RESPONSE OF SUPRAOPTIC NEUROENDOCRINE CELLS TO LINEAR CHANGES OF PLASMA OSMOLALITY IN UNANESTHETIZED SHEEP

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1969

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1976

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ACKNOWLEDGEMENTS

Sincere appreciation is extended to Dr. David P. Jennings for serving as major adviser during my graduate studies and for providing guidance, facilities and support for the research described in this dissertation.

Further gratitude is expressed to Dr. John H. Venable for his support and council while serving as chairman of my advisory committee.

The author is grateful also for encouragement and assistance provided by Drs. Calvin G. Beames, Richard J. Orts and Paul A. McCollum.

Appreciation is expressed to the Department of Medicine and Surgery for suggestions and cooperation in anesthesia, surgery and especially radiological support throughout the course of this study. I am thankful also for the computer facilities provided by the School of Mechanical and Aerospace Engineering.

Financial aid and support for this study were provided by the National Science Foundation (Summer Research Award, 1974), USPHS GRS RR05567, NIH NS11978 and the Department of Physiological Sciences.

Finally, a very special expression of appreciation is extended to my wife Vera and daughter Kerri. Without their encouragement and understanding this endeavor would not have been possible.

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CHAPTER I

INTRODUCTION

Hypothalamic regulation of hormonal secretions from the hypophysis is well documented and constitutes so-called "neuroendocrine systems" (Beyer and Sawyer, 1969; Cross, 1973). However the neuronal mechanisms responsible for the control and release of hormone from the mammalian hypothalamus have not been identified. A major assumption motivating this investigation is that an understanding of the magnocellular neuroendocrine system controlling blood antidiuretic hormone and oxytocin levels will provide insight to basic mechanisms which underly operation of all neuroendocrine systems. Furthermore it seems inevitable that gross neuroanatomical analogies will give way to detailed explanations at the functional level of neuroendocrine cells, including their electrophysiology and functional coordination with other neuroendocrine cells, interneurons and receptors.

As early as 1947, Verney demonstrated that an increase in the osmolality of brain blood could elicit the release of antidiuretic hormone (ADH). This implied the existence of specialized neurons, within the brain, sensitive to changes in osmotic pressure. It now seems certain that the neuroendocrine system responsible for synthesis and release of both ADH and oxytocin is localized within the supraoptic nucleus (SON), internuclear zone of Greving (INZ) and the paraventricular nucleus (PVN) (Sachs and Takabatake, 1964; Sachs, et al., 1969; Thorn, 1970; Hayward,

1975; Jewell and Verney, 1957).

Neurons of the SON and PVN were the first hypothalamic units to be recorded (Cross and Green, 1959); since they are readily identifiable (Yagi, et al., 1966), neuroendocrine cells of these nuclei have often been used to associate neural activity with hormone release.

Numerous differences of nomenclature are present in the literature pertaining to the cells of the SON, INZ and PVN. As early as 1960 Ortman called these cells neurosecretory cells and defined them as true neurons with axons and dendrites in which secretory activity could be morphologically demonstrated. Dellman (1973) stated that the main function of neurosecretory neurons was to produce and release chemical mediators which reach their target organs indirectly through the blood circulation or directly through synaptoid contacts. These definitions could conceivably then include all neurons as being neurosecretory. Dellman went on to point out that in "classic" neurosecretory neurons the chemical mediators are octapeptides and truly hormonal in character. Cross, et al. (1975) introduced the term endocrine neuron to emphasize concern only with hormone forming nerve cells that pass their products into the vascular system. Probably the most specific definition is that used by Hayward and Jennings (1973a). They called the neurosecretory cells of the SON, INZ, and PVN magnocellular neuroendocrine cells. This definition is particularly useful since it provides a distinction between this neuroendocrine system and that which is involved in synthesis, transport and secretion of hypothalamic releasing factors and adenohypophysial regulation, namely the parvocellular neuroendocrine system.

Hayward and Jennings (1973a) recorded single unit activity of magnocellular neuroendocrine cells in the hypothalamus of unanesthetized

monkeys and observed three basic patterns of spontaneous activity: silent, continuously active and low frequency bursting. However it was not known if these represented functional cell types or merely different levels of excitatory drive. In isolated rat neurohypohyses Dreifuss, et al. (1971) noted that a given number of action potentials were most effective in releasing hormone when they occurred close together in time implying that rate is more important than the total number of spikes (action potentials). Wakerley and Lincoln (1973) in a similar study in urethane anesthetized rats concluded that an explosive burst of paraventricular unit activity brought on the release of oxytocin just prior to milk ejection. Furthermore, Hayward and Jennings (1973b) noted that a five second pulse of hypertonic sodium chloride into the common carotid artery of unanesthetized monkeys evoked a burst of activity followed by inhibition in identified supraoptic cells. It is surprising in view of these findings that most conclusions drawn are still based on analysis of mean firing rate which may not be of great physiological importance (Uttall, 1972).

To date the behavior of identified supraoptic magnocellular neuroendocrine cells has not been studied during a slow continuous rise in plasma osmolality. The research in this dissertation is designed to test the following hypotheses, in unanesthetized sheep:

1. the spontaneous firing patterns of magnocellular neuroendocrine cells previously recorded in the rat and monkey are exhibited by magnocellular neuroendocrine cells of the unanesthetized sheep;
2. neuroendocrine cells do not exhibit all firing patterns of neurons recorded in hypothalamic and septal areas;
3. the activity of a magnocellular neuroendocrine cell evoked by

osmotic drive is inversely related to cell size of the magnocellular neuroendocrine cell in question;

4. that activity patterns exhibited by magnocellular neuroendocrine cells are dynamically and/or tonically related to changes in plasma osmolality; and

5. a single neuroendocrine cell can exhibit all patterns of activity found in supraoptic neuroendocrine cells depending on the level of osmotic stimulation.

Since behavioral state seems to be an important factor in regulation of ADH (Verney, 1947; Hayward and Jennings, 1973d), the study of neuronal activity in an unanesthetized animal appears imperative. Almost all studies done previously have been conducted on anesthetized animals; however most anesthetics depress pituitary secretion (Beyer and Sawyer, 1969). Conversely, urethane, one of the most commonly used anesthetics in neurophysiological research, produces high blood levels of ADH and milk ejection activity, possibly by a direct action on neurosecretory nerve terminals (Dyball, 1971; Dyball and Dyer, 1971). Ginsburg and Brown (1957) noted that urethane altered the ratio of oxytocin and ADH in the neural lobe as well as increased plasma levels of these hormones. Furthermore anesthetics often block the milk ejection reflex and therefore any response to teat stimulation may be unspecific. Low doses of urethane depress the number of active hypothalamic units (Hayward and Vincent, 1970). Spontaneous firing patterns in urethane anesthetized rats were poorly correlated with blood ADH and milk ejection activity (Dyball, 1971). In fact, Hayward and Vincent (1970) noted that about one-half the anesthetizing dose of urethane converts continuously active magnocellular neuroendocrine cells of the SON to silent phases in the

unanesthetized monkey and depresses their responsiveness to osmotic stimuli. Later studies (Novin and Durham, 1973) achieved similar results when the investigators concluded that urethane blocked the reflex activation of magnocellular neuroendocrine cells to orthodromic stimuli possibly by stimulation of a central monoaminergic pathway. Antidromic facilitation in the PVN was also blocked but antidromic inhibition was not. Therefore, although informative, the use of anesthetized preparations in the study of hypothalamic unit activity presents serious drawbacks. For these reasons considerable effort was expended in order to record from magnocellular neuroendocrine cells in unanesthetized behaving sheep.

An intracarotid pulse of hypertonic solution is a good means of evoking the release of ADH and the excitation of single neurons although it suffers from serious disadvantages. Holland, et al. (1959) demonstrated that a pulse injection of hypertonic sodium chloride altered both blood pressure and EEG pattern. These changes may bring about a non-specific unit response. It also releases oxytocin (Abrahams and Pickford, 1954) and may induce sympathetic nervous system discharge (Holland, et al., 1959). Normal water and electrolyte balance does not involve such dramatic changes in osmolality. In addition, osmolalities at the osmoreceptive zone are impossible to measure and therefore a quantitative relation between neuronal firing and plasma osmolality cannot be developed. Therefore the problem of magnocellular neuroendocrine cell control of ADH was approached using changes in plasma osmolality which could be quantitated.

The technique of single unit recording although suffering from disadvantages in acquiring adequate sample sizes has the advantage that

results are easy to interpret since there is no doubt that they represent action potentials generated by neurons close to the recording microelectrode (Morrell, 1967). It therefore seems reasonable that recording of single neuron activity should be the method of choice for studying hypothalamic cells and their responses to environmental changes.

CHAPTER II

REVIEW OF LITERATURE

This chapter is not intended to represent an exhaustive review of the literature. The intent is rather to introduce the reader to the problem and to familiarize him with the current status in this area of investigation. Sections have been included which are meant to introduce the reader to humoral and neuronal mechanisms of the maintenance of ADH levels.

Structure and Organization of the Supraoptic Nucleus

Magnocellular neuroendocrine cells of the richly vascularized supraoptic nucleus evolve from the paired nucleus preopticus, pars magnocellularis, of cyclostomes and fish (Crosby and Showers, 1969). In many animals the SON consists of two to four parts linked by strands of scattered cells (clustered around and along blood vessels) between the nucleus proper and the PVN. In general, the main portion of the SON follows the dorsolateral border of the optic chiasm and optic tract. Another portion lies at the ventromedial edge of the optic tract (Haymaker, et al., 1969).

Eränko (1951) and later Rechardt (1969) demonstrated the presence of both light and dark cells in the SON, the difference being the number of free ribosomes (Rechardt, 1969). The light cells are larger and circular

or oval with large pale eccentric nuclei. The smaller dark cells, relatively few in number, are scattered among the light cells and have a fusiform shape with smaller and less electron dense nuclei.

Vesicles of neurosecretory material are found in the somata, axons and terminals of supraoptic magnocellular neuroendocrine cells and have diameters of 1000 to 3000 angstroms (Sachs, et al., 1969; Dyer, et al., 1973). These large neurosecretory vesicles are present in addition to common cellular organelles. They may contain ADH or oxytocin, and carrier proteins, neurophysin-II or -I, respectively (McNeilly, et al., 1972a,b). Synthesis and liberation of these peptide mediators led to their classification as peptidergic neurons (Thorn, 1970; Dellman, 1973).

A characteristic of these peptidergic neurons is the presence of large (two microns or more) dilatations. These Herring bodies may contain various types of vesicles, lysosomes and tubular formations. The significance of Herring bodies is still in doubt although an autophagic process seems likely (Dellman, 1973).

By following retrograde degeneration in the SON after section of the infundibular stalk in the monkey, Magoun and Ranson (1939) concluded that the main body of each SON contained 30,000 to 40,000 cells. In addition they observed that eighty per cent of the cells were lost following sections that were placed through the median eminence whereas only seventy per cent were lost following transection below the median eminence. This localization of magnocellular neuroendocrine cell terminals in the median eminence has been confirmed by recent immunocytochemical techniques which have shown that in addition to forming the supraopticohypophysial tract, axons of supraoptic magnocellular neuroendocrine cells also terminate in both the zona interna and zona externa of the median eminence and border

on the primary portal plexus in man, monkey, cow, guinea pig and mouse (Silverman and Zimmerman, 1975; Zimmerman, et al., 1974).

Synthesis, Transport, Storage and Release of Neurohypophysial Hormones

The concept that ADH and its carrier protein neurophysin-II are synthesized in neurons of the SON, INZ and PVN of the hypothalamus is well established (Bargmann and Scharrer, 1951; Sachs and Takabatake, 1964; Fawcett, et al., 1968; Sachs, et al., 1969; and Thorn, 1970). The unmyelinated nerve fibers forming the hypothalamo-hypophysial tract are practically all derived from the supraoptico-hypophysial tract (Sachs, et al., 1969; Thorn, 1970). In the neurohypophysis, terminals of these fibers are separated from capillary basement membranes by well defined perivascular spaces which are characteristic of secretory organs in general. It seems certain that the initial step in the ADH neurosecretory process, biosynthesis and packaging of ADH into neurosecretory vesicles (Bargmann and Scharrer, 1951), occurs in the somata of neurons of the supraoptico-hypophysial tract and involves a protein molecule (Sachs and Takabatake, 1964). In vitro studies (Sachs, 1967) have shown that hypothalamic tissue can incorporate cysteine-^{35}S into protein while the distal hypothalamic neurohypophysial system does not, implying that axons cannot synthesize neurosecretory material.

Isotope studies have provided the most evidence for a precursor model of ADH synthesis (Sachs, 1967; Sachs, et al., 1969; Cross, et al., 1975). After infusion of cysteine-^{35}S into the third ventricle, labeled ADH is not found in association with ribosomes but appears packed in granules ready for transport after a delay of ninety minutes between the

incorporation of label into peptide and the appearance of ADH (Jones and Pickering, 1972; Sachs, 1967). In fact, puromycin if given prior to the cysteine-^{35}S will prevent the appearance of radioactive hormone.

Sachs, et al. (1969) suggested that the biosynthetic process forming a precursor of ADH also forms a neurophysin precursor, and that the release of octapeptide takes place in the maturation of the large (1000 to 3000 angstroms) neurosecretory vesicles. As stated by Cross, et al. (1975), three criteria should be met if hormone and neurophysin arise from the same process. One, it does appear that synthesis rates for ADH and neurophysin are the same under all conditions, and two, claims of specific neurophysins for each neurohypophysial hormone are now well founded (McNeilly, et al., 1972a,b; Dean, et al., 1968). Only the third prediction needs substantiation, that of an active neurosecretory vesicle enzyme "maturase" which promotes the conversion of the precursor to hormone and neurophysin. The function of neurophysin is currently unknown but since hormone and neurophysin associate readily it may function as a carrier molecule to keep the hormone within the vesicles as they are transported down the axons and are stored in the magnocellular neuroendocrine cell terminals (Ginsburg, 1968).

The stimulus to hormone synthesis is a point of active investigation. It does not appear that an acute secretory stimulus causes an increase in biosynthetic activity but a prolonged chronic stimulus such as dehydration may increase ADH synthesis (Sachs, et al., 1969).

Whatever the origin of the material found in the neurosecretory vesicle it must be transported to the magnocellular neuroendocrine cell terminals before it can be released into the blood circulation. This second step of the neurosecretory process may be simply protoplasmic flow

(Bargmann and Scharrer, 1951), pressure exerted by hormone synthesis in the soma, or pulsation of the oligodendroglia (Sloper, 1966). A more likely explanation includes a neurotubular function since colchicine has been shown to block axonal flow in the magnocellular neurohypophysial system (Flament-Durand and Dustin, 1972). The rate of transport nevertheless is very rapid, neurosecretory material accumulating in the posterior pituitary only one-half hour after initiation of transport (Jones and Pickering, 1972; Norstrom and Sjostrand, 1971), implying a rate of one to four millimeters per hour. Fast axoplasmic flow in the range of 40- to 400 mm per day has now been demonstrated in several systems (Lubinska, 1975).

The last step in the neurosecretory process outlined by Bargmann and Scharrer (1951) is release of the hormone into the blood circulation upon appropriate stimulation. The stimulus for ADH release is action potential generation and conduction in the magnocellular neuroendocrine cell by common sodium dependent and tetrodotoxin sensitive spike mechanisms (Dreifuss, et al., 1971). Evidence has now accumulated which indicates that calcium is essential for the excitation-secretion coupling (Douglas and Poisner, 1964; Russell and Thorn, 1974a,b; Nakazato and Douglas, 1974; Müller, et al., 1975). Calcium is essential whether terminal depolarization occurs by electrical stimulation or high extracellular potassium concentrations (Douglas and Poisner, 1964; Fawcett, et al., 1968; Dreifuss, et al., 1971).

The bulk of a neurosecretory vesicle is made up of neurophysin-hormone complexes (Fawcett, et al., 1968). The observation that small changes in calcium concentrations detach ADH from its protein carrier neurophysin-II led to elaboration of the complex dissociation hypothesis

for release of ADH occurring at deploarization of the magnocellular neuroendocrine cell terminal (Ginsburg and Ireland, 1966). Under this hypothesis calcium detaches the ADH from its carrier and the hormone then passes through the barriers into the blood stream.

Probably the most tenable hypothesis is the cellular mechanism for ADH release. According to this theory an action potential at the magnocellular neuroendocrine cell terminal triggers reverse pinocytosis. Contents of the neurosecretory vesicle are discharged to the exterior while the vesicular membrane is retained (Douglas, 1968). There does not appear to be a fixed ratio of hormone to neurophysin release (Fawcett, et al., 1968), although recent evidence indicates that the release of neurophysin does parallel that of ADH in response to a stimulus for ADH release (Cheng, et al., 1972a,b; Forsling, et al., 1973; McNeilly, et al., 1972a,b). This may reflect free ADH and neurophysin in the cytoplasm (Barer, et al., 1963; Ginsburg, 1968), or differential binding by the neurophysin molecule (Wuu and Saffran, 1969).

Evidence for the cellular mechanism of ADH release is both chemical and morphological. Electron microscope studies in the hamster (Douglas, et al., 1971) and in the rat (Dreifuss, et al., 1974; Dempsey, et al., 1974) have revealed exocytotic images. Edwards, et al. (1973) using the enzymes lactic acid dehydrogenase and adenylate kinase as markers of axoplasm revealed that a strong stimulus for the release of ADH did markedly increase plasma ADH with no increase in either lactic acid dehydrogenase or adenylate kinase.

Data have also been presented that indicate distribution of ADH in the magnocellular neuroendocrine cell terminal into at least two pools. This material has been reviewed by Cross, et al. (1975) and only a

cursory view will be presented here. Pulse labelling techniques indicate that most of the hormone is present in the larger less accessible pool. Acute stimulation elicits the release of only a small portion of the readily accessible pool which amounts to approximately two percent of the total hormone in the neural lobe.

Preferential Localization of Antidiuretic Hormone and Oxytocin Within the Supraoptic Nucleus

Recent evidence indicates that in at least two species (rat and cow) ADH- and oxytocin-producing neurons are distributed preferentially in both the supraoptic and paraventricular nuclei (Swaab, et al., 1975; Vandesande and Dierickx, 1975). Immunoenzyme stains of both magnocellular nuclei revealed that oxytocin containing cells were localized more in the rostral part and ADH containing cells more in the caudal part.

In addition Vandesande and Dierickx (1975) found that both hypothalamic magnocellular nuclei of the rat contained ADH and oxytocin neurons in about the same number. Swaab, et al. (1975) observed also in the rat that the two hormones are found in both nuclei in similar percentages but due to the large size of the supraoptic nucleus it had about 2.5 more oxytocin containing cells. These data do not support the classical view of a functional separation of the supraoptic nucleus and paraventricular nucleus.

Control of Antidiuretic Hormone Release

Osmotic

In 1947 Verney demonstrated that short term infusions (5-20 seconds) of hypertonic sodium chloride into the common carotid artery would

decrease the urine flow rate of a conscious dog in a water diuresis. He proposed that the release of ADH from the neurohypophysis into the blood was triggered by osmoreceptors located somewhere in the brain (anterior hypothalamus) in the distribution of the common carotid artery. Verney also hypothesized that the osmoreceptors were not stimulated by a change in body fluid tonicity per se but by a change in extracellular fluid tonicity which in turn affected the volume of the receptors. Injections of hypertonic sodium salts, sucrose, and glucose caused an antidiuresis in hydrated dogs while hypertonic urea had no effect due to its high diffusibility or to damage to the blood brain barrier (Hayward and Jennings, 1973c).

Later work using the techniques of electrical stimulation (Harris, 1955), arterial ligation (Jewell and Verney, 1957), hypothalamic deafferentation (Sundsten and Sawyer, 1961; Woods, et al., 1966), and single unit recording (Hayward and Vincent, 1970), narrowed the osmoreceptor zone to the SON and its immediate perinuclear zone.

Andersson, et al. (1967) used injections into the third ventricle of goats to study regulation of ADH secretion. They found that both hypertonic sodium chloride and ammonium chloride produced antidiuretic effects and presumably an increase in ADH release. In addition, the antidiuretic effect of ammonium chloride was greater than sodium chloride and there was no effect of d-glucose. A similar study (Olsson, 1969) showed that an intraventricular infusion of sucrose had no effect on water diuresis or urine osmolality in female goats and that the antidiuretic effect of ammonium chloride remained even when an isotonic solution was administered. A later study in sheep (Olsson and MacDonald, 1970) demonstrated an antidiuresis when hypertonic sodium chloride was given via the carotid

artery. However this osmotic stimulus could be overridden in the presence of a water diuresis.

Shimizu, et al. (1973) established a quantitative relationship between plasma osmolality and plasma ADH. Using pentobarbital anesthetized dogs these investigators observed that plasma ADH increased progressively in response to an increasing plasma osmolality caused by an intrajugular infusion of hypertonic sodium chloride. A rectilinear relationship was found to exist between plasma ADH and plasma osmolality in the range studied (270-330 mOsm/kg). A similar relationship has been observed in unanesthetized rats (Dunn, et al., 1973), where plasma ADH increased two to four times with each 1% increase in plasma osmolality.

A highly significant and direct correlation between plasma osmolality and plasma ADH has been observed in healthy recumbent adults (Robertson, 1974). A 1% change in plasma osmolality was sufficient to evoke a 1 pg/ml change in plasma ADH. Changes of this magnitude are large enough to cause pronounced changes in urine osmolality. A significant correlation between plasma ADH and urine osmolality was also found.

The previous discussion has dealt with the response of plasma ADH to increases in plasma osmolality. Arndt (1965) and Arndt and Gauer (1965) demonstrated that a water diuresis normally follows an infusion of water into a carotid loop of conscious dogs. This implied an inhibition of ADH secretion by hypotonicity and confirmed the osmoreceptor concepts of Verney as important in urine flow in the dog.

Zehr, et al. (1969) followed plasma ADH and plasma osmolality down to control values in unanesthetized ewes following a 72-hour water deprivation. They observed that plasma osmolality decreased to control values four hours after the beginning of hydration but that plasma ADH had

decreased to control values two and one-half hours after the beginning of hydration. This is suggestive of a dynamic component in the ADH regulatory mechanism. However, these times were not found to be statistically significant. A follow up study (Johnson, et al., 1970), where plasma osmolality decreased with no change in either left atrial pressure or mean arterial blood pressure, showed a significant decrease in plasma ADH with a large and significant increase in urine flow. These investigators concluded that a decrease in plasma osmolality of 3.5 mOsm/kg could block the release of ADH.

Volumetric

Along with the osmotic effects on ADH first noted by Verney (1947), volumetric effects also appear important (Henry and Gauer, 1951; Atkins and Pearce, 1959; Weinstein, et al., 1960; Baratz and Ingraham, 1960; Thorn, 1970). Henry and Gauer (1951) were among the first to observe that hemorrhage decreased urine flow rate, presumably due to an increase in ADH release (Ginsburg and Heller, 1953; Weinstein, et al., 1960; Baratz and Ingraham, 1960). Many investigators have noted that a hemorrhage equivalent to an 8 to 10% decrease in blood volume can increase plasma ADH in both anesthetized and unanesthetized animals (Dunn, et al., 1973; Henry, et al., 1968; Goetz, et al., 1974; Johnson, et al., 1970; Share, 1968; Szepanska-Sadowska, 1972).

Shade and Share (1975a,b) studied the effect of a slow non-hypotensive hemorrhage on plasma ADH in anesthetized dogs. Blood volume was first expanded to a value 17 percent greater than that in normovolemic dogs. Arterial pressures and plasma osmolalities were maintained at control values in both groups. They observed the same significant

correlation between plasma ADH and blood volume for normovolemic and expanded dogs. In other words acute volume expansion did not alter volume control of ADH.

Forsling, et al. (1973) in pentobarbital anesthetized rats noted that hemorrhage released not only ADH but its carrier protein, neurophysin-II as well, and that there was a direct relation between plasma levels of these substances. On the basis of these investigations the authors tentatively assumed that one molecule of neurophysin is released for each molecule of hormone. McNeilly, et al. (1972b) noted in the goat that plasma hormone and neurophysin levels were closely related after both experimental and physiological stimuli.

An 8% hemorrhage is known to block the water diuresis produced by intracarotid injection of distilled water into unanesthetized trained dogs (Arndt, 1965; Arndt and Gauer, 1965). Conversely, increases in blood volume have been shown to increase urine flow (Atkins and Pearce, 1959; Baratz and Ingraham, 1960; Henry, et al., 1956; Henry and Pearce, 1956; Ledsome and Linden, 1968).

Zehr, et al. (1969) and Johnson, et al. (1970) observed that left atrial pressure, an indirect estimate of blood volume, was particularly suited to studies in unanesthetized ewes. They noted that a three day water deprivation or a 10% decrease in blood volume (with no change in plasma osmolality) decreased left atrial pressure by 5 cm of water and increased plasma ADH to 4 microUnits/ml from a control value of 1.7 microUnits/ml. Similarly in the goat (McNeilly, et al., 1972b) a low left atrial pressure induced by hemorrhage increased ADH secretion. In man, an isovolemic decrease in arterial blood pressure will increase ADH only if the decrease is greater than 5 to 10% of the resting recumbent

value (Robertson, 1974).

In the dog within a few minutes following a large hemorrhage and maintenance of arterial blood pressure at 50 mm Hg there is a massive release of ADH into the blood (Weinstein, et al., 1960). Antidiuretic activity peaks at two to five minutes post bleeding. This initial rapid secretory response is not maintained and the rate of hormone release decreased in spite of a continuing hypovolemia again implying a dynamic component in the ADH regulatory mechanism.

In contrast to the rather large changes in blood volume discussed previously, Claybaugh and Share (1973) noted in urethane and chloralose anesthetized dogs that a decrease in blood volume as small as 2.6% could affect the system controlling ADH release. However, as Goetz, et al. (1974) pointed out, blood loss during preparative surgery could have elevated plasma ADH levels prior to the beginning of the experimental hemorrhage.

Henry, et al. (1956) were the first to use a balloon to distend the left atrium in an attempt to demonstrate the presence of volume receptors. They found that a continuous left atrial distension produces a transient diuresis that returns to control values after thirty minutes (cf. Lawrence, et al., 1973). This was the first evidence that atrial stretch receptors might be involved in regulating ADH release. Since that time additional evidence has accumulated to substantially support the idea that acute distension of the left atrium increases the activity of stretch receptors and this activity is transmitted via the vagus to decrease ADH release (Share, 1965; Ledsome and Linden, 1968; Zehr, et al., 1969; Johnson, et al., 1969, 1970; Szcepanska-Sadowska, 1972; Kinney and DiScala, 1972; Share and Claybaugh, 1972; Claybaugh and Share, 1973;

Gillespie, et al., 1973; Lawrence, et al., 1973; Kappagoda, et al., 1974a). The mechanism appears to involve the stimulation of subendocardial stretch receptors, the majority of which lie at the junctions of the pulmonary veins and the left atrium (Coleridge, et al., 1957). There appear to be two functional types of receptors involved (each type found in both atria) although they have not been differentiated histologically (Goetz, et al., 1975). The natural stimulus for type A receptors may be atrial tension while that for type B is definitely atrial volume (Paintal, 1973). Electrical activity of type A receptor occurs during the a wave of the atrial pressure curve. Less is known about the type A receptors because their scarcity makes study difficult. The activity of type B receptors is associated with the v wave of the atrial pressure tracing and is linearly related to blood volume. Type B receptors are therefore considered to be the receptors which have the most effect on ADH release. Their afferent fibers travel in the vago-sympathetic trunk (Szcepanska-Sadowska, 1972). It also appears that there may be ventricular receptors which are connected with nonmyelinated fibers in the right vagus and which may inhibit normally excitatory influences on ADH release (Harris and Spyer, 1973).

The effect on urine flow of stimulating right atrial receptors (unencapsulated nerve endings) appears to be qualitatively the same as that brought about by stimulation of left atrial receptors. Kappagoda, et al. (1973) found that distension of a balloon in the lumen of the right atrium of chloralose anesthetized dogs and at the superior vena caval-right atrial junction produced an increase in both urine flow rate and sodium excretion.

Although the previous discussion presents a rather strong case for

left atrial distension producing a decrease in antidiuretic activity, the area remains one of active interest. Using chloralose anesthetized dogs Kappagoda, et al. (1974a) distended balloons in the left atrium to produce a diuresis and measured ADH via bioassay. They found no correlation between diuresis and ADH activity of the plasma and suggested that a diuretic agent might be involved. In a follow up study (Kappagoda, et al., 1974b) similar maneuvers were employed; intravenous ADH was found to suppress the evoked diuresis. The bioassay faithfully detected changes in plasma antidiuretic activity resulting from these injections. These workers concluded that a decrease in plasma ADH level did not accompany the diuretic response to left atrial distension in their preparation

Osmotic and Volumetric

The strong role for left atrial regulation of ADH release presented thus far is diminished when one compares osmotic and volume stimuli separately and simultaneously. Work in unanesthetized ewes (Zehr, et al., 1969) demonstrated that osmoreceptor activity becomes more prominent as stimulus intensity increased. Changes in plasma osmolality within the range of 2 to 3% could be modified by increasing left atrial pressure; however, a progressively greater role is played by the osmoreceptor system at osmolality changes greater than 2 to 3%. A subsequent study (Johnson, et al., 1970) using simultaneous and separate osmotic and volume stimuli led to the conclusion that neither receptor system was dominant over the other. However, as pointed out by Goetz, et al. (1975), changes in plasma osmolality of approximately 1% were being compared with changes in blood volume of 10%. In view of this it appears that the osmoreceptor system plays a much greater role in day to day

regulation of ADH secretion. Similar studies in rats (Dunn, et al., 1973) and in man (Moses, et al., 1967; Moses and Miller, 1971; Robertson, 1974) support this concept. In other words, the magnitude of the necessary changes in blood volume and the highly variable response seem to argue against a primary role for blood volume control of ADH secretion. It appears more likely that left atrial pressure is a modulator of the osmotic response, and the increase in ADH after sufficient hemorrhage may not be a physiological response but is only elicited to adapt the cardiovascular system to a significant blood loss (Goetz, et al., 1974).

Along the same lines evidence has accumulated that the distribution of blood within the body can influence receptors that normally block ADH release (Segar and Moore, 1968; Robertson, 1974). ADH levels in human plasma vary with position and are maximal when the subject is erect and minimal when supine. Similarly, Hayward and Baker (1969) produced a diuresis in a variety of animals by preoptic cooling. The diuresis was presumably due to an inhibition of ADH release by an increased central blood volume caused by peripheral vasoconstriction due to the preoptic cooling.

Other Influences

Magnocellular neuroendocrine cells of the supraoptic nucleus have rich connections with the limbic system and brain stem as demonstrated by electrical stimulation (Aulsebrook and Holland, 1969; Hayward, 1972; Koizumi and Yamashita, 1972; Negoro, et al., 1973b; Slotnick and Rothballer, 1964). It therefore seems likely that a multitude of inputs could influence the release of ADH. It has been noted that pain (Verney, 1947; Ginsburg, 1966) as well as osmotic stress can produce an

antidiuretic response; therefore behavioral state may be important in hormone release. Indeed Hayward and Jennings (1973d) observed that behavioral state (pain) was an important correlate to magnocellular neuroendocrine cell activity.

Neuronal Properties of Magnocellular Neuroendocrine Cells

This section is intended to discuss evidence which supports claims that magnocellular neuroendocrine cells are truly neuronal in character.

At the light microscope level, magnocellular neuroendocrine cells of the supraoptic nucleus exhibit features similar to neurons of the central nervous system, including neurofilaments and Nissl substance (Scharrer and Scharrer, 1954).

Electron microscope studies (Rechardt, 1969) of magnocellular neuroendocrine cells of the supraoptic nucleus revealed that cells of this nucleus receive all three of the basic types of synapses: axodendritic, axosomatic and axoaxonic. Dendrites were usually densely covered with synapses which contained large granular, small granular and agranular vesicles. Axoaxonal synapses were rare and always found near a capillary.

Electrophysiological investigation of the neuronal character of supraoptic magnocellular neuroendocrine cells began when von Euler (1953) recorded slow dc osmopotentials from anesthetized cats given injections of hypertonic saline or glucose. Later, Cross and Green (1959), who were the first to record from single units in the hypothalamus, noted that action potentials of supraoptic neurons were similar in shape to those recorded from other brain areas.

Intracellular recording of magnocellular neuroendocrine cells was

first done in the preoptic nucleus of anesthetized goldfish by Kandel (1964). Resting membrane potential, on the average, was found to be 50 mV. Action potentials up to 117 mV in magnitude and up to 10 msec (average 3.5 msec) duration were observed. Action potential duration in mammalian magnocellular neuroendocrine cells is slightly less, averaging 2.7 msec (Novin, et al., 1970). These were further characterized by a prominent diphasic hyperpolarizing afterpotential consisting of a small and brief first phase followed by a larger and longer lasting second phase. These magnocellular neuroendocrine cells spontaneously fired at a rate of two to eight spikes per second and could be stimulated to sustain a slow firing rate in response to constant current. This behavior is similar to that of spinal motoneurons which may also have a prominent hyperpolarizing afterpotential.

Both orthodromic and antidromic potentials were evoked in the magnocellular neuroendocrine cells of the goldfish. By stimulating the olfactory tract Kandel (1964) was able to evoke a long latency depolarizing synaptic potential (EPSP) in the neuroendocrine cell which was graded and could trigger an action potential if a threshold voltage was reached. Adequate stimulation of the pituitary stalk could evoke an antidromic potential or an inhibitory post synaptic potential (latency slightly longer than antidromic spike) if the stimulus intensity was subthreshold for activation of the neuron. These observations led Kandel to conclude that neuroendocrine cells have electrical membrane properties similar to other neurons. Antidromic activation also enabled Kandel to calculate a mean conduction velocity from conduction distance and measurement of antidromic latency. A conduction velocity of 0.46 m/sec was found which was similar to that observed earlier also in neuroendocrine cells of fish

(Potter and Loewenstein, 1955).

The earliest attempt to extend the technique of antidromic identification to cells of the mammalian SON was that of Yagi, et al. in 1966. They were able to record spontaneous as well as antidromic impulses evoked by stimulation of the pituitary stalk of the rat both in vivo and in vitro. Since processes of these cells project to the neurohypophysis, this was an effective demonstration that mammalian magnocellular neuroendocrine cells can generate and conduct action potentials. These investigators were able to go one step further in vitro. They noted that the observed excitability disappeared in the absence of sodium (a result later confirmed by Dreifuss, et al., 1971 and Ishida, 1970) and in the presence of high potassium, thus again suggesting that magnocellular neuroendocrine cells have membrane properties similar to those of non-neuroendocrine neurons. In addition, on the basis of antidromic latency and an estimation of conduction distance (Eccles, et al., 1958), they calculated conduction velocity of these fibers to be approximately 1 m/sec. Conduction velocities similar to these observed by Yagi, et al. (1966) have been observed in goldfish (Kandel, 1964; Hayward, 1974), cats (Koizumi and Yamashita, 1972), dogs (Koizumi and Yamashita, 1972), monkeys (Hayward and Jennings, 1973a), and rabbit (Sundsten, et al., 1970; Novin, et al., 1970).

Intracellular recording of mammalian magnocellular neuroendocrine cells (Sundsten, et al., 1970; Novin, et al., 1970; Koizumi and Yamashita, 1972) revealed a resting membrane potential of 40 to 80 mV and often a complete separation of A and B components of the antidromic spike. The complete separation and long interval between the A and B spikes can be accentuated by shortening the interval between two stimuli

(Novin, et al., 1970) and may be due to the presence of recurrent axon collateral inhibition which has been demonstrated in both the SON and PVN (Sundsten, et al., 1970; Novin, et al., 1970; Koizumi and Yamashita, 1972; Dreifuss and Kelly, 1972). Novin, et al. (1970) hypothesized that the absolute refractory period of the B spike may be greater than that of the A spike resulting in this separation. Similar results are seen in pyramidal tract neurons. The height of the B spike is also about twice the magnitude of the A spike, a phenomenon noted in lateral geniculate neurons (Bishop, et al., 1962).

Stimulation of the pituitary stalk has been shown to set up a hyperpolarization of the somatic membrane at intensities subthreshold for antidromic activation (Kandel, 1964; Koizumi and Yamashita, 1972; Dreifuss and Kelly, 1972). This hyperpolarization is of considerable magnitude and duration (80-125 msec) and may delay or prevent invasion of the soma by late antidromic spikes. Antidromic stimulation leading to inhibition of spontaneous activity has now been demonstrated in several species (Kandel, 1964; Koizumi and Yamashita, 1972; Dreifuss and Kelly, 1972; Driefuss, et al., 1973; Sundsten, et al., 1970; Novin, et al., 1970; Hayward and Jennings, 1973a). Again this may be due to the presence of recurrent axon collaterals with or without imposition of an inhibitory interneuron.

Morphological support for the existence of recurrent collaterals is limited. Branching of nerve fibers of the hypothalamo-neurohypophysial tract has been described (Christ, 1966). Degeneration studies in the rat (Olivecrona, 1957) also support the concept of recurrent outflow of supraoptic and paraventricular neurons. Hayward (1974) using the technique of procion yellow marking to study magnocellular cells of the

goldfish preoptic nucleus described three cell types, one of which was characterized by multiple branched axons (cell type I).

Koizumi and Yamashita (1972) recorded intracellularly from single neurons in or near the SON of anesthetized cats and dogs and found that some fired 5 to 7 spikes at a rate of 500 to 800 spikes per second when excited by pituitary stalk stimulation. Weaker stimulation produced fewer spikes. These cells may be inhibitory interneurons between the recurrent collateral branches and magnocellular neuroendocrine cell somata.

In summary, magnocellular neuroendocrine cells exhibit no properties not reported for other neurons of the central nervous system. They generate action potentials, respond to strength duration parameters as expected (Sundsten et al., 1970) and have conduction velocities in the range of mammalian type C fibers. An approach to this system as a neuronal system should lead to new views concerning the control of all neuroendocrine systems.

Electrical Activity and Hormone Release

As mentioned earlier, Verney (1947) introduced the technique of an intracarotid injection of hypertonic saline as a means of releasing ADH. The zone of specialized brain cells sensitive to changes in osmotic pressure was later (Jewell and Verney, 1957) localized in the hypothalamus and thought to be in the area of the supraoptic nucleus.

von Euler (1953) sought to correlate electrical activity of these cells with pituitary secretion. He recorded slow potential changes in the SON region under conditions which were conducive to ADH release from the neurohypophysis. The dc shifts were interpreted to be due to a summation of generator potentials (similar to that of peripheral receptors),

or of potential changes from neurons activated by osmoreceptors.

Although enlightening, the work of von Euler suffered from the fact that the meaning and origin of dc potential changes are still unknown. Shortly afterwards recording of single supraoptic neurons was done in the urethane anesthetized rabbit (Cross and Green, 1959). Electrical activity in these cells was increased by stimuli which also promoted the release of ADH from the neurohypophysis. Hypertonic NaCl and glucose affected the spontaneous rate of discharge of many neurons. Neurons responding to osmotic stimuli were highly specific and rarely responded to non-noxious arousing stimuli. This is quite in line with the proposal of von Euler (1953); a neuron is an osmoreceptor only if it responds specifically to osmotic stimuli.

Not only do supraoptic magnocellular neuroendocrine cells respond to an increase in osmotic pressure with an increase in firing rate, they also show slow spontaneous activity in the absence of any apparent stimulation (0.1 to 5.0 spikes/sec in the chloralose anesthetized cat (Koizumi, et al., 1964)). In addition, intracarotid distilled water has been shown to block firing of these supraoptic magnocellular neuroendocrine neurons (Vincent, et al., 1972). The slower firing of these neurons is significant in the sense that hypothalamic island preparations fire faster than those in control animals (Novin and Durham, 1969) and still respond to changes in the osmotic condition (Woods, et al., 1966; Sundsten and Sawyer, 1961). This may be a reflection of volume inhibition. Often the neuronal response to an osmotic stimulus was stronger in the island preparation (Novin and Durham, 1969).

Hayward and Vincent (1970) were the first to record extracellularly single units of the SON and their responses to intracarotid injections of

hypertonic NaCl and other arousing stimuli in the conscious behaving rhesus monkey. They sought to determine if previous results in anesthetized animals and in vitro could be observed in the unanesthetized animal. In addition, they attempted to distinguish between osmoreceptors a and magnocellular neuroendocrine cells in the region of the SON. Fifty percent of the neurons recorded by these investigators were specifically sensitive to osmotic changes while the remainder were sensitive to both sensory arousing stimuli and osmotic changes. Cells responding to both osmotic and sensory stimuli were called nonspecific osmosensitive cells; both excitatory (34%) and inhibitory (16%) responses were observed. Specific osmosensitive cells responded to intracarotid injections of hypertonic sodium chloride but rarely responded to nonnoxious arousing stimuli. Of the specific osmosensitive cells 30% responded monophasically with either a shortlived accelerated firing (20%) or inhibition (10%). These monophasic osmosensitive cells were found mainly in the perinuclear zone of the SON, and were postulated to be the osmoreceptors as defined by von Euler (1953). The remainder (20%) of the specific osmosensitive cells responded biphasically (a period of excitation followed by inhibition) to an intracarotid pulse of hypertonic saline. This pattern of neuronal firing may be significant to the normal maintenance of ADH levels as has been suggested (Harris, et al., 1975; Wakerley, et al., 1975; Walters and Hatton, 1974; Arnauld, et al., 1974, 1975). These cells were regarded as the neuroendocrine cells responsible for synthesizing and releasing ADH. A serious drawback however to the study of Hayward and Vincent (1970) was the fact that identification of these cells was based strictly on histological location and osmotically evoked discharge patterns; therefore one cannot be certain that the biphasic

cells actually had their terminals in the posterior pituitary.

In a follow-up study, Vincent, et al. (1972) observed an inhibition of antidromically identified SON units during water drinking and an opposite response to osmotic stress in the unanesthetized monkey. These investigators also noted that some of these antidromically identified cells did show patterns of excitation followed by inhibition. Hayward and Jennings (1973b) confirmed these findings when they recorded from identified neurons in unanesthetized monkeys. Their work corresponded exactly with that of Hayward and Vincent (1970). Specific biphasic osmosensitive responses occurred only in identified magnocellular neurons, and monophasic nonspecific responses occurred only in nonidentified cells. It may be that biphasic responses emerge when excitatory drive increases beyond a certain threshold and collateral branches (discussed previously) lead to the excitation-inhibition sequence of bursting noted in the monkey (Hayward and Vincent, 1970; Hayward and Jennings, 1973a,b) and in the rat (Wakerley and Lincoln, 1973; Wakerley, et al., 1975; Harris, et al., 1975).

Dyball (1971) used the technique of antidromic identification to study supraoptic and paraventricular neurons in the urethane anesthetized rat. It was observed that an intracarotid injection of 0.25 ml of 1 M sodium chloride accelerated discharge of SON units and elevated plasma ADH to a peak concentration of 1300 microUnits/ml in 3 minutes. These results support the concept that the release of ADH is triggered by a discharge of action potentials down magnocellular neuroendocrine cell axons.

Knowing that 2% sodium chloride substituted for drinking water would release both ADH and oxytocin (Jones and Pickering, 1969), Dyball and

Pountney (1971, 1973) studied antidromically identified cells in urethane anesthetized rats which had drank 2% sodium chloride ad libitum for three days prior to neuronal recording. Compared to controls they observed a significant increase in the firing rate of both nuclei which was associated with a depletion of neural lobe hormone.

Blood Volume and Hypothalamic Unit Activity

Little information is available concerning the effects of changes in blood volume or left atrial pressure on hypothalamic unit activity.

Menninger and Frazier (1972) studied the effects of changes in blood volume and left atrial distension on the electrical activity of non-identified hypothalamic neurons in urethane anesthetized cats. A total of nineteen cells were tested for response to both balloon inflation and injection of hypertonic sodium chloride. Eight of these neurons responded in a manner consistent with the differential effects of volume and osmotic stimuli. This study then neither refutes nor supports the concept of differential effects of osmotic and volume stimuli.

A more recent investigation, Wakerley, et al. (1975), studied the effects of a hemorrhage of 15% blood volume on phasic PVN neurons of urethane anesthetized rats. The stimulus was observed to convert the phasically active neurons to continuously active or to increase their burst duration. The authors concluded that phasic activity is a functional state of the cell, and that during a stimulus many phasic cells are synchronized to produce a periodic discharge of neurohypophysial hormone. This synchronization may be a modulating mechanism of hormone release.

Spontaneous and Evoked Firing Patterns

In previous sections mention has been made of continuously active cells, burster cells, silent cells, and biphasic and monophasic responses. The functional significance of these firing patterns is unknown.

Many studies have now confirmed that an optimal frequency for stimulation of the pituitary stalk to produce hormone release is in the neighborhood of 40 spikes per second (Harris, et al., 1969; Ishida, 1970; Sundsten, et al., 1970; Dreifuss, et al., 1971). Hormone output also decreases markedly above and below this frequency. Sundsten, et al. (1970) noted in anesthetized rats that the lowest frequency of stimulation which would produce a milk ejection response was 15 spikes per second and that the magnitude of this parameter did not increase when the frequency was increased above 100 spikes per second.

In vitro studies (Dreifuss, et al., 1971) have shown that at frequencies less than 35 spikes per second hormone release depends on the number of action potentials and their frequency. At frequencies greater than 35 spikes per second numbers of action potentials are less effective and frequency dependence is less obvious. The authors concluded that a discrete number of action potentials released the most hormone when they occurred close together in time.

Again in anesthetized animals, Dyball (1971) studied the responses of antidromically identified units in both the SON and PVN during injections of sodium chloride. A high proportion of the units in both nuclei were firing at a rate of less than 1 spike per second. Changes in action potential activity of the SON were associated with hormone release; however, both excitatory and inhibitory responses were observed. It was generally found that units firing spontaneously at rates less than two

per second were excited and that units firing spontaneously at rates greater than 2 per second were inhibited by intracarotid hypertonic sodium chloride. In addition, blood ADH levels did not return to control levels at the same rate as firing rate of the individual neurons.

Many laboratories have now demonstrated that a substantial number (up to 32%) of magnocellular neuroendocrine cells fire intermittently in a pattern called phasic or bursting in both anesthetized (Wakerley and Lincoln, 1971a; Negoro and Holland, 1972; Negoro, et al., 1973a,b; Wakerley and Lincoln, 1973; Walters and Hatton, 1974; Dyball and Pountney, 1973; Harris, et al., 1975; Wakerley, et al., 1975) and unanesthetized animals (Hayward and Jennings, 1973a,b; Arnauld, et al., 1974, 1975). Although the data were collected from differently prepared animals under widely varying experimental conditions, characteristics of bursts are remarkably similar.

Responses of antidromically identified cells of the paraventricular nucleus to pain, vaginal distension and suckling have been studied (Wakerley and Lincoln, 1973; Negoro, et al., 1973a,b) in anesthetized rats. It was concluded that suckling did not directly modulate neuronal activity but could influence the generation of phasic activity. A burst firing rate of 40 to 80 spikes/sec was reached during the bursts that preceded milk ejection. The excitatory response was again followed by a period of inhibited firing which was found to increase as the firing rate of the responsive cell increased. Graded milk ejection responses with periods of excitation below a threshold for hormone release were not observed.

Harris, et al. (1975) studied the response of antidromically identified supraoptic magnocellular neuroendocrine cells to bilateral occlusion of the common carotid arteries; a stimulus known to release ADH (Clark

and Rocha e Silva, 1967). Phasic neurons responded with increased burst firing rates four to nine seconds after the start of occlusion. On the basis of these and previous data showing that approximately 50% of continuously active cells were excited during milk ejection (Lincoln and Wakerley, 1974), the authors suggested that phasic neurons might represent a distinct class of ADH secreting neurons and that irregular neurons were secreting oxytocin.

Phasic cells have also been implicated as being ADH neurons in other studies (Dyball and Pountney, 1973). Activity of these cells increased in sodium chloride treated rats but rarely exhibited the 20- to 40-fold increase in firing rate of paraventricular neurons noted by Wakerley and Lincoln (1973). Substituting 2% NaCl for drinking water, Dyball and Pountney noted that in treated rats the interburst interval had increased as well as the mean burst firing rate. Although these effects are in an opposite direction, the result was an overall increase in mean firing rate.

In unanesthetized female rhesus monkeys, antidromically identified magnocellular neuroendocrine cells exhibited three discrete patterns of spontaneous firing distributed randomly in the SON and INZ (Hayward and Jennings, 1973a,b). Silent cells (3%) were discovered only by stimulation of the pituitary stalk. The most common pattern (63%) of firing was that described as continuously active. Twenty-one percent of the cells exhibited a pattern of firing described as low frequency bursting. All three firing pattern types were found to be specifically osmosensitive with a biphasic response. Two of these patterns, continuously active and silent, have been described elsewhere in the hypothalamus and are not therefore unique to the hypothalamic magnocellular neuroendocrine system.

However cells of the class of low frequency bursters do appear to be limited to neuroendocrine systems, especially in invertebrates (Kater and Kaneko, 1972; Strumwasser, 1971; Stennakre and Tauc, 1969). Hayward and Jennings hypothesized that specific input connections might be responsible for each firing pattern type which may reflect cellular secretion of specific neurohypophysial hormones and neurophysins. In addition, the bursting pattern may be related to an inhibitory recurrent collateral pathway. An alternate hypothesis was that each firing pattern represented a secretory state of a cell. In other words, synthesis and transport without release occurs in silent cells, continuously active cells maintain basal hormone levels and low frequency bursting cells are releasing pulsatile amounts of hormone (see also Sachs, et al., 1969). In fact, it was observed that osmotic loading could convert some silent cells to continuously active and some continuously active cells to low frequency bursting patterns of firing (Hayward and Jennings, 1973c; Hayward and Murgas, 1973).

Similar conclusions were reached in a recent study of the response of phasic paraventricular neurons to hemorrhage amounting to 15% blood volume (Wakerley, et al., 1975), a stimulus probably more physiologic than 5 second intracarotid pulses of hypertonic saline. Hemorrhage was found to increase overall mean firing rate by increasing burst duration as well as intraburst frequency, although the latter effect was not as consistent as the former. These investigators, like Hayward and Jennings, felt that the pattern of discharge described as phasic is a functional state of activity of a neurosecretory cell which can at other times be continuously active or silent. Furthermore, it appeared to the authors that a synchronization (normally absent) of phasic cells occurs under the

appropriate stimulus. This synchronization (overlapping of activity) appears unavoidable as burst duration increases. A possible mechanism then of the regulation of hormone release is this modulation of burst duration which could provide a pulsatile discharge of neurohypophysial hormones. In addition, they proposed that neurons synthesizing and releasing oxytocin were the ones whose firing was characterized by a high frequency discharge during suckling. ADH release in turn is determined by the frequency and duration of the burst of the phasic cells.

Walters and Hatton (1974) used a water deprivation from 0 to 5 days to study the effects of a progressive dehydration on histologically identified SON neurons. The overall firing rate of neurons in the SON was seen to change significantly during the deprivation (days 1, 3, and 4 significantly greater than day 0 with days 2 and 5 intermediate). In addition, the percentage of neurons firing phasically changed with water deprivation. The highest percentages were observed at days 0 and 2 and the lowest at day 5. The mean firing rate of these neurons was again significantly higher at days 1, 3, and 4 with 2 and 5 being intermediate. No significant differences in burst duration or interburst intervals were noted although there was a trend for burst duration to be shorter in deprived animals. A highly significant increase in burst mean firing rate was observed during deprivation which the authors believed reflected the progressive dehydration. Supraoptic neurons were observed to be more likely phasic than nearby cells.

A concurrent study had one distinct advantage over the one just discussed (Walters and Hatton, 1974). Arnauld, et al. (1974, 1975) studied the effects of prolonged water deprivation on antidromically identified magnocellular neuroendocrine cells of the SON of unanesthetized female

rhesus monkeys. Antidromic identification has the advantage of definite recognition of neuroendocrine cells where histological identification of cells in the nucleus does not distinguish between interneurons and magnocellular neuroendocrine cells. Like previous studies, Arnauld, et al. found that an overall increase in mean firing rate of supraoptic neuroendocrine cells occurred with dehydration and decreased upon rehydration. At plasma osmolalities less than 300 mOsm/kg they observed that most cells were spontaneously active in a pattern much like the continuously active cells of Hayward and Jennings (1973a,b) with only about 13% firing phasically. Upon water removal the ratio of phasic to continuously active patterns of firing increased as well as the overall mean firing rate. One mechanism for the increased firing rate involves these phasic cells. Both the burst duration and burst firing rate increased following water removal. At osmolalities greater than 310 mOsm/kg, continuously active patterns of firing appeared which had fluctuations similar to the phasic cells. With further increases in plasma osmolality, the proportion of this type of cell increased. On the basis of these data the authors, like others, suggested a switching in firing pattern from continuously active to phasic with the appropriate stimulus.

Genesis of Firing Patterns

The previous section indicated that neurons of both the paraventricular and supraoptic nuclei have similar spectra of firing patterns ranging from silent to continuously active to bursting. A question regarding the functional significance of the spontaneous patterns of firing is obvious and the answer probably lies in an understanding of the modes of their generation.

Some authors have suggested that these patterns of neuronal activity represent a specific hormonal state, each releasing a different neurohypophysial peptide and its specific carrier protein (Hayward and Jennings, 1973b; Cross, et al., 1975; Harris, et al., 1975; Wakerley, et al., 1975). Other authors have suggested that the patterns of firing represent different secretory states in which each type of activity is performing a function specific to a particular aspect of hormonal regulation (Hayward and Jennings, 1973c; Sachs, et al., 1969).

Hayward and Jennings (1973a) suggested that the bursting pattern may be related to a recurrent collateral inhibitory pathway whose existence was discussed earlier. Perhaps the reduced hormone output at pituitary stalk stimulation frequencies greater than 35 per second is due to an enhanced recurrent collateral inhibition and not due to reduced impulse transmissions as suggested by Dreifuss, et al. (1971).

Specific neural inputs were also suggested as possible mechanisms for firing pattern genesis by the work of Wakerley and Lincoln (1973). They noted that the explosive increase in paraventricular unit activity preceding milk ejection did not occur as a result of a cue from the suckling pups and therefore must be a reflexive event. They further suggested that the biphasic pattern was suggestive of positive and negative feedback effects due to intrinsic neuronal wiring of the neurosecretory cells (recurrent collaterals). This concept is further supported by the work of Negoro and Holland (1973a,b) which demonstrated that the attainment of a higher firing rate resulted in an increased length of the following inhibitory period.

The generation of succeeding bursts may be due to a post inhibitory rebound as indicated by the work of Hayward and Jennings (1973d). The

authors did not discuss this phenomenon although post inhibitory rebound appears likely from inspection of figure 1 of the cited work.

It seems reasonable then to propose that the bursting pattern of firing originates by an increased excitatory drive which sets up a volley of impulses in the neuroendocrine cell. These impulses are fed back in an inhibitory manner to produce the period of hyperpolarization and absence of cell firing recorded intracellularly and extracellularly, respectively. The post inhibitory rebound as well as the subsequent excitatory drive may be important in generating subsequent bursts.

Generation of the silent and continously active firng patterns may be random noise or the type of activity resulting from a particular threshold of excitation for that cell.

Modeling of Firing Patterns

The making of models often provides answers to problems which may seem insurmountable when approached by current experimental techniques. The extensive review by Harmon and Lewis (1966) sets forth three advantages of modeling to physiologists. Firstly, it provides a convenient means of testing hypotheses. Secondly, it may often synthesize diverse data into a unified picture, and thirdly, it can provide direction for further physiological experiments. This section will deal with the genesis of bursting patterns of firing and their relation to other spontaneous activity patterns where applicable. In most cases qualitative and not quantiative examples are discussed.

Burns (1954, 1955) modeled afterbursts observed in the cortex of the cat. These afterbursts of cortical neurons last up to one hour and can be elicited in response to strong stimulation of the cat cortex. The

author proposed that this was due to a differential repolarization of the neurons and used electrical analogs to quantitatively test his hypothesis. The major assumption was that the two ends of cortical neurons have different time courses for their repolarization, the central ends repolarizing more slowly than the distal ends. The potential difference between these two points then might accumulate to a point where current flow between them is greater than threshold and excitation can occur. Burns' model closely approximated his physiological observations.

A later study (Andersen, et al., 1966) used a digital computer to simulate a neuronal network of 80 cells. Two basic assumptions were used. One, all cells had a random probability of discharge when not affected by other cells and two, this probability of discharge changed in a certain number of cells when one neuron fired. The resulting discharge patterns generated by this model paralleled those of neurons recorded from the animal thalamus. A transient burstlike activity ensued following the start of the network and was followed by a random fluctuation in the number of active cells and later periods of rhythmic activity occurred which were similar to physiological data. The factors most important to the rhythmic activity in the model were found to be post-inhibitory rebound and the degree of collateral inhibition to neighboring cells.

MacGregor and Palasek (1974) modeled rhythmic activity having a period of 600 to 1300 msec which depended primarily on the rate of recovery from refractoriness in individual cells from mutually exciting pools of neurons. The rate of bursting was found to be affected by the average level of random background activity. It was also found that the burst duration and spikes per burst depended on the number of

interneuronal connections, connection strength and the magnitude of change of the conductance to potassium. The bursts were found to merge giving an overall appearance of marginal periodicity when background activity was increased and other parameters were held at normal values. Rhythmic activity could be activated initially from random background activity or by the presence of a leaky membrane in some or all cells.

Two papers have recently addressed the problem of periodicity with models which include recurrent inhibition (Perkel, et al., 1974; Wigstrom, 1974). Perkel, et al. introduced the concept that the connections will establish a regularly repeating firing pattern if one of the cells is a pacemaker. In addition, a relatively large postinhibitory rebound will lead to indefinite periods of bursting from synaptic input when no pacemaker is present. The alternative concept of a domination principle was introduced by Wigstrom. The model incorporated excitatory, inhibitory and unspecified input with all connections random. It was found that diverse patterns of output were subject to a process by which the largest component was reinforced and all others were suppressed. Furthermore, additional input had little or no effect after the initial decision was made.

The true mechanism of burst genesis observed in mammalian neuroendocrine cells is still in doubt. The above models do shed some light on what parameters of the neuronal pool may be important to phasic discharge. Aspects of neuroendocrine cell function particularly significant may be recurrent inhibition, postinhibitory rebound and dynamic processing of input, as suggested by the models of Perkel, et al. (1974), Wigstrom (1974) and Andersen (1966).

CHAPTER III

MATERIALS AND METHODS

Animal Preparation

Southdown ewes (4-9 years of age) were conditioned to a recording stanchion prior to any surgery. Sodium thiamylal (Surital, Parke-Davis) was used for induction of anesthesia and introduction of an endotracheal tube. Anesthesia was maintained by connecting the endotracheal tube to an Ohio 300 D/O Deluxe Anesthesia Ventilator Machine and Vaporizer for methoxyflurane gas (Metofane, Pitman-Moore).

Animals were placed in a stereotaxic headholder (Baltimore Instrument Company) modified for sheep and a craniotomy performed without excising the dura mater at Fr 30.0, Right 3.0 (Rogers, 1976) which would accommodate a bone wax filled stainless steel cylinder with an outside diameter of 20 mm (Trent Wells, Inc.). Subsequent craniotomies were performed for placement of stainless steel epidural platform bolts and biparietal silver-silver chloride ball electrodes for electrocorticographic recording. The cylinder, bolts and electrodes were cemented to the cranium and all craniotomies sealed with dental cement (Caulk Grip Cement) to prevent pressure fluctuations and minimize sepsis in the cranium. Periorbital stainless steel electrodes for recording extraocular eye movements were also implanted. These and the EEG electrodes were led to, and fixed at a receptacle (Amphenol 2NO52) on an elevated lucite platform held permanently above the scalp by the four stainless

steel epidural platform bolts (Baker, et al., 1968). Also fixed to the lucite platform were receptacles for a 10-pin dc preamplifier and power input and unit output leads (Amphenol 223-1205).

Pituitary Stimulating Electrode Placement

After completion of the above preparation, a titanium micropositioner (Trent Wells, Inc.) with a specially designed pituitary electrode guide (Figure 1) was fixed to the implanted stainless steel cylinder. A radiograph was taken which allowed calculation of the correct coordinates for future implantation (relative to the location of the permanently implanted cylinder) of a pair of insulated (Isonel 31 Ins. Var., Schenectady Chemicals, Inc.) tungsten pituitary stimulating electrodes with 2 mm tips bared across the hypophyseal stalk. Radiographic landmarks were the sella turcica and optic foramen.

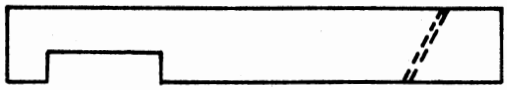
Following radiology (4-7 days), a second craniotomy was performed for placement of pituitary stimulating electrodes. Pituitary guide tubes and stimulating electrodes were stereotaxically lowered through the electrode guide apparatus from above at a rostral-caudal angle of 29°. The electrodes and their guide tubes were then firmly fixed to the cranium and platform with dental cement.

Arterial and Venous Cannulation

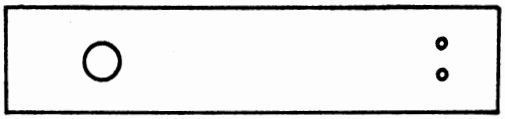
The carotid artery was cannulated through a cut end of the thyrolaryngeal artery and fixed without interruption of blood flow in the common carotid artery. This cannula was used for introduction of a 5 to 10 second pulse of hypertonic NaCl. Pulse injections of hypertonic solutions into the common carotid artery proved unsatisfactory (see Chapter

Figure 1. Scale Drawing (1:1) of Pituitary Stimulating Electrode Guide Apparatus

(A) left lateral view; (B) top view; (C) bottom view; (D) three dimensional view.



(A)



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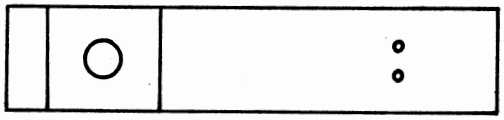

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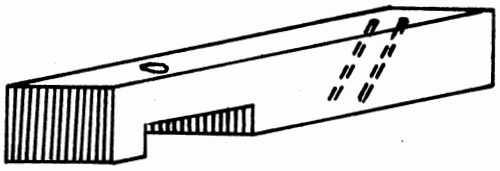


(D)

IV) and jugular veins were cannulated in subsequent sheep.

Cannulae (Dow Corning Silastic) were placed in both external jugular veins (right, 0.078 inches inside diameter and left, 0.062 inches inside diameter) and threaded about 6 inches toward the heart. A small bead of silicone medical adhesive (Dow Corning) served to anchor each cannula by suture to subcutaneous tissues. These cannulae continued subcutaneously to the head where they were capped with hypodermic needles and fixed to the lucite platform (Baker, et al., 1968). Both cannulae were filled with heparinized saline (1000 U/ml) and flushed daily with one-half ml of the same solution. All wounds were closed with Vetaphil suture. The completed preparation is illustrated schematically in Figure 2.

Confirmation of Pituitary Stimulating Electrode Placement

Correct pituitary electrode placement was confirmed the next day by the production of an antidiuresis in response to pituitary stalk stimulation. A Foley catheter (Fr. 12) was placed in the bladder and the experimental animal was then hydrated by an intravenous infusion of hypotonic saline (0.45% NaCl) at a rate of 3 ml per minute until a stable urine flow was achieved. The pituitary stalk was then stimulated (Grass Model S4) with square wave pulses of 2 msec duration, 2-8 mA constant current (ELS CS-1) intensity at a rate of 40 per second for 5 seconds every 30 seconds for 5 minutes. Stimulation of correctly placed pituitary electrodes resulted in both an increase in urine osmolality and a decrease in urine flow rate. A typical response is illustrated in Figure 3.

Figure 2. Diagrammatic View of the Hydraulic Microdrive, Pituitary Stimulating Electrodes, and the Carotid and Venous Cannulae on the Head of a Sheep

A cranial platform holding the stereotaxic bone fixed adapter cylinder and capped arterial and venous cannulae was chronically fixed above the scalp on four bolts cemented in the skull. Bipolar pituitary stimulating electrodes were stereotaxically lowered from above at a 29° angle and permanently fixed to the skull and platform with dental cement. The silicon rubber cannula was placed into the common carotid artery through the cut end of the thyrolaryngeal artery and fixed without interruption of flow in the common carotid artery. The silicon rubber cannulae were threaded under the skin of the neck and head to the cranial platform. Labels: l.l.t., leur-lok tip with rubber caps for the arterial and venous cannulae; r.e., recording electrode; p.e., pituitary electrode; i.m.a., internal maxillary artery; e.m.a., external maxillary artery; cc., carotid cannula; j.c., jugular cannula; t.a., thyrolaryngeal artery; c.a., carotid artery; j.v., jugular vein.

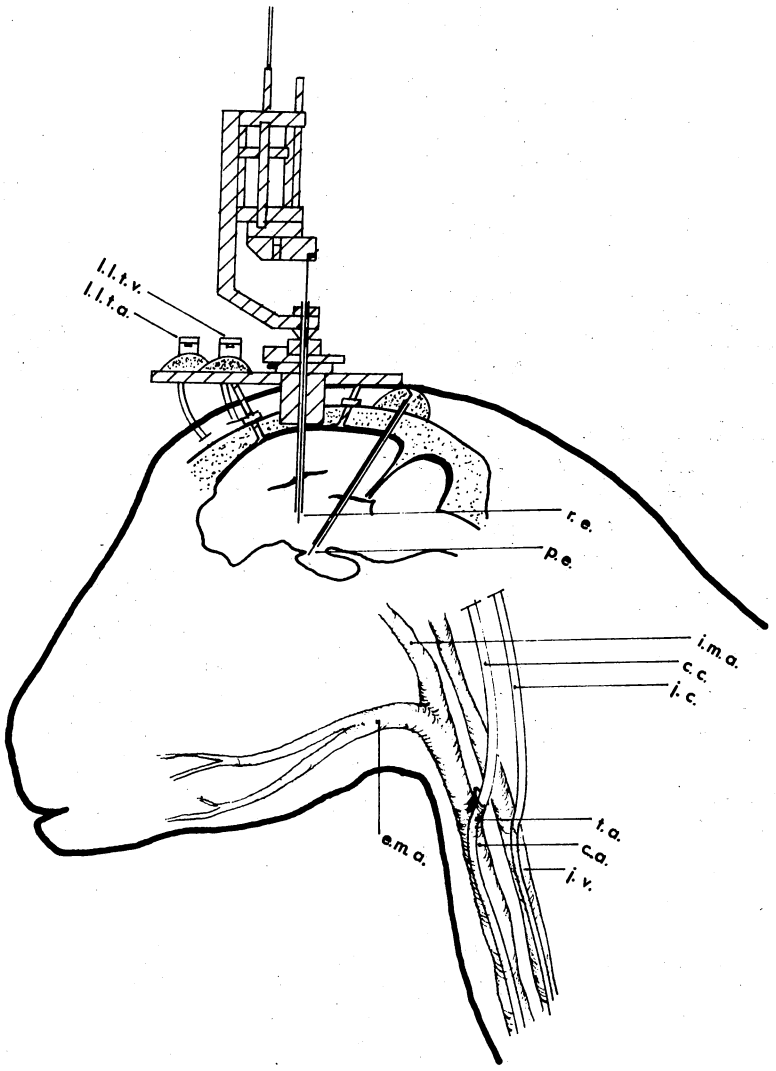
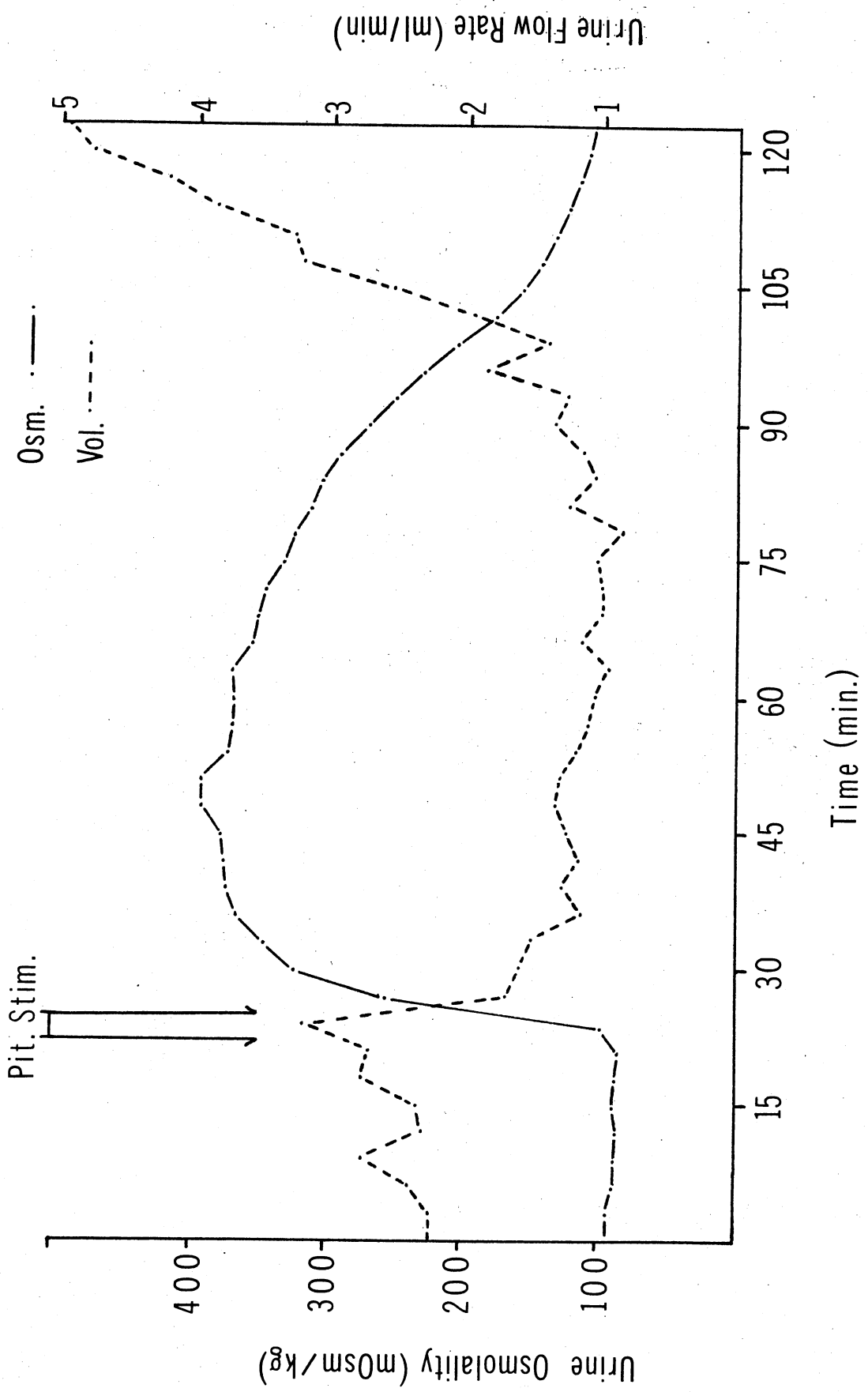

l.l.t.v.
l.l.t.a.
r.e.
p.e.
i.m.a.
c.c.
i.c.
e.m.a.
t.a.
c.a.
i.v.

Figure 3. Antidiuresis Produced in a Hydrated Sheep by Pituitary Stalk Stimulation

Pit. Stim., pituitary stalk stimulation; Osm., urine osmolality (mOsm/kg); Vol., urine flow rate (ml/min).


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

Left Atrial Cannulation

Since left atrial pressure has been implicated in the control of ADH, left atrial catheters were placed in 5 sheep in order to follow left atrial pressure during an osmotic forcing (to be discussed later). A silicone rubber catheter with a radiopaque marker was placed in the left atrial appendage by means of a left thoracotomy in the fifth intercostal space and secured by means of a purse string suture through the appendage. The radiopaque marker served to locate the left atrial appendage for later transducer placement. The cannula was filled with heparinized saline (1000 U/ml) and retracted so that a collar of silicone medical adhesive abutted against the purse string tie.

Single Unit Recording

Following recovery from surgery, as indicated by eating at preoperative levels, the sheep was isolated from the investigator and recording equipment in a recording chamber. Behavior was constantly monitored during the course of an experimental period by means of a television monitor system (camera SONY AVC-3000 and monitor CVM-920U). The head was held painlessly by a nose band, head supporting platform and neck strap which allowed the sheep to behave normally throughout the duration of the experiment.

Tungsten microelectrodes were constructed (after Hubel, 1957) from 0.008 inch diameter, 10-12 cm long tungsten wires. Tip diameters of one micron or less were made by electrolytic etching and polishing in a solution of saturated potassium nitrite. Microelectrodes were then insulated to within 10 to 20 microns of the tip with four coats of Isonel 31 insulating varnish (Schenectady Chemicals, Inc.). A capacitance meter

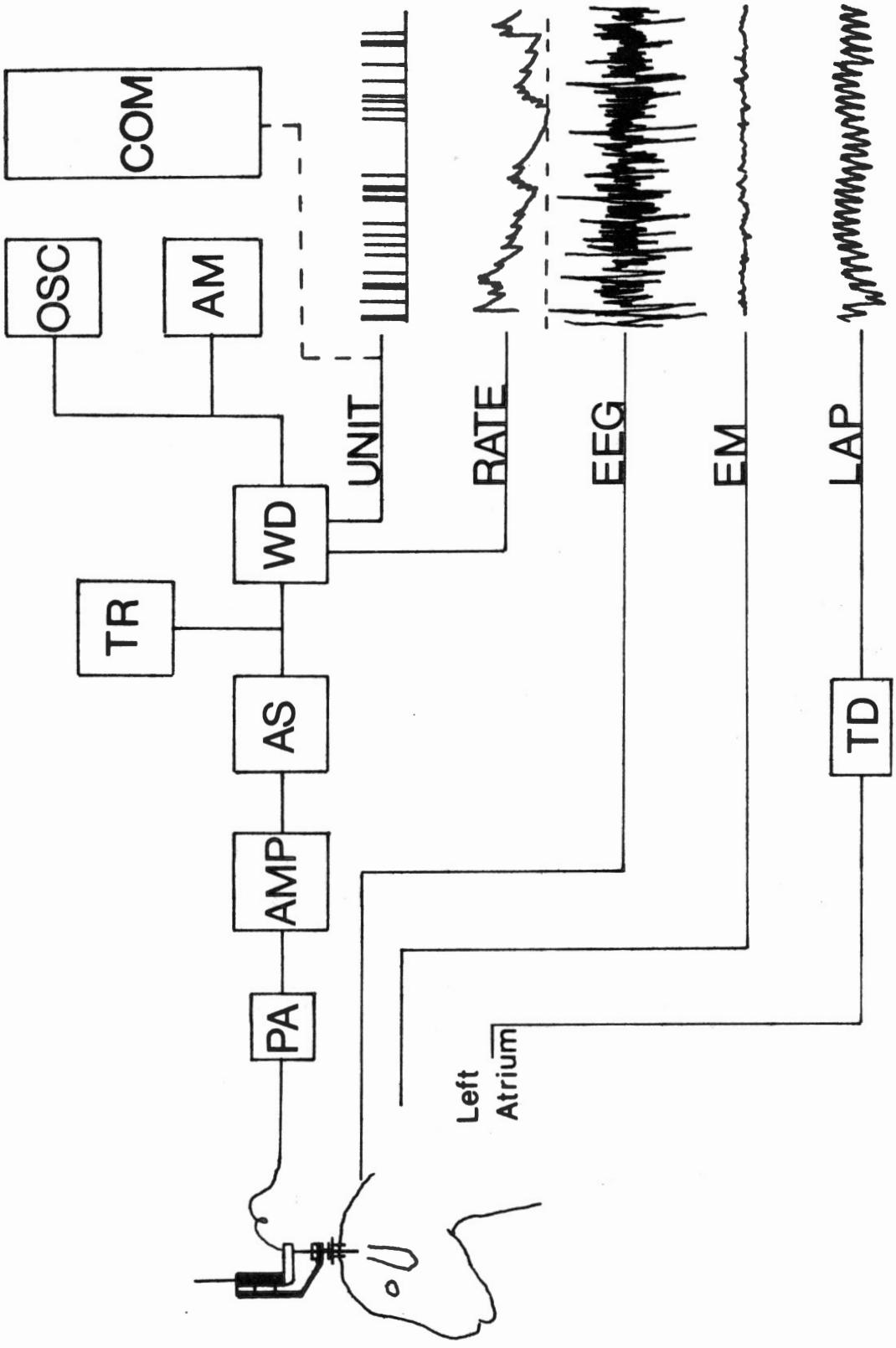
(Tektronix Type 130 L-C Meter) was then used to measure tip capacitance (50-80 pf acceptable) and uninsulated tip length (Bak, 1967). A tungsten microelectrode was then lowered within a 22 gauge stainless steel guide tube through the Starr guide of the titanium micropositioner and bone wax of the stainless steel cylinder to a position 5-10 mm above the SON. The electrode was then lowered out of the protective guide tube into the SON and optic chiasm by means of a calibrated hydraulic micromanipulator (Trent Wells, Inc.).

Extracellular single unit activity was amplified by the dc preamplifier on the lucite platform and led into a high gain ac coupled band pass amplifier (F. Haer). This amplified activity was directed to a window discriminator, audiomonitor (Grass AM3), magnetic tape recorder (SONY TC 366) and oscilloscope monitor (Tektronix 5103N/D13). The magnetic tape recordings could later be replayed for analysis and photography. Two outputs from the window discriminator were led to an ink writing polygraph (Grass Model 7). These were a one to one pulse output and an analog output proportional to neuronal discharge rate. These were recorded simultaneously with EEG and eye movement. The polygraph written records could then be correlated with behavior and unit recording from the hypothalamus. The written records were also used to subjectively classify neuron discharge patterns, by visual inspection, into one of seven types. A flow diagram of this recording system is illustrated in Figure 4.

Single units were tested for antidromic activation by stimulation of the pituitary stalk with single square wave pulses of 2 msec duration and 2-8 mA intensity. A unit was considered to be an identified magnocellular neuroendocrine cell if, in response to pituitary stalk stimulation, it

Figure 4. 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; COM, computer; TD, blood pressure transducer; EEG, electrocorticogram; EM, eye movement; LAP, left atrial pressure.


COM
OSC
AM
TR
WD
AS
AMP
PA
UNIT
RATE
EEG
EM
LAP
TD
Left
Atrium

exhibited a stable wave form and latency, followed high frequency stimuli (100 per second) and/or collided with a spontaneously occurring antidromic spike. An antidromically evoked spike and collision are illustrated in Figure 5.

After detection and isolation of a stable neuron its spontaneous activity was recorded for a period of 5-15 minutes. At that time an infusion of hypertonic saline (1.2 M sodium chloride) was employed to "force" a change in plasma osmolality of about 10 mOsm/kg (9.46 $\pm$ 1.60) in 10 minutes or a 20 mOsm/kg (17.79 $\pm$ 2.95) in 20 minutes. Osmolalities were determined with an Advanced Instruments, Inc. osmometer. In eighteen cases left atrial pressure was also monitored through the left atrial catheter by means of a Statham P23DC transducer. Mean changes of plasma osmolality (mOsm/kg $\pm$ SEM) and mean changes of left atrial pressure (cm H_2O $\pm$ SEM) are plotted in Figure 6.

Unit activity was followed after termination of the forcing to observe behavior of the magnocellular neuroendocrine cell during the return of plasma osmolality towards control levels. Recording duration for a single neuron ranged from 5 minutes to 4 hours, averaging about 1 hour.

Localization of Recording Sites

At the conclusion of experimentation the animal was terminally anesthetized and deposits of iron from stainless steel lesion electrodes were stereotaxically placed at known intervals. The brain was then perfused via the carotid arteries with saline containing 2% sodium ferrocyanide and 10% formalin. The brain was exposed ventrally to allow visual inspection of pituitary electrode placement. In animals from which antidromically identified magnocellular neuroendocrine cells had

Figure 5. An Antidromically Evoked Spike and Collision

Labels: r.e., recording electrode; s.e., stimulating electrode; O.C., optic chiasm; Trace 1, spontaneously occurring action potential; Trace 2, action potential evoked by stimulation of the pituitary stalk; Trace 3, antidromic field resulting from collision of spontaneously occurring action potential with evoked action potential; Trace 4, Trace 3 at a higher magnification.

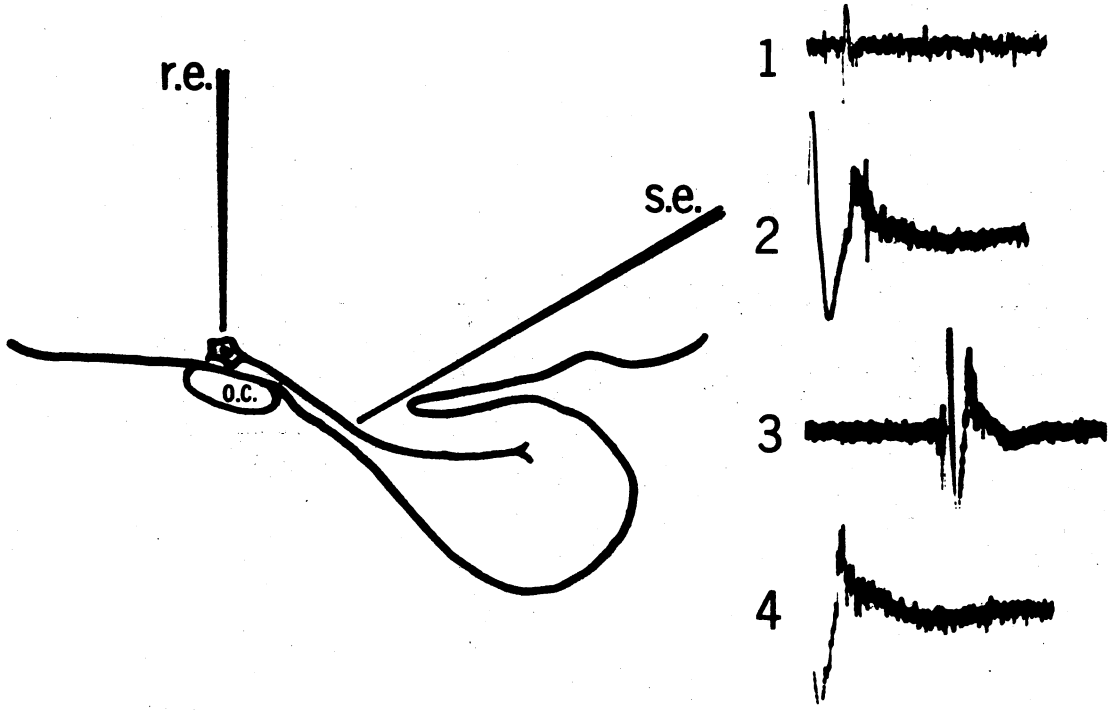
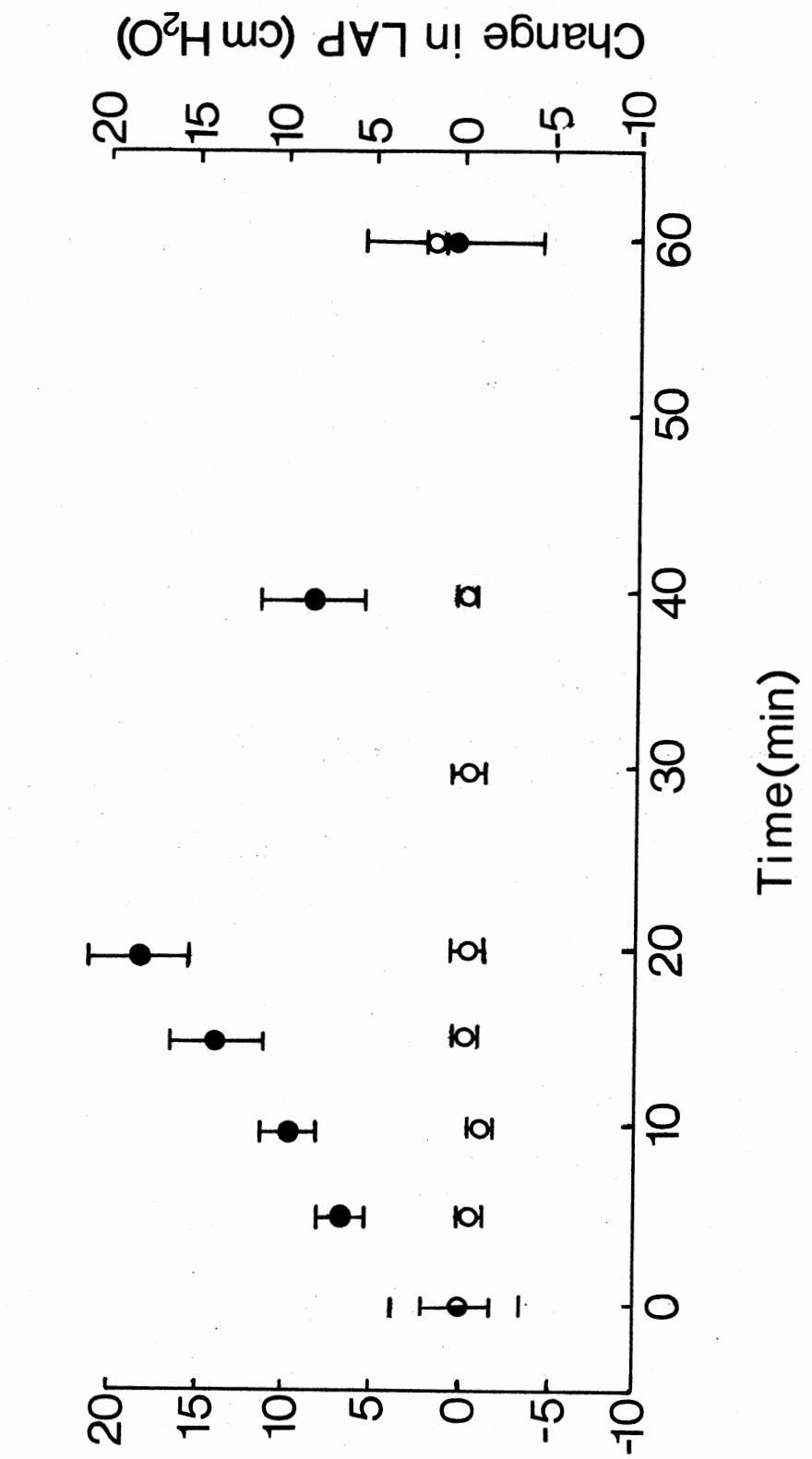

r.e.
s.e.
o.c.
1
2
3
4

Figure 6. Mean Response of Plasma Osmolality and Left Atrial Pressure to a Hypertonic Forcing

Labels: ●, change in plasma osmolality (mOsm/kg); O, change in left atrial pressure (cm/H_2O).


Change in Osmolality (mOsm/kg)
20
15
10
5
0
-5
-10
0
10
20
30
40
50
60
Time(min)
Change in LAP (cm H_2O)
20
15
10
5
0
-5
-10

been recorded, accurate drawings were made of electrode tip placement so that estimates of conduction distance could be made. Knowing antidromic latency and conduction distance, an estimate of conduction velocity for magnocellular neuroendocrine cells was calculated (Eccles, et al., 1958). The brain was then removed and frozen sections of the diencephalon were cut at 100 microns in the stereotaxic plane. Location of the Prussian blue spots (Figure 7) aided in reconstruction of electrode tracts and placement of unit locations on outline drawings of the areas sectioned.

Data Analysis

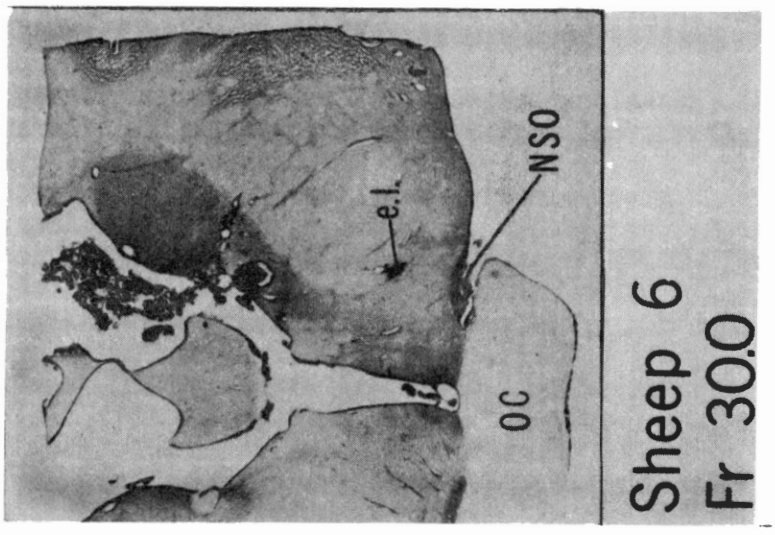
Analysis of data began when a unit was isolated from baseline activity. After noting the recording period to be further analyzed, magnetic tape sections were replayed and unit action potentials led into the window discriminator. Short duration (0.5 msec) square wave pulse outputs of the window discriminator triggered by unit action potentials were further recorded on magnetic tape and used for subsequent computer analysis.

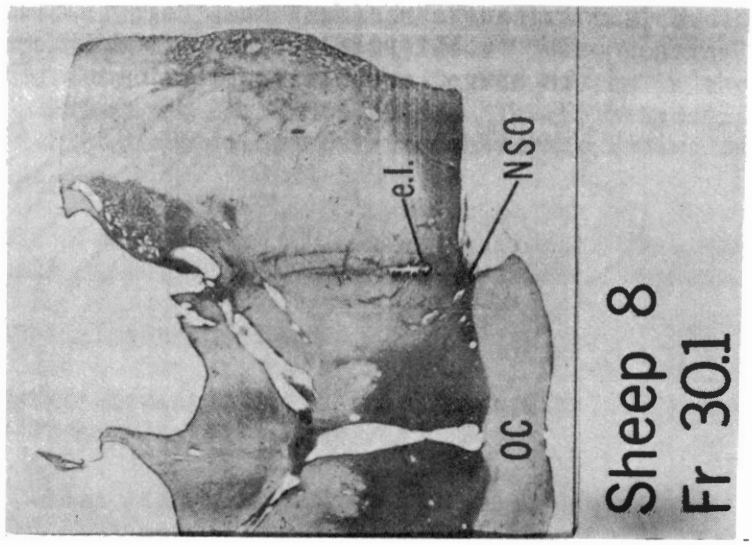
Some computer analysis was performed by Mr. Mike Davis at the Brain Research Institute, University of California, Los Angeles. The program directed a PDP 8 Digital Computer to calculate mean firing rate, mean interspike interval, standard deviation, coefficient of variation and histogram of any desired order. The coefficient of variation as calculated by the computer program is the reciprocal of that discussed in textbooks of statistics. Some or all of this data was used to classify a neuron discharge pattern into one of seven types.

Subsequent computer analysis was performed on the campus of Oklahoma State University. An Interdata 7-16 computer system with a Centrionic

Figure 7. Lesion Verification of Recording Sites in Two Sheep

Prussian blue spots were made after passing a dc current through stainless steel electrodes supported from the recording platform. The lesions were placed 3 mm above the optic chiasm level (determined physiologically with recording electrodes) to ensure their visibility. Frontal coordinates for these lesions are Fr. 30.0 (Sheep 6) and Fr. 30.1 (Sheep 8). Labels: e.l., electrode lesion; NSO, supraoptic nucleus, OC, optic chiasm.


e.l.
NSO
OC
Sheep 6
Fr 30.0


e.l.
NSO
OC
Sheep 8
Fr 30.1

Line Printer (capable of generating plots) was programmed to calculate mean firing rate, mean interspike interval and its standard deviation, mean instantaneous rate and its standard deviation and confidence limits regarding increasing or decreasing interspike interval length (trend analysis). The line printer also plotted interspike interval histograms and cumulative time histograms. Mean firing rate was calculated by taking the reciprocal of the mean interspike interval. Mean instantaneous rate is the firing rate calculated by averaging the reciprocals of each interspike interval.

Trend analysis, based on the Rank Correlation Method of Kendall (1970), calculated the confidence limits for the tendency of interspike intervals to progressively increase or decrease spontaneously or in response to an osmotic forcing. The value K_a, produced by the trend analysis was used as a measure of osmosensitivity. Trend analysis was calculated from successive bins of the cumulative time histogram or instantaneous rate plot. These values were either used alone to define osmosensitivity or divided by the change in mOsm/kg for the analysis period.

Since neuronal response to a continuous linear change in plasma osmolality was the major concern of this dissertation, it was imperative that a method for displaying this response be designed. Some experimental periods ran into hours, therefore large amounts of magnetic tape and polygraph tracings were accumulated. The need for compressing spike trains into a form which displayed the neuronal response without the presence of a large amount of material was obvious. Chung, et al. (1974) designed a simple device for interspike interval analysis. The instrument designed and constructed for display of this data is similar in

theory. A binary counter was used to generate a linear voltage change amounting to a series of discrete jumps. This voltage change was used to drive a single sweep of the oscilloscope lasting up to 40 minutes. The vertical deflection scale amounted to a logrithmic voltage change across a capacitor. Pulses from the window discriminator served as input to the device which generated a one to one output to the z-axis of the oscilloscope. Interspike interval then was expressed on the vertical scale in a logrithmic fashion while spike occurrence appeared as a "dot" generated by input to the z-axis. Spike trains up to 40 minutes in duration could then be displayed as a single oscilloscope sweep. This enabled one to observe spontaneous activity patterns as well as changes evoked by a hypertonic forcing.

Statistical Analysis

Analysis of variance (one-way classification) and Duncan's new multiple range test were employed to find significant differences where more than two means were to be compared. Least significant ranges for groups with unequal replication were found by the method described in Steele and Torrie (1960).

Statistical analysis involved comparisons between antidromically identified magnocellular neuroendocrine cells and nonidentified cells located outside the supraoptic nuclear region (defined to be within 1 mm of the histologic boundaries of the SON). Nonidentified cells were not included in the analysis due to the possibility that they might be magnocellular neuroendocrine cells whose axons were not stimulated by the electrodes across the hypophyseal stalk.

A chi-square analysis of counts was used to approximate the

probability that the distribution of counts was due to chance for the parameter in question (e.g., distribution of firing pattern, sleep-waking and sensory responsiveness within an area). Yates' correction for continuity was employed with this analysis to obtain a more exact probability value from the chi-square table.

The t test for nonpaired experiments was employed where comparisons between two means were to be made.

The correlation coefficient, r, was calculated to determine the degree of reliability of the relationship between two variables (conduction velocity and measures of osmosensitivity).

CHAPTER IV

RESULTS

Spike trains from over 125 neurons were recorded from hypothalamic and septal areas of 10 unanesthetized sheep. Of these, 116 were found suitable for further analysis. Spike trains from 75 supraoptic magnocellular neuroendocrine cells were studied in 8 animals. The low number of neuroendocrine cells recorded "per sheep" was due to several factors. Two major problems were difficulty in obtaining optimal pituitary stimulating electrode placement and recording from a well isolated neuroendocrine cell throughout the necessarily long recording period which included blood sampling and behavioral testing. Other problems encountered included long term anesthesia in a ruminant and the maintenance of patent chronic jugular cannulae. A large stimulus artifact which sometimes obscured antidromically evoked action potentials was introduced by necessary placement of the stimulus isolation unit several feet from the experimental animal.

Categorization of Firing Patterns

Activity of all recorded units could be categorized into one of seven firing patterns ranging from silent through periodic bursting to continuously active. Categorization was based on some or all of the following parameters: mean spontaneous firing rate, mean interspike interval, interspike interval mode, interspike interval standard deviation,

interspike interval histogram shape and coefficient of variation. Bursting cells were further categorized on the basis of burst duration, interburst interval, period, spikes per burst and burst mean firing rate. Means ± SEM of the parameters used for classification of spike trains into firing pattern types are listed in Tables I and II.

Silent cells (3%) were discovered only by stimulation of the hypophyseal stalk which evoked an antidromic potential in the soma. These cells were found only in the supraoptic nuclear region.

Continuously active slow cells (21%) were found in both the supraoptic nuclear region and widespread hypothalamic and septal areas. The mean firing rate was defined to be less than one spike per second. The interspike interval histogram tended to have a Poisson distribution (assymetrical unimodal peak) with the mode generally less than the mean interspike interval. Several long intervals were usually present in the spike train. The coefficient of variation was always found to be greater than 0.5.

Low frequency bursting cells (14%) fired in relatively long duration (2-15 seconds) low frequency bursts (2-8 spikes per second) and had a period from 6 to 50 seconds. The mean firing rate was usually greater than 1 spike per second and the interspike interval histogram showed a narrow unimodal peak with the mode greater than 60 milliseconds. Any low frequency bursting train of action potentials could have singly occurring spikes during the interburst interval although these were uncommon. The coefficient of variation was usually less than 0.5. These cells were also found in both the supraoptic nuclear region and in hypothalamic and septal areas. Mean burst characteristics are listed in Table II.

Continuously active fast cells (45%) were defined to have a train of

TABLE I

MEAN PARAMETERS[1] OF SPONTANEOUS FIRING PATTERNS

Cell Type	MFR[2]	ISI[2]	Mode[2]	CV[2]	MIR[2,3]
CAS[2]	.42 ± .05 n=37	7.05 ± 2.15 n=37	364.29 ± 123.98 n=7	.80 ± .04 n=20	19.92 ± 5.89 n=20
LFB[2]	1.57 ± .20 n=25	.86 ± .09 n=25	140.00 ± 18.28 n=9	.46 ± .08 n=24	6.43 ± 1.57 n=8
CAF[2]	4.84 ± .44 n=81	.34 ± .03 n=81	84.70 ± 8.97 n=23	.92 ± .17 n=68	17.98 ± 3.85 n=29
HFB[2]	3.24 ± .51 n=11	.39 ± .06 n=11	13.78 ± 4.08 n=9	.36 ± .08 n=10	
CAB[2]	7.13 ± 1.03 n=6	.15 ± .02 n=6	40.67 ± 20.57 n=3	.42 ± .10 n=6	
CAR[2]	5.02 ± .83 n=15	.25 ± .03 n=15	214.00 ± 62.00 n=5	3.19 ± 1.15 n=13	

[1]Means ± standard error of the mean.

[2]Abbreviations used are: MFR, overall mean firing rate; ISI, interspike interval; Mode, mean interspike interval mode; CV, coefficient of variation; MIR, mean instantaneous rate.

[3]Available for antidromically identified neurons only.

TABLE II

MEAN PARAMETERS[1] OF SPONTANEOUS BURSTING PATTERNS

Cell Type	MFR[2]	BD[2]	S/B[2]	BMFR[2]	IBI[2]	Pd[2]	n
AD+ LFB[2]	1.26 ± .15	9.76 ± 3.05	33.99 ± 9.01	4.03 ± .70	20.86 ± 4.40	30.75 ± 6.78	(MFR = 11) 8
AD- LFB[2]	1.83 ± .36	6.22 ± 1.23	23.23 ± 6.84	4.05 ± .51	12.43 ± 2.63	18.65 ± 3.24	(MFR = 9) 14
HFB[2]	3.14 ± .57	.57 ± .11	9.22 ± 1.76	19.19 ± 4.20	1.71 ± .23	2.29 ± .31	(MFR = 6) 11
CAB[2]	6.62 ± .97	.66 ± .14	7.09 ± 1.52	11.37 ± 1.07	.34 ± .03	.99 ± .15	(MFR = 5) 6

[1]Means ± standard error of the mean.

[2]Abbreviations used are: MFR, overall mean firing rate; BD, burst duration; S/B, spikes per burst; BMFR, burst mean firing rate; IBI, interburst interval; Pd, period; AD+ LFB, antidromically identified low frequency burster; AD- LFB, nonidentified low frequency burster located outside the supraoptic nuclear region; HFB, high frequency burster; CAB, continously active burster.

singly occurring spikes having a mean firing rate greater than one spike per second. The interspike interval histogram like that of the continuously active slow cells was assymetrical with a unimodal peak also tending toward a Poisson distribution. In contrast to the continuously active slow cell the interspike interval histogram was broad. The mode again was generally less than the mean. This firing pattern type can have some clusters (less than 10 spikes per second) and may exhibit irregular cyclic variations in mean firing rate. Cells in both the supraoptic nuclear region and hypothalamic and septal areas exhibited this pattern of firing.

High frequency bursting cells (6%) exhibited short duration (0.3 to 5 seconds) high frequency (greater than 10 spikes per second) bursts usually characterized by irregular periods not less than twice the burst duration. The interspike interval histogram showed a narrow unimodal peak with a random distribution of longer intervals and a mode of 5 to 40 milliseconds. Singly occurring spikes did occur and the standard deviation of the interspike interval was greater than 1000. Cells exhibiting the high frequency bursting pattern of firing were found in both the supraoptic nuclear region and in hypothalamic and septal areas. Mean burst characteristics can be found in Table II. No magnocellular neuroendocrine cell exhibited this pattern of firing.

Continuously active bursting cells (3%) were found to fire in short rapidly occurring bursts giving a superficial appearance of continuous activity with few, if any, singly occurring spikes. The interspike interval histogram was bimodal with a relatively short interspike interval mode of 10 to 70 milliseconds. The standard deviation of the interspike interval was large. Cells exhibiting the continuously active

bursting pattern of firing were found in both the supraoptic nuclear region and in hypothalamic and septal areas. Mean burst characteristics can be found in Table II. No magnocellular neuroendocrine cell exhibited this pattern of firing.

Cells exhibiting the continuously active regular pattern of firing (8%) exhibited a train of single spikes occurring at regular intervals and had a mean firing rate of 5.02 $\pm$ 0.83 (SEM). The interspike interval histogram had a narrow symmetrical modal peak with the mode being nearly equal to the mean and a small standard deviation. The coefficient of variation was greater than 1.0. This firing pattern was also found in both the supraoptic nuclear region and hypothalamic and septal areas. No magnocellular neuroendocrine cell exhibited this pattern of firing.

Example spike trains and interspike interval histograms for each firing pattern type (with the exception of silent which shows no spontaneous activity) are shown in Figures 8 and 9.

Spike trains for the same cells are shown in Figures 10 through 15 as they are generated by the dot raster display.

Stability of Firing Patterns

Stability of firing patterns was defined as the ratio of time spent in a firing pattern divided by the total recording time of that neuron. Most neurons had a stability of 1.

The continuously active patterns of firing were the least stable. Very slow firing cells were often placed in the silent category until closer inspection revealed the presence of occasional spikes. Other cells exhibiting the continuously active slow pattern of firing fired in clusters giving the appearance of occasional low frequency bursting.

Figure 8. Example Spike Trains and Interspike Interval Histograms of Three Continuously Active Neurons

A, continuously active slow; B, continuously active fast; C, continuously active regular. Labels: EEG, electrocorticogram; B.W., bin width in milliseconds.

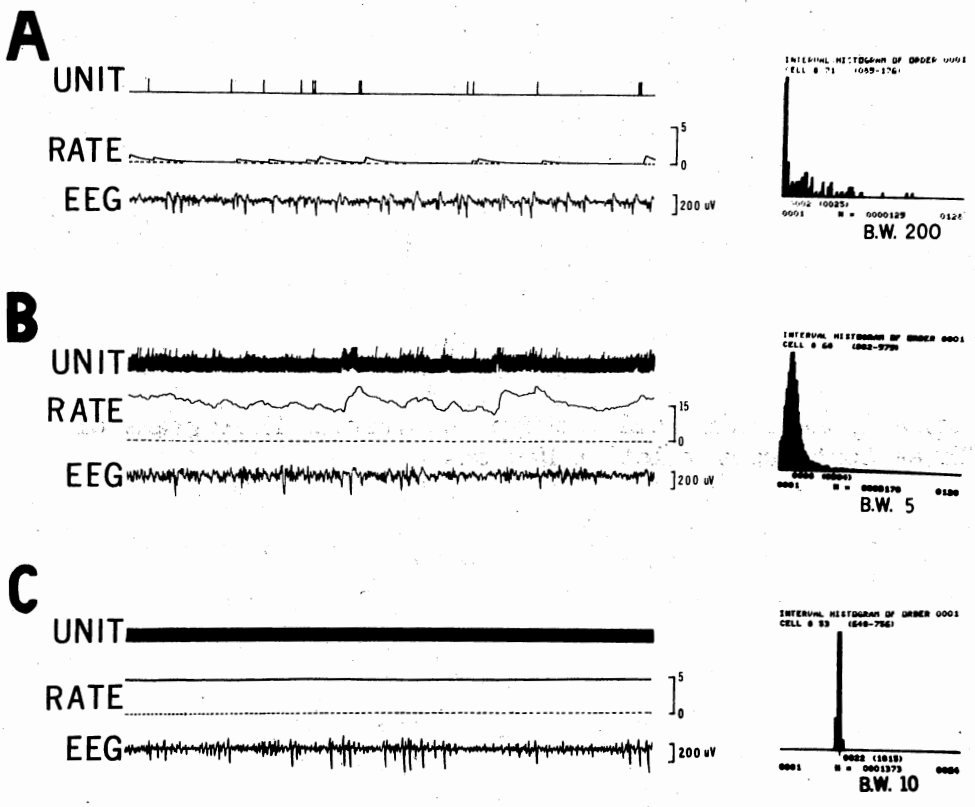

A
UNIT
RATE
EEG
5
0
200 uV
B.W. 200
B
UNIT
RATE
EEG
15
0
200 uV
B.W. 5
C
UNIT
RATE
EEG
5
0
200 uV
B.W. 10

Figure 9. Example Spike Trains and Interspike Interval Histograms of Three Bursting Neurons

A, low frequency bursting neuron; B, high frequency bursting neuron; C, continuously active bursting neuron. Labels: EEG, electrocorticogram; B.W., bin width in milliseconds.

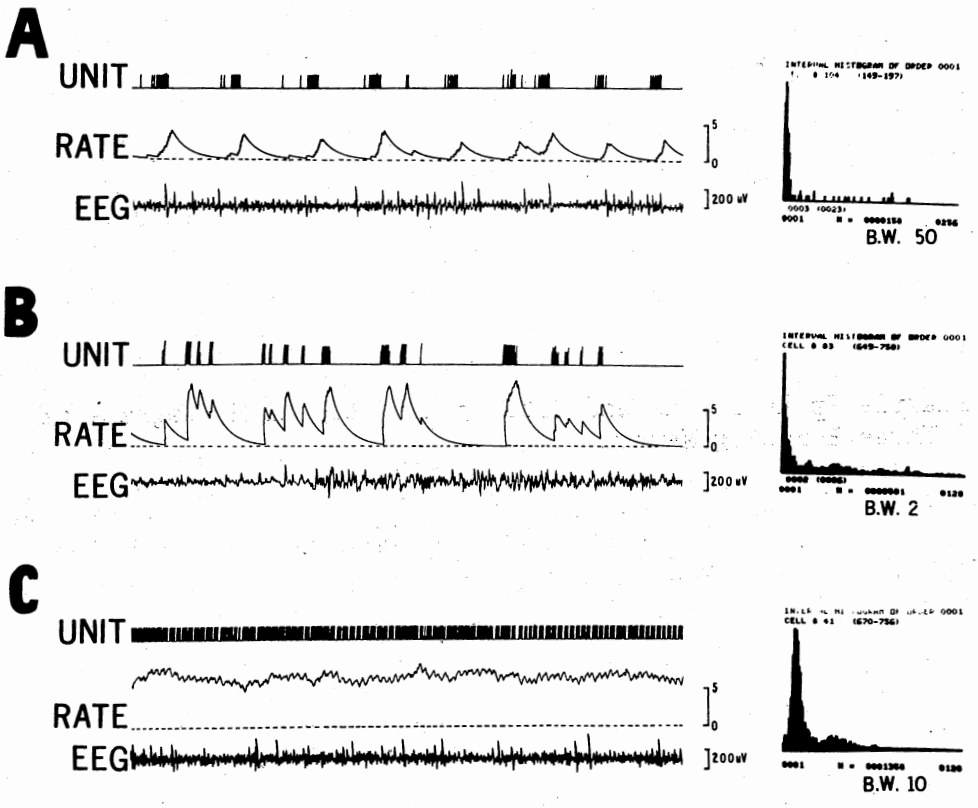

A
UNIT
RATE
EEG
5
0
200 uV
B.W. 50
B
UNIT
RATE
EEG
5
0
200 uV
B.W. 2
C
UNIT
RATE
EEG
5
0
200 uV
B.W. 10

Figure 10. Dot Raster Display of a Continuously Active Slow Cell

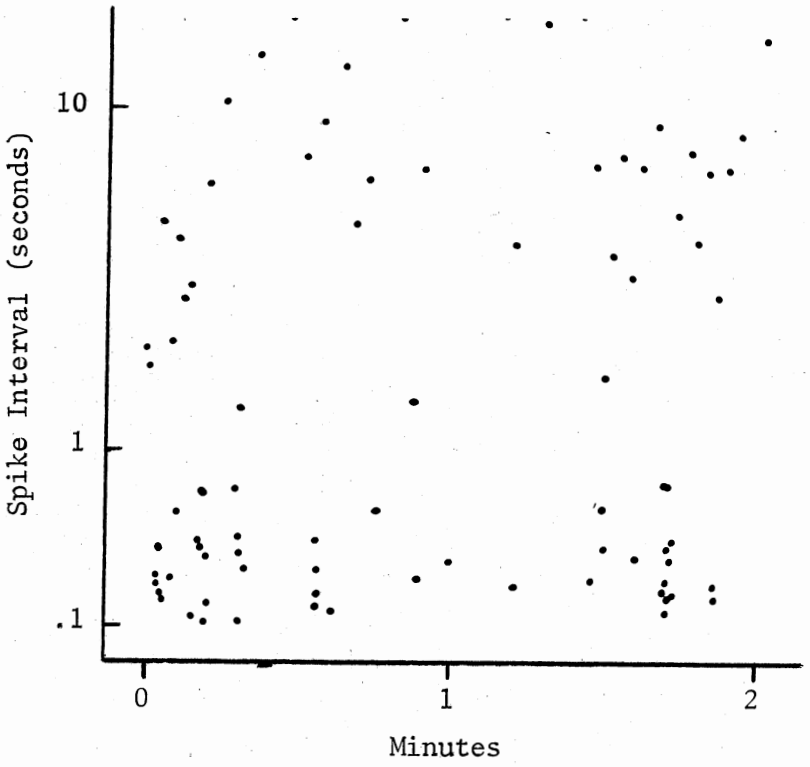

Spike Interval (seconds)
10
1
.1
0
1
2
Minutes

Figure 11. Dot Raster Display of a Low Frequency Bursting Cell

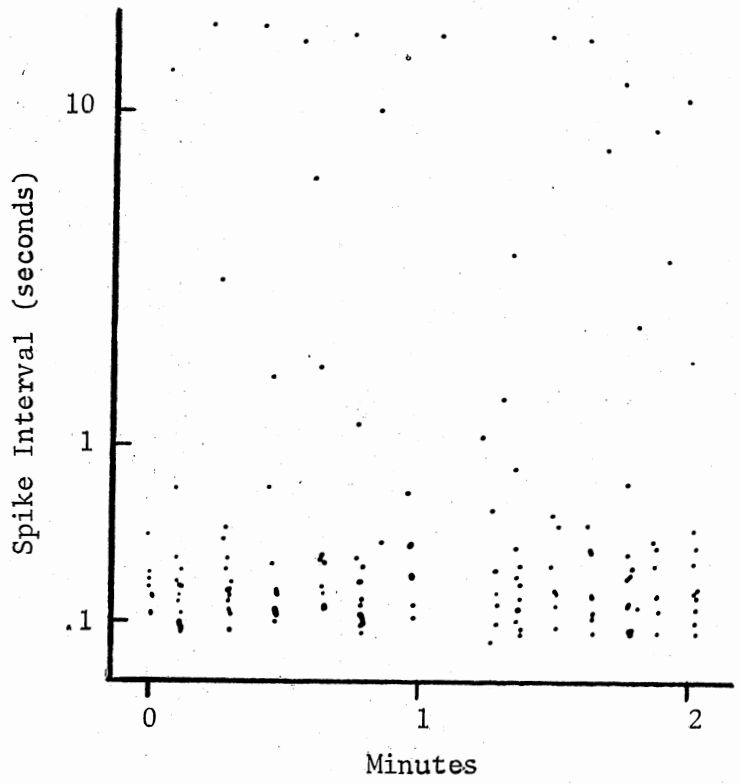

Spike Interval (seconds)
10
1
.1
0
1
2
Minutes

Figure 12. Dot Raster Display of a Continuously Active Fast Cell

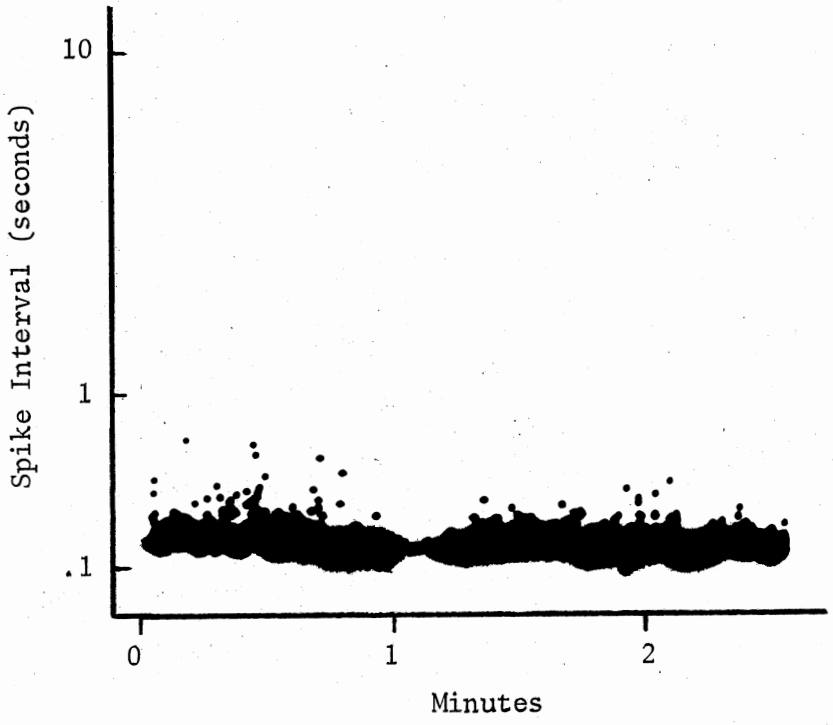

10
1
.1
Spike Interval (seconds)
0
1
2
Minutes

Figure 13. Dot Raster Display of a High Frequency Bursting Cell

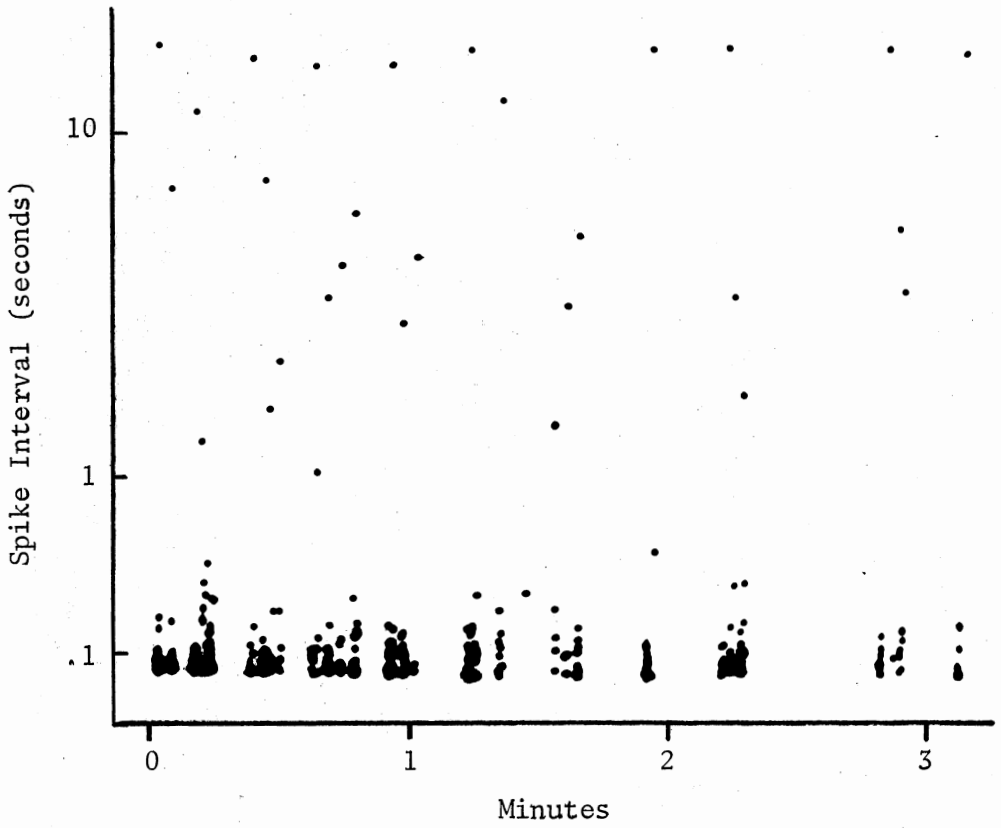

Spike Interval (seconds)
10
1
.1
0
1
2
3
Minutes

Figure 14. Dot Raster Display of a Continuously Active Bursting Cell

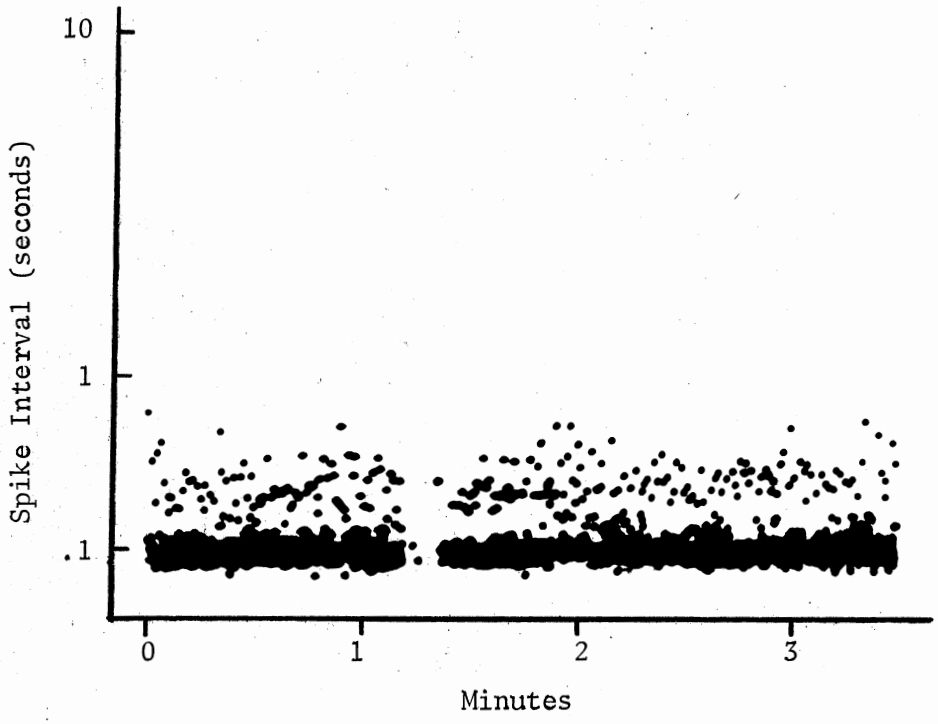
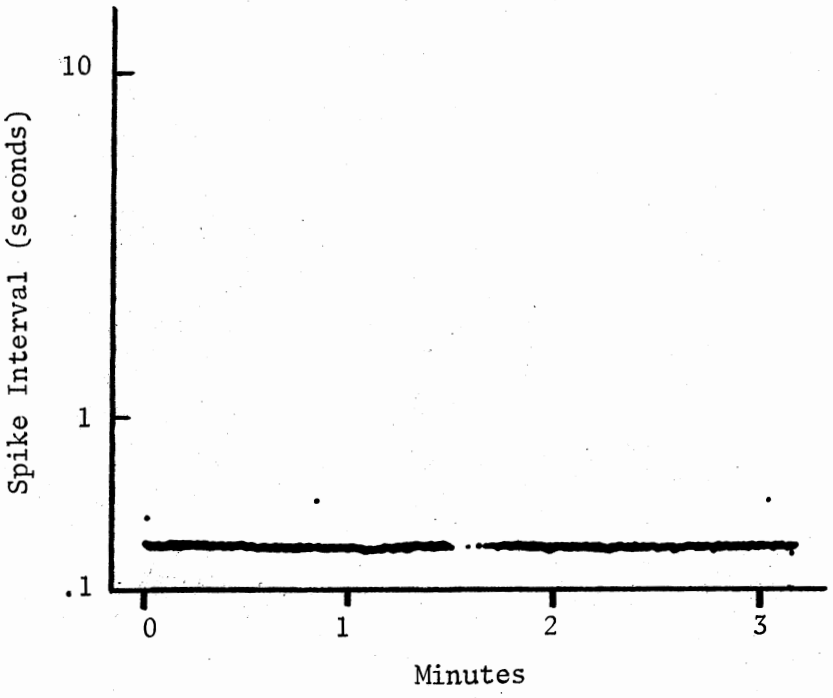

10
1
.1
Spike Interval (seconds)
0
1
2
3
Minutes

Figure 15. Dot Raster Display of a Continuously Active Regular Cell


Spike Interval (seconds)
10
1
.1
0
1
2
3
Minutes

Cells firing in a continuously active fast pattern also generated clusters of spikes giving the train an appearance of continuously active bursting for a short segment.

The low frequency bursting pattern of firing at times could appear to be continuously active slow or continuously active fast since singly occurring spikes were sometimes present in these trains.

Only the continuously active regular pattern of firing was more stable than high frequency bursting and continuously active bursting patterns of activity. These patterns only changed level of firing rate in response to a stimulus (to be discussed later). Continuously active regular cells rarely responded to any stimulus or changed their pattern of firing for any reason.

Statistical Analysis of Firing Pattern Parameters

Analysis of variance for each parameter used to classify spike trains into a pattern of activity demonstrated significant differences ($P < .05$) in most cases. Significant differences were not observed in some cases where the mean values "look" different. This may be due to heterogeneity of variances or lack of sensitivity of the Duncan Multiple Range test for separation of means.

The mean firing rate of continuously active bursting cells was found to be significantly greater ($P < .05$) than the mean firing rate of all other cells. The mean firing rate of continuously active regular cells was significantly greater ($P < .05$) than that of low frequency bursting and continuously active slow cells. Continuously active fast cells had a mean firing rate greater ($P < .05$) than that of either the low frequency bursting cells or continuously active slow cells. The mean firing rate

of high frequency bursting cells was significantly greater ($P < .05$) than the mean firing rate of continuously active slow cells. No other significant differences were found (Table III).

The mean interspike interval of continuously active slow spike trains was significantly greater ($P < .05$) than the mean interspike intervals of all other firing patterns. No other significant differences were found (Table IV).

Interspike interval modes of each activity pattern were significantly different ($P < .05$) in each comparison with the exception that continuously active bursting was not different from either continuously active fast or high frequency bursting (Table V).

The coefficient of variation for continuously active regular spike trains was significantly greater ($P < .05$) than the coefficient of variation for all other firing patterns. No other significant differences were found (Table VI).

Mean instantaneous rate was compared for continuously active slow, low frequency bursting and continuously active fast firing patterns. No significant differences were found (Table VII).

Statistical Analysis of Bursting Pattern Parameters

Parameters used to classify spike trains into one of the bursting patterns of activity were mean firing rate, period, burst duration, burst mean firing rate, spikes per burst, and interburst interval. Comparisons were made between continuously active bursting, high frequency bursting, nonidentified low frequency bursting cells outside the supraoptic nuclear region and antidromically identified low frequency bursting cells.

TABLE III

COMPARISON OF MEAN FIRING RATES[1] OF SPONTANEOUS FIRING PATTERNS

CAB[2]	CAR[2]	CAF[2]	HFB[2]	LFB[2]	CAS[2]
7.13	5.02	4.84	3.24	1.57	0.42

[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 ($P < .05$).

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

TABLE IV

COMPARISON OF MEAN INTERSPIKE INTERVALS[1] OF SPONTANEOUS FIRING PATTERNS

CAS[2]	LFB[2]	HFB[2]	CAF[2]	CAR[2]	CAB[2]
7.05	.86	.39	.34	.25	.15

[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 ($P < .05$).

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

TABLE V

COMPARISON OF MEAN INTERSPIKE INTERVAL MODES[1] OF SPONTANEOUS FIRING PATTERNS

CAS[2]	CAR[2]	LFB[2]	CAF[2]	CAB[2]	HFB[2]
364.29	214.00	140.00	84.70	40.67	13.78

[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 ($P < .05$).

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

TABLE VI

COMPARISON OF MEAN COEFFICIENTS OF VARIATION[1] OF SPONTANEOUS FIRING PATTERNS

CAR[2]	CAF[2]	CAS[2]	LFB[2]	CAB[2]	HFB[2]
3.19	.92	.80	.46	.42	.36

[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 ($P < .05$).

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

TABLE VII

COMPARISON OF MEAN INSTANTANEOUS RATES[1,2] OF SPONTANEOUS FIRING PATTERNS

CAS[3]	CAF[3]	LFB[3]
19.92	17.98	6.43

[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 ($P < .05$).

[2]Available for identified neuroendocrine cells only.

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

Means $\pm$ SEM for these parameters are listed in Table II.

The mean firing rate of continuously active bursting cells was found to be significantly greater ($P < .05$) than the mean firing rate of all other firing pattern types considered (Table VIII). High frequency bursting cells had a significantly greater ($P < .05$) mean firing rate than antidromically identified low frequency bursting cells. No other significant differences were found.

The mean burst durations of antidromically identified low frequency bursting cells and of antidromically negative low frequency bursting cells were found to be significantly greater ($P < .05$) than all other means to which they were compared. The mean burst durations of the low frequency bursting cells were not significantly different. Similarly, no significant difference was found when mean burst durations of high frequency bursting and continuously active bursting cells were compared (Table IX).

The number of spikes per burst (Table X) in the antidromically identified low frequency bursting cells was significantly greater ($P < .05$) than that of all other bursting spike trains except for non-identified low frequency bursting cells outside the supraoptic nuclear region. No other significant differences were found.

The burst mean firing rate of high frequency bursting neurons and of continuously active bursting neurons were significantly higher ($P < .05$) than the burst mean firing rates of both groups of low frequency bursting neurons. Burst mean firing rate of high frequency bursting neurons was not different from that of continuously active bursting neurons. Similarly burst mean firing rates of the two groups of low frequency bursting neurons were not significantly different (Table XI).

TABLE VIII

COMPARISON OF MEAN FIRING RATES[1] OF SPONTANEOUS BURSTING PATTERNS

CAB[2]	HFB[2]	AD- LFB[2]	AD+ LFB[2]
6.62	3.14	1.83	1.26

[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 ($P < .05$).

[2]Abbreviations used are: CAB, continuously active burster; HFB, high frequency burster; AD- LFB, nonidentified low frequency burster located outside the supraoptic nuclear region; AD+ LFB, antidromically identified low frequency burster.

TABLE IX

COMPARISON OF MEAN BURST DURATIONS[1] OF SPONTANEOUS BURSTING PATTERNS

AD+ LFB[2]	AD- LFB[2]	CAB[2]	HFB[2]
9.76	6.22	.66	.57

[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 ($P < .05$).

[2]Abbreviations used are: CAB, continuously active burster; HFB, high frequency burster; AD- LFB, nonidentified low frequency burster located outside the supraoptic nuclear region; AD+ LFB, antidromically identified low frequency burster.

TABLE X

COMPARISON OF MEAN SPIKES PER BURST[1] OF SPONTANEOUS BURSTING PATTERNS

AD+ LFB[2]	AD- LFB[2]	HFB[2]	CAB[2]
33.99	23.23	9.22	7.09

[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 ($P < .05$).

[2]Abbreviations used are: CAB, continuously active burster; HFB, high frequency burster; AD- LFB, nonidentified low frequency burster located outside the supraoptic nuclear region; AD+ LFB, antidromically identified low frequency burster.

TABLE XI

COMPARISON OF MEAN BURST MEAN FIRING RATES[1] OF SPONTANEOUS BURSTING PATTERNS

HFB[2]	CAB[2]	AD- LFB[2]	AD+ LFB[2]
19.19	11.37	4.05	4.03

[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 ($P < .05$).

[2]Abbreviations used are: CAB, continuously active burster; HFB, high frequency burster; AD- LFB, nonidentified low frequency burster located outside the supraoptic nuclear region; AD+ LFB, antidromically identified low frequency burster.

Comparison of mean interburst interval (Table XII) and mean period (Table XIII) revealed that these parameters of antidromically identified low frequency bursting cells were significantly greater ($P < .05$) than those of all other neurons. In addition these parameters of antidromically negative low frequency bursting cells were significantly greater ($P < .05$) than those of the high frequency bursting and continuously active bursting spike trains. Comparison of mean interburst interval and mean period revealed no significant differences between high frequency bursting and continuously active bursting firing patterns.

Distribution of Firing Patterns Within the Hypothalamus

That firing patterns are not distributed equally between antidromically identified neurons and antidromically negative neurons outside the supraoptic nuclear region is apparent from inspection of Table XIV. This distribution may not be completely accurate since sampling was not entirely random but based on firing patterns particularly interesting to the investigator. No magnocellular neuroendocrine cell exhibited a continuously active regular, high frequency bursting or continuously active bursting pattern of firing although all these patterns were found within the limits of the supraoptic nuclear region.

Figures 16, 17, and 18 depict location of each cell recorded on a schematic drawing of brain stem cross sections.

A chi-square analysis of counts was used to approximate the probability that the distribution of firing patterns within the hypothalamus is due to chance. The numbers of antidromically positive cells were compared with the numbers of antidromically negative cells located

TABLE XII

COMPARISON OF MEAN INTERBURST INTERVALS[1] OF SPONTANEOUS BURSTING PATTERNS

AD+ LFB[2]	AD- LFB[2]	HFB[2]	CAB[2]
20.86	12.43	1.71	0.34

[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 ($P < .05$).

[2]Abbreviations used are: CAB, continuously active burster; HFB, high frequency burster; AD- LFB, nonidentified low frequency burster located outside the supraoptic nuclear region; AD+ LFB, antidromically identified low frequency burster.

TABLE XIII

COMPARISON OF MEAN PERIODS[1] OF SPONTANEOUS BURSTING PATTERNS

AD+ LFB[2]	AD- LFB[2]	HFB[2]	CAB[2]
30.75	18.65	2.29	.99

[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 ($P < .05$).

[2]Abbreviations used are: CAB, continuously active burster; HFB, high frequency burster; AD- LFB, nonidentified low frequency burster located outside the supraoptic nuclear region; AD+ LFB, antidromically identified low frequency burster.

TABLE XIV

COUNTS OF SPONTANEOUS FIRING PATTERN TYPES IN THREE GROUPS OF NEURONS IN THE HYPOTHALAMUS

Firing Pattern	AD- not SON[1]	AD- SON[1]	AD+ SON[1]	Total
S[1]	0	0	6	6
CAS[1]	9	8	24	41
LFB[1]	9	5	12	26
CAF[1]	39	13	33	85
HFB[1]	6	5	0	11
CAB[1]	5	1	0	6
CAR[1]	11	5	0	16
Total	79	37	75	191

[1]Abbreviations used are: AD- not SON, neurons outside the supraoptic nucleus not antidromically identified; AD- SON, neurons located in the supraoptic nucleus not antidromically identified; AD+ SON, antidromically identified supraoptic neuroendocrine cell; S, silent; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; HFB, high frequency burster; CAB, continuously active burster; CAR, continuously active regular.

Figure 16. Schematic of Recording Sites: Frontal 31.0 to 34.0

Symbols: X, HFB (high frequency burster); ●, CAS (continuously active slow); Δ, LFB (low frequency burster); ⊠, CAB (continuously active burster); □, CAR (continuously active regular); O, CAF (continuously active fast); ▲, S (silent). Labels: VL, lateral ventricle; SR, septal region; C, caudate; CO, optic chiasm; VIII, third ventricle; THAL, thalamus; MT, mammilothalamic tract; FX, fornix; TO, optic tract; NSO, supraoptic nucleus.

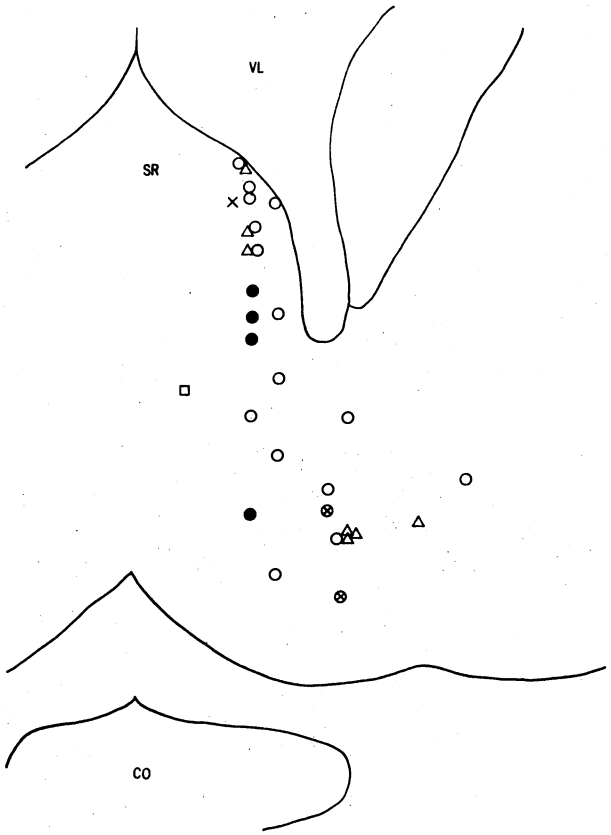

VL
SR
CO

Figure 17. Schematic of Recording Sites: Frontal 27.0 to 31.0

Symbols: X, HFB (high frequency burster); ●, CAS (continuously active slow); Δ, LFB (low frequency burster); ⊠, CAB (continuously active burster); □, CAR (continuously active regular); O, CAF (continuously active fast); ▲, S (silent). Labels: VL, lateral ventricle; SR, septal region; C, caudate; CO, optic chiasm; VIII, third ventricle; THAL, thalamus; MT, mammilothalamic tract; FX, fornix; TO, optic tract; NSO, supraoptic nucleus.

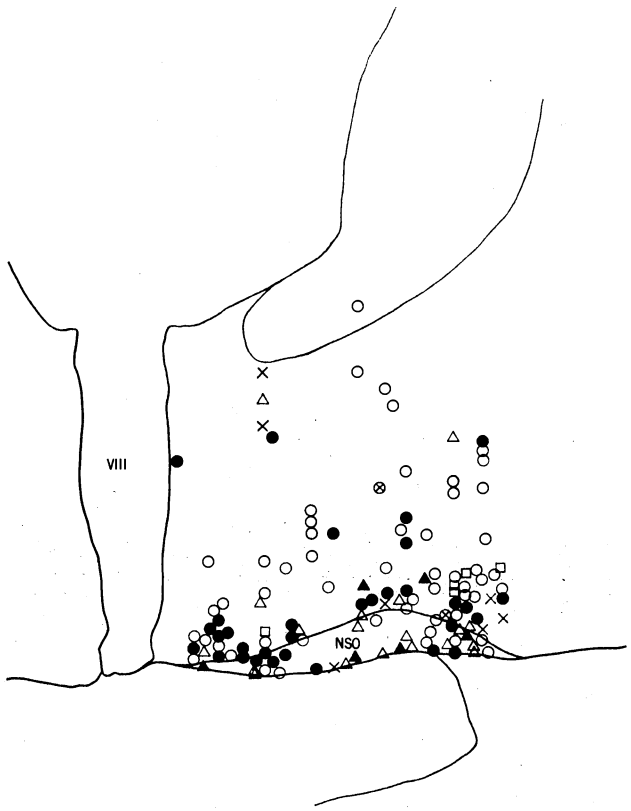
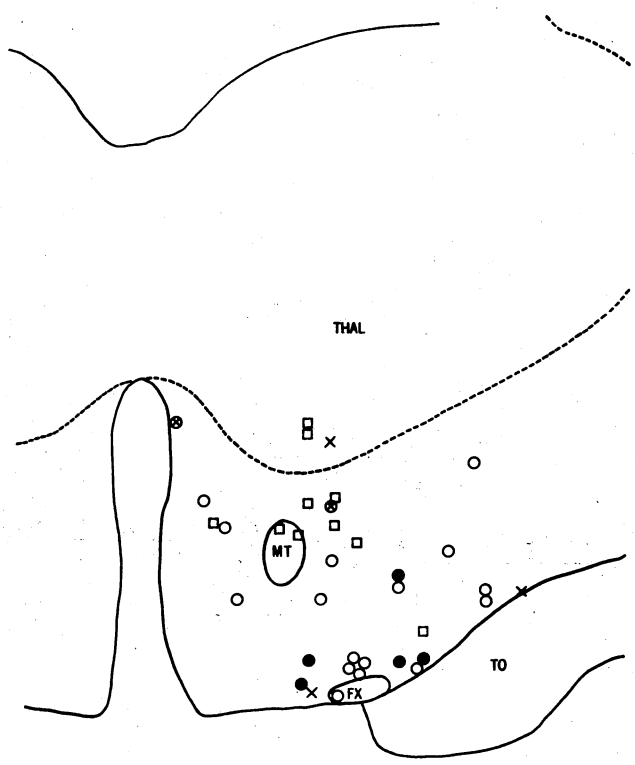

VIII
NSO

Figure 18. Schematic of Recording Sites: Frontal 24.0 to 27.0

Symbols: X, HFB (high frequency burster); ●, CAS (continuously active slow); Δ, LFB (low frequency burster); ⊠, CAB (continuously active burster); □, CAR (continuously active regular); O, CAF (continuously active fast); ▲, S (silent). Labels: VL, lateral ventricle; SR, septal region; C, caudate; CO, optic chiasm; VIII, third ventricle; THAL, thalamus; MT, mammilothalamic tract; FX, fornix; TO, optic tract; NSO, supraoptic nucleus.


THAL
MT
FX
TO

outside the supraoptic nuclear region (Table XV). The probability that the observed distribution of all cell types in the two areas could occur by chance was less than .001. The probability that the observed distribution of silent and high frequency bursting cells was due to chance was found to be less than .05. The probability that the observed distribution of continuously active slow and continuously active regular cells was due to chance was found to be less than .01. The probability that the observed distribution of low frequency bursting and continuously active fast cells was due to chance was found to be less than .90. The probability the observed distribution of continuously active bursting cells was due to chance was found to be less than .10.

An illustration of percentages of the total cell number found within antidromically identified, nonantidromically identified SON neurons and hypothalamic and septal areas is shown in Figure 19.

Statistical Analysis of Firing Pattern Parameters Between Areas

The t test for non paired experiments was used to test for significant differences between mean firing rates, interspike intervals, modes and coefficients of variation of continuously active slow, low frequency bursting and continuously active fast activity patterns found in magnocellular neuroendocrine cells and in antidromically negative neurons located outside the supraoptic nuclear region. Means for these parameters are listed in Table XVI. Values for "t" and significance levels for these comparisons are given in Table XVII.

Antidromically positive cells exhibiting the continuously active slow firing pattern had significantly greater modes ($P < .001$) and

TABLE XV

CHI-SQUARE ANALYSIS OF SPONTANEOUS FIRING PATTERN TYPE DISTRIBUTION

Firing Pattern	X^2[1]	P
S[1]	4.51	< .05
CAS[1]	6.64	< .01
LFB[1]	0.33	< .90
CAF[1]	0.14	< .90
HFB[1]	3.85	< .05
CAB[1]	2.89	< .10
CAR[1]	8.74	< .01
Total	27.10	< .001

[1]Abbreviations used are: X^2, calculated value of chi-square; S, silent; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; HFB, high frequency burster; CAB, continuously active burster; CAR, continuously active regular.

Figure 19. Percentages of Firing Pattern Types in Three Groups of Neurons of the Hypothalamus

Abbreviations used are: S, silent; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; HFB, high frequency burster; CAB, continuously active burster; CAR, continuously active regular; NSO AD+, antidromically identified supraoptic neuroendocrine cells; NSO, nonidentifed cells in the supraoptic nucleus; Hyp & Spt, neurons recorded from hypothalamic areas exclusive of the supraoptic nucleus and its perinuclear zone.

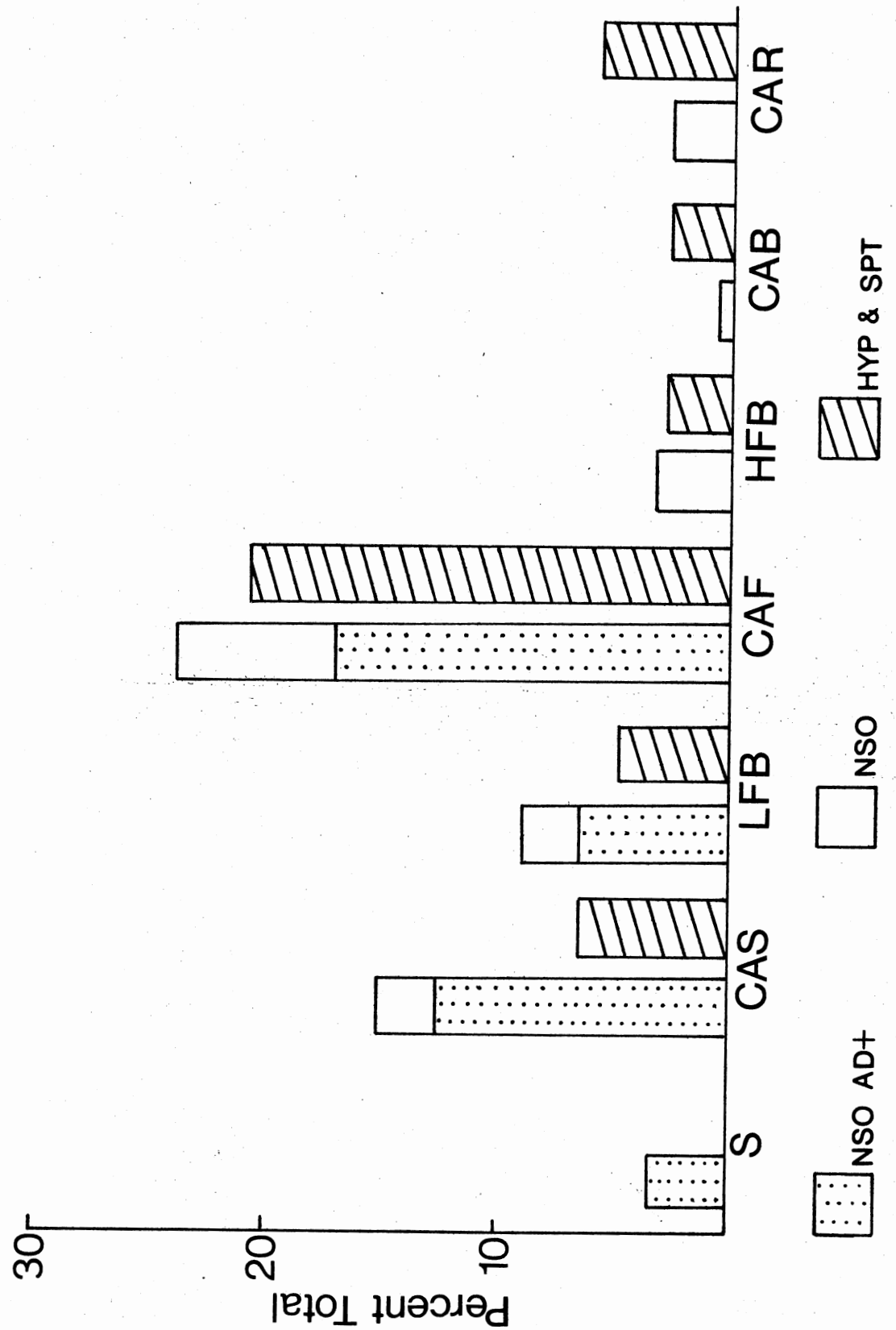

Percent Total
30
20
10
S
CAS
LFB
CAF
HFB
CAB
CAR
NSO AD+
NSO
HYP & SPT

TABLE XVI

MEAN SPONTANEOUS FIRING PATTERN PARAMETERS OF NEURONS IN TWO AREAS OF THE HYPOTHALAMUS[1]

Firing Pattern	AD+[2]				AD-[2]			
	MFR[2]	ISI[2]	Mode[2]	CV[2]	MFR	ISI	Mode	CV
CAS[2]	.38±.08 n=20	10.54±3.87 n=20	2.70±.23 n=8	.78±.07 n=11	.48±.10 n=9	2.72±.47 n=9	.13±.02 n=2	.78±.09 n=5
LFB[2]	1.26±.16 n=11	.94±.14 n=11	.13±.025 n=2	.37±.04 n=10	1.83±.38 n=9	.73±.13 n=9	.15±.03 n=3	.43±.06 n=9
CAF[2]	4.13±.54 n=31	.38±.04 n=31	.31±.13 n=23	.95±.08 n=28	5.17±.67 n=38	.31±.03 n=38	.08±.01 n=18	.61±.10 n=31

[1]Means ± standard errors of the mean.

[2]Abbreviations used are: AD+, antidromically identified supraoptic neuroendocrine cells; AD-, nonidentified cells outside the supraoptic nucleus; MFR, mean firing rate; ISI, mean interspike interval; Mode, mean interspike interval mode; CV, mean coefficient of variation; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast.

TABLE XVII

COMPARISON OF MEAN FIRING PATTERN PARAMETERS BETWEEN TWO AREAS OF THE HYPOTHALAMUS[1]

Firing Pattern	MFR[2]	ISI[2]	Mode[2]	CV[2]
CAS[2]	t = 0.811	t = 2.114	t = 11.30	t = 1.018
	NS	P < .05	P < .001	NS
LFB[2]	t = 1.5378	t = 1.712	t = 0.843	t = 0.9573
	NS	NS	NS	NS
CAF[2]	t = 1.2438	t = 1.3285	t = 1.81	t = 2.7272
	NS	NS	NS	P < .01

[1]Comparison by nonpaired t test.

[2]Abbreviations used are: MFR, mean firing rate; ISI, mean interspike interval; Mode, mean interspike interval mode; CV, mean coefficient of variation; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast.

interspike intervals ($P < .05$) than did the continuously active slow firing cells found outside the supraoptic nuclear region.

No significant differences were found between any of the parameters of the low frequency bursting firing patterns of the two areas.

The only parameter of the continuously active fast firing pattern found to be significantly different between the two areas was the coefficient of variation with that of the antidromically identified neuroendocrine cells being significantly greater ($P < .01$) than that of the antidromically negative cells found outside the supraoptic nuclear region.

Sleep-Waking and Sensory Responsiveness of Two Groups of Neurons in the Hypothalamus

Antidromically Identified Neuroendocrine Cells

Sixty-five neuroendocrine cells were tested for responsiveness to either intravenous or intracarotid infusions of hypertonic NaCl. A cell was considered responsive to an intracarotid injection of hypertonic NaCl if it changed its firing rate by 20% during the period of the injection. Cells were considered responsive to intravenous infusions of hypertonic NaCl if trend analysis revealed a K_a of 95% or greater. Of all cells tested, 64.6% were osmosensitive.

Thirty-three of these identified neuroendocrine cells were tested for responsiveness to one or all of a battery of sensory arousing stimuli. These stimuli included such things as sound, touch, light, odor and vaginal distension. No identified neuroendocrine cell responded to any of these stimuli.

Two neuroendocrine cells were tested for responsiveness to changes

in sleep-waking state. Neither of these two neuroendocrine cells responded.

Nonidentified Neurons Outside the Supraoptic Nuclear Region

Only 19.2% of the 62 cells tested with osmotic stimuli were affected. Both excitation and inhibition were observed.

Eleven (14.7%) of the 75 cells tested to sensory arousing stimuli in this area responded with either a decrease or increase in mean firing rate.

Six (31.6%) of 19 cells tested to sleep-waking changes were sensitive with either an excitatory or inhibitory response.

These data are summarized in Table XVIII.

A chi-square test (Table XIX) was employed to approximate the probability that the observed frequency of occurrence of responsive cells was equal between antidromically identified cells and nonidentified cells located outside the supraoptic nuclear region.

The null hypothesis that cells responsive to sensory arousing stimuli are distributed evenly in each area was not rejected. A similar hypothesis regarding cells responsive to changes in sleep-waking state was also not rejected.

The null hypothesis that cells responsive to osmotic stimuli are distributed evenly between the SON and other areas of the hypothalamus was rejected at the 99.9% confidence level. Osmotically sensitive magnocellular neuroendocrine cells outnumbered the osmotically insensitive magnocellular neuroendocrine cells while the reverse was the case for nonidentified neurons located outside the supraoptic nuclear region.

TABLE XVIII

SLEEP-WAKING AND SENSORY RESPONSIVENESS OF FIRING PATTERN TYPES LOCATED IN THE SUPRAOPTIC NUCLEUS AND HYPOTHALAMIC AND SEPTAL AREAS

Area	Test[1]	Total		S[3]		CAS[3]		LFB[3]		CAF[3]		HFB[3]		CAB[3]		CAR[3]	
		#T[2]	%R[2]	#T	%R	#T	%R	#T	%R	#T	%R	#T	%R	#T	%R	#T	%R
SON[4]	Osm	33	0	2	0	11	0	13	0	7	0	0	0	0	0	0	0
AD+	Sens	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
	SDW	65	65	6	50	20	50	13	77	26	77	0	0	0	0	0	0
SON	Osm	28	21	0	0	6	0	4	25	8	25	6	50	1	0	3	0
AD-	Sens	3	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
	SDW	25	36	0	0	5	20	5	40	8	38	4	50	1	100	2	0
Hyp	Osm	75	15	0	0	11	18	9	11	36	11	6	50	5	0	8	13
&	Sens	19	32	0	0	1	100	3	33	9	11	3	67	1	0	2	50
Spt	SDW	62	19	0	0	10	0	9	22	32	19	5	80	4	0	2	50

[1]Osm, osmotic, stimuli consisted of intravenous or intracarotid infusions of NaCl; Sens, sensory arousal, transient response to EEG arousal associated with nonosmotic sensory stimuli; SDW, sleep, drowsy, and waking behavioral state changes as determined by EEG, eye movement, eye lid position, body movement arousal threshold, and posture.

[2]#T, number of neurons tested; %R, percent of tested neurons which responded.

[3]Abbreviations used are: S, silent, CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; HFB, high frequency burster; CAB, continuously active burster; CAR, continuously active regular.

[4]SON, neurons recorded from within the supraoptic nucleus and its immediate perinuclear zone (within 1 mm of the histologic boundaries of the SON); AD+, antidromically identified supraoptic neuroendocrine cells; AD-, nonidentified cells outside the supraoptic nucleus; Hyp and Spt, neurons recorded from hypothalamic areas exclusive of the supraoptic nucleus and its perinuclear zone.

TABLE XIX

CHI-SQUARE ANALYSIS OF RESPONSIVE NEURONS IN TWO AREAS OF THE HYPOTHALAMUS

Test[1]	X^2[2]	P
Osmotic	14.3189	< .001
Sensory	3.5801	< .10
SDW	0.0186	----
Total	17.9176	< .001

[1]Osmotic, stimuli consisted of intravenous or intracarotid infusions of NaCl; Sensory, transient response to EEG arousal associated with non-osmotic sensory stimuli; SDW, sleep, droswy, and waking behavioral state changes as determined by EEG, eye movement, eye lid position, body movement arousal threshold, and posture.

[2]X^2, calculated value of chi-square.

Sleep-Waking and Sensory Responsiveness of Firing Pattern Types Within an Area

Osmotic Sensitivity

Numbers of osmotically sensitive magnocellular neuroendocrine cells were not found to be distributed unevenly when compared by the X^2 method (Table XX). However in all firing patterns considered, the number of responsive cells was equal to or greater than the number of nonresponsive cells in all cases. This is in contrast to sensory arousal and sleep-waking state changes where no responsive cells were found. Fifty percent of all cells exhibiting the silent or continuously active slow pattern of firing were found to be osmosensitive. Seventy-seven of the neuroendocrine cells exhibiting low frequency bursting or continuously active fast patterns of firing were found to be osmosensitive. The response of 1 low frequency bursting cell was negative while 6 responses of continuously active fast cells were negative.

In contrast to magnocellular neuroendocrine cells, antidromically negative neurons outside the supraoptic nuclear region had no response more often than a response in all cases except for neurons exhibiting a high frequency bursting pattern. Continuously active slow and continuously active bursting cells were not sensitive to osmotic stimuli. Low frequency bursting cells were both excited and inhibited by osmotic stimuli. Of the continuously active fast, 6.2% were inhibited while 12.6% were excited in response to osmotic stimuli. Four of five high frequency bursting cells and 1 of 2 continuously active regular cells were excited. Calculated X^2 (Table XXI) for high frequency bursting cells was 7.8125 indicating that the probability of this distribution

TABLE XX

CHI-SQUARE ANALYSIS OF IDENTIFIED NEUROENDOCRINE CELLS SENSITIVE TO OSMOTIC STIMULI

Firing Pattern	X^2[1]	P
S[1]	.18750	< .75
CAS[1]	1.62430	< .25
LFB[1]	.27830	< .75
CAF[1]	.90870	< .50
Total	2.99880	< .50

[1]Abbreviations used are: X^2, calculated value of chi-square; S, silent; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast.

TABLE XXI

CHI-SQUARE ANALYSIS OF NEURONS OUTSIDE THE SUPRAOPTIC NUCLEAR REGION SENSITIVE TO OSMOTIC STIMULI

Firing Pattern	X^2[1]	P
CAS[1]	1.2736	.50
LFB[1]	.0290	.90
CAF[1]	.0180	---
HFB[1]	7.8125	.01
CAB[1]	.1406	.75
CAR[1]	.0313	.75
Total	9.3050	.10

[1]Abbreviations used are: X^2, calculated value of chi-square; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; HFB, high frequency burster; CAB, continuously active burster; CAR, continuously active regular.

occurring by chance was less than .01.

Figure 20 depicts the response or lack of response of different continuously active cells to various external sensory arousal stimuli and to intracarotid injections of 0.6 M NaCl.

Figure 21 depicts the response or lack of response of 2 firing pattern types (continuously active slow and low frequency bursting) to osmotic and/or sensory arousing stimuli.

Figure 22 illustrates the change in firing rate and burst period for a high frequency bursting cell in response to sensory arousing stimuli and behavioral state changes.

Sensory Arousal Sensitivity

No identified magnocellular neuroendocrine cell responded to sensory arousal and therefore no statistical analysis was performed.

The null hypothesis was not rejected when a comparison was made of responsive and nonresponsive numbers of nonidentified neurons located outside the supraoptic nuclear region. Seventy-five nonidentified cells located outside the supraoptic nuclear region were tested for responsiveness to sensory arousal stimuli. Two continuously active cells were found to be sensitive (1 excitatory and 1 inhibitory response). One low frequency bursting cell was excited by these stimuli. Four continuously active fast cells were found to be responsive (one case of inhibition). Three of 6 high frequency bursting cells were excited. One continuously active regular cell and no continuously active bursting cells were excited. Calculation of X^2 for high frequency bursting cells indicated a probability of less than .10 that a larger value could be obtained by chance (Table XXII). In all cases except that of high frequency bursting

Figure 20. Segments of Spontaneous and Evoked Activity From Three Different Types of Continuously Active Neurons

Part A is an example of a CAF (continuously active fast) neuron. Note the irregularity of its interspike intervals and its nonspecific increased mean firing rate in response to a 1 ml intracarotid injection of .6 M NaCl and to sound. Part B demonstrates the absolute regularity of interspike intervals exhibited by a CAR (continuously active regular) neuron and its typical nonresponsiveness to sensory stimuli. Part C is an example of a CAB (continuously active bursting) neuron that tends to fire in bursts of action potentials. EEG, bi-parietal cortical electroencephalogram.

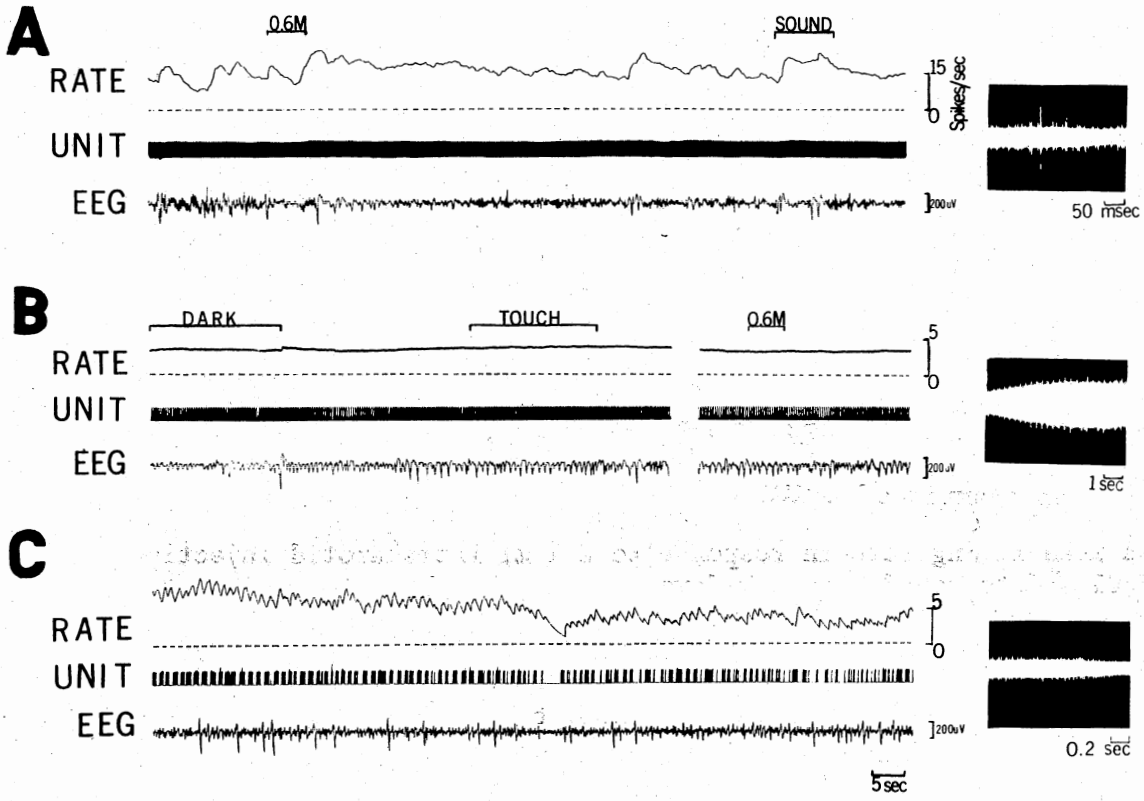

A
0.6M
SOUND
RATE
UNIT
EEG
15
0
Spikes/sec
200 uV
50 msec
B
DARK
TOUCH
0.6M
RATE
UNIT
EEG
5
0
200 uV
1 sec
C
RATE
UNIT
EEG
5
0
200uV
5 sec
0.2 sec

Figure 21. Segments of Spontaneous and Evoked Activity Recorded From Three Different Neurons in the Supraoptic Nucleus

Part A demonstrates a CAS (continuously active slow) pattern of firing. Note the tendency of this neuron towards "low frequency bursting"; this was observed in several neurons exhibiting this pattern. Part B shows three typical low frequency bursts characteristic of the LFB (low frequency bursting) pattern of firing. Three of the six antidromically identified neuroendocrine cells in this study were of this nature. Part C shows another type of bursting activity which was referred to as HFB (high frequency bursting) on the basis of estimated burst mean firing rates. This neuron had longer duration bursts (2-4 sec) and interburst intervals (5-8 sec) than was typical of other HFB cells recorded (see Figure 22); it was nonresponsive to sensory stimuli.

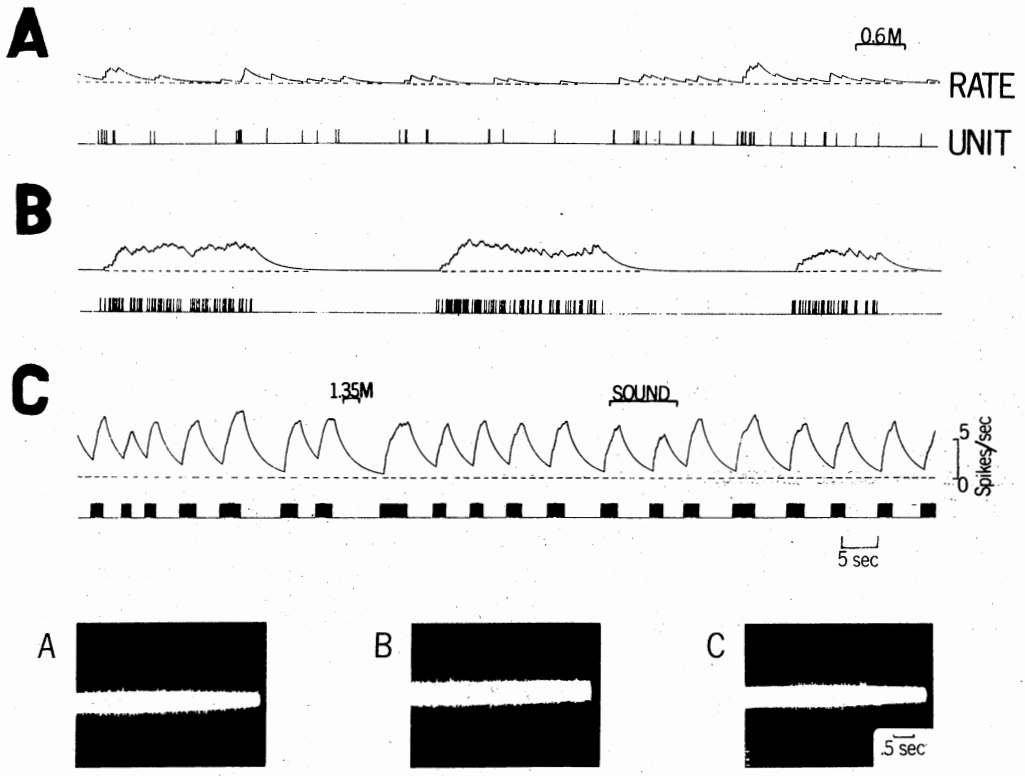

A
0.6M
RATE
UNIT
B
C
1.35M
SOUND
5
0
Spikes/sec
5 sec
A
B
C
.5 sec

Figure 22. Segments of Spontaneous and Evoked Activity Recorded From a High Frequency Bursting Neuron

A typical HFB (high frequency bursting) firing pattern is demonstrated during waking (A) and sleeping (B). Note this dorsal hypothalamic unit's increased burst firing rate in response to touch. Photographs of this unit are shown in Parts C and D. EEG, bi-parietal cortical electroencephalogram; EM, eye movement.

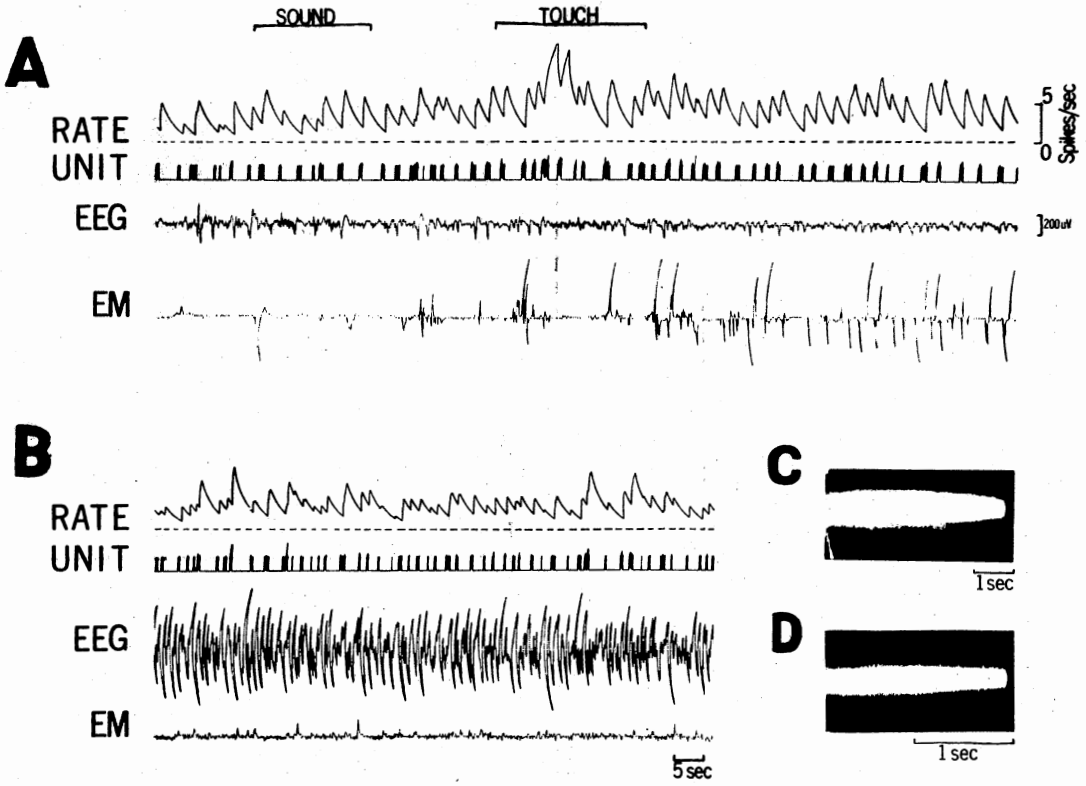

A
SOUND
TOUCH
RATE
UNIT
EEG
EM
5
0
Spikes/sec
200uV
B
RATE
UNIT
EEG
EM
5 sec
C
1 sec
D
1 sec

TABLE XXII

CHI-SQUARE ANALYSIS OF NEURONS OUTSIDE THE SUPRAOPTIC NUCLEAR REGION RESPONSIVE AND NOT RESPONSIVE TO SENSORY AROUSAL STIMULI

Firing Pattern	X^2[1]	P
CAS[1]	.0073	---
LFB[1]	.0360	< .90
CAF[1]	.1758	< .75
HFB[1]	3.3464	< .10
CAB[1]	.0664	< .90
CAR[1]	.0882	< .90
Total	3.7201	< .75

Abbreviations used are: X^2, calculated value of chi-square; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; HFB, high frequency burster; CAB, continuously active burster; CAR, continuously active regular.

cells the number of nonresponsive cells was greater than the number of responsive cells.

Sleep-Waking State Sensitivity

No identified magnocellular neuroendocrine cell responded to changes in sleep-waking state and therefore calculations of X^2 were not possible.

No differences in distribution of cells responsive to behavioral state changes were found in nonidentified cells located outside the supraoptic nuclear region (Table XXIII). The only continuously active slow cell tested was inhibited. One of 3 low frequency bursting cells tested responded (also an inhibition). One of 9 continuously active fast cells tested responded with an increase in its mean firing rate. Both responses of high frequency bursting cells were inhibitory. No continuously active bursting cells responded. One of 2 continuously active regular cells tested was excited. All firing patterns except continuously active slow and high frequency bursting had greater or equal numbers of nonresponsive cells than responsive cells.

Conduction Velocity of Identified Neuroendocrine Cells

Estimates of conduction velocity for axons of identified neuroendocrine cells were calculated from known antidromic latencies and estimates of conduction distance from electrodes across the hypophyseal stalk (Table XXIV).

The mean latency for all cells was found to be 12.47 $\pm$ .62 (SEM) milliseconds while the mean conduction velocity was .59 $\pm$ .03 (SEM) meters per second. Silent cells were found to have the fastest

TABLE XXIII

CHI-SQUARE ANALYSIS OF NEURONS OUTSIDE THE SUPRAOPTIC NUCLEAR REGION RESPONSIVE AND NOT RESPONSIVE TO SLEEP-WAKING CHANGES

Firing Pattern	X^2[1]	P
CAS[1]	.1905	< .75
LFB[1]	.2540	< .75
CAF[1]	.8762	< .50
HFB[1]	.5714	< .50
CAB[1]	.1904	< .75
CAR[1]	.0238	< .90
Total	2.1063	< .90

[1]Abbreviations used are: X^2, calculated value of chi-square; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; HFB, high frequency burster; CAB, continuously active burster; CAR, continuously active regular.

TABLE XXIV

MEAN LATENCIES AND CONDUCTION VELOCITIES OF IDENTIFIED NEUROENDOCRINE CELL FIRING PATTERN TYPES[1]

Firing Pattern	Latency[2]	CV[2]
S[2]	12.77 ± 2.27 n=6	.72 ± .22 n=6
CAS[2]	12.86 ± 0.85 n=21	.61 ± .06 n=21
LFB[2]	12.58 ± 1.09 n=12	.53 ± .06 n=12
CAF[2]	12.16 ± 1.16 n=32	.63 ± .05 n=31
All Cells	12.47 ± 0.62 n=71	.59 ± .03 n=68

[1]Means ± standard errors of the mean.

[2]Abbreviations used are: Latency, mean antidromic latency of identified neuroendocrine cells; CV, conduction velocity; S, silent, CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast.

conduction velocities while low frequency bursting cells had the slowest. Analysis of variance revealed no significant differences between conduction velocities of any firing pattern types (Table XXV).

Osmotic Sensitivity of Identified Neuroendocrine Cells

Intracarotid Injections

Antidromically identified neuroendocrine cells were initially tested with a 5-10 second pulse of 1.2 M NaCl. This stimulus proved to be less than satisfactory for three reasons: 1) cells were only slightly responsive to these stimuli probably due to a dampening and dilution of the stimulus solution because of the extensive branching (rete mirabile) of the arterial supply to the brain of the sheep; 2) the stimulus intensity could not be measured and a quantitative relationship between neuronal activity and osmotic pressure could not be developed; and 3) because of the arterial supply to the face of the sheep, intracarotid injections of hypertonic NaCl caused behavioral distress to the animal. The results of one of these tests are illustrated in Figure 23. Note the EEG arousal evoked by this injection.

Intravenous Infusions

Of the 75 neurons identified antidromically, 61 were tested for responsiveness to a slow intravenous infusion of hypertonic (1.2 M) NaCl. Thirty-eight neurons (62.3%) were found to change their activity in response to the osmotic stimulus. Thirty-one (81.6%) of the responsive neuroendocrine cells were excited in response to this stimulus and seven (18.4%) were inhibited by the osmotic forcing. Twenty-three (37.7%)

TABLE XXV

COMPARISON OF MEAN CONDUCTION VELOCITIES OF IDENTIFIED NEUROENDOCRINE CELL FIRING PATTERN TYPES[1]

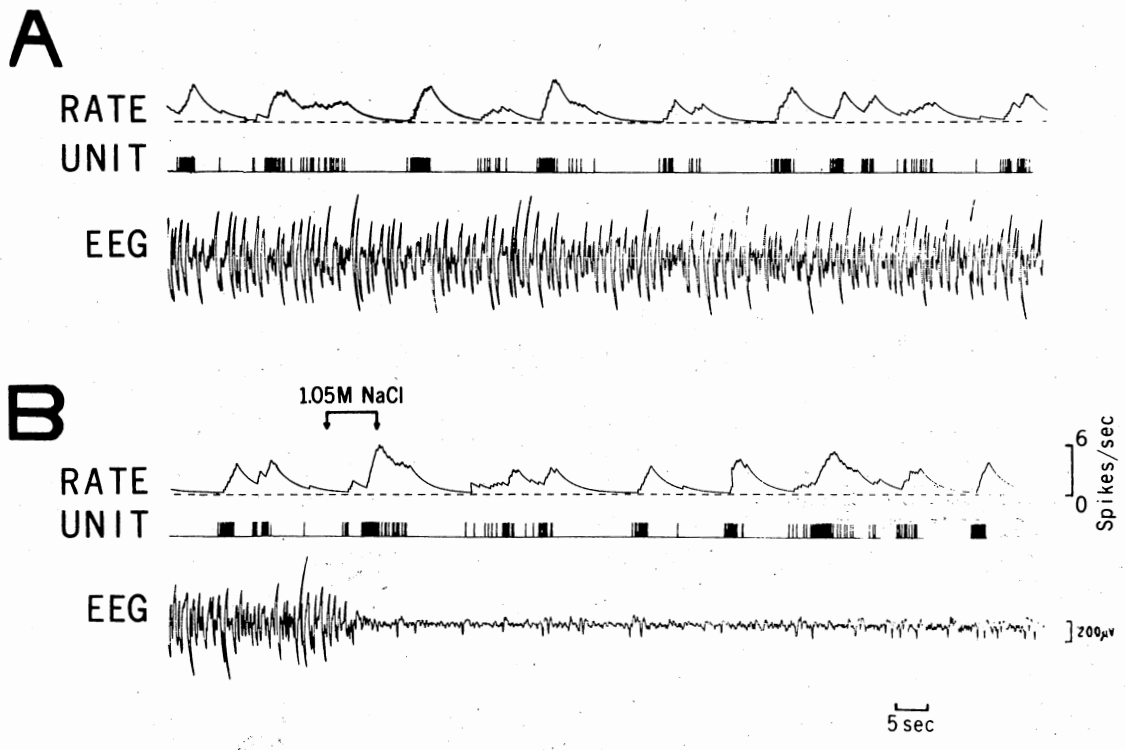
S[2]	CAF[2]	CAS[2]	LFB[2]
.72	.63	.61	.53

[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 ($P < .05$).

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

Figure 23. The Effects of Hypertonic NaCl and Sleep-Waking Behavior on the Activity of a Low Frequency Bursting Neuroendocrine Cell in the Supraoptic Nucleus of an Unanesthetized Sheep

Part A was recorded during a period of sleep (high voltage - slow wave EEG) and is an example of the LFB (low frequency bursting) behavior typically exhibited by these cells. In Part B, a 1 ml intracarotid injection of 1.05 M NaCl administered during an interburst interval evoked a slightly higher frequency burst than normal. Also associated with the injection was arousal of the sheep as indicated by the change in EEG pattern to low voltage - fast activity. Note that the spontaneous bursting behavior of this neuron was not apparently altered between sleeping and waking of the animal. Labels: EEG, bi-parietal cortical electroencephalogram; Rate, analog output proportional to the discharge; Unit, a one-to-one pulse output from the pulse height discriminator.


A
RATE
UNIT
EEG
B
1.05M NaCl
RATE
UNIT
EEG
6
0
Spikes/sec
200μv
5 sec

magnocellular neuroendocrine cells showed no change in their activity in response to the forcing.

It was possible to obtain, by computer analysis, a "trend" for increasing or decreasing interval length in response to these forcings. These trends were grouped by firing patterns, averaged (Table XXVI) and the means tested for significant differences. No significant differences were found between trends of any two firing pattern types (Tables XXVII and XXVIII).

From each trend (K_{aH} and K_{aR}) and change in mOsm/kg for the trend analysis period, an osmosensitivity was calculated for each neuron, as described in Chapter III. These values were grouped by firing pattern type, averaged (Table XXVI), and tested for significant differences between the means. No significant differences were found between osmosensitivities of any firing pattern type (Tables XXIX and XXX).

Correlation of Osmotic Sensitivity and Conduction Velocity

Osmosensitivity as defined in Chapter III was the ratio of K_a from the computer analysis to the change in plasma osmolality for the period of intravenous infusion analyzed.

The correlation coefficient, r, was calculated for conduction velocities and osmosensitivity values determined from both cumulative time histogram trend analysis and mean instantaneous rate trend analysis (Table XXXI).

Trend, based on successive bins of the cumulative time histogram, (K_a, tendency for interval length to decrease or increase) was found to be highly correlated ($r = -0.4365$, $P < .025$) with calculated conduction

TABLE XXVI

MEAN TRENDS AND OSMOSENSITIVITIES OF TESTED IDENTIFIED NEUROENDOCRINE CELL FIRING PATTERN TYPES[1]

Firing Pattern	K_{aH}[2]	K_{aR}[2]	$K_{aH}/\Delta Osm$[2]	$K_{aR}/\Delta Osm$[2]
S[2]	4.9900±1.2304 n=2	3.5695±0.2055 n=2	.3245±.1614 n=2	.2130±0.0500 n=2
CAS[2]	4.0800±1.2143 n=10	4.1780±1.4002 n=6	.4595±.1474 n=6	.3922±0.1352 n=6
LFB[2]	5.5700±1.6616 n=6	5.5356±2.0672 n=5	.4400±.1800 n=5	.4578±0.1498 n=5
CAF[2]	1.0500±1.1747 n=22	1.4435±0.9166 n=14	.2424±.1603 n=14	-2.1875±2.4219 n=15

[1]Means ± standard errors of the mean.

[2]Abbreviations used are: K_{aH}, mean trend based on histogram plot; K_{aR}, mean trend based on instanteous rate plot; $K_{aH}/\Delta Osm$, mean trend based on histogram plot divided by the change in osmolality for the analysis period; $K_{aR}/\Delta Osm$, mean trend based on instanteous rate plot divided by the change in osmolality for the analysis period; S, silent, CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast.

TABLE XXVII

COMPARISON OF MEAN TRENDS (K_{aH}) OF OSMOTICALLY TESTED IDENTIFIED NEUROENDOCRINE CELL FIRING PATTERN TYPES[1]

LFB[2]	S[2]	CAS[2]	CAF[2]
5.57	4.99	4.08	1.05

[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 ($P < .05$).

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

TABLE XXVIII

COMPARISON OF MEAN TRENDS(K_{aR}) OF OSMOTICALLY TESTED IDENTIFIED NEUROENDOCRINE CELL FIRING PATTERN TYPES[1]

LFB[2]	CAS[2]	S[2]	CAF[2]
5.5356	4.1780	3.5695	1.4435

[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 ($P < .05$).

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

TABLE XXIX

COMPARISON OF MEAN OSMOSENSITIVITIES ($K_{aH}/\Delta Osm$) OF IDENTIFIED NEUROENDOCRINE CELL FIRING PATTERN TYPES[1]

CAS[2]	LFB[2]	S[2]	CAF[2]
.4595	.4400	.3245	.2424

[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 ($P < .05$).

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

TABLE XXX

COMPARISON OF MEAN OSMOSENSITIVITIES ($K_{aR}/\Delta Osm$) OF IDENTIFIED NEUROENDOCRINE CELL FIRING PATTERN TYPES[1]

LFB[2]	CAS[2]	S[2]	CAF[2]
.4578	.3922	.2130	-2.1875

[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 ($P < .05$).

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

TABLE XXXI

CORRELATION OF OSMOSENSITIVITY AND CONDUCTION VELOCITY[1]

Osmosensitivity	CV
K_{aH}	r = -0.4365 t = 2.4260 P < .025
K_{aR}	r = -0.3597 t = 1.9273 P < .100
$K_{aH}/\Delta Osm$	r = -0.2940 t = 1.5381 P < .200
$K_{aR}/\Delta Osm$	r = -0.2746 t = 1.4278 P < .200

[1]Abbreviations used are: CV, conduction velocity; K_{aH}, mean trend based on histogram plot; K_{aR}, mean trend based on instantaneous rate plot; $K_{aH}/\Delta Osm$, mean trend based on histogram plot divided by the change in osmolality for the analysis period; $K_{aR}/\Delta Osm$, mean trend based on instantaneous rate plot divided by the change in osmolality for the analysis period; r, calculated correlation coefficient.

velocity. Trend based on the mean instantaneous rate plot was also well correlated ($r = -0.3597$, $P < .100$) with calculated conduction velocity. Osmosensitivities ($K_{aH}/\Delta Osm$ and $K_{aR}/\Delta Osm$) were not well correlated with calculated conduction velocity ($r = -0.2940$ and $r = -0.2746$, respectively, $P < .200$). Values of the correlation coefficients, r, can be found in Table XXXI.

Tonic and Dynamic Osmotic Sensitivity of Identified Neuroendocrine Cells

It was possible to classify 21 of the osmotically forced cells as dynamically or tonically osmosensitive based on their response to the change in plasma osmolality.

Seven cells (33%) were classified as tonically osmosensitive cells. The change in mean firing rate of these cells was proportional to the change in absolute value of plasma osmolality evoked by the osmotic forcing.

Fourteen cells (67%) were classified as dynamically osmosensitive neurons. They appeared sensitive primarily to the rate of change of plasma osmolality. Firing rates of these neurons (33%) were compared during and after the osmotic forcing where equal values of plasma osmolality occurred. If the value of the firing rate during the rise in plasma osmolality was greater than the value at the same point while plasma osmolality was decreasing, the cell was said to be dynamically osmosensitive. If the firing rates at the two points were equal, the cell was said to be tonically osmosensitive. Other neurons (33%) were classified as dynamically osmosensitive if their firing rate was greater during a faster rate of change of plasma osmolality than during a slower

rate of change of plasma osmolality.

A chi-square analysis was again employed to approximate the probability that tonic and dynamic sensitivity is equal within a single cell type. Again, this null hypothesis was not rejected for any firing pattern (Table XXXII). Numbers of dynamic cells within a firing pattern were found to be greater than numbers of tonic cells in all cases except for neurons exhibiting the continuously active fast pattern of firing. No silent or low frequency bursting cells were found to be tonically osmosensitive.

Figure 24 illustrates the response of a tonically osmosensitive continuously active slow cell to a 10 minute intravenous infusion of 1.2 M NaCl. Plasma osmolality increased by 10 mOsm/kg in 10 minutes while firing rate increased by 2.38 spikes per second.

Figure 25 illustrates the response of a dynamically osmosensitive continuously active slow cell to a 20 minute intravenous infusion of 1.2 M NaCl. Plasma osmolality reached a peak value of 291 mOsm/kg at 10 minutes after initiation of the forcing. This was an increase of 17 mOsm/kg over the control value of 274 mOsm/kg. Mean firing rate reached a peak value of 7.53 spikes per second, also at 10 minutes after initiation of the forcing. This was an increase of 7.09 spikes per second over the control value of 0.44 spikes per second. After 5 minutes of hypertonic forcing the neuron exhibited an overall appearance of continuously active fast pattern of firing and the plasma osmolality was 279 mOsm/kg. At 20 minutes after initiation of the hypertonic forcing, plasma osmolality had decreased from the peak value to 279 mOsm/kg. At this point this antidromically identified neuroendocrine cell was exhibiting a low frequency bursting pattern of firing with a mean firing rate of

TABLE XXXII

CHI-SQUARE ANALYSIS OF TONIC AND DYNAMIC CELLS WITHIN A FIRING PATTERN TYPE[1]

Firing Pattern	No. Tonic	No. Dynamic	X^2	P
S	0	1	0.1905	.75
CAS	2	3	0.0357	.90
LFB	0	6	1.6875	.25
CAF	5	4	0.6964	.50
Total	7	14	2.6101	.50

[1]Abbreviations used are: X^2, calculated value of chi-square; S, silent; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast.

Figure 24. Response of a Tonically Osmosensitive Neuroendocrine Cell to a Seven Minute Hypertonic Forcing

Labels: Posm, plasma osmolality (mOsm/kg); Mfr, overall mean firing rate (spikes/second); Calibration Bar, 0 to 5 spikes/second; Start, begin hypertonic forcing; Stop, end hypertonic forcing.

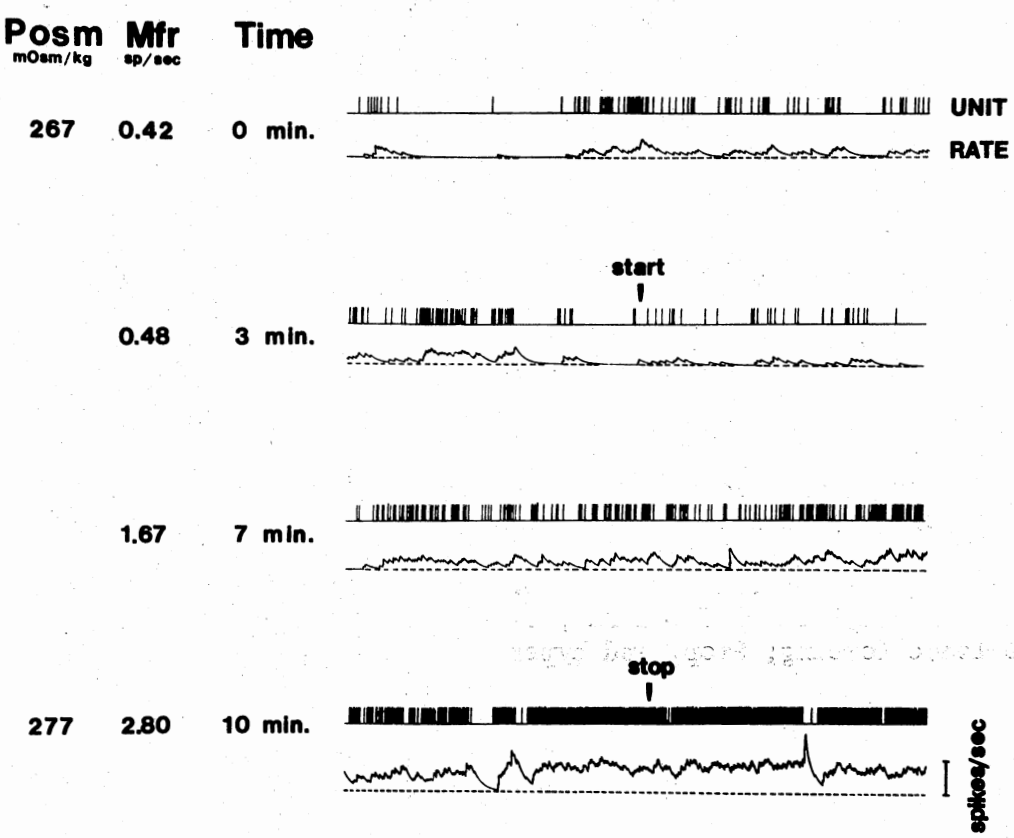
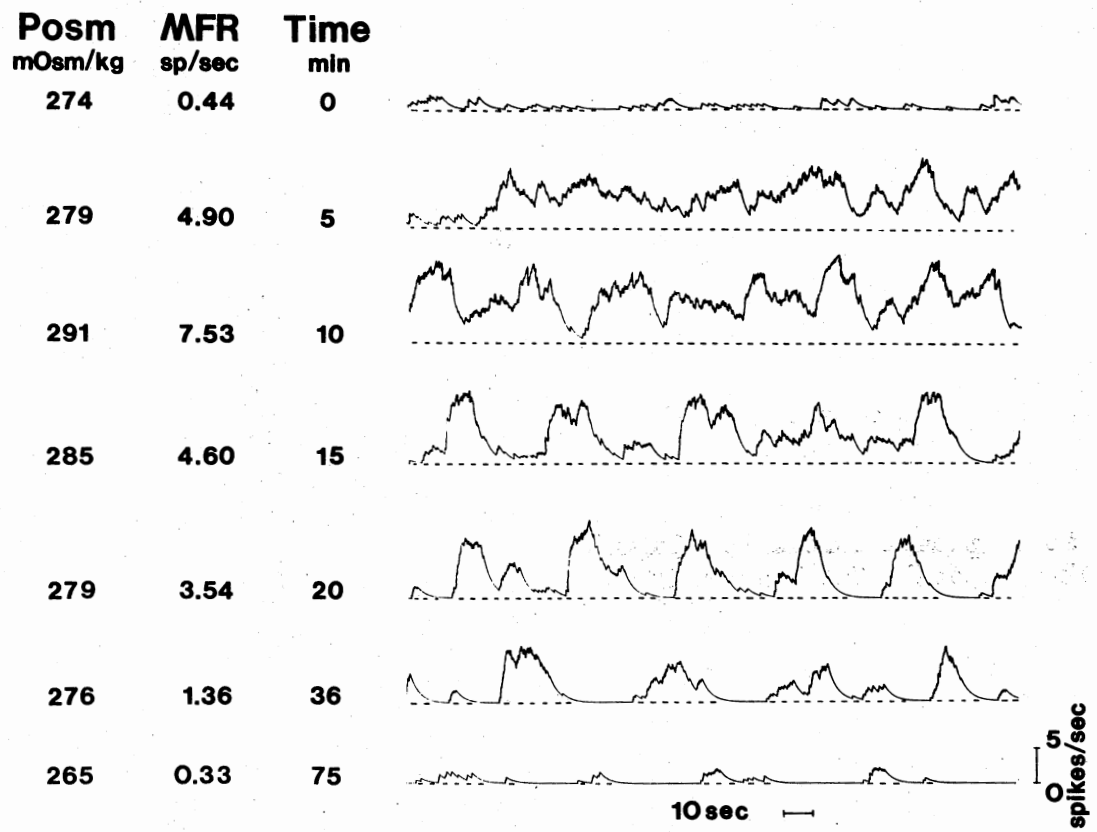

Posm
mOsm/kg
Mfr
sp/sec
Time
267
0.42
0 min.
UNIT
RATE
start
0.48
3 min.
1.67
7 min.
stop
277
2.80
10 min.
spikes/sec

Figure 25. Response of a Dynamically Osmosensitive Continuously Active Slow Neuroendocrine Cell to a Twenty Minute Hypertonic Forcing

Labels: Posm, plasma osmolality (mOsm/kg); MFR, overall mean firing rate (spikes/second); Calibration Bar, 0 to 5 spikes/second.


Posm
mOsm/kg
MFR
sp/sec
Time
min
274
0.44
0
279
4.90
5
291
7.53
10
285
4.60
15
279
3.54
20
276
1.36
36
265
0.33
75
5
0
spikes/sec
10 sec

3.54 spikes per second. This is an effective demonstration of dynamic sensitivity. The behavior of this neuron is not exclusively dependent upon the absolute value of plasma osmolality but upon the direction of change of plasma osmolality.

Figure 26 illustrates the dot raster display of the train of action potentials recorded from the same neuron as presented in Figure 25. On the same time base as the dot raster display is a plot of the course of plasma osmolality during and after the forcing period. Changes in firing pattern from continuously active slow to continuously active fast during the maximum change in plasma osmolality and from continuously active fast to low frequency bursting during the decline of plasma osmolality to control values are evident on inspection of the dot raster display.

Figure 27 illustrates the response of a dynamically osmosensitive low frequency bursting neuroendocrine cell to a 17 minute intravenous infusion of 1.2 M NaCl. After an unusual initial inhibition this neuroendocrine cell, like the previous one, exhibited a continuously active fast pattern of firing with a marginal appearance of periodic activity. Plasma osmolality and mean firing rate again reached simultaneous peaks (291 mOsm/kg and 2.87 spikes per second, respectively) at 15 minutes after initiation of the osmotic forcing. Although plasma osmolality did not change from 15 to 25 minutes after initiation of the forcing, the marginal periodicity of the continuously active fast pattern of firing emerged into a more definitive, slower, low frequency bursting activity pattern again indicating neuronal sensitivity to direction of change of plasma osmolality. This neuroendocrine cell although dynamically osmosensitive was not as osmosensitive as the previous (Figure 25), increasing its mean firing rate only 41% as compared to 945% for the

Figure 26. Dot Raster Display of the Response of a Dynamically Osmosensitive Neuroendocrine Cell to a Twenty Minute Hypertonic Forcing

Overall mean firing rate, spikes/second, (MFR) and plasma osmolality, mOsm/kg, (Posm), are plotted below for comparison. Note changes in firing pattern from slow irregular spontaneous activity to continuously active fast (CAF) during maximum Posm change and low frequency bursting (LFB) during the decline of Posm to control values.

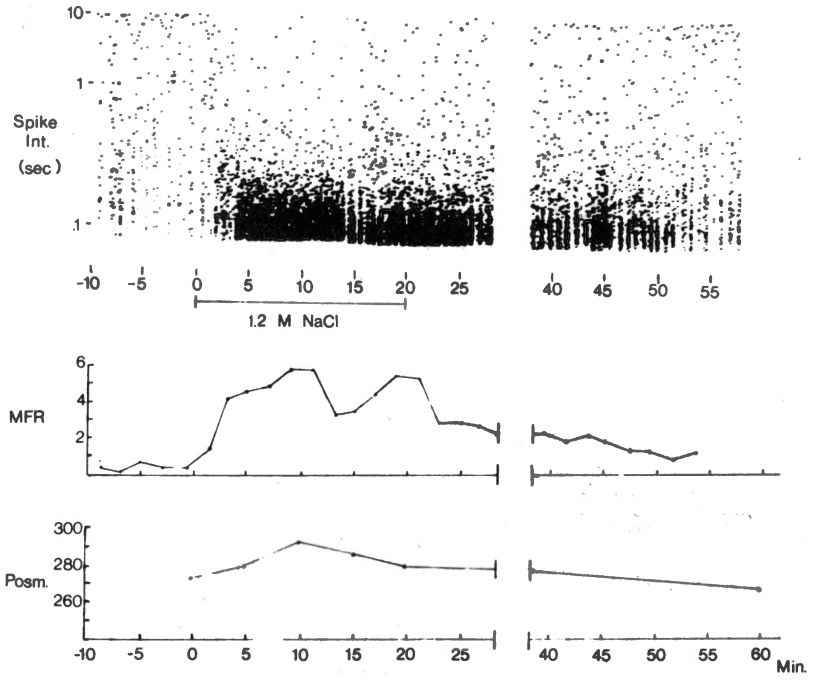
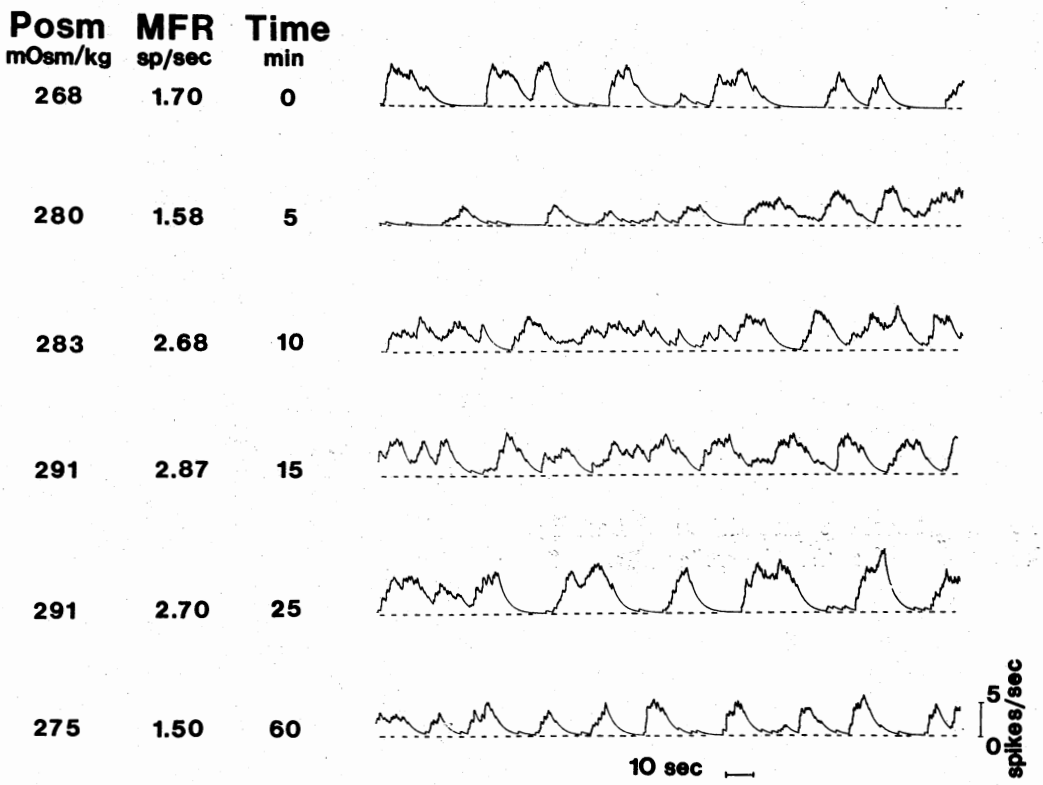

10
1
.1
Spike
Int.
(sec)
-10
-5
0
5
10
15
20
25
40
45
50
55
1.2 M NaCl
6
4
2
MFR
300
280
260
Posm.
-10
-5
0
5
10
15
20
25
40
45
50
55
60
Min.

Figure 27. Response of a Dynamically Osmosensitive Low Frequency Bursting Neuroendocrine Cell to a Seventeen Minute Hypertonic Forcing

Labels: Posm, plasma osmolality (mOsm/kg); MFR, overall mean firing rate (spikes/second); Calibration Bar, 0 to 5 spikes/second.


Posm
mOsm/kg
MFR
sp/sec
Time
min
268
1.70
0
280
1.58
5
283
2.68
10
291
2.87
15
291
2.70
25
275
1.50
60
5
0
spikes/sec
10 sec

neuron discussed previously.

Tonically and dynamically osmosensitive cells were tested for differences between osmosensitivity based on histogram trend analysis and on instantaneous rate trend analysis. No significant differences in osmosensitivity were found between tonically and dynamically responsive cells (Table XXXIII). Calculated conduction velocities were also compared for tonic and dynamic cells. Again no significant differences were found (Table XXXIV).

Osmotic Sensitivity of Rostral and Caudal Areas of the Supraoptic Nucleus

The SON was arbitrarily divided into rostral and caudal portions (frontal coordinates greater than 29.0 and less than 29.0, respectively). An outline drawing of the SON and its relation to the optic chiasm and optic tract are presented in Figure 28 in a frontal plane transverse to that of electrode penetration. Trends and osmosensitivities were grouped by area (rostral and caudal), averaged (Table XXXV), and tested for significant differences between areas. In all cases osmosensitivity of the caudal area was greater than osmosensitivity of the rostral area. However, a statistically significant difference was found only for the case of osmosensitivity based on instantaneous rate trend (Table XXXVI).

Neuroendocrine cells with significant positive trends ($K_a \geq 95\%$) in response to the forcing were found to be more numerous in the caudal portion than the rostral portion of the SON (63% and 55%, respectively). Neuroendocrine cells with significant negative trends ($K_a \geq 95\%$) in response to the forcing were found to be more numerous in the rostral area than in the caudal area (18% and 15%, respectively).

TABLE XXXIII

COMPARISON OF MEAN TRENDS AND OSMOSENSITIVITIES OF TONICALLY AND DYNAMICALLY SENSITIVE IDENTIFIED NEUROENDOCRINE CELLS

		Dynamic Cells			
		K_{aH}[1]	K_{aR}[1]	$K_{aH}/\Delta Osm$[1]	$K_{aR}/\Delta Osm$[1]
Tonic Cells	K_{aH}	t = 0.5551 n=19	---	---	---
	K_{aR}	---	t = 1.4214 n=19	---	---
	$K_{aH}/\Delta Osm$	---	---	t = 0.2285 n=19	---
	$K_{aR}/\Delta Osm$	---	---	---	t = 0.7281 n=19

[1]Abbreviations used are: K_{aH}, mean trend based on histogram plot; K_{aR}, mean trend based on instantaneous rate plot; $K_{aH}/\Delta Osm$, mean trend based on histogram plot divided by the change in osmolality for the analysis period; $K_{aR}/\Delta Osm$, mean trend based on instantaneous rate plot divided by the change in osmolality for the analysis period.

TABLE XXXIV

COMPARISON OF MEAN CONDUCTION VELOCITIES OF TONICALLY AND DYNAMICALLY SENSITIVE IDENTIFIED NEUROENDOCRINE CELLS[1]

		Dynamic Cells CV[2]
Tonic Cells	CV	t = 0.3843 n=19

[1]Comparison by nonpaired t test.

[2]Abbreviations used are: CV, conduction velocity.

Figure 28. Schematic Drawing of the Relationship of the Supraoptic Nucleus to the Optic Chiasm and Tract

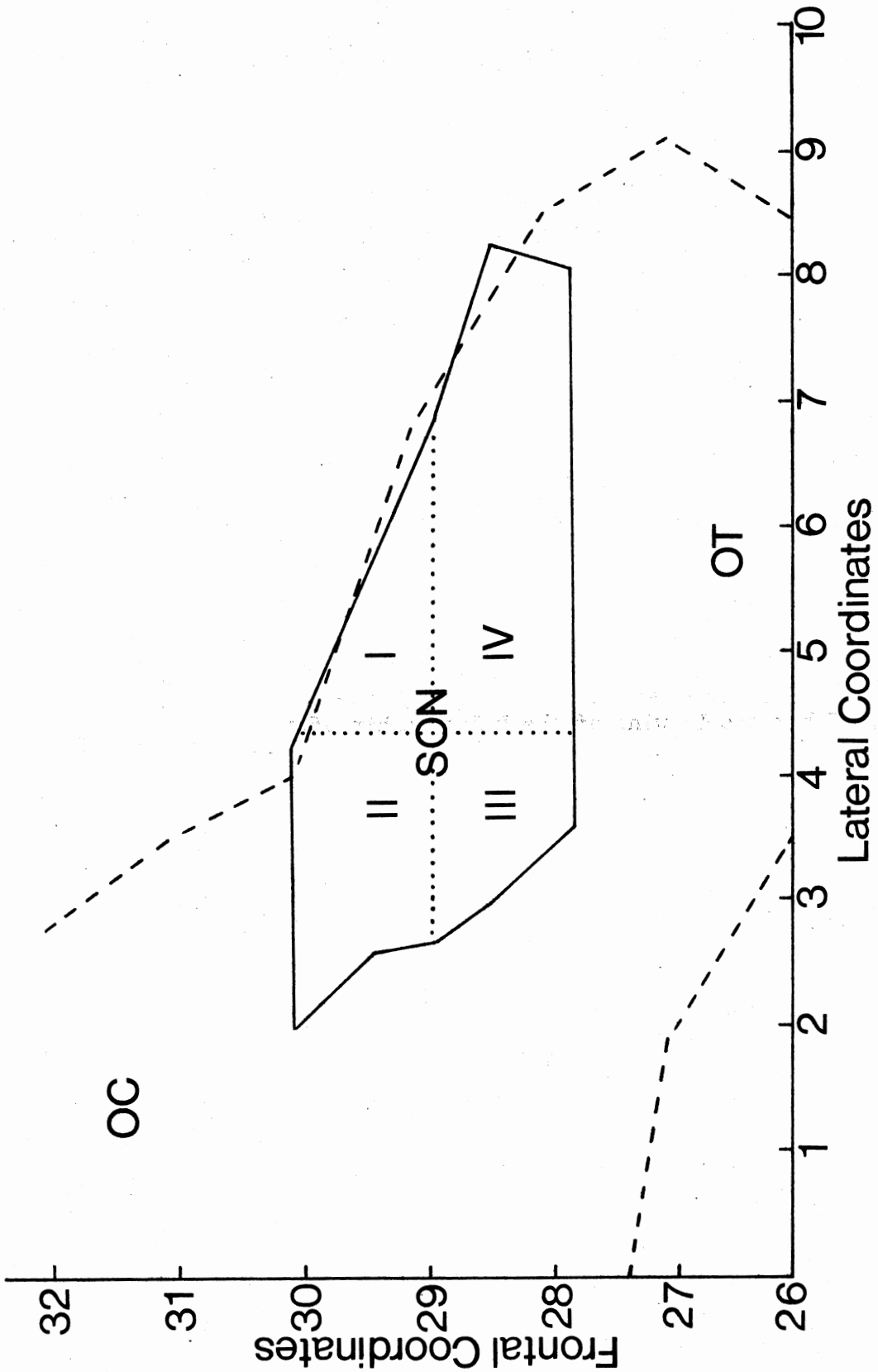

Frontal Coordinates
32
31
30
29
28
27
26
Lateral Coordinates
1
2
3
4
5
6
7
8
9
10
OC
OT
SON
I
II
III
IV

TABLE XXXV

CHARACTERISTICS OF ROSTRAL AND CAUDAL AREAS OF THE SUPRAOPTIC NUCLEUS[1]

	Rostral Areas I & II	Caudal Areas III & IV
K_{aH}[2]	2.5057 ± .1183 n=9	4.2511 ± 2.2011 n=19
K_{aR}[2]	1.7544 ± .0699 n=9	3.3161 ± 0.1027 n=19
$K_{aH}/\Delta Osm$[2]	0.1280 ± .1330 n=9	0.4360 ± 0.1250 n=18
$K_{aR}/\Delta Osm$[2]	0.0820 ± .0790 n=9	0.3800 ± 0.1100 n=18
Number of Tonic Cells	3 (37.5%)	4 (36.4%)
Number of Dynamic Cells	5 (62.5%)	7 (63.6%)
% S[2]	11	6
% CAS[2]	22	22
% LFB[2]	11	22
% CAF[2]	56	50
$+SK_{aH}$[2]	55%	63%
$-SK_{aH}$[2]	18%	15%
$+SK_{aR}$[2]	45%	50%
$-SK_{aR}$[2]	9%	15%

[1]Means ± standard errors of the mean.

[2]Abbreviations used are: K_{aH}, mean trend based on histogram plot; K_{aR}, mean trend based on instantaneous rate plot; $K_{aH}/\Delta Osm$, mean trend based on histogram plot divided by the change in osmolality for the analysis period; $K_{aR}/\Delta Osm$, mean trend based on instantaneous rate plot divided by the change in osmolality for the analysis period; S, silent; CAS, continuously active slow; LFB, low frequency burster; CAF, continuously active fast; $+SK_{aH}$, percent of neuroendocrine cells with ≥ 95% confidence of positive trend based on cumulative time histogram; $-SK_{aH}$, percent of neuroendocrine cells with ≥ 95% confidence of negative trend based on cumulative time histogram; $+SK_{aR}$, percent of neuroendocrine cells with ≥ 95% confidence of positive trend based on instantaneous rate plot; $-SK_{aR}$, percent of neuroendocrine cells with ≥ 95% confidence of negative trend based on instantaneous rate plot.

TABLE XXXVI

COMPARISON OF MEAN TRENDS AND OSMOSENSITIVITIES OF ROSTRAL AND CAUDAL AREAS OF THE SUPRAOPTIC NUCLEUS[1]

	t	P	n
K_{aH}[2]	1.1778	NS	28
K_{aR}[2]	1.2798	NS	28
$K_{aH}/\Delta Osm$[2]	1.8330	NS	27
$K_{aR}/\Delta Osm$[2]	2.5000	< .025	27

[1]Comparison by nonpaired t test.

[2]Abbreviations used are: K_{aH}, mean trend based on histogram plot; K_{aR}, mean trend based on instantaneous rate plot; $K_{aH}/\Delta Osm$, mean trend based on histogram plot divided by the change in osmolality for the analysis period; $K_{aR}/\Delta Osm$, mean trend based on instantaneous rate plot divided by the change in osmolality for the analysis period.

CHAPTER V

DISCUSSION

Assignment of a specific function to a particular firing pattern type has been implicated (Hayward and Vincent, 1970; Cross, et al., 1975; Wakerley, et al., 1975). The material in this dissertation goes further, first describing spontaneous firing patterns then their responsiveness to different kinds of stimuli.

It was determined that spontaneous firing patterns of neurons recorded from hypothalamic and septal areas could be categorized into seven types: silent, continuously active slow, low frequency bursting, continuously active fast; high frequency bursting, continuously active bursting and continuously active regular. Statistical analysis of the parameters used to classify a pattern of activity into one of the seven types did confirm that differences do exist between firing patterns of these neurons. Spontaneous firing patterns of identified neuroendocrine cells ranged from silent (no spontaneous activity) through periodic activity (low frequency bursting) to continuous activity. No identified neuroendocrine cell exhibited a high frequency bursting, continuously active bursting or continuously active regular pattern of firing. It was also observed that neurons do not spontaneously change their pattern of firing.

Since neurons were recorded in unanesthetized animals prepared for chronic recording, it can be said that these firing patterns are not the

result of surgical stress or anesthesia.

Nonidentified neurons in widespread hypothalamic and septal areas were found to exhibit all firing patterns described except silent. No response could be evoked if axons of these neurons did not pass through the hypophyseal stalk. Nonidentified neurons located outside the supraoptic nuclear region were more responsive to sensory arousing stimuli and changes in behavioral state and less responsive to osmotic stimuli than were magnocellular neuroendocrine cells.

High frequency bursting cells have been previously described (Hayward and Jennings, 1973; Koizumi and Yamashita, 1972) and their function as neuroendocrine "Renshaw" cells has been implicated. No magnocellular neuroendocrine cell was found to exhibit this pattern of firing. Hayward and Jennings (1973) described seven of these cells which were located at the junction of the SON and optic tract. All were inhibited by intracarotid injections of hypertonic NaCl. Eleven high frequency bursting cells were recorded in this study. Five were recorded in the supraoptic nuclear region but not in the supraoptic nucleus itself. Both excitatory and inhibitory responses were recorded from these cells in response to osmotic stimuli. Other nonidentified cells located in the supraoptic nuclear region were not as sensitive to osmotic stimuli as the high frequency bursting cell and may be "wired" to another system not involved with osmotic regulation of antidiuretic hormone levels. These data then neither refute nor support the hypothesis that high frequency bursting cells are neuroendocrine "Renshaw" cells. If high frequency bursting cells are indeed neuroendocrine "Renshaw" cells, they may be responsible for the alternating periods of activity observed in low frequency bursting magnocellular neuroendocrine cells. Why some high

frequency bursting cells are inhibited by osmotic stimuli is unknown. It may be that an osmoreceptor (may or may not be the magnocellular neuroendocrine cell) which transduces osmotic pressure into action potentials both excites the neuroendocrine cell and inhibits the high frequency bursting cell by collateral axons. This was observed in two cases in the unanesthetized sheep. Excitement of the high frequency bursting cell probably arises from stimulation by a collateral axon of the magnocellular neuroendocrine cell. In support of this, high frequency bursting cells often respond to stalk stimulation with a burst of 4 or 5 action potentials at a frequency of 200-600 spikes per second (see also Hayward and Jennings, 1973; Koizumi and Yamashita, 1972). High frequency bursting cells were very stable, never changing their basic pattern of firing.

Nonidentified low frequency bursting neurons were also found to exist outside the supraoptic nuclear region. These cells were not found to respond as often to osmotic stimuli as did identified low frequency bursting magnocellular neuroendocrine cells. The idea that low frequency bursting neurons are strictly ADH-producing cells is therefore not supported. The function of these neurons is unknown.

Continuously active regular cells have not been described in the mammalian nervous system, although they have been described in the nervous systems of invertebrates such as Aplysia (Kogan, 1973; Kim, 1973). It is generally noted in the nervous system that with evolution, the proportion of nonsteady neurons increases. The variable response of these neurons with a variety of synaptic inputs depends on previously existing conditions. Continuously active regular cells on the other hand behave deterministically when driven by afferent input. This may explain the remarkable unresponsiveness observed for these neurons.

Only four of the seven described firing patterns were found to be exhibited by identified magnocellular neuroendocrine cells: silent, continuously active slow, low frequency bursting and continuously active fast. These are the same patterns of firing found in magnocellular neuroendocrine cells of other species (rat and monkey). Magnocellular neuroendocrine cells were totally unresponsive to sensory arousing stimuli and changes in sleep-waking state, but highly responsive to osmotic stimuli. Of all identified neuroendocrine cells, 62.3% were osmosensitive. Of these, 84.2% (or 51% of all magnocellular neuroendocrine cells) were excited by osmotic stimuli. The patterns of firing exhibited by magnocellular neuroendocrine cells (except continuously active fast) had the slowest firing rates of any cell type recorded. Neuroendocrine cells did not fire in the short, high frequency clusters of action potentials as did the high frequency bursting and continuously active bursting cells.

Magnocellular neuroendocrine cells were found to conduct action potentials at a velocity generally less than 1 m/sec, a result consistent with the work of other investigators. Eight identified neuroendocrine cells were not located within the histologic boundaries of the SON but were recorded above it in the INZ of Greving. According to the Henneman size principle, neurons with small axon diameters have a high input resistance and therefore are recruited into activity prior to neurons with larger axon diameters. Conduction velocity of a neuron is known to be directly related to the diameter of its axon. On the basis of the relation of axon diameter and conduction velocity and the Henneman size principle, it was hypothesized at the initiation of this study that cells exhibiting a rapid spontaneous mean firing rate and therefore a low

threshold for recruitment to activity, would have the slowest conduction velocity. This was not found to be the case. The slowest firing neurons, silent, did have the highest conduction velocity, but this relationship did not hold true for the other patterns of firing. However, a significant correlation ($P < .025$) of conduction velocity and osmosensitivity (K_{aH}) was observed.

A slow intravenous infusion of hypertonic NaCl was found to be a suitable stimulus for excitement of magnocellular neuroendocrine cells. In addition, these infusions permitted quantitation of the osmotic stimulus and correlation with neuronal activity. Some antidromically negative neurons were observed during osmotic forcing and were found to be insensitive to this stimulus. The response of antidromically identified neuroendocrine cells then is a specific osmosensitive response.

In response to intravenous infusions of hypertonic NaCl magnocellular neuroendocrine cells behaved in three ways: inhibition, no change and excitement. Hypertonic forcing not only excited spontaneously active neuroendocrine cells but could recruit silent cells into activity, change the firing pattern of spontaneously active neuroendocrine cells and synchronize bursting of a "pool" of neuroendocrine cells. It was observed that most cells during a forcing had a marginal periodicity which emerged after termination of the forcing into a low frequency bursting pattern of firing. Few dynamically osmosensitive neurons were ever found to return immediately to a continuously active slow pattern of firing during the decline of plasma osmolality toward control values. These cells first went through the low frequency bursting pattern and then eventually to continuously active slow after the forcing. The hypertonic

forcing appears therefore to provide a stimulus for initiation of low frequency bursting in these neurons. This bursting appeared to be synchronized since many times multiunit activity "sounded" and "looked" as if bursting of several neurons was simultaneous. This synchronization could be provided by auto-, lateral and/or recurrent inhibition or excitation with neighboring neuroendocrine cells. Indeed, Lafarga, et al. (1975) noted morphologically the presence of cholinergic axosomatic synapses in the SON with synaptic terminals that are shared by two neural somas. Structures of this sort could permit interneuronal coordination and discharge.

Arnauld, et al. (1975) noted that with progressive dehydration (5 day water deprivation) systematic changes in action potential firing occurred. At control osmolality the majority of neurons fired slowly and irregularly. As dehydration progressed the number of phasic cells increased while the number of slow, irregular cells decreased. With further increased in plasma osmolality the number of phasic cells decreased and the number of continuously active fast cells increased. Whether or not these changes in firing pattern with increasing plasma osmolality were due to interneuronal coordination is unknown.

Computer analysis made it possible to measure osmosensitivity in four ways. Two of these were trend analysis based on cumulative time histogram and instantaneous rate plot. The others were based on the trend analyses and the respective change in osmolality for the analysis period. These estimates of osmosensitivity were not found to be statistically different between firing pattern types. However, in all estimates continuously active fast cells were the least osmosensitive while either continuously active slow or low frequency bursting cells appeared

to be the most osmosensitive. It may be that continuously active fast cells had the lowest threshold for osmotic stimulation and were therefore firing at or near their maximum rate. If this is the case and the Henneman size principle does apply to neuroendocrine neurons, one would expect continuously active fast cells to have the slowest conduction velocity of neuroendocrine cell firing patter types. As mentioned earlier, this was not the case.

Calculation of the correlation coefficient did reveal, in two cases, a likely correlation between calculated conduction velocity and osmosensitivity (K_{aH} and K_{aR}). When osmosensitivity ($K_{aH}/\Delta Osm$ and $K_{aR}/\Delta Osm$) was correlated with conduction velocity, the relationship was not substantiated. The reason for the poorer correlation when the change in osmolality is taken into account is unknown, but it may be that any change in plasma osmolality is a stimulus while magnitude of the change is less important.

Cells that responded in a positive manner to the hypertonic forcing did so in one of two ways, tonically or dynamically. About twice as many cells were classified as dynamic as were classified as tonic (67% and 33%, respectively).

Dynamic sensitivity may be a mechanism necessary to prevent an "overshoot" of antidiuretic hormone past the amount necessary for maintenance of osmotic and/or volumetric homeostasis. As mentioned in Chapter II, plasma ADH levels return to control values prior to either osmotic or volumetric stimuli. Although not discussed by Wakerley, et al. (1975), it appears from inspection of Figure 1 of the cited work that identified phasic neuroendocrine cells of the PVN are dynamically sensitive to changes in arterial blood pressure (induced by

hemorrhage). In fact one neuroendocrine cell was totally inhibited when reinfusion of blood was begun, although arterial blood pressure remained below control levels until reinfusion was complete.

Tonically osmosensitive cells on the other hand may be more sensitive to absolute value of plasma osmolality and/or blood volume rather than direction of their change.

As reviewed in Chapter II, there is a preferential localization of antidiuretic hormone-producing and oxytocin-producing neurons within both the supraoptic and paraventricular nuclei. It was noted in the present study that the caudal portion of the SON did have more osmotically excited neurons and fewer osmotically inhibited neurons than the rostral portion.

The observation that osmosensitive neuroendocrine cells were not limited to a particular pattern of firing and that more than one firing pattern could be exhibited in response to a stimulus by a single neuroendocrine cell seem to support the idea of a specific functional state rather than specific secretory or hormonal states as hypothesized by other authors. Approximately 50% of the identified magnocellular neuroendocrine cells studied were found to be excited by an osmotic stimulus while the other 50% were either not affected or inhibited. These data do not support a nuclear theory of organization of the hypothalamic magnocellular neurosecretory system, but rather a cellular theory. In other words, some neurons of the supraoptic nucleus may be related to antidiuretic hormone synthesis and release while others may be involved in synthesis and release of oxytocin or other hypothalamic peptides.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The purpose of this study was to categorize and gain knowledge concerning the functional significance of firing patterns recorded from magnocellular neuroendocrine cells of unanesthetized sheep. Previous investigators have hypothesized that these firing patterns represent specific secretory states (i.e., release, synthesis, transport or storage) or specific hormonal states (i.e., antidiuretic hormone-producing or oxytocin-producing) of these neurons. For the present study it was hypothesized that these firing patterns represent specific functional states existing in the neuron at the time it was recorded. It was further hypothesized that the functional state of a neuroendocrine cell depends on stimulus intensity and cell size.

It was determined that all neuronal firing patterns recorded from random areas of the hypothalamus and septal areas could be categorized into seven types (silen, continuously active slow, low frequency bursting, continuously active fast, high frequency bursting, continuously active bursting and continuously active regular). Although neurons exhibiting these patterns were distributed randomly throughout most hypothalamic and septal areas, antidromically identified supraoptic neuroendocrine cells exhibited only silent, continuously active slow, low frequency bursting and continuously active fast activity.

The response of supraoptic neuroendocrine cells to a 20 mOsm/kg

increase in plasma osmolality evoked by a 20 minute infusion of hypertonic NaCl was studied. Of the neuroendocrine cells tested, 51% were excited by the increase in plasma osmolality, 11% were inhibited and 38% were not affected. Of those cells that were excited, 33% exhibited tonic osmosensitive properties (sensitive to the absolute value of plasma osmolality) while 67% exhibited dynamic osmosensitive properties. Cells defined as dynamically osmosensitive increased their overall activity while plasma osmolality was increasing, but returned towards prestimulus behavior if plasma osmolality held steady at a high value or decreased towards control levels. Neuroendocrine cells exhibiting spontaneous low frequency bursting were always dynamically osmosensitive.

Osmotic forcing could evoke all spontaneous patterns of activity found in supraoptic neuroendocrine cells in a single neuroendocrine cell. Continuously active slow cells were driven to low frequency bursting and eventually to continuously active fast activity during maximum osmotic forcing. During the decline of plasma osmolality many neurons exhibited low frequency bursting even though they discharged continuously during control and increasing plasma osmolality periods.

Cell size (as indicated by conduction velocity) was found to be significantly correlated with osmosensitivity if the total absolute change in plasma osmolality was not taken into account. This indicates that the Henneman size principle may be a factor in determining osmosensitivity of neuroendocrine cells. Correlation without absolute change in osmolality taken into account further indicates that direction and rate of change of plasma osmolality may be more important than the absolute value of plasma osmolality change.

The cellular theory of organization of this hypothalamic

magnocellular neurosecretory system is supported by this investigation. Of the neuroendocrine cells tested, 51% were excited by increasing plasma osmolality. The remaining cells were either inhibited or not affected by the hypertonic forcing. These inhibited or unaffected supraoptic neuroendocrine cells are presumably concerned with regulation of oxytocin or other hypothalamic peptides. In agreement with recent immunocytochemical studies, the caudal area of the SON had more osmotically excited cells (ADH neurons?) and fewer osmotically inhibited cells (oxytocin neurons?) than the rostral area.

In conclusion, these studies demonstrated that:

1. the recorded supraoptic neuroendocrine cells discharge in only four of the categorized firing pattern types;
2. a single neuroendocrine cell may exhibit all patterns of activity found in supraoptic neuroendocrine cells depending on the level of osmotic stimulation;
3. 51% of the neuroendocrine cells tested were excited by increasing plasma osmolality;
4. there is preferential localization of osmosensitive neuroendocrine cells in the caudal half of the SON;
5. no significant differences exist in osmosensitivity of neuroendocrine cells spontaneously exhibiting any of the firing patterns found in neuroendocrine cells;
6. relative osmosensitivity of neuroendocrine cells may be related to the Henneman size principle; and
7. the neuroendocrine system regulating ADH secretion has both tonic and dynamic components.

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VITA

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