and early attempts failed (Boer, 1976); however, investigators

have recently succeeded in recording from single neurons for long periods in the conscious rat (Summerlee, et al., 1979).

Relatively few studies of antidromically identified neuroendocrine cells have been performed in unanesthetized animals of any species. Hayward and Vincent (1970), first using unanesthetized monkeys, found that identified magnocellular neuroendocrine cells were biphasically responsive to osmotic stimuli. Hayward and Jennings (1973a, b) recorded in the SON and internuclear zone of unanesthetized monkeys and described three functional types of firing patterns in AD identified neuroendocrine cells. Thirty-three percent of these cells were specifically osmosensitive. They responded to a five second intracarotid injection of hypertonic saline with a biphasic excitation and inhibition response followed by return to baseline rate 30-50 seconds following the injection. This response tends to substantiate other electrophysiologic evidence for recurrent inhibition of these neuroendocrine cells. Further study with the same animal model showed a lack of responsiveness of the AD identified neurons to alteration in sleep-waking behavior and an excitation in most cells in response to painful stimuli or the conditioned expectation of such stimuli (Hayward and Jennings, 1973c).

Haskins, et al. (1975) showed several firing patterns in identified cells of the SON in unanesthetized ewes, but did not find a correlation between osmotic excitation, firing pattern, or plasma osmolality. Jennings, et al. (1978), again using unanesthetized ewes, found 51% of antidromically identified SON neurons to be excited, 12% to be inhibited, and 39% to be unaffected by slow intrajugular infusions of hypertonic saline; again no correlation of osmosensitivity and

spontaneous firing pattern was shown. However, they also demonstrated that 80% of the continuously active slow and continuously active fast discharging neurons entered a low frequency bursting pattern during or following osmotic loading (Jennings, et al., 1978). These findings are consistent with the distribution of firing patterns recorded in unanesthetized monkeys following various degrees of dehydration (Arnauld, et al., 1974).

In summation, the literature indicates a number of stimuli that release oxytocin and vasopressin, not necessarily exclusively, one or the other, but rather preferentially according to the stimulus applied. Vasopressin is preferentially released in response to increased plasma osmolality, decreased blood volume, and emotional disturbance or pain. Oxytocin is preferentially released, depending on reproductive or endocrine status, in response to suckling, milking, vaginal distension, odors of a male, and vulvar stimulation. Anesthesia alters the release of both hormones, however, as does the sleep-waking status of the animal in the case of the milk-ejection reflex.

Activity of antidromically identified neuroendocrine cells has been monitored in association with these stimuli known to cause neurohypophyseal hormone release, but primarily in anesthetized animals. The responsiveness of these cells to vasopressinergic stimuli and oxytocinergic stimuli seems to be about evenly split between the two categories of stimuli, 50% of the cells being excited by each and a variable percentage being inhibited. The responsiveness is not, however, specific to one category or the other, since both oxytocinergic and vasopressinergic stimuli may excite a particular cell. The magnitude of responsiveness of the cells depends on the type of

stimulus applied and its intensity. Limited studies of unanesthetized animals confirm these conclusions in general.

CHAPTER III

MATERIALS AND METHODS

The techniques used in both the magnocellular neuroendocrine cell activity experiments and the measurement of evoked hormone release are described here. Most of the techniques used for recording single unit activity are identical to or modified from the techniques used by Haskins (1976).

Magnocellular Neuroendocrine Activity

Animal Preparation

Pure-bred Southdown ewes, two to nine years of age, were medicated with atropine sulfate and induced to general anesthesia with sodium thiamylal (Surital, Parke-Davis). A cuffed endotracheal tube was positioned and connected to a Fluotec^{R} vaporizer for fluothane anesthesia (Halothane, Ayerst Laboratories). The animals were placed in a stereotaxic headholder and a craniotomy performed at coordinates of frontal 30.0 mm, lateral 0.0 mm (Rogers, 1976) which would accommodate a bone wax-filled stainless steel cylinder 20 mm in outside diameter (Trent Wells, Inc.). Craniotomies for placement of four stainless steel epidural platform bolts were performed. Cylinder and bolts were secured and all craniotomies sealed with dental cement (Caulk Grip Cement).

Following this preparation, a titanium micropositioner (Trent

Wells, Inc.) was attached to the implanted cylinder and a specially designed pituitary electrode guide block was affixed to the micropositioner. A lateral radiograph was taken with 22 ga stainless steel tubing passing through the guide block at a 29° rostral-caudal angle. The radiograph was examined to determine if the electrodes, passing through the stainless steel tubing, would pass into the sella tursica. If not, the position of the guide block was altered by calculated forward or backward movement of the micropositioner. Finally, an additional craniotomy was performed and the tungsten pituitary electrodes were passed through the guide block to the sella tursica. Lateral position of the electrodes confirmed at the time of surgery by determination of the threshold of eyeball movement evoked by electrical stimulation of the ipsilateral occulomotor nerve as it passed just lateral to the sella tursica. Lateral movement of the electrodes was never found to be necessary. The electrodes and guide tubes were fixed to the platform bolts and cranium with dental cement. Eventually, after considerable practice, all craniotomies were performed in a single surgical procedure.

Cannulae (Dow Corning Silastic) were placed in both external jugulars after blunt dissection of the veins, threaded approximately eight inches toward the heart, and secured by suture attached to the cannulae by silicone medical adhesive (Dow Corning). Cannulae were capped with Injection Caps (Becton-Dickinson), filled with heparinized saline (1,000 U/ml), and flushed daily with one-half ml of the same solution. This technique was later replaced by a percutaneous technique allowing relatively easy removal of non-patent cannulae and replacement with new cannuale. Polyethylene cannulae (P.E. 205

Medline) were inserted into the veins through thin-walled 12 ga hypodermic needles placed in the jugulars. Cannulae were capped with 16 ga hypodermic needle hubs and secured by suture attached to the hubs. A schematic of the completed preparation is shown in Figure 1.

Estradiol implants were prepared using a method described by Roberts (1973). Silastic tubing (0.052 in ID x 0.095 in OD, Dow Corning) was cut into 9 cm lengths and one end plugged with Silicone Sealer (Dow Corning). The 9 cm lengths were autoclaved and, with aseptic techniques, were filled with 17 β-estradiol (ICN Pharmaceuticals, Inc.) for 9 cm of their length. The other end was then sealed with Silicone Sealer. Two of the completed implants were placed between scapulae of each ewe, each approximately 5 cm from the dorsal midline.

Confirmation of Pituitary Stimulating Electrode Placement

Correct pituitary electrode position was confirmed in ewes by antidiuretic response to electrical stimulation of the pituitary stalk. The ewes were catheterized with a Foley catheter (Fr. 12) and hydrated by intravenous infusion of hypotonic saline (0.45% NaCl) at 3 ml per minute until a stable urine flow was achieved. Square wave pulses of 2 msec duration, 2-8 mA constant current intensity (Frederick Haer, Pulsar 6i) were applied to the pituitary stimulating electrodes at 50 Hz for 5 seconds every 30 seconds for 5 minutes. If the electrodes were placed correctly, an increase in urine osmolarity and a decrease in urine flow rate was obtained (Figure 2).

Figure 1. Diagrammatic View of the Hydraulic Microdrive, Pituitary Stimulating Electrodes, and Venous Cannula on the Head of a Sheep

Labels: r.e., recording electrode; p.e. pituitary electrode; i.m.a., internal maxillary artery; e.m.a., external maxillary artery; t.a., thyrolaryngeal artery; c.a., carotid artery; j.v., jugular vein; j.c., jugular cannula. Modified from Haskins (1976).

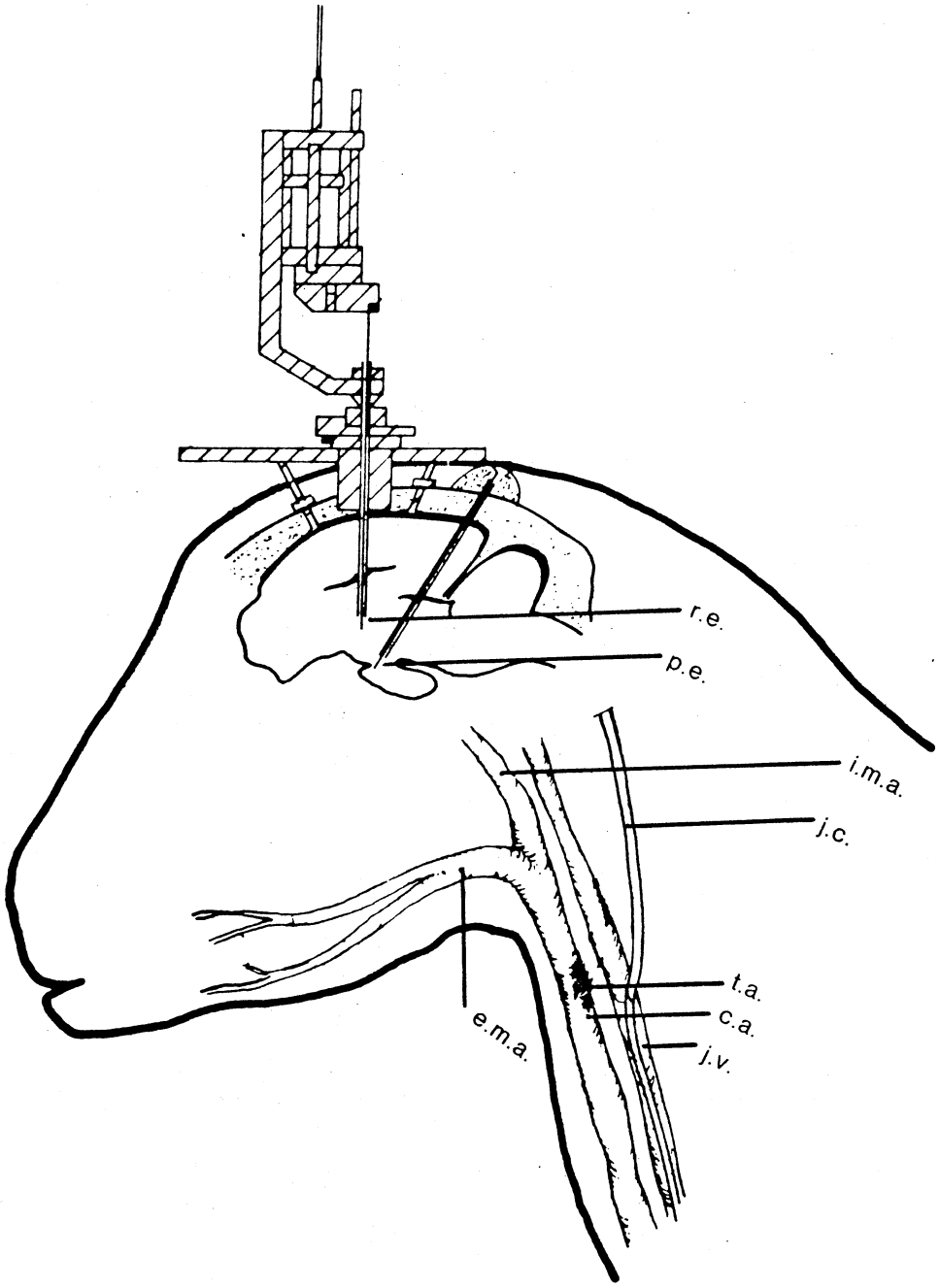
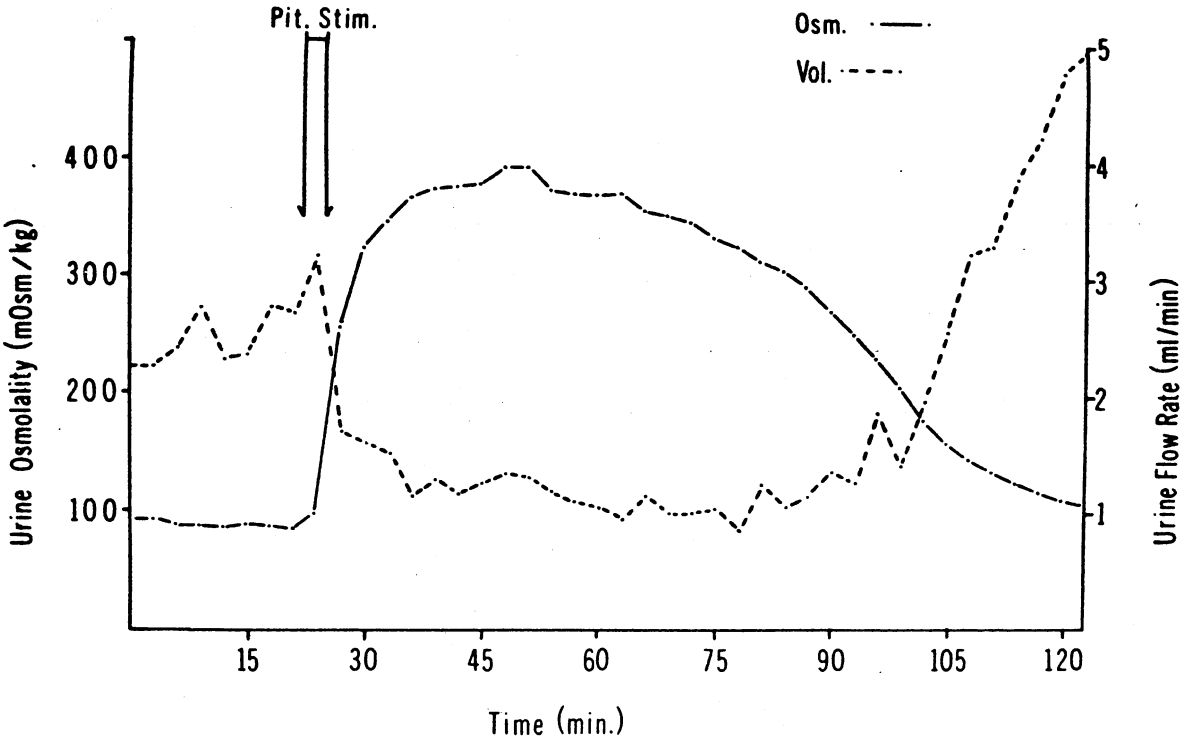

r.e.
p.e.
i.m.a.
j.c.
t.a.
c.a.
j.v.
e.m.a.

Figure 2. Antidiuresis Produced in a Hydrated Sheep by Pituitary Stalk Simulation

Pit. Stim., pituitary stalk stimulation; Osm, urine osmolarity (mosm/kg); Vol., urine flow rate (ml/min). Modified from Haskins (1976).


Pit. Stim.
Osm.
Vol.
Urine Osmolality (mOsm/kg)
400
300
200
100
Urine Flow Rate (ml/min)
5
4
3
2
1
15
30
45
60
75
90
105
120
Time (min.)

Construction of Microelectrodes

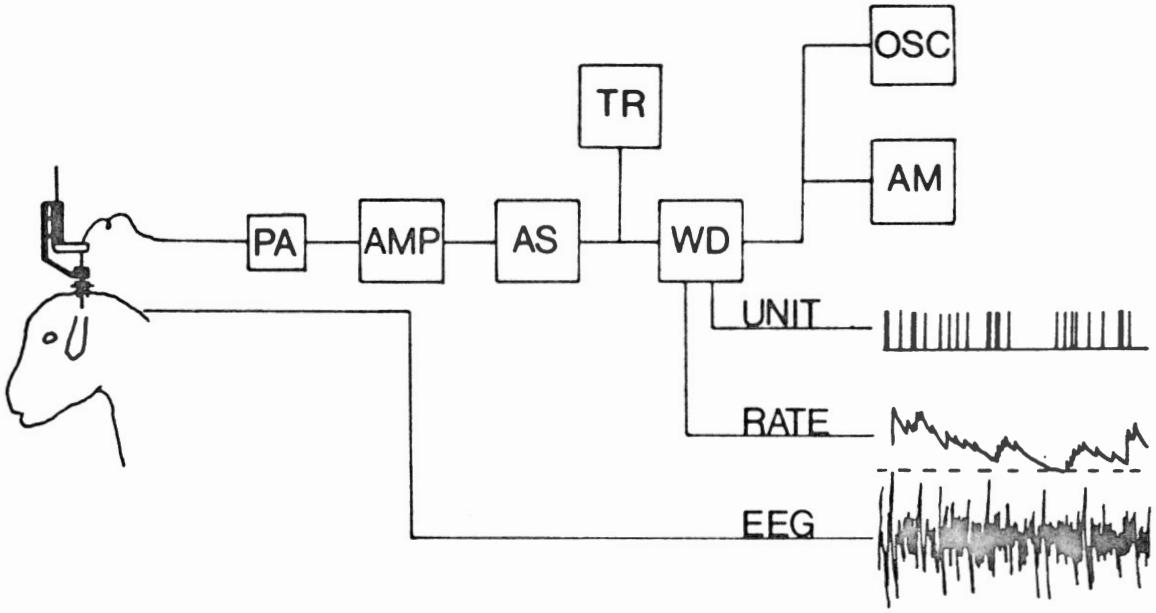
Microelectrodes were constructed by methods modified from those described by Hubel (1957). Tungsten wires, 0.203 mm in diameter and 12 cm long, were etched and polished electrolytically in a saturated potassium nitrite solution to provide tip diameters of one micron or less. Following this, the electrodes were insulated to within 10 to 20 microns of the tip with four coats of Isonel 31 insulating varnish (Schenectady Chemicals, Inc.). A Tektronix Type 130 L-C Meter was then used to measure tip capacitance (50-80 pf acceptable) and uninsulated tip length (Bak, 1967).

Single Unit Recording

Fourteen days following completion of the estradiol implantation, the ewe, having free access to water, was restrained by a nose band, a neck band, and a canvas sling. A microelectrode was inserted into a 22 ga stainless steel guide tube placed into a calibrated hydraulic micromanipulator (Trent Wells, Inc.). The guide tube and electrode were lowered through the Starr guide of the micromanipulator to 5-10 mm above the position of the SON. The microelectrode was then lowered and neuron activity was amplified by a miniaturized preamplifier (Frederick Haer), and an oscilloscope monitor (Tektronix 5103 N/D13). The magnetic tape recordings were kept for data analysis. Outputs from the window discriminator were led to a pen polygraph (Grass Model 7) and recorded as a one-to-one pulse output and as an analog output proportional to neuron firing rate. A diagram of this recording system is illustrated in Figure 3. The analog rate meter was later replaced with a Rate/Interval Analyzer (Frederick Haer) whose output could be

Figure 3. Flow Diagram of Single Unit Recording System

Labels: PA, dc preamplifier; AMP, high gain band pass amplifier; AS, artifact suppressor; TR, tape recorder; WD, window discriminator; OSC, oscilloscope; AM, audio monitor; EEG, electrocorticogram. Modified from Haskins (1976).


OSC
TR
AM
PA
AMP
AS
WD
UNIT
RATE
EEG

displayed via the pen polygraph or the oscilloscope monitor.

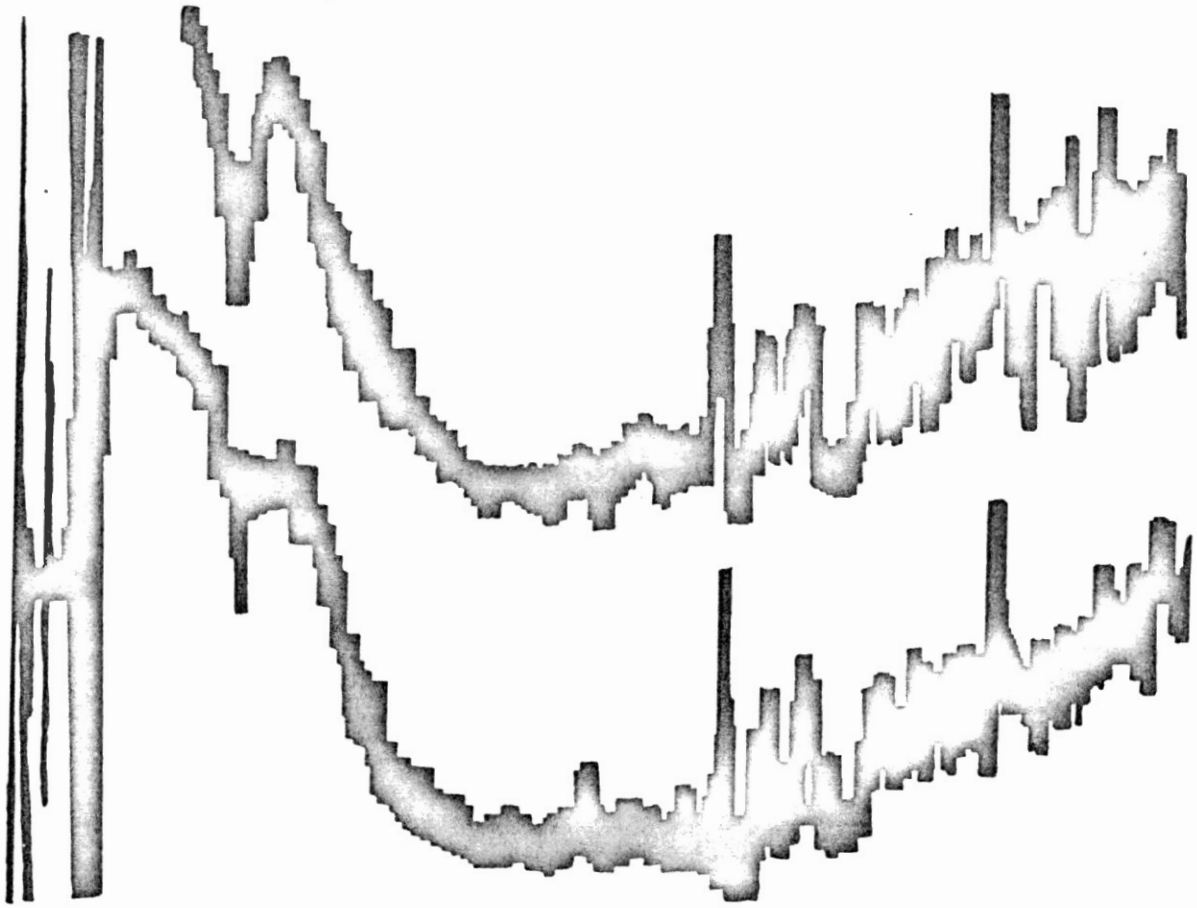
Magnocellular neuroendocrine cells were identified by antidromic response to pituitary stalk stimulation by square wave pulses from a Pulsar 6i stimulator (Frederick Haer), 2 msec duration and 2-8 mA intensity. A constant latency of response to the repeated antidromic response identified the neuron activity to be that of a magnocellular neuroendocrine cell (Figure 4). Most cells were also identified by self-evoked collision.

Upon identification, the activity of the unit was recorded for a minimum of 200 spikes or two minutes if the unit was silent or slow-firing and a period of one minute or more was recorded between every stimulus. One minute of manual tactile stimulation of the dorsum of the back of the ewe followed, and the activity of the unit was monitored. Two minutes of vaginal distension followed, performed by placing a balloon in the vagina of the ewe and rhythmically inflating and deflating the balloon with a syringe (60 cc of air).

During a number of experiments, two minutes of rhythmic, manual massage of the vulva followed or replaced vaginal distension. Vulvar massage was often repeated after one minute, or was repeated following one minute of exposure to the odor of sexually mature ram's urine and/or one minute of exposure to the odor of sexually mature ram's feces. In some experiments ram odors preceeded the first vulvar massage. The effect of one minute exposure to acetic acid and ammonium sulfide odors was also monitored.

Urine was collected weekly as a free-flowing sample, as was fecal material, from Suffolk, Dorset, and Southdown rams known to have bred for at least one season. Samples were frozen and thawed an hour prior

Figure 4. Mutually Isolatable Antidromically Evoked Spikes



to expected use, put in a bracket attached to a rod, and placed, uncapped, directly beneath the ewe's nose at the time of stimulation.

Following the last period of vaginal distension or vulvar massage, an infusion of 1.2 molar sodium chloride was begun via the intrajugular cannula. The infusion was at the rate of 6-8 milliliters per minute and continued for ten minutes. For three cells, however, the infusion was rapid (50 ml/min) for only three minutes, and for some other cells the slow infusion was terminated after six or more minutes when obvious response was noted or when the unit activity being recorded was lost. Unit activity was also recorded during a 10-15 percent hemorrhage in three cases, and 6% dextran infusion in four cases.

A summary of the basic protocol is as follows. One minute tactile stimulation, and two minutes of vaginal distension or vulvar massage, which may be repeated following exposure to ram urine or fecal odor; this is finally followed by a ten minute, slow infusion of 1.2 molar sodium chloride. All stimuli are separated by a minimum period of one minute (Table I).

Confirmation of Recording Sites

Following a series of experiments, the health or behavior of the ewe would deteriorate. At this time, the animal was terminally anesthetized with sodium pentabartital (Nembutal, Abbott Laboratories). Prussian blue lesions were placed in the presumed region of the SON and rostral to it by passing current through stainless steel electrodes. The brain was perfused via the carotid arteries with 2 liters of normal saline followed by a solution of 2% sodium ferricyanide and 10% formalin. The brain was exposed ventrally to allow visual inspection

TABLE I

PROTOCOL FOR STIMULUS APPLICATION DURING
AD-IDENTIFIED UNIT RECORDING

Event	Time (min)
Control	0- 2
Tactile Stimulation	2- 3
Control	3- 4
Urine or Fecal Odor (optional)	4- 5
Control	5- 6
Vulvar Massage or Vaginal Distension	6- 8
Control	8- 9
Urine or Fecal Odor (optional)	9-10
Control	10-11
Vulvar Massage (optional)	11-13
Control	13-14
15% Hemorrhage (optional)	14-20
Reinfusion	20-26
Control	26-27
1.2 M NaCl Infusion	27-37
Dextran Infusion (optional)	37-44

of pituitary electrode placement. The brain was removed and frozen sections were cut at 100 microns in the stereotaxic plane in the diencephalic region. Location of the Prussian blue spots in relation to the location of the SON were used to confirm the recording sites.

Measurement of Evoked Plasma Oxytocin and Plasma Vasopressin

This series of experiments utilized the estradiol-implanted ewe and radioimmunoassays for oxytocin and vasopressin developed and applied by Dr. Richard Weitzman (Weitzman, 1977) to measure plasma oxytocin and vasopressin levels in association with those stimuli used in the study of magnocellular neuroendocrine cells described earlier in this section. Odors used included horn pit, anal, and preputial swabs as well as urine and feces.

At least two weeks following estradiol implantation, both jugular veins were cannulated using the percutaneous technique described earlier. One jugular cannula was fitted with a 3-way valve to which two syringes were attached, one used for sampling and one filled with sterile physiological saline for flushing the cannula. A balloon was placed in the vagina of the ewe at least fifteen minutes prior to the experiment. Each ewe was placed in a restraining stanchion, and an 85-minute protocol ensued (Table II).

A second series of experiments was undertaken which included only ram odor exposure and vaginal distension or vulvar massage or only vaginal distension or vulvar massage (Table III).

The blood samples were packed in ice until the end of an experiment. The samples were then centrifuged at 15,000 rpm at zero degrees

TABLE II

FULL PROTOCOL FOR EVOKING OF AND SAMPLING FOR PLASMA OXYTOCIN AND VASOPRESSIN

Event	Time (min)	Sample	Time (min)
Ram Odor Exposure	0	1	0
		2	5
Vaginal Distension	11-13	3	10
or		4	12
Vulvar		5	14
		6	20
		7	27
15% Hemorrhage	28-31	8	30
		9	33
Reinfuse Blood	34-40	10	38
		11	43
		12	55
		13	59
Hypertonic NaCl (1.2 MO)	60-70	14	65
		15	71
		16	76
		17	85

TABLE III

ABBREVIATED PROTOCOL FOR EVOKING OF AND SAMPLING FOR PLASMA OXYTOCIN AND VASOPRESSIN

Event	Time (min)	Sample	Time (min)
Ram Odor Exposure (optional)	0	1	0
		2	5
		3	10
Vaginal Distension or Vulvar Massage	10-12	4	10.5
		5	11
		6	12
		7	15
		8	20

centigrade. An aliquot of each was taken and the remainder transferred to borosilicate tubes, frozen, and packed in dry ice. After variable lengths of storage, samples were sent to Dr. Richard Weitzman for radioimmunoassay of vasopressin and oxytocin.

Data Analysis

Unit Analysis

The pen polygraph records of each spike train were examined. Several spike trains were excluded from analysis due to extensive recording artifact. The magnetic tape records of the unit activity of the remaining spike trains were then examined. With suitable amplification it was seen that the AD stimulation record of a spike train frequently contained two or even three mutually isolatable AD-identified units, each showing its characteristic invariant latency of response. The amplitude of each AD-identified unit and the amplitude of any fluctuation of the baseline were measured. The width of the spike for each unit at the baseline was observed at high oscilloscope sweep speeds. The window discriminator was then set, to isolate a single AD-identified unit from all other electrical activity, and the entire spike train was played back. Finally, after evidence was sufficient to show that a given unit could be well isolated, the unit activity was played through the window discriminator. The output of the window discriminator was recorded on magnetic tape in one minute segments corresponding to control periods and applied stimuli.

The recorded window discriminator output was played back through an amplifier to the window discriminator. This output was then lead to the rate/interval analyzer and the signal averager, obtaining an analog

rate output and a spike count, respectively. Mean firing rate was calculated for each minute, and the number of intervals exhibiting rates of over 10 Hz were counted for each minute.

Two parameters were used to describe the response of AD-identified cells. The use of the mean firing rate is traditional in neurophysiological investigations (Lincoln and Wakerley, 1974). The use of mean intervals over 10 Hz is unique, but supported by a recent investigation showing significant hormone release in response to regular electrical stimulation at 10 Hz (Dutton and Dyball, 1979).

By observing the variance in mean firing rate (MFR) and in number of intervals with rates over 10 Hz (high frequency intervals, HFI) within control periods, a definition for enhancement or inhibition was decided upon for each of the two methods of measurement. During a stimulus, 50% increase or decrease in MFR was used as a criterion for enhancement or inhibition, respectively, while 25% increase or decrease in HFI was used as a criterion for enhancement or inhibition based on the rate measurements. This information, along with visual inspection of the rate meter and unit output tracings, was also used to classify firing patterns as silent, low frequency bursting (LFB), continuously active fast (CAF), or continuously active slow (CAS) according to the definition of Jennings, et al. (1978).

One way and two way analysis of variance were used to determine means, mean changes, and significance. Duncan's multiple range test and linear regression were also used to further describe data.

Oxytocin and Vasopressin Data

Mean value of each sample value for oxytocin or vasopressin was

tested for significance against the pooled mean of those samples taken prior to stimulation by the students' t test.

CHAPTER IV

RESULTS

Magnocellular Neuroendocrine Activity

During the course of this investigation ninety-seven spike trains were recorded from eleven estradiol-implanted, unanesthetized Southdown ewes. Twenty-six of these spike trains were selected for analysis based on lack of recording artifact, containing one or more AD-identified units isolatable by the window discriminator, and a sufficient length of recording to enable one or more stimuli to be applied. Forty-two AD-identified magnocellular neuroendocrine cells were isolated from these spike trains.

Spontaneous Firing Patterns

The spontaneous activity of the cells was used to classify each cell into one of seven spontaneous firing patterns by methods modified from those described by Haskins (1976). The most numerous category was found to be the continuously active slow (CAS) firing pattern with 52.38% of the total followed by the low frequency bursting (LFB) firing pattern with 19.04%; silent and continuously active fast (CAF) firing patterns each constituted 14.28% of the total. No continuously active regular (CAR), continuously active bursting (CAB), or high frequency bursting (HFB) firing patterns were found (Table IV). Examples of the firing patterns are shown in Figure 5.

TABLE IV

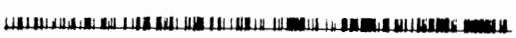
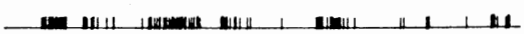
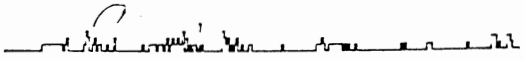
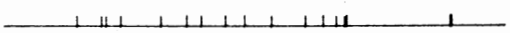
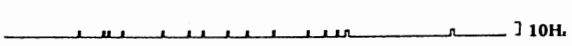
NUMBER AND PERCENT OF CELLS WITHIN SPONTANEOUS FIRING PATTERNS[1]

Spontaneous Firing Pattern	Number of Cells	Percent of Total
Silent	6	14.28
CAS	22	52.38
CAF	6	14.28
LFB	8	19.04
CAR	0	0.00
CAB	0	0.00
HFB	0	0.00

[1]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting; CAR, continuously active regular; CAB, continuously active bursting; HFB, high frequency bursting.

Figure 5. Examples of Spontaneous Firing Patterns Found Among Antidromically Identified Magnocellular Neuroendocrine Cells

Abbreviations used are: CAF, continuously active fast; LFB, low frequency bursting; CAS, continuously active slow; MFR, mean firing rate; Hz, Hertz.

Spontaneous Pattern Classification	MFR		
CAF	3.267	Unit	
		Rate	
LFB	1.567	Unit	
		Rate	
CAS	0.2833	Unit	
		Rate	 10H.

The mean firing rates (MFR) and mean high frequency intervals (HFI) were compared between spontaneous firing patterns by one-way analysis of variance and Duncan's multiple range test. As in all cases in this investigation, the spike trains were divided into one minute segments, and the individual spikes and the intervals with frequencies greater than 10 Hertz were counted in each segment.

The mean firing rate of each firing pattern was mutually different from that of each other firing pattern at the 0.05 level of significance. The silent pattern exhibited the lowest mean firing rate (0 Hz) and the CAF pattern exhibited the highest (1.8360 Hz) (Table V).

The mean HFI of the silent and CAS firing patterns were found to be significantly different from those of the CAF and LFB firing patterns, but the mean HFI of the silent firing pattern was not significantly different from that of the LFB firing pattern. The mean HFI's of the CAF and LFB firing patterns were considerably higher than that of the silent and CAS firing patterns (Table V).

Vasopressin-Evoking Stimuli

Two stimuli well known to evoke release of vasopressin are hemorrhage and intrajugular hypertonic saline infusion (Hayward, 1977). The responsiveness of AD-identified magnocellular neuroendocrine cells to 10-15% hemorrhage and/or intrajugular infusion of 1.2 molar sodium chloride was examined. Figure 6 shows the response of a cell to both hemorrhage and intrajugular infusion of 1.2 M sodium chloride.

A stimulus documented to inhibit vasopressin release is plasma volume expansion by dextran infusion (Zehr, et al., 1969). The response of a few AD-identified magnocellular neuroendocrine cells to

TABLE V

COMPARISON OF MEAN HIGH FREQUENCY INTERVALS AND MEAN FIRING RATES OF SPONTANEOUS FIRING PATTERNS[1,2]

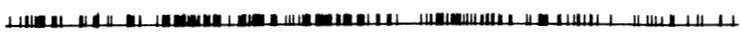
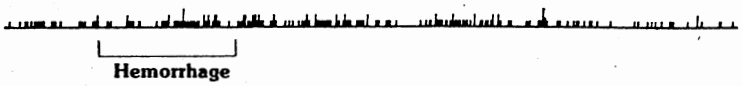
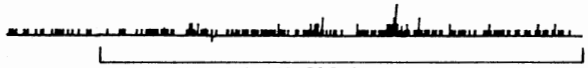
	Silent	CAS	CAF	LFB
HFI	0	1.1529	15.4583	18.3571
MFR	0	0.2127	1.8360	1.2234

[1]Any two means underscored by the same line are not different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting; HFI, high frequency intervals; MFR, mean firing rate.

Figure 6. Response of an Antidromically Identified Magnocellular Neuroendocrine Cell to Hemorrhage and Hypertonic Saline Infusion

Abbreviations used are: PS MFR, post-stimulus mean firing rate; PS HFI, post-stimulus mean high frequency intervals; Hz, Hertz.

Time (min)	PS MFR	PS HFI		
0	0.6587	0.5	Unit	
			Rate	
				Hemorrhage
16	0.5262	0.14	Unit	
			Rate	 10H.
				1.2M NaCl Infusion
				1 min.

intrajugular infusion of 6% dextran was investigated.

Hemorrhage. The response of three AD-identified cells to a 10-15% hemorrhage was examined. By the HFI definition, only one cell (33.33%) was enhanced, an LFB cell. By the MFR, three cells (100%) were enhanced, one LFB and two CAS cells. No cells were inhibited (Table VI).

Dextran. The response of four AD-identified cells to a slow intrajugular infusion of 5% dextran was examined. One cell (35.00%), a CAF pattern cell, was enhanced by dextran infusion by the HFI definition, while one cell (35.00%), a CAS pattern cell, was inhibited by the definition. No cells were affected according to the MFR definition (Table VII).

Hypertonic Sodium Chloride Infusion. The response of 22 AD-identified magnocellular neuroendocrine cells to intrajugular infusion of 1.2 M sodium chloride was investigated. By the HFI definition, eight cells (36.36%) were enhanced, and four cells (18.18%) were inhibited. By the MFR definition 10 cells (45.45%) were enhanced, and four cells (18.18%) were inhibited (Table VIII). Within each of the definitions, analysis of variance and Duncan's multiple range test showed the parameters of the enhanced, unaffected, and inhibited cells to be mutually significantly different (Table XXII and Table XXIII, Appendix).

Analyzing response according to spontaneous firing reveals that silent, CAS, and LFB cells were about equally enhanced with 33.33%, 38.46%, and 40.00%, respectively within the HFI definition. All of the CAF cells and 40.00% of the LFB pattern cells were inhibited according to the HFI definition (Table VIII). By the MFR definition the CAS

TABLE VI

RESPONSIVENESS OF CELLS TO HEMORRHAGE[1]

Spontaneous Firing Pattern	nIHF	%IFH	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enhanced			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	2	100.00	0.0238
CAF	0	0.00	--	0	0.00	--
LFB	1	100.00	7.0000	1	100.00	0.3072
All Types	1	33.33	7.0000	3	100.00	0.1183
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	0	0.00	--
CAF	0	0.00	--	0	0.00	--
LFB	0	0.00	--	0	0.00	--
All Types	0	0.00	--	0	0.00	--

[1]Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

TABLE VII

RESPONSIVENESS OF CELLS TO DEXTRAN INFUSION[1]

Spontaneous Firing Pattern	nIHF	%IFH	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enhanced			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	0	0.00	--
CAF	1	50.00	1.2500	0	0.00	--
LFB	0	0.00	--	0	0.00	--
All Types	1	25.00	1.2500	0	0.00	--
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	1	50.00	-3.2857	0	0.00	--
CAF	0	0.00	--	0	0.00	--
LFB	0	0.00	--	0	0.00	--
All Types	1	25.00	-3.2857	0	0.00	--

[1]Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

TABLE VIII

RESPONSIVENESS OF CELLS TO 1.2 M SODIUM CHLORIDE INFUSION[1]

Spontaneous Firing Pattern	nIHF	%IHF	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enhanced			
Silent	1	33.33	0.1000	1	33.33	0.1466
CAS	5	38.46	1.2647	7	58.33	0.2111
CAF	0	0.00	--	1	50.00	0.6003
LFB	2	40.00	4.6000	1	20.00	0.3650
All Types	8	36.36	1.9529	10	45.45	0.2590
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	2	16.67	-0.1943
CAF	2	100.00	-13.5000	0	0.00	--
LFB	2	40.00	- 1.3889	2	40.00	-0.9126
All Types	4	18.18	- 7.4445	4	18.18	-0.5535

[1]Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

cells were most frequently enhanced (58.33%), followed by the CAF (50.00%), silent (33.33%), and LFB (20.00%) cells. The LFB pattern cells were most frequently inhibited (40.00%), followed by the CAS cells (16.67%) (Table VIII). The mean changes in HFI were all mutually significantly different among firing patterns (Table XXIII, Appendix), while the mean change in MFR of the unaffected cells was not different from that of the inhibited cells within the CAS and LFB groups (Table XXII, Appendix).

Oxytocin-Evoking Stimuli

Two stimuli, well documented to evoke release of oxytocin, are vulvar massage and vaginal distension (Tindal, 1974). The responsiveness of AD-identified cells to single or sequential periods of vulvar massage and vaginal distension was investigated using two definitions. The MFR definition uses a 50% increase or decrease in MFR from that of a control period as the criterion for enhancement or inhibition, respectively. The HFI definition uses a 25% increase or decrease in HFI from that of a control period as the criterion for enhancement or inhibition, respectively. Figure 7 shows a silent cell's enhancement by both vaginal distension and vulvar massage by both definitions. There was no tactile response, indicating a specific response to these oxytocin-evoking stimuli. Those cells exhibiting a tactile response were excluded from analysis for responsiveness to vulvar massage and vaginal distension, being classified as nonspecific in their response.

Vaginal Distension. The responsiveness of nineteen AD-identified magnocellular neuroendocrine cells to vaginal distension was examined. By both MFR and HFI definitions, 15.79% of the cells were enhanced by

Figure 7. Response of an Antidromically Identified Magnocellular Neuroendocrine Cell to Vulvar Massage and Vaginal Distension

Abbreviations used are: PS MFR, post-stimulus mean firing rate; PS HFI, post-stimulus mean high frequency intervals; Hz, Hertz.

Time (min)	PS MFR	PS HFI		
0	0	0	Unit	
			Rate	tactile
19	1.1667	4	Unit	
			Rate	vulvar massage
21.5	0.6917	0.5	Unit	
			Rate	] 10H. vaginal distension

vaginal distension and 10.53% of the cells were inhibited (Table IX), leaving 73.68% of the cells unaffected.

Analysis by one-way analysis of variance and Duncan's multiple range test shows the change in MFR of the enhanced, unaffected, and inhibited cells to be mutually significantly different (Table XXIV, Appendix). This significance is maintained within the individual firing patterns in spite of the reduction of sample size to be analyzed in each.

The same analysis performed on the changes of HFI showed the enhanced cells were not significantly different from the unaffected, but the inhibited cells exhibited a change of HFI different from both enhanced and unaffected (Table XXV, Appendix). In this case, analysis by spontaneous firing pattern showed no significant difference between the change of HFI of any groups.

The maximum number of cells enhanced within any spontaneous firing pattern category was one cell by either definition, and the maximum number inhibited was two cells making any comparison of frequency of responsiveness between firing patterns suspect (Table IX).

Ram Odors. The responsiveness of nine AD-identified magnocellular neuroendocrine cells to one minute exposure to ram fecal odor was examined. No cell responsive to ammonium sulfide odor or acetic acid odor was included in the analysis. Two cells (22.22%) were enhanced in the HFI definition and three cells (33.33%) were enhanced in the MFR definition. Only one cell (11.11%) was inhibited in the HFI definition (Table X). Analysis by spontaneous firing pattern was hampered by the small sample size; however, one CAF cell (100%) and one LFB cell (50%) were enhanced in the HFI definition, while one LFB cell was inhibited

TABLE IX

RESPONSIVENESS OF CELLS TO VAGINAL DISTENSION[1]

Spontaneous Firing Pattern	nIHF	%IHF	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enchanced			
Silent	1	25.00	0.5000	1	25.00	0.6916
CAS	0	0.00	--	1	5.26	0.2091
CAF	1	50.00	2.0000	0	0.00	--
LFB	1	20.00	2.0000	1	20.00	1.3464
All Types	3	15.79	1.2500	3	15.79	0.9099
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	1	5.26	- 0.5000	2	10.53	-0.2536
CAF	1	50.00	-25.0000	0	0.00	--
LFB	0	0.00	--	0	0.00	--
All Types	2	10.53	-12.7500	2	10.53	-0.2536

[1]Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

TABLE X

RESPONSIVENESS OF CELLS TO RAM FECAL ODOR[1]

Spontaneous Firing Pattern	nIHF	%IHF	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enhanced			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	2	40.00	0.1396
CAF	1	100.00	1.0000	0	0.00	--
LFB	1	50.00	3.0000	1	50.00	0.1000
All Types	2	22.22	2.0000	3	33.33	0.1197
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	0	0.00	--
CAF	0	0.00	--	0	0.00	--
LFB	1	50.00	-1.0000	0	0.00	--
All Types	1	11.11	-1.0000	0	0.00	--

[1]Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

(50%). Two CAS cells (40%) and one LFB cell (50%) were enhanced in the MFR definition. No significant difference between groups were found by analysis of variance, the student's "t" test, or Duncan's multiple range test among spontaneous firing patterns. The pooled mean HFI of the enhanced group was found to be significantly different from that of the unaffected (Table XXVI and Table XXVII, Appendix).

The responsiveness of 11 AD-identified magnocellular neuroendocrine cells to one minute exposure to ram urine was examined. Only one cell (9.09%) was enhanced in the HFI definition, an LFB cell, while two cells (18.18%) were inhibited within the same definition. No cells were enhanced within the MFR definition and four cells (36.36%) were inhibited within the definition. All were CAS cells (Table XI). No significant differences were found between groups by analysis of variance, Duncan's multiple range test, or the student's "t" test (Table XXVIII and Table XXIX, Appendix).

Vulvar Massage. The responsiveness of AD-identified cells to periods of vulvar massage was investigated using both the MFR definition (plus or minus 50%) and the HFI definition (plus or minus 25%). Single or sequential periods of vulvar massage, preceeded or not preceeded by ram urine or fecal odor, were applied to the ewes and the results were analyzed. Vulvar massage replaced vaginal distension late in the investigation due to the distressed behavior exhibited by ewes to vaginal distension.

The first period of vulvar massage was analyzed, ignoring the effect of odor exposure, on 32 cells. By the MFR definition, 25.00% of the cells studied were enhanced and 15.63% were inhibited (Table XII), leaving 59.39% unaffected. The mean change in MFR of the enhanced

TABLE XI

RESPONSIVENESS OF CELLS TO RAM URINE ODOR[1]

Spontaneous Firing Pattern	nIHF	%IHF	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enhanced			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	0	0.00	--
CAF	0	0.00	--	0	0.00	--
LFB	1	100.00	3.0000	0	0.00	--
All Types	1	9.09	3.0000	0	0.00	--
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	1	12.50	-1.0000	4	50.00	-0.1111
CAF	1	50.00	-3.0000	0	0.00	--
LFB	0	0.00	--	0	0.00	--
All Types	2	18.18	-2.0000	4	36.36	-0.1111

Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

TABLE XII

RESPONSIVENESS OF CELLS TO ONE PERIOD OF VULVAR MASSAGE[1]

Spontaneous Firing Pattern	nIHF	%IHF	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enhanced			
Silent	1	25.00	26.0000	1	25.00	2.4340
CAS	2	11.76	1.0000	4	25.53	0.0650
CAF	3	60.00	9.6000	2	40.00	0.7400
LFB	1	16.67	13.5000	1	16.67	0.5333
All Types	7	21.88	8.7500	8	25.00	0.5092
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	4	23.55	- 1.6000	5	29.41	-0.1002
CAF	0	0.00	--	0	0.00	--
LFB	1	16.67	-22.0000	0	0.00	--
All Types	5	15.63	- 5.6800	5	15.63	-0.1002

Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

cells is significantly different from those of the unaffected and inhibited cells, although the mean change of MFR of the unaffected cells is not significantly different from that of the inhibited cells (Table XXX, Appendix). The mean change in HFI of the enhanced cells is also significantly higher than that of the unaffected and inhibited groups (Table XXXI, Appendix), and within the HFI definition 21.88% of the cells were enhanced and 15.63% were inhibited, leaving 62.49% unaffected (Table XII). Grouping the cells by spontaneous firing pattern shows the CAF cells to be most frequently enhanced (IHF, 60%; IMFR, 40%) and never inhibited. The CAS and LFB cells were least frequently enhanced and most frequently inhibited within the HFI definition, while the silent and LFB cells were least frequently enhanced and the CAS cells most frequently inhibited within the MFR definition (Table XII). Analysis by Duncan's multiple range test and analysis of variance showed mutual significant differences between groups among spontaneous firing patterns within both the MFR definition and the HFI definition with the exceptions of the CAS within the MFR definition and the LFB within the HFI definition (Table XXX and Table XXXI, Appendix).

Ram Odor Effect on Response to Vulvar Massage. An interesting association between two types of stimuli was noted during analysis. The responsiveness of 12 AD-identified magnocellular neuroendocrine cells to vulvar massage preceeded by exposure to ram odors was examined. Ram fecal and/or urine odor preceeded vulvar massage by approximately one minute. Within the HFI definition 25.00% of the cells were enhanced and 8.33% of the cells were inhibited, leaving 66.67% unaffected (Table XIII). This is substantially the same as vulvar massage without odor exposure. The MFR definition, however,

TABLE XIII

RESPONSIVENESS OF CELLS TO VULVAR MASSAGE PRECEDED BY ODOR[1]

Spontaneous Firing Pattern	nIHF	%IHF	$\bar{x}$	nIMFR	%IMFR	$\bar{x}$
			Enhanced			
Silent	1	100.00	8.0000	1	100.00	2.0833
CAS	1	14.29	1.0000	5	71.43	0.0650
CAF	1	33.33	2.0000	2	66.67	0.5167
LFB	0	0.00	--	1	100.00	0.4334
All Types	3	25.00	3.2500	9	75.00	0.1991
			Inhibited			
Silent	0	0.00	--	0	0.00	--
CAS	0	0.00	--	0	0.00	--
CAF	0	0.00	--	0	0.00	--
LFB	1	100.00	-22.0000	0	0.00	--
All Types	1	8.33	-22.0000	0	0.00	--

[1]Abbreviations used are: nIHF, number in category by high frequency interval definition; %IHF, percent in category by high frequency interval definition; $\bar{x}$, average; nIMFR, number in category by mean firing rate definition; %IMFR, percent in category by mean firing rate definition; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

showed 75.00% of the cells to be enhanced and none inhibited (Table XIII). This was found to be a significantly greater grequency of enhancement than that exhibited without odor exposure when analyzed by the chi square test for association.

By the HFI definition only one cell was enhanced in the silent (100%), CAS (14.29%), and CAF (33.33%) patterns, while one LFB (100%) was inhibited. By the MFR definition, however, one silent (100%), one LFB (100%), two CAF (66.67%), and five CAS (71.43%) cells were enhanced, and none were inhibited (Table XIII). Figure 8 shows the effect of odor on responsiveness to vulvar massage.

Analysis of variance showed both the mean change in HFI and mean change in MFR of all cells evoked by vulvar massage preceeded by ram odor to be higher than those evoked by vulvar massage not preceeded by odor, but this difference was not found to be significant (Table XIV).

Summary of the Effects of Oxytocin-Evoking and Vasopressin-Evoking Stimuli

According to the MFR definition, the stimulus most effective in enhancing firing rate is hemorrhage (100%) followed in descending order by 1.2 M sodium chloride infusion (45.45%), vulvar massage (34.38%), ram fecal odor (33.33%), vaginal distension (10.00%), ram urine odor (0.00%), and dextran infusion (0.00%). It should be noted that the effect of hemorrhage and dextran infusion was tested on only three cells and four cells, respectively. The CAS and CAF pattern cells are more frequently responsive to 1.2 M sodium chloride infusion and vulvar massage than silent or LFB pattern cells. The few cells enhanced by hemorrhage, dextran infusion, and vaginal distension make spontaneous

Figure 8. Response of an Antidromically Identified Magnocellular Neuroendocrine Cell to Vulvar Massage, Ram Urine Odor, and Vulvar Massage Preceeded by Ram Odor

Abbreviations used are: PS MFR, post-stimulus mean firing rate; PS HFI, post-stimulus mean high frequency intervals; Hz, Hertz.

Time (min)	PS MFR	PS HFI	
0	1.3000	7.5	Unit / Rate — vulvar massage
3.5	1.0500	4.0	Unit / Rate — urine odor
5.5	1.5417	9.5	Unit / Rate — vulvar massage; 10H.

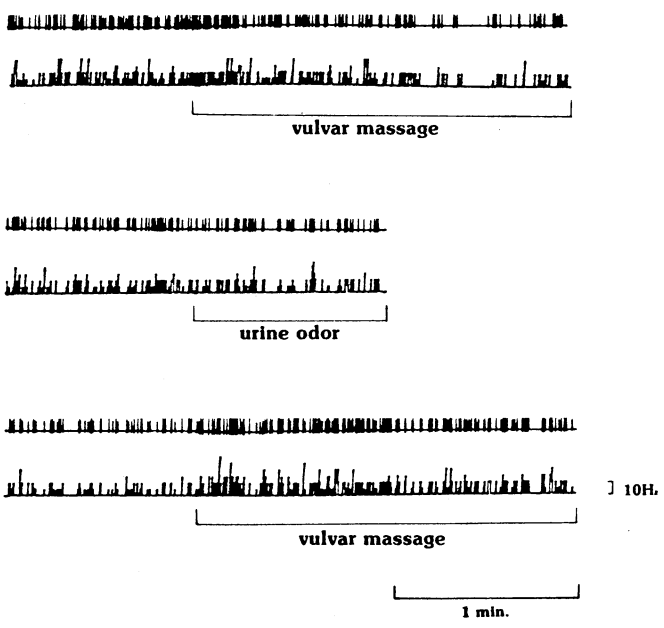


TABLE XIV

COMPARISON OF RESPONSIVENESS OF CELLS TO VULVAR MASSAGE PRECEDED BY AND NOT PRECEDED BY RAM ODORS[1,2]

	Preceded by Odor	Not Preceded by Odor
IHF	2.0526	1.3000
IMFR	0.2775	0.0746

[1]Abbreviations used are: IHF, high frequency interval definition; IMFR, mean firing rate definition.

[2]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

firing pattern comparisons of enhancement difficult. None of the stimuli inhibited cells with notable frequency, but ram urine odor inhibited cells most frequently (36.36%), followed by 1.2 M sodium chloride infusion (18.18%), vulvar massage (15.63%), vaginal distension (6.67%), dextran infusion (0.00%), ram fecal odor (0.00%), and hemorrhage (0.00%). The LFB pattern cells were most frequently inhibited (40.00%) by 1.2 M sodium chloride infusion, and only CAS pattern cells were inhibited by vulvar massage and vaginal distension (Table XV).

According to the HFI definition, the stimulus most effective in enhancing high frequency intervals is 1.2 M sodium chloride infusion (35.35%), followed in descending order by hemorrhage (33.33%), vulvar massage (28.13%), dextran infusion (35.00%), ram fecal odor (22.22%), vaginal distension (10.00%), and ram urine odor (0.09%). Again, it should be noted that the responsiveness of only three cells was tested to hemorrhage and only four cells to dextran. The LFB pattern cells were most frequently enhanced by 1.2 M sodium chloride (40.00%) and hemorrhage (100%), while the CAF pattern cells were most frequently enhanced by vaginal distension (50.00%) and vulvar massage (60.00%). The CAS pattern cells were responsive to both 1.2 M sodium chloride infusion (38.46%) and vulvar massage (23.53%), as were the silent pattern cells, 33.33% and 25.00%, respectively. Inhibition of cells to the stimuli was shown in response to 1.2 M sodium chloride (18.18%), dextran (25.00%), vaginal distension (6.66%), and vulvar massage (15.63%). The LFB (50.00%) and CAF (100%) cells were most frequently inhibited by 1.2 M sodium chloride infusion. The CAS pattern cells were most frequently inhibited by dextran infusion (50.00%) and vulvar

TABLE XV

RESPONSIVENESS OF CELLS TO ALL MAJOR STIMULI MEASURED BY MEAN FIRING RATE[1]

Spontaneous Firing Pattern	1.2 M NaCl		Hemorrhage		Dextran		Vaginal Distension		Vulvar Massage	
	n	%	n	%	n		n	%	n	%
					Enhanced					
Silent	1	33.33	0	0.00	0	0.00	1	25.00	1	25.00
CAS	7	53.85	2	100.00	0	0.00	1	5.26	6	35.29
CAF	1	50.00	0	0.00	0	0.00	0	0.00	3	60.00
LFB	1	20.00	1	100.00	0	0.00	1	20.00	1	16.67
All Types	10	45.45	3	100.00	0	0.00	3	10.00	11	34.38
					Inhibited					
Silent	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
CAS	2	16.67	0	0.00	0	0.00	2	10.53	5	29.41
CAF	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
LFB	2	40.00	0	0.00	0	0.00	0	0.00	0	0.00
All Types	4	18.18	0	0.00	0	0.00	2	6.67	5	15.63

[1]Abbreviations used are: 1.2 M NaCl, 1.2 molar sodium chloride; n, number of cells in category; %, percent of cells in category; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

massage (23.53%). The CAF pattern cells (50.00%) were most frequently inhibited by dextran infusion (50.00%) and vulvar massage (23.53%). The CAF pattern cells (50.00%) were most frequently inhibited by vaginal distension (Table XVI).

Sequential Oxytocin-Evoking and Vasopressin-Evoking Stimuli

The response of twenty-one AD-identified cells to sequential oxytocin-evoking (vulvar massage and/or vaginal distension) and vasopressin-evoking (hemorrhage and/or 1.2 M sodium chloride infusion) stimuli. Two cells were not included in the analysis due to non-specific response to tactile stimulation. Figures 9, 10, and 11 illustrate examples of specific responsiveness to oxytocin-evoking stimuli (Figure 9) and vasopressin-evoking stimuli (Figure 10), and finally responsiveness to both (Figure 11).

By the MFR definition, the largest categories were those enhanced by both oxytocin- and vasopressin evoking stimuli (26.32%) and those unaffected by any stimulus (25.32%). Oxytocin-evoking stimuli enhanced 47.37% of the cells, while vasopressin-evoking stimuli enhanced 42.00% of the cells, with 63.16% of the cells being enhanced by at least one stimulus. Subtracting those cells enhanced by both categories of stimuli, oxytocin-evoking stimuli specifically enhanced 21.05% of the cells, and vasopressin-evoking stimuli specifically enhanced 15.79% of the cells.

No CAS pattern cells were unaffected. Eighty percent were enhanced by at least one stimulus, 20% specifically enhanced by vasopressin-evoking stimuli and 30% specifically enhanced by

TABLE XVI

RESPONSIVENESS OF CELLS TO ALL MAJOR STIMULI MEASURED BY HIGH FREQUENCY INTERVALS[1]

Spontaneous Firing Pattern	1.2 M NaCl		Hemorrhage		Dextran		Vaginal Distension		Vulvar Massage	
	n	%	n	%	n	%	n	%	n	%
					Enhanced					
Silent	1	33.33	0	0.00	0	0.00	1	25.00	1	25.00
CAS	5	38.46	0	0.00	0	0.00	0	0.00	4	23.53
CAF	0	0.00	0	0.00	1	50.00	1	50.00	3	60.00
LFB	2	40.00	1	100.00	0	0.00	1	20.00	1	16.67
All Types	8	36.36	1	33.33	1	25.00	3	10.00	9	28.13
					Inhibited					
Silent	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
CAS	0	0.00	0	0.00	1	50.00	1	5.26	4	23.53
CAF	2	100.00	0	0.00	0	0.00	1	50.00	0	0.00
LFB	2	40.00	0	0.00	0	0.00	0	0.00	1	16.67
All Types	4	18.18	0	0.00	1	25.00	2	6.67	5	15.63

[1]Abbreviations used are: 1.2 M NaCl, 1.2 molar sodium chloride; n, number of cells in category; %, percent of cells in category; CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

Figure 9. Specific Enhancement of an Antidromically Identified Magnocellular Neuroendocrine Cell by an Oxytocin-Evoking Stimulus

Abbreviations used are: PS MRS, post-stimulus mean firing rate; PS HFI, post-stimulus mean high frequency intervals; Hz, Hertz.

Time (min)	PS MFR	PS HFI	
0	0.4063	0	Unit
			Rate
			tactile
1.5	0.8000	4	Unit
			Rate
			vaginal distension
10.5	0.2000	0.6	Unit
			Rate
			10Hz
			1.2M NaCl Infusion
			1 min.

Figure 10. Specific Enhancement of an Antidromically Identified Magnocellular Neuroendocrine Cell by a Vasopressin-Evoking Stimulus

Abbreviations used are: PS MFR, post-stimulus mean firing rate; PS HFI, post-stimulus mean high frequency intervals; Hz, Hertz.

Time (min)	PS MFR	PS HFI	
0	0	0	Unit Rate tactile
3	0	0	Unit Rate vulvar massage
9	0.1067	0.1	Unit Rate 10H. 1.2M NaCl Infusion 1 min.

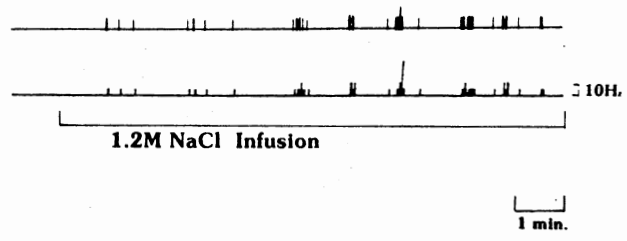
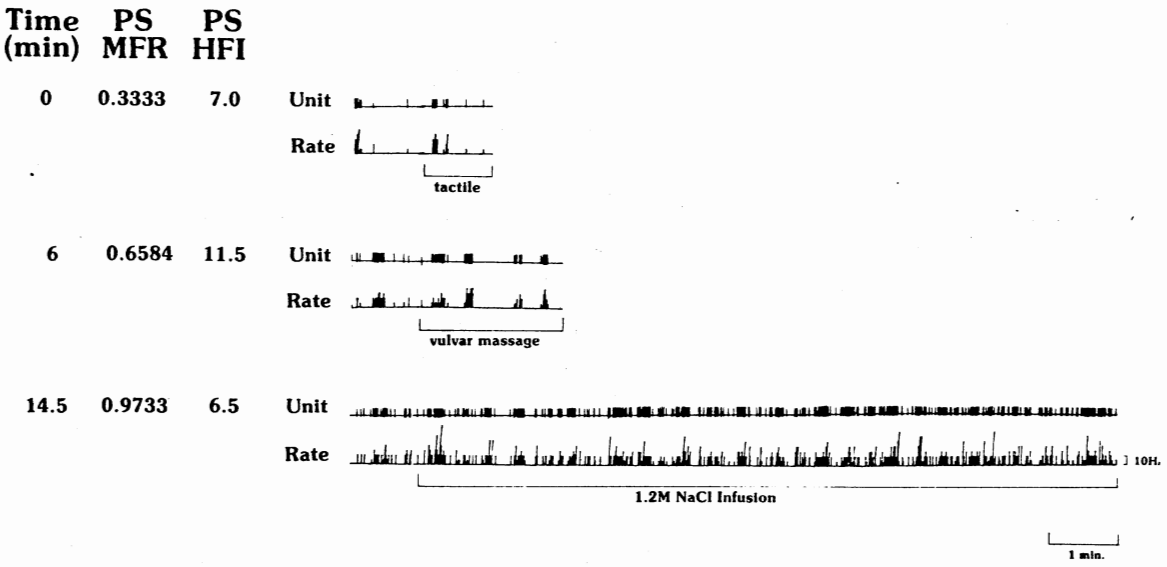


Figure 11. Enhancement of an Antidromically Identified Magnocellular Neuroendocrine Cell by Both Oxytocin-Evoking and Vasopressin-Evoking Stimuli

Abbreviations used are: PS MFR, post-stimulus mean firing rate; PS HFI, post-stimulus mean high frequency intervals; Hz, Hertz.


Time (min)
PS MFR
PS HFI
0
0.3333
7.0
Unit
Rate
tactile
6
0.6584
11.5
Unit
Rate
vulvar massage
14.5
0.9733
6.5
Unit
Rate
10H.
1.2M NaCl Infusion
1 min.

oxytocin-evoking stimuli. Twenty percent of the CAS pattern cells were specifically inhibited by vasopressin-evoking stimuli. No silent cell was enhanced by oxytocin-evoking stimuli, one was enhanced (33.33%) by a vasopressin-evoking stimulus, and 66.67% were unaffected. One CAF cell was enhanced (50.00%) by both oxytocin- and vasopressin-evoking stimuli and one was unaffected. One LFB cell (25.00%) was enhanced by both types of stimuli, one was enhanced specifically by oxytocin-evoking stimuli and inhibited by vasopressin-evoking stimuli, and two (50.00%) were unaffected (Table XVII).

By the HFI definition, 47.37% of the cells examined were enhanced by at least one stimulus. Vasopressin-evoking stimuli enhanced 26.32% of the cells, and 15.79% were enhanced specifically. Oxytocin-evoking stimuli enhanced 31.59% of the cells, and 21.06% were enhanced specifically. These are the same percentages found to be specifically enhanced as those found by the MFR definition; they are not, however, the same cells. Utilizing the HFI definition, 60% of the CAS cells were unaffected by any stimulus, 10% were specifically enhanced by oxytocin-evoking stimuli, 20% were specifically enhanced by vasopressin-evoking stimuli, and 10% were enhanced by both categories of stimuli. One (33.33%) silent cell was specifically enhanced by vasopressin-evoking, and two (66.67%) were unaffected. All CAF cells were specifically enhanced by oxytocin-evoking stimuli and inhibited by vasopressin-evoking stimuli. No LFB cells were unaffected, one cell (25.00%) was enhanced by oxytocin-evoking stimuli, one was specifically inhibited by vasopressin-evoking stimuli, and one was inhibited by both categories of stimuli (Table XVIII).

TABLE XVII

RESPONSIVENESS OF CELLS TO SEQUENTIAL OXYTOCIN-EVOKING AND VASOPRESSIN-EVOKING STIMULI MEASURED BY MEAN FIRING RATE[1]

Oxytocin Stimuli	Spontaneous Firing Pattern	Vasopressin Stimuli					
		Enhanced		Unaffected		Inhibited	
		Number	Percent	Number	Percent	Number	Percent
Enhanced	Silent	0	0.00	0	0.00	0	0.00
	CAS	3	30.00	3	30.00	0	0.00
	CAF	1	50.00	0	0.00	0	0.00
	LFB	1	25.00	0	0.00	1	25.00
	All Types	5	26.32	3	15.79	1	5.26
Unaffected	Silent	1	33.33	2	66.67	0	0.00
	CAS	1	10.00	0	0.00	2	20.00
	CAF	0	0.00	1	50.00	0	0.00
	LFB	0	0.00	2	50.00	0	0.00
	All Types	2	10.53	5	26.32	2	10.53
Inhibited	Silent	0	0.00	0	0.00	0	0.00
	CAS	1	10.00	0	0.00	0	0.00
	CAF	0	0.00	0	0.00	0	0.00
	LFB	0	0.00	0	0.00	0	0.00
	All Types	1	5.26	0	0.00	0	0.00

[1]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

TABLE XVIII

RESPONSIVENESS OF CELLS TO SEQUENTIAL OXYTOCIN-EVOKING AND VASOPRESSIN-EVOKING STIMULI MEASURED BY HIGH FREQUENCY INTERVALS[1]

Oxytocin Stimuli	Spontaneous Firing Pattern	Vasopressin Stimuli					
		Enhanced		Unaffected		Inhibited	
		Number	Percent	Number	Percent	Number	Percent
Enhanced	Silent	0	0.00	0	0.00	0	0.00
	CAS	1	10.00	1	10.00	0	0.00
	CAF	0	0.00	0	0.00	2	100.00
	LFB	1	25.00	1	25.00	0	0.00
	All Types	2	10.53	2	10.53	2	10.53
Unaffected	Silent	1	33.33	2	66.67	0	0.00
	CAS	2	20.00	6	60.00	0	0.00
	CAF	0	0.00	0	0.00	0	0.00
	LFB	0	0.00	0	0.00	1	25.00
	All Types	3	15.79	8	42.11	1	5.26
Inhibited	Silent	0	0.00	0	0.00	0	0.00
	CAS	0	0.00	0	0.00	0	0.00
	CAF	0	0.00	0	0.00	0	0.00
	LFB	0	0.00	0	0.00	1	25.00
	All Types	0	0.00	0	0.00	1	5.26

[1]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

Evoked Plasma Oxytocin and Vasopressin

A total of thirty-eight experiments were performed to determine the plasma oxytocin and vasopressin evoked by ram odors, vaginal distension, vulvar massage, hemorrhage, and 1.2 M sodium chloride infusion.

A series of sixteen experiments applied vaginal distension, hemorrhage, and hypertonic (1.2 M) saline infusion to unanesthetized ewes. Significant elevations of plasma vasopressin were found two minutes after vaginal distension (133.75% increase), two minutes post-hemorrhage (32.59% increase), during reinfusion (621.20% increase), three minutes after reinfusion (64.93% increase), and during and up to fifteen minutes after hypertonic saline infusion (193.32% to 558.50% increase). Significant elevations of plasma oxytocin were found at the end of vaginal distension (69.61% increase), five minutes after beginning hypertonic saline infusion (122.92% increase), and six minutes after completion of hypertonic saline infusion (117.56% increase) (Table XIX).

A series of twelve experiments were performed to determine the effect of ram odors on plasma oxytocin and vasopressin levels. The mean change in both oxytocin and vasopressin levels was significant, a 985.81% and 160.66% increase, respectively (Table XX).

In a series of six experiments, the effect of ram odors on plasma oxytocin evoked by vulvar massage was tested. In spite of the small number of replications, vulvar massage preceeded by odor significantly elevated plasma oxytocin levels (15.64% to 94.76% increase), but no such increase was found without odor (Table XXI).

TABLE XIX

COMPARISON OF MEAN PLASMA OXYTOCIN LEVELS WITH MEAN PLASMA VASOPRESSIN LEVELS EVOKED BY VAGINAL DISTENSION, HEMORRHAGE, AND HYPERTONIC SALINE INFUSION[1]

Event	Sample Time (min)	Mean Plasma Vasopressin (μU/ml)	Mean Plasma Oxytocin (μU/ml)
Control	0-10	0.1212	1.6224
Vaginal Distension	12	0.1362	2.7517*
Post Vaginal	14	0.2833*	1.9417
Post Vaginal	20	0.1252	1.7617
Post Vaginal	27	0.1105	1.4583
Hemorrhage	30	0.1166	1.4533
Post Hemorrhage	33	0.1607*	1.7900
Reinfusion	38	0.8741*	2.3017
Post Reinfusion	43	0.1999*	2.9567
Post Reinfusion	55	0.9940	3.1680
Post Reinfusion	59	0.1255	3.2550
Hypertonic Saline	65	0.3555*	3.6167*
Hypertonic Saline	71	0.6921*	3.4467
Post Saline	76	0.4809*	3.5767*
Post Saline	85	0.7981*	2.7333

[1]An asterisk indicates a significant difference from the control value ($P < 0.05$).

TABLE XX

MEAN PLASMA HORMONE LEVELS EVOKED BY RAM ODORS[1]

Event	Mean Plasma Oxytocin (μU/ml)	Percent Change	Mean Plasma Vasopressin (μU/ml)	Percent Change
Control	0.4586	--	0.0633	--
Ram Odor	4.9800*	985.91	0.1650*	160.66

[1]An asterisk indicated a significant difference from the control value ($P < 0.05$).

TABLE XXI

MEAN PLASMA OXYTOCIN LEVELS EVOKED BY VULVAR MASSAGE WITH AND WITHOUT EXPOSURE TO RAM ODORS[1]

Event	Sample Time (min)	Ram Odor Mean Plasma Oxytocin (μU/ml)	No Ram Odor Mean Plasma Oxytocin (μU/ml)
Control	0-10	2.5467	0.9456
Vulvar Massage	10.5	2.9450*	0.3900
Vulvar Massage	11	2.5650	0.4100
Vulvar Massage	12	4.9600*	0.4067
Post Massage	15	2.5000	2.0500
Post Massage	20	2.5250	0.5133

[1]An asterisk indicates a significant difference from the control value ($P < 0.05$).

CHAPTER V

DISCUSSION

This investigation is unique in several aspects. Currently, it is the only study which examines, in unanesthetized animals, the response of antidromically identified magnocellular neuroendocrine cells to sequential stimuli known to evoke release of oxytocin and vasopressin. Such examination provides valuable information concerning convergence of sensory stimuli on magnocellular neuroendocrine cells. This is also the only study to examine the effect of ram odors and vulvar massage on the activity of AD-identified magnocellular neuroendocrine cells.

The plasma levels of oxytocin and vasopressin associated with the stimuli used indicate convergence of the stimuli on both oxytocin- and vasopressin-containing neurons. Specifically, vaginal distension, hypertonic saline infusion, and ram odors were found to be convergent stimuli by evoking release of both oxytocin and vasopressin. Hemorrhage (10-15%) evoked significant vasopressin elevation only, but other literature indicates that 25% hemorrhage evokes elevation of both oxytocin and vasopressin (Weitzman, 1978), indicating that it, too, is a convergent stimulus. Vulvar massage may only be speculated upon at this time. No vasopressin values were obtained, but vulvar massage is possibly a convergent stimulus in terms of hormone release. The stimuli used evoke release of the neurohypophyseal hormones, but the question of the degree of specificity and convergence of the stimuli on

individual magnocellular neuroendocrine cells is not answered by hormone assays.

A question that is answered, however, is interesting in light of recent research. It has been found that synchronous EEG patterns, associated with slow wave sleep, drowsiness, or anesthesia, are required for milk ejection in rats (Lincoln, et al., 1980); however, in the unanesthetized, estradiol-implanted ewe it becomes obvious that slow wave sleep is not required for oxytocin release or neuroendocrine cell activation in response to genital stimulation since ewes exhibited an active and occasionally resentful pattern of behavior in response to vulvar massage and, particularly, to vaginal distension.

Analysis of spontaneous firing patterns of AD-identified cells revealed no continuously active regular, continuously active bursting, or high frequency bursting patterns. This agrees with the findings of Jennings, et al. (1978), further supporting the concept of only four patterns existing spontaneously among AD-identified cells in the supraoptic nucleus (SON): silent, continuously active slow (CAS), continuously active fast (CAF), and low frequency bursting (LFB). The frequency distribution of patterns found in the current study was significantly different from that observed by Jennings, et al. (1978). This may reflect a difference in the methods of searching for AD-identified cells between investigators or an effect of the estradiol implants.

Analysis of the change in mean firing rates and in mean high frequency intervals within enhanced, unaffected, and inhibited groups of cells by their respective definitions generally showed significant differences between groups. This significance shows the definitions to

be meaningful and adequate for description of the response of the AD-identified magnocellular neuroendocrine cells to stimuli in the unanesthetized, estradiol-implanted ewe. Cells were found to be somewhat more frequently enhanced according to the mean firing rate (MFR) definition than by the high frequency interval (HFI) definition, particularly the CAS cells. The MFR definition, then, seems to be more sensitive to change, but does not necessarily indicate the release of hormone by the cell. The HFI definition, while less sensitive to change, is a parameter shown to be compatible with hormone release in vitro (Dutton and Dyball, 1979).

It has been demonstrated that cells identified as "oxytocin" cells by responsiveness to suckling also respond to vasopressin-evoking stimuli in the anesthetized rat (Poulain and Wakerley, 1977), and the data from the unanesthetized ewe supports this observation. However, in the unanesthetized, estradiol-implanted ewe, response to oxytocin- or vasopressin-evoking stimuli specifically and response to the two types of stimuli nonspecifically were seen using both the HFI and MFR definitions.

The existence of inhibitory influences on some magnocellular neuroendocrine cells was demonstrated in the unanesthetized ewe. Either oxytocin- or vasopressin-evoking stimuli or both caused inhibition of some cells. Inhibition of the firing of supraoptic neuroendocrine cells associated with vasopressin-evoking stimuli has been shown in the past (Dyball and Koizumi, 1969); however, the data from the unanesthetized ewes suggest a degree of reciprocal inhibition between cells enhanced or unaffected by vasopressin-evoking stimuli and cells enhanced or unaffected by oxytocin-evoking stimuli.

Analysis of response according to spontaneous firing patterns revealed the LFB cells to be notably responsive (enhanced or inhibited) to vasopressin-evoking stimuli, and the CAF cells to be notably enhanced by oxytocin-evoking stimuli and inhibited by vasopressin-evoking stimuli. The CAS and silent cells were responsive to both types of stimuli, specifically or nonspecifically. This is supportive of the hypothesis of Jennings, et al. (1978) that LFB cells are vasopressin-containing cells. It also leads to the formation of new hypotheses: that CAF cells are oxytocin-containing cells and that CAS and silent cells are either oxytocin- or vasopressin-containing cells at a low level of excitation. This is similar to and supported by data from anesthetized rats which suggested continuous firing patterns to be associated with oxytocin-containing cells and phasic (bursting) firing patterns to be associated with vasopressin-containing cells (Brimble and Dyball, 1977; Poulain and Wakerley, 1977). LFB cells, however, have been demonstrated to pass into a CAF firing pattern under the influence of osmotic stimuli (Hayward and Jennings, 1973b). This indicates that neither firing pattern is necessarily entirely specific for one hormone.

Olfactory inputs to the supraoptic nuclei have been described (Hayward and Smith, 1963), and the odors of rams have been shown to cause oxytocin release in ewes (McNeilly and Ducker, 1972). In a single study, odor of female rats has been shown to enhance the activity of AD-identified cells of the paraventricular nucleus in anesthetized male rats (Young, 1976). While showing oxytocin and vasopressin release and enhancement of firing of magnocellular neuroendocrine cells in response to ram odors, this investigation

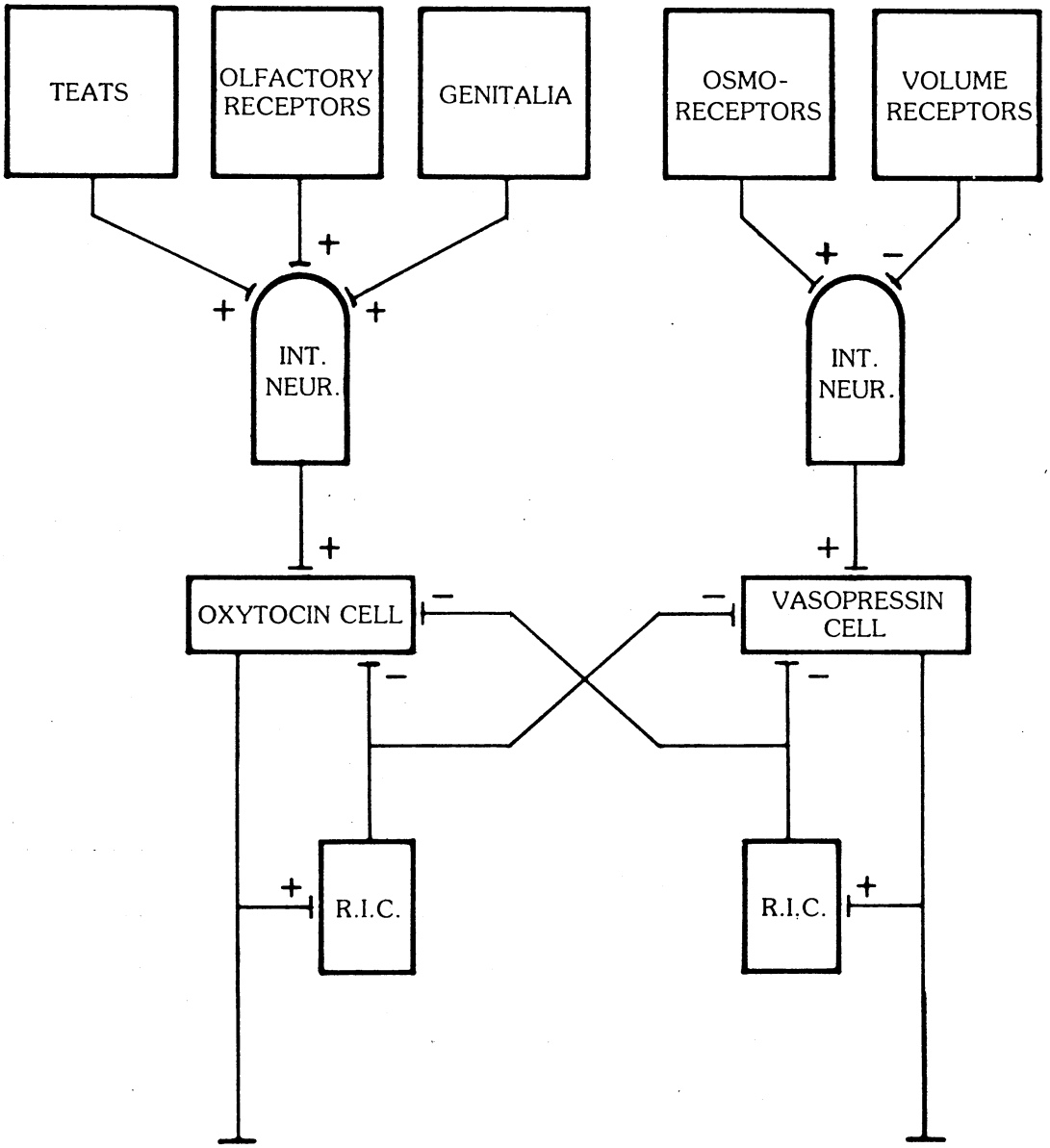
demonstrates an increased probability of cells to be enhanced by vulvar massage when the massage is preceeded by ram odor. This may be an additive effect, or odor may increase the sensitivity of neuroendocrine cell systems to subsequent appropriate stimuli.

On the basis of the data obtained in this investigation and on the basis of previous investigations a model of cellular interactions of the hypothalamo-neurohypophyseal system may be proposed (Figure 12). Excitatory input on oxytocin-containing cells includes stimulation of the teats (Poulain and Wakerley, 1977), genitalia, and olfactory receptors in appropriate fashion. Excitatory input on vasopressin-containing cells includes osmotic and volume receptor stimulation in appropriate fashion. Recurrent inhibitory collaterals have been demonstrated by antidromic inhibition (Kandel, 1964; Kelly and Dreifuss, 1970; Koizumi and Yamashita, 1972; Hayward and Jennings, 1973a). Inhibition and reciprocal inhibition have been demonstrated in this study. Convergence of the various stimuli is evident, and may be a result of converging neural pathways (not shown in Figure 12) or of a rebound excitation following inhibition. Rebound excitation following a five second intracarotid injection of hypertonic sodium chloride has been demonstrated in AD-identified neuroendocrine cells in the unanesthetized monkey (Hayward and Jennings, 1973b).

It also may be that the release of both oxytocin and vasopressin in response to several stimuli is a response to relatively high stimulus intensity. The attempt in this investigation to apply stimuli of an intensity likely to be normally encountered by sheep may have been the cause of a demonstrated specific response of some AD-identified cells to only one type of stimulus. Other investigators have

Figure 12. Simplified Model of Cellular Interactions of the Hypothalamo-Neurohypophyseal System

Abbreviations used are: Int. Neur., interneurons; R.I.C., recurrent inhibitory collateral.


TEATS
OLFACTORY RECEPTORS
GENITALIA
OSMO-RECEPTORS
VOLUME RECEPTORS
INT. NEUR.
INT. NEUR.
OXYTOCIN CELL
VASOPRESSIN CELL
R.I.C.
R.I.C.

demonstrated that some AD-identified cells respond to suckling, an oxytocin-evoking stimulus, while others do not. They also demonstrated that all AD-identified cells respond to intraperitoneal injection of hypertonic saline (Poulain and Wakerley, 1977). This could be a demonstration that suckling is a stimulus of normal intensity while intraperitoneal hypertonic saline injection is a stimulus of high intensity.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The unanesthetized, estradiol-implanted Southdown ewe was used to evaluate oxytocin and vasopressin release and supraoptic magnocellular neuroendocrine cell responsiveness to stimuli known to alter plasma oxytocin and vasopressin levels. Hormone levels were measured by radioimmunoassay by Dr. Richard Weitzman. The activity of antidromically-identified supraoptic cells was recorded from ewes stereotaxically implanted with pituitary stimulating electrodes and a bone wax-filled cylinder directly above the supraoptic nuclei by the techniques described by Haskins (1976). The stimuli applied included ram odors, vulvar massage, vaginal distension, intrajugular infusion of six percent dextran and 1.2 M sodium chloride, and 10-15% hemmorhage.

All stimuli with the exceptions of hemmorhage and vulvar massage were shown to cause significant elevations of both plasma oxytocin and plasma vasopressin. Hemmorhage caused significant elevation of plasma vasopressin only, while vulvar massage caused significant elevation of plasma oxytocin with no vasopressin levels measured. This indicates a degree of convergence of stimuli upon the hypothalamo-neurohypophyseal system. Hemorrhage (10-15%) and vulvar massage may exhibit some specificity for vasopressin release or oxytocin release, respectively. It was also shown that vulvar massage was most effective in causing elevation of plasma oxytocin when preceeded by ram odors.

Enhancement and inhibition were defined in two ways, using mean firing rate and using intervals with rates greater than 10 Hz. These definitions were found to be statistically valid. According to these definitions all stimuli except dextran infusion enhanced or inhibited some cells. Among cells exposed to sequential oxytocin-evoking (vulvar massage, vaginal distension) and vasopressin-evoking (hemorrhage, hypertonic saline infusion) stimuli, specific enhancement by each type of stimulus and nonspecific enhancement by both types of stimuli were shown. This indicates a degree of convergence and of specificity in the response of the SON magnocellular neuroendocrine cells to the stimuli applied in this investigation. Inhibition of cells by both oxytocin- and vasopressin-evoking stimuli was observed, suggesting a degree of reciprocal inhibition between oxytocin- and vasopressin-containing cells.

Antidromically-identified neuroendocrine cells were classified by spontaneous firing pattern. Only four types were found: silent, continuously active slow (CAS), continuously active fast (CAF), and low frequency bursting (LFB). This agrees with a prior investigation in the unanesthetized ewe (Jennings, et al., 1978), but differs in frequency distribution of the firing types. Of these types, the LFB cells were frequently responsive to vasopressin-evoking stimuli, and the CAF cells were most frequently enhanced by oxytocin stimuli. This indicates a possibility that the LFB pattern may be primarily associated with vasopressin-containing cells and that the CAF pattern may be primarily associated with oxytocin-containing cells.

Another noteworthy finding was that exposure to ram odors prior to vulvar massage significantly increased the frequency of enhancement of

the AD-identified cells to vulvar massage. This may be interpreted as an additive effect of the two stimuli or as a pre-conditioning of the cells resulting in an increased sensitivity to subsequent appropriate stimuli.

The data obtained in this investigation and in previous investigations were used to propose a model of cellular interaction of the hypothalamo-neurohypophyseal system.

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APPENDIX

STATISTICAL ANALYSIS OF DEFINITIONS OF ENHANCEMENT AND INHIBITION

TABLE XXII

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO 1.2 M SODIUM CHLORIDE INFUSION MEASURED BY MEAN FIRING RATE[1,2]

	Enhanced	Unaffected	Inhibited
Silent	0.1466	0.0000	---[3]
CAS	0.2111	0.0150	0.1943
CAF	0.6003	---[3]	---[3]
LFB	0.3650	- 0.6644	- 0.9126
All Types	0.2590	- 0.2435	- 0.5535

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXIII

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO 1.2 M SODIUM CHLORIDE INFUSION MEASURED BY HIGH FREQUENCY INTERVALS[1,2]

	Enhanced	Unaffected	Inhibited
Silent	0.1000	0.0000	---[3]
CAS	1.2647	- 0.5000	---[3]
CAF	---[3]	---[3]	-13.5000
LFB	4.6000	0.2000	- 1.3889
All Types	1.9529	- 0.2300	- 7.4445

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXIV

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO VAGINAL DISTENSION MEASURED BY MEAN FIRING RATE[1,2]

	Enhanced	Unaffected	Inhibited
Silent	0.6916	0.0000	---[3]
CAS	0.2091	0.0058	- 0.2536
CAF	---[3]	---[3]	---[3]
LFB	1.3464	0.0000	---[3]
All Types	0.9099	0.0056	- 0.2536

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXV

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO VAGINAL DISTENSION MEASURED BY HIGH FREQUENCY INTERVALS[1,2]

	Enhanced	Unaffected	Inhibited
Silent	0.5000	0.0000	---[3]
CAS	---[3]	0.0000[3]	- 0.5000[3]
CAF	2.0000[3]	---[3]	-25.0000[3]
LFB	2.0000[3]	0.0000[3]	---[3]
All Types	1.2500	- 0.1111	-12.7500

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXVI

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO RAM FECAL ODOR MEASURED BY MEAN FIRING RATE[1,2]

	Enhanced	Unaffected	Inhibited
Silent	---[3]	---[3]	---[3]
CAS	0.1396	0.0000[3]	---[3]
CAF	---[3]	- 0.0166[3]	---[3]
LFB	0.1000	0.0547	---[3]
All Types	0.1197	- 0.0494	---[3]

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXVII

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO RAM FECAL ODOR MEASURED BY HIGH FREQUENCY INTERVALS[1,2]

	Enhanced	Unaffected	Inhibited
Silent	---[3]	---[3]	---[3]
CAS	---[3]	0.0000[3]	---[3]
CAF	1.0000[3]	---[3]	---[3]
LFB	3.0000[3]	- 1.0000[3]	- 2.0000[3]
All Types	2.0000	- 0.2857	- 2.0000[3]

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXVIII

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO RAM URINE ODOR MEASURED BY MEAN FIRING RATE[1,2]

	Enhanced	Unaffected	Inhibited
Silent	---[3]	---[3]	---[3]
CAS	---[3]	- 0.0878[3]	- 0.1111[3]
CAF	---[3]	0.3750[3]	---[3]
LFB	---[3]	0.0500[3]	---[3]
All Types	---[3]	- 0.0899	- 0.1111

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXIX

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO RAM URINE ODOR MEASURED BY HIGH FREQUENCY INTERVALS[1,2]

	Enhanced	Unaffected	Inhibited
Silent	---[3]	---[3]	---[3]
CAS	---[3]	- 0.1667[3]	- 1.0000[3]
CAF	---[3]	0.0000[3]	- 3.0000[3]
LFB	3.0000[3]	---[3]	---[3]
All Types	3.0000	- 0.1667	- 2.0000

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXX

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO VULVAR MASSAGE MEASURED BY MEAN FIRING RATE[1,2]

	Enhanced	Unaffected	Inhibited
Silent	2.4340	0.0000	---[3]
CAS	0.0650	- 0.0025	- 0.1002
CAF	0.7400	- 0.0985	---[3]
LFB	0.5333[3]	0.0772[3]	---[3]
All Types	0.5092	- 0.0040	- 0.1002

[1]Any two means underscored by the same line are not significantly different. Any two means not underscored by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

TABLE XXXI

COMPARISON BETWEEN SPONTANEOUS FIRING PATTERNS OF MEAN RESPONSIVENESS TO VULVAR MASSAGE MEASURED BY HIGH FREQUENCY INTERVALS[1,2]

	Enhanced	Unaffected	Inhibited
Silent	26.0000	0.0000	---[3]
CAS	1.0000	0.0000	- 1.6000
CAF	9.6000	- 2.0000	---[3]
LFB	13.5000	-14.0000	-22.0000[3]
All Types	8.7500	- 1.7241	- 5.6800

[1]Any two means underscored by the same line are not significantly different. Any two means not underscroed by the same line are significantly different ($P < 0.05$).

[2]Abbreviations used are: CAS, continuously active slow; CAF, continuously active fast; LFB, low frequency bursting.

[3]An insufficient number of cells were present in this category to permit statistical analysis.

VITA[2]

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