ELECTRICAL ACTIVITY OF SUPRAOPTIC NEURONS IN WATER DEPRIVED RATS DURING SLOW INTRAGASTRIC INFUSIONS OF WATER

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
CHARLES THOMAS BENNETT
1971

LIBRARY
Michigan State
University

This is to certify that the

thesis entitled
ELECTRICAL ACTIVITY OF SUPRAOPTIC NEURONS IN WATER DEPRIVED RATS DURING SLOW INTRAGASTRIC INFUSIONS OF WATER

presented by

Charles Thomas Bennett

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Psychology

Glenn I. Hatton

Date 9/21/71

O-7639

6722

ABSTRACT

ELECTRICAL ACTIVITY OF SUPRAOPTIC NEURONS IN WATER DEPRIVED RATS DURING SLOW INTRAGASTRIC INFUSIONS OF WATER

By

Charles Thomas Bennett

It is known that the supraoptic nucleus (SON) produces at least the humoral precursor of vasopressin, the antidiuretic hormone (ADH). The nature of the relationship between electrical activity of SON neurons to changes in concentration of body fluids has not been clearly defined. Therefore, to more precisely understand this relationship, changes in activity of neurons in the SON and anterior hypothalamus (AH) of rats were monitored during changes in tonicity of body fluids. In Experiment I, rats were placed on a 23.5 hr water deprivation schedule for five days. On the fifth day, before access to water, the rats were anesthetized and prepared as if for unit recording. They then received a 10 cm^3 gastric water load at 1 cm^3/min. This rate is similar to the rate of water ingestion of awake rats under similar dehydration conditions. At four minute intervals for 24 min from the beginning of the water load plasma osmolality (P_{osm}), plasma volume (P_v). and water absorption were measured. It was found that by 11 min of absorption time P_{osm} decreased significantly 3%, while 2.5 cm^3 of water had been absorbed. P_v, on the other hand, did not

change until 18 min of absorption time.

In Experiment II, rats were treated just as those were in the previous study, but during the water load, unit activity in the SON and AH were monitored. Following the stomach load in some rats, a 1 cm^3 16% NaCl injection was administered subcutaneously (SC). The following changes in unit activity were observed:

SON unit activity was found to be significantly faster in the water deprived rat, as compared to rats on an ad libitum food and water schedule.

A transient, short latency increase in SON unit activity was observed in response to initial filling of the stomach with water. Following this, when P_{osm} began to significantly decrease, unit activity in the SON began to also significantly decrease below baseline levels.

In response to a SC hypertonic saline injection, a short latency, transient increase in SON unit activity was seen. This increase occurred during a time period when awake rats, treated in a similar fashion, exhibit a marked pain reaction. By seven minutes post-injection, when P_{osm} has been shown to significantly increase, SON unit activity became significantly faster.

AH cells, treated in the same fashion, did not show similar changes in unit activity in response to rapid changes in P_{osm}. This is true depsite the fact that following a period of water deprivation, unit activity in the AH was significantly faster than in animals on an ad libitum

feeding and drinking schedule.

If (a) the initial short latency, transient changes in SON unit activity following the water load or injection can be attributed to peripheral stimuli, and, if (b) the secondary changes in unit activity can be attributed to shifts in P_{osm}, then this is apparently the first reported evidence for convergence of stimuli that are potent releasers of ADH.

ELECTRICAL ACTIVITY OF SUPRAOPTIC NEURONS IN WATER DEPRIVED RATS DURING SLOW INTRAGASTRIC INFUSIONS OF WATER

By

Charles Thomas Bennett

A Thesis

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Psychology

1971

Acknowledgments

I want to express deep appreciation to Dr. Glenn I. Hatton who guided my graduate training.

I wish to also acknowledge Dr. John I. Johnson, Dr. Lawrence I. O'Kelly, and Dr. Ralph Pax who helped direct this thesis.

Important to the completion of this thesis was the assistance of the Department of Psychology and the Laboratory for Comparative Neurology, which supplied necessary photographic and data analysis equipment.

I want to thank Mrs. John Haight for histological assistance.

I want to express special thanks to Diane Bennett who spent many late hours typing and doing the basic statistics.

This thesis was completed while the author was supported by a predoctoral fellowship from the National Institute of Mental Health, no. MH 48474. The research was supported by a grant from the National Institute of Neurological Diseases and Stroke, no. NS 09140 to Dr. Glenn I. Hatton.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures Page

Figures Page

LIST OF APPENDICES

INTRODUCTION

Ingestion and excretion of water are two primary control mechanisms most mammals employ to insure a proper water balance. When water is freely available, animals usually have few problems maintaining normal hydration levels. To do this, the volume of water lost must equal, for the most part, the volume of water ingested. But, if ingestion is restricted for any reason, the amount of water lost may exceed the ingested volume (Chew, 1965). As a result, an animal incurs a negative water balance, with a consequent increase in the tonicity of body fluids, and a decrease in their volume (Wolf, 1958).

To maintain life, it is imperative that an animal be able to limit these changes. One way to do this is by the conservation of body water. While there are several ways an animal can conserve body water, one primary method is to reduce renal water loss.

There are two hormonal systems which can limit the amount of water excreted by the kidney. However, these hormones are released as the result of essentially different stimuli. As renal blood pressure decreases (which can be a consequence of the decrease in blood volume) aldosterone is released. This hormone promotes the renal retention of sodium.

Then, as blood sodium levels increase, water tends to be retained in the body (Pitts, 1968). But also, as a result of excessive water loss, blood tonicity increases. This can result in the increased circulating titers of vasopressin, or the antidiuretic hormone (ADH). This hormone has the effect of increasing the reabsorption of water in the proximal tubules of the kidney (Pitts, 1968).

The control of the release of either aldosterone or ADH is not completely understood. Of special interest here, however, is the regulatory system controlling vascular ADH titers. It is believed that there are two primary system variables (blood volume and tonicity) that are potent stimuli for the release of ADH. Changes in the former are detected by baroreceptors in the carotid arteries and left atrium (Share, 1968). As blood volume decreases these receptors mediate an increased release of ADH into the blood (Bland, 1963). The exact neural pathways which subserve this system have not been clearly established (Barker, Crayton, and Nicoll, 1971).

It is believed that hyperosmolality of the blood will result in increased release of ADH; and, that these changes in blood tonicity are detected by osmosensitive neurons located in the vascular bed of the carotid artery (Verney, 1947). There is some evidence to indicate that there may also be hepatic osmosensitive cells important to the release of ADH (Haberich, 1965 and 1968; Lydtin, 1969). These investigators have reported that as tonicity of the blood

perfusing the liver increases, there is an increased release of ADH. How important the role of these latter receptors are to the daily regulation of ADH secretion has not, however, been established.

The importance of the hypothalamo-hypophyseal system to the elaboration of ADH had been established many years ago (Fisher, Ingram, and Ranson, 1938; Harris, 1947; Verney, 1947). On the basis of data reported in these works, it has generally been concluded that elements in the hypothalamo-hypophyseal system become activated as the result of increases in effective osmotic pressure (EOP) of body fluids. As this activation takes place, ADH (or, its humoral precursor) is released into the blood.

Since that time, histochemical (Belislin, Bisset, and Halder, 1967) and anatomical (Howe and Jewell, 1959) studies have indicated that the supraoptico-hypophyseal system is responsible for the production and release of ADH.

Early electrophysiological investigations (Von Euler, 1953) indicated that the supraoptic nucleus (SON) might be be detecting changes in EOP. His work indicated that there are slow wave D.C. potential shifts following intacarotid (IC) injections of hypertonic solutions. On the basis of these "osmopotentials" he concluded that the SON might be a detector component of the ADH release system.

A few years later, Cross and Green (1959) recorded from single neurons in this area. They reported that cells in the SON were osmosensitive (ie., the firing frequency of

certain cells changed with a short, reliable latency following an osmotic stimulus).

Since Cross and Green's now classic study, many investigators have substantiated the fact that at least some of the cells in the SON are osmosensitive (Brooks, Koizumi, and Zebalios, 1966; Brooks, Ushiyama, and Lange, 1962; Cross and Silver, 1966; Dyball, 1969; Dyball and Koizumi, 1969; Ishikawa, Koizumi, and Brooks, 1966a; Ishikawa, Koizumi, and Brooks, 1966B; Joynt, 1964; Koizumi, Ishikawa, and Brooks, 1964; Wang, 1969). However, not until recently (Dyball, 1969) has anyone attempted to correlate changes in SON unit activity with the release of ADH. Dyball reported that shortly after SON unit activity increased, there was an elevation of ADH titers in jugular blood.

The fact remains, though, that none of the studies cited above, in themselves, demonstrate that the SON actually is a detector of osmotic pressure shifts. It could be that activity changes in SON neurons, seen in response to EOP shifts, are merely the result of influences impinging on them from other neurons. If this is the case, the true detectors cells are probably located within some subcortical area. This notion has some support because isolated, decorticated brains seem capable of producing ADH in response to EOP changes (Cross and Kitay, 1967; Saito, Yoshida, and Nakao, 1969; Suda, Koizumi, and Brooks, 1963; Sundsten and Sawyer, 1961; Woods, Bard, and Bleir, 1966).

The problems of demonstrating that the supraoptic nucleus is actually the detector component of this system are enormous. Because the area of the hypothalamus is highly vascularized (Haymaker, 1969), any attempt to surgically isolate structures within it will disrupt the blood supply, and increase the difficulty in interpreting the results.

As indicated above, it is debatable whether or not the SON actually is an osmodetector. Despite this, and as indicated earlier, the supraoptico-hypophyseal system seems to be the effector organ of the ADH release system. However, essentially all that is known about the electrophysiological properties of this effector organ is that there are osmosensitive neurons in it. This is true despite the fact that there are at least three stimuli (blood volume, blood tonicity, and pain, Pitts, 1968) which elicit the release of vasopressin. Yet, if we are to better understand the neurophysiological correlates of ADH release, we must know how cells in the SON code the stimuli which impinge upon it; whether these stimuli impinge directly on the neurons, or via nervous pathways from other loci.

The purpose of this thesis, in part, is to determine more precisely the manner in which SON neurons respond to osmotic stimuli. To accomplish this, traditional methodology could not be used. Apparently, without exception, in every reported study dealing with the osmosensitivity of SON neurons, osmotic stimuli (hypertonic solutions) have been

presented via intracortid (IC) cannulae. No one has apparently even recorded from SON neurons in water deprived animals. Further, no one has apparently monitored SON units in water deprived animals as they are being rehydrated by means of oral water loads. And, apparently, no one has even actually measured blood tonicity while concurrently recording from cells in the hypothalamus.

If we are to understand the changes in SON unit activity of awake animals, it would seem reasonable to monitor these neurons under conditions which most closely approximate those an animal might actually encounter. One of the purposes of the thesis, then, is to determine changes in SON unit activity of deprived animals, while they are being rehydrated with a gastric load of water.

It has been reported that, after a period of water deprivation, rats, when given access to water, will drink at the approximate rate of 2 cm^3/min (Hatton and Bennett, 1970). This rate is, generally, maintained until almost 3 cm^3 of water has been absorbed and plasma osmolality (P_{osm}) has decreased at least 1-2%. With this information, it is possible to understand more clearly the relationship of SON unit activity to changes in drinking behavior. For, if changes in P_{osm} and water absorption are being measured while recording from SON units (as a water load is being given), then the time relationship between the cessation of drinking and changes in SON unit activity may be approximated.

Verney (1947) reported that an increase in diuresis begins when concentration of body fluids decreases as little as 1-2%. It would be expected, then, that similar changes in P_{osm} should result in both the cessation of drinking and changes in unit activity of the SON. This hypothesis will be tested in the present investigation.

Specifically, then, the purposes of this thesis are to (a) establish whether there are consistent, correlated changes in plasma osmolality and unit activity of the SON, and, (b) determine the time relationship of these changes to the P_{osm} thresholds that are known to bring about the cessation of drinking.

To accomplish this, in Experiment I animals were placed on a water deprivation schedule. They were then anesthetized, prepared as if for unit recording, and given a stomach load of water at a rate similar to normal ingestion rates. Changes in plasma osmolality, plasma volume (P_v), water absorption, and heart rate were measured.

In Experiment II, SON units were monitored under these same conditions. Some of these animals also received a common thirst-producing stimulus, a subcutaneous hypertonic saline injection.

These procedures (water load and saline injection) bring about dramatic changes in blood tonicity. As a result, only one cell was recorded from each animal. This procedure

has obvious limitations. But these are outweighed by the fact that changes in unit activity could be compared to changes in tonicity of body fluids that are similar to those that might naturally occur.

EXPERIMENT I

INTRODUCTION

In this study, rats were placed on a water deprivation schedule for five says. Before access to water on the fifth day, they were anesthetized and prepared as if for unit recording. They then received a gastric water load. Changes in P_{osm}. P_v, water absorption, and heart rate were monitored.

With this data, some of the concommitant neural changes were then examined in Experiment II.

METHOD

Subjects

The animals used were 40 naive, male albino rats of the Holtzman strain, 100-110 days old at the beginning of the experimental treatments. They were housed in individual cages under conditions of constant light and given Wayne Mouse Breeder Blox ad libitum. While there was no humidity control, temperature was maintained between 22-25°C.

Procedure

After arrival into the laboratory, the rats were given at least three days of free access to food and water in

their home cages. The animals were then placed on a 23.5 hr water deprivation schedule. During their 0.5 hr access to water, drinking bottles were placed on the rats' home cages.

Before access to water on the fifth day, four rats were randomly assigned to each of the following treatment conditions:

Ten Min Pre-Load Group--rats which were anesthetized with Dial-urethane (Ciba). The anesthetic was given intraperitoneally (IP); the dosage being 0.7 cm^3/kg plus a supplementary dose of 0.02 cm^3. In no case was any other supplementary dose given.

Once anesthetized, a 2 cm^3 heart blood sample was taken from the left ventricle of their surgically exposed hearts. Following this, stomachs, small intestines, caecum-colons (taken as one) were clamped, removed, and stripped of excess lipomal and mysenteric tissue. The organs were weighed wet, then dried for approximately 24 hr at 100°C. This procedure has been shown to remove all tissue fluids (Bennett, 1969).

Immediately upon removal the blood was centrifuged at 3300rpm for 4 min. The plasma was then withdrawn, protein concentration determined by refractometry, and the remaining plasma sample was frozen in sealed glass vials for later osmometric determinations in a freezing point osmometer.

0 Min Group--rats which were anesthetized and then

prepared as if for unit recording. This preparation included the following: (a) positioning of a PE 50 (Intramedic) stomach tube, (b) positioning sub-dermal cardiac electrodes, the ground being attached to the stereotaxic apparatus, (c) placing the rat in the stereotaxic instrument, (d) positioning a rectal thermistor thermometer, (e) wrapping the animal with a heating tape to maintain body temperature, (f) exposing and cleaning the skull. Following these procedures, a ten minute sample of heart rate was taken. To do this, cardiac bioelectric activity was amplified through a low level pre-amplifier, then displayed on an oscilloscope and recorded on magnetic tape. (See Appendix A for a detailed description.) When the baseline record of heart rate was taken, the animals were treated as the 10 Min Pre-Load Group was.

Three, 6, and 9 Min Groups--rats which were treated just like the 0 Min Group, except that 0.5 hr following the surgical preparation for recording was completed, they received stomach loads of 3, 6, and 9 cm^3, respectively, of tap water at room temperature. The load was delivered manually at the rate of 1 cm^3/min. Ten minutes prior to and then during the load, heart rate was monitored and recorded on magnetic tape. Body temperature was maintained throughout the procedures at $37\pm1^{\circ}C$. This was done through a regulated D.C. power supply. Immediately following the load, the animals were treated the same as the 10 Min Pre-Load Group.

Twelve, 15, 18, 21, and 24 Min Groups--rats which were

treated as the previous groups were, but received a 10 cm^3 load of water delivered at the rate of 1 cm^3/min. Then 12, 15, 18, 21, and 24 min, respectively, from the beginning of the load, they were removed and treated like the 10 Min Pre-Load Group. Heart rate was also recorded 10 min prior to and then during and after the water load.

Determination of water absorption. A detailed description is presented elsewhere (Bennett, 1969; Hatton and Bennett 1970). Essentially, the procedure was the following: Fluid loss from the guts of the 10 Min Pre-Load Group provided the amount of fluid in the tissues. Fluid loss from the guts of animals which had been stomach loaded provided the amount of fluid in the tissues and lumen. Fluid loss from the guts of animals which had been loaded, minus that from animals that had not been loaded provided the amount of fluid in the lumen of the intestinal tract. Therefore, the amount of water absorbed is given by the following formula:

$$A_j = I_j - TFW_j$$

Where A=amount of water absorbed
I=amount of water loaded
TFW=amount of total water in the lumen of the small intestine.

Determination of heart rate. Actual frequency of heart rate was determined by an electronic counting system. (A detailed description is presented in Appendix A.)

RESULTS AND DISCUSSION

In Table 1 are shown the mean (±S.E.) amount of water remaining in the stomachs and small intestines of the various groups. Of interest here is that the maximum amount of fluid remaining in the stomach does not occur until approximately the 9 min sampling.

Figure 1 shows the mean (±S.E.) amount of water absorbed as a function of time for the groups receiving the stomach load. The double abscissa (in this, and the following figures) is necessary because while a given animal was removed at (for example) 9 min, and after 9 cm^3 of water had been loaded, approximately 2 min elapsed during which time surgical procedures were performed. During these two minutes, water that had been loaded could be absorbed and bring about changes in blood tonicity.

The 3 Min Group had significantly ($p<0.05$) more water in their intestinal tract than would be expected on the basis of the 3 cm^3 that had been loaded. These data indicate that fluid had entered the intestinal lumen, either from the peritoneal cavity or from the vascular system, or both. By 6 min, the amount of water loaded equalled the amount of water in the intestinal tract. This would mean that the fluid that had entered the lumen previously had begun to flux back into the blood and/or abdominal cavity.

Not until 9 min had a significant amount of water been

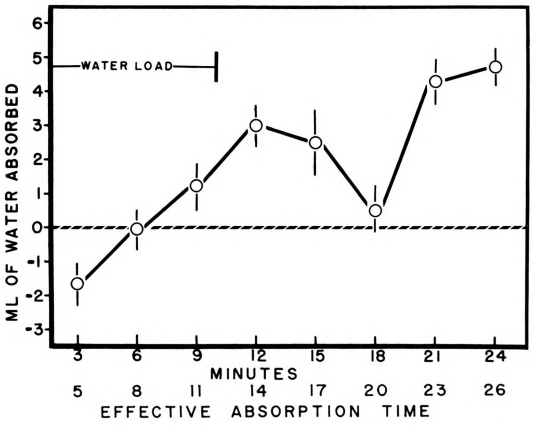
Table 1

Amount of water remaining in the stomachs and small intestines of the animals in Experiment I.

Group	Amount in Stomach (cm^3)	Amount in Small intestine (cm^3)
3 Min	3.0 ± 0.17	1.7 ± 0.54
6 Min	3.8 ± 0.33	1.8 ± 0.69
9 Min	6.4 ± 0.48	1.4 ± 0.68
12 Min	4.3 ± 0.68	2.6 ± 0.36
15 Min	5.2 ± 0.84	2.3 ± 0.76
18 Min	6.2 ± 0.82	2.4 ± 0.50
21 Min	3.7 ± 0.72	2.1 ± 0.69
24 Min	3.6 ± 0.82	1.7 ± 0.68

Figure 1.

Indicated are the mean (±S.E.) amount of water absorbed as a function of minutes. See text for an explanation of the double abscissa. N=4 per point.


WATER LOAD
ML OF WATER ABSORBED
6
5
4
3
2
1
0
-1
-2
-3
3
6
9
12
15
18
21
24
MINUTES
5
8
11
14
17
20
23
26
EFFECTIVE ABSORPTION TIME

absorbed. However, by the 18 min sampling, significantly more water was in the intestinal tract than at either the 9 or 12 min sampling. The implication is that fluid again began to flow back into the small intestine. Shortly after this, as indicated by the 21 min sampling, water again began to be absorbed into the blood.

Figure 2 shows the mean ($\pm$S.E.) P_{osm} levels as a function of time before, during, and after the stomach load of water. Though there is a slight decrease in P_{osm} by 5 min of effective absorption time, no significnat decrease in plasma concentration occurred until 11 min. By that time, P_{osm} had decreased approximately 3%. There was then no statistically significant change until 24 min following the beginning of the load, when P_{osm} again tended to decrease. The magnitude and time course of the first significant decrease observed, here, is similar to that reported for awake, drinking rats under similar hydration conditions (Hatton and Bennett, 1970).

Figure 3 shows the mean ($\pm$S.E.) change in heart rate as a function of time during and after the load of water. There is an initial small decrease in heart rate following the beginning of the load. But, by the time the load is completed (and, when plasma osmolality and stomach fluid volume decrease), heart rate returns to baseline levels.

Increases in heart rate during stomach loads have been reported (O'Kelly, Hatton, Tucker, and Westall, 1965). However, the loads in their study were given at a rather rapid rate. But, despite this, 30 min following a 3% water

Figure 2.

Indicated are the mean ($\pm$S.E.) levels of plasma osmolality for the different groups as a function of minutes. See text for an explanation of the double abscissa. N=4 per point.

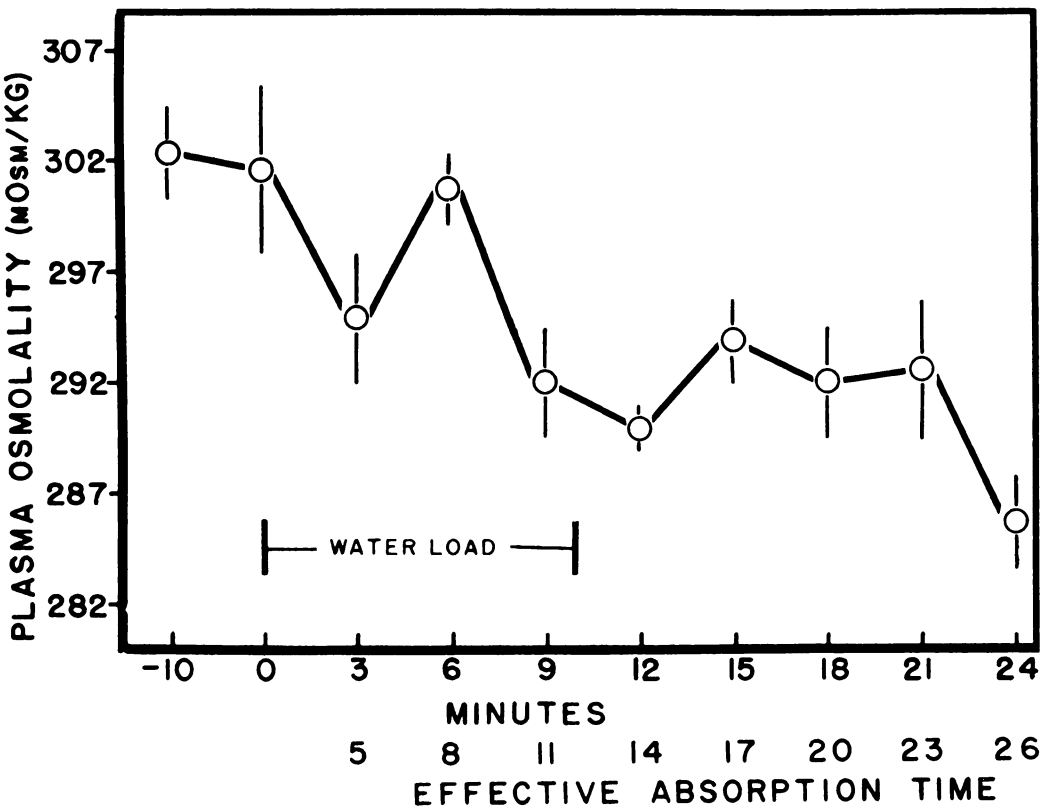
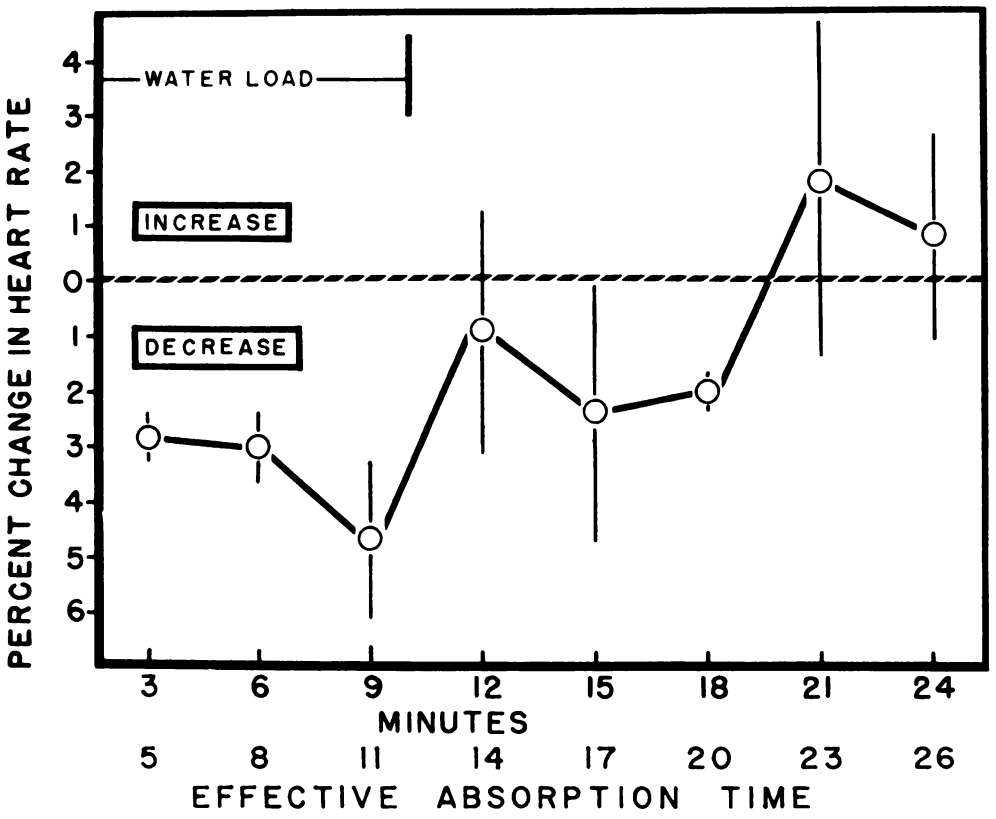

PLASMA OSMOLALITY (MOSM/KG)
307
302
297
292
287
282
WATER LOAD
-10
0
3
6
9
12
15
18
21
24
MINUTES
5
8
11
14
17
20
23
26
EFFECTIVE ABSORPTION TIME

Figure 3.

Indicated are the mean (±S.E.) percent changes in heart rate for the various groups. Change in heart rate for each rat was calculated from their respective mean baseline rates. See text for explanation of the double abscissa. N=4 per point.


WATER LOAD
INCREASE
DECREASE
PERCENT CHANGE IN HEART RATE
4
3
2
1
0
1
2
3
4
5
6
3
6
9
12
15
18
21
24
MINUTES
5
8
11
14
17
20
23
26
EFFECTIVE ABSORPTION TIME

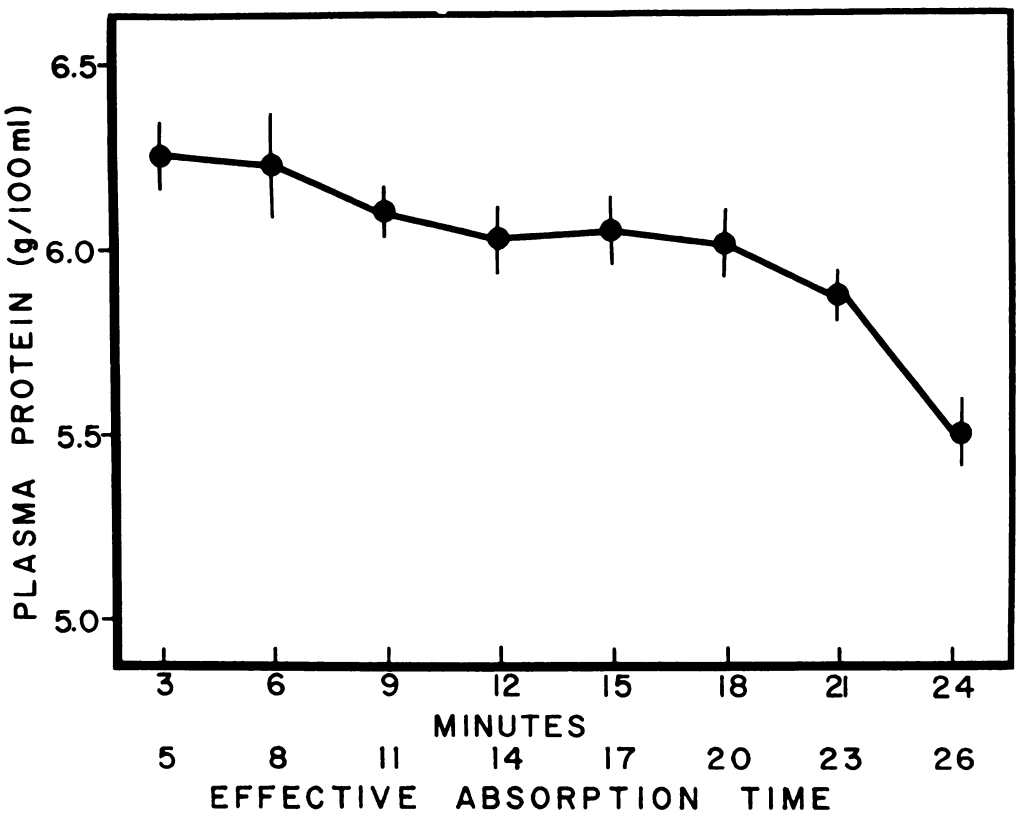
load (comparable to the 10 cm^3 load given here), heart rate had returned to near baseline levels, as it did in this study.

Mean P_v changes ($\pm$S.E.) as reflected by plasma protein concentration are shown in Figure 4 during and after the stomach load. The magnitude of change in plasma volume are greater than those reported for awake, drinking rats under similar dehydration conditions (Hatton and Bennett, 1970). In addition, the absolute level of plasma protein concentration is lower than that reported for normal, deprived rats. This difference in hemodilution might be attributed to the effect of being under anesthesia for approximatley 45 min before experimental treatments had begun.

Also, in the present study, P_v decreases occurred several minutes after the first significant change reported by Hatton and Bennett. This could be caused by the initial hemodilution that was observed in these animals.

Figure 4.

Indicated are the mean (±S.E.) plasma protein concentrations for each group as a function of minutes. See text for an explanation of the double abscissa. N=4 per point.


PLASMA PROTEIN (g/100ml)
6.5
6.0
5.5
5.0
3
6
9
12
15
18
21
24
MINUTES
5
8
11
14
17
20
23
26
EFFECTIVE ABSORPTION TIME

EXPERIMENT II

INTRODUCTION

In the previous experiment, changes in P_{osm}, P_v, water absorption and heart rate were established in response to a water load. This was done in rats prepared for unit recording. In the present study, animals were treated as those in Experiment I, but during the water loads, unit activity in the SON was actually recorded.

Twenty-five minutes after the water load was begun, a 1 cm^3 16% subcutaneous (SC) NaCl injection was administered to some rats. Awake rats, under similar hydration conditions, will drink in approximately 6 min following such an injection, and, when P_{osm} has increased 2-4% (Hatton and Almli, 1969).

Following experimental manipulations, changes in unit activity examined in relation to the decreases in P_{osm} reported in the previous study, and the increases in P_{osm} following the NaCl injection reported by Hatton and Almli.

METHOD

Subjects

Animals used were 84 male albino rats of the Holtzman strain, 100-110 days old when the experimental treatments

began. The rats were maintained under conditions similar to those in the previous study. During experimental treatments, 15 animals died, and are not included in the data.

General recording procedures. These procedures were generally the same for each treatment condition.

The animals were anesthetized as in the previous study. Once recording procedures were begun, no supplementary doses were given.

The animals were then prepared for unit recording. This preparation included: (a) the positioning of a PE 50 (Intramedic) stomach tube; (b) implanting a sub-dermal cardiac electrode (the ground was attached to the stereotaxic apparatus); (c) positioning a rectal thermister thermometer for recording body temperature; (d) wrapping the animal in heating tape to maintain body temperature; (e) the placement of the animal in a stereotaxic instrument; (f) the exposing of the dura through a trephine hole in the calvarium; (g) the removal of the dura and covering the exposed neural tissue with mineral oil. Body temperature was maintained as in the previous study.

Once an animal was surgically prepared, a glass insulated microelectrode (exposed tip, 15-40μ; widest shaft diameter, 40-60μ) was lowered at DeGroot coordinates 9.8 AP, $\pm$1.4 Lat, and 8.5 DV. The skull was positioned horizontally in the apparatus.

Once the electrode was lowered into the brain, neural

activity was amplified by a low level pre-amplifier. It was then visually displayed on a dual beam oscilloscope (as was bioelectric activity of the heart). When experimental treatments began, unit and cardiac electrical activity was recorded on magnetic tape. (See Appendix A for a detailed description of the amplification and recording system)

After the microelectrode was lowered to at least 8.5 mm from the top of the cortex, isolation of a single unit was attempted. This procedure was the following: The electrode was slowly lowered manually. Once the extracellularly recorded action potentials of a given cell were at least 50μv above baseline noise (which usually was 25-50μv) the lowering of the electrode was stopped. If the amplitude of the action potential was sufficient to reliably trigger the sweep of the oscilloscope, the cell was considered to be isolated.

Following this, the various experimental treatments were begun.

Immediately following recording, a small D.C. cathodal (20μa for 5 sec) marking lesion was made through the tip of the recording electrode. Following lesioning, the animals were perfused with physiological saline and then a 10% formo-saline solution. The brains were removed and stored in the latter solution. Prior to frozen sectioning, some brains were placed for 2-3 days in sucro-formaline solution. Once the brains were sectioned, they were stained in cresyl violet

or thionin. The recording sites were then verified.

Treatment conditions. Once the rats arrived in the laboratory, they received at least three days of free access to food and water. At this time they were assigned to one of the two basic treatment conditions: Ad Libitum or Deprived. (See Table 2.)

Following the three day adaptation period, the rats in the Ad Libitum Group received at least five more days of free access to food and water. These animals were then anesthetized and prepared for unit recording. The electrode was lowered to the predicted location of the SON. Once a cell was isolated, ten minutes of activity was recorded before a marking lesion was made. Two to four cells were recorded from each animal of this group.

The animals in the Deprived Condition were placed on a 23.5 hr water deprivation schedule similar to the one used in Experiment I. Then on Day 5 of the deprivation schedule, prior to access to water, the animals were anesthetized and prepared for unit recording. Prior to actual recording, the rats in the Deprived Condition were further subdivided into one of the following treatment conditions:

Deprived Group--once a cell was electrically isolated, 49 min of activity was recorded on magnetic tape. A small marking lesion was then made.

Load Only Group--once a cell was isolated, in the approximate area of the SON, 10 min of baseline activity was

Table 2

Treatment conditions

Group	Treatment
Ad Libitum Group	Five days of free access to food and water before unit recording.
Deprivation conditions	
Deprived Group	Five days on 23.5 hr water deprivation schedule before unit recording.
Load Only Group	Deprivation period, plus 10 cm^3 gastric water load during unit recording.
Load-Injected Group	Deprivation period plus water load, plus 1 cm^3 16% NaCl SC injection.

recorded. Immediately following this, a 10 cm^3 stomach load of water at room temperature was manually delivered at 1 cm^3/min. For 39 min from the beginning of the load, unit activity was then recorded. A marking lesion was then made.

Load-Injected Group--animals which were treated as the Load Only Group were, but, 24 min from the beginning of the load a 1 cm^3 16% NaCl subcutaneous (SC) injection was given in the lower back. Fifteen more minutes of activity were then recorded. A small marking lesion was then made.

Frequency analysis. Determination of frequency of unit activity and heart rate was determined by electronic counters. (See Appendix A for a detailed description of this data analysis system.)

RESULTS AND DISCUSSION

Heart rate

Figure 5 shows the mean percent changes (±S.E.) for heart rate during and after the water load, and following the NaCl injection for six animals randomly sampled from the Load-Injected Group. A random sample was used here merely for convenience sake. The changes in heart rate seen in these animals are comparable to those seen in the animals in Experiment I (cf. Figure 3). There is an initial significant ($p<0.05$) bradycardia which is maintained until 10 min, at which time heart rate returns to near baseline levels.

In the first min following the injection, there is a significant ($p<0.05$) tachycardia. Heart rate then continues to increase until approximately 10 min following the injection.

Figure 6 shows the mean (±S.E.) percent changes in heart rate for the first minute following the beginning of the load. Immediately following the beginning of the water load, a bradycardia ($p<0.05$) is seen.

In Figure 7 are shown the mean (±S.E.) changes in heart rate per ten seconds immediately following the injection. In contrast to the bradycardia seen in the early portions of the stomach load, the tachycardia observed following the injection develops apparently more gradually.

It is well known that there are gastric stretch receptors that contribute to the vagal afferents (Paintal, 1954, 1963).

Figure 5.

Indicated are the mean (±S.E.) percent changes in heart rate for six animals selected at random from the Load-Injected Group. During the stomach load, change in heart rate for each rat was calculated from their respective mean baseline rates. During the injection, the change was calculated from each rat's mean minute rate for the last minute of the load period. N=6.

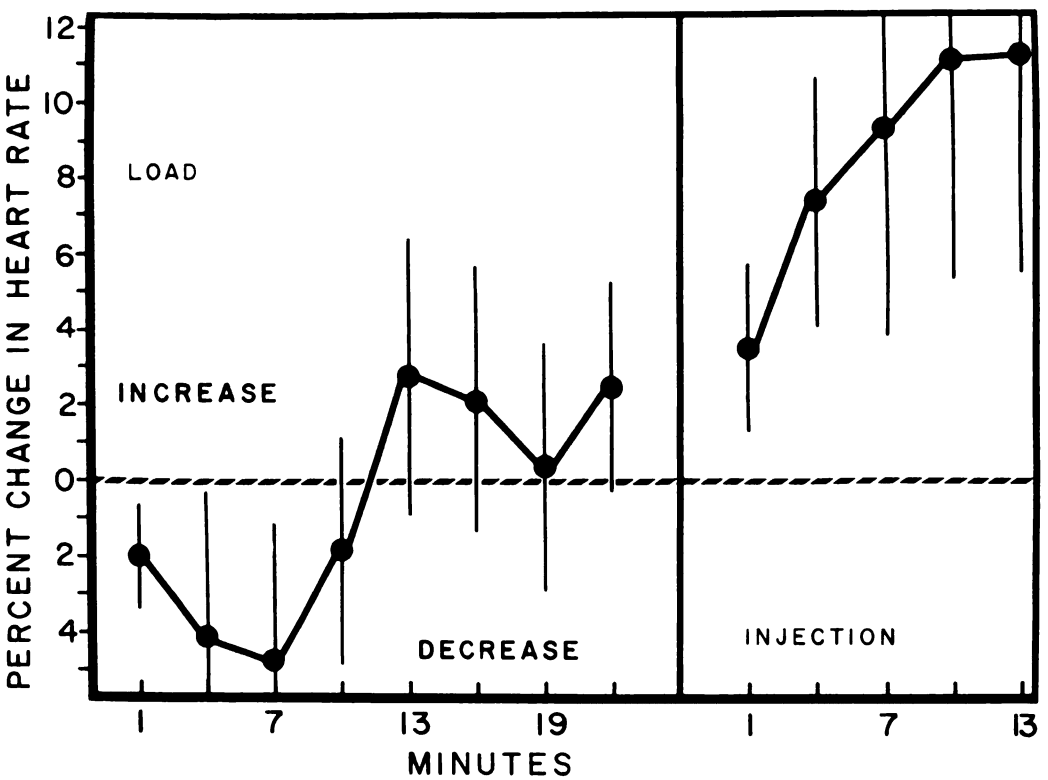

PERCENT CHANGE IN HEART RATE
12
10
8
6
4
2
0
2
4
LOAD
INCREASE
DECREASE
INJECTION
1
7
13
19
1
7
13
MINUTES

Figure 6.

Indicated are the mean (±S.E.) percent changes in heart rate per ten seconds in the first minute of the stomach load. The animals are the same as those used in Figure 5. N=6.

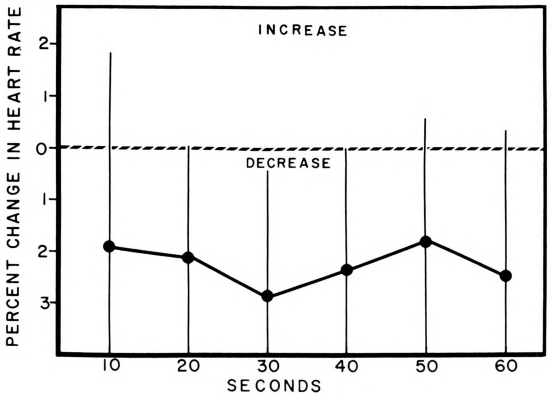
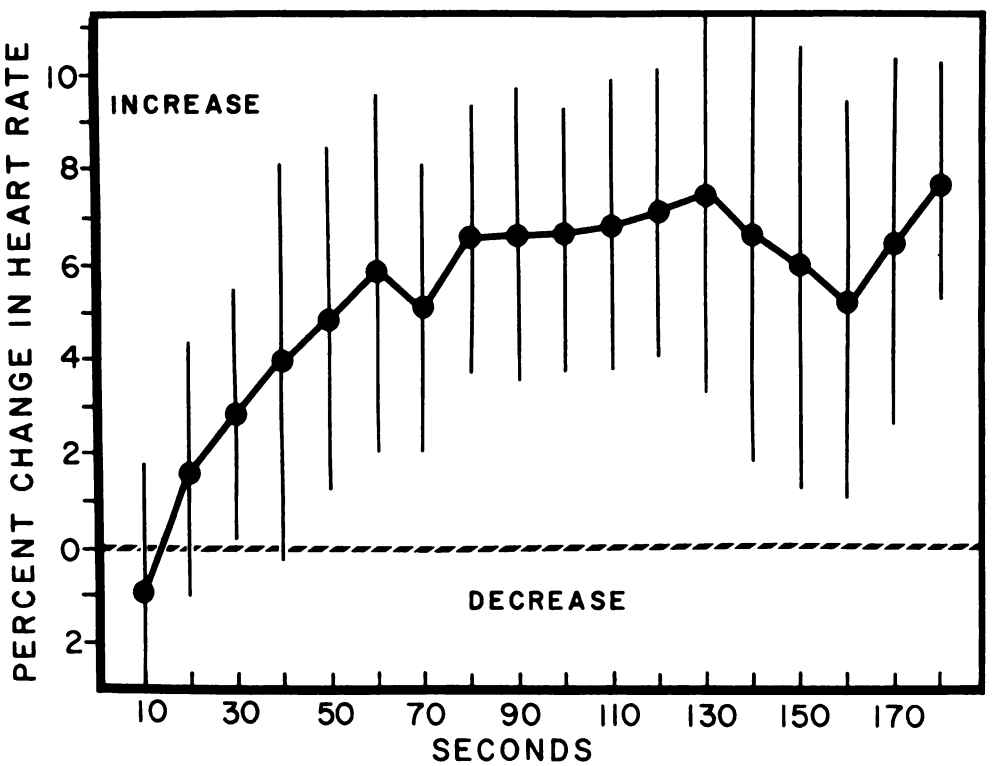

INCREASE
DECREASE
PERCENT CHANGE IN HEART RATE
2
1
0
1
2
3
10
20
30
40
50
60
SECONDS

Figure 7.

Indicated are the mean (±S.E.) percent changes in heart rate per ten seconds for the first three minutes following the saline injection. The animals are the same as those used in Figure 5. N=6.


PERCENT CHANGE IN HEART RATE
10
8
6
4
2
0
2
INCREASE
DECREASE
10
30
50
70
90
110
130
150
170
SECONDS

The changes in heart rate that are seen following the beginning of the load could be the result of increased activity in such mechanoreceptors. However, in addition to these vagal afferents, it is known that abdominal sympathetic afferents may influence heart rate (Sarnoff and Yamada, 1959). But because of the lack of evidence, it is unclear whether the bradycardia and the tachycardia seen in the present study are reflexively mediated through either one, or both, of the abdominal afferents.

Neural activity

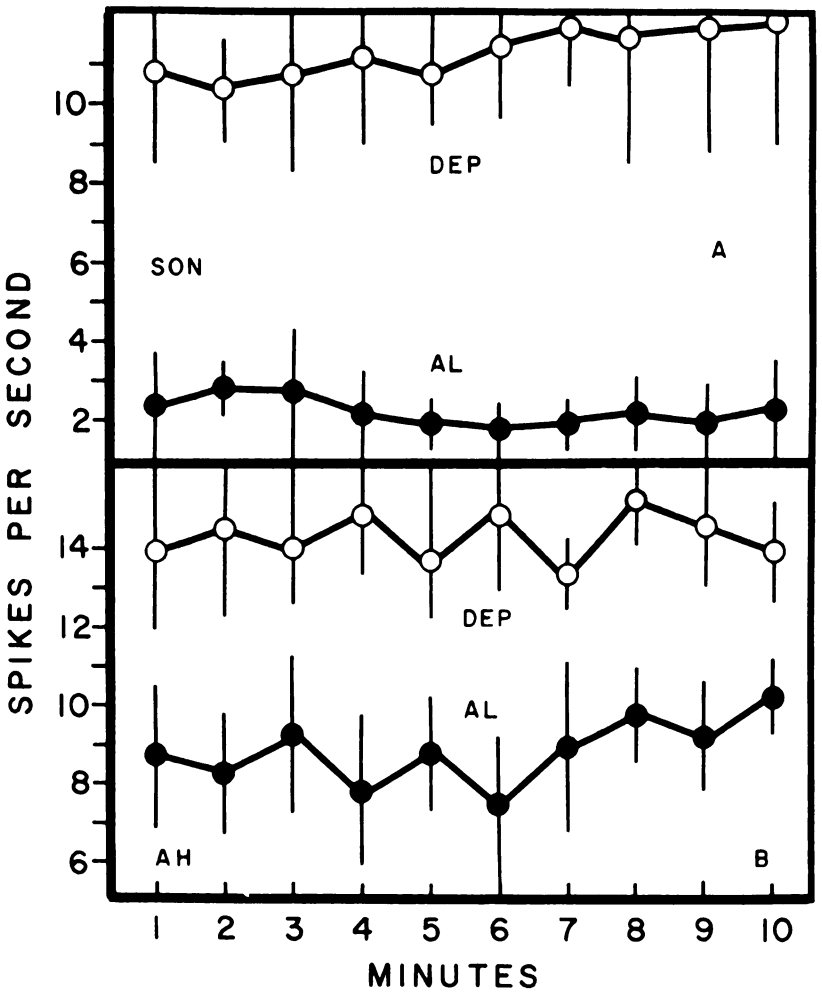
In Figure 8a are shown the mean($\pm$S.E.) spikes per second for a 10 min sample of activity of SON cells recorded from deprived rats, and, rats on an ad libitum food and water regimen. The overall mean from the deprived animals is 11.9 ± 0.2 spikes per second; and, the mean from the ad libitum animals is 2.4 ± 0.1 spikes per second. The difference is statistically significant ($p<0.05$). This is clear evidence that SON unit activity is faster after a period of water deprivation.

In Figure 8b is the mean minute ($\pm$S.E.) spikes per second for cells located in the anterior hypothalamus (AH), but outside of the SON. The upper line is the mean minute activity of cells recorded from animals that had been on a water deprivation schedule (overall mean is 14.3 ± 0.2 spikes/second). The lower line indicates the rate of firing of AH cells in rats that were on an ad libitum feeding and

Figure 8.

In panel A are indicated the mean (±S.E.) spikes per second per minute for 12 SON cells recorded from water deprived rats (○—○), and, 7 SON cells recorded from rats on an ad libitum food and water schedule (●—●).

In panel B are indicated the mean (±S.E.) spikes per second per minute for 17 AH cells recorded from water deprived rats (○—○), and, 8 AH cells recorded from rats on an ad libitum food and water schedule (●—●).


SPIKES PER SECOND
10
8
6
4
2
DEP
SON
A
AL
14
12
10
8
6
DEP
AL
AH
B
1 2 3 4 5 6 7 8 9 10
MINUTES

drinking schedule (overall mean is 8.9 ± 0.2 spikes per second). The mean difference between those two groups is significant ($p<0.05$).

As is indicated in panel 8a, SON cells are apparently differentially affected by the water deprivation procedure. This might be expected on the basis of their presumed role in the elaboration of ADH in the dehydrated state, but more about this point later.

Because of the rather heterogeneous population of cells that are represented in panel 8b, it would be hazardous to make any statements about the effect of water deprivation on unit activity of any specific cell in the AH. But, generally, water deprivation apparently does result in significant increases in AH cellular activity.

Figure 9 shows the mean ($\pm$S.E.) activity in spikes per second for 49 min of cells in the AH and SON. The mean minute activity of the two SON cells recorded here is slightly slower than that reported for deprived rats in Figure 8a. However, the mean minute activity of the SON cells in Figure 9 is significantly faster than the cells recorded from in animals of the Ad Libitum Condition (cf. Figure 8a). But, most importantly, there are no dramatic shifts in activity of these cells during the 49 min recording session. Then, any changes that are seen following other experimental treatments, might be attributed to the effects of those manipulations.

Figure 9.

Indicated are the mean (±S.E.) spikes per second per minute for five AH cells (●—●) and two SON cells (○—○). These cells were recorded from rats that had been water deprived.

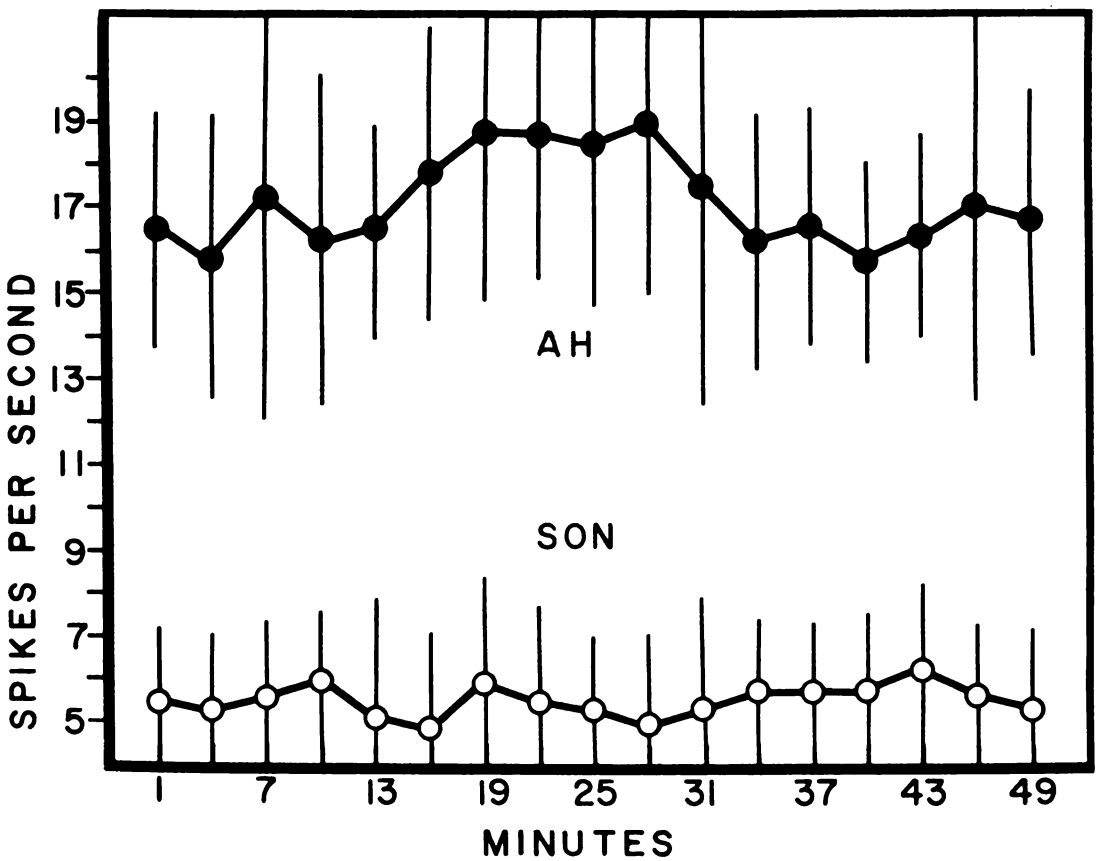

SPIKES PER SECOND
19
17
15
13
11
9
7
5
AH
SON
1
7
13
19
25
31
37
43
49
MINUTES

Figure 10a shows the mean (±S.E.) activity in spikes per second of 5 AH cells of rats that were subjected to the water load, then recorded from for 29 more minutes. The mean minute activity of these cells during baseline is 11.1±0.4 spikes per second. The mean minute activity of these cells during the water load period is 10.1±0.1 spikes per second. Taking into account the variability about these means, the activity during baseline is not different than that during the load period. However, each mean firing rate per minute during the load is below the mean rate per minute during the baseline. The probability of this occuring on the basis of chance is very small ($p<0.01$). Exactly why this occurs is unclear. It seems reasonable, however, that it is related to water entering the stomach.

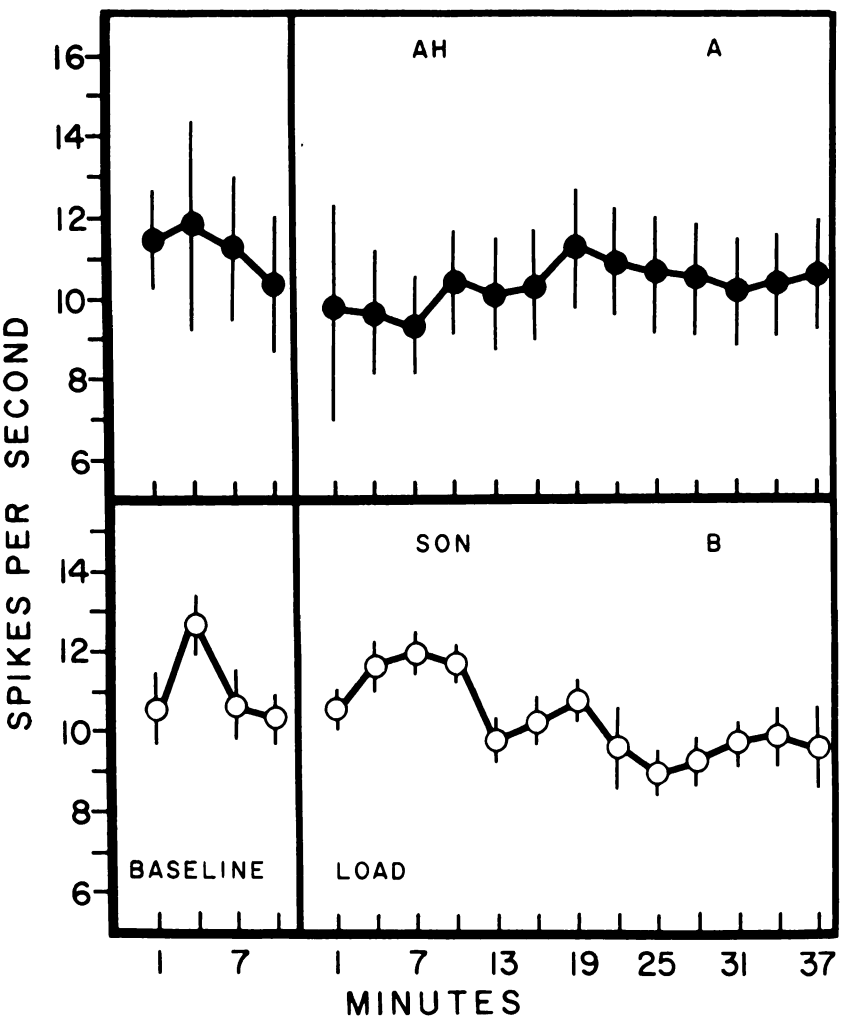
In experiment I, it was reported that P_{osm} significantly decreases by the 11th minute of effective absorption time. No change in AH mean minute firing rate is seen during that period following the water load. This is interpreted to mean that the rapid P_{osm} changes that occurred following the stomach load of water did not effect the firing rate of these AH cells.

In Figure 10b is indicated the mean (±S.E.) minute firing rate in spikes per second for three cells in the SON treated the same as those cells in Figure 10a. The mean minute firing rate of these cells during baseline is 10.9±0.6 spikes per second. Mean minute firing rate of these cells increases

Figure 10.

In panel A are indicated the mean (±S.E.) spikes per second for 15 AH cells in the Load Only Group as a function of minutes. The animals from which these cells were recorded had been water deprived and then received a 10 cm^3 gastric water load at the rate of 1 cm^3/min.

In panel B are indicated the mean (±S.E.) spikes per second for 4 SON cells. The animasl from which these cells were recorded were treated as those in panel A.


AH
A
SON
B
BASELINE
LOAD
SPIKES PER SECOND
16
14
12
10
8
6
14
12
10
8
6
1
7
1
7
13
19
25
31
37
MINUTES

slightly in the early portions of the load. By the thirteenth minute following the beginning of the load, mean minute firing rate is below that seen during baseline. For the remainder of the recording session, mean activity per minute of these cells never becomes as fast as it was during baseline. It is interesting that activity of these cells decreases below baseline by the 13th minute (which corresponds favorably with the time when P_{osm} decreases occur).

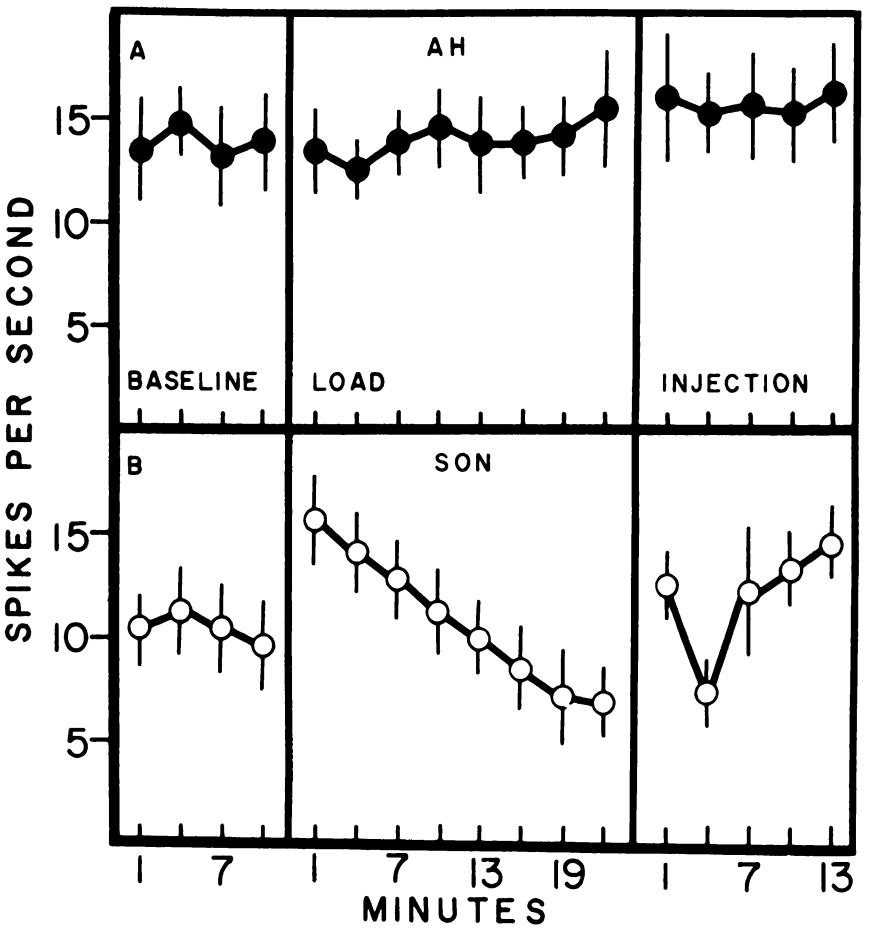
In Figure 11a is the mean ($\pm$S.E.) minute activity in spikes per second of 17 AH cells. Twelve minutes following the completion of the water load, a 1 cm^3 16% NaCl SC injection was administered. As was the case with the AH cells of the Load Only Group, the mean minute activity of these cells, during the load period, is below that seen during baseline. Because this decrease occurs within the first minute, it seems reasonable that this depression in frequency is the result of water entering the stomach. Most important here, however, is the fact no significant change in mean activity which might be attributed to changes in P_{osm}, occurred either during the load, or following the injection.

In contrast to this is the mean frequency per minute of 12 SON cells plotted in panel 11b. These cells were treated as those in panel 11a were. There is an initial increase in activity which gradually decreases throughout the remainder of the load procedure. The animals from which these cells were recorded were given (as those in panel 11a

Figure 11.

In panel A are indicated the mean (±S.E.) spikes per second for 17 AH cells as a function of minutes. These cells were recorded from animals that had been water deprived. As indicated, they received a 10cm^3 gastric load of water and a 1 cm^3 16% NaCl SC injection.

In panel B are indicated the mean (±S.E.) spikes per second for 12 SON cells. The animals from which these cells were recorded were treated as those in panel A.


A
AH
B
SON
BASELINE
LOAD
INJECTION
SPIKES PER SECOND
15
10
5
15
10
5
1
7
1
7
13
19
1
7
13
MINUTES

were) a hypertonic saline injection. Immediately following the injection, there was a significant increase in mean minute firing rate of these cells. By four minutes, this increase had been attenuated to some degree. But, by seven minutes, the cells' activity had again increased significantly.

Figure 12 indicates the median (Q_1 and Q_3) percent change from baseline for the SON cells depicted in 11b, which showed a significant change in mean minute firing rate. All but two of these cells showed a median percent increase in the first minute. This trend continued until minute seven, by which time activity of the cells was actually decreasing. By 13 min, all but one of the cells was firing below baseline. On the basis of random distribution, the changes in firing rates seen at 13 min would be expected only 5% of the time. Throughout the remaining portion of the session, the activity of the cells tended to decrease. It is interesting, of course, that by thirteen minutes P_{osm} had been shown to have significantly decreased.

In the right had side of Figure 12 are the median percent changes that occur following the injection: (a) the initial increase immediately following the injection, (b) the decrease by four minutes, and, then, (c) the dramatic increase by seven minutes. As pointed out earlier, it has been shown that there is a significant increase in P_{osm} following such an injection.

In Figure 13 are indicated the mean ($\pm$S.E.) firing

Figure 12.

Indicated are the median (Q_1 and Q_3) percent changes of 9 of the cells shown in Figure 11. These 9 cells showed a significant decrease in firing rate during the load period. Each cell's percent change during the load was calculated on the basis of each cell's respective mean baseline firing rate. Percent change following the injection was calculated from each cells mean minute rate during the last minute of the load period.

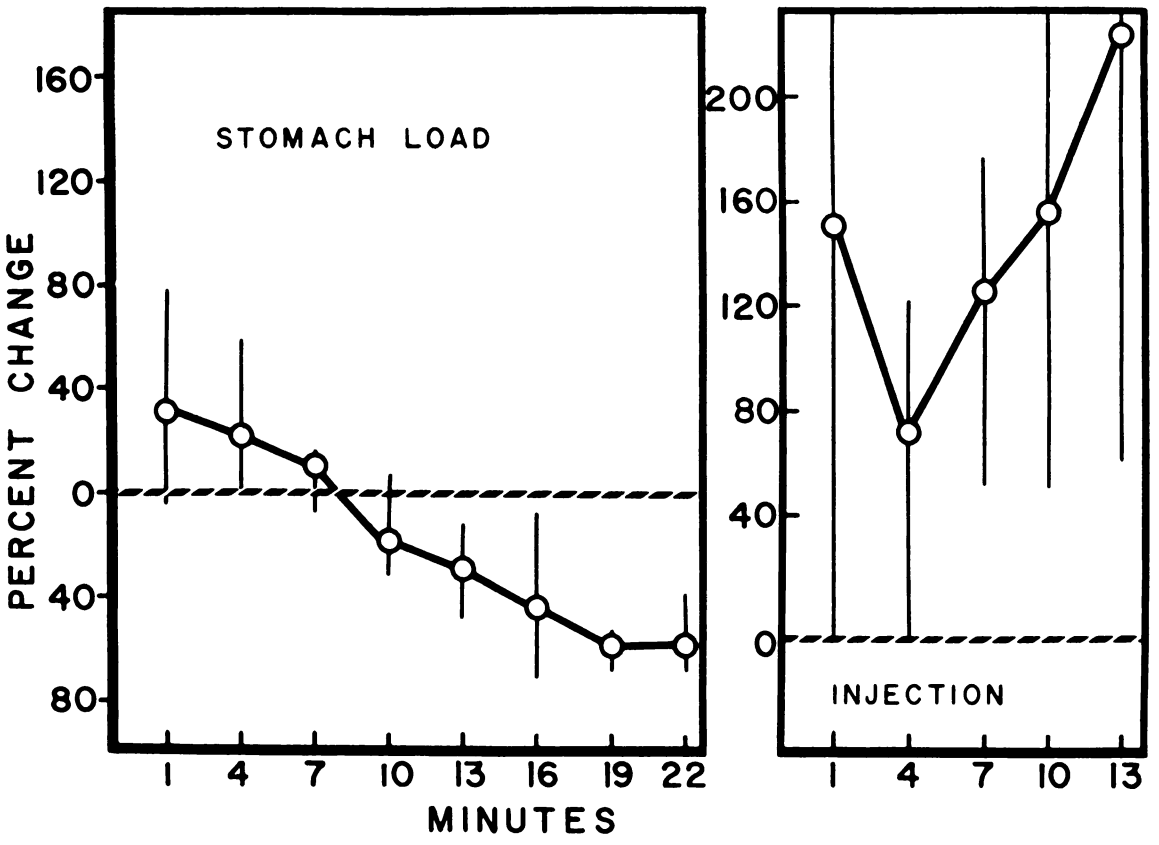
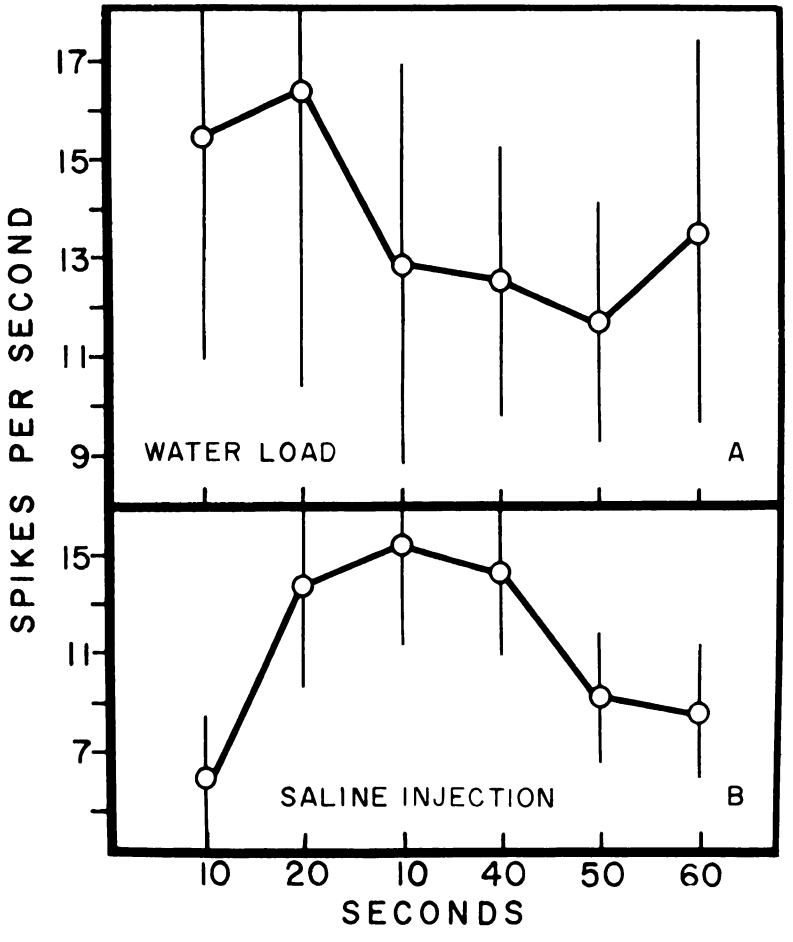

STOMACH LOAD
INJECTION
PERCENT CHANGE
MINUTES
160
120
80
40
0
40
80
200
160
120
80
40
0
1
4
7
10
13
16
19
22
1
4
7
10
13

Figure 13.

Indicated are the mean (±S.E.) spikes per second per ten second time bins of the first minute following the beginning of the water load (panel A) and injection (panel B). These are the same cells that are depicted in Figure 11.


SPIKES PER SECOND
17
15
13
11
9
WATER LOAD
A
15
11
7
SALINE INJECTION
B
10
20
10
40
50
60
SECONDS

rates of SON cells immediately following the beginning of the stomach load (panel a) and the injection (panel b). In Figure 14 are the mean (±S.E.) percent changes during these same periods. The change in firing rate following the beginning of the load is more rapid, and transient, than that following the injection. The changes in SON unit activity that occur during these periods will be discussed in more detail later.

Histology

Figure 15 shows portions of diagrams of a rat brain at de Groot planes 6.6-8.3 AP. The black circles indicate location of marking lesions which indicate that the cell being recorded from was probably a SON neuron. The open circles indicate marking lesions at the various other recording sites. All animals from which these cells had been recorded from were of the Ad Libitum Group.

Figure 16 indicates location of marking lesions in diagrams of a rat brain at de Groot planes 7.0-8.2 AP. Black circles again indicate location of recording sites that are probably within the SON. Open circles indicate other recording sites. Animals from which these cells had been recorded from were in the Deprived Group.

Figure 17 indicates diagrams of a rat brain at de Groot planes 6.6-7.8 AP. Black circles indicate marking lesions of recroding sites probably within the SON. Open circles indicate other recording sites. All animals from which these cells were recorded from were in the Load Only Group.

Figure 14.

Indicated are the mean (±S.E.) percent changes in firing rate per ten seconds for the first minute following the beginning of the water load (panel A) and injection (panel B). These are the same cells depicted in Figure 13.

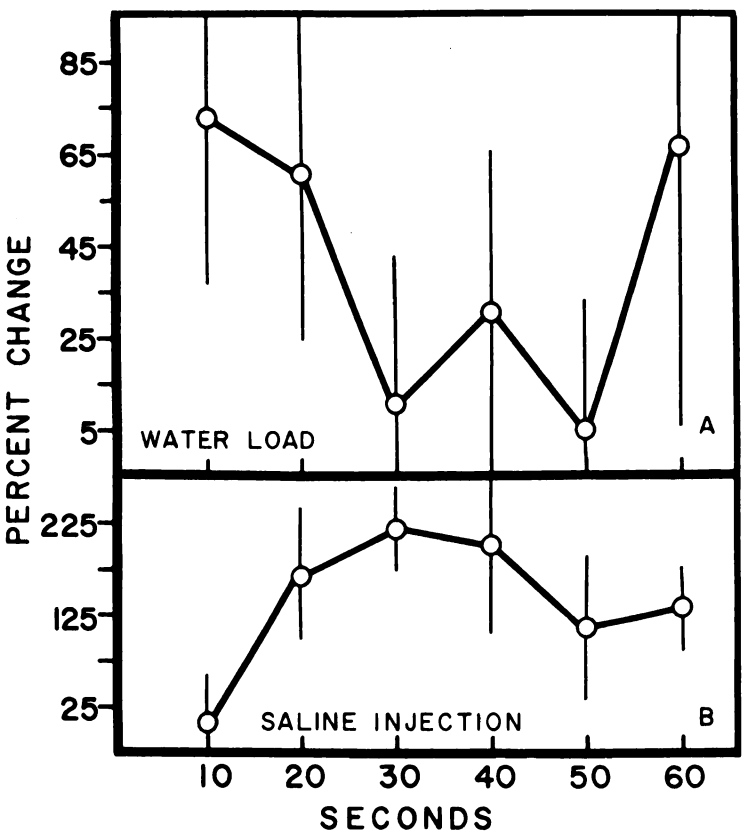

85
65
45
25
5
WATER LOAD
A
225
125
25
SALINE INJECTION
B
PERCENT CHANGE
10
20
30
40
50
60
SECONDS

Figure 15.

Indicated are portions of diagrams of a rat brain at de Groot planes 6.6-8.2 AP. The black circles indicate location of marking lesions which indicate that the cell being recorded from was probably within the SON. The open circles indicate marking lesions at various other recording sites. All animals from which these cells had been recorded were of the Ad Libitum Group.

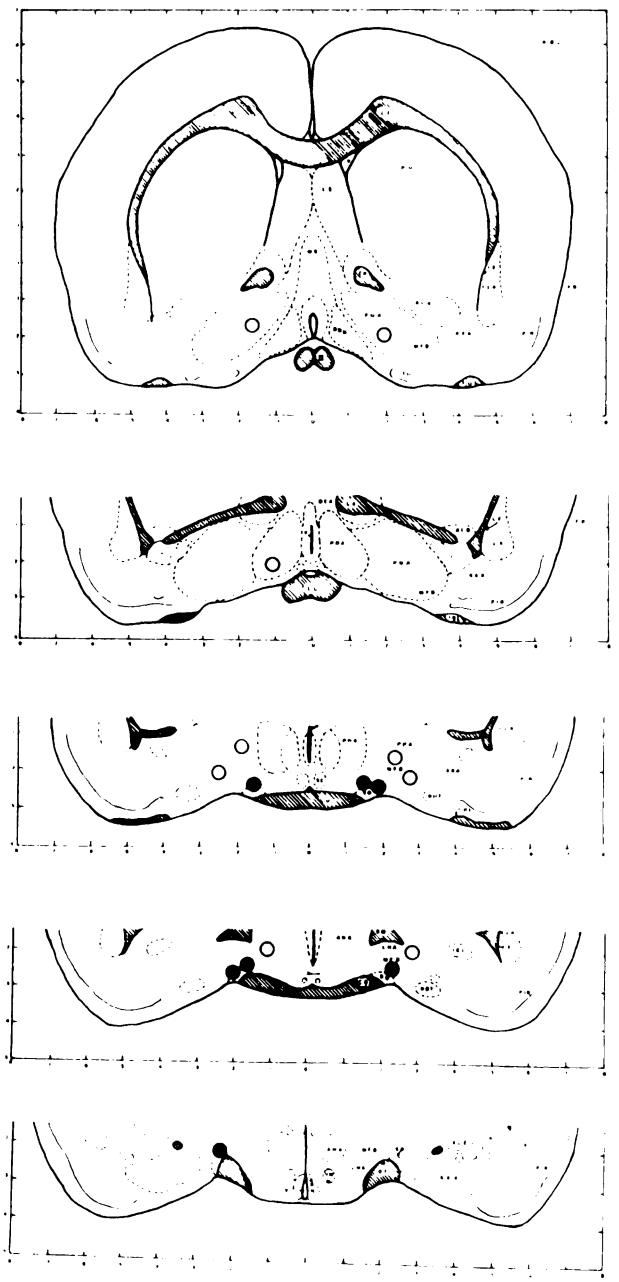


Figure 16.

Indicated are the location of marking lesions in diagrams of a rat brain at de Groot planes t.0-8.2 AP. Black circles indicate location of recording sites that are probably within the SON. Open circles indicate other recording sites. Animals from which these cells had been recorded were in the Deprived Group.

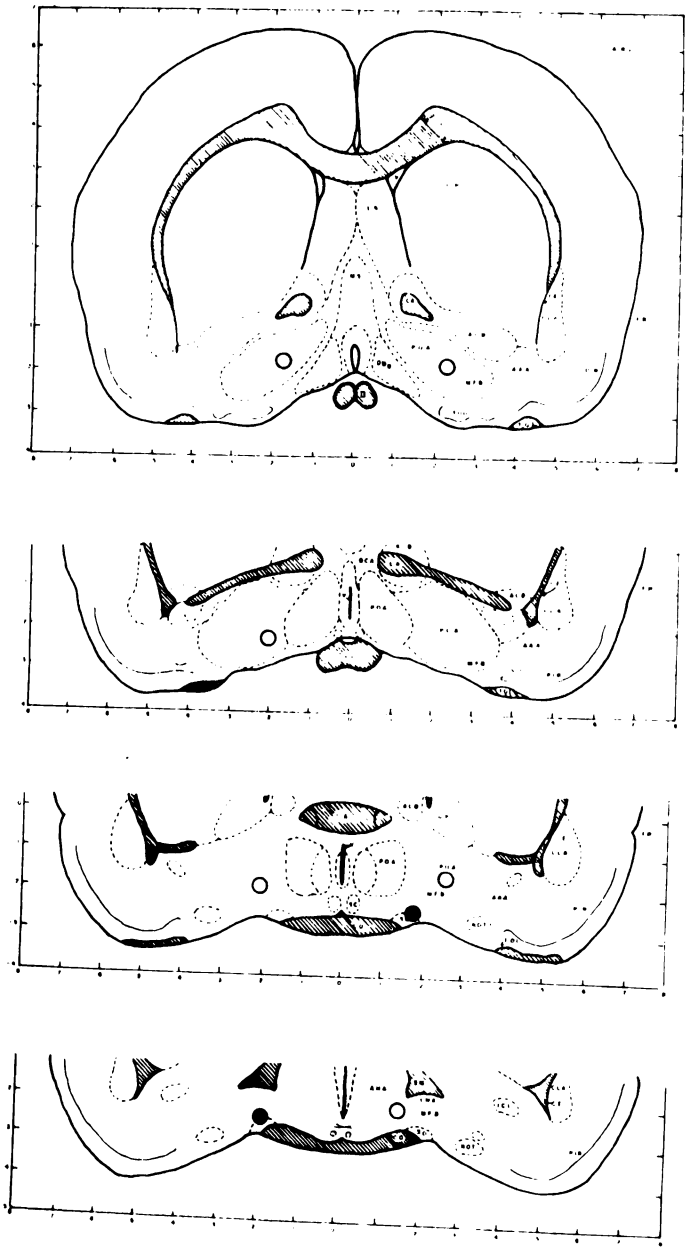
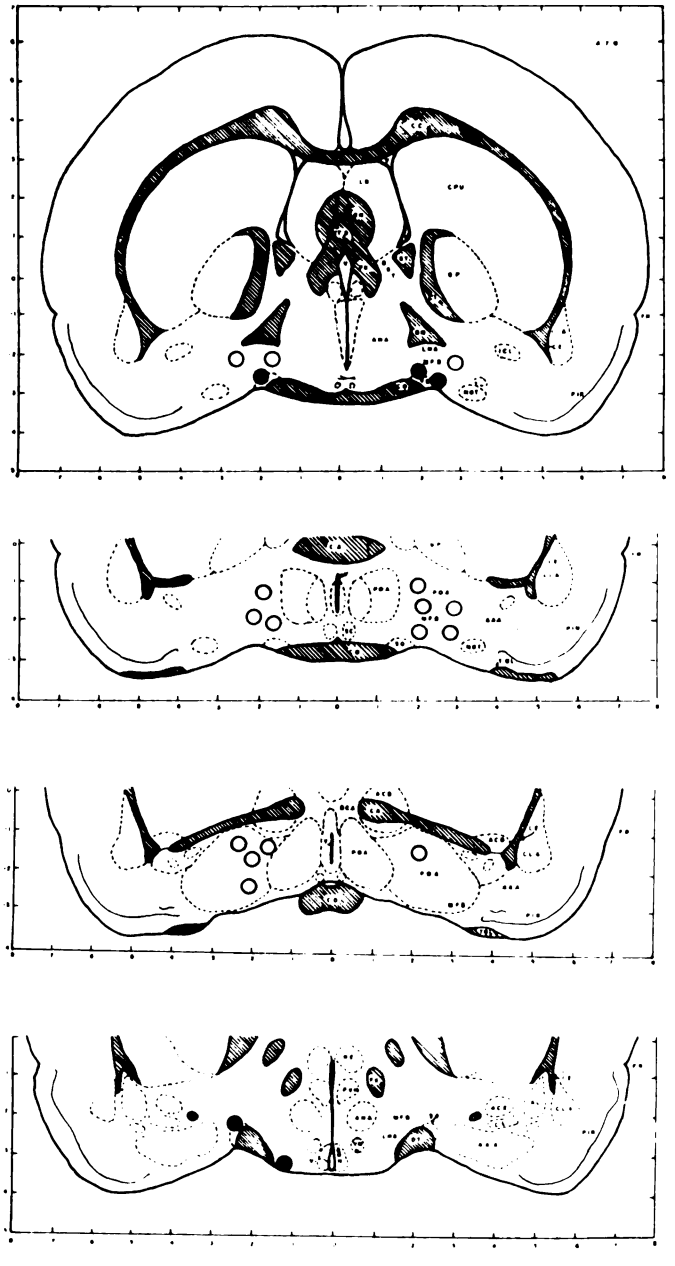


Figure 17.

Indicated are diagrams of a rat brain at de Goot planes 6.6-7.8 AP. Black circles indicate marking lesions of recording sites probably within the SON. Open circles indicate other recording sites. All animals from which these cells were recorded were in the Load Only Group.



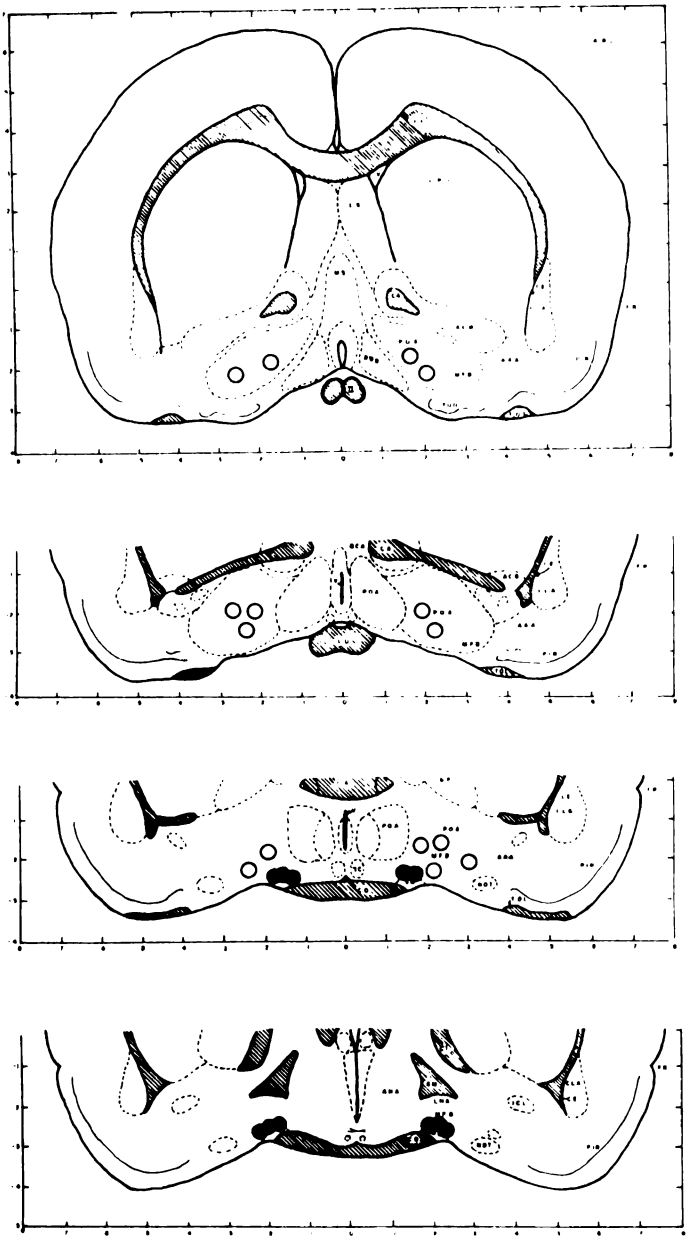
In the bottom section is indicated a recording site probably within the tuberal portion of the SON. Interesting is that this cell responded as did the anterior SON cells.

Figure 19 indicates diagrams of a rat brain cut at de Groot planes 7.0-8.2 AP. Black circles indicate probable SON recording sites. Open circles indicate neurons which were recorded from in areas outside the SON. Animals from which these cells were recorded from were in the Load Injected Group.

In Appendix A are photomicrographs of some of these recording sites.

Figure 18.

Indicated are diagrams of a rat brain cut at de Groot planes 7.0-8.2 AP. Black circles indicate probable SON recording sites. Open circles indicate neurons which were recorded from in areas outside the SON. Animals from which these cells were recorded from were in the Load Injected Group.



GENERAL DISCUSSION

In Experiment I, changes in P_{osm}, P_v, water absorption, and heart rate were measured in water deprived animals which had received a stomach load of water. In addition, these animals were anesthetized and prepared for unit recording. As was pointed out, the time course and magnitude of changes that occur, during and after the water load, in P_{osm} and water absorption are comparable to those that occur in awake, drinking rats under similar deprivation conditions. In Experiment II, unit activity in the anterior hypothalamus of rats (under conditions similar to the first study) was recorded.

Unit activity changes in response to water loads

SON activity. Mean minute firing rates of SON cells are significantly faster in a deprived animal than in one given free access to food and water. This was also true with other AH cells. Cells within the SON are extremely homogeneous in respect to their cellular morphology (Rechardt, 1969). On the basis of this, it seems reasonable that the difference in firing rates is not the result of a sampling bias, but actually reflects the fact that increases in tonicity of body fluids result in faster firing rates of SON cells.

It has been well documented that the production of an action potential by a neuron requires metabolic energy (Ochs,

1965). The notion that SON cells increase their metabolic activity during periods of water stress receives some support from histochemical (Watt, 1970) and anatomical studies (Hatton, Johnson, and Malatesta, 1971). And the increases in metabolic activity of SON cells, indicated by these studies might be the result, in part, of the increased firing rate of these cells. The increased protein synthesis indicated by the studies cited above might also reflect the increased production of ADH which is a polypeptide hormone.

The initial increases in unit activity following the beginning of the stomach load were unexpected. Because of the short latency with which the increase in firing rates occur, it is likely that this response is mediated through gastric stretch receptor stimulation. Activity of chemoreceptors in the stomach should not be ruled out. However, apparently no evidence has been gathered for such receptors responding to water (Paintal, 1963).

As pointed out earlier, there is abundant evidence that baroreceptors in the stomach respond to passive distention. There is, however, apparently no evidence on the relationship of these baroreceptors to SON unit activity. It has been shown, however, that palpation of the surgically exposed stomach will result in dramatic increases in ADH titers in the blood (Moran and Zimmermann, 1969). In any case, whether this effect might be mediated through vagal or sympathetic afferents has apparently not been determined.

Because gastric mechanoreceptors have different thresholds (Paintal, 1963), it would not be unlikely that they are mediating (in part, at least) the early changes in SON activity. In any case, their influence on the SON is probably rather transitory. By 7 min, following the beginning of the stomach load, the frequency of firing of SON units has decreased to near baseline levels (See Figure 20), but, complete gastric filling had not yet taken place (cf. Table 1).

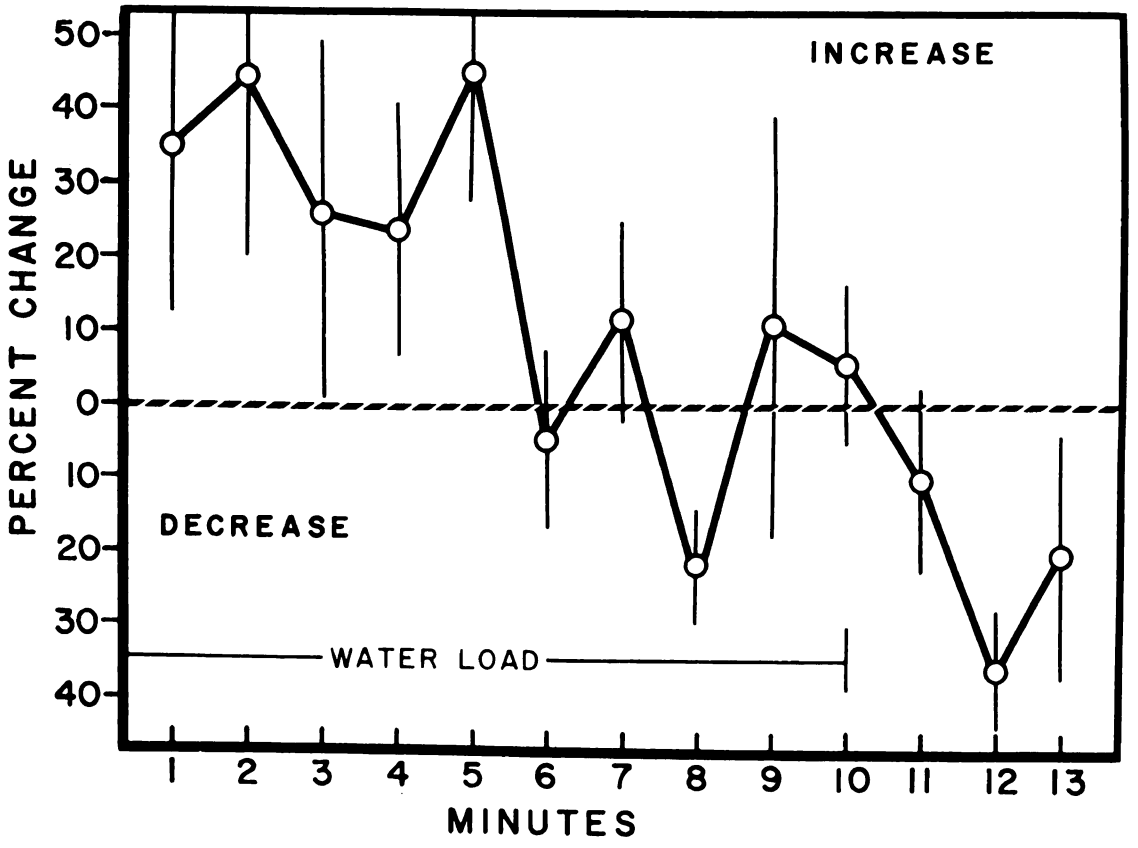
It should be appreciated, though, that the initial increase in firing rates of SON cells, following the beginning of the load, occurred when only 0.25 cm^3 of water had entered the stomach.

Following the first phasic increase in unit activity following the beginning of the water load, there is no significant change in mean rate of firing of SON cells until 12 minutes. By this time most cells are firing below baseline. Then generally, as seen in Figure 11b, the activity of these cells tends to continue to decrease. Interesting, of course, is the fact that by 11 min of effective absorption time there is a significant 3% decrease in P_{osm}. It would seem reasonable that the decrease in P_{osm} is at least contributing to the decrease in SON activity.

P_v on the other hand, as indicated by protein concentration, does not shift until approximately 18 min, So, it seems unlikely that P_v, <u>per se</u>, is bringing about the changes in activity that are seen at 12 min.

Figure 19.

Indicated are the mean (±S.E.) percent changes in firing rate for 13 minutes following the beginning of the water load. These are the same cells that are depicted in Figure 11b.


INCREASE
DECREASE
WATER LOAD
PERCENT CHANGE
50
40
30
20
10
0
10
20
30
40
1
2
3
4
5
6
7
8
9
10
11
12
13
MINUTES

The difficulty in attributing the decrease in SON activity to P_{osm} decreases, or to changes in stomach fluid volume should be appreciated. Because changes in P_{osm} and gastric fluid volume are occurring at approximately the same time, the role that each plays in the decrease of SON activity is unclear. But, because unit activity in the SON returns to baseline levels by 7 min after the load starts, it seems reasonable that the change in rate seen at 12 min is the result of P_{osm} decreases. If, then, the decrease in SON activity below baseline rates can be attributed to decreases in P_{osm} changes, then the magnitude of the decrease in P_{osm} (3%) during this period might be considered to be a plasma osmotic pressure threshold.

AH activity. Following five days on a water deprivation schedule, mean firing rates per minute of AH cells were significantly faster than that seen in the Ad Libitum Group. But, in contrast to this, and as indicated in Figures 10 and 11, AH cells generally show no dramatic changes in mean firing rates per minute as the result of fairly rapid alterations in the tonicity of the plasma. There were, however, three cells in the Preoptic Area (POA) which did show a change in firing in response to the water load. Once the load began, there was a slight significant decrease in the rate of these cells, then a gradual increase. However, no other cellular activity changes were recorded. In contrast to this, other investigators have found decreases in POA activity in response

to either gastric (Thorton, 1969) or IC water loads (Brooks, Koizumi, and Zebalios, 1966; Ishikawa, Koizumi, and Brooks, 1966b; Joynt, 1964).

The basis for the discrepancy between this study and those which have reported such findings is unclear. Most investigators in the area do not report their exact recording sites. So any comparison of exact recording sites and changes in cellular activity is impossible. The studies that have reported histological findings have used goats and cats. But, because of the species differences, anatomical comparisons between these is made difficult. In addition, these investigators did not monitor the osmotic state of their animals. As a result, it is difficult to make any quantitative statements about the change in blood tonicity which their manipulations produced.

The lack of evidence of AH cells responding to decreases in tonicity of the blood in the present study should not be taken to mean that no such cells exist. Because of the relatively few number of cells investigated in the present study, it would not be at all suprising if some of these cells were not isolated.

There is also a significant procedural difference between other recording studies investigating neural osmosensitivity and the present study. Only one other study apparently has employed the procedure of recording during and after a stomach load of water (Thornton, 1969). In this

study, 3 cells in the POA were found to decrease frequency to the load. It is not clear, however, whether or not these responses were to decreases in tonicity of the body fluids.

Changes in unit activity in response to hypertonic injections

SON activity. Cells in the SON showed a clear and consistent reaction to the hypertonic injection. In the first minute following a similar subcutaneous injection, most awake rats exhibit a marked pain response (ie., squealing, biting at the injection site, etc.). During this time period, SON cells showed a marked significant increase in firing rate. During the time period when the pain reaction is subsiding in awake animals (usually within a minute or so) the activity of these cells has markedly decreased. Interesting is that nocioceptive stimuli have been shown to result in a marked antidiuresis and release of ADH into the blood (Verney, 1947; Tata and Buzalkov, 1966; Moran and Zimmermann, 1969). Because of the short latency and transitory nature of the changes in firing rates of these cells (following the injection), it seems reasonable to assume that these changes are the result of the saline injection acting upon peripheral sensory receptors.

The central pathways which might mediate this response are quite obscure. Mills and Wang (1964) have shown, though, that ADH is released in response to peripheral pain; and, that this response is probably not mediated through the

medial lemniscal system. Their evidence further indicates that nociceptive stimuli (radial nerve stimulation) resulting in ADH release probably pass by way of the extralemniscal pathway to the centre median nucleus of the thalamus. However, the pathways that might act upon the SON directly have not been established.

The transitory increase in firing rates of these cells after the injection is followed by a significant and maintained (throughout the recording session) increase in activity (Figure 12). In awake rats under similar hydration conditions, it has been shown that there are usually significant 2-3% increases in P_{osm} by 6 min following such an injection (Hatton and Almli, 1969). Coincident with the increase in P_{osm}, the rats initiate drinking.

It seems reasonable to assume the increase in SON activity seen by the 7th minute is the result of shifts in osmotic pressure of body fluids. Because the changes in firing rates are also temporally coincident with a 2-4% increase in P_{osm}, it might be assumed that this change represents a threshold for firing rates of these cells.

AH unit activity. As indicated earlier, no mean minute changes in activity of AH cells was observed following the SC injection of saline. However, other investigators have found cells in these general areas that have responded (increased frequency) following intracarotid (IC) injections of hypertonic solutions. Here, again, there is a difficulty in

determining the apparent discrepancy. There is apparently only one other study which reported the use of SC NaCl injections while recording from the hypothalamus (Hatton and Almli, 1970). These investigators reported one cell in the posterior hypothalamus that increased its firing rate following the injection. Despite this, no clear statements should be made on the basis of the present data, with regard to the presence (or absence) of AH cells sensitive to rapid increases in tonicity of body fluids.

Review of changes in unit activity in response to water loads and saline injections

In general, the following changes were observed in the animals studied: (a) As the load began, there was a short latency hypothalamic and cardiac response; the former being an increase in SON unit activity, the latter being a bradycardia. This response is probably mediated via gastric stretch receptors. As pointed out, if this is true, these receptors are responding to 0.25 cm^3 entering the stomach. As the water load proceeds, the stomach continues to fill until approximately 11 min of effective absorption time. Until this time, a clear bradycardia is seen. As the contents in the stomach diminish, heart rate returns to near baseline levels. On the other hand, firing rate of SON cells returns to near baseline levels by 7 min.

(b) By 12 min post-load, these SON cells decreased their firing rate significantly below baseline. This period also

corresponds to the first significant decrease in P_{osm}. Because the initial neural response to the load had subsided by 7 min, the decrease at 12 min might be the result of the change in P_{osm}. Under these conditions, it would not be expected that the change in SON activity were due to P_v changes because no significant change in P_v was seen until approximately 18 min of effective absorption time.

If the decrease in unit activity below baseline was the result of P_{osm} shifts, then, the 3% decrease in P_{osm} seen at this time could be considered a threshold for decreased firing frequency.

(c) Approximately 25 min after the beginning of the load, a 1 cm^3 16% NaCl injection was given to some animals. Following the injections, there was a tachycardia and a transient increase in rate of firing of SON cells. By 4 min, this increase had attenuated. But, by 7 min, the cells' activity tended to again increase, coincident with a 2-4% increase in P_{osm}. Also during this period, heart rate again tended to increase.

It seems reasonable that this two phase change in unit activity is the result : (1) of peripheral stimuli (resulting from the injection) causing the initial transient change in firing rate, and, (2) of a change in tonicity of body fluids resulting in the second more sustained rise in firing rate.

While it seems reasonable to attribute some of the changes

in activity of SON cells to changes in tonicity of the body fluids, it is difficult, on the basis of the present data, to determine the influence on unit activity of peripheral stimuli versus that of P_{osm}.

Also on the basis of the data, it is impossible to determine the actual nature of the osmotic influence on activity of the SON cells. It could be that changes in EOP of the extracellular fluid are directly affecting unit activity in the SON. On the other hand, EOP changes could be detected by neural elements located in other areas of the central or peripheral nervous system. There is no sound evidence that any given area in the CNS affects SON unit activity (in response to osmotic pressure changes). There is, however, evidence that osmoreceptors in the hepatic circulation can alter SON unit activity (Metveeva and Osipovich, 1970). And, as has been pointed out earlier, changes in concentration of the hepatic circulation can bring about changes in diuresis.

Of course, because blood pressure was not recorded in the present studies, its possible influence on SON activity cannot be completely assessed.

Relationship of SON activity to drinking behavior.

Hatton and Bennett (1970) reported that significant decreases in P_{osm} may result in the cessation of drinking. These investigators also reported that water deprived rats, when given access to water will drink for approximately 10 min, by which time 3 cm^3 of water has been absorbed and

P_{osm} has decreased 1-2%. In the present study, similar changes in P_{osm} and water absorption were reported. Also, during this time period SON unit activity decreases.

The temporal relationship between SON unit activity and the cessation of drinking is interesting. This correlation, of course, should not necessarily be taken to mean that the SON plays a role in bringing about the cessation of drinking.

It is well documented that interruption of the supra-optico-hypophyseal system results in a syndrome called diabetes insipidus (Fisher, Ingram, and Ranson, 1938); when, following such lesions, the observed polydipsia is secondary to the resulting polyuria. However, in some cases the polydipsia is thought to be primary. This is true because (a) in some cases the polydipsia actually proceeds the polyuria (Smith and McCann, 1962 a and b), and, (b) the polydipsia occurs in nephrectomized animals (Rolls, 1970). If the SON plays no role in the initiation or cessation of drinking, then drinking thresholds following SON lesions should not be disrupted. However, this has not apparently been measured.

As stated earlier (p. 7), it was expected that SON unit activity would be closely correlated with changes in drinking behavior. This was expected not because the SON necessarily plays a role in drinking behavior, but because similar changes in P_{osm} result in changes in both the release of ADH and drinking.

Stimulus convergence

In addition to changes in P_{osm} and blood volume, it has been shown that peripheral stimuli (like gastric palpation and pain) will result in the increased release of ADH. There has been, however, apparently no reported evidence that these different stimuli act on the same SON neurons.

In the present study, the data indicated that changes in gastric filling, stimuli that can elicit pain, and changes in P_{osm} can apparently alter the firing rates of single SON neurons. If this data is supported, it is, then, the first reported evidence that the stimuli which are potent releasers of ADH converge on single SON neurons.

Heart rate

There was a short latency bradycardia following the beginning of the water load in both experiments. This bradycardia is maintained as the stomach fills, but, then, gradually diminishes. This presumably occurs as the result of gastric stretch receptors initially being activated, adapting, and then diminishing in activity as the amount of fluid in the lumen of the stomach decreases. It should be pointed out that if activation of gastric mechanoreceptors mediates the bradycardia, they do so, initially, in response to 0.25 cm^3 of water entering the stomach.

The tachycardia seen following the injection is a little more difficult to interpret. It develops rather gradually

over the first 3 min, by which time it stabilizes. But, by 7 min, it begins to increase and then plateaus at a higher level by 10 min (cf. Figure 5 and 7). There are apparently no comparable data on which to assume whether these two phases in the changes in heart rate are the result of two processes or not.

It has been well documented that intravascular infusions of hypertonic (or isotonic) solutions will result in a tachycardia (Bard, 1968). The second increase in heart rate is, however, coincident with an increase in P_{osm}, presumably the result of the hypertonic saline injection.

Summary

SON unit activity was found to be significantly faster in the water deprived rat, as compared to rats on an ad libitum food and water schedule.

A transient, short latency increase in SON unit activity was observed in response to initial filling of the stomach with water. Following this, when P_{osm} began to significantly decrease, unit activity in the SON also decreased significantly below baseline levels.

In response to a SC hypertonic saline injection, a short latency, transient increase in SON unit activity was seen. This increase ocurred during a time period when many awake rats, treated in a similar fashion, exhibit a marked pain reaction. By seven minutes post-injection, when P_{osm}

has been shown to significantly increase. SON unit activity became significnatly faster.

AH cells, treated in the same fashion, did not show similar changes in unit activity in response to rapid changes in P_{osm}. This is true despite the fact that following a period of water deprivation, unit activity in the AH was significantly faster than in animals on an ad libitum feeding and drinking schedule.

If (a) the initial short latency, transient changes in SON unit activity, following the water load or injection, can be attributed to peripheral stimuli, and, if (b) the secondary changes in unit activity can be attributed to shifts in P_{osm}, then this is apparently the first reported evidence for convergence on SON sells of stimuli that elicit the release of ADH.

Addendum

In the Load Only and Load Injection Groups, 16 SON cells were recorded from. Of these, 12 showed transient increases in firing rates during the first 10 seconds of the water load. The other four cells showed no increases in firing rate until approximately the second minute of the load.

It is unclear whether the increase in firing rate of the 12 SON cells was mediated neurally or by changes in blood pressure. But, on the basis of their reaction to water initially entering the stomach, there seems to be two populations of SON cells, one set that shows a rather rapid change, and, one a much slower.

LIST OF REFERENCES

LIST OF REFERENCES

Bard, P., Control of systemic circulation, In Medical Physiology, 12th edition, St. Louis: C.V. Mosby, Inc., 1968, pp. 178-208.

Barker, J.L., Crayton, J. W., and Nicoll, R.A., Supraoptic neurosecretory cells: Autonomic modulation, Science, 1971, 171:206-207.

Beleslin, D., Bisset, G.W., Halder, J., and Polak, R.L., The release of vasopressin without oxytocin in response to haemorrhage, Proceedings of the Royal Society, London, Series B, 1967, 166:443-456.

Bennett, C.T. Water absorption and changes in plasma osmotic pressure as determinants of the satiation of thirst, M.A. Thesis, Michigan State University, 1969.

Bland, J.H., Clinical Metabolism of Body Water and Electrolytes, Philadelphia: W.B. Saunders, Co., 1963.

Brooks, C.McC., Koizumi, K., and Zebalios, G.A., A study of factors controlling activity of neurons within the paraventricular, supraoptic, and ventromedian nucei of the hypothalamus. Acta Physiologica Latino Americana, 1966, 16:83-118.

Brooks, C.McC., Ushyiyama, J. and Lange, G., Reactions of neurons in or near the supraoptic nucleus. American Journal of Physiology, 1962, 202:487-490.

Chew, R.M., Water metabolism of mammals, In Physiological Mammology, Mayer, W.V. and Van Gelder, R.G., (Eds.), New York: Academic Press, 1965, 44-197.

Cross, B.A. and Green J.D., Activity of single neurons in the hypothalamus: Effect of osmotic stimuli, Journal of Physiology, London, 1959, 148:554-569.

Cross, B.A. and Kitay, J.I., Unit activity in diencephalic islands, Experimental Neurology, 1967, 19:316-330.

Cross, B.A. and Silver, I.A., Electrophysiological studies on the hypothalamus, British Medical Bulletin, 1966, 254-260.

Dyball, R.E.J., The time relation between the secretory and electrical responses of supraoptic neurones to osmotic stimuli, Journal of Phsyiology, London, 1969, 203:67-68P.

Dyball, R.E.J. and Koizumi, K., Electrical activity in the SON and PVN associated with neural hypophyseal release. Journal of Physiology, London, 1969, 201:711-722.

Fisher, C., Ingram, W.R., and Ranson, S.W., Diabetes insipidus and the neuro-hormonal control of water balance: a contribution to the structure and function of the hypothalamico-hypophyseal system. Ann Arbor: Edward Bros.,1938.

Haberich, F.J., Osmoreception in the portal circulation, Federation Proceedings, 1968, 27:1137-1141.

Haberich, F.J., Aziz, O., and Nowacki, P.E., Uber einen osmoreceptorish tatigen mechanismus in der leber, Pflugers Archiv, 1965, 285:73-89.

Harris, G.W., The innervation and actions of the neurohypophysis; an investigation using the method of remote-control stimulation, Philosophical Transactions, 1947, B232:385-441.

Hatton, G.I. and Almli, C.R., Plasma osmotic pressure and volume changes as determinants of drinking thresholds, Physiology and Behavior, 1969, 4:207-214.

Hatton, G.I. and Almli, C.R., Localization of osmotically sensitive cells functioning in the initiation of drinking behavior. Third Annual Winter Conference on Brain Research, Aspen, Colo., 1970.

Hatton, G.I. and Bennett, C.T., Satiation of thirst and termination of drinking: Roles of plasma osmolality and absorption, Physiology and Behavior, 1970, 5:479-487.

Hatton, G.I., Johnson, J.I., and Malatesta, C., Supraoptic nuclei of rodents adapted for mesic and xeric environments: numbers of cells, multiple nucleoli and their distribution. Submitted for publication.

Haymaker, W., Blood supply of the human hypothalamus, In The Hypothalamus, Haymaker, W., Anderson, E., and Nauta, W.J.H., (Eds.), New York: C.C. Thomas, 1969, pp.210-218.

Howe, A. and Jewell, P.A., Effects of water deprivation upon the neurosecretory material of the desert rat (Meriones meriones) compared with the laboratory rat, Journal of Endocrinology, 1959, 18:118-124.

Ishikawa, T., Koizumi, K., and Brooks, McC.,Electrical activity recorded from the pituitary stalk of the cat. American Journal of Physiology, 1966, 210:427-431.

Ishikawa, T., Koizumi, K., and Brooks, McC., Activity of NSO neurons of the hypothalamus, Neurology, 1966, 16: 101-102.

Joynt, R.J., Functional significance of osmosensitive units in the anterior hypothalamus, Neurology, 1964, 14:584-590.

Koizumi, K., Ishikawa, T., and Brooks, McC., Control of activity of neurons in the supraoptic nucleus. Journal of Physiology, London, 1964, 27:878-892.

Lydtin, H., Untersuchungen uber mechanismen der osmo- und volumenregulation. II. Untersuchungen uber den einfluss intrvenos, intraportal and oral zugefuhrter hypotoner kochsalslosungen auf die diurese des hundes, Zeitshrift fur Gesamte Experimentelle Medezin, 1969, 149:193-210.

Metveeva, M.B. and Osipovich, N.V., Unit activity in supra-optic and paraventricular nuclei during stimulation of the osmoreceptive zone of the liver in cats. Fiziologicheskii Zhurnal S.S.S.R. Imeni I.M. Sechanov, 1970, 56:324-331. As translated in Neuroscience Translations, 1970, 14:26-32.

Mills, E. and Wang, S.C., Liberation of antidiuretic hormone: Location of ascending pathways. American Journal of Physiology, 1964, 207:1399-1404.

Moran, W.H. and Zimmermann, B., Mechanisms of antidiuretic hormone (ADH) control of importance to the surgical patient, Surgery, 1967, 62:639-644.

Ochs, S., Elements of Neurophysiology, New York: J. Wiley and Sons, Inc., 1965.

O'Kelly, L.I., Hatton, G.I., Tucker, L., and Westall, D., Water regulation in the rat: Heart rate as a function of hydration, anesthesia, and association with reinforcement, Journal of Comparative and Physiological Psychology, 1965, 59:159-165.

Paintal A.S., A study of gastric stretch receptors. Their role in the peripheral mechanisms of satiation of hunger and thirst, Journal of Physiology, London, 1954, 126:225-270.

Paintal, A.S., Vagal afferent fiber, Ergibnesse der Physiologie 1963, 52:76-156.

Pickford, M., Neurohypophysis-antidiuretic (vasopressor) and oxytocic hormones, In The Hypothalamus, Haymaker, W., Anderson, E., Nauta, W.J.H., (Eds.), New York: C.C. Thomas, 1969, pp. 463-505.

Pitts, R.F., Physiology of the Kidney and Body Fluids, Chicago: Medical Yearbook Publishers, Inc., 1968.

Rechardt, L., Electron microscopic and histochemical observations on the supraoptic nucleus of normal and dehydrated rats. Acta Physiologica Scandanavia, Supplement, 329, 1969.

Saito, T., Yoshida, S., and Nakao, K., Release of ADH from neurohypophysis in response to hemorrhage and infusion of hypertonic saline in dogs. Endocrinology, 1969, 85:72-78.

Sarnoff, D. and Yamada, K., Evidence for reflex control of arterial pressure from abdominal receptors with special references to the pancrease, Circulation Research, 1959, 7:325-335.

Share, L., Control of plasma ADH titer in hemorrhage: Role of atrial and artrial receptors, American Journal of Physiology, 1968, 215:1384-1389.

Suda, I., Koizumi, K., and Brooks, McC., A study of unitary activity in the supraoptic nucleus of the hypothalamus, Japanese Journal of Physiology, 1963, 13:374-385.

Sundsten, J.W. and Sawyer, C.H., Osmotic activation of neurohypophyseal homone release in rabbits with hypothalamic islands, Experimental Neurology, 1961, 4:545-561.

Tata, P.S. and Buzalkov, D., Vasopressin studies in the rat. III. Inability of ethanol anesthesia to prevent ADH secretion due to pain and hemorrhage, Pflugers Archiv, 1966, 290: 294-297.

Thornton, L.W., Effects of repeated infusions of hypertonic saline solutions on the electrical activity of hypothalamic neural units in goats, Ph.D. Thesis, Michigan State University, 1969.

Von Euler, C.A., A preliminary note on slow hypothalamic osmopotentials, Acta Physiologica Scandanavia, 1953, 29: 133-136.

Verney, E.B., The antidiuretic hormone and factors which determine its release. Proceedings of the Royal Society, London, Series B, 1947, 135:25-106.

Wang, M.B., The distribution and control of osmosensitive cells within the hypothalamus of the opossum, Didelphis virginiana, Neuroendocrinology, 1969, 21:51-67.

Watt, R.M., Metabolic activity in single supraoptic neurones and its relation to osmotic stimulation, Brain Research, 1970, 21:443-447.

Wolf, A.V., Thirst, Springfield: C.C. Thomas, 1958.

Woods, J.W., Bard, P., and Bleir, D., Functional capacity of the de-afferented hypothalamus: Water balance and responses to osmotic stimuli in the decerebrate cat and rat, Journal of Neurophysiology, 1966, 29:751-767.

APPENDIX A

Apparatus

Equipment and materials used in making microelectrodes.

1. Stainless steel tubing, 26 gauge, Superior Tube Co.
2. Tungsten wire, 0.005", Sylvania.
3. Glass tubing, Corning Glass.
4. Hydrofluoric acid, Merck, Inc.
5. Micrometer eyepiece, x8, Zeiss.
6. Stereozoom dissecting microscope, Nikon.

Equipment used in blood analysis.

1. Centrifuge, International Equipment Co.
2. Refractometer, American Optical Co., Instrument Div.
3. Freezing point osmometer, Precision Instrument Corp.

Equipment and materials used in bioelectric data recording and analysis.

1. Stereotaxic instrument, Kopf.
2. Preamplifiers, 122, Textronix.
3. Power supply, 125, Textronix.
4. Oscilloscope, 502A, Textronix.
5. Tape recorder, 1028, Magnecord.
6. Electronic counter, 5512A, Hewlett Packard.
7. Printout counter, Digitron.
8. Audiomonitor, AM8, Grass Instruments.
9. Audiomonitor, AM5-B, Grass Instruments.
10. Thermistor thermometer, Yellow Springs Instruments.
11. Micromanipulator, Narishige.
12. Magnetic tape, Scotch, 1.5 mil acetate, $\frac{1}{4}$"x2500'.
13. Stimulator, S8-Cr, Grass Instruments.
14. Stimulus isolation unit, SIU 4678, Grass Instruments.

15. Technical Measurement Corp., Mneumotron Div.,
    Amplitude discriminator 605
    Peak detector 607
    Amplitude to time discriminator 606
    Computer of averaged transients 400B

16. Oscilloscope, 565, Textronix

17. Lesion maker, G. Connors.

APPENDIX B
Raw Data

Experiment I

Plasma Osmolality, mOsm/kg

Presurgery	0 Min	3 Min	6 Min
300	311	292	298
307	291	290	304
303	302	299	301
299	303	300	301

9 Min	12 Min	15 Min	18 Min
291	290	294	289
287	287	293	288
296	287	297	295
295	293	290	296

21 Min	24 Min
287	279
289	290
293	292
301	286

Protein concentration, g/100ml

Presurgery	0 Min	3 Min	6 Min
6.3	6.3	6.3	6.6
6.3	5.9	6.3	6.3
6.3	6.2	6.3	6.1
6.3	6.3	6.2	5.8

9 Min	12 Min	15 Min	18 Min
6.4	6.1	6.3	6.2
6.0	6.4	6.1	6.4
6.0	5.9	5.9	6.0
6.1	5.7	5.7	5.6

21 Min	24 Min
5.8	5.4
6.2	5.9
5.6	5.8
5.9	5.1

Experiment I

Water absorbed, ml

3 Min	6Min	9 Min	12 Min
-1.93	1.23	-1.63	2.53
-3.03	-1.03	0.83	2.13
-2.33	1.33	2.93	4.83
-3.93	-2.73	1.43	2.83

15 Min	18 Min	21 Min	24 Min
6.13	0.83	5.23	3.73
1.23	-1.37	5.43	6.73
2.23	-1.37	4.43	4.53
0.63	3.63	2.13	3.73

Percent change in heart rate

3 Min	6 Min	9 Min	12 Min
2.9	2.1	7.2	10.4
2.0	2.8	2.6	10.1
3.2	4.9	7.2	1.0
3.4	2.5	1.8	7.0

15 Min	18 Min	21 Min	24 Min
12.8	1.7	1.0	7.7
7.9	2.0	14.7	6.8
1.0	1.7	12.7	1.1
5.4	2.6	8.8	1.0

Experiment II

Unit activity, Animals in the Ad Libitum Group, Spikes/second Anterior Hypothalamic cells

Cell No.	Minute 1	2	3	4	5	6	7	8	9	10
74-1	5.2	2.7	2.3	1.9	8.2	7.2	9.1	9.1	8.5	9.2
72-1	4.4	4.2	4.3	4.4	4.8	4.7	4.8	4.3	4.3	4.5
72-3	4.7	4.8	4.2	5.3	5.8	4.8	4.5	4.9	4.7	4.6
72-4	3.6	3.7	2.2	3.8	4.3	5.2	5.8	5.6	5.3	4.9
68-1	13.1	13.0	11.3	11.7	12.4	13.3	11.9	12.3	13.2	13.7
68-3	8.7	7.8	11.5	7.6	8.3	9.7	10.2	10.3	11.2	11.2
58-1	15.1	16.3	16.3	17.4	12.1	17.1	15.2	15.2	15.8	15.3
58-2	15.0	12.3	12.4	13.3	16.2	13.5	14.3	14.3	12.9	13.8
56-1	8.7	8.8	8.6	7.3	6.6	8.7	6.9	6.6	8.2	9.5
56-3	24.2	22.3	22.2	16.5	15.5	15.2	15.7	14.2	15.3	20.2
52-3	0.5	1.1	1.3	1.3	1.3	1.1	1.0	1.1	0.9	0.9
57-1	9.8	8.2	6.4	9.1	8.9	9.2	9.0	8.6	8.8	8.8

Unit activity, animals in the Ad Libitum Group, Spikes/second Supraoptic Nucleus cells

Cell No.	Minute 1	2	3	4	5	6	7	8	9	10
74	2.4	2.1	2.2	2.1	1.9	1.6	1.6	0.9	0.6	1.6
72	0.4	1.4	1.9	1.6	0.4	0.7	0.7	0.8	1.4	1.3
68-1	1.3	2.1	2.3	3.2	2.2	1.6	2.2	2.3	2.3	2.2
58-1	8.7	5.7	7.6	5.1	3.5	3.5	2.7	3.8	3.5	3.8
56	0.4	1.2	0.5	0.7	0.5	0.4	0.5	0.6	1.0	0.6
57	4.7	5.2	5.4	4.3	5.3	5.2	5.8	6.2	1.4	5.9
53	0.6	1.3	1.2	1.7	1.8	1.8	1.3	1.2	0.6	1.1

Unit activity, animals in the Deprived Group, Spikes/second Anterior Hypothalamic cells

Cell No.	Minute 1	4	7	10	13	16	19	22	25	28
49	11.5	2.4	7.5	6.0	7.7	16.9	6.1	10.0	12.5	10.1
48	12.5	15.0	15.0	14.0	12.5	12.8	14.8	16.0	10.6	9.8
47	12.5	11.8	11.2	10.9	12.9	10.6	10.2	12.5	22.3	22.6
45	15.7	15.0	16.8	14.9	15.4	15.4	35.1	24.4	29.7	38.0
44	30.0	35.7	37.7	35.0	34.4	35.6	36.3	39.7		

Cell No.	Minute 31	34	37	40	43	46
49	7.0	7.9	7.5	5.6	6.2	7.0
48	9.1	11.0	9.8	10.0	10.3	9.9
47	22.1	22.5	22.7	21.0	22.2	20.2
45	22.5	22.9	22.5	22.1	22.7	32.7

Experiment II

Unit activity, animals in the Deprived Group, Spikes/second Supraoptic Nucleus cells

Cell No.	Minute 1	4	7	10	13	16	19	22	25	28
65	7.1	7.2	7.7	7.6	9.1	7.7	8.2	9.1	8.2	8.5
62	3.1	3.1	3.1	3.5	3.1	2.5	1.9	2.6	2.9	2.5

Cell No.	Minute 31	34	37	40	43	46	49
65	8.2	9.1	10.1	9.3	8.8	9.7	9.0
62	2.1	2.0	2.7	3.0	3.2	3.8	3.6

Unit activity, animals in the Load-Injected group, Spikes/second, Anterior Hypothalamic cells

Cell No.	Minute 1	4	7	10	1	4	7	10	13	16
14	6.2	5.1	4.8	4.3	3.6	2.0	2.1	2.5	3.7	3.0
16	9.6	9.9	10.6	11.0	11.4	10.8	10.6	10.9	10.4	11.2
17	20.2	16.2	18.1	20.4	14.0	11.6	9.2	6.6	5.3	5.4
18	46.4	47.8	56.2	69.1	45.7	40.1	42.6	38.2	37.5	41.9
21	21.8	19.2	19.8	21.1	22.2	22.9	21.8	20.9	21.6	21.2
23	10.6	13.4	12.6	8.4	11.6	11.9	12.7	15.8	13.3	13.6
24	12.6	6.6	6.9	6.8	4.8	2.1	5.6	6.7	4.8	5.9
26	16.7	22.4	9.5	2.7	1.6	15.2	16.7	15.0	13.9	18.8
27	27.4	26.6	27.8	22.9	23.2	18.3	7.3	21.2	16.8	22.5
28	3.9	8.8	8.4	7.9	11.7	5.2	8.6	9.4	10.6	9.1
29	8.0	17.0	8.9	6.8	4.7	9.6	16.9	18.6	9.9	15.5
38	8.9	6.9	7.6	7.5	9.2	9.7	8.9	8.3	5.9	5.6
13	5.1	7.8	3.2	10.2	12.4	2.8	1.9	4.3	6.7	3.0
10	2.5	2.1	1.9	1.3	1.9	2.2	1.8	1.6	1.8	2.1
7	9.2	9.3	10.4	9.7	9.0	7.8	8.2	1.8	8.8	10.1
4	22.3	17.4	17.3	18.8	16.2	15.8	15.9	15.4	14.9	14.4
3	6.1	7.8	5.9	6.2	5.8	8.8	6.9	8.4	8.0	5.9

Experiment II

Unit activity, animals in the Load-Injected Group, spikes/second (cont.), Anterior Hypothalamic cells

Cell No.	Minute 19	22	1	4	7	10	13
14	2.8	3.0	9.9	4.4	3.2	2.3	2.7
16	10.8	11.5	10.4	8.7	11.1	10.6	9.5
17	5.2	5.6	12.1	8.2	2.6	2.4	2.7
18	39.1	38.4	33.9	37.6	39.7	41.1	37.7
21	23.9	21.0	20.1	20.3	17.7	22.9	26.1
23	14.3	13.9	2.7	6.4	6.8	6.4	5.2
24	5.5	5.1	25.8	10.2	25.6	9.2	21.0
26	9.2	15.4	1.9	1.8	0.9	1.4	1.2
27	19.6	20.4	10.1	10.4	9.7	11.2	11.1
28	8.4	10.3	13.5	13.8	15.2	12.2	11.9
29	21.0	24.9	9.6	6.8	6.5	10.4	8.5
38	8.4	10.0					
13	3.4	10.6					
10	2.3	2.1					
7	9.4	9.4					
4	13.9	14.4					
3	7.5	7.5					

Unit activity, animals in the Load-Injected Group, spikes/second, Supraoptic Nucleus cells

Cell No.	Minute 1	4	7	10	1	4	7	10	13	16
15	21.1	19.9	21.3	19.4	18.9	20.5	17.9	14.0	20.7	18.7
19	9.5	6.2	10.8	7.5	12.9	13.1	9.1	10.3	6.6	8.0
30-1	7.5	6.3	6.9	7.5	8.0	6.7	5.8	3.6	3.0	1.0
30-2	12.2	9.2	13.5	14.5	16.2	22.5	11.1	8.5	3.1	3.9
32-1	4.5	4.0	5.6	4.7	11.5	12.3	5.0	2.5	1.9	0.9
32-2	14.1	17.7	19.2	15.2	18.6	17.7	20.9	21.2	22.0	18.0
33	3.5	3.6	3.9	4.4	2.5	2.8	2.3	4.9	3.0	1.9
34-1	26.7	30.5	25.7	27.0	52.7	32.3	30.7	30.9	16.7	6.5
34-2	10.5	12.3	11.5	13.1	13.7	15.6	14.5	12.6	8.7	8.2
35-1	1.2	3.9	2.5	3.8	16.1	1.1	6.4	7.2	5.4	2.9
35-2	13.6	13.0	19.0	18.9	5.0	11.3	19.4	14.4	12.9	8.9
36	3.4	4.0	3.1	3.9	5.1	5.3	3.1	0.9	1.2	2.9

Experiment II

Unit activity, animals in the Load-Injected Group, spikes/second, Supraoptic cells (cont.)

Cell No.	Minute 19	22	1	4	7	10	13
15	14.3	13.9	12.2	6.1	7.1	4.0	7.2
19	4.7	6.0	11.2	3.2	5.2	4.6	6.0
30-1	4.6	4.0	18.0	1.5	12.0	19.0	17.2
30-2	4.4	2.6	8.5	0.8	5.0	9.0	8.1
32-1	0.5	0.3	32.5	13.7	22.7	21.0	31.2
32-2	11.0	19.7	4.5	0.2	2.5	3.5	1.7
33	2.5	3.0	13.1	18.3	28.6	30.2	29.7
34-1	15.1	17.0	6.0	8.6	15.9	24.6	27.2
34-2	4.7	7.0	7.6	2.3	3.2	3.3	7.0
35-1	1.2	1.1	1.9	2.8	4.0	6.9	9.2
35-2	5.5	5.6	11.9	9.5	13.3	16.5	20.4
36	2.7	1.2	2.2	0.9	1.2	2.2	3.6

Unit activity, animals in the Load-Only Group, spikes/second, Anterior Hypothalamic cells

Cell No.	Minute 1	4	7	10	1	4	7	10	13	16
73	17.8	19.6	18.4	15.9	14.8	12.6	10.1	8.1	9.0	6.3
69	16.0	22.5	9.8	2.5	0.8	15.0	17.0	15.0	14.2	14.1
61	1.5	2.2	2.1	2.3	2.4	2.3	1.2	0.5	0.9	0.8
60	27.5	28.8	27.6	22.7	22.9	17.8	7.4	23.1	16.7	22.5
57	3.7	9.1	8.8	7.6	12.0	5.0	8.1	9.8	10.6	9.2
46	10.4	13.3	12.5	8.0	11.9	11.9	12.7	15.9	13.0	13.3
43	12.7	7.0	6.9	6.9	5.0	2.1	5.9	6.9	4.9	6.0
59-1	6.0	7.5	6.8	6.3	5.8	7.5	8.5	7.0	8.8	8.7
59-2	2.5	1.1	1.1	1.1	2.0	2.0	2.3	1.2	1.0	1.8
40-1	21.5	17.5	17.5	16.2	16.4	17.7	16.0	16.0	16.0	15.0
40-2	9.0	9.1	9.2	8.7	9.0	10.0	7.6	8.5	8.5	8.7
39	2.3	2.6	2.8	2.8	4.8	1.6	1.0	0.7	1.0	6.2
9	8.2	9.8	8.6	11.1	11.4	2.9	2.1	8.1	9.9	11.2
8	11.2	8.9	8.7	8.6	5.2	5.8	7.1	8.1	9.1	11.1
6	21.7	19.9	20.3	20.3	21.3	22.0	21.0	20.3	21.3	21.0

Experiment II

Unit activity, animals in the Load-Only Group, spikes/second, Anterior Hypothalamic cells (cont.)

Cell No.	Minute 19	22	25	28	31	34	37
73	3.7	3.5	2.9	3.2	2.7	2.9	2.5
69	9.0	15.3	2.2	1.1	1.7	2.1	2.1
61	1.2	2.0	9.8	7.1	7.1	10.1	8.6
60	19.9	20.1	1.5	1.5	0.1	1.0	0.1
57	8.4	10.3	16.0	16.0	15.1	12.0	12.0
46	14.7	14.1	10.0	10.5	9.8	11.5	11.5
43	5.7	5.0	6.2	6.9	7.3	7.8	7.6
59-1	5.9	7.9	14.3	14.3	14.6	14.9	11.6
59-2	1.9	2.0	12.1	11.2	9.3	9.2	8.4
40-1	15.0	8.0	23.0	24.3	24.0	22.6	25.9
40-2	10.0	9.5					
39	7.4	5.3					
9	14.1	14.6					
8	12.2	12.2					
6	23.3	23.3					

Unit activity, animals in the Load-Only Group, spikes/second, Supraoptic Nucleus Cells

Cell No.	Minute 1	4	7	10	1	4	7	10	13	16
67	12.7	11.9	12.8	11.6	11.3	12.3	10.7	8.4	11.1	12.3
63	7.9	17.4	9.0	6.7	4.8	9.9	17.0	18.5	9.9	15.4
50	9.2	8.1	7.1	7.5	6.5	8.2	7.1	8.5	7.9	8.0
10	12.3	12.6	11.4	13.1	14.9	16.9	12.2	9.4	9.1	10.1

Cell No.	Minute 19	22	25	28	31	34	37
67	11.2	8.4	8.3	7.0	7.0	7.8	7.1
63	21.1	25.0	7.0	9.6	10.3	10.9	10.3
50	8.0	6.9	8.5				
10	12.1	12.3					

Figure 20.

Shown is a photograph of a frontal cut of a rat brain embedded in celloiden and stained in thionin. Indicated is the supraoptic nucleus (SO), suprachiasmatic nucleus (SC), and the optic chiasm (OC).

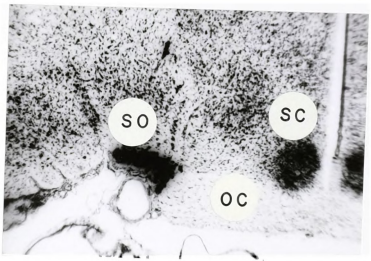
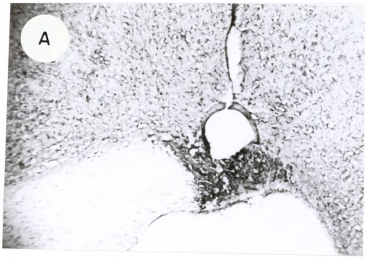

SO
SC
OC
I MM

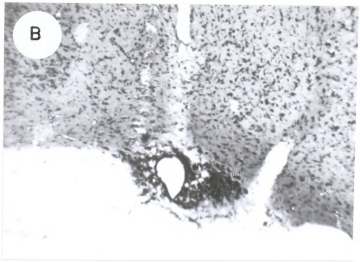
Figure 21.

A. Shown is a photograph of a frozen section of a rat brain stained in thionin and cut in the same plane as Figure 20. Encroaching on the SO is a marking lesion indicating a recording site.

B. Shown is a photograph of a frozen section of a rat brain stained in thionin and cut in the same plane as Figure 20. Encroaching on the SO is another marking lesion indicating a recording site.



1 MM

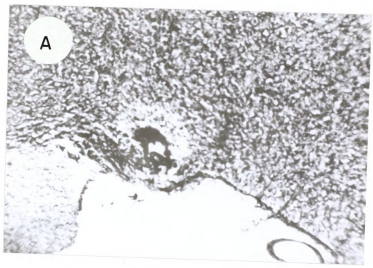


1 MM

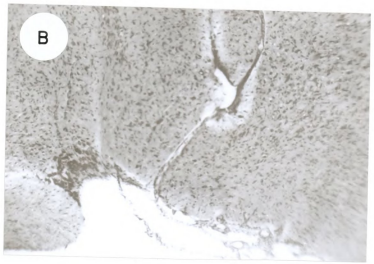
Figure 22.

A. Shown is a photograph of a frozen section of a rat brain stained in thionin and cut in the same plane as the previous photographs. Encroaching on the SO is a marking lesion.

B. Shown is a photograph of a frozen section of a rat brain stained in thionin and cut in the same plane as Figure 20. Seen is a marking lesion located in the preoptic area.



1 MM



1 MM

MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 03057 7385