EFFECTS OF REPEATED INFUSIONS OF HYPERTONIC SALINE SOLUTIONS ON THE ELECTRICAL ACTIVITY OF HYPOTHALAMIC NEURAL UNITS OF GOATS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
LARRY WILLIAM THORNTON
1969

This is to certify that the

thesis entitled

EFFECTS OF REPEATED INFUSIONS
OF HYPERTONIC SALINE SOLUTIONS ON
THE ELECTRICAL ACTIVITY OF
HYPOTHALAMIC NEURAL UNITS OF GOATS

presented by

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has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Psychology

Glenn I. Hatton
Major professor

Date 22 December 1969

O-169

ABSTRACT

EFFECTS OF REPEATED INFUSIONS OF HYPERTONIC SALINE SOLUTIONS ON THE ELECTRICAL ACTIVITY OF HYPOTHALAMIC NEURAL UNITS OF GOATS

BY

Larry William Thornton

Areas of the hypothalamus have been shown to participate in parts of the motor sequence leading to water intake, and to underlie motivational components of thirst leading to the acquisition of an instrumental response sequence. Under some circumstances, the internal condition of an animal is in an adequate state to elicit drinking behavior, but the animal does not have immediate access to water. Maintained high blood NaCl concentration is related to a state of prolonged water need. This study investigated some of the characteristics of the internal mechanism that is sensitive to prolonged water need. In order to learn more about the mechanism, within the hypothalamus, that signals the need for water, electrical activity of single cells was recorded and analyzed.

To determine the long term effects of high levels of blood NaCl concentrations on the electrical activity of hypothalamic osmosensitive neural units of goats, spike discharge activity of hypothalamic units was recorded after each of three infusions of 50cc of 16% NaCl solutions. Eight neural units showed a decrease in spike discharge frequency, while two units showed an increase in spike discharge frequency following an increase in blood NaCl concentration. All osmosensitive units showed a response to an increase in blood NaCl

concentration which persisted after an increment in blood salinity. Five of the neural units showed a graded response of long duration to increments in blood NaCl concentration. Eight of the hypothalamic neural units showed a response to water loaded into the stomach of the goats.

If the cells sampled in this study participate in the control of drinking behavior, then the results of this experiment indicate that the hypothalamic activity signaling the need for water does not adapt over prolonged periods of thirst. Also, the activity of some of the hypothalamic cells may signal different degrees of thirst. Finally, the activity of some of the hypothalamic cells alter their activity when water need is reduced.

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A THESIS

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

DOCTOR OF PHILOSOPHY

Department of Psychology

1969

ACKNOWLEDGEMENTS

I express deep appreciation to: Jeffrey Licht, Earl Walker, and Mark Wiltrakis to whom I'm indepted for their assistance throughout the research project; to Peter Bates, Edwin Rubel, and John Wright whose assistance was invaluable throughout a large part of the experiment; to C. Robert Almli, C. Thomas Bennett, G. I. Hatton, Russel Newell, and Andrew Harton who rendered assistance when extra help was necessary; and the Department of Psychology and Laboratory of Comparative Neurology who provided support to carry out the research.

I express thanks to: Dr. Glenn I. Hatton who served as chairman of the thesis committee and guided my graduate program; to Dr. Lawrence O'Kelly, Dr. Ralph Pax, and Dr. Robert Raisler who served on the thesis committee.

I express special thanks to: Dr. John I. Johnson who provided support and time to make it possible for me to reify the idea into the deed; and to Sandy Thornton who baked cakes for the midnight watch, extracted brains, did the histology, and typed the many versions of the thesis.

I dedicate this thesis to the Goat God.

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Introduction

Verney (1947) and Andersson (1952) showed that areas in the anterior hypothalamus participate in the regulation of intake and output of water. Since their studies, many different experimental approaches have been used to locate and study the effects of hypertonic solutions on the water regulatory system in different animals.

B. Andersson observed drinking behavior after infusing small amounts of NaCl solutions into the brain, electrically stimulating cells in different parts of the hypothalamus, or lesioning parts of the hypothalamus. Andersson et al. (1951, 1952, 1953) has demonstrated that drinking behavior is elicited in goats when NaCl solutions are placed in direct contact with cells around the paraventricular nucleus of the hypothalamus. Also, Andersson (1955) has shown that drinking behavior can be elicited in goats when areas between the fornix and the mammillothalamic tract of the hypothalamus are electrically stimulated. Lesions of hypothalamic areas between the fornix and mammillothalamic tract were followed by partial or complete adipsia in goats and dogs (Andersson, 1957). Goats acquire a "new" motor sequence (climbing stairs) to get access to water while being electrically stimulated in the hypothalamus (Andersson, 1956). These studies by Andersson show that there is a neural substrate in the hypothalamus of the goat underlying parts of the motor sequence leading to water intake, and underlying motivational components of thirst leading to the acquisition of an instrumental response sequence.

Andersson's research is designed to study the effects of electrical and osmotic stimulation of the hypothalamus on drinking behavior when the goat is given immediate access to water. Under some circumstances, the internal condition of an animal is in an adequate state to elicit drinking behavior, but the animal does not have immediate access to water. Studies in the literature suggest that changes in blood NaCl concentration continue to affect drinking behavior of animals when access to water is delayed after the adequate stimulus that usually results in drinking behavior. When rats are not given access to water for six hours after either a lcc injection of 16% NaCl or a .87% NaCl solution, they approach water more rapidly and drink more water after the 16% injection than after the .87% injection (Thornton, 1966). Fitzsimons (1963) demonstrated that there is no difference in the change in body weight after drinking between rats who had immediate access to water after a hypertonic injection and rats who had access to water 24 hours after a hypertonic injection. Both studies suggest that there are internal mechanisms that are sensitive to water need over periods of delayed access to water. The study of electrical activity of hypothalamic cells in the neuralsubstrate underlying drinking behavior (as defined by the results of Andersson's research) is an indicator of the characteristics of the internal mechanisms that are sensitive to prolonged water need.

Other studies are designed to locate and study electrically active neural units in the anterior hypothalamus while infusing hypertonic solutions into the carotid artery (Cross and Green, 1959; Joynt, 1964). These studies sampled the electrical activity of osmosensitive neural units over short periods of time (14 seconds to 60 seconds). The present experiment was designed to sample the electrical activity of neural units over longer periods of time and to repeat the infusions several times. The experiment represents an initial effort to furnish information on the effects of changes in blood salinity on the electrical activity of neural units in the goat anterior hypothalamus and to assess possible changes in the electrical response of neural units over extended periods of time after repeated infusions of hypertonic saline solutions.

Specifically, this study is centered around the characteristics of direction, gradation, and duration of the electrical response of osmosensitive neural units before, during, and for an extended period of time after changes in blood salinity.

Direction refers to an increase and/or decrease of the electrical response (spikes/unit time) after an infusion of hypertonic saline solution.

The gradation of electrical responses to increases in blood salinity was proposed by Von Euler (1953) as a criterion for an osmosensitive nerual unit located in the anterior hypothalamus. Gradation is a term referring to the effect on the electrical responses after repeated infusions of hypertonic saline solutions. If the electrical response of an osmosensitive unit is graded to increments in blood NaCl concentrations, then each successive infusion of saline solution should affect the electrical response of the neural unit in steps. Each step should be in the same direction and of greater magnitude than the previous step. Cross and Green (1959) and Joynt (1964) report that the electrical responses of osmosensitive neural units in the anterior hypothalamus of rabbits and cats show the characteristic of gradation after repeated infusions of hypertonic saline solutions.

Transitoriness is a term referring to the duration of the observed electrical response of the osmosensitive neural unit after an infusion of hypertonic saline solution. If the response changes during the infusion and then returns to preinfusion level, then the electrical response is characterized as transitory. A modification of this transitory characteristic is a residual effect. A residual effect is an electrical response that is less than the initial transitory effect but greater than the pre-infusion level and persists after the increment in blood NaCl concentration. Cross and Green (1959) and Joynt (1964) indicate that increases of blood salinity have transitory effects on the electrical responses of osmosensitive neural units in the anterior hypothalamus. Their results might be due to the small amounts of NaCl solutions that they infused into the carotid artery during each stimulation.

In this experiment, more NaCl was infused during each stimulation, and each infusion was repeated three times for each osmosensitive neural unit under observation. If a certain increment in blood NaCl concentration is necessary before the electrical response is nontransitory, then pushing the neural unit to its limits should increase the probability of a longer lasting electrical response to increases in blood NaCl concentration.

Threshold is defined as the amount of infused NaCl solution needed to cause a lasting electrical response. Von Euler (1953) proposed evidence of threshold as a criterion for an osmosensitive neural unit in the anterior hypothalamus.

The purpose of this experiment is to determine if goat osmosensitive neural units in the anterior hypothalamus have a graded electrical response after repeated increases in blood salinity, and, if the electrical response to an increase in blood salinity is transitory when activity is sampled over a long period of time. Goats were used in the experiment to extend Andersson's (1952) findings that osmosensitive areas are located in the hypothalamus of goats.

Method

Subjects

Information for this study was obtained from five male domestic goats (*Capra hircus*, Toggenburg strain). The goats were obtained from a goat herd maintained by the Endocrine Research Unit at Michigan State University. They weighed (40.7, 36.2, 33.0, 33.6, and 32.6 kg.). Their age was between one and two years. The goats were food deprived the day before surgery.

The animals were initially anesthetized intraperitoneally with dial-urethane (157 mg of urethane and 49 mg of diallybarbituric acid per kilogram of body weight). One-half hour prior to the dial-urethane administration, the animals were injected with 1cc (50 mg) of promazine hydrochlorine (Sparine®). Throughout an experiment, additional dial-urethane anesthesia was limited to one-fourth of the original dosage every eight hours. Sodium pentobarbital (20 mg/kg) was used when anesthesia was required during the eight hour interval between dial-urethane injections. The animals were tracheotomized and maintained on artificial respiration throughout the experiment. After the head was cemented into a holder, the left neo-cortex was exposed for access. The left carotic artery was cannulated with Intramedic® polyethylene #90 tubing which was filled with heperinized physiological saline and connected to a constant infusion pump. The animals' rectal temperature and heart rate were monitored throughout the experiment. The animals' temperatures varied between 33-36°C during the experiments.

Apparatus

Glass insulated tungsten microelectrodes were used for recording. The recording electrodes were modifications of the Hubel (1957) tungsten microelectrode. The electrodes were uninsulated for 30 to 100 microns from the tip. The shaft diameter was 40 to 80 microns at a point one millimeter from the tip of the electrode. The electrode signals were amplified by an A. C. preamplifier and the vertical amplifier of the oscilloscope. During the experiment the signals were recorded on magnetic tape. After the completion of the experiment, the signals were converted to standard pulses and put into a small computer which gave the number of spikes per 10 seconds.

Procedure

Electrodes were driven manually by means of a micromanipulator through the opening in the skull. When the electrode was presumed to arrive at a site in the hypothalamus, a unit was selected whose spike activity seemed to be relatively stable. Baseline activity was recorded and infusions began.

To determine if the relationship between increasing blood tonicity and neural unit activity was graded, 50cc of 16% sodium chloride solution was infused at a rate of 5cc/minute into the carotid artery three times in sequence, with 50 minutes between each infusion. A magnetic tape record was taken of the electrical activity before, during, and immediately after the infusion, and 20 minutes after the completion of the infusion. The independent variable was the amount of saline infused while recording from the neural unit. The dependent variable was the total number of spikes per 10 seconds during and after the infusion of the saline. The same procedure was used to determine if the neural unit response to the infusion of saline was transitory. The overall procedure for each unit consisted of three infusions of 50cc of 16% saline solution followed by water loaded into the stomach after the series of three infusions. Magnetic tape records were taken of the neural unit electrical activity before, during, after each infusion, and after 3 liters of water were placed in the stomach.

Data Analysis

Eight records of electrical activity were recorded on tape for each hypothalamic neural unit. Each record was for a certain length of time, according to the schedule below. There was a period of time between each record when nothing was recorded. Refer to Figure 1 for a diagram of the following schedule. The periods of recording and non-recording for each neural unit were as follows:

1. A 15 minute period of recording followed by a 5 minute period of non-recording.

Record 1 is referred to as the pre-infusion period.

2. A 10 minute period of recording, during which 50cc of 16% NaCl solution was infused during the first 10 minutes, followed by a 15 minute period of non-recording.

Record 2 is referred to as the first infusion period.

3. A 10 minute period of recording followed by a 5 minute period of non-recording.

Record 3 is referred to as the post-first-infusion period.

4. A 10 minute period of recording, during which 50cc of 16% NaCl was infused, followed by a 15 minute period of non-recording.

Record 4 is referred to as the second infusion period.

5. A 10 minute period of recording followed by a 5 minute period of non-recording.

Record 5 is referred to as the post-second-infusion period.

6. A 10 minute period of recording, during which 50cc of 16% NaCl was infused, followed by a 15 minute period of non-recording.

Record 6 is referred to as the third infusion period.

7. A 10 minute period of recording followed by the period of time needed to load 3 liters of water into the stomach of the goat.

Record 7 is referred to as the post-third-infusion period.

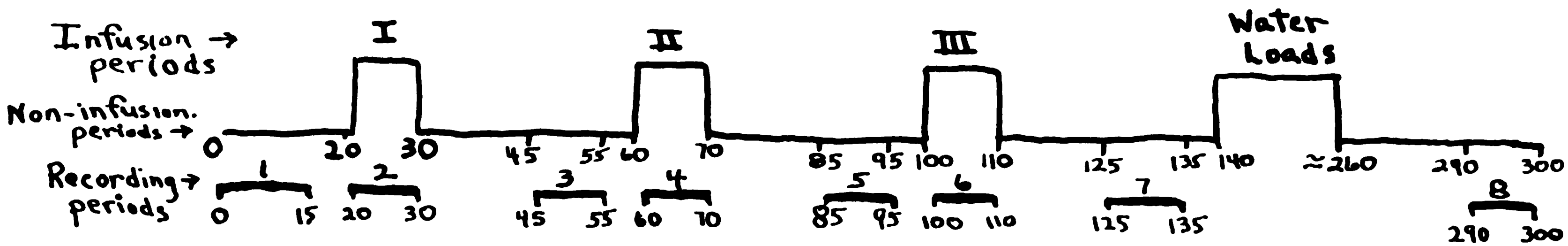
8. A 10 minute period of recording.

Record 8 is referred to as the post-water-load period.

The electrical activity from each of the tape records of the periods listed above was put into a Computer of Average Transients. The computer was programmed to count the number of unit discharges occuring in successive ten second intervals. The computer only counted unit spike discharges of a predetermined amplitude. The amplitude was determined so that only the spike activity under study was counted. The computer output was printed on paper by a tele-typewriter. Periods of recorded unit electrical activity that lasted for 15 minutes had 90 ten second intervals. Periods of recorded electrical activity that lasted for 10 minutes had 60 ten

Figure 1 shows the experimental design for each hypothalamic neural unit. Infusion periods and non-infusion periods are super-imposed on the time (minutes) of the experiment on the neural unit. I, II and III refer to the periods of infusion of 50cc of 16% NaCl solution into the carotid artery. 0, 20, 30, etc. refer to the successive minutes of the experiment. The period of time in which 3 liters of water was loaded into the stomach is represented at the right of the figure. Periods of recording the electrical activity of the neural unit is represented below the periods of infusion and non-infusion. The numeral 1 refers to the tape record before starting the series of infusions. The numerals 2, 4 and 6 refer to the tape records during the infusions. The numerals 3, 5 and 7 refer to the tape records after the infusions. The numeral 8 refers to the tape record after the completion of the water load.

Figure 1


Infusion periods →
I
II
III
Water Loads
Non-infusion periods →
0
20
30
45
55
60
70
85
95
100
110
125
135
140
≈260
290
300
Recording periods →
1
0
15
2
20
30
3
45
55
4
60
70
5
85
95
6
100
110
7
125
135
8
290
300

second intervals. The unit of observation in analyzing electrical activity is a ten second interval.

The dependent variable used in analyzing the electrical activity of the neural unit was the mean number of spike discharges per second during each ten second interval. The average discharge frequency was found by dividing the number of spike discharges occuring in a ten second interval by 10.

Results

The overall effects of repeated hypertonic saline infusions and stomach water loading on the discharge frequencies of ten hypothalamic neural units are shown in Figure 2. Figures 15 through 24 in Appendix B show the effects of repeated infusions and loading water into the stomach on the variability of spike discharge frequency of each unit. The figures are histograms showing the distribution of unit discharge frequency over each of the recorded samples of unit activity before, during, and after each infusion. They show the proportion of ten second intervals of the recorded sample of unit activity that had a certain discharge frequency specified in mean number of spikes per second.

The descriptive statistic used to compare the different treatment periods was the median discharge frequency. The average number of spike discharges per second during each ten second interval of a 10 or 15 minute treatment was found. The median discharge frequency refers to the particular mean spikes/second above which and below which fifty percent of the ten second intervals' discharge frequencies fell.

Table 1 lists the median discharge frequencies and semi-interquartile ranges of the pre-infusion electrical activity of ten hypothalamic units. Experiments were done on more than one unit in two of the goats; consequently the goats had a history of infusions and stomach loading prior to the observation of activity of some units. Five units were observed from goats who had previous infusions and stomach loading of water.

Figure 2 shows the overall effects of hypertonic saline infusions and stomach water loading on the spike discharge frequencies of ten hypothalamic neural units. Each panel represents a different neural unit. The median average spike frequency is represented on the vertical side of each panel. The different periods of recording are represented under the bottom panel. Each point within the panel represents the median average discharge frequency of a unit during each successive period of recording, i.e., pre-infusion, first infusion, post-first-infusion, second infusion, post-second-infusion, third infusion, post-third-infusion, post-water-load. Units are grouped so that similar behaviors are next to each other.

Figura 2

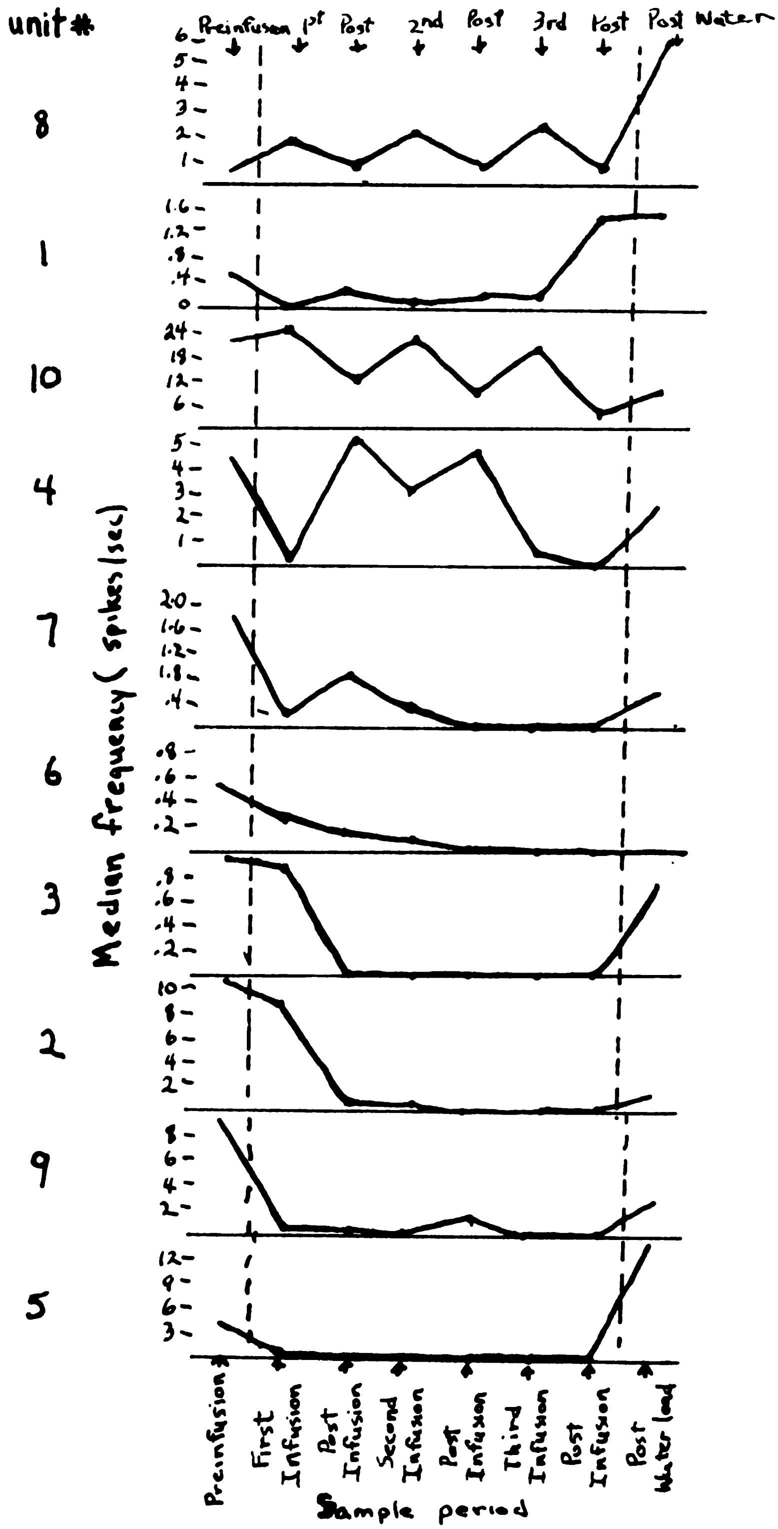

unit#
Preinfusion
1st
Post
2nd
Post
3rd
Post
Post Water
8
1
10
4
7
6
3
2
9
5
Median frequency (spikes/sec)
Preinfusion
First Infusion
Post Infusion
Second Infusion
Post Infusion
Third Infusion
Post Infusion
Post Water load
Sample period

Table 1

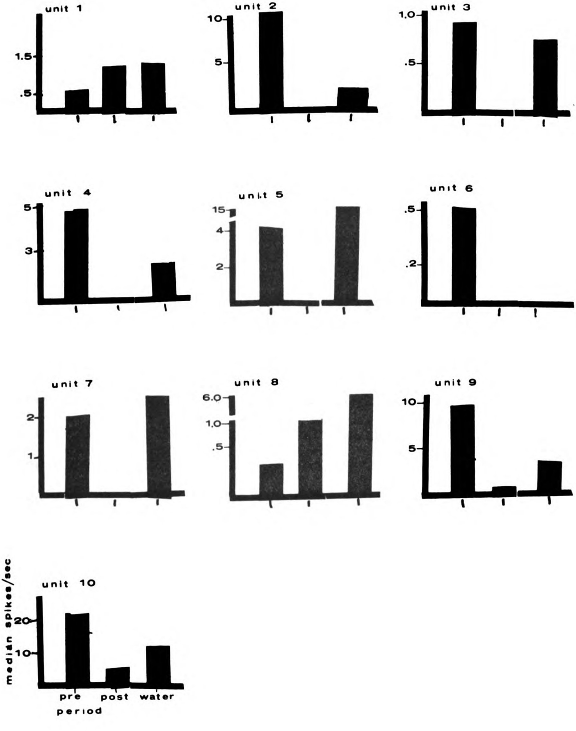
Medians and Semi-interquartile ranges of the pre-infusion electrical activity of ten hypothalamic neural units.

Unit number	Previous infusions and stomach loading into the goat.	Median ($\bar{x}$ spikes/sec.)	Semi-interquartile Range ($\bar{x}$ spikes/sec.)
1	No	.49	.18
2	No	10.28	.40
3	No	.93	.20
4	No	4.45	.26
5	Yes	4.19	.48
6	Yes	.52	.18
7	No	1.90	.32
8	Yes	.29	.16
9	Yes	9.20	1.26
10	Yes	21.18	2.51

The osmosensitivity of a neural unit was determined by comparing the median spike discharge frequency of the unit before the first infusion with the median spike discharge frequency after the third infusion. If the discharge frequency of a neural unit after the final increase in blood salinity was different from the pre-infusion unit discharge frequency, then the neural unit was considered an osmosensitive neural unit. Refer to Figure 3. Ten units were sampled All units were affected by the combined salt load of the three infusions.

Figure 3 shows the total effect of three infusions of hypertonic saline solution and the water load on the discharge frequency of each neural unit. The histograms show the median average firing frequency as a function of the treatment time that the electrical activity was sampled. Pre refers to the electrical activity of the unit before beginning the series of three infusions. Post refers to a ten minute record of activity after the completion of the three infusions. Water refers to a ten minute record of activity after the water load. The median refers to the particular $\bar{x}$ spikes/second above which and below which fifty percent of the ten second intervals' average discharge frequencies fell. For example, if the median firing frequency of unit 1 before infusions was .4 spikes/second, then fifty percent of the ten second intervals during the time of recorded activity had an average discharge frequency equal to or less than .4 spikes/second.

FIGURE 3


unit 1
1.5
.5
unit 2
10
5
unit 3
1.0
.5
unit 4
5
3
unit 5
15
4
2
unit 6
.5
.2
unit 7
2
1
unit 8
6.0
1.0
.5
unit 9
10
5
unit 10
20
10
median spikes/sec
pre
post
water
period

The direction of the effect of increased blood NaCl concentration on the discharge frequency was determined by seeing if the change in unit discharge frequency after the final increase in blood salinity was above or below the pre-infusion discharge frequency. Two units increased their discharge frequency, while eight units decreased their discharge frequency after the final increase in blood salinity.

To determine the duration of the effect of each infusion, the median average discharge frequencies before, during, and after each infusion were compared. Refer to Figure 4. Only effects which were in the same direction as the discharge frequency after the third infusion were considered to be due to increased salinity. If the change in discharge frequency during infusion was in the direction opposite to the total effect of the salt load on the unit (as indicated by the difference between pre-infusion discharge frequency and post-third-infusion discharge frequency) then the question of duration of saline effects could not be determined. If the change in discharge frequency during the infusion was in the same direction as the total effect of the salt load on the unit, but the discharge frequency of the unit returned to or above the pre-infusion discharge frequency after the termination of the infusion, the effect of the saline was considered transitory. If both the records of unit activity during infusion and after the infusion were in the same direction as the effect of the total salt load on the unit, the saline effect was considered to be non-transitory or of long duration. Table 2 lists the classification of each unit.

Figure 4 shows the duration of the effect of each infusion of hypertonic saline solution on the spike discharge frequencies of osmosensitive hypothalamic units. Each of the ten histograms represent the spike discharge activity of an osmosensitive hypothalamic unit. There are three triads of bars or medians in each histogram. Each triad represents the effects of a hypothalamic saline infusion on the discharge activity of the osmosensitive unit. The three bars of a triad represent the median average discharge frequency ($\bar{x}$ spikes/second) of the osmosensitive unit during different periods of recorded electrical activity. The first bar represents the median average discharge frequency during a period of time before the infusion. The second bar represents the median average discharge frequency during the infusion. The third bar represents the median average discharged frequency during a period of time after the termination of the infusion. The median refers to the particular value ($\bar{x}$ spikes/second) above which and below which fifty percent of the ten second intervals fell during the period of recorded activity. For example, if the median average discharge frequency of unit 2 for the period of recorded electrical activity occuring before infusion 3 was 0.0 spikes/second, then fifty percent of the ten second intervals during the period of recorded activity had an average spike discharge frequency equal to or less than 0.0 spikes/second. The arrow indicates the threshold infusion which resulted in an effect of long duration.

FIGURE 4a

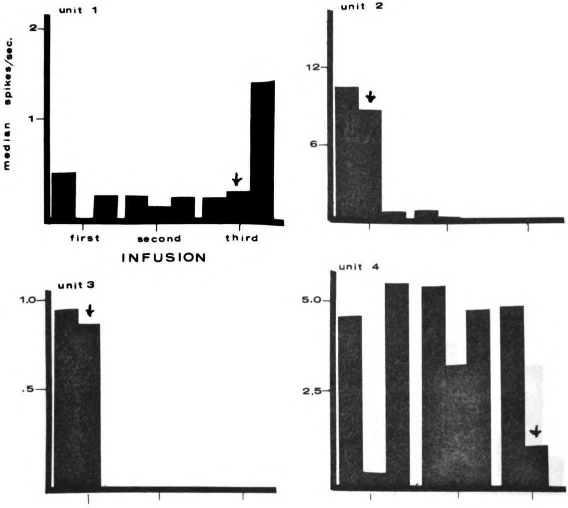

unit 1
2
1
median spikes/sec.
first
second
third
INFUSION
unit 2
12
6
unit 3
1.0
.5
unit 4
5.0
2.5

FIGURE 4b

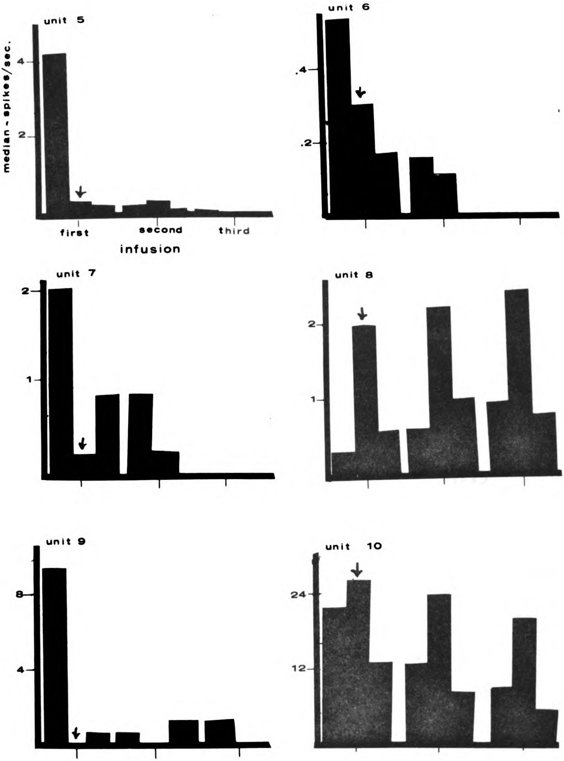

unit 5
median - spikes/sec.
4
2
first
second
third
infusion
unit 6
.4
.2
unit 7
2
1
unit 8
2
1
unit 9
8
4
unit 10
24
12

Table 2

Duration of saline effects due to each infusion. (The number under each category indicates the first, second, or third infusion.)

Unit #	Transitory	Non-transitory	Indeterminate
1		3	1,2
2		1,2,3	
3		1,2,3	
4	1,2	3	
5		1,3	2
6		1,2,3	
7		1,2,3	
8	3	1,2	
9	2	1,3	
10			1,2,3

The present experiment was designed to determine the effects of increased blood NaCl concentration for an extended period of time after the infusion. Infusion threshold is defined as the number of infusions of 50cc of 16% NaCl necessary to get a change in spike discharge frequency that persists after the termination of an infusion. The infusion which is followed by a change in spike discharge frequency after the termination of the infusion and which is in the same direction as the total salt load effect is called the threshold infusion. Refer to Figure 4. The infusion threshold was infusion one, for units # 2,3,5,6,7,8,9 and 10. The infusion threshold was infusion three, for units # 1 and 4.

Gradation of saline effects was determined by comparing the pre-infusion, post-first-infusion, post-second-infusion, and post-third-infusion discharge frequencies. In order to be considered graded, the spike discharge frequency of the unit, after each infusion, must be different from the saline effect after the previous infusion and in the same direction as the total salt load effect. The question of gradation is only considered after the threshold infusion that was followed by effects of long duration. Refer to Figure 5. The effects of repeated saline infusions are considered graded for five units - units 2,5,6,7 and 10. Unit 2,6 and 10, reached their limit of change before the completion of the series of infusions, but one infusion demonstrated gradation of saline effects. Unit 3 reached its limit of change after the first infusion. Blood salinity did not reach threshold until infusion 3 for units 1 and 4. The effect of repeated infusions was irregular for units 8 and 9.

Figure 5 shows the cumulative effects of repeated infusions of hypertonic saline infusions on the spike discharge frequencies of osmosensitive hypothalamic units. Each histogram of the ten histograms represents a hypothalamic osmosensitive unit. Each histogram has four bars or median. The four bars represent the cumulative effects of repeated hypertonic saline infusions on the spike discharge frequency of the osmosensitive unit after the termination of the infusion. The four bars represent the median average spike discharge frequency ($\bar{x}$ spikes/second) of the osmosensitive unit during different periods of electrical activity. The first bar represents the median average discharge frequency during a period of time before beginning the series of infusions. The second, third, and fourth bar represent the median average discharge frequency of the period of electrical activity after the termination of the first, second, and third infusion, respectively. The median average discharge frequency refers to the particular value ($\bar{x}$ spikes/second) above which and below which fifty percent of the ten second intervals fell during the period of recorded activity.

FIGURE 5a

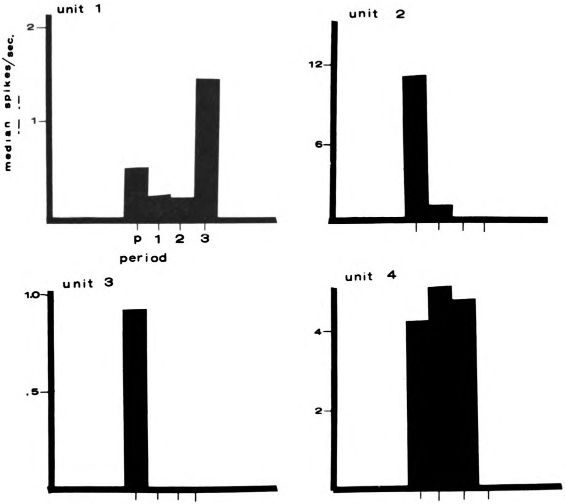
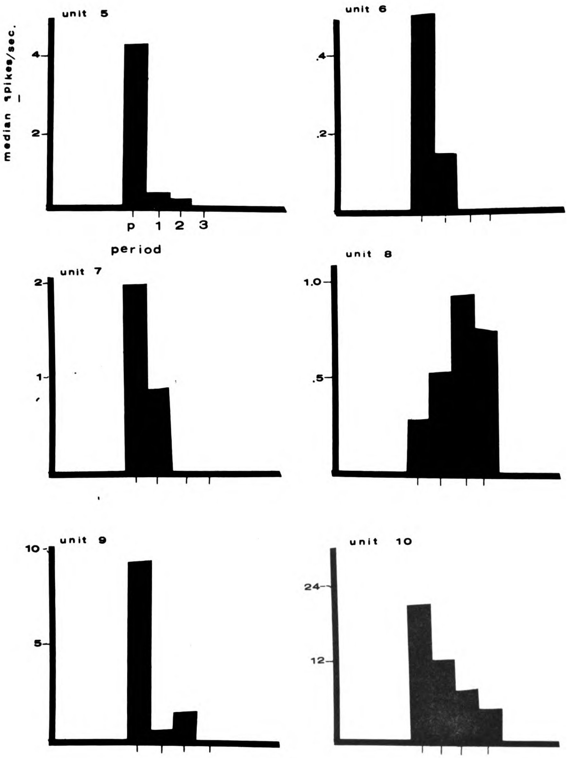

unit 1
2
1
median spikes/sec.
p 1 2 3
period
unit 2
12
6
unit 3
1.0
.5
unit 4
4
2

FIGURE 5b


unit 5
median spikes/sec.
4
2
p 1 2 3
period
unit 6
.4
.2
unit 7
2
1
unit 8
1.0
.5
unit 9
10
5
unit 10
24
12

Water loaded into the stomach affected eight of the ten units. Refer to Figure 3. For seven of the eight affected units, the spike discharge frequency changed in the direction opposite to that of the salt load.

An attempt was made to locate the bottom of the electrode track for each neural unit. A tracing was made of the brain section showing the lower limit of each electrode track. Figures 6 through 14 show the tracings of the hypothalamic regions in which the bottom of the electrode puncture for each of the ten units was located. The approximate locations of the units were classified according to four regions of the hypothalamus: (1) Pre-optic region, (2) Supra-optic region, (3) Tuberal region, and (4) Mammillary region. The regions are defined in an anterior-posterior direction, as follows: (1) Pre-optic region is anterior to the optic chiasma; (2) Supra-optic region is above the optic chiasma; (3) Tuberal region is from the optic chiasma to the mammillary body; and, (4) the Mammillary region is around the mammillary body. There are no lateral limits in the definition of the regions.

Four units were located in the pre-optic region. Two units were located in the tuberal region. One unit was located in the mammillary region. Table 3 summarizes the approximate location of each neural unit and the effects of changes in blood salinity on unit spike discharge activity.

The characteristics of the units in the supraoptic region were similar in that they showed a decreased response of long duration to the first increment of blood NaCl concentration, showed graded responses to repeated increments of blood NaCl concentration, and showed a response to a stomach load of 3 liters of water in a direction opposite to the response to increased blood NaCl concentration.

Table 3

Summary of anatomical location and characteristics of unit discharge frequency following repeated increases in blood salinity and water loaded into the stomach.

Unit #	Location	Direction	Threshold	Gradation	Direction of stomach water load effect
1	Mammillary region	increase	third	------	------
2	Supraoptic region	decrease	first	yes	increase
3	Tuberal region	decrease	first	limited	increase
4	Tuberal region	decrease	third	------	increase
5	Supraoptic region	decrease	first	yes	increase
6	Preoptic region	decrease	first	yes	------
7	Supraoptic region	decrease	first	yes	increase
8	Preoptic region	increase	first	irregular	increase
9	Preoptic region	decrease	first	irregular	increase
10	Preoptic region	decrease	first	yes	increase

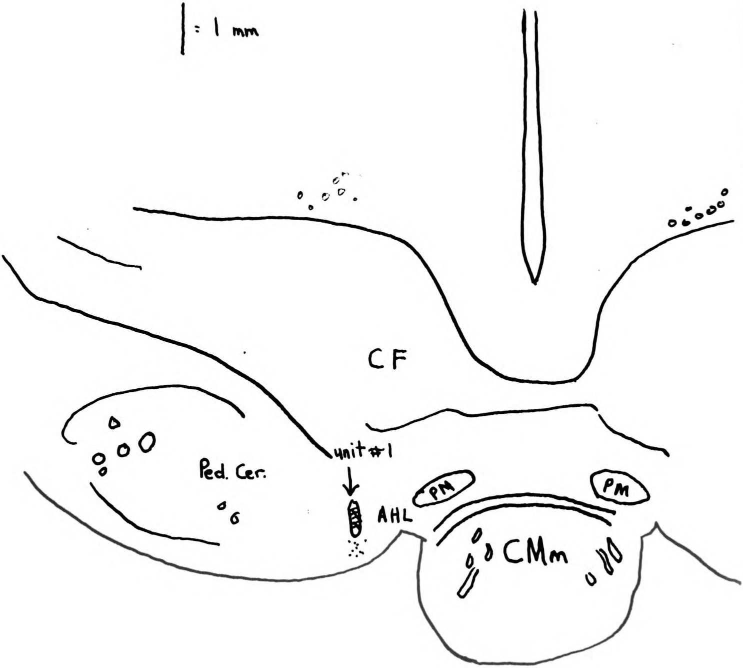
The following section is made up of figures (Figures 6-14) and ten photomicrographs. Each figure represents a coronal section of the hypothalamus of a goat brain. The figure is a tracing of the section showing the approximate location of the microelectrode tip during the recording of the neural unit electrical activity. The arrow in each figure indicates the representation of the electrode track and unit number. The magnification for tracing each section was X 14. Following each figure is the photomicrograph of the brain section showing the electrode track. The magnification is X 15.

Key to abbreviations used in tracings

of brain sections (Richard, 1967).

AHA	-	area hypothalamia anterior
AHL	-	area hypothalamia lateralis
APO	-	area preoptica
A. Sept.	-	area septalis
A. Sept. L	-	area septalis lateralis
CA	-	commissura anterior
CF	-	campi foreli
C int.	-	capsula interna
C Mm	-	corpus mammillare mediale
Ch. O	-	chiasma opticum
FMT	-	fasiculus mammillothalamicus
FR	-	fasiculus retroflexus
FX	-	fornix
NHA	-	nucleus hypothalamicus anterior
NHDM	-	nucleus hypothalamicus dorsalis medialis
NHVM	-	nucleus hypothalamicus ventralis medialis
NPV	-	nucleus paraventricularis hypothalami
NSO	-	nucleus supraopticus
Ped. Cer.	-	pedunculus cerebri
PM	-	pedunculus mammillaris
RE	-	nucleus reuiens
RET	-	nucleus reticularis
SCH	-	nucleus suprachiasmaticus
TO	-	tractus opticus

Figure 6



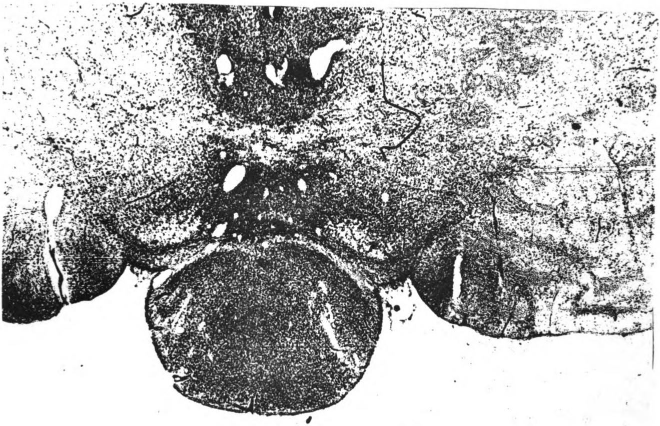
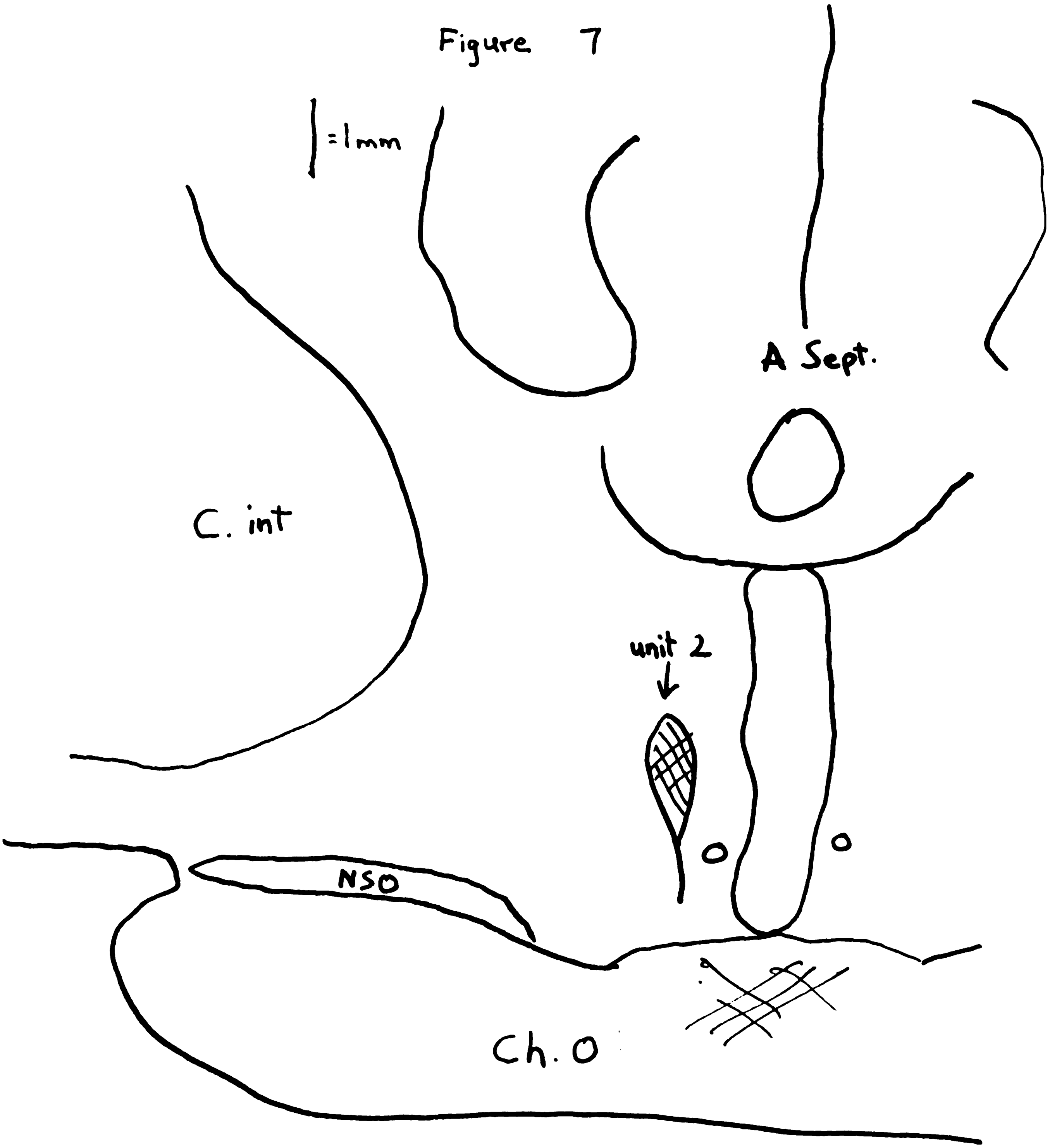


Figure 7



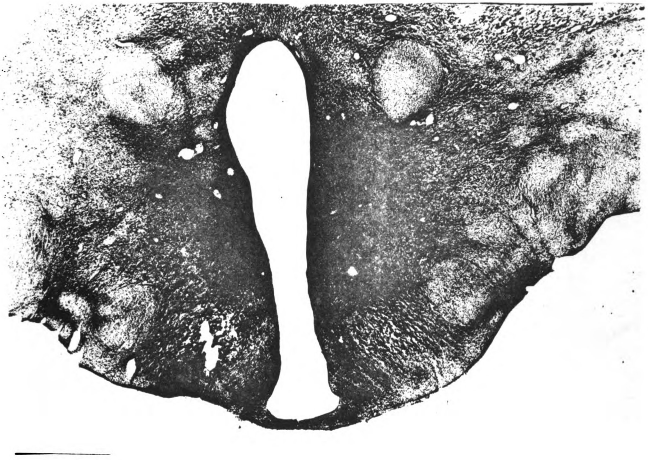
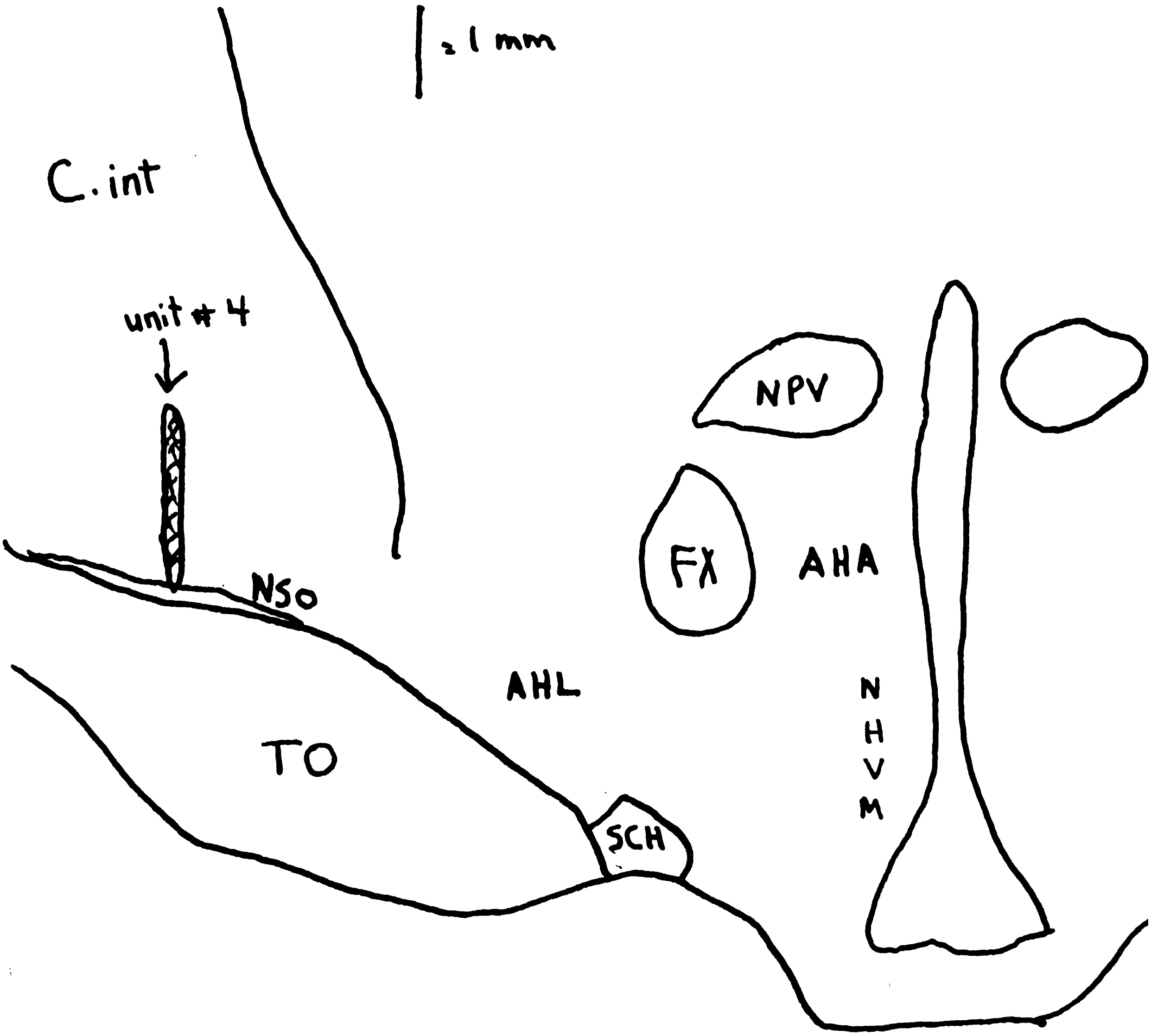


Figure 9



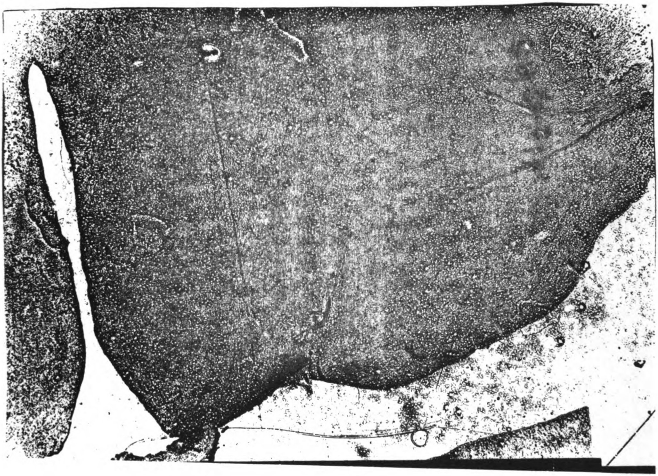
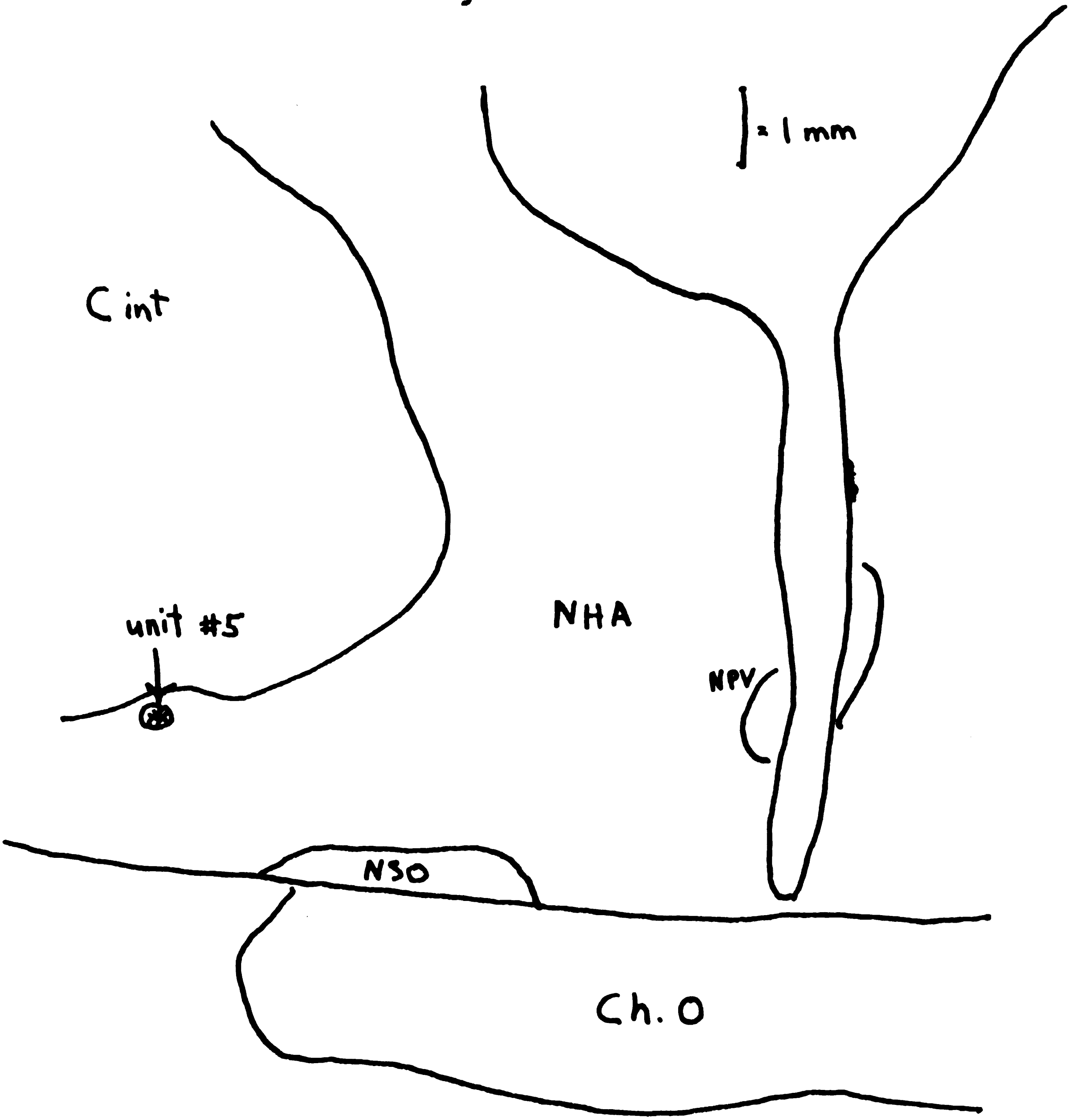


Figure 10



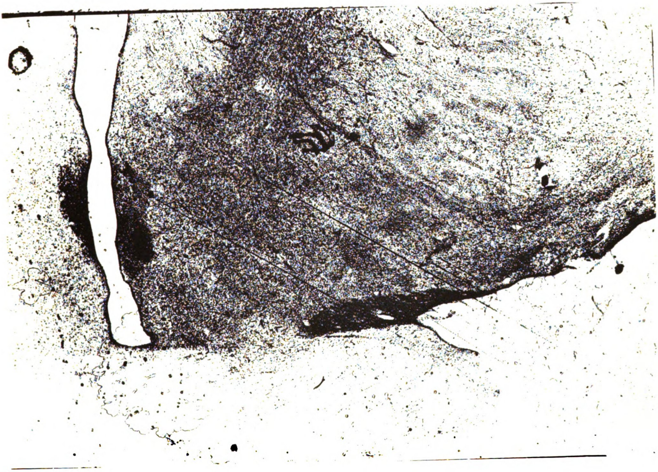
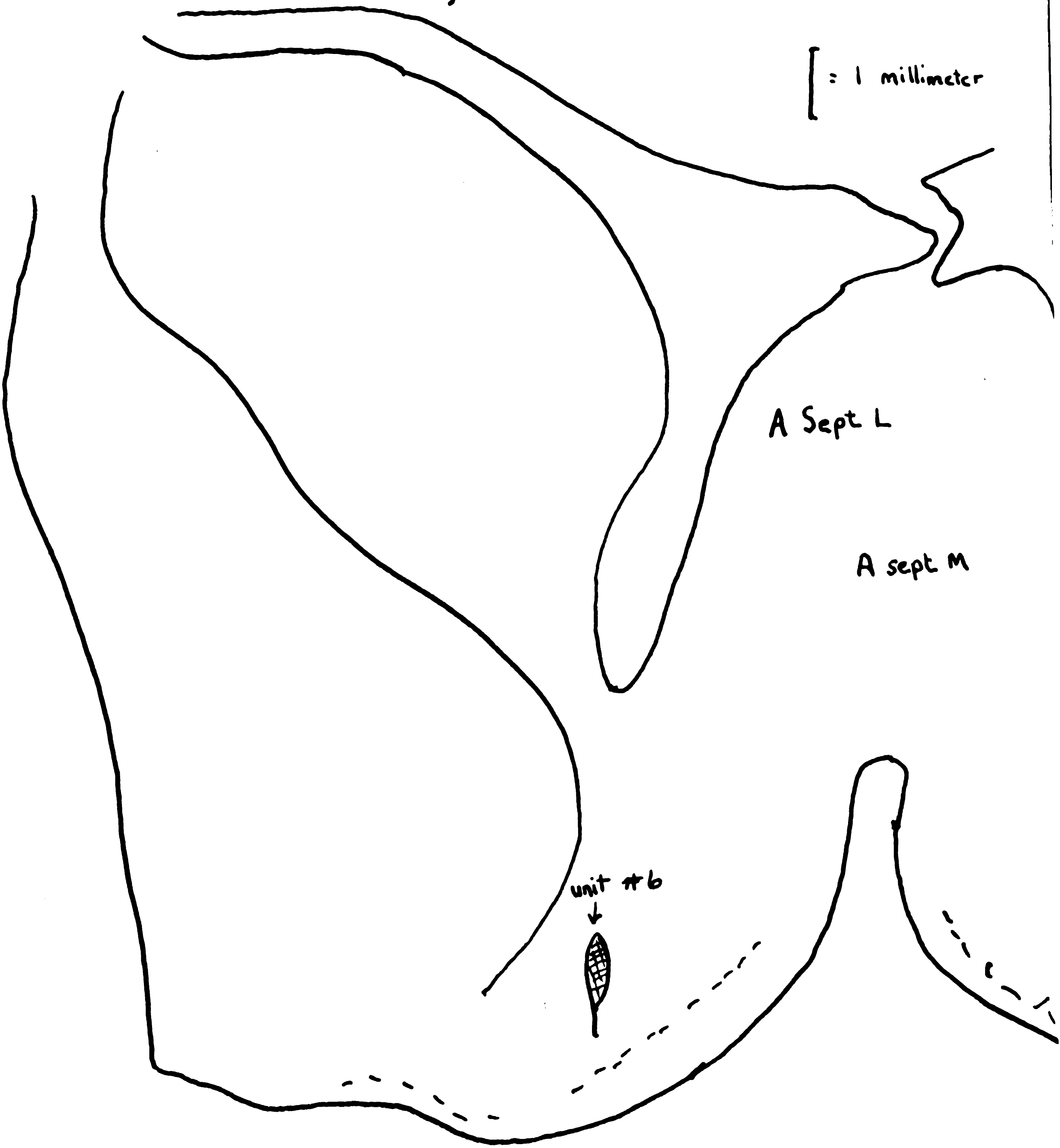


Figure 11



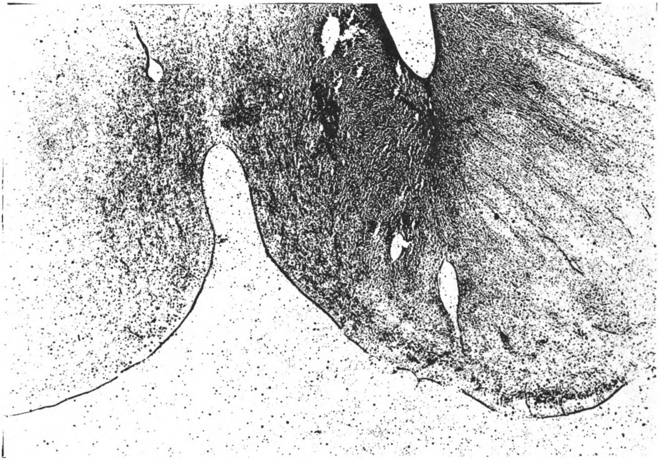
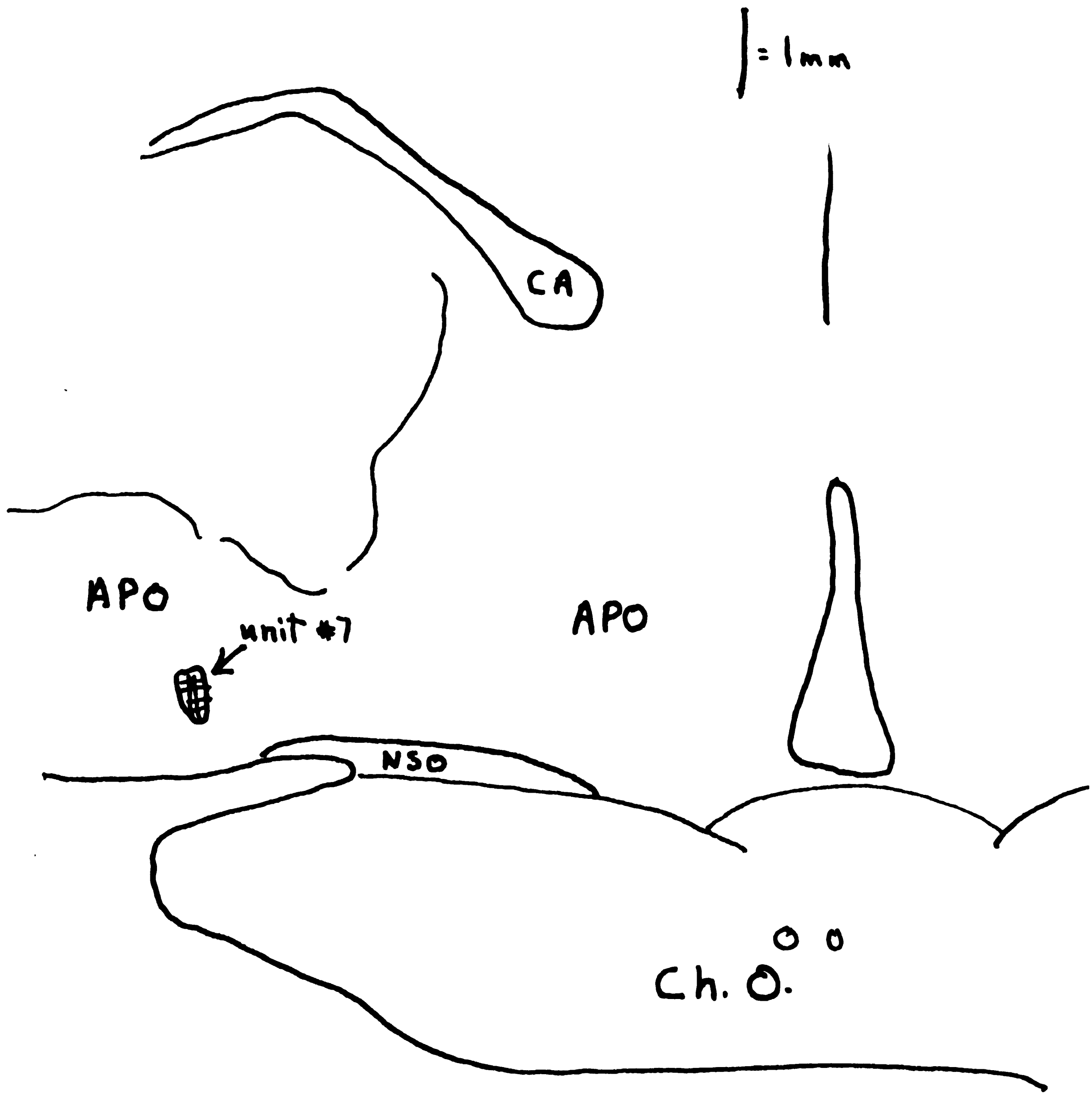


Figure 12



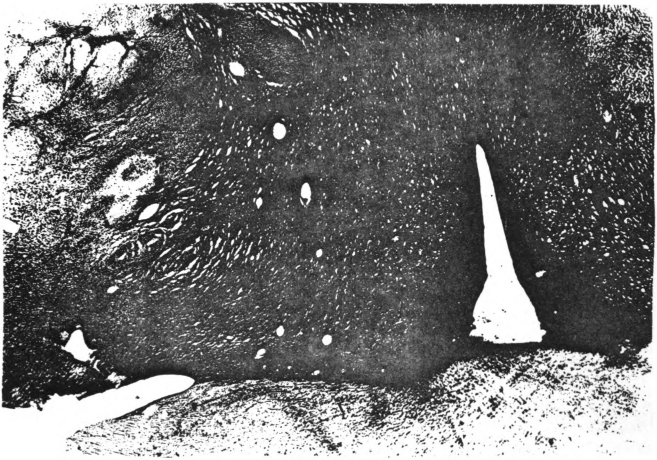
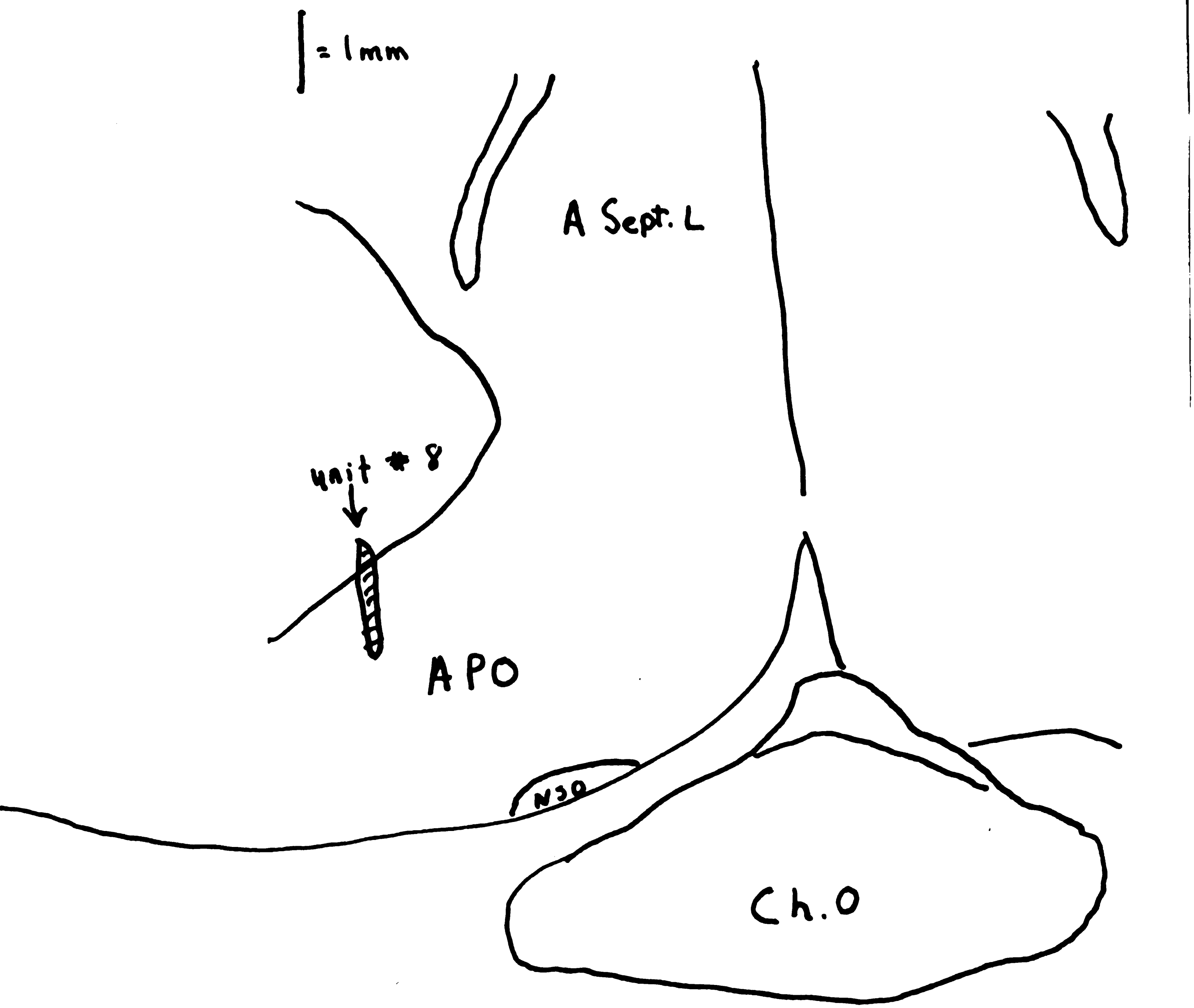


Figure 13



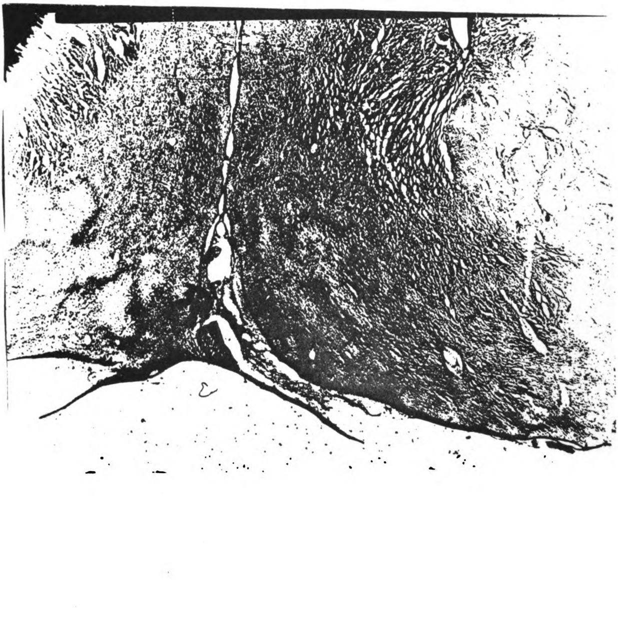
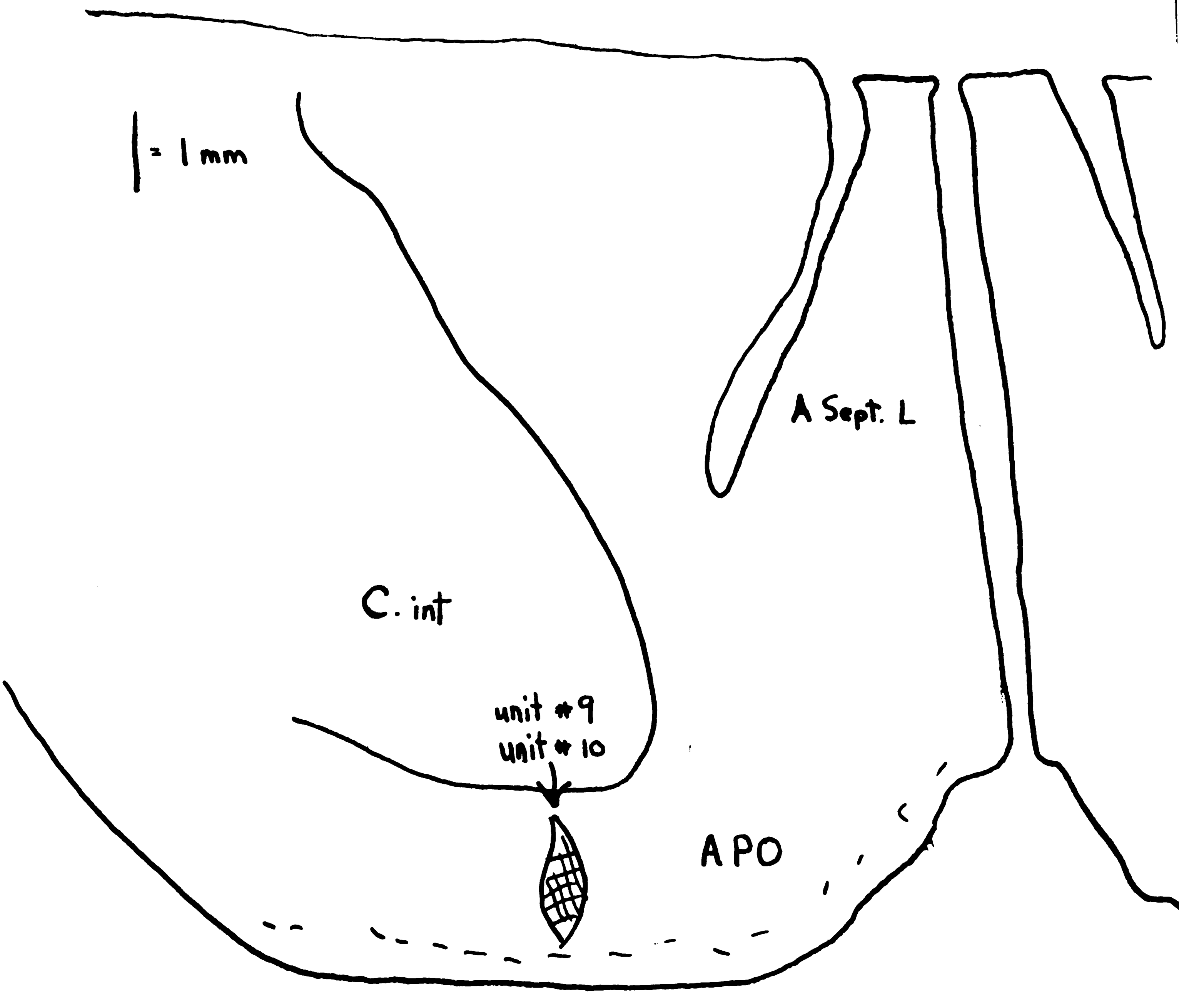
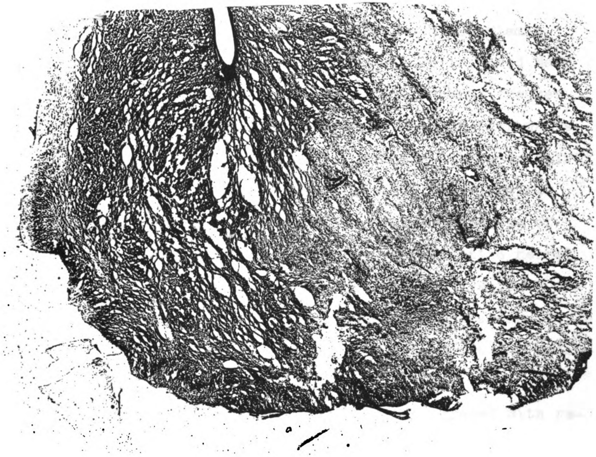


Figure 14





Discussion

The problem under investigation in this study was the long-term effects of increments in blood NaCl concentration on the discharge patterns of osmosensitive hypothalamic neural units. The results indicate the following answers to the questions presented in the introduction:

1. Do the responses of osmosensitive hypothalamic neural units persist after a change in blood NaCl concentrations? The change in discharge frequencies of all ten osmosensitive units to increments in blood NaCl levels persisted for twenty to thirty minutes after the change in blood NaCl levels.
2. What is the direction of change of responses of long duration? Eight of the osmosensitive neural units showed a decrease in discharge frequency after the increase in blood NaCl concentration, while two osmosensitive neural units showed an increase in discharge frequency.
3. Are the responses of long duration graded with repeated increments in blood NaCl concentrations? All of the osmosensitive neural units did not show a graded response to increments in blood NaCl concentration. Five of the ten units showed the characteristic of gradation of response to changes in stimulus intensity.

The remainder of this section will be a more detailed discussion of the response characteristics of duration, direction, and gradation, of an osmosensitive hypothalamic neural unit. Further, findings about the effects of water loaded into the goat's stomach on the responses of osmosensitive hypothalamic neural units will be discussed. The findings from the results of the locations of the units will be discussed. Finally, hypotheses suggested by the results and future areas of research will be discussed.

Duration of the response

The findings of other research looking at the effects of changes in extracellular NaCl concentration levels on the water regulatory system suggest that the activity of parts of the neural substrate underlying the control of water consumption and ADH release should persist for long periods of time.

It has been demonstrated that the rate of release of ADH does not accomodate in dogs when high blood NaCl levels are maintained for 40 minutes (Verney, 1947). Fitzsimons (1963) interprets results of an experiment as showing that receptors for thirst do not adapt. He demonstrated that when water was withheld for 24 hours after injection of NaCl solution into nephrectomized rats, there was no difference in the change in weight between animals who drank after 24 hours and animals who drank immediately after the injection. Both of these findings suggest that osmosensitive units in the hypothalamus may respond as long as blood concentration of NaCl is higher than normal. The results of the present experiment show, that in all osmosensitive neural units sampled, a response to an increment in blood NaCl concentration persists for 20 to 30 minutes after the change in blood NaCl concentration was present. This persistent response occurred if the change in blood NaCl concentration was high enough.

Several studies have shown that the electrical responses of different hypothalamic areas are transient after an increase in blood NaCl concentration (Brooks, Ushiyama, and Lange, 1962; Cross and Green, 1959; Koizumi, Ishikawa, and Brooks, 1964; Nakayma, 1955; Novin and Durham, 1969; Sawyer and Gernandt, 1956; Von Euler, 1953). A possible reason for the difference in the findings from this experiment and theirs is that the increase in blood NaCl concentration from the infusions in their experiments was not large enough to maintain the blood NaCl concentration above the threshold value. The results of this experiment indicate that the amount of NaCl solution infused into the blood can determine if a response to an increment in blood NaCl concentration persists after the termination of the infusion. While eight of the units showed a response to an increase in blood NaCl concentration after the first infusion of 50cc of 16% NaCl solution, two of the units did not show a persistent response until after three infusions or 150cc of 16% NaCl solution.

<u>Direction</u>

All neural units whose electrical activity was sampled were affected by the increase in blood NaCl concentration. Eight of the units decreased their spike discharge frequency, while two units increased their spike discharge frequency. The units were located throughout the hypothalamus. One of the units which increased its activity to increased blood NaCl concentration was located in the pre-optic area of the hypothalamus, while the other unit was located in the mammillary area of the hypothalamus. Many studies show that, in general, the units in the supraoptic nucleus increase their activity with an increase in the blood NaCl concentration. (Brooks, Ushiyama, and Lange, 1962; Cross and Green, 1959; Joynt, 1964; Novin and Durham, 1969; Zeballos, Wang, Koizumi, and Brooks, 1967). In this study, none of the units were located in the supraoptic nucleus. Cross and Green (1959) report that units in the paraventricular nucleus decrease their activity. Also Suda et al. (1963) report that the spontaneous activity of supraoptic units increased their activity when an island of hypothalamus containing the supraoptic nucleus was isolated from other neural connections. They interpret their results as providing evidence that cells in the supraoptic nucleus are under inhibitory control from other neural structures. Since the supraoptic nucleus seems to be the final neural area involved in the release of ADH it seems reasonable that cells in the supraoptic nucleus show an increase in their activity with an increase in blood NaCl concentration. Wayner and Kahan (1969) have reported that, in general, units in other parts of the hypothalamus, thalamus, and midbrain also show an increase in activity with an increase in blood NaCl concentration.

The difference between the findings in this experiment and others gives support to a conclusion made by Cross (1964).

"Lability of unit discharges in the hypothalamus tends to erode confidence in the concreteness of the 'centres' demarcated by the methods of stimulation and ablation." (p. 163.)

The findings of the present study suggest that, when activity is sampled over a longer period of time than in previous studies, more extra-supraoptic units, in general, decrease their activity with an increase in blood NaCl concentration, than increase their activity.

Gradation

Novin and Durham (1969) and Von Euler (1953) have stated that in order for a unit to be considered an osmoreceptor it must show the property of responding to increments in the concentration of NaCl in the blood. The results from both of their studies demonstrate that the magnitude of slow potentials in the supraoptic nucleus varies proportionally with blood NaCl concentration. Several studies have shown that the changes in spike discharge frequency is proportional to increments in blood NaCl concentration, during and shortly after the injection of NaCl solutions into the blood. (Cross and Green 1959; Joynt, 1964). The results of this experiment show that in five of the ten osmosensitive units the change in discharge frequency was graded to increments in blood NaCl concentrations for 20 to 30 minutes after the termination of the infusion of NaCl solutions into the blood. There are osmosensitive units in the hypothalamus that continue to respond to different concentrations of NaCl in the blood after the initiation of blood NaCl concentration change.

Five osmosensitive units did not show the property of gradation of response with repeated increases in blood NaCl concentration. Two of the units did not show a persistent response to an increment in blood NaCl concentration until after the third or final infusion. One unit reached its limit of response change after the first infusion. Two units showed irregular responses to repeated increments in blood NaCl concentration. Not all of the osmosensitive units showed the property of gradation of response.

Water loaded into the stomach

Eight of the ten units showed a change in discharge frequency after loading water into the stomach of the goat. Seven of the eight units showed a response in the direction opposite to the response to increased blood NaCl concentration. The response to the water load persisted through the ten minutes after the completion of loading water into the stomach. Other studies report different results. Generally, they report that osmosensitive units do not respond to water infused into the blood (Brooks, Ushiyama, and Lange, 1962; Cross and Green, 1959; Joynt, 1964).

Some studies have reported that slow potentials show a change in the opposite direction if water is infused into the blood. (Nakayama, 1955; Novin and Durham, 1969).

Some experiments demonstrate that water infused into the carotid artery has an effect on electrical activity of osmosensitive neural units while other experiments reported no effect from water infused into the carotid. The differences in results of the different experiments might be due to the salinity of the blood prior to infusing water into the carotid artery. If the blood NaCl concentration remains above the threshold concentration to which a neural unit is sensitive, then the unit could respond to water diluting the blood. Many experiments infuse small amounts of NaCl solution into the blood before looking at the effects of water infusion on the unit activity. If the small infusions of NaCl solution are not large enough to keep the blood at a high NaCl concentration, then the units will not respond. In this study, all units continued to respond to increased blood NaCl concentrations after the termination of the infusions. If the continued response of the unit indicates that the stimulus is above threshold, then the probability is high that the unit will respond to decrement in blood NaCl concentration due to loading water into the stomach. The finding suggested by the results of this experiment indicates that the blood NaCl concentration has to be above the threshold amount at which a unit responds to increased blood salinity before a response to a decrease in blood salinity can be demonstrated.

Maintained activity

There is also interest in the 'maintained activity' of units. The present study reports results of the maintained activity for 15 minutes prior to beginning the series of infusions. Five of the ten units sampled cannot be compared to the findings of other studies because there was a previous history of changing blood NaCl concentrations. The average discharge frequency of the remaining five units varied between .49 spikes per second to 10.28 spikes per second.

The findings of this experiment are in agreement with others, which report unit maintained activities from less than 1 spike per second to 40 spikes per second (Cross and Silver, 1966).

<u>Future research</u>

The finding that all units, sampled in this experiment, eventually responded to increased blood concentration and the finding that the units were located in diverse areas of the hypothalamus suggest a hypothesis about sensitivity of hypothalamic neurons. It is possible that all neural units are sensitive to an increment in blood NaCl concentration, if the increment is large enough. The hypothesis suggests a research design in which the response of hypothalamic units in different areas are measured as a function of small increments in blood NaCl concentration. The procedural difference of this design from previous studies is to increase blood NaCl concentration until the unit electrical activity is affected. It is predicted that the blood NaCl concentration to which the cells in the supraoptic nucleus would respond would be lower than other areas of the hypothalamus. It would be interesting to see the results of measuring differential sensitivities of hypothalamic units to increments in blood NaCl concentrations.

It was found that the procedure of loading water into the stomach of the goat eventually affected the electrical activity of osmosensitive units when the blood NaCl concentration was large. Further research is necessary to determine how the water in the stomach affected the unit electrical activity. Can the findings, that water loaded into the stomach was related to a change in discharge frequency of an osmosensitive unit, be repeated by infusing water directly into the blood. A better research design is necessary to determine if the units are responding to the water loaded or to the water absorbed or both. It appears from conflicting findings in the literature and the findings of this experiment that the blood NaCl concentration before infusing water into the blood is of critical importance. It is hypothesized that if the blood NaCl concentration is large enough that an osmosensitive unit will respond to a decrease in blood NaCl concentration. If the blood NaCl concentration is not large enough, the electrical activity of an osmosensitive unit will not be sensitive to a decrease in blood NaCl concentration.

The characteristics of the changes in electrical activity of osmosensitive units that did not show a graded response to repeated increments of blood NaCl concentrations are interesting. One of the units reached its limit of response change after the threshold infusion. Other units also reached a limit of response change before the completion of the repeated increments in blood NaCl concentration. These findings suggest the possibility that within certain ranges of blood NaCl concentration, osmosensitive units show a graded response to increments in blood NaCl concentration. An example of this possible characteristic is shown by unit 10 in Figure 5b. If the blood NaCl concentration is larger than the upper limit of that range, the unit does not show an increased change with an increment in blood NaCl concentration. An example of this characteristic is shown by unit 3 in Figure 5a.

Finally, two units showed an irregular response to repeated increments in blood NaCl concentration which resulted in a clear response of the unit. These findings suggest the possibility that some osmosensitive units show an all-or-nothing response to an increment in blood NaCl concentration. Below a certain concentration of NaCl in the blood, the osmosensitive unit is not sensitive to changes in blood salinity. Above the same concentration of NaCl in the blood, the unit discharge frequency changes to a new level. Any further changes in blood salinity do not change the response of this type of osmosensitive unit. The unit discharges only in response to the blood salinity being below or above a certain concentration. This suggestion is similar to Corbit's (1969) proposal that parts of the neural regulatory system underlying drinking behavior function in an all-or-none manner.

This experiment has demonstrated that some neural units throughout the hypothalamus are sensitive to changes in blood NaCl concentrations for an extended period of time after the termination of the infusion of NaCl solutions into the blood. Some of the units showed a graded response to increments in blood NaCl concentration for these extended time periods. Also, there are osmosensitive units in the hypothalamus which respond to water loaded into the stomach by changing its discharge frequency in the direction opposite to the response to increments in blood NaCl concentration.

One of the purposes of this experiment was to study characteristics of internal mechanisms that underlie a prolonged need for water. Maintaining a high blood NaCl concentration for a long period of time is related to a state of prolonged water need. Recording and analyzing the electrical activity of hypothalamic cells during periods of maintained high blood NaCl concentration is a way of studying characteristics of internal mechanisms that underlie a prolonged need for water. To conclude that an osmosensitive unit in the hypothalamus participates in drinking behavior is speculation, since there is no way at present to clearly identify a single cell in the hypothalamus as a neural cell directly participating in the control of drinding behavior. If the cells sampled in this experiment participate in the control of drinking behavior, then the electrical activity that signals the need for water in the hypothalamus does not adapt over prolonged periods of thirst. Also, for at least some of the hypothalamic cells, the electrical activity signals different degrees of thirst. Finally, the electrical activity of some of the hypothalamic cells signal the reduction of the need for water.

Summary

1. All neural units whose electrical activity was sampled were affected by increased blood salinity. Eight of the units decreased their activity, while two increased their activity.
2. The changes in spike discharge frequency of the ten neural units persisted for at least 20 to 30 minutes after the termination of the final change in blood salinity.
3. Two of the ten units required a larger change in blood salinity than the other eight units before showing a change in discharge frequency of long duration.
4. The responses of five of the ten neural units were graded to repeated increases in blood salinity.
5. Four units reached their limit of response change before the completion of the series of changes in blood salinity.
6. Eight of the ten units showed a change in discharge frequency after loading water into the stomach of the goat. Seven of the eight units showed a response in the opposite direction of the response to increased blood NaCl concentration.
7. The units showing a response to increased blood salinity were located in different regions of the hypothalamus.

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APPENDICES

Appendix A

Apparatus used for infusing solutions

1. Compact infusion pump.
   Harvard Apparatus Co., Inc.

2. Sodium Heparin.
   Panheprin®, Abbott Laboratories.

3. Polyethylene tubing.
   Inside diameter = .034"
   Outside diameter = .050".
   Intramedic ® PE 90.

Determination of volume and concentration of hypertonic saline solutions

The amount of NaCl in the solutions infused into the blood were determined by equations developed by Wolf (1950). Given the body weight and blood volume, the equations predict the change in blood NaCl concentration following the infusion of a salt load into the blood. The equations predict that an infusion of 50cc of 16% NaCl would result in a 2.9 to 3.1% change in blood NaCl concentration, given the weights of the goats used in this experiment.

Solutions were maintained at 34° centigrade in a water bath prior to infusion.

Apparatus and materials used in manufacture of microelectrodes

1. Tungsten wire.

2. Pyrex glass tubing.

3. The wire was sharpened with current passing through potassium nitrate.

4. Sharpened wire was insulated with glass by means of a vertical pipette puller (Model 700c, David Kopf Instruments.)

5. Glass was etched away from tip of electrode with hydrofluoric acid.

Apparatus used for respiration

1. Respirator, Bodine Electric Company, Type NSH-34 RH.

2. Rate of 15 cycles/minute, and volume of 300cc per cycle.

Apparatus used for recording electrical activity

1. Tungsten microelectrode.

2. Preamplifier.
Voltage gain = X1000.
Frequency range = 80 hertz to 40,000 hertz.
Tektronix, Type FM 122.

3. Vertical amplifier of dual beam oscilloscope.
Output voltage from vertical amplifier varied between 2 and 6 volts.

4. Signals monitored with audio monitor.
Grass Instrument Company, Model AM 5

5. Electrical activity recorded on Scotch recording tape (Low print, 1.5 Mil Acetate) by a Magnecord tape recorder, model 1028.

Apparatus used to analyze recorded electrical activity

1. Magnecord tape recorder, Model 1028.

2. Tektronix 502 A Dual Beam Oscilloscopes.

3. Peak Detector.
Baseline is adjustable from 0.5 to 10.0 volts. Window is adjustable from 0.0 to 10.0 volts above the baseline.
Frequency range is 100 hertz to 1500 hertz.
Technical Measurement Corporation, Model 607.

4. Computer of Average Transients.
Mnemotron Corporation, CAT 400 B.

5. Stimulator.
Grass Instruments Company, Model S 4.

6. Teletype Type-Punch-Read Unit.
Technical Measurement Corporation, Model 535.

Apparatus and materials used for histology

1. Animal perfused with 10% formalin solution.

2. Block of hypothalamus from goat brain frozen and 50 u sections cut with a microtome (American Optical Company, Model 860).

3. Sections mounted on glass slides and stained with cresylecht violet solution.

Appendix B

Frequency histograms of different records of electrical activity of each neural unit

Appendix B

The following section is made up of ten pages of figures (15 - 24). Each page of histograms represent the effects of the repeated infusions of hypertonic saline solutions and stomach water loading on the discharge frequency of a hypothalamic neural unit. Each page is made up of eight histograms. The histograms represent distributions of average discharge frequencies for periods of time before, during and for an extended period of time after each infusion of hypertonic saline into the common carotid artery and for a period of time after loading water into the stomach. Each period of time represented by the histogram is either 10 or 15 minutes. Each 10 or 15 minute period is divided into 60 or 90 ten second intervals. A discharge frequency for a particular ten second interval is expressed in mean number of spikes per second ($\bar{x}$ spikes/second). Each histogram presents the proportion of ten second intervals of a specified period of time represented by the histogram in which the neural unit has a certain average discharge frequency. For example, a neural unit might have had an average discharge frequency of two spikes/second for 20% of the ten second intervals during the first infusion of hypertonic saline into the carotid artery.

Figure 15

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #1. The neural unit activity was recorded from goat #12. There were 0 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the mammillary region of the hypothalamus. Refer to Figure 6.

Pre-infusion refers to the electrical activity of the neural unit #1 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is .49 spikes/second. The semi-interquartile range is 0.18 spikes/second.

First infusion refers to the electrical activity of unit #1 during 10 minutes of infusion. The median average discharge frequency is 0 spikes/second. The semi-interquartile range is 0.05 spikes/second.

Post-first-infusion refers to the electrical activity of unit #1 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0.23 spikes/second. The semi-interquartile range is 0.16 spikes/second.

Second infusion refers to the electrical activity of unit #1 during 10 minutes of infusion. The median average discharge frequency is .10 spikes/second. The semi-interquartile range is .10 spikes/second.

Post-second-infusion refers to the electrical activity of unit #1 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 0.20 spikes/second. The semi-interquartile range is 0.22 spikes/second.

Third infusion refers to the electrical activity of unit #1 during 10 minutes of infusion. The median discharge frequency is 0.23 spikes/second. The semi-interquartile range is 0.16 spikes/second.

Post-third-infusion refers to the electrical activity of unit #1 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 1.45 spikes/second. The semi-interquartile range of average discharge frequencies is 1.86 spikes/second.

Post-water-load refers to the electrical activity of unit #1 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 1.47 spikes/second. The semi-interquartile range is 0.33 spikes/second.

Figure 15

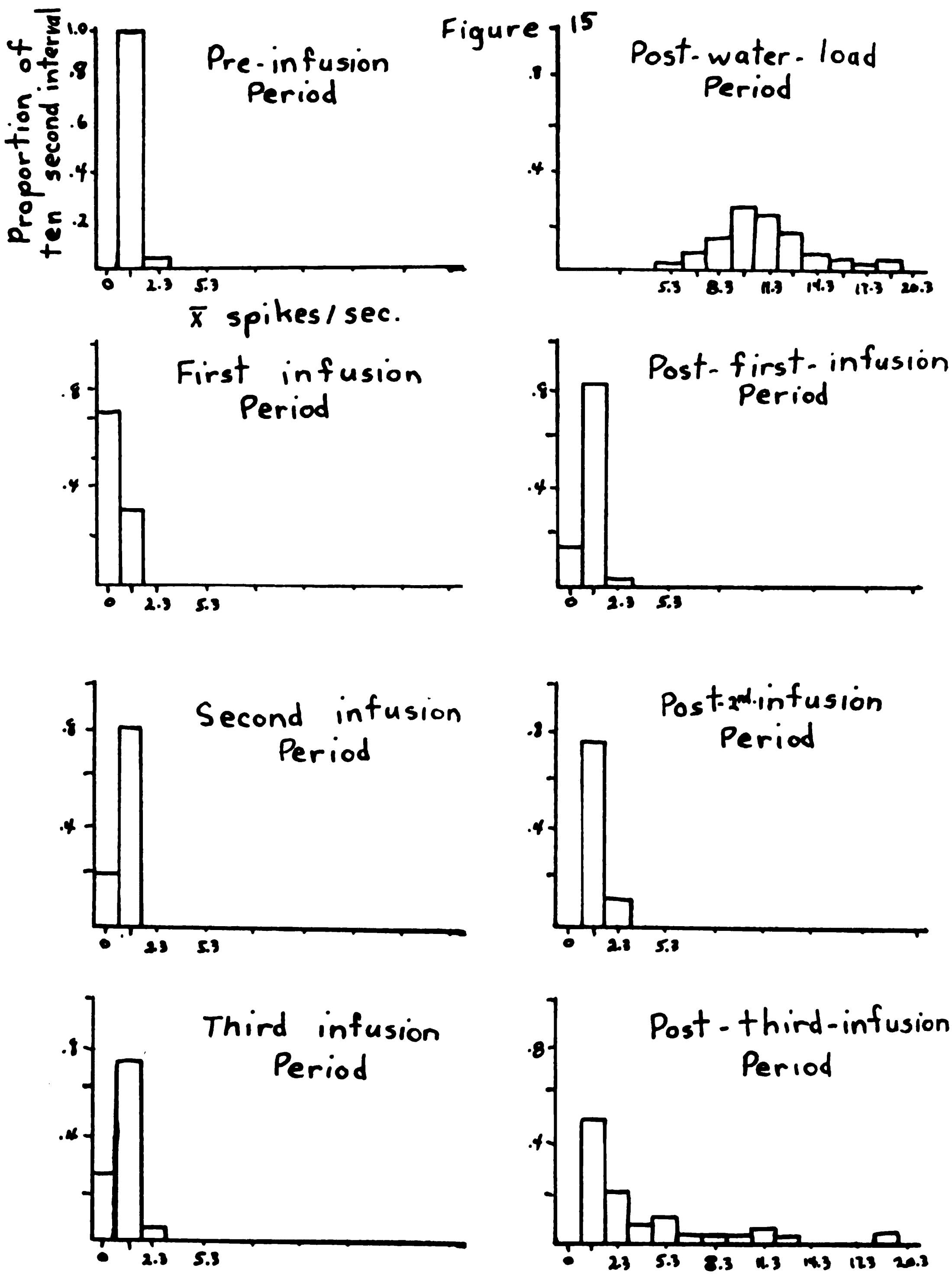

Proportion of ten second interval
1.0
.8
.6
.4
.2
Pre-infusion Period
0
2.3
5.3
x̄ spikes/sec.
Post-water-load Period
.8
.4
5.3
8.3
11.3
14.3
17.3
20.3
First infusion Period
Post-first-infusion Period
Second infusion Period
Post-2nd-infusion Period
Third infusion Period
Post-third-infusion Period

Figure 16

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #2. The neural unit activity was recorded from goat #14. There were 0 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the supra optic region of the hypothalamus. Refer to Figure 7.

Pre-infusion refers to the electrical activity of the neural unit #2 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 10.28 spikes/second. The semi-interquartile range is 0.40 spikes/second.

First infusion refers to the electrical activity of unit #2 during 10 minutes of infusion. The median average discharge frequency is 8.45 spikes/second. The semi-interquartile range is 0.41 spikes/second.

Post-first-infusion refers to the electrical activity of unit #2 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0.62 spikes/second. The semi-interquartile range is 0.23 spikes/second.

Second infusion refers to the electrical activity of unit #2 during 10 minutes of infusion. The median average discharge frequency is 0.22 spikes/second. The semi-interquartile range is 0.18 spikes/second.

Post-second-infusion refers to the electrical activity of unit #2 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.08 spikes/second.

Third infusion refers to the electrical activity of unit #2 during 10 minutes of infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.0 spikes/second.

Post-third-infusion refers to the electrical activity of unit #2 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0.0 spikes/second. The semi-interquartile range of average discharge frequencies is 0.0 spikes/second.

Post-water-load refers to the electrical activity of unit #2 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 1.42 spikes/second. The semi-interquartile range is 0.19 spikes/second.

Figure 16

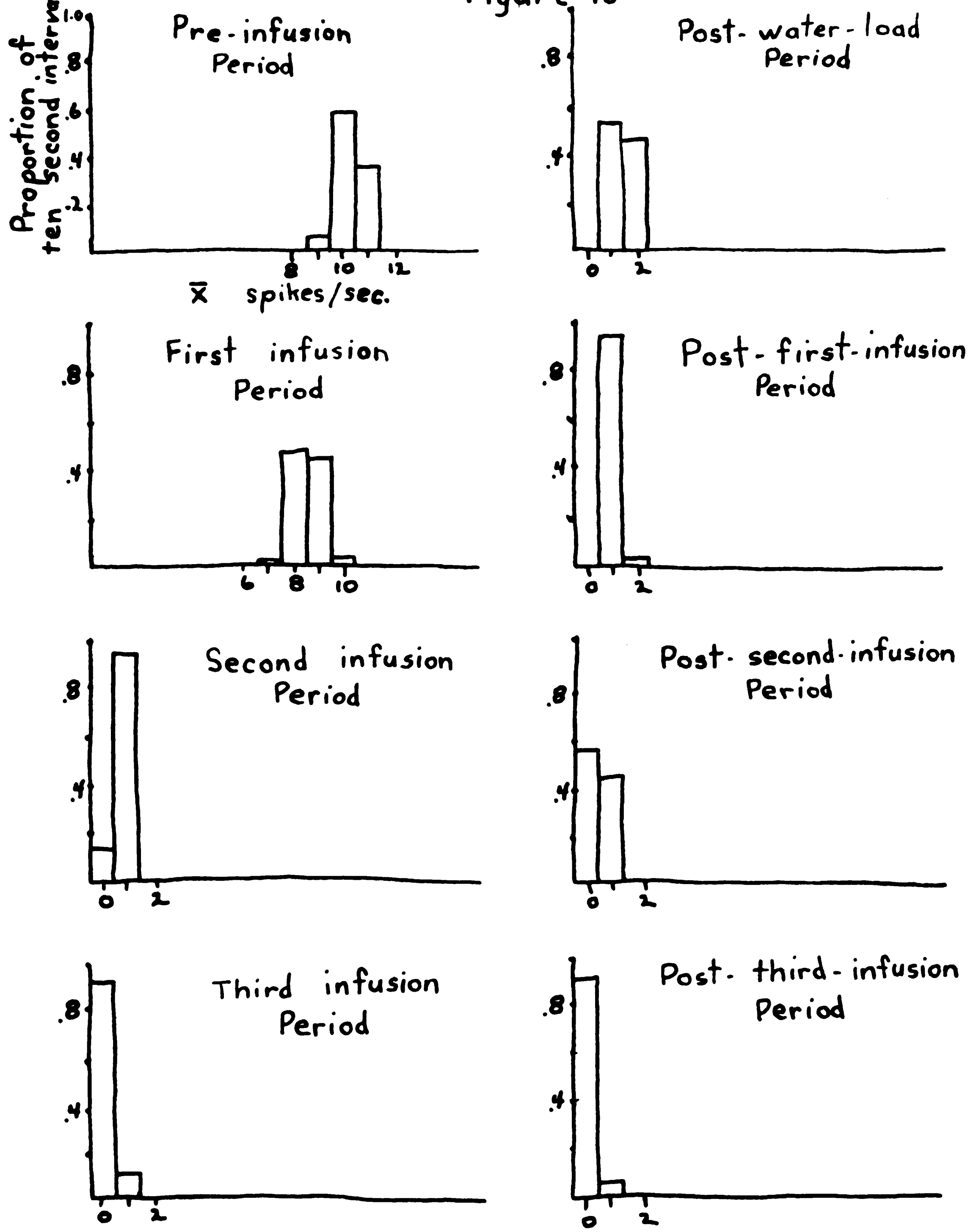

Proportion of ten second intervals
1.0
.8
.6
.4
.2
Pre-infusion Period
8
10
12
X̄ spikes/sec.
Post-water-load Period
.8
.4
0
2
First infusion Period
.8
.4
6
8
10
Post-first-infusion Period
.8
.4
0
2
Second infusion Period
.8
.4
0
2
Post-second-infusion Period
.8
.4
0
2
Third infusion Period
.8
.4
0
2
Post-third-infusion Period
.8
.4
0
2

Figure 17

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #3. The neural unit activity was recorded from goat #15. There were 0 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the tuberal region of the hypothalamus. Refer to Figure 8.

Pre-infusion refers to the electrical activity of the neural unit #3 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 0.93 spikes/second. The semi-interquartile range is 0.20 spikes/second.

First infusion refers to the electrical activity of unit #3 during 10 minutes of infusion. The median average discharge frequency is 0.88 spikes/second. The semi-interquartile range is 0.16 spikes/second.

Post-first-infusion refers to the electrical activity of unit #3 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0 spikes/second. The semi-interquartile range is 0 spikes/second.

Second infusion refers to the electrical activity of unit #3 during 10 minutes of infusion. The median average discharge frequency is 0 spikes/second. The semi-interquartile range is 0 spikes/second.

Post-second-infusion refers to the electrical activity of unit #3 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.0 spikes/second.

Third infusion refers to the electrical activity of unit #3 during 10 minutes of infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.0 spikes/second.

Post-third-infusion refers to the electrical activity of unit #3 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0.0 spikes/second.

Post-water-load refers to the electrical activity of unit #3 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 0.77 spikes/second. The semi-interquartile range is 0.18 spikes/second.

Figure 17

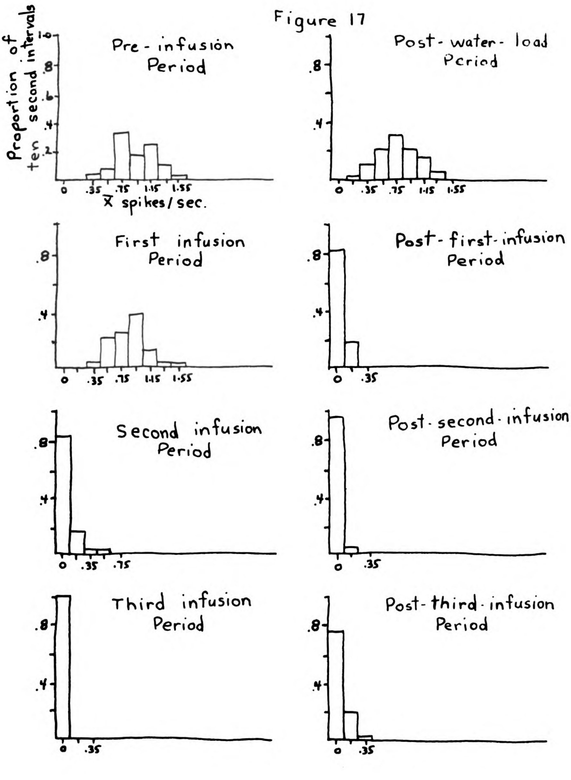

Proportion of ten second intervals
Pre-infusion Period
Post-water-load Period
First infusion Period
Post-first-infusion Period
Second infusion Period
Post-second-infusion Period
Third infusion Period
Post-third-infusion Period
X̄ spikes/sec.
1.0
.8
.6
.4
.2
0
.35
.75
1.15
1.55

Figure 18

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #4. The neural unit activity was recorded from goat #16. There were 0 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the tuberal region of the hypothalamus. Refer to Figure 9.

Pre-infusion refers to the electrical activity of the neural unit #4 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 4.45 spikes/second. The semi-interquartile range is 0.26 spikes/second.

First infusion refers to the electrical activity of unit #4 during 10 minutes of infusion. The median average discharge frequency is 0.41 spikes/second. The semi-interquartile range is 1.22 spikes/second.

Post-first-infusion refers to the electrical activity of unit #4 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 5.40 spikes/second. The semi-interquartile range is 0.44 spikes/second.

Second infusion refers to the electrical activity of unit #4 during 10 minutes of infusion. The median average discharge frequency is 3.10 spikes/second. The semi-interquartile range is 0.93 spikes/second.

Post-second-infusion refers to the electrical activity of unit #4 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 4.78 spikes/second. The semi-interquartile range is 0.51 spikes/second.

Third infusion refers to the electrical activity of unit #4 during 10 minutes of infusion. The median discharge frequency is 0.53 spikes/second. The semi-interquartile range is 0.31 spikes/second.

Post-third-infusion refers to the electrical activity of unit #4 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0 spikes/second. The semi-interquartile range of average discharge frequencies is 0.12 spikes/second.

Post-water-load refers to the electrical activity of unit #4 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 2.45 spikes/second. The semi-interquartile range is 0.79 spikes/second.

Figure 18

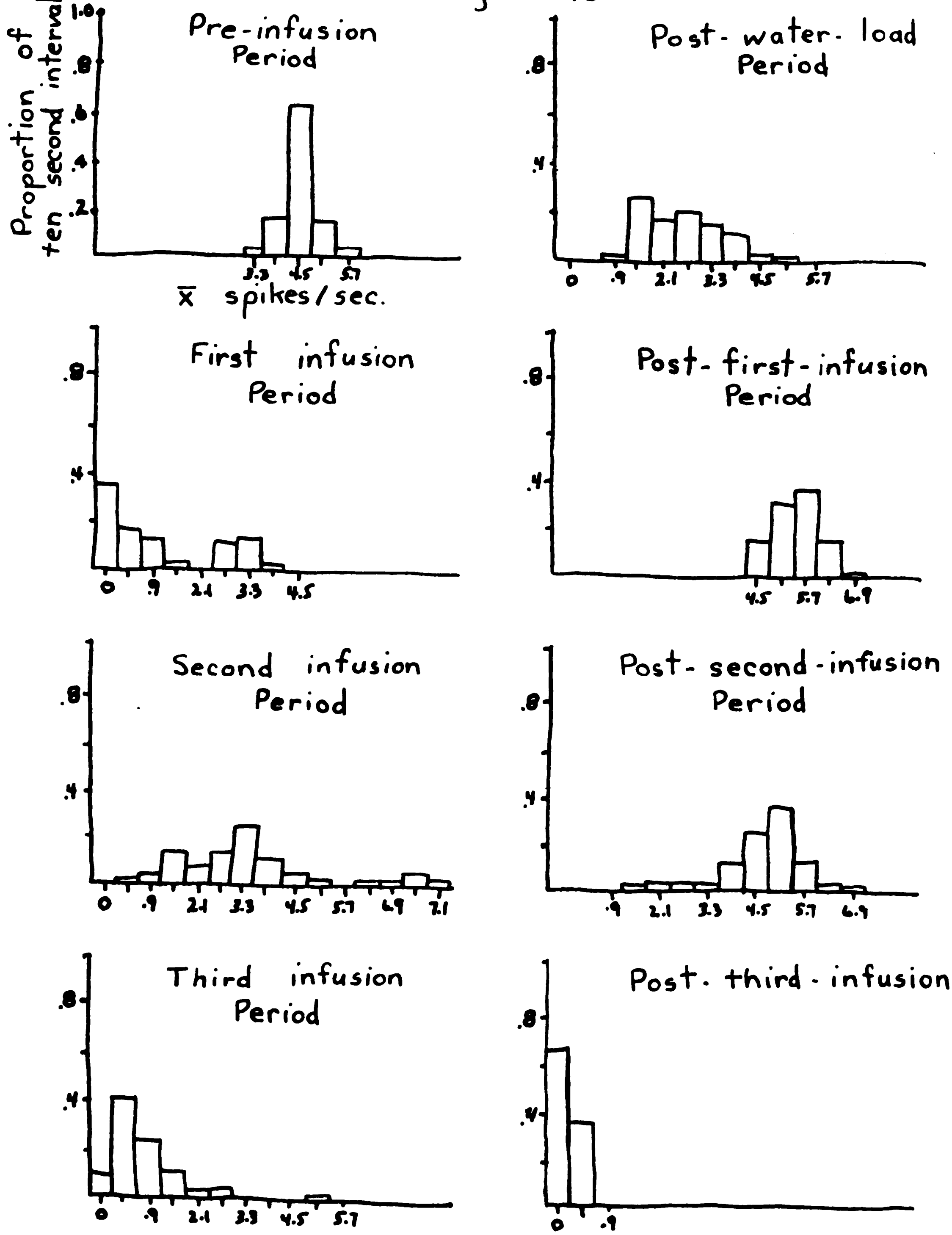

Proportion of ten second intervals
1.0
.8
.6
.4
.2
Pre-infusion Period
3.3 4.5 5.7
x̄ spikes/sec.
Post-water-load Period
.8
.4
0 .9 2.1 3.3 4.5 5.7
First infusion Period
.8
.4
0 .9 2.1 3.3 4.5
Post-first-infusion Period
.8
.4
4.5 5.7 6.9
Second infusion Period
.8
.4
0 .9 2.1 3.3 4.5 5.7 6.9 7.1
Post-second-infusion Period
.8
.4
.9 2.1 3.3 4.5 5.7 6.9
Third infusion Period
.8
.4
0 .9 2.1 3.3 4.5 5.7
Post-third-infusion
.8
.4
0 .9

Figure 19

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #5. The neural unit activity was recorded from goat #16. There were 1 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the supraoptic region of the hypothalamus. Refer to Figure 10.
Pre-infusion refers to the electrical activity of the neural unit #5 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 4.19 spikes/second. The semi-interquartile range is 0.48 spikes/second.
First infusion refers to the electrical activity of unit #5 during 10 minutes of infusion. The median average discharge frequency is 0.34 spikes/second. The semi-interquartile range is 0.38 spikes/second.
Post-first-infusion refers to the electrical activity of unit #5 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0.25 spikes/second. The semi-interquartile range is 0.10 spikes/second.
Second infusion refers to the electrical activity of unit #5 during 10 minutes of infusion. The median average discharge frequency is 0.38 spikes/second. The semi-interquartile range is 0.25 spikes/second.
Post-second-infusion refers to the electrical activity of unit #5 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 0.12 spikes/second. The semi-interquartile range is 0.14 spikes/second.
Third infusion refers to the electrical activity of unit #5 during 10 minutes of infusion. The median discharge frequency is 0 spikes/second. The semi-interquartile range is 0 spikes/second.
Post-third-infusion refers to the electrical activity of unit #5 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0 spikes/second. The semi-interquartile range of average discharge frequencies is 0 spikes/second.
Post-water-load refers to the electrical activity of unit #5 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 14.26 spikes/second. The semi-interquartile range is 0.54 spikes/second.

Figure 19

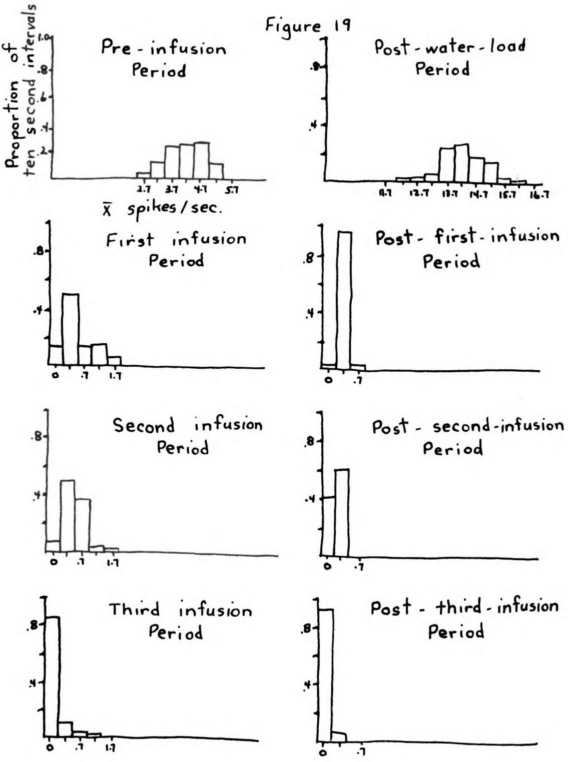

Proportion of ten second intervals
Pre - infusion Period
1.0
.8
.6
.4
.2
2.7 3.7 4.7 5.7
x̄ spikes / sec.
Post - water - load Period
.8
.4
11.7 12.7 13.7 14.7 15.7 16.7
First infusion Period
.8
.4
0 .7 1.7
Post - first - infusion Period
.8
.4
0 .7
Second infusion Period
.8
.4
0 .7 1.7
Post - second - infusion Period
.8
.4
0 .7
Third infusion Period
.8
.4
0 .7 1.7
Post - third - infusion Period
.8
.4
0 .7

Figure 20

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #6. The neural unit activity was recorded from goat #16. There were 2 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the preoptic region of the hypothalamus. Refer to Figure 11.

Pre-infusion refers to the electrical activity of the neural unit #6 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 0.52 spikes/second. The semi-interquartile range is 0.18 spikes/second.

First infusion refers to the electrical activity of unit #6 during 10 minutes of infusion. The median average discharge frequency is 0.29 spikes/second. The semi-interquartile range is 0.12 spikes/second.

Post-first-infusion refers to the electrical activity of unit #6 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0.15 spikes/second. The semi-interquartile range is 0.13 spikes/second.

Second infusion refers to the electrical activity of unit #6 during 10 minutes of infusion. The median average discharge frequency is 0.10 spikes/second. The semi-interquartile range is 0.08 spikes/second.

Post-second-infusion refers to the electrical activity of unit #6 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.04 spikes/second.

Third infusion refers to the electrical activity of unit #6 during 10 minutes of infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.0 spikes/second.

Post-third-infusion refers to the electrical activity of unit #6 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0.0 spikes/second. The semi-interquartile range of average discharge frequencies is 0.0 spikes/second.

Post-water-load refers to the electrical activity of unit #6 occuring 10 to 20 minutes after stomach loading 3000c of water. The median average discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.06 spikes/second.

Figure 20

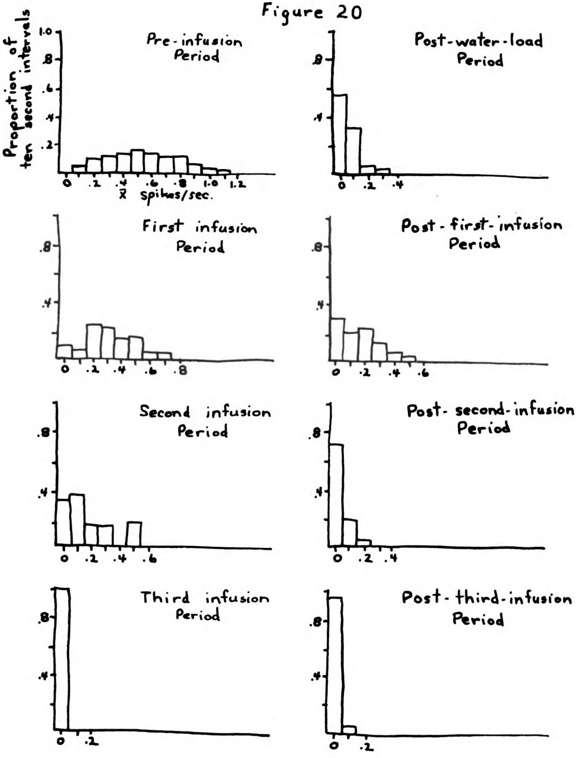

Proportion of ten second intervals
1.0
.8
.6
.4
.2
Pre-infusion Period
0 .2 .4 .6 .8 1.0 1.2
x̄ spikes/sec.
Post-water-load Period
.8
.4
0 .2 .4
First infusion Period
.8
.4
0 .2 .4 .6 .8
Post-first-infusion Period
.8
.4
0 .2 .4 .6
Second infusion Period
.8
.4
0 .2 .4 .6
Post-second-infusion Period
.8
.4
0 .2 .4
Third infusion Period
.8
.4
0 .2
Post-third-infusion Period
.8
.4
0 .2

Figure 21

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #7. The neural unit activity was recorded from goat #18. There were 0 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the supraoptic region of the hypothalamus. Refer to Figure 12.

Pre-infusion refers to the electrical activity of the neural unit #7 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 1.90 spikes/second. The semi-interquartile range is 0.32 spikes/second.

First infusion refers to the electrical activity of unit #7 during 10 minutes of infusion. The median average discharge frequency is 0.20 spikes/second. The semi-interquartile range is 0.48 spikes/second.

Post-first-infusion refers to the electrical activity of unit #7 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0.81 spikes/second. The semi-interquartile range is 0.16 spikes/second.

Second infusion refers to the electrical activity of unit #7 during 10 minutes of infusion. The median average discharge frequency is 0.25 spikes/second. The semi-interquartile range is 0.25 spikes/second.

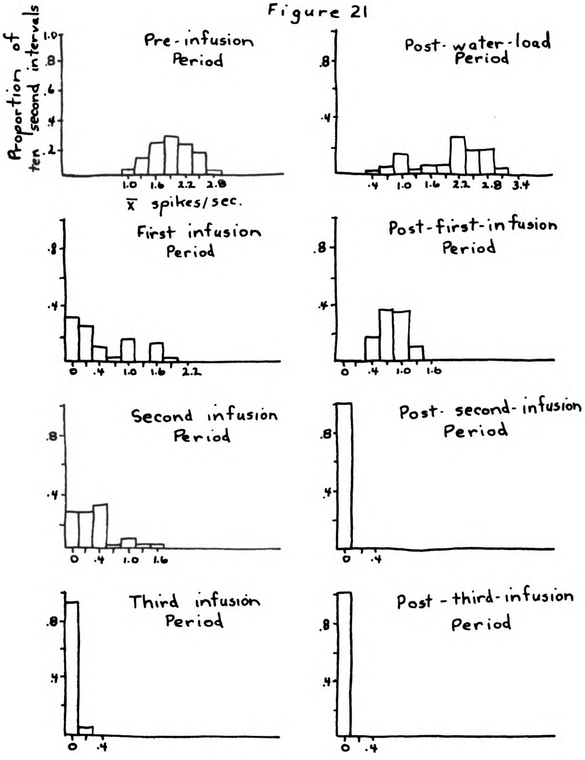
Post-second-infusion refers to the electrical activity of unit #7 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.0 spikes/second.

Third infusion refers to the electrical activity of unit #7 during 10 minutes of infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.0 spikes/second.

Post-third-infusion refers to the electrical activity of unit #7 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0.0 spikes/second. The semi-interquartile range of average discharge frequencies is 0.0 spikes/second.

Post-water-load refers to the electrical activity of unit #7 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 2.26 spikes/second. The semi-interquartile range is 0.48 spikes/second.

Figure 21



Proportion of ten second intervals
Pre-infusion Period
1.0
.8
.6
.4
.2
1.0 1.6 2.2 2.8
x̄ spikes/sec.
Post-water-load Period
.8
.4
.4 1.0 1.6 2.2 2.8 3.4
First infusion Period
.8
.4
0 .4 1.0 1.6 2.2
Post-first-infusion Period
.8
.4
0 .4 1.0 1.6
Second infusion Period
.8
.4
0 .4 1.0 1.6
Post-second-infusion Period
.8
.4
0 .4
Third infusion Period
.8
.4
0 .4
Post-third-infusion Period
.8
.4
0 .4

Figure 22

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #8. The neural unit activity was recorded from goat #18. There were 1 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 1 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the preoptic region of the hypothalamus. Refer to Figure 13.
Pre-infusion refers to the electrical activity of the neural unit #8 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 0.29 spikes/second. The semi-interquartile range is 0.16 spikes/second.
First infusion refers to the electrical activity of unit #8 during 10 minutes of infusion. The median average discharge frequency is 1.95 spikes/second. The semi-interquartile range is 0.92 spikes/second.
Post-first-infusion refers to the electrical activity of unit #8 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0.53 spikes/second. The semi-interquartile range is 0.54 spikes/second.
Second infusion refers to the electrical activity of unit #8 during 10 minutes of infusion. The median average discharge frequency is 2.23 spikes/second. The semi-interquartile range is 0.62 spikes/second.
Post-second-infusion refers to the electrical activity of unit #8 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 0.92 spikes/second. The semi-interquartile range is 0.19 spikes/second.
Third infusion refers to the electrical activity of unit #8 during 10 minutes of infusion. The median discharge frequency is 2.41 spikes/second. The semi-interquartile range is 0.90 spikes/second.
Post-third-infusion refers to the electrical activity of unit #8 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0.76 spikes/second. The semi-interquartile range of average discharge frequencies is 0.28 spikes/second.
Post-water-load refers to the electrical activity of unit #8 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 5.91 spikes/second. The semi-interquratile range is 0.56 spikes/second.

Figure 22

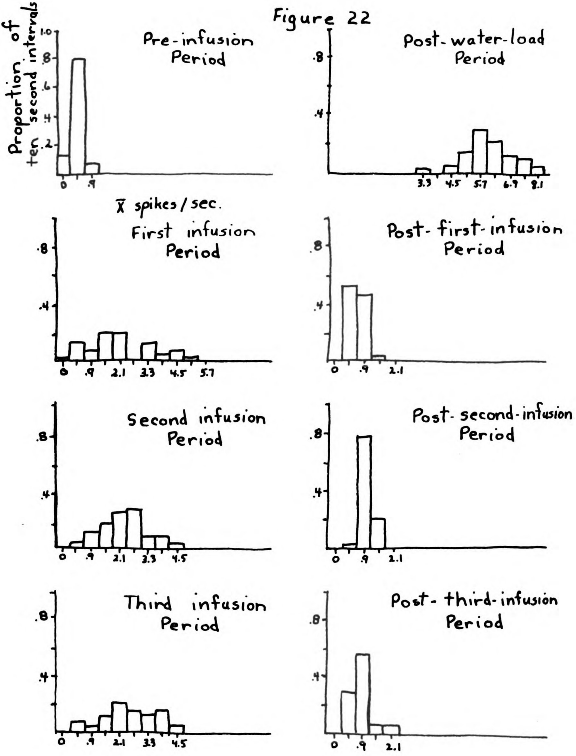

Proportion of ten second intervals
Pre-infusion Period
Post-water-load Period
X̄ spikes/sec.
First infusion Period
Post-first-infusion Period
Second infusion Period
Post-second-infusion Period
Third infusion Period
Post-third-infusion Period

Figure 23

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #9. The neural unit activity was recorded from goat #18. There were 1 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 2 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the preoptic region of the hypothalamus. Refer to Figure 14.
Pre-infusion refers to the electrical activity of the neural unit #9 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 9.20 spikes/second. The semi-interquartile range is 1.26 spikes/second.
First infusion refers to the electrical activity of unit #9 during 10 minutes of infusion. The median average discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.16 spikes/second.
Post-first-infusion refers to the electrical activity of unit #9 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 0.35 spikes/second. The semi-interquartile range is 0.32 spikes/second.
Second infusion refers to the electrical activity of unit #9 during 10 minutes of infusion. The median average discharge frequency is 0 spikes/second. The semi-interquartile range is 0 spikes/second.
Post-second-infusion refers to the electrical activity of unit #9 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 1.39 spikes/second. The semi-interquartile range is 0.58 spikes/second.
Third infusion refers to the electrical activity of unit #9 during 10 minutes of infusion. The median discharge frequency is 0.0 spikes/second. The semi-interquartile range is 0.17 spikes/second.
Post-third-infusion refers to the electrical activity of unit #9 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 0.0 spikes/second. The semi-interquartile range of average discharge frequencies is 0.0 spikes/second.
Post-water-load refers to the electrical activity of unit #9 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 2.95 spikes/second. The semi-interquartile range is 3.35 spikes/second.

Figure 23

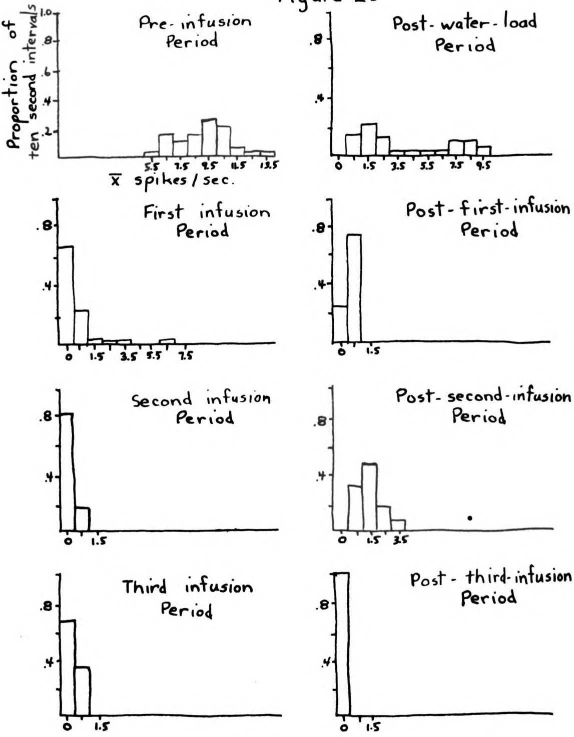
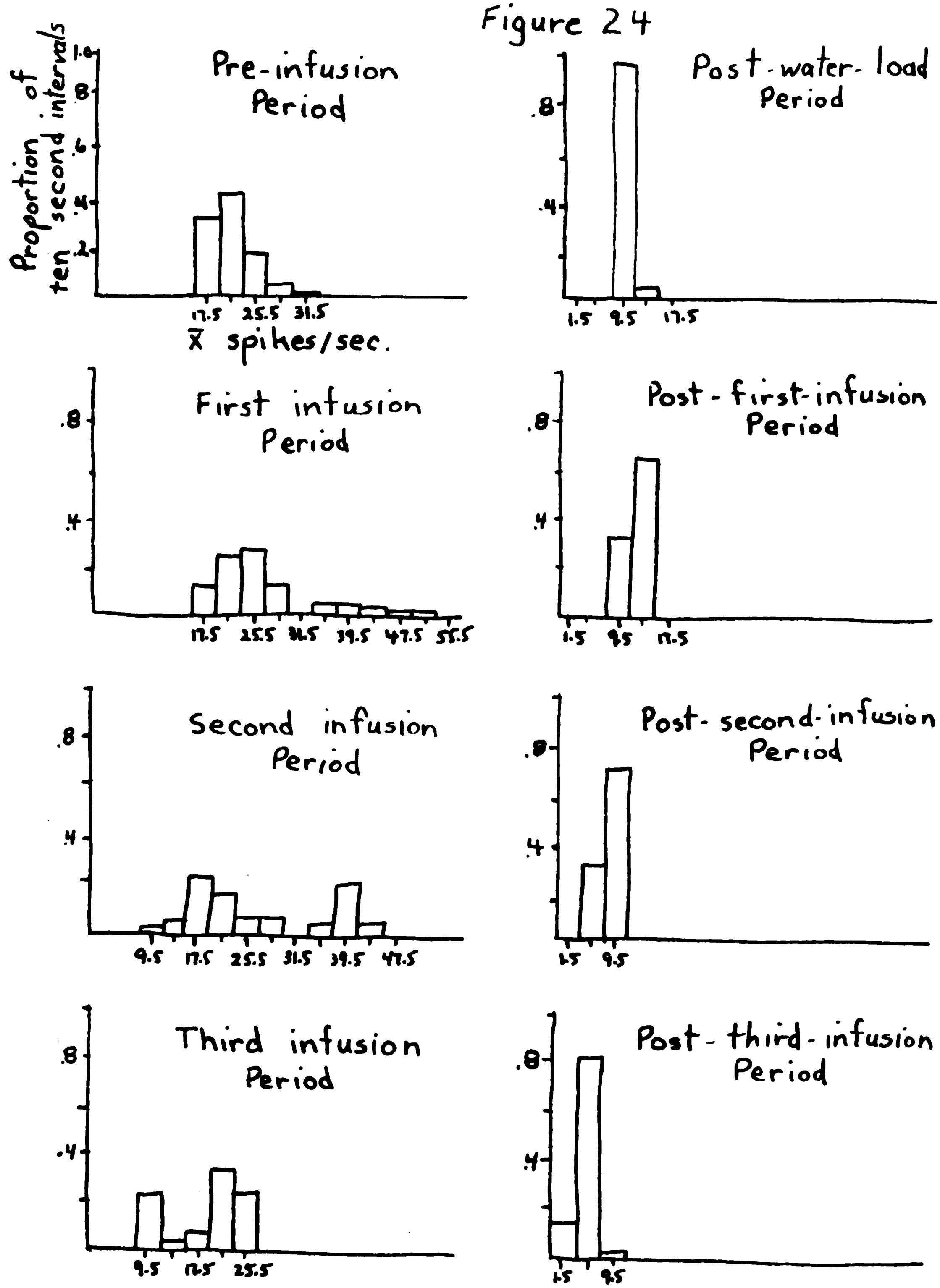

Proportion of ten second intervals
Pre-infusion Period
x̄ spikes / sec.
Post-water-load Period
First infusion Period
Post-first-infusion Period
Second infusion Period
Post-second-infusion Period
Third infusion Period
Post-third-infusion Period

Figure 24

The histograms represent the effect of repeated infusions of hypertonic saline and stomach water load on the discharge frequency of neural unit #10. The neural unit activity was recorded from goat #18. There were 2 series of three hypertonic saline infusions followed by water stomach loads prior to the observations of this unit. This unit was 1 of 2 units recorded from the same electrode site. The electrode tip was estimated after histology to be in the preoptic region of the hypothalamus. Refer to Figure 15.
Pre-infusion refers to the electrical activity of the neural unit #10 prior to beginning the series of infusions. The histogram represents 15 minutes of tape recorded electrical activity. The median average discharge frequency is 21.18 spikes/second. The semi-interquartile range is 2.51 spikes/second.
First infusion refers to the electrical activity of unit #10 during 10 minutes of infusion. The median average discharge frequency is 25.14 spikes/second. The semi-interquartile range is 4.32 spikes/second.
Post-first-infusion refers to the electrical activity of unit #10 occuring 20 to 30 minutes after the termination of the first infusion. The median average discharge frequency is 12.5 spikes/second. The semi-interquartile range is 1.8 spikes/second.
Second infusion refers to the electrical activity of unit #10 during 10 minutes of infusion. The median average discharge frequency is 23.14 spikes/second. The semi-interquartile range is 10.72 spikes/second.
Post-second-infusion refers to the electrical activity of unit #10 occuring 20 to 30 minutes after the termination of the second infusion. The median discharge frequency is 8.64 spikes/second. The semi-interquartile range is 1.62 spikes/second.
Third infusion refers to the electrical activity of unit #10 during 10 minutes of infusion. The median discharge frequency is 20.95 spikes/second. The semi-interquartile range is 5.46 spikes/second.
Post-third-infusion refers to the electrical activity of unit #10 occuring 20 to 30 minutes after the termination of the third infusion. The median average discharge frequency is 5.18 spikes/second. The semi-interquartile range of average discharge frequencies is 1.20 spikes/second.
Post-water-load refers to the electrical activity of unit #10 occuring 10 to 20 minutes after stomach loading 3000cc of water. The median average discharge frequency is 9.6 spikes/second. The semi-interquartile range is 1.06 spikes/second.

Figure 24



Proportion of ten second intervals
1.0
.8
.6
.4
.2
Pre-infusion Period
17.5
25.5
31.5
X̄ spikes/sec.
Post-water-load Period
.8
.4
1.5
9.5
17.5
First infusion Period
.8
.4
17.5
25.5
31.5
39.5
47.5
55.5
Post-first-infusion Period
.8
.4
1.5
9.5
17.5
Second infusion Period
.8
.4
9.5
17.5
25.5
31.5
39.5
47.5
Post-second-infusion Period
.8
.4
1.5
9.5
Third infusion Period
.8
.4
9.5
17.5
25.5
Post-third-infusion Period
.8
.4
1.5
9.5

Appendix C

Raw Data

Unit 1

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
2	9	8	1	1	1	2	2	2	1	1
4	5	8	3	0	0	1	2	2	1	4
5	6	8	4	0	0	1	1	2	1	2
6	2	2	4	0	0	0	4	6	2	6
6	7	3	2	0	0	4	3	2	4	6
1	7	4	5	0	0	0	2	4	2	13
7	9	3	7	1	1	6	7	3	1	10
5	4	9	5	0	0	1	1	6	0	1
6	5	5	1	0	0	5	5	1	1	7
6	4	3	0	0	0	0	5	2	0	6
6	4	1	0	0	0	3	3	4	1	9
8	1	6	0	0	0	0	4	1	2	1
8	7	2	0	0	0	3	3	4	1	3
4	3	4	0	0	2	0	2	0	3	9
4	10	4	0	0	13	6	3	3	0	3
4	2	2	0	0	10	4	0	1	1	6
7	8	5	0	0	12	2	1	7	0	3
3	4	6	0	0	15	3	7	1	1	8
8	25	6	0	0	24	2	1	3	1	34
3	4	3	0	0	23	2	1	3	4	20
5	7	2	1	0	27	7	3	4	5	13
3	7	5	2	0	36	0	8	1	0	9
6	8	5	1	0	35	4	0	2	0	5
5	5	5	0	0	48	1	1	1	1	4
2	1	4	0	1	41	2	11	0	0	1
6	5	2	0	0	34	0	3	2	1	1
4	5	3	1	0	25	5	3	2	1	3
2	4	19	0	0	36	2	7	0	4	6
8	3	6	1	0	26	2	1	1	0	0
7	12	4	1	0	21	1	2	2	0	3

Unit 1 (cont'd.)

Post-second infusion		Third infusion			Post-Third infusion		Post-water load	
0	1	7	3	3	3	14	25	21
3	5	11	3	6	14	18	23	12
2	3	3	2	3	8	25	30	35
1	3	1	0	1	17	49	14	18
0	3	0	1	4	11	66	13	28
2	1	0	3	0	8	109	23	27
3	2	2	1	2	5	4	20	24
7	3	4	0	1	7	4	30	27
1	0	3	2	4	9	5	33	13
3	1	1	3	5	2	9	25	22
1	4	3	4	3	10	4	17	15
0	2	3	4	6	8	24	17	21
16	8	3	16	3	55	13	21	23
3	1	1	3	5	57	18	27	19
4	0	3	3	16	8	22	19	37
12	7	0	4	9	12	21	23	27
14	16	3	0	8	25	27	19	16
6	8	5	6	5	103	32	20	19
2	15	2	1	3	192	133	21	23
2	7	2	1	9	89	43	21	22
9	5	4	3	2	46	118	20	38
1	9	1	3	3	51	43	25	21
0	11	0	4	9	42	13	19	17
0	31	0	0	4	50	4	24	17
2	16	0	6	7	32	7	24	18
0	17	0	2	1	4	12	25	23
1	8	0	1	6	11	8	10	25
2	9	2	0	6	3	28	17	25
0	3	0	2	9	9	25	32	23
3	1	2	5	13	21	5	21	30

Unit 2

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
100	92	98	89	83	77	7	8	1	0	0
96	101	103	86	86	85	6	13	2	3	0
98	100	102	87	78	93	6	3	4	3	0
99	106	103	81	82	87	13	4	5	0	1
92	105	101	84	77	95	16	4	4	3	5
95	103	102	78	79	85	7	4	4	4	1
94	102	103	83	83	87	9	9	2	3	4
100	104	104	80	72	75	12	4	2	1	2
95	111	101	86	79	83	4	4	3	1	0
96	107	109	84	80	81	5	7	1	1	5
96	107	99	91	81	81	3	7	4	0	2
97	112	103	90	79	90	17	9	2	0	3
99	104	100	90	80	93	6	6	1	6	5
96	106	99	87	77	95	7	2	1	2	0
97	109	101	86	76	102	9	1	3	4	6
97	108	106	86	81	100	12	5	0	1	3
92	104	101	90	81	110	7	4	3	0	3
99	103	112	89	80	101	12	12	2	2	1
103	110	107	92	85	98	7	8	1	3	3
102	112	112	88	80	101	5	5	2	2	3
103	109	97	83	87	106	8	5	0	5	4
102	110	111	95	84	105	5	9	2	0	0
102	114	108	89	85	108	4	6	9	2	5
97	107	102	82	88	102	9	5	4	3	0
97	110	100	92	91	98	8	3	1	5	1
107	111	100	87	87	98	12	1	1	1	0
105	110	100	94	75	94	6	2	4	3	3
108	108	108	88	78	88	8	5	2	3	1
104	110	110	79	93	85	11	1	4	2	0
103	106	110	89	95	89	3	6	1	3	0

Unit 2 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
0	0	0	0	0	0	0	15	15
3	1	0	0	0	0	0	10	16
0	0	1	0	0	0	0	10	17
0	0	0	0	0	0	0	12	17
4	1	0	0	0	0	1	12	16
2	0	0	0	0	0	0	12	13
2	0	0	0	1	1	1	9	15
3	0	1	0	0	0	0	16	15
1	0	0	0	1	0	0	7	17
1	0	0	0	1	0	0	10	17
1	0	1	0	0	0	0	11	14
2	0	0	0	2	0	0	14	16
2	0	0	0	0	0	0	10	13
0	0	0	0	0	1	0	14	16
1	0	0	0	3	0	0	11	20
2	0	1	0	0	0	0	12	14
4	0	0	0	1	0	0	17	15
2	0	0	0	0	0	0	13	11
1	0	0	0	4	0	0	14	16
1	0	0	0	0	0	0	14	14
2	0	0	0	1	0	0	18	17
4	0	3	0	0	0	0	10	13
2	0	0	0	0	0	0	18	16
1	0	0	0	1	0	0	13	12
2	0	0	0	0	0	0	16	16
0	0	0	0	3	0	0	20	14
1	0	0	0	0	0	0	12	13
1	0	0	0	2	0	0	13	15
1	0	3	0	1	0	0	14	18
0	0	0	0	1	1	0	13	17

Unit 3

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
8	12	8	12	7	10	0	0	0	0	1
11	10	11	9	8	7	0	0	0	0	0
5	14	8	7	9	14	0	1	3	0	0
12	7	15	9	6	10	0	1	0	0	0
12	8	10	10	6	5	1	0	0	0	1
9	3	11	6	8	1	0	0	1	0	0
9	10	8	8	6	4	1	0	0	0	1
7	7	8	9	7	4	0	0	5	0	0
7	11	6	9	6	1	0	0	0	0	0
8	11	8	8	9	0	0	1	1	0	0
14	10	9	9	7	0	0	0	0	0	0
9	13	12	6	10	0	1	0	0	0	0
7	7	10	11	8	0	2	0	0	0	0
10	10	8	8	10	0	0	0	0	0	0
7	14	7	7	10	0	0	0	1	0	0
11	11	11	9	10	0	0	0	0	0	1
16	12	12	5	11	0	2	0	0	0	0
7	7	14	8	6	0	1	1	1	0	0
11	7	7	11	9	0	0	0	0	0	2
8	10	8	11	9	0	0	0	0	0	0
9	8	6	6	8	0	0	0	0	0	0
12	4	7	10	9	0	0	0	0	0	0
7	14	5	6	9	0	0	0	0	0	0
10	6	11	11	14	0	0	0	1	0	0
10	13	6	9	6	0	0	0	0	1	0
11	8	11	9	4	0	0	0	1	0	0
4	6	3	9	15	0	0	1	1	0	0
13	8	13	8	9	0	0	0	0	0	1
10	11	7	10	6	0	0	0	0	0	0
12	12	12	12	11	0	0	0	1	0	1

Unit 3 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
0	0	0	0	0	1	0	4	10
0	0	0	0	0	0	1	6	8
0	0	0	0	0	0	0	10	6
0	0	0	0	0	0	2	14	12
0	0	0	0	0	3	1	8	6
0	0	0	0	0	0	1	6	6
0	0	0	0	0	1	0	6	8
0	0	0	0	0	0	0	8	14
0	0	0	0	0	0	1	4	6
0	0	0	0	0	1	1	10	14
0	0	0	0	0	0	1	9	6
0	0	0	0	0	0	0	12	7
0	1	0	0	1	0	0	12	9
0	0	0	0	0	0	0	7	12
0	0	0	0	0	0	1	8	4
0	0	0	0	0	0	0	12	8
0	0	0	0	1	0	0	12	8
0	0	0	0	1	0	0	8	8
0	0	0	0	0	0	0	10	7
0	0	0	0	1	1	0	4	10
0	0	0	0	1	0	0	9	12
0	0	0	0	0	0	0	8	6
0	1	0	0	0	0	0	10	12
0	0	0	0	0	0	0	6	8
0	0	0	0	0	0	0	7	4
0	0	0	0	0	0	0	9	10
0	0	0	0	0	0	0	4	7
0	0	0	0	0	0	0	6	10
0	0	0	0	0	0	0	6	7
0	0	0	0	0	0	0	1	8

Unit 4

Each number represents the number of spikes per successive ten second interval (Read down Columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
49	46	50	39	0	7	47	49	73	27	44
43	46	38	33	0	5	56	56	78	26	37
46	45	48	33	0	12	55	53	87	25	35
43	47	44	32	0	12	50	58	75	13	38
46	43	42	33	0	10	51	56	73	22	41
44	45	45	32	0	15	48	45	67	29	38
45	47	38	33	0	13	54	57	64	33	43
46	48	35	30	0	13	57	47	53	30	43
49	44	44	26	0	8	57	53	52	31	41
39	45	40	24	10	0	57	42	43	33	39
45	45	44	26	17	3	55	45	32	31	38
40	45	51	27	14	8	50	55	29	42	33
40	41	41	28	8	5	60	46	18	31	34
45	42	46	28	1	9	51	56	14	43	41
43	46	41	23	4	11	52	50	13	34	32
46	45	40	30	9	19	56	56	16	35	38
42	45	46	27	3	23	42	57	5	35	35
45	42	57	10	0	28	57	57	6	31	40
48	46	46	0	1	34	45	54	4	32	43
43	45	49	0	1	36	60	51	9	37	42
41	41	48	0	2	46	50	58	9	35	41
42	54	46	0	1	51	50	47	12	37	51
47	50	41	0	2	58	64	42	18	36	42
40	39	47	0	1	53	49	53	23	35	42
50	45	46	0	4	57	59	52	25	40	45
53	42	50	0	3	55	68	62	24	39	41
47	42	43	0	7	39	63	51	18	26	42
50	46	42	0	6	40	58	62	14	35	45
50	46	42	0	6	45	51	62	17	35	41
43	46	39	0	7	40	60	61	16	39	42

Unit 4 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
56	50	49	4	18	1	0	15	39
60	20	29	0	21	0	1	16	17
56	15	18	0	10	0	0	17	24
63	33	27	3	16	0	0	16	23
67	24	21	1	16	2	0	14	26
57	23	18	2	21	0	0	18	30
52	29	12	2	13	0	5	13	35
48	44	26	1	11	1	0	17	20
57	47	7	2	16	1	0	16	30
52	48	5	1	17	1	0	19	36
56	33	11	2	20	0	2	16	29
52	49	8	1	14	0	2	19	14
47	47	12	3	13	0	3	24	26
52	52	12	6	15	0	0	13	35
41	44	10	2	16	0	1	15	23
56	51	8	5	16	0	0	37	36
46	46	7	6	16	0	1	20	37
41	53	8	3	18	1	1	20	27
45	50	4	9	18	0	2	43	31
44	56	3	6	17	0	2	25	24
43	40	2	4	19	1	0	30	37
40	41	0	5	20	0	0	16	16
46	44	2	10	23	1	0	30	41
40	47	0	10	21	1	1	21	14
50	46	0	12	20	0	0	31	44
51	52	2	15	19	0	0	19	31
47	41	3	11	23	0	0	33	50
58	52	1	13	21	1	0	23	27
49	78	0	10	22	0	1	26	26
52	51	1	17	15	0	0	14	22

Unit 5

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
43	46	47	16	3	1	2	2	8	6	1
38	44	38	14	6	2	3	2	15	1	3
39	33	44	10	2	3	3	2	13	1	4
51	47	44	12	3	6	3	1	6	3	2
49	51	37	19	2	2	3	0	6	5	11
51	49	34	15	0	2	2	6	9	4	5
42	46	30	14	2	7	2	1	9	6	8
45	46	38	14	3	4	3	1	11	2	9
48	46	33	12	2	4	2	3	9	6	8
47	48	39	18	2	2	3	3	8	3	8
41	45	46	19	4	5	3	2	2	0	4
40	38	28	10	0	3	1	1	2	3	3
51	50	35	8	5	5	3	1	2	3	6
36	52	31	12	1	4	2	1	4	1	2
50	49	38	11	1	5	1	3	4	2	2
43	41	40	9	1	5	3	2	3	8	2
46	52	40	7	1	7	3	2	3	0	3
34	41	39	7	1	6	2	2	3	3	4
43	43	28	7	0	3	2	2	1	3	4
41	43	45	3	1	8	3	1	2	8	2
46	51	44	2	2	5	4	2	3	6	4
47	49	38	5	1	8	2	2	8	3	2
43	37	36	1	1	4	2	3	0	5	4
36	37	40	2	3	4	2	3	1	2	4
51	46	48	3	0	6	1	1	0	2	5
44	46	37	1	1	5	2	2	6	6	13
38	40	33	0	1	3	2	0	6	5	4
44	40	32	2	0	8	1	0	5	2	2
35	37	32	0	0	2	3	2	0	1	4
39	32	25	2	2	4	3	1	1	8	4

Unit 5 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
2	2	5	0	0	0	0	139	153
0	0	6	0	1	0	0	141	135
2	0	2	0	0	0	0	147	138
2	3	6	0	0	0	0	141	142
1	2	1	0	0	0	1	139	153
0	0	0	0	0	1	0	125	141
2	1	1	0	0	0	0	132	141
0	0	10	0	0	0	0	124	138
1	0	0	0	0	0	0	147	148
2	0	0	0	0	0	0	138	137
0	2	0	0	0	0	0	133	148
1	1	0	0	0	0	0	142	142
1	1	0	0	0	0	0	137	151
1	2	0	0	0	0	0	161	148
0	2	0	0	0	1	0	138	149
0	2	2	0	0	0	0	156	142
0	2	0	0	0	0	0	155	150
1	0	1	0	0	1	0	150	150
1	3	1	0	0	0	0	153	154
2	1	0	0	0	0	1	134	142
0	2	0	0	0	0	0	138	141
1	2	0	0	0	0	0	136	149
0	0	0	0	0	0	0	152	140
2	0	0	0	0	0	0	142	145
0	2	0	0	0	0	0	136	140
0	3	0	0	0	0	0	146	141
2	0	0	0	0	0	0	151	142
0	0	0	0	0	0	0	140	138
0	1	0	0	0	0	0	146	147
1	1	0	0	0	0	0	139	138

Unit 6

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
4	9	8	2	5	2	3	0	2	0	0
3	4	3	0	0	3	1	0	0	1	1
5	10	2	5	5	9	2	4	0	3	1
9	6	5	3	5	2	5	4	0	1	0
6	9	3	3	3	5	2	1	3	0	0
8	6	7	1	1	3	0	3	0	1	2
5	7	3	3	2	3	3	2	1	1	1
11	8	1	3	2	3	0	4	1	1	0
5	8	2	4	5	1	0	2	0	1	0
2	7	7	5	2	5	0	2	2	3	0
1	2	1	7	3	1	3	4	0	1	0
6	6	4	3	3	3	2	1	0	3	0
5	7	2	2	2	3	0	5	0	2	0
5	8	6	3	4	4	5	0	1	1	1
5	7	2	4	2	5	2	2	1	1	0
9	6	1	2	2	0	3	0	1	1	1
6	5	3	4	4	2	0	2	1	0	0
4	6	3	2	4	5	1	1	5	3	1
5	2	2	5	3	2	0	1	1	0	0
8	7	5	0	2	1	0	1	2	3	0
7	4	4	6	1	1	3	1	2	3	0
8	5	7	2	3	5	2	4	3	0	0
3	4	4	2	6	5	0	1	1	2	0
4	6	7	0	4	2	0	0	1	1	1
2	5	4	2	4	3	0	1	0	2	0
10	5	5	0	5	2	1	3	2	0	0
8	8	5	7	1	5	2	2	0	0	3
9	4	6	3	3	3	0	2	2	1	0
6	3	4	5	4	4	1	3	3	0	1
8	3	3	0	1	2	0	2	1	0	1

Unit 6 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
0	0	0	0	1	0	0	1	0
0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0
0	0	0	0	0	1	0	1	0
1	0	0	0	0	0	0	0	1
1	0	0	0	0	0	0	0	3
2	0	0	0	0	0	0	1	0
2	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	1	2
0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	1
1	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	1
1	0	0	0	0	0	0	1	1
1	0	0	0	0	0	0	0	0
2	1	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	2
1	0	0	0	0	0	1	1	1
1	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	2
1	0	0	0	0	0	0	0	1
1	0	0	0	0	0	0	0	2
0	0	0	0	0	0	1	0	1
0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	1	0

Unit 7

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
16	26	23	3	0	14	14	4	3	3	0
19	27	20	0	0	10	9	6	3	2	0
24	26	22	0	0	6	6	7	3	3	0
12	24	20	2	0	12	11	8	1	1	0
19	22	19	0	0	11	9	13	2	0	0
14	14	11	0	0	15	10	7	4	1	0
16	23	12	0	1	15	7	4	0	1	2
22	25	20	1	3	18	6	5	5	3	0
18	25	23	4	9	15	7	5	4	0	0
17	16	9	2	17	14	11	5	3	1	0
20	25	21	2	19	19	9	6	2	6	0
20	19	17	2	16	17	10	8	3	4	0
15	17	15	2	16	15	7	10	5	4	0
14	20	17	3	16	15	11	6	2	5	1
19	21	14	0	15	13	8	7	3	2	1
21	25	17	0	11	20	11	6	11	2	0
20	21	16	0	10	20	9	3	4	3	0
22	19	20	2	11	19	10	3	10	0	0
19	22	17	0	16	20	14	5	11	2	0
25	17	19	1	16	15	6	4	12	0	2
22	23	11	0	10	19	10	13	15	0	0
21	24	14	1	10	9	7	12	5	0	0
16	20	18	2	9	19	7	11	15	1	0
19	20	17	3	6	14	12	11	12	0	0
24	24	12	1	11	22	4	9	11	0	0
19	22	14	0	11	18	9	8	1	0	0
22	20	15	2	7	22	6	4	11	0	0
25	16	15	2	17	16	7	7	8	0	0
18	17	13	1	5	17	10	9	0	0	0
21	24	14	0	10	17	10	11	2	0	0

Unit 7 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
0	0	0	0	0	0	0	9	21
0	0	0	0	0	0	0	14	24
0	0	1	0	0	0	0	10	27
0	0	0	0	0	0	0	10	26
0	0	0	0	0	0	0	11	38
0	0	0	0	0	0	0	23	22
0	0	0	0	0	0	0	23	25
0	0	0	0	0	0	0	22	25
0	0	0	0	0	0	0	13	20
0	0	1	0	2	0	0	3	19
0	0	0	0	0	0	0	7	8
0	0	0	0	0	0	0	22	10
0	0	0	0	0	0	0	22	24
0	0	0	0	0	0	0	23	23
0	0	0	0	0	0	0	25	20
0	0	0	0	0	0	0	22	26
0	0	0	0	0	0	0	23	25
0	0	0	0	0	0	0	25	21
0	0	0	0	0	0	0	25	28
0	0	0	0	0	0	0	11	17
0	0	1	0	0	0	0	11	15
0	0	0	0	0	0	0	16	21
0	0	0	0	0	0	0	27	31
0	0	0	0	0	0	0	23	27
0	0	0	0	0	0	0	21	28
0	0	0	0	0	0	0	29	27
0	0	0	0	0	0	0	27	27
0	0	0	0	0	0	0	25	31
0	0	0	0	0	0	0	23	29
0	0	0	0	0	0	0	10	27

Unit 8

Each number represents the number of spikes per successive ten second interval (Read down Columns)

Pre-infusion			First infusion			Post-first infusion		Second infusion		
4	2	0	6	34	12	2	10	5	42	10
7	3	0	2	36	15	10	3	7	41	11
5	6	0	1	30	16	9	2	9	40	9
5	3	2	4	34	5	4	3	6	38	13
2	5	3	1	31	11	2	7	6	25	10
3	4	4	2	25	11	9	4	5	31	6
0	2	3	3	22	14	5	9	10	34	7
1	5	5	4	15	12	3	10	14	26	2
5	3	4	10	14	12	5	3	21	21	4
7	8	4	12	29	9	3	5	9	30	3
4	3	2	3	19	13	8	2	11	16	2
6	1	0	10	16	6	7	6	20	21	6
1	1	3	11	17	10	5	5	23	26	4
1	1	1	12	24	10	3	6	22	25	3
5	1	6	12	23	7	10	11	22	23	3
3	1	0	20	19	11	6	12	24	15	9
3	3	1	21	13	5	6	5	30	22	6
4	0	3	40	16	10	8	7	29	24	6
1	3	3	47	18	8	6	5	27	24	5
2	0	2	42	23	6	7	9	27	12	7
1	2	2	31	25	10	2	1	22	17	9
1	4	3	43	22	4	4	9	24	23	3
5	3	3	48	24	1	5	4	24	13	5
3	0	4	49	21	12	10	12	23	15	4
3	6	3	45	20	9	10	5	26	27	5
2	3	1	27	11	5	8	5	30	24	5
3	1	2	0	16	5	4	1	29	16	3
4	2	0	32	15	2	4	6	21	15	9
5	2	2	37	18	7	5	11	39	23	9
4	6	2	33	17	3	5	10	37	16	4

Unit 8 (oont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
3	1	3	39	9	12	8	63	69
2	2	4	38	22	18	5	54	66
3	3	2	33	11	19	11	75	54
3	2	5	20	17	23	5	58	65
7	10	3	19	15	21	9	59	61
3	4	11	20	12	4	9	56	63
1	3	18	22	8	7	9	70	56
3	4	23	22	11	8	8	56	64
7	3	23	24	5	11	15	58	53
5	2	28	24	4	5	11	70	53
3	3	28	11	5	7	9	59	72
2	2	31	11	8	16	7	77	62
0	3	34	23	9	6	5	47	44
3	3	33	24	7	8	2	62	79
6	5	30	15	2	16	2	32	62
3	6	36	22	2	3	4	57	65
6	7	36	17	13	15	5	53	55
5	5	31	23	7	10	4	60	63
4	5	42	15	3	8	5	50	58
5	7	38	16	5	5	7	55	88
7	3	37	23	6	7	1	71	74
4	3	32	24	5	6	10	53	52
6	5	37	11	4	3	6	59	77
8	1	43	16	7	10	9	57	52
5	3	42	16	6	3	5	70	45
3	5	40	25	4	11	6	63	66
5	3	41	24	3	8	7	50	50
4	3	35	16	5	4	10	54	63
1	6	45	24	8	7	8	59	60
1	3	37	19	4	10	7	76	58

Unit 9

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
74	76	94	65	0	2	4	3	0	2	1
111	88	100	19	0	4	2	1	0	0	2
82	97	102	20	0	3	3	1	0	1	2
103	113	97	31	0	4	2	0	0	0	2
109	73	95	15	0	7	1	0	0	0	6
93	81	104	7	0	4	1	2	0	1	1
63	99	83	2	1	4	2	3	1	0	6
61	72	95	1	0	7	1	0	0	0	4
22	78	80	0	0	6	2	2	0	0	4
62	78	108	0	0	3	2	2	0	0	2
63	68	111	0	0	4	2	0	0	0	2
84	63	112	0	0	7	2	1	0	1	2
109	69	86	0	0	9	2	1	0	0	0
103	84	94	3	0	6	1	1	0	0	0
69	73	91	0	0	10	2	0	0	0	2
67	99	100	0	0	4	2	1	0	0	2
87	93	105	0	0	6	0	2	0	0	1
89	69	93	0	0	5	2	0	1	0	2
86	95	93	1	1	2	1	1	0	0	0
96	135	84	2	1	8	1	0	0	0	1
93	127	67	0	1	4	4	0	0	0	1
60	97	99	0	0	2	0	0	0	0	2
56	62	99	0	1	2	1	0	0	0	0
55	94	107	0	0	4	1	1	0	0	0
96	105	109	0	1	5	0	1	1	0	0
126	113	105	1	0	1	1	1	0	1	0
104	113	100	0	0	2	1	0	1	0	0
97	104	75	0	0	3	4	1	1	0	0
93	78	81	1	0	1	2	0	0	0	0
91	101	86	0	0	2	1	1	1	0	1

Unit 9 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
38	9	0	1	4	0	0	99	10
20	17	4	1	4	0	0	94	17
12	30	1	0	0	0	0	114	28
16	28	0	0	11	0	0	113	26
20	31	1	1	8	0	0	119	48
19	23	1	0	17	0	0	112	88
11	16	1	0	28	0	0	113	26
7	38	0	0	26	0	0	107	97
17	10	0	0	48	0	0	100	26
15	8	0	1	14	0	0	100	50
18	22	0	0	10	0	0	99	63
13	22	1	1	2	0	0	107	71
4	15	1	0	0	0	0	88	28
10	8	0	0	10	0	0	107	21
11	10	0	0	1	0	0	93	24
23	11	0	0	10	0	0	97	16
12	18	0	0	4	0	0	86	14
18	6	0	0	7	0	0	86	33
15	5	1	0	10	0	0	89	22
14	1	0	0	6	0	0	80	30
17	9	0	0	4	0	0	47	38
29	5	1	1	16	0	0	71	23
14	4	1	0	24	0	0	63	16
31	17	1	0	6	0	0	50	28
9	8	4	0	14	0	0	26	31
21	7	1	0	16	0	0	31	17
23	6	0	1	10	0	0	38	30
11	13	1	0	2	0	0	22	13
8	4	4	0	17	0	0	17	29
16	2	0	0	24	0	0	38	17

Unit 10

Each number represents the number of spikes per successive ten second interval (Read down columns)

Pre-infusion			First infusion			Post-first-infusion		Second infusion		
299	254	217	319	279	186	129	111	114	274	123
348	239	187	339	280	179	120	122	101	265	117
231	206	162	311	273	184	117	116	130	223	118
225	207	245	292	287	176	128	108	156	211	126
263	220	196	319	272	186	127	125	254	238	109
258	209	168	288	291	184	108	110	358	215	122
229	227	157	316	252	170	129	109	402	212	101
247	217	191	392	287	148	147	112	388	206	107
272	191	211	456	262	178	113	115	408	200	115
220	219	180	480	274	154	123	105	433	193	105
242	185	173	550	255	166	129	104	424	200	84
251	173	228	557	280	163	121	144	411	186	111
242	188	210	511	267	140	121	132	401	193	102
258	183	209	420	300	149	133	125	437	201	108
256	174	221	395	280	158	96	107	425	209	97
186	248	214	436	246	162	132	125	436	194	102
226	203	182	439	291	154	116	132	450	196	109
200	254	180	401	248	144	110	132	422	216	106
230	262	209	388	224	151	135	124	423	191	105
289	224	174	324	246	145	135	114	434	182	111
228	211	173	335	242	146	114	128	419	185	86
234	226	224	321	237	148	120	145	401	216	96
282	216	174	313	235	133	102	131	379	150	96
182	204	203	281	231	139	103	127	368	194	101
191	201	197	305	226	152	115	119	298	192	89
206	190	187	280	224	147	130	118	283	178	88
258	170	180	299	199	142	101	114	305	153	82
252	185	192	282	207	160	110	131	260	158	77
280	212	184	266	209	168	124	124	290	129	103
259	228	194	241	233	123	123	128	289	140	107

Unit 10 (cont'd.)

Post-second infusion		Third infusion			Post-third infusion		Post-water load	
102	83	95	234	204	46	47	98	87
97	97	91	242	223	59	43	103	85
105	70	114	208	226	49	47	105	89
103	66	101	233	219	57	50	87	91
98	70	103	205	214	53	52	116	82
103	78	97	227	231	49	45	95	101
78	84	106	226	216	51	27	110	100
88	71	95	237	222	55	39	102	97
84	93	104	233	241	46	42	100	85
84	88	93	239	219	36	35	95	101
83	82	84	236	213	41	40	116	100
90	52	101	255	212	65	32	103	96
72	89	93	227	185	40	33	95	89
85	76	98	267	227	76	39	105	91
67	80	102	258	216	38	58	111	85
84	69	144	238	216	36	39	86	88
67	78	221	238	216	35	31	92	102
75	84	153	255	215	47	42	112	101
66	73	161	255	205	38	39	93	96
74	67	178	243	209	35	32	107	90
76	72	181	260	194	47	34	118	92
87	71	170	233	207	44	32	105	81
99	100	219	231	198	44	36	105	105
76	83	213	213	188	46	35	89	98
90	82	193	246	186	43	38	99	106
86	75	210	228	188	50	36	91	91
86	77	221	237	194	53	32	89	105
64	65	196	251	162	56	28	87	103
96	91	195	221	216	48	37	93	99
86	60	226	223	195	45	46	103	103