PROLONGED ACUTE THIRST: SOME NEURAL, PHYSIOLOGICAL, AND BEHAVIORAL CORRELATES IN RATS

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ABSTRACT

PROLONGED ACUTE THIRST:
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BEHAVIORAL CORRELATES IN RATS

By

James Karl Walters

Drastic changes in drinking behavior are known to follow interruption of the axons from cells of the supraoptic nucleus (SON) of the hypothalamus. Animals with lesions in the median eminence, which sever the supraoptico-hypophyseal tracts, drink huge quantities of water daily. This condition led to the realization that cells of the SON are crucial for maintaining proper body water balance and may even be part of an internal sensing system.

The antidiuretic hormone (ADH) is produced by these neurons and migrates down their axons to be stored and released in the posterior pituitary. ADH acts to promote water reabsorption at the kidneys. Without it, considerable water passes out of the body as urine and increased drinking results. Due to this water saving function, the SON plays an integral part in an animal's ability to adapt to periods of prolonged acute thirst resulting from water deprivation.

Uncertainty exists, however, as to precisely what changes

take place in blood volume and tonicity, the major ADH liberating stimuli, during extended water deprivation and how these changes are related to the firing rates of single SON neurons. In addition, little is known of the relationship between SON unit activity, ADH release, and the phenomenon of multiple nucleoli in SON cells when an animal remains thirsty for a considerable length of time. Therefore, two interrelated experiments were carried out to investigate some of the neural, physiological, and behavioral events taking place during prolonged acute thirst in rats.

Using water deprivation to operationally define thirst, Experiment I involved monitoring the physiological and behavioral adaptation of rats allowed free access to food and water and rats deprived of water for 1, 2, 3, 4, or 5 days. Six independent groups of six rats each were kept in individual metabolism cages for daily determination of food intake, body weight, and volume of urine excreted. A sample of each animal's urine was saved for later determination of sodium and potassium concentrations and total solids content. Also, a blood sample was taken from all rats so that serum osmolality and protein concentration could be measured.

In Experiment II, ten minutes of maintained activity were recorded from SON and surrounding anterior hypothalamic (AH) neurons in rats deprived of water for 0, 1, 2, 3, 4, or 5 days. After being anesthetized, each animal was stereotaxically positioned and surgically prepared for single unit recording. As many units as possible were isolated in a

three hour recording session, and all were marked with a lesion made through the tip of the recording electrode. A blood sample was taken at the end of each recording session. Brains were preserved for later histological verification of recording sites.

The physiological and behavioral data of Experiments I and II provided a clearer picture of many of the adaptive regulatory processes called into play to help maintain the constancy of the internal environment when an animal is without water for a prolonged period. For the albino rat there was a decreased urine flow which was probably due to a lowered glomerular filtration rate at the kidneys. An increased urine concentration also occurred as a result of ADH release from the posterior pituitary. Food intake gradually ceased, thus preventing additional solutes from entering the body and further concentrating the body fluids. And finally, there was a retention of sodium which is necessary to maintain water balance and preserve life. These processes reached their maximal effectiveness by the third waterless day and helped to preserve plasma volume while at the same time stabilizing the increased body fluid osmolality.

Concerning neural activity, Experiment II found that the mean SON cell firing rate increased after one day of acute thirst and stayed near that level for the remaining four days of water deprivation. This paralleled ADH levels found by others in the urine of rats during a similar time span and adds circumstantial evidence to the hypothesis that ADH

release is related to SON cell firing rates. In addition, many SON cells fired intermittently, suggesting that ADH may be released in bursts. Plasma osmotic pressure continued to increase for four days before stabilizing, while both SON unit activity and serum protein concentration (an inverse index of plasma volume) increased for just one day and then remained relatively constant. Thus, SON cells apparently responded more to volume than to osmotic stimuli. AH units showed no significant firing rate changes under the same conditions.

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James Karl Walters

A Thesis

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To My Parents

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INTRODUCTION

Drastic changes in drinking behavior are known to follow interruption of the axons from cells of the supraoptic nucleus (SON) of the hypothalamus. In their classic 1938 study, Fisher, Ingram, & Ranson found that animals with lesions in the median eminence, which severed the supraoptico-hypophyseal tracts, drank huge quantities of water daily. This condition, known as diabetes insipidus, led to the realization that cells of the SON are crucial for maintaining proper body water balance. This is due to the fact that antidiuretic hormone (ADH) is produced by these cells and migrates down their axons, perhaps attached to a carrier molecule, to be stored and released in the neurohypophysis (Bargmann & Scharrer, 1951; Scharrer & Scharrer, 1954). ADH is an octapeptide hormone which acts primarily on the collecting ducts of the kidneys to promote water reabsorption (Dicker, 1970). Without it, considerable water passes out of the body as urine. The deficit must then be made up by an increased water intake. Knowing this, one might expect the supraoptico-hypophyseal system to play an integral part in an animal's ability to adapt to periods of water restriction, and such is apparently the case (Pickford, 1969, p. 469).

A variety of stimuli can cause ADH to be liberated from SON axon terminals in the posterior pituitary. Among these are pain, emotional stress, and many drugs (Thorn, 1958). Under normal circumstances though, the major stimuli for ADH release are 1) cellular dehydration caused by increases in the effective osmotic pressure (EOP) of plasma, and 2) decreases in the extracellular fluid volume (Share, 1967).

Concerning the former of these two stimuli, cellular dehydration, Verney (1947) showed that salt solutions infused into the carotid artery of dogs would reduce a water diuresis. He and his co-workers attributed this to ADH release and showed that it resulted from an increased EOP of plasma acting on cells of the hypothalamus, not on the pituitary itself (Verney, 1954; Jewell & Verney, 1957). Since then, numerous workers have searched for the "osmodetectors" postulated by Verney. Using single unit recording techniques, many studies have shown cells in and around the SON to be osmosensitive (Cross & Green, 1959; Brooks, Ushiyama & Lange, 1962; Koizumi, Ishikawa, & Brooks, 1964; Cross & Silver, 1966; Brooks, 1966; Hayward & Vincent, 1970). Recently a group of investigators has suggested that, for primates at least, the true osmodetectors are located in the perinuclear zone within one millimeter of the SON, while SON cells themselves are basically secretory (Vincent & Hayward, 1970; Vincent, Arnauld, & Bioulac, 1972; Vincent, Arnauld, & Nicolescu-Catargi, 1972). Whether this

is true or not remains to be firmly established.

With regard to the other stimulus for ADH release, decreased extracellular fluid volume, considerably less experimental work has been done. But it has been shown that hemorrhage can be a potent ADH releaser (Share, 1961, 1969; Ardnt, 1969) as can the sequestering of water in the body due to polyethelene glycol injection (Stricker, 1969). These volume influences may be exerted through afferents arising in the left atrium and the low pressure side of the circulatory system (Henry, Gupta, Meehan, Sinclair, & Share, 1968). To date, however, no single unit recording studies have been aimed at assessing specifically the effects of volume manipulations on SON neurons. Menninger & Frazier (1972) varied left atrial stretch and found changes in stretch to affect only part of the various hypothalamic cells responsive to osmotic stimuli, while changes in osmotic pressure affected all cells responsive to stretch. They concluded that osmotic stimuli may have greater control of hypothalamic unit activity, and possibly ADH release, than volume stimuli. Other investigators have employed different methods and reached similar conclusions concerning ADH release (Moses, 1963; Forsling, Martin, & Burton, 1971).

For years, a tempting hypothesis to couple these stimuli to ADH secretion has been the idea that they cause an increased frequency of impulses traveling down SON axons resulting in hormone release (Scharrer & Scharrer, 1954; Cross & Silver, 1966; Douglas & Poisner, 1964). Proof

for this hypothesis is non-existent (Share & Claybaugh, 1972). Some reasonable circumstantial evidence has been provided by many unit recording studies, however, especially that of Dyball (1969) who used independent groups of rats to correlate ADH levels with SON activity after hypertonic saline injections.

Considering that ADH can reduce renal water loss and help to concentrate the urine, there should be a high demand for ADH during dehydration (Pickford, 1969, p. 469). This suggestion is supported by studies showing increased levels of ADH in the urine (Ames & Van Dyke, 1950; Dicker & Nunn, 1957; Miller & Moses, 1971) and plasma (Little & Radford, 1964) of rats during extended water deprivation. Unfortunately though, these studies have not definitely determined the exact function for ADH release when rats are without water. This is mainly due to the imprecision of ADH assay methods and the questionable practice of using systemic blood or urine levels as indicators of ADH release from the neurohypophysis.

Evidence of increased ADH production, as indexed by an increase in the percentage of SON cells with multiple nucleoli, has also been discovered in acutely water deprived rats (Hatton & Walters, submitted for publication). The relationship, if any, between ADH production (or multiple nucleoli) and SON cell firing rates remains obscure. However, this study did indicate that volume stimuli may be quite important activators of the SON.

Obviously, any condition producing acute thirst, such as prolonged water deprivation, would cause changes in blood volume and tonicity, the major ADH liberating stimuli. Conflicting reports exist, however, as to precisely what changes do take place in these stimuli during an extended period without water (Little & Radford, 1964; Kutscher, 1965, 1966; Hatton, 1971). In addition, no one has ever attempted to determine the relationship of SON unit activity to blood volume, blood osmotic pressure, or ADH levels in acutely water deprived animals. Bennett (1971) did record from SON cells of rats adapted to a 23.5 hour water deprivation schedule. He found SON cell firing rates to be increased at a time when blood osmotic pressure was increased and blood volume decreased. No information was available to him concerning ADH levels of such animals though, and he was more concerned with osmotic than volume stimuli.

Therefore, two interrelated experiments were carried out to investigate the relationship of SON neuronal activity to those physiological and behavioral events taking place during prolonged acute thirst in rats. Using water deprivation to operationally define thirst, Experiment I involved monitoring the physiological and behavioral adaptation of rats during a five day period without water. In Experiment II, the activity of single SON neurons was recorded in rats having free access to food and water and in those deprived of water for 1, 2, 3, 4, or 5 days. It was the purpose of these studies to answer the following questions:

1. What changes take place in blood volume and osmotic pressure during five days of water deprivation?

2. Do SON cell firing rates in acutely water deprived animals follow volume or osmotic stimuli more closely?

3. What is the relationship between SON unit activity and ADH release when water is withheld?

4. Is SON neuronal activity related to the phenomenon of multiple nucleoli in SON cells?

5. What behavioral and physiological mechanisms come into play during acute water deprivation to maintain an animal's body water and electrolyte balance?

6. What is the time course of those events occurring during a rat's adaptation to prolonged acute thirst?

EXPERIMENT I

This study was carried out to determine the effects of an extended period of water deprivation on a number of variables known to be crucial for body water and electrolyte balance. Six independent groups of rats, run in squads of three animals each, were deprived of water for 0, 1, 2, 3, 4, or 5 days. During this time intake, output, and blood measures were recorded in order to assess precisely the deprivation effects.

METHOD

Subjects

Thirty-six male albino rats of the Holtzman strain were used. At 100 days of age they were placed in individual metabolism cages under conditions of constant light where powdered Wayne Mouse Breeder Blox and tap water were available ad libitum.

Metabolism cages

The stainless steel cage living dimensions measured 26.5 cm. x 20.5 cm. x 16.5 cm. high. A 50 cm. tall base supported each cage. There were 18 such metabolism units arranged on 3

tables, 6 per table. All cages were kept in the same room which was maintained at 22-25°C, although no humidity control was available. Also housed in that room were 12 other rats in metabolism cages with drinkometers connected. They were subjects in a separate experiment, however.

Procedure

During a seven day adaption period each rat was handled for one minute and then weighed at approximately the same time each day. Beginning on the eighth day and continuing for the remainder of the experiment, 24 hour measures of food intake, water intake (when water was available to the animals), body weight, and urine volume output were recorded for each animal. These measures were taken at 1800-1900 hours for the first set of 18 animals and at 1500-1600 hours for the second set, which was run approximately two months after the first. A daily sample of each rat's urine was also saved for later determination of sodium and potassium concentration by flame photometer and total solids by use of a refractometer. (See Appendix A for a detailed description of equipment.)

Following a 10 day baseline period, three animals were randomly assigned to the Day-0 group; blood samples were obtained from them at the time of day when other measures were taken. In addition, the water bottles were removed from all other animals on this day. Thus all remaining rats were deprived of water starting on day 17, but food continued

to be freely available.

After one day of water deprivation, three of the remaining animals were randomly assigned to the Day-1 deprived group and blood samples obtained from them. After two days of water deprivation, three animals were rendomly assigned to the Day-2 deprived group and blood samples taken. A similar procedure was followed for the Day-3, Day-4, and Day-5 water deprived groups.

Blood samples were obtained by decapitation. When all measures had been taken for an animal it was weighed and then quickly decapitated in the same room rather than being returned to its cage as usual. Trunk blood was funnelled into a tube which was then centrifuged at 3300 RPM for five minutes. The serum was drawn off and a few drops immediately analyzed for protein in a refractometer. The remaining serum was placed in a glass vial for later determination of osmolality by freezing point osmometry and sodium and potassium concentrations by flame photometry. All serum samples were frozen within one to three minutes after removal from the centrifuge.

RESULTS

Body metabolism data for each of the six groups are plotted in Figures 1-10. Each point in these figures represents a mean 24 hour reading for the six animals randomly assigned to that group. Bars representing the estimated standard error (S.E.) of the mean are also included around each mean.

One-way analyses of variance were performed to determine if baseline differences existed between the six groups on any of the variables measured. Using the last day of free access to food and water as a baseline, no significant differences ($p < .25$) were found. It was not possible, however, to test for such differences in serum measures, since only one blood sample was taken from each animal.

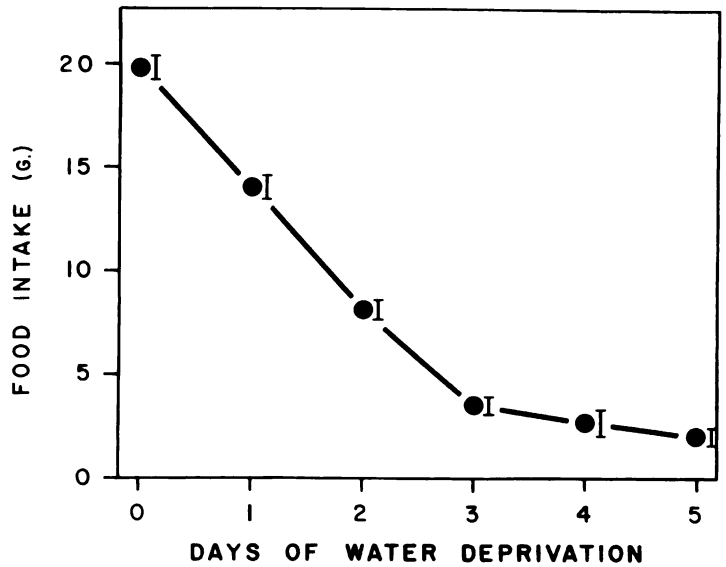
Figure 1 shows the mean ($\pm$ S.E.) 24 hour food intake for each group. A statistically reliable decrease in food intake occurred during five days of water deprivation ($F = 201.26$, df 5/30, $p < .001$). There was a drop from 19.8 grams for the Day-0 group to only 3.5 grams during the third day of water deprivation. Food intake continued to decrease, but only very slightly, during the fourth (2.7g.) and fifth (2.0g.) days of deprivation.

Volume of urine excreted for each group across days of water deprivation is presented in Figure 2. Five days of deprivation caused a significant decrease in urine volume output ($F = 25.08$, df 5/30, $p < .001$). The Day-0 group excreted 12.5 ml. of urine, while the Day-3 group excreted 2.9 ml. Again, as for food intake, only small changes occurred after the third day.

The urine electrolyte data (Figures 3 and 4) revealed a significant decrease in urine sodium concentration ($F = 17.45$, df 5/30, $p < .001$) and a significant rise in urine potassium concentration ($F = 4.74$, df 5/30, $p < .005$) when all six groups were included in the analysis. However, the concentrations of both electrolytes were elevated on the first day.

Figure 1. Mean (± S.E.) food consumption as a function of days of water deprivation.

Figure 2. Mean (± S.E.) volume of urine excreted as a function of days of water deprivation.


FOOD INTAKE (G.)
20
15
10
5
0
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION

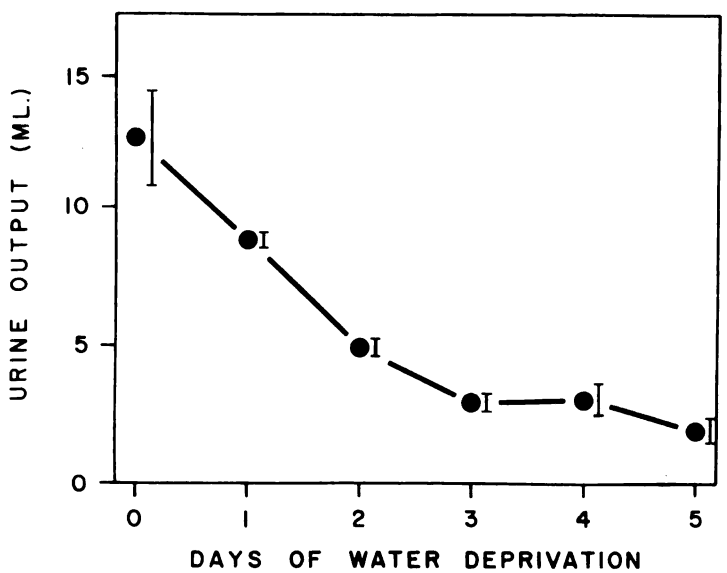
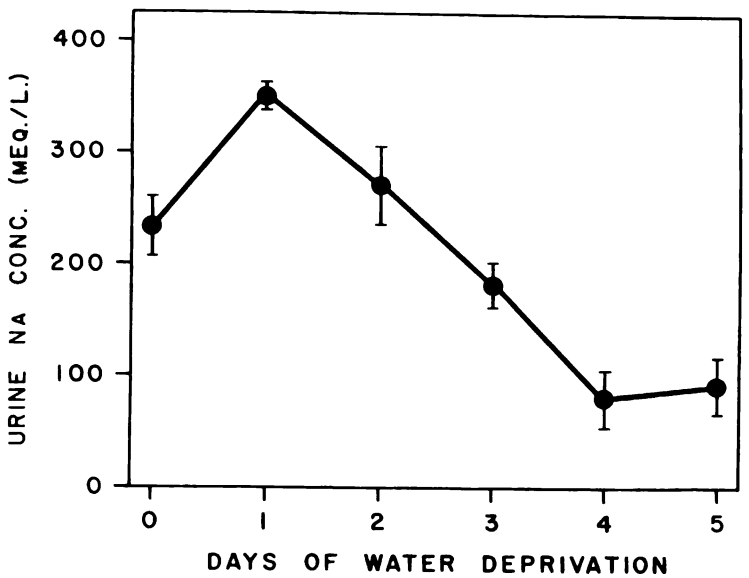
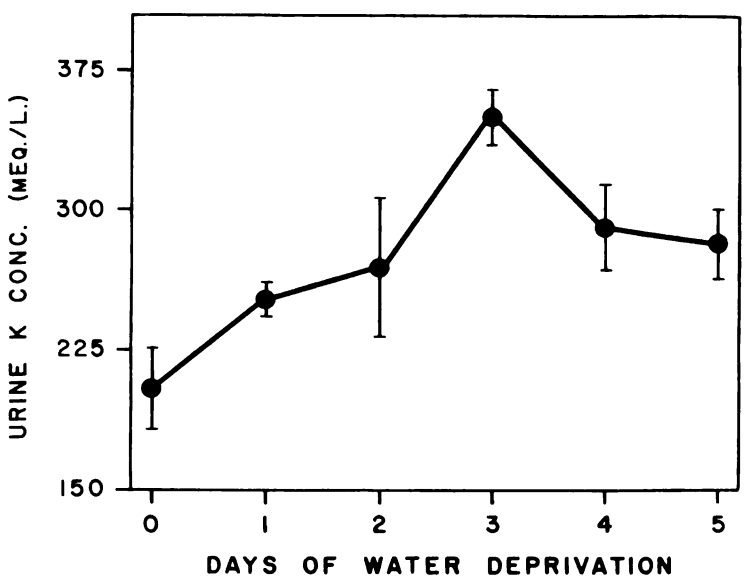

URINE OUTPUT (ML.)
15
10
5
0
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION

Figure 3. Mean (± S.E.) urine sodium concentration as a function of days of water deprivation.

Figure 4. Mean (± S.E.) urine potassium concentration as a function of days of water deprivation.


URINE NA CONC. (MEQ./L.)
400
300
200
100
0
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION


URINE K CONC. (MEQ./L.)
375
300
225
150
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION

Urine sodium increased from 233.67 mEq./L. for the Day-0 group to 349.00 mEq./L. for the Day-1 deprived group. Then from day one to day four there was a linear sodium concentration decrease to a value of 79.50 mEq./L. The fifth day of deprivation resulted in little sodium change. Urine potassium concentration rose from a Day-0 value of 203.03 mEq./L. to a Day-1 value of 252.00 mEq./L. Mean potassium concentration remained relatively constant for the rest of the deprivation period except for an elevation to 351.83 mEq./L. for the Day-3 group.

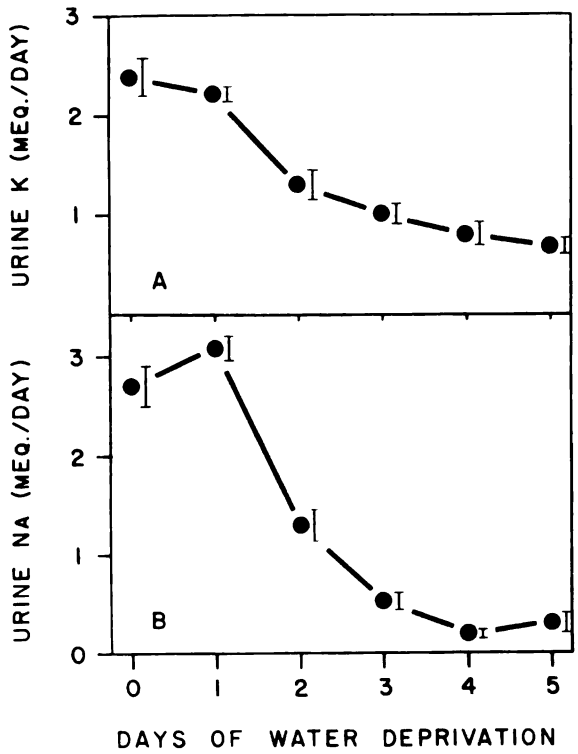
Panel B of Figure 5 shows that the function for total daily urine sodium output was similar to that for urine sodium concentration (Figure 3). Again there was a statistically reliable decrease during five days of dehydration ($F = 107.85$, df 5/30, $p < .001$) but an increase on day one. Mean total sodium output for the Day-0 group was 2.7 mEq./day while the Day-1 group mean was 3.1 mEq./day. Sodium output then continued to decrease to 0.2 mEq./day by four days of deprivation, changing little on the fifth day. In contrast to the general increase in urine potassium concentration shown in Figure 4, a pronounced and significant ($F = 36.56$, df 5/30, $p < .001$) decrease in total daily urine potassium output occurred during five waterless days (Figure 5, Panel A). A steady mean decrease was evident from a Day-0 value of 2.4 mEq./day to a Day-5 group mean of 0.7 mEq./day.

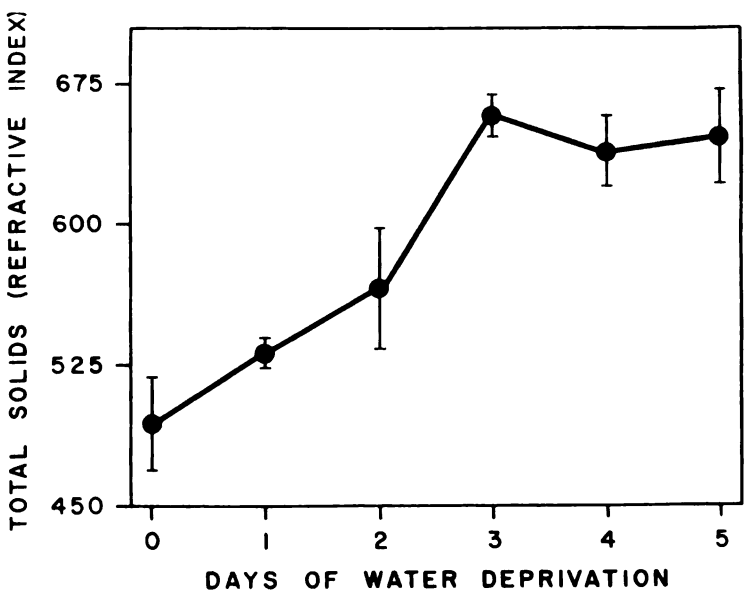
With regard to urine total solids (refractive index), acute water deprivation resulted in a significant increase in

Figure 5. Panel A indicates mean (± S.E.) total urinary potassium excreted as a function of days of water deprivation.

Panel B indicates mean (± S.E.) total urinary sodium excreted as a function of days of water deprivation.

Figure 6. Mean (± S.E.) urinary total solids as a function of days of water deprivation. Only the last three numbers from the refractive index are presented on the ordinate of this figure; full readings would take the form 1.3 _ _ _.


URINE K (MEQ./DAY)
3
2
1
A
URINE NA (MEQ./DAY)
3
2
1
0
B
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION


TOTAL SOLIDS (REFRACTIVE INDEX)
675
600
525
450
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION

this measure ($F = 11.15$, df 5/30, $p < .001$). Figure 6 shows a rise from 1.3493 for the Day-0 group to 1.3657 for the Day-3 group. There were no further changes in urine total solids during the fourth (1.3638) or fifth (1.3646) days.

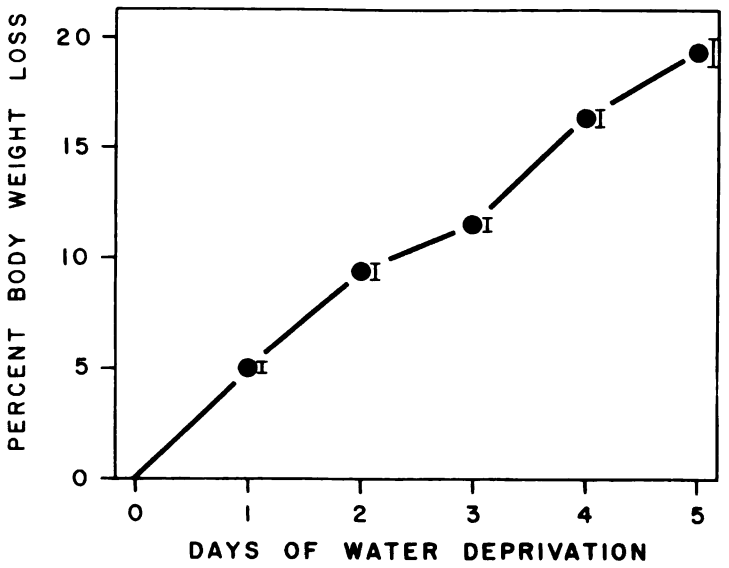
Body weight loss for each group during the five day water deprivation period is presented as a percentage of their own Day-0 body weight in Figure 7. A significant and linear increase in percent body weight loss occurred ($F = 179.94$, df 4/25, $p < .001$). Mean body weight losses were 5.0%, 9.4%, 11.8%, 16.4%, and 19.3% for the Day-1, -2, -3, -4, and -5 groups, respectively.

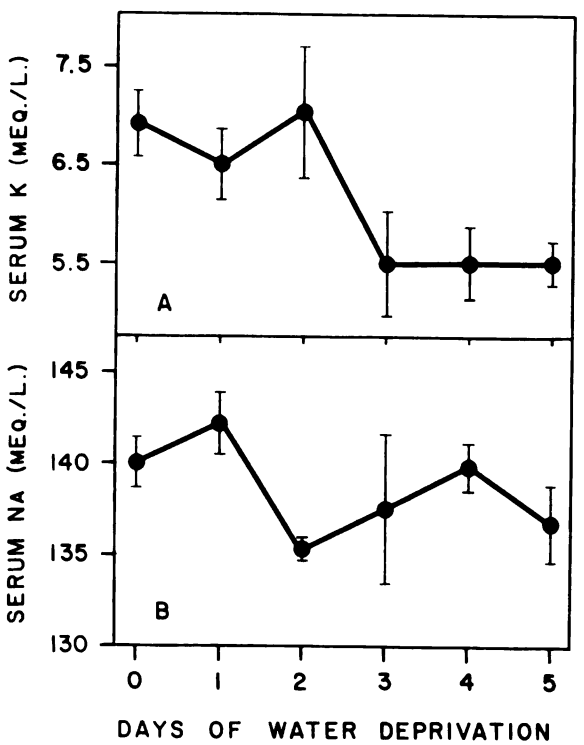
Turning now to the serum measures, Panel B of Figure 8 shows that the range for mean serum sodium concentration was small during five days of deprivation, and a one-way analysis of variance showed no significant effects of the water deprivation treatment ($F = 1.37$, df 5/30, $p < .25$). However, the Day-2 deprived group mean of 135.33 mEq./L. was considerably below the Day-1 mean of 142.17 mEq./L. Significance was obtained in a one-way analysis of variance which included only the Day-0, Day-1 and Day-2 deprived groups ($F = 7.31$, df 2/15, $p < .001$). Although such an analysis is not statistically sound since the means had already been tested, it does imply that there was a definite decrease in serum sodium concentration during two days of deprivation. This decrease was masked in the overall analysis due to variability on later days. Mean serum potassium concentration (Figure 8, Panel A) did show a significant decrease during the five day span of

Figure 7. Mean (± S.E.) percent body weight loss as a function of days of water deprivation.

Figure 8. Panel A indicates mean (± S.E.) serum potassium concentration as a function of days of water deprivation.

Panel B indicates mean (± S.E.) serum sodium concentration as a function of days of water deprivation.


PERCENT BODY WEIGHT LOSS
0
5
10
15
20
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION

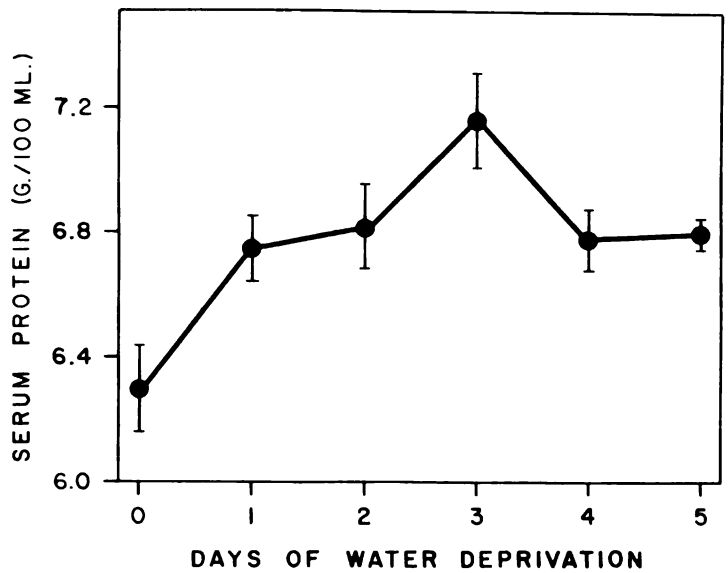

SERUM K (MEQ./L.)
7.5
6.5
5.5
A
SERUM NA (MEQ./L.)
145
140
135
130
B
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION

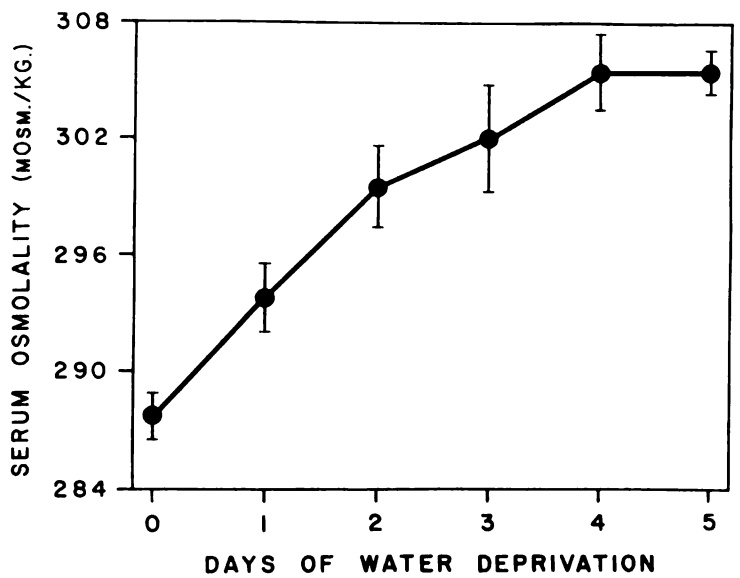
water deprivation ($F = 3.01$, df 5/30, $p < .025$). It remained relatively stable for the Day-1 and Day-2 groups then decreased from a Day-2 mean of 7.0 mEq./L. to 5.5 mEq./L. for the Day-3 group. It remained there for both the Day-4 and Day-5 groups.

Figure 9 presents mean ($\pm$ S.E.) serum protein concentration as a function of days without water. (This measure can also be converted to serum specific gravity.) There was a significant increase in serum protein with deprivation ($F = 6.01$, df 5/30, $p < .001$). The Day-0 group averaged 6.32 g./100 ml. while the Day-1 deprived group mean was 6.75 g./100 ml. Serum protein remained near this level throughout the deprivation period except for an elevation to 7.17 g./100 ml. on the third day. Mean serum osmotic pressure (Figure 10) also increased significantly during water deprivation ($F = 12.57$, df 5/30, $p < .001$). The Day-0 group mean was 287.8 mOsm./kg. From there osmotic pressure increased steadily to a mean of 305.5 mOsm./kg. after four days of water deprivation. The fifth day mean was the same as the fourth.

Figure 9. Mean (± S.E.) serum protein concentration as a function of days of water deprivation.

Figure 10. Mean (± S.E.) serum osmolality as a function of days of water deprivation.


SERUM PROTEIN (G./100 ML.)
7.2
6.8
6.4
6.0
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION


SERUM OSMOLALITY (MOSM./KG.)
308
302
296
290
284
0
1
2
3
4
5
DAYS OF WATER DEPRIVATION

EXPERIMENT II

Cells of the supraoptic nucleus produce ADH, which is known to play a role in maintaining body water balance during water deprivation (Pickford, 1969, p.469). Since these neurosecretory cells also generate action potentials (Cross & Green, 1959; Bennett, 1971), Experiment II was carried out to determine the firing rates of SON neurons in animals having free access to food and water and in animals deprived of water for up to five days. Firing rates were then related to blood and body metabolism data in water deprived animals.

METHOD

Subjects

Forty-three male Holtzman albino rats, 100-160 days old and 305-460 grams on the day of recording, were used as subjects. All animals were housed in individual cages under constant light. They had pellets of Wayne Mouse Breeder Blox and tap water available ad libitum for at least ten days after arrival and prior to experimentation.

Procedure

Ten minutes of maintained activity were recorded from

supraoptic and surrounding anterior hypothalamic neurons in rats deprived of water for 0, 1, 2, 3, 4, or 5 days. All water deprived subjects were weighed and had their water bottles removed one to five days before the time at which they were anesthetized for single unit recording. On the recording date each rat was again weighed and given an IP injection of 0.7 g./kg. Dial-urethane anesthesia. Supplementary doses of 0.05 g. were occassionally needed to completely anesthetize the animal. After 15-30 minutes, the head and chest of each rat was shaved with electric clippers. A needle electrode serving as a heart lead was placed under the skin of the chest and taped down. Ear pins were then inserted and the rat stereotaxically positioned. After surgical exposure of the skull, it was cleaned and adjusted to a horizontal position. Two overlapping 0.4 mm. holes were trephined in the skull to expose the brain; the dura was removed and neural tissue covered with mineral oil. Only animals which were completely anesthetized and had minimal bleeding were used as subjects, since both pain (Verney, 1947) and hemorrhage (Belesum, Bisset, Haldar, & Pocak, 1967) are known to cause ADH release.

Next, each subject was wrapped in a variable temperature heating coil after which a rectal thermister was inserted 7-8 cm. Body temperature was maintained at 36-38°C throughout the recording session. All leads were connected to the recording apparatus, and a tungsten microelectrode with glass insulation (except for a 10-30u exposed tip) was lowered

approximately 8.5 mm. below the cortex to the region of the supraoptic nucleus. Neural and cardiac activity were amplified by a low-level preamplifier and visually displayed on a dual beam oscilloscope. Neural activity was monitored auditorily with a loud speaker. Once the action potentials of a given cell were sufficiently above baseline noise to reliably trigger the sweep of the oscilloscope, the cell was considered to be isolated. At this time 10 minutes of maintained single unit activity were recorded on magnetic tape; cardiac activity was also recorded. An on-line counter was available to record the rates of impulses whose amplitude exceeded the threshold of the oscilloscope trigger. (See Appendix A for a description of the equipment.)

Each recording site was marked with a small D.C. cathodal lesion (usually 20 μa for five seconds) made through the tip of the recording electrode. A maximum of six electrode punctures were made bilaterally in each animal, three per side, in order to isolate as many supraoptic units as possible during a recording session. In some cases more than one unit was recorded per electrode puncture. Within three hours or less of the time at which anesthesia was administered, the recording session was ended and a blood sample obtained in a heparin coated syringe by heart puncture. The animal was then perfused with physiological saline followed by a 10% formalin solution to await frozen sectioning. Sections were stained with cresyl violet and recording sites verified histologically.

RESULTS

Neural activity

Activity was recorded from a total of 129 single units, 46 within the anterior portion of the supraoptic nucleus (SON) and 83 in other anterior hypothalamic (AH) areas. Some animals contributed one or more SON units only; some contributed one or more AH units only; and most contributed one or more units of each type. Table 1 gives the exact number and types of units for each treatment condition.

Figure 11 presents a schematic representation of the SON in coronal section at four different coordinates when the skull is positioned horizontally. The number adjacent to a section indicates the number of mm. anterior to ear bar zero at which that cut was made. SON units were distributed on both sides of the brain between the most posterior (7.8) and most anterior (9.0) of these coordinates. The majority were isolated between 8.2 and 8.6, however. Anterior hypothalamic units were usually distributed within one and one-half mm. of the SON in medial, lateral, anterior, or dorsal directions. See Appendix C for more precise localization of recording sites.

In Figure 12 are plotted the mean ($\pm$ S.E.) firing rates in spikes per second for both SON and AH units at 0, 1, 2, 3, 4, and 5 days of water deprivation. These means are not statistically independent, however, since several units in one or both locations at each day may have been contributed by the same animal. Thus, the assumption of independence is

Figure 11. Schematic representation of the SON at four different coordinates when the skull is positioned horizontally. The number adjacent to a section indicates the number of mm. anterior to ear bar zero at which that cut was made. SON: supraoptic nucleus, PV: paraventricular nucleus, FX: fornix, CO: optic chiasm, OT: optic tract, SC: suprachiasmatic nucleus, V: ventricle III.

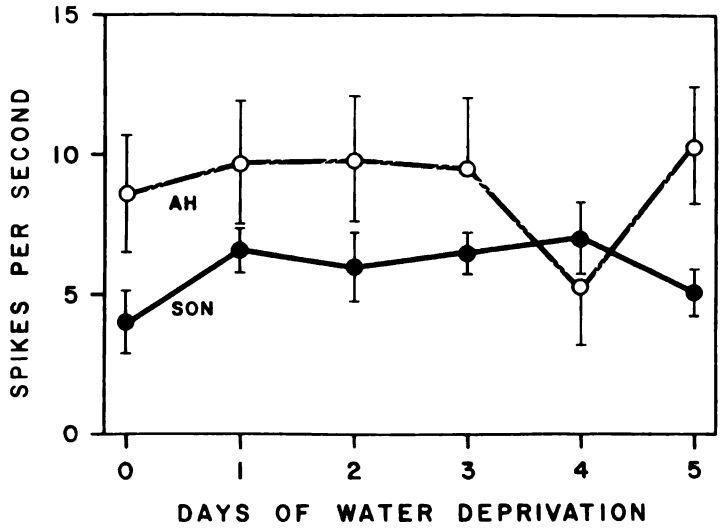
violated in all statistical tests performed on these data. Figure 13 also presents mean (± S.E.) firing rates of SON and AH cells as a function of days of water deprivation. Here the means for each day are independent of one another. This was accomplished by randomly choosing only one SON unit and one AH unit to represent an animal in those cases where several units of each type had been recorded.

For both non-independent (Figure 12) and independent (Figure 13) means, SON cells increased their firing rates from days zero to one. The non-independent increase was from 4.02 to 6.58 spikes per second, while the independent increase was from 4.21 to 7.17 spikes per second. These differences both proved to be significant when a one-tailed t-test for independent groups was used ($t = 1.91$, df 14, $p < .05$ with non-independence and $t = 2.44$, df 9, $p < .025$ with independence). A one-tailed test was applied because previous research (Bennett, 1971) had indicated that both SON and AH units significantly increase their firing rates when rats are adapted to a water deprivation schedule. Both independent and non-independent SON means remained near the one day level on days two, three, and four. Each decreased somewhat on day five, however, with the non-independent rate falling to 5.05 spikes per second and the independent rate falling to 5.55 spikes per second. Nevertheless, one-way analyses of variance failed to find any significant differences in either case during days one through five.

It is interesting to note the similarity of the mean SON cell firing rate curves regardless of the degree of

Figure 12. Mean (± S.E.) spikes per second as a function of days of water deprivation for SON (●—●) and AH (○—○) units. All such units are included so the means at each day are non-independent. Table 1 gives the number of units for each location at each day of deprivation.

Figure 13. Mean (± S.E.) spikes per second as a function of days of water deprivation for SON (●—●) and AH (○—○) units. A maximum of one unit per animal per location is included so that the means at each day are independent. There are 5, 6, 3, 6, 3, and 4 SON units and 9, 6, 7, 6, 5, and 6 AH units at days 0, 1, 2, 3, 4, and 5, respectively.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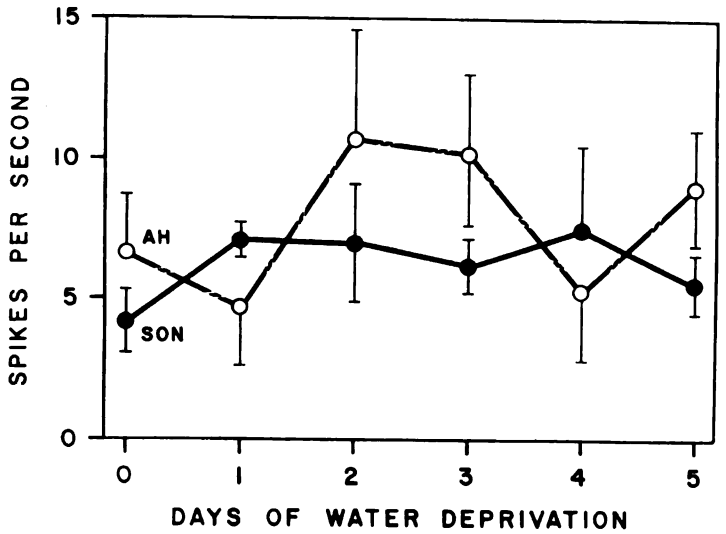
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independence. This reflects the fact that SON cells showed small variability in their mean firing rates, which only ranged from 0.21 to 13.23 spikes per second.

With regard to AH cell firing rates, neither the non-independent (Figure 12) not the independent (Figure 13) means changed significantly from days zero to one. There was a mean increase from 8.60 to 9.70 spikes per second with non-independence, while a decrease from 6.64 to 4.68 spikes per second was evident with independence. The non-independent mean AH cell firing rates remained rather stable from days one to five with the exception of a decrease to 5.27 spikes per second at day four. However, it should be observed that there was a much smaller number of units (6) at this day than at any other day (12-18 units). The independent mean AH firing rates varied considerably across days, increasing to 10.66 spikes per second on day two and remaining near that level on day three. Mean rate then dropped to 5.35 spikes per second on day four and finally increased to 9.01 spikes per second on day five. One-way analyses of variance performed in both the non-independent and independent cases resulted in no significant differences for AH units during days one through five.

Comparison of the mean firing rates of SON vs. AH units gave different results depending on whether independent (Figure 13) or non-independent (Figure 12) means were used. A t-test for differences between means showed that the mean firing rates of SON cells were significantly slower than the mean firing rates of AH cells ($t = 2.98$, $df\ 5$, $p < .025$) in

the non-independent case. Such was not true for independent means. One-tailed tests were used here since Bennett (1971) had previously found SON units to fire slower than AH units. The firing rates of AH cells tended to vary considerably, ranging from 0.12 to 34.59 spikes per second, and large samples may be required for accurate estimation of their mean rate. Unfortunately, the sample size was reduced considerably in order to carry out an independent test.

An interesting difference between SON and AH units was observed in the characteristic firing patterns of these neurons. A number of units were seen to cease firing for periods ranging from 10 to 30 seconds. Such units were referred to as being intermittent in their firing pattern. (A 10 second interval was the smallest quantifiable time unit, since the digital counter which recorded spikes printed out every 10 seconds.)

Table 1 shows that 24 of the 46 SON units (52%) were of the intermittent type, while only 13 of the 83 AH units (16%) were intermittent. These figures include all SON and AH units, several of which were recorded from single animals. When a two by two Chi-square test was carried out on these data, it was found that SON units were significantly more likely to be of the intermittent type than were AH units ($X^2 = 17.54$, df 1, $p < .001$). Since the Chi-square assumption of independence was violated here, a similar but independent test was also done. Approximately half of the 43 subjects were randomly (where possible) assigned to the SON category

Table 1

Summary of the number and types of units for each water deprivation condition.

Days of water deprivation	Number of animals	Number of SON units		Number of AH units	
		Total	Intermittent	Total	Intermittent
0	10	8	6	18	3
1	7	8	4	18	3
2	7	7	4	14	0
3	8	8	4	12	4
4	5	9	5	6	2
5	6	6	1	15	1
TOTAL	43	46	24	83	13

and half to the AH category. Then one SON unit or one AH unit from each animal was randomly selected and categorized as being intermittent or not. As a result of this procedure, 11 of 21 SON units (52%) and four of 22 AH units (18%) were classified as intermittent. Again, SON units proved significantly more likely than AH units to be intermittent ($X^2 = 4.13$, df 1, $p < .05$).

Table 1 makes it apparent that the percentages of intermittent AH and SON units were not the same at each day of deprivation. There seemed to be a rather systemic decrease in the percentage of SON intermittent units across days. Unfortunately though, those differences which do exist are not statistically testable due to the small number of subjects available per group for an independent test.

Blood and body weight data

Turning now to other physiological measures, Table 2 gives a summary of changes in plasma osmolality, plasma protein concentration, and body weight for animals in the recording experiment. Since some animals had units in just one location, means ($\pm$ S.E.) are given separately for animals with SON units and animals with AH units at each day of deprivation. Animals with both SON and AH units are represented in all means.

During the five day span, mean plasma osmolality increased significantly from 292.6 to 321.8 mOsm./kg. for animals with SON units ($F = 11.59$, df 5/19, $p < .001$). A

Table 2

Mean plasma osmolality, plasma protein concentration, and percent body weight loss for animals with SON units and animals with AH units.

	Days of water deprivation					
	0	1	2	3	4	5
Animals with SON units:						
Mean (± S.E.) plasma osmolality (mOsm./kg.)	292.6 ± 0.9	300.2 ± 3.5	309.7 ± 5.9	309.6 ± 3.0	319.0 ± 1.0	321.8 ± 1.9
Mean (± S.E.) plasma protein (g./100 ml.)	5.3 ± 0.2	6.2 ± 0.1	6.4 ± 0.1	6.3 ± 0.2	6.2 ± 0.2	6.1 ± 0.2
Mean (± S.E.) body wt. loss (% of ad lib)		5.4 ± 0.5	8.4 ± 0.4	10.4 ± 0.5	14.3 ± 0.5	14.5 ± 0.4
Animals with AH units:						
Mean (± S.E.) plasma osmolality (mOsm./kg.)	293.6 ± 2.7	301.3 ± 3.3	308.1 ± 4.0	311.8 ± 1.7	319.0 ± 0.5	320.6 ± 3.3
Mean (± S.E.) plasma protein (g./100 ml.)	5.4 ± 0.1	6.2 ± 0.1	6.6 ± 0.1	6.3 ± 0.2	6.2 ± 0.2	6.0 ± 0.2
Mean (± S.E.) body wt. loss (% of ad lib)		5.7 ± 0.3	8.2 ± 0.4	10.7 ± 0.6	14.4 ± 0.4	14.7 ± 0.4

similar significant osmolality increase from 293.6 to 320.6 mOsm./kg. was apparent for animals contributing AH units ($F = 4.90$, df 5/29, $p < .005$). Osmotic pressure levels for recording animals were higher than those of animals in Experiment I (Figure 10) under all treatment conditions.

With regard to plasma protein, SON animals showed a mean increase from 5.3 g./100 ml. ad lib to 6.6 g./100 ml. at two days of deprivation. There was then a decrease to 6.1 g./100 ml. at five days. A one-way analysis of variance revealed significant differences ($F = 3.33$, df 5/21, $p < .025$). Likewise, significant changes occurred in the plasma protein levels of AH animals ($F = 13.00$, df 5/31, $p < .001$). From an ad lib value of 5.4 g./100 ml., an increase was evident to a two day peak of 6.6 g./100 ml. This was followed by a decrease to 6.0 g./100 ml. at five days. Plasma protein levels for recording animals were somewhat lower than those for the subjects of Experiment I (Figure 9), and the peak occurred at day two instead of day three.

Finally, Table 2 shows that percent body weight loss increased significantly from 5.4 to 14.5% and from 5.7 to 14.7% for animals with SON units ($F = 59.58$, df 4/19, $p < .001$) and animals with AH units ($F = 83.02$, df 4/25, $p < .001$), respectively.

Mean plasma osmolality, mean plasma protein, and mean percent body weight loss functions were very much alike for animals with SON units and animals with AH units, indicating that both types of units were receiving similar stimulation.

DISCUSSION

Body Metabolism Data

In viewing Experiment I broadly, it is apparent that the Holtzman laboratory rat has reached its maximal adaptation to a condition of prolonged acute thirst by the third waterless day. Most of the variables measured, including food intake, volume of urine excreted, urine total solids, daily urine sodium and potassium output, serum sodium and potassium concentrations, and serum osmolality, had all essentially stabilized by that time. Further changes might occur with more extended deprivation, but it is doubtful whether additional useful knowledge concerning the rat's adaptive ability would be gained with such a deteriorating preparation.

More specifically speaking, the data of Experiments I and II confirm, for the most part, and extend the work of previous investigators concerning the physiological picture of water deprivation. It has been known for quite some time (Verplank & Hayes, 1953) that rats eat less food than normal when kept without water. The present results indicate that by three days of dehydration they have essentially stopped eating completely, or at least this is true of those animals in Experiment I who were fed a dry powdered diet. The few

grams of food which Figure 1 shows them to have consumed on days three, four, and five represent mostly uncontrollable spillage into the metabolism cage pan.

These rats continued to lose weight almost linearly during the five day deprivation span, and their mean body weight loss values are nearly the same as those reported by Kutscher (1965) at two and four days of water deprivation. Although actual food intake was not determined for the recording animals of Experiment II, body weight losses were measured. The weight loss functions for these animals were somewhat attenuated in comparison to that for the rats of Experiment I. In addition, their mean body weight losses leveled off after four days of dehydration, rather than continuing to increase. Since the subjects of the two experiments were housed in separate rooms, conditions such as room temperature or humidity may have produced these differences. Another contributing factor may have been the consistency of the diet, however, for Experiment I rats recieved powdered Mouse Breeder Blox while Experiment II rats recieved Breeder Blox pellets. Some food was also observed in the stomachs of many recording animals even at the extended deprivation times. This evidence supports the notion that the amount of food consumed and the degree of body weight loss during dehydration are partially functions of the form in which food is given to an animal.

With regard to the volume of urine excreted under water deprivation conditions, Experiment I confirms the

findings of Dicker & Nunn (1957) who also showed that rats respond to dehydration with a decreasing urine output until an asymptote is reached. Their rats reached that plateau at day two, though, while the urine output of rats in Experiment I did not level off until day three. Individual differences may account for this minor discrepency. They suggest that the urine flow, which is necessitated by the obligatory excretion of toxic solutes, is primarily determined by the glomerular filtration rate (GFR) rather than by the release of ADH from the posterior pituitary. Dicker (1957) reiterates this conclusion, stating that the decreased GFR probably results from plasma volume reductions. Their arguments are supported by an experiment of Miller & Moses (1972) which showed that even Brattleboro rats with hereditary hypothalamic diabetes insipidus could drastically reduce their urine output when water was withheld. Such rats were unable to concentrate their urine nearly as well as normal rats, however.

Despite the fact that ADH may not be necessary for decreasing urine flow during dehydration, there is an abundance of morphological (Hatton & Walters, submitted for publication; Watt, 1970; Rechardt, 1969) electrophysiological (Bennett, 1971) and horomonal evidence (Vilhardt, 1970; Little & Radford, 1964) of increased supraoptico-hypophyseal activity in thirsting animals. These findings, together with that of increased SON cell firing rates during acute water deprivation as revealed by Experiment II, suggest

that ADH does have an important role to play in the renal concentration of urine during hydropenia. In all liklihood both the GFR and ADH concentration are important factors. Lee & Williams (1972) suggest that ADH may not only promote water reabsorption, but may also enhance sodium reabsorption, thus increasing the interstitial osmotic gradient of the kidney and helping to concentrate the urine.

The overall trend of decreased urine sodium concentration and decreased daily sodium output found in Experiment I is compatible with Lee & Williams' suggestion. It also confirms and extends the findings of Dicker & Nunn (1957) concerning sodium excretion. However, a number of other factors also provide explanations for the decrease in urinary sodium which is a necessary condition for maintaining the extracellular fluid (ECF) volume. Both a lowered GFR (Lee & Williams, 1972) and an enhanced aldosterone secretion (Pitts, 1968, p. 219), well established to occur with dehydration, promote greater sodium reabsorption from the kidney tubules. ADH may also play an indirect part in this increased sodium reabsorption. For although the renin-angiotensin system is the chief stimulator of aldosterone secretion, Pitts (1968, p. 219) states that high levels of ADH may stimulate ACTH release which in turn effects a secretion of aldosterone from the adrenal gland, or ADH may act directly on the adrenal itself, liberating aldosterone. In addition to these factors, the voluntary starvation of

water deprived animals results in less sodium intake, and consequently, less sodium output.

Why urine sodium concentration and output both increased on the first day of deprivation remains an interesting question. This finding conflicts with Dicker & Nunn's 1957 investigation. It may have been due in part to the fact that the animals consumed a relatively large quantity of food (approximately two-thirds of their normal amount) on the first deprivation day, although they had no water available to dilute their body fluids. Thus, their kidneys may have eliminated some of the excess sodium in an attempt to reduce the ECF concentration and maintain the intracellular fluid (ICF) volume.

The data for urine potassium excretion is more easily accounted for. Since potassium secretion into the kidney tubules is partially coupled to sodium reabsorption from the tubules (Pitts, 1968, p. 113), an increased urine concentration of potassium would be expected to accompany a decreased sodium concentration. This result might also be explained by the fact that potassium, the chief intracellular cation, is probably liberated, along with proteins and other constituents, as cells die and are broken down to provide energy during dehydration. Any excess potassium would be rapidly removed in the urine, while the proteins would remain in the blood since they are not normally filtered by the kidney. Such a phenomenon could help to explain the striking resemblance of the urine potassium concentration

function (Figure 4) to the serum protein concentration function of Figure 9.

The decreasing daily output of urine potassium across days of water deprivation for Experiment I is quite similar to that found by Dicker & Nunn (1957). The dehydration induced decrease in food intake and lowered GFR seem to account for this, since there would be less potassium in the animal's body for excretion while less is being filtered.

Turning now to urine total solids (refractive index), a measure which takes into account all solutes in the urine, not just sodium and potassium, it is obvious that maximal urine concentration is reached by the third waterless day. This finding agrees well with that of Lee & Williams (1972) who showed that urine osmolality increased linearly for the first three days of deprivation, just as total solids did in Experiment I. They failed to take measurements beyond three days, however. Evidently by the third day of dehydration the limit of the urine concentrating mechanism of the rat kidney has been reached. The increase in total solids must have been primarily a result of increased urine potassium, and especially urea, concentrations, since urine sodium concentration fell with extended water deprivation.

A similar situation was found for serum solute concentration as determined by osmolality. A rather steady rise in serum osmolality was apparent for the first three days of dehydration, after which time no further major

increases occurred. Thus, serum osmolality increased with prolonged water deprivation even though serum sodium exhibited no overall significant changes and serum potassium decreased slightly. Potassium changes were probably caused by the increased secretion of this solute into the renal tubules as considerable sodium was reabsorbed. Obviously, the elevated serum osmolality must have been due to an increase in the concentration of some solute(s) other than these. One highly likely candidate is urea, while another possibility is increased serum protein concentration resulting from ingested food combined with ECF volume depletion and cell destruction. As food intake nearly ended by the third day, shutting off a major source of protein, serum osmolality also leveled off.

The serum sodium function is somewhat similar to that obtained by Jones & Pickering (1969). They too found a slight increase at day one, a large decrease at day two, and then a further increase during extended deprivation. In contrast though, their observed plasma sodium levels increased significantly and paralleled, almost exactly, the function they obtained for plasma osmolality. In Experiment I it was found that serum sodium was maintained within narrow limits during five days of deprivation with no overall significant changes. Also, little similarity was evident between serum osmolality and serum sodium. It is difficult to reconcile the results of the present study with those of Jones & Pickering.

In any case, the serum osmolality curve obtained in Experiment I seems to conform well with the bulk of the data collected by other investigators. The plasma osmolality function of Jones & Pickering, on the other hand, apparently stands alone. Like the results of Kutscher (1966) who observed a steady mean plasma osmolality increase to 60 hours of deprivation, serum osmolality also displayed a monotonic mean increase for three days before leveling off. In spite of this increase though, mean osmolalities proved not to be significantly different, as revealed by highly overlapping S.E. bars, at two and three days of water deprivation. Hatton found exactly this in 1971.

Regarding plasma volume, as inversely indexed by plasma protein concentration, Hatton (1971) observed it also to remain the same at two and three days of water deprivation. The serum protein data of Experiment I (Figure 9) contradict his finding, while the plasma protein data of recording animals in Experiment II (Table 2) confirm it. Basically, the curves for serum and plasma protein are very similar, except for an unusually high serum protein reading at day three. This elevation is difficult to explain, but may have resulted from trouble encountered in decapitating several of the three day deprived animals of Experiment I. In any case, the results from both studies conflict with the findings of Kutscher (1965), who showed plasma volume to be less at four days of deprivation than two. In both of the present experiments, plasma volume was down after one

day of deprivation and remained relatively stable thereafter. Similar results were obtained by Hatton & Walters (submitted for publication) and Watt (1970). Evidently after the initial volume depletion some regulatory processes take place to preserve plasma volume. The loss of sodium in the urine at the first day of deprivation and its subsequent retention on later days is compatible with the plasma volume data. Perhaps a day or so is necessary for the aldosterone system to come into full play and begin defending the ECF volume challenge posed by dehydration. In addition, water may move out of the cells due to the increased ECF concentration, and interstitial water may move into the plasma. Unfortunately though, protein concentration is undoubtly not the best index of plasma volume, since food intake and perhaps other factors may influence this measure. Despite the fact that Kutscher (1971) showed it to be correlated with plasma volume, some of the discrepancies may be due to the possible inaccuracy of protein concentration estimation of volume.

In summary now, it is apparent that the body metabolism data of Experiments I and II have provided a clearer picture of many of the adaptive regulatory processes called into play to help maintain the constancy of the internal environment when an animal is deprived of water for a prolonged period. For the albino rat there is a decreased urine flow which is probably due to a lowered GFR. An increased urine concentration also occurs as ADH is liberated from the

neurohypophysis. Food intake gradually ceases, thus preventing additional solutes from entering the body and further concentrating the body fluids. And finally, there is a retention of those solutes, especially sodium, which are necessary to maintain water balance and preserve life. These processes have reached their maximum effectiveness by the third waterless day and help to preserve plasma volume while at the same time stabilizing the increased body fluid osmolality. Coincident with the physiological changes would be a search for water and subsequent drinking to restore the imbalance if water were available (Hatton & Almli, 1969; Hatton & Ives, 1969; Hatton & Thornton, 1968).

Neural Activity

Several problems present themselves in the interpretation of the single unit data of Experiment II. Despite the homogeneity of the SON on casual observation, several investigators have observed different types of cells within SON including: light vs. dark (Rechardt, 1969) round vs. angular (Hatton & Walters, submitted for publication) and those with single vs. multiple nucleoli (Hatton, Johnson & Malatesta, 1971). Since even a small marking lesion destroys many cells, it was impossible to tell the types of cells from which SON recordings were made. Any marking lesion, the majority of which encroached on SON, was considered to have designated an SON recording site. Any other marking lesion, the majority of which was not within SON, was considered to have designated an AH recording

site. The limit of localization of cells in this study was at least 0.1 mm. It is important to remember that the AH category includes a very heterogenous population of cells from the anterior portion of the hypothalamus. Cells of the preoptic area, medial forebrain bundle, anterior lateral hypothalamus, and anterior hypothalamic area are all included. With these limitations in mind, it is now possible to discuss the implications of the data.

The finding that mean AH and SON cell firing rates were significantly different with non-independence but failed to reach significance when an independent sample was taken is probably due to the large variability in the firing rates of AH cells. This reflects the heterogeneous population from which AH cells were drawn and the fact that the mean is a statistic heavily influenced by extreme values (Games & Klare, 1967, p. 97). A large sample, as was achieved with non-independence, may be necessary for accurate determination of mean AH cell firing rates. Bennett (1971) found AH cells to fire faster than SON cells.

Unlike Bennett's data, however, AH cells of Experiment II did not significantly increase their firing rates during deprivation in either the independent or the non-independent cases. This result is somewhat surprising, for many investigators have found a high percentage of anterior hypothalamic neurons to be osmosensitive (Joynt, 1964; Brooks, Ishikawa, & Lu, 1966; Hayward & Vincent, 1970; Menninger & Frazier, 1972). Curiously, many of the AH cells recorded

in Experiment II were located in the so-called perinuclear zone within one mm. of SON. This is precisely the area where several workers have supposedly localized the "osmoreceptors" of the ADH system of monkeys (Vincent & Hayward, 1970; Vincent, Arnauld & Bioulac, 1972; Vincent, Arnauld & Nicolescu-Catargi, 1972). The reason for this discrepancy remains unclear, but it may be due, in part, to species differences or to the water deprivation conditions used.

As for SON units, they were found to increase their mean firing rate on the first waterless day and remain near that rate for the rest of the deprivation span. This occurred in both independent and non-independent cases, confirming the work of Bennett (1971) who found SON cells of rats adapted to a water deprivation schedule to fire faster than those of ad lib rats. Considering that numerous investigators have hypothesized a relationship between increased SON activity and increased ADH secretion from the posterior pituitary (Douglas & Poisner, 1964; Cross & Silver, 1966; Dyball & Koizumi, 1969; Dyball, 1969), such a result may indicate a high but constant ADH release during water deprivation. This interpretation is in agreement with the finding of Miller & Moses (1971) that the urinary excretion of ADH increased rapidly following dehydration and remained at the same level throughout a four day deprivation period. If this most recent radioimmunoassay study is valid and if urinary ADH excretion is

representative of pituitary release, then Experiment II offers further circumstantial evidence for a link between SON firing rates and ADH release.

In contradiction stand older bioassay studies of the antidiuretic activity (ADA) of urine which showed steadily increasing urine ADA with extended deprivation (Ames & Van Dyke, 1950; Dicker & Nunn, 1957). Also, Little & Radford's 1964 experiment demonstrating an increasing ADA of plasma to 48 hours and then a leveling off at 72 hours, is somewhat inconsistent with the results of Experiment II. What is obviously still needed is a thorough study of ADH release during extended water deprivation.

Interestingly, the function for SON unit activity across days of water deprivation bears a striking similarity to the function obtained by Hatton & Walters (submitted for publication) for multiple nucleoli in SON cells during the same time span. That multiple nucleoli in SON cells may be related to the increased metabolic activity required for increased neuron firing, rather than to hormone production, is a plausible hypothesis. The question still awaits the proper experiment for answer.

Related to the suggestion that ADH release is coupled to SON neuronal activity is the observation that SON units were significantly more likely than AH units to be of the intermittent variety, shutting off for periods of 10-30 seconds. These cells were often seen to shut off for a while, then gradually build up to a very high frequency of

firing before gradually slowing down to a standstill again. No previous study, to my knowledge, has reported such a consistent finding for SON units. Strangely, most experimenters have reported little or nothing at all about firing patterns. Either this intermittent pattern has been overlooked previously, or it is peculiar to SON cells of acutely water deprived rats. One possible interpretation for the phenomenon is that ADH is released in bursts from some SON terminals of the neuropophysis; a critical frequency might even have to be reached before any hormone is released at all. Whatever the interpretation, this finding presents another way to dichotomize SON neurons--those with intermittent firing patterns vs. those which fire steadily. An explanation for the fact that a small number of AH cells were intermittent might be provided by the study of Peterson (1966), who showed that neurosecretory type cells exist in various hypothalamic areas. It is also thought that cells producing anterior pituitary releasing factors may be scattered throughout the hypothalamus (Ganong, 1969). Intermittent AH cells could possibly have been some such secretory neurons, or they may have been true SON cells scattered above the main nuclear complex. The criterion for judging whether or not a unit was intermittent may also have spuriously increased the number of intermittent AH units. Many of these cells had extremely slow base firing rates. This greatly enhanced the probability that they would stop firing during at least one ten second interval and be classified as intermittent.

Finally, and perhaps most importantly, is the relationship between SON unit activity and the major stimuli for ADH release--increased effective osmotic pressure (EOP) of the plasma (causing cellular dehydration) and decreased ECF volume. The data of Experiment II revealed that plasma osmotic pressure continued to increase for four days before stabilizing, while both SON unit activity and serum protein concentration increased for just one day and then remained relatively constant. This finding supports the notion that SON cells were responding in direct proportion to a volume depletion stimulus and agrees with a study by Stricker (1969) which showed the excretion of a water load to be inhibited in direct proportion to the degree of hypovolemia induced by subcutaneous injection of polyethylene glycol. Also, the discovery of volume sensitive afferents from the low pressure side of the circulatory system (Henry, Gauer, & Reeves, 1956; Henry, et. al., 1968) and the fact that small hemorrhage can release ADH (Arndt, 1965; Henry, et. al., 1968) both lend additional credence to such a suggestion.

In contradiction stands evidence indicating that osmotic stimuli may be more potent than volume stimuli in activating hypothalamic neurons (Menninger & Frazier, 1972) and in releasing ADH (Moses, 1963; Forsling, Martin & Burton, 1971). A possible explanation for this discrepancy is that plasma or serum EOP, rather than the total osmotic pressure as revealed by osmolality, must be increased before SON cells are osmotically activated to fire and ADH is released.

Although plasma sodium concentration could not be determined for recording animals, the serum sodium function of Experiment I showed no significant changes with five days of deprivation. If anything, there was a drop in serum sodium at day two. Since the sodium concentration of the blood is an excellent correlate of EOP (Welt, 1965, p. 768) one might assume that the SON was receiving little effective osmotic stimulation under the "natural" condition of water deprivation. Thus, SON cells may well have been responding primarily to volume depletion stimuli rather than to cellular dehydration.

A different interpretation is afforded by the fact that there seems to be a level of ADH secretion which produces maximal antidiuresis (Gauer & Tata, 1966). It is possible that the maximal secretory rate necessary of SON cells was reached after just one day of acute water deprivation, so there was no need for further firing rate increases. Nevertheless, Experiment II provides circumstantial evidence that, under normal conditions, volume stimuli may be more important activators of SON cells, and possibly ADH release, than was previously thought.

To summarize Experiment II, it was found that the mean SON cell firing rate increased after one waterless day and stayed near that level for the remaining four days of water deprivation. This paralleled ADH levels found by others in the urine of rats during a similar time span and adds circumstantial evidence to the hypothesis that ADH

release is related to SON cell firing rates. Many SON cells fired intermittently, suggesting that ADH may be released in bursts. SON cells apparently responded more to volume than to osmotic stimuli. Meanwhile, control AH cells outside the SON showed no significant rate changes with deprivation, although they were found to fire faster than SON cells when a large, non-independent sample was obtained.

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APPENDIX A:

Apparatus

Equipment, Suppliers, and Unit Prices

Body metabolism recording

1. Individual metabolism cage with base, Acme Metal Products, $45.00.
2. Animal balance, Ohaus, $74.75.

Sampling and analysis of blood and urine

1. Rat guillotine, Stoelting Co., $80.00.
2. Refractometer, American Optical, $125.00.
3. Freezing point osmometer #2007, Precision Systems Inc., $1495.00.
4. Flame photometer #143, Instrumentation Laboratories, $2390.00.
5. Centrifuge, International Equipment Co., $270.00

Microelectrode manufacture

1. Stainless steel tubing (26 g., .018" I.D. with .00425" wall), Superior Tube Co., $90.81/1000 pcs.
2. Tungsten wire (.005" diam.), Sylvania Corp., $10.00/reel.
3. Glass tubing (.032" O.D., .006" wall), Corning Glass, $45.00/3 lbs.
4. Pipett puller, David Kopf Instruments, $425.00.
5. Nikon stereozoom dissecting microscope, E. H. Sargent & Co., $467.00.
6. Unitron microscope #42024, E. H. Sargent & Co., $135.00.

Bioelectric data recording and analysis

1. Stellar stereotaxic instrument, Stoelting Co., $750.00.
2. Preamplifier #122, Tektronix Inc., $165.00.
3. Power supply #125, Tektronix Inc., $335.00.
4. Dual beam oscilloscope #502A, Tekronix Inc., $1365.00.

5. Magnecord tape recorder #1028, Main Electronics, $1196.00.

6. Electronic frequency counter #5512A, Hewlett Packard, $1050.00.

7. Digital printer, Digitron Corp., $988.00.

8. Audiomonitor #AM8 with speaker, Grass Instruments, $157.00.

9. Thermister thermometer, Yellow Springs Instruments, $124.00.

10. Magnetic Tape (1.5 mm. acetate, 1/4" x 2500'), 3M Company, $6.96/reel.

11. Lesion maker, Psychology Department Electronics Shop.

12. Dental drill #18 with foot pedal, Buffalo Dental Mfg. Co., $93.00.

APPENDIX B:

Raw Data

Raw Data - Experiment I

Food Intake (g.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
19	13	8	3	1	2
22	15	7	2	2	3
20	14	10	4	4	2
20	12	9	5	5	1
19	14	7	3	3	1
19	16	8	4	1	3
Mean					
19.83	14.00	8.17	3.50	2.67	2.00
S.E.					
±0.48	±0.58	±0.48	±0.43	±0.67	±0.36

Urine volume output (ml.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
19.0	8.0	4.0	3.0	4.0	1.5
10.0	9.0	6.0	3.5	4.5	2.0
10.0	8.0	5.0	3.0	2.5	4.0
11.0	9.0	4.5	2.0	1.0	1.0
8.0	10.0	6.0	2.0	2.0	1.0
17.0	9.0	4.0	4.0	4.0	2.0
Mean					
12.50	8.83	4.92	2.92	3.00	1.92
S.E.					
±1.803	±0.31	±0.38	±0.33	±0.56	±0.46

Urine Na concentration (mEq./L.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
142	368	384	182	32	85
270	364	240	108	74	198
244	336	127	186	100	17
316	314	306	139	192	108
260	348	256	232	50	34
170	364	307	240	29	104
Mean					
233.67	349.00	270.00	181.17	79.50	91.00
S.E.					
±26.68	±8.58	±35.22	±20.98	±25.02	±26.25

Raw Data - Experiment I (cont'd)

Urine K concentration (mEq./L.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
125	280	372	397	256	328
235	250	257	305	278	326
262	266	111	313	299	262
247	222	304	356	376	269
200	233	268	392	320	206
154	261	303	348	210	303
Mean					
203.83	252.00	269.17	351.83	289.83	282.33
S.E.					
±22.309	±8.81	±35.64	±35.09	±23.15	±19.01

Urine K output (mEq./day)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
2.38	2.24	1.49	1.19	1.02	0.49
2.35	2.25	1.54	1.07	1.25	0.65
2.62	2.13	0.56	0.94	0.75	1.05
2.72	2.00	1.37	0.71	0.38	0.67
1.60	2.33	1.61	0.78	0.64	0.62
2.62	2.35	1.21	1.39	0.84	0.61
Mean					
2.38	2.22	1.30	1.01	0.81	0.68
S.E.					
±0.17	±0.06	±0.16	±0.10	±0.12	±0.08

Urine Na output (mEq./day)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
2.70	2.94	1.54	0.55	0.13	0.13
2.70	3.28	1.44	0.38	0.33	0.40
2.44	2.69	0.64	0.56	0.25	0.68
3.48	2.83	1.38	0.28	0.19	0.27
2.08	3.48	1.54	0.46	0.10	0.10
2.89	3.28	1.23	0.96	0.12	0.21
Mean					
2.72	3.08	1.30	0.53	0.19	0.30
S.E.					
±0.19	±0.13	±0.14	±0.10	±0.04	±0.09

Raw Data - Experiment I (cont'd)

Urine total solids

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
1.3436	1.3560	1.3416	1.3640	1.3596	1.3696
1.3528	1.3506	1.3554	1.3670	1.3614	1.3610
1.3540	1.3538	1.3582	1.3642	1.3714	1.3676
1.3500	1.3538	1.3644	1.3676	1.3626	1.3570
1.3502	1.3530	1.3590	1.3622	1.3674	1.3636
1.3450	1.3514	1.3606	1.3694	1.3604	1.3690
Mean					
1.3492	1.3531	1.3565	1.3657	1.3638	1.3646
S.E.					
±0.0017	±0.0008	±0.0032	±0.0011	±0.0019	±0.0025

Percent body weight loss

	Day-1	Day-2	Day-3	Day-4	Day-5
	5.5	8.8	11.5	17.1	17.8
	4.0	9.8	13.2	16.1	18.5
	4.5	8.0	11.6	16.0	21.8
	5.4	9.1	11.7	15.8	19.2
	5.5	10.1	11.8	15.7	20.9
	5.1	10.3	11.2	17.6	17.4
Mean					
	5.00	9.35	11.83	16.38	19.27
S.E.	±0.25	±0.36	±0.29	±0.32	±0.72

Serum K concentration (mEq.L.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
6	7	6	7	6	5
6	8	6	7	5	6
8	6	7	5	7	6
7	6	10	4	5	5
7	6	7	5	5	6
7	6	6	5	5	5
Mean					
6.83	6.50	7.00	5.50	5.50	5.50
S.E.					
±0.31	±0.34	±0.63	±0.50	±0.34	±0.22

Raw Data - Experiment I (cont'd)

Serum Na concentration (mEq.L.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
137	146	134	138	138	141
137	139	136	142	136	141
140	140	136	139	145	138
141	137	136	118	139	135
146	144	137	146	142	138
139	147	133	142	139	127
Mean					
140	142	135	138	140	137
S.E.					
±1.4	±1.7	±0.6	±4.1	±1.3	±2.1

Serum protein (g./100 ml.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
6.7	6.7	6.7	6.9	6.8	6.8
6.4	6.8	6.8	6.7	6.6	6.6
6.7	6.9	6.4	7.4	6.8	6.7
5.9	6.6	7.3	7.0	7.1	6.9
6.1	6.4	6.8	7.4	6.5	6.9
6.1	7.1	6.9	7.6	6.9	6.9
Mean					
6.3	6.8	6.8	7.2	6.8	6.8
S.E.					
±0.1	±0.1	±0.1	±0.1	±0.1	±0.1

Serum osmolality (mOsm./kg.)

Day-0	Day-1	Day-2	Day-3	Day-4	Day-5
292	295	295	300	305	306
284	290	300	294	305	304
287	292	308	299	298	307
290	294	302	298	311	299
287	302	294	311	303	307
287	290	298	310	311	310
Mean					
288	294	300	302	306	306
S.E.					
±1.2	±1.8	±2.1	±2.8	±2.0	±1.5

Raw Data - Experiment II

Sub. #	Days of Water Dep.	O.P.	Prot.	% B.W. loss	Unit Number	Mean Rate	Intermit.	Loc.
12	0	292	4.9	----	1	4.00	no	SON*
					2	1.36	no	AH*
					3	19.90	no	AH
18	0	290	5.9	----	1	8.50	no	SON
					2	8.52	yes	SON*
20	0	292	5.3	----	1	14.24	no	AH*
					2	0.12	yes	AH
					3	1.77	no	AH
					4	5.80	no	AH
					5	16.22	no	AH
23	0	294	5.3	----	1	2.38	yes	SON
					2	2.16	yes	SON*
					3	0.94	no	AH*
					4	6.98	no	AH
25	0	288	6.3	----	1	5.56	no	AH*
					2	9.48	no	AH
26	0	301	5.4	----	1	33.87	no	AH
					2	4.91	no	AH*
					3	0.95	yes	AH
28	0	295	5.4	----	1	7.80	no	AH*
					2	2.42	yes	SON*
29	0	288	5.2	----	1	3.25	no	AH*
54	0	301	5.2	----	1	0.21	yes	SON
					2	3.97	yes	SON*
					3	19.21	no	AH*
58	0	292	5.2	----	1	2.47	yes	AH*
9	1	304	6.6	7.4	1	8.17	yes	SON*
13	1	310	6.2	5.5	1	0.18	yes	AH*
					2	4.48	yes	SON*
					3	30.46	no	AH
					4	12.08	no	AH
					5	21.68	no	AH
					6	9.19	no	AH
					7	16.72	no	AH
14	1	300	5.8	5.4	1	7.50	yes	SON*
					2	7.40	no	AH*
19	1	292	6.4	3.6	1	1.28	no	AH*
					2	8.17	yes	SON*
					3	9.55	no	AH
					4	2.26	no	SON
21	1	307	6.3	5.6	1	7.05	no	AH
					2	0.63	no	AH*
					3	7.91	no	SON*
22	1	299	6.2	4.7	1	4.87	no	AH
					2	27.22	no	AH

Raw Data - Experiment II (cont'd)

Sub. #	Days of Water Dep.	O.P.	Prot.	% B.W. loss	Unit Number	Mean Rate	Intermit.	Loc.
					3	0.72	no	AH
					4	2.07	yes	AH*
24	1	300	6.2	5.2	1	6.85	no	AH
					2	7.34	no	SON
					3	6.78	no	SON*
					4	0.22	yes	AH
					5	16.51	no	AH*
30	2	304	6.6	8.8	1	5.68	no	AH
					2	2.36	no	AH
					3	11.04	no	AH*
31	2	310	7.1	8.5	1	17.93	no	AH
					2	7.94	no	AH*
					3	6.07	no	AH
32	2	306	6.5	8.5	1	9.04	no	AH*
33	2	---	---	6.2	1	1.04	no	AH*
					2	8.32	no	AH
34	2	314	6.6	9.1	1	18.34	no	AH
					2	7.02	no	AH*
					3	7.09	no	SON
					4	3.36	yes	SON*
					5	4.59	no	AH
35	2	298	6.2	8.3	1	4.11	yes	SON
					2	33.05	no	AH*
					3	10.86	no	SON*
					4	8.47	yes	SON
53	2	317	6.4	7.9	1	6.74	no	SON*
					2	1.29	yes	SON
					3	5.51	no	AH*
36	3	316	7.2	9.6	1	16.36	no	AH*
37	3	315	6.9	9.4	1	10.83	no	SON*
39	3	316	6.3	12.2	1	4.59	no	SON*
					2	7.55	no	AH*
					3	10.86	no	AH
40	3	309	6.3	12.3	1	14.11	no	AH*
46	3	299	6.5	10.3	1	4.41	yes	SON*
47	3	310	6.0	11.3	1	2.77	no	AH*
					2	0.06	yes	AH
					3	6.64	yes	SON*
49	3	308	6.3	8.8	1	18.06	no	AH*
					2	10.11	no	AH
					3	0.38	yes	AH
					4	5.29	no	SON*

Raw Data - Experiment II (cont'd)

Sub. #	Days of Water Dep.	O.P.	Prot.	% B.W. loss	Unit Number	Mean Rate	Intermit.	Loc.
50	3	---	5.9	10.1	1	6.89	yes	SON
					2	2.52	no	AH*
					3	5.31	yes	SON*
					4	28.96	no	AH
					5	7.75	no	SON
					6	2.06	yes	AH
38	4	---	---	14.0	1	2.78	no	AH*
44	4	318	5.7	14.8	1	2.76	no	SON*
					2	0.13	yes	AH*
					3	4.94	no	SON
					4	10.96	no	SON
					5	3.33	yes	SON
					6	2.43	yes	SON
48	4	---	6.5	14.8	1	2.74	no	AH*
					2	6.48	yes	SON*
51	4	319	6.3	15.3	1	4.87	yes	AH
					2	14.63	no	AH*
56	4	320	6.3	13.2	1	10.58	yes	SON
					2	13.23	no	SON*
					3	6.45	no	AH*
					4	8.33	yes	SON
42	5	323	6.6	14.4	1	4.88	yes	SON*
					2	2.53	no	AH
					3	15.34	no	AH*
45	5	317	6.0	16.4	1	8.14	no	AH
					2	14.86	no	AH
					3	9.36	no	AH*
					4	6.45	no	AH
52	5	324	5.8	15.5	1	5.95	no	SON
					2	10.00	no	AH
					3	6.10	no	SON*
					4	34.59	no	AH
					5	13.04	no	AH*
					6	1.15	no	AH
55	5	324	6.2	14.4	1	8.11	no	SON*
					2	10.34	no	AH*
57	5	320	5.6	13.6	1	2.46	yes	AH*
					2	14.08	no	AH
					3	9.39	no	AH
59	5	316	5.7	13.7	1	3.52	no	AH*
					2	3.11	no	SON*
					3	2.15	no	SON

* Units used for independent determinations of mean rate.

APPENDIX C:

Histology

Figure 14. Shown is a photomicrograph of a normal cresyl violet stained coronal section through a rat's supraoptic nucleus. This 40 µm. section is approximately 8.2 mm. anterior to ear bar zero when the skull is positioned horizontally. SON: supraoptic nucleus, PV: paraventricular nucleus, FX: fornix, CO: optic chiasm, V: ventricle III, bv: blood vessel.

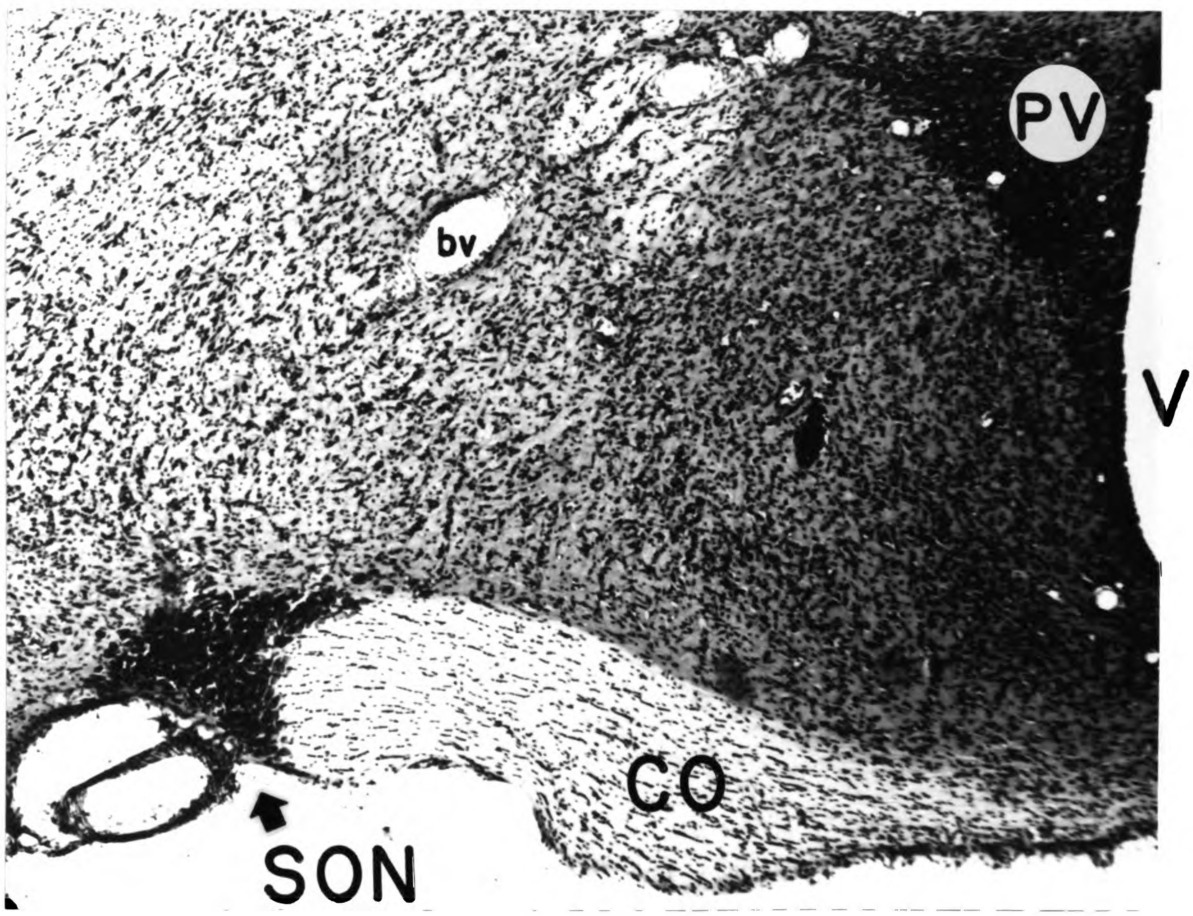
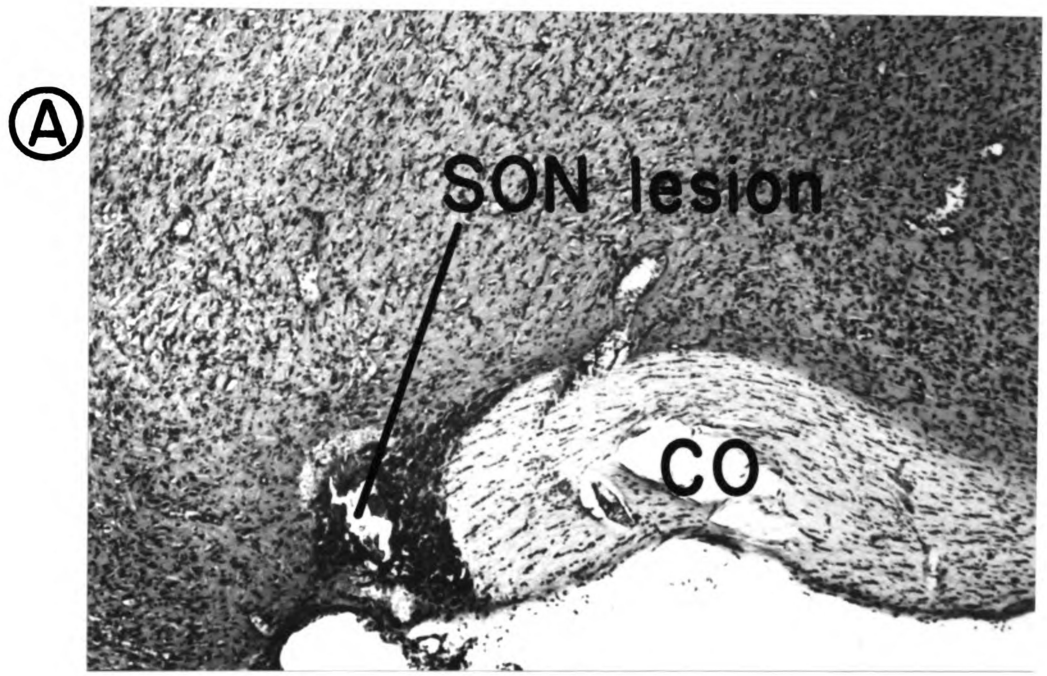

PV
bv
V
CO
SON
0.5 mm

Figure 15. Panel A shows a photomicrograph of a cresyl voilet stained coronal section from an animal contributing a supraoptic unit. Indicated is one marking lesion within SON revealing a recording site. SON: supraoptic nucleus, CO: optic chiasm.

Panel B shows a similar photomicrograph from a recording animal which contributed AH units. Indicated are two marking lesions in the anterior hypothalamus directly above SON which show control recording sites. AH: anterior hypothalamus, CO: optic chiasm.



0.5 mm

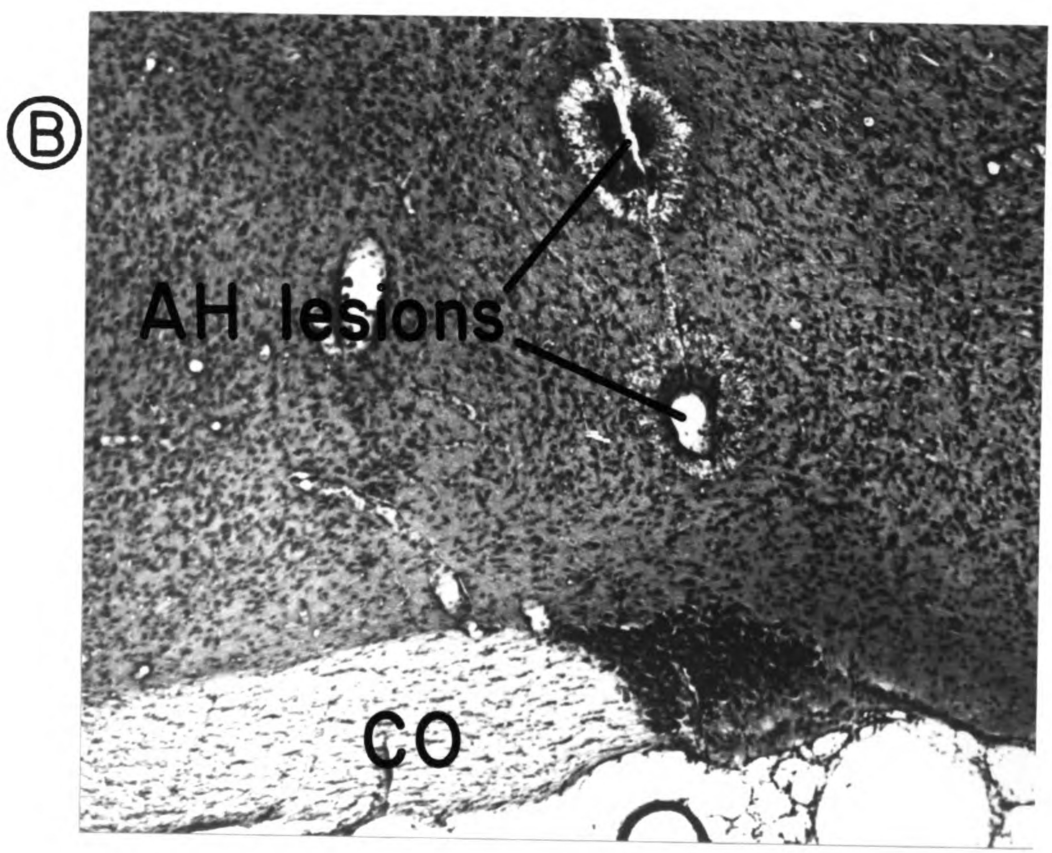


Figure 16. Shown is the same schematic representation of SON as in Figure 11. Refer to that figure for abbreviations. Nearly all AH units were located within one and one-half mm. of SON in the dorsal and anterior directions and within less than one mm. of SON in the medial and lateral directions. Stipling on two of the sections exemplifies the area, though AH units were <u>not</u> confined solely to the planes of those two sections. AH and SON units were recorded on both sides of the brain. The number of units located at each of these planes of section was as follows:

posterior to SON	(0 SON 0 AH)
7.8 ±0.2 mm.	(0 SON 5 AH)
8.2 ±0.2 mm.	(25 SON 29 AH)
8.6 ±0.2 mm	(20 SON 21 AH)
9.0 ±0.2 mm.	(1 SON 19 AH)
anterior to SON	(0 SON 9 AH)
TOTAL	46 SON 83 AH