ELECTRICAL ACTIVITY OF SINGLE CELLS IN THE SEPTAL AREA OF RATS RESULTING FROM CHANGES IN PLASMA VOLUME AND OSMOLALITY

Thesis for the Degree of M. A.

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JOHN G. BRIDGE

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ABSTRACT

ELECTRICAL ACTIVITY OF SINGLE CELLS IN THE SEPTAL AREA OF RATS RESULTING FROM CHANGES IN PLASMA VOLUME AND OSMOLALITY

By

John G. Bridge

The septal nucleus is an area of the brain that has been implicated in a number of different activities. Stimulation of the septal area in humans has been shown to reduce awareness of pain, have a general calming effect, and elicit sexual thoughts and/or feelings. In some species septal lesions interfere with performance in one choice (go - no go) situations, but not with two choice tasks, such as choosing the correct alley in a T-maze. Some investigators have found septal lesions to interfere with learning of reversal tasks; other report enhancement of reversal performance. The "septal rage syndrome" is a classical symptom of lesions of the rat or cat septum.

Involvement in water regulation is perhaps the function of the septum most frequently investigated. There is, for example, evidence that septal lesions

increase water ingestion and septal stimulation inhibits ongoing drinking. Other evidence indicates that septal cells respond to stimuli of one recognized component of thirst (reduction of blood volume), but not to another (an increase in blood osmotic pressure). To further explore the role of the septum in water regulation, discharges of single septal cells during alterations of blood volume and osmotic pressure were observed.

In the first experiment firing rates of septal cells in response to the hypovolemic component of dehydration were monitored. Rats on ad libitum food and water schedules were anesthetized and blood was extracted from the femoral vein. During the extraction cells characteristically increased discharge rates. After the extraction, cells in rats that had large (5 cm^3) extractions decreased firing rates while cells of rats that received moderate size (2-3.5 cm^3) extractions displayed no typical discharge pattern.

In experiment 2 septal activity in 8 water deprived and 8 water satiated rats was monitored for 1 hour. After a 10 minute base rate recording period the deprived rats were stomach loaded with water; 20 minutes after the

stomach load they were injected subcutaneously with 1 cm^3 16% NaCl. With this series of manipulations firing rates of septal cells were recorded as the dehydrated rat became relatively hydrated and then dehydrated again. Satiated rats were injected with the saline solution at the end of the 10 minute base rate recording period, and 20 minutes later stomach loaded with water. With this procedure the satiated rat became relatively dehydrated and then hydrated again. Firing rates of cells in deprived rats and firing rates of cells in satiated rats did not differ significantly during the base rate period. However, 13 of the 16 cells discharged faster during the dehydrated period than during the hydrated period ($p < .02$, sign test).

To observe responses of septal cells to changes in blood osmotic pressure, solutions of hypertonic, isotonic, or hypertonic saline were infused into the carotid artery. Isotonic injections offered an index of the effect of the injection *per se*, while deviations from this index during hypotonic or hypertonic injections indicated changes in firing rates coincidental with changes in osmotic pressure. Injections characteristically increased firing rates; increases after hypertonic injections were usually the

largest, while increases after hypotonic injections were usually smallest.

There are septal cells that are responsive to plasma volume depletion (femoral vein extractions, experiment 1), changes in osmotic pressure (carotid injections, experiment 3), and combinations of these stimuli (stomach loads of water and hypertonic injections, experiment 2). In general, cells fired faster during hyperosmotic than during hypoosmotic blood conditions. Cells also usually discharged faster during blood hypervolemia than blood hypovolemia. This combination of results was not anticipated--since hypovolemia and hypertonicity are both conditions of the water deprived state, it was expected that these conditions would both increase or both decrease firing rates of septal cells.

Approved
Glenn J. Sutton
25 Feb. 1972

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INTRODUCTION

During the past two decades, the role of the lateral and medial septal nucleus in water regulation has been the topic of numerous experiments. However, these investigations have used ablation or stimulation techniques; none used extracellular single unit recording. A number of issues remain unresolved, apparently because the experimental designs inherent in lesion and stimulation studies prohibit a direct analysis of the role of septal cells in water regulation.

The first and most basic issue is still in question: Are septal cells involved in water regulation? In more operationally definable terms this might be asked: Do septal cells alter firing rates in response to changes in blood volume or osmotic pressure? There is mounting indirect evidence that they do, but some quite different theories are tenable.

For example, the septum has been considered an inhibitor of ongoing behavior; the septal lesioned animal

is unable to inhibit ongoing behavior (drinking as well as other behavior). This theory has been supported by experiments that demonstrate Passive Avoidance Response (PAR) deficits in septal lesioned animals (Kaada, Rasmussen, and Kveim, 1962; McCleary, 1961). But in 1965, Harvey and Hunt found that septal lesioned rats could maximize water rewards on Differential Reinforcement of Law rates (DRL), and that differential water pre-loads equalize bar pressing on Fixed Interval (FI) and Continuous Reinforcement (CRF) schedules with water as a reward. It was, perhaps, that study that turned attention to the lateral and medial septum as areas directly involved in water regulation. To date, evidence for this comes from a number of perspectives: a) lesions in both lateral and medial septal regions produce hyperdipsia (Blass and Hanson, 1970; Harvey and Hunt, 1965); b) deprivation enhances and satiation diminishes septal self-stimulation (Brady, Boren, Conrad, and Sidman, 1957); c) cholinergic stimulation of the lateral septum increases both food and water intake (Fisher and Coury, 1962) and carbachol stimulation of the medial septum increases drinking and decreases eating (Grossman, 1964).

Hyperdipsia following septal lesions is a highly consistent phenomenon, while increments in food ingestion and the septal rage syndrome are not consistent results. Lubar, Schaefer, and Wells (1969) found that hyperdipsic effects are strongest with lesions in the middle and posterior third of the septum (particularly in the anterior two-thirds of the posterior third of the septum). Effects of lesions in the anterior septum, they showed, are much less than those of posterior lesions on water intake, urine output, and urine osmolality. Injections of pitressin tannate returned these measures to near normal levels.

This literature that indicates septal cells probably are involved in water regulation contains a basis for a hypothesis about the direction of the changes in firing rate as an animal goes from one hydration state to another. Since septal self-stimulation is enhanced by deprivation and inhibited by satiation, and septal lesions produce hyperdipsia, an intuitively attractive hypothesis would be that the septum is a drinking inhibitory center, and activity of septal cells decreases during deprivation and increases during satiation.

Another line of reasoning leads to the opposite hypothesis, that septal cell firing decreases during satiation and increases during deprivation. Cross and Green (1959), Dyball and Koizumi (1968), and Suda, Koizumi, and Brooks (1963) have established that the cells of the supraoptic nucleus increase activity and ADH release increases in response to increased blood tonicity. Hayward and Smith (1963) have demonstrated that septal stimulation (and stimulation of other limbic structures) causes pituitary release of ADH. With this line of reasoning one can account for polydipsia in septal lesioned rats: without excitatory stimulation from the septal nucleus, supraoptic activity diminishes, ADH release decreases, and diuresis leads to additional water ingestion. From this perspective it appears that septal cells should be hyperactive during deprivation and hypoactive during satiation. With these two arguments leading to different conclusions about the relative discharge rate of septal cells during deprivation and satiation, a direct measurement of this phenomenon would be helpful.

While these first two questions involve (a) whether or not septal cells are involved in water regulation, and (b) if so, whether the cells are more active during

deprivation or satistion, a different area of research has indicated that the septum may be sensitive to vascular volume shifts, but not to osmotic pressure changes. Blass and Hanson (1970) injected septal lesioned rats and normal rats with either polyethylene glycol (PG) to induce hypovolemia, hypertonic saline to cause blood hypersmolality, or isotonic saline (preceded by 6 hrs. food and water deprivation), and then measured intakes at 15, 30, 60, 90, and 120 minutes post injection. Lesions damaged both lateral and medial septal areas extensively. The PG septal rats drank more than the PG normal rats in the first 15 minutes, but intakes were identical from then on. In the hypertonic saline group, septals drank less than normals in the first 15 minutes, but more during the 30, 60, 90, and 120 minute periods; however, none of these differences appears significant. Finally, in the deprivation and isotonic saline group, septals drank more than controls at each interval. Blass and Hanson interpreted these data as evidence that hyperdipsia in septal rats is in response to hypovolemia, but not to hypersmolality. They also measured hematocrit and serum sodium, and concluded that normal and septal rats differ on these measures neither in ad lib nor in PG injected states. To Blass and Hanson this indicated

that septal hyperdipsia is not a result of a shift in body fluid balance; that is, the septal rats are drinking more than normal rats in response to similar blood volume and osmotic conditions.

A few procedural anomalies and some more recent experiments cast doubt on the results and interpretations of the Blass and Hanson study. Almli (1970) noted that hypertonicity accompanies hypovolemia when the sample is taken as the animal initiates drinking, even when a "pure" volume manipulation such as hemorrhage is made. Perhaps rats in Blass and Hanson's hypovolemia group were, in fact, responding to hypertonic cues that follow a hypovolemic manipulation. One disappointment in the Blass and Hanson article is that they used very small N's and rarely reported significance levels. Finally, Wishart and Mogenson (1970) referenced an unpublished study that fails to confirm that septal rats over-respond to hypovolemia. For these and other minor reasons, the Blass and Hanson article has been discredited to the extent that the hypothesis that septal cells are sensitive to vascular volume changes but not to osmotic pressure changes should be carefully examined.

The present study is designed to investigate the questions raised above. These are:

(a) How do septal cells respond to changes in the hydration state of the animal?

(b) How does hemorrhagic hypovolemia alter the firing rates of septal cells?

(c) What is the response of septal cells to carotid injections of hypo-, iso-, and hypertonic saline injections?

The firing rates of septal cells as a function of different hydration states were determined by two methods. The first compared the firing rates of septal cells under ad lib food and water conditions, 23.5 hour water deprivation, and satiation (immediately after the 0.5 hour drink period). In the second method, firing rates before, during, and after both stomach loads of water and subcutaneous injections of hypertonic saline were examined. To analyze the Blass and Hanson proposal that septal cells respond to vascular volume shifts but not to osmotic pressure changes, units were recorded before, during, and after either injections of saline solutions into the common carotid artery

or extractions of blood from the femoral vein. Heart rates were monitored during all experiments. Histological slides of the recording sites were made with either 40 micron frozen sections and cresyl violet stain, or 25 micron celloidin sections and Heidenhain, Weil, or Thionin stain.

EXPERIMENT 1

Pilot data indicated that in determining the response of septal cells to the volume component of dehydration stimuli a femoral vein extract was superior to the tail cut method; in addition to resulting in greater blood flow than tail cuts, femoral vein extracts allowed the experimenter to extract blood at a constant predetermined rate.

Method

Subjects

Subjects were 18 male albino rats, 105-125 days old and 350-425 grams on the recording date, supplied by Holtzman Company of Madison, Wisconsin. They were housed in single cages with constant light and ad lib food and water.

Procedure

Prior to surgery all subjects received 0.7 g/kg Dial-urethane anesthetic; occasionally, supplementary doses of 0.03-0.07 g were necessary to anesthetize the animal completely. Each rat was shaved on the crown, chest, and abdomen with electric clippers. The femoral vein was exposed and a 0.025 o.d. x 0.012 i.d. 22 cm. catheter was inserted approximately six to seven cm. toward the heart by passing it through the vein wall in a 17 gauge needle and tying the vein on the proximal side of the insertion, and the needle then removed. The catheter was filled with sodium heparin and the outer end joined to a heparinized syringe by a 26 ga. stainless steel needle. Once the surgery was complete, the wound was closed and held with wound clips. A needle electrode served as a heart lead and was subcutaneously onto the chest; both the heart lead and the catheter were prevented from slipping by affixing them to the skin with masking tape. Next, ear pins were inserted, and the rat stereotaxically positioned. Body temperature was maintained at 36-38°C. Each subject was wrapped in a variable temperature heating coil and both the rat and the coil were wrapped in a towel; temperature was monitored with a rectal thermister inserted to approximately 7-8 cm.

After the skull was surgically exposed, a 0.4 cm. hole was trephined, dura mater removed, and a tungsten electrode with glass insulation (except for a 5-8 micron exposed tip) was lowered to the septum. Leads from the electrode and the heart probe went to a junction box with off-lesion-record options. In the lesion position, current could be supplied to the electrode tip for a marking lesion (20 μA anodal current for five seconds); in the recording position, impulses from the electrode and the heart lead went to channel one and channel two, respectively, of a dual beam oscilloscope and to a stereo tape recorder, via a low level preamplifier set to amplify times 1000. Impulses were monitored auditorily with a loudspeaker, and an on-line counter was available to record the rates of impulses whose amplitude exceeded the threshold of the oscilloscope trigger.

Once a cell was sufficiently isolated (the signal-to-noise ratio allowed reliable recording of the unit discharge), magnetic tape records were taken during the following procedure:

1. Five minutes of base rate.
2. A two to five minute extract period in which 2-5 cm^3 of blood were extracted at a rate of approximately 1.0 cm^3 per minute.
3. A 50 minute post-extract period.

Results

Distribution of recording sites on the anterior-posterior dimension is shown in Figure 1; for recording sites of individual cells, see Appendix B. The cytoarchitectural demarcation of the lateral and medial septum (shown by the DeGroot atlas used in this appendix) was particularly vivid with Heidenhain and Weil fiber staining techniques, revealing the medial area to be a much more fiberous portion of the septum than is the lateral region. The results of experiment 1, expressed in percent of base rate, are summarized in Figures 2-4. The control group was comprised of three normal control rats and three surgery controls that had the femoral vein exposed and a catheter inserted but no blood drawn. Because the normal controls and surgery controls were similar in firing patterns throughout the one hour recording session, they were combined to form the control group of N = 6 in Figure 2. As the graph indicates, at no point did the mean discharge rate differ reliably from its original five minute base rate. But there was a tendency for these cells to increase in rate over the entire time of the recording session. Statistically significant differences in rates may have

Figure 1.--Indicated are maximum, 0.5 mm lateral, and 1.0 mm lateral sagittal projections of the rat septum. Below are confirmed recording sites for each of three experiments in the anterior-posterior dimension. Adapted from de Groot, 1959.

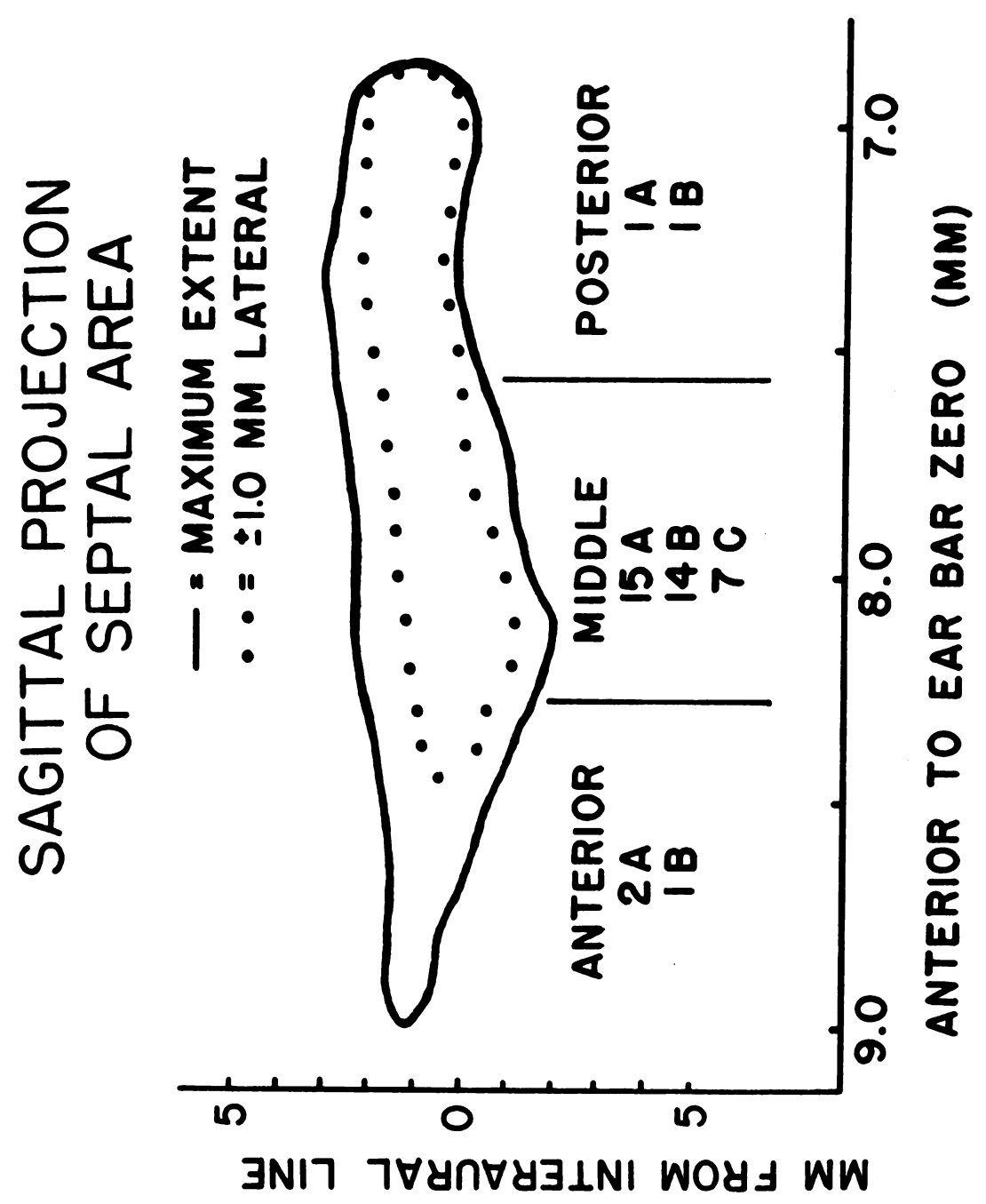

SAGITTAL PROJECTION
OF SEPTAL AREA
— = MAXIMUM EXTENT
• • = ±1.0 MM LATERAL
ANTERIOR
2A
1B
MIDDLE
15A
14B
7C
POSTERIOR
1A
1B
9.0
8.0
7.0
ANTERIOR TO EAR BAR ZERO (MM)
5
0
5
MM FROM INTERAURAL LINE

Figure 2.--Indicated are the mean (± S.E.) percent changes from a five minute mean base rate. N = 6.

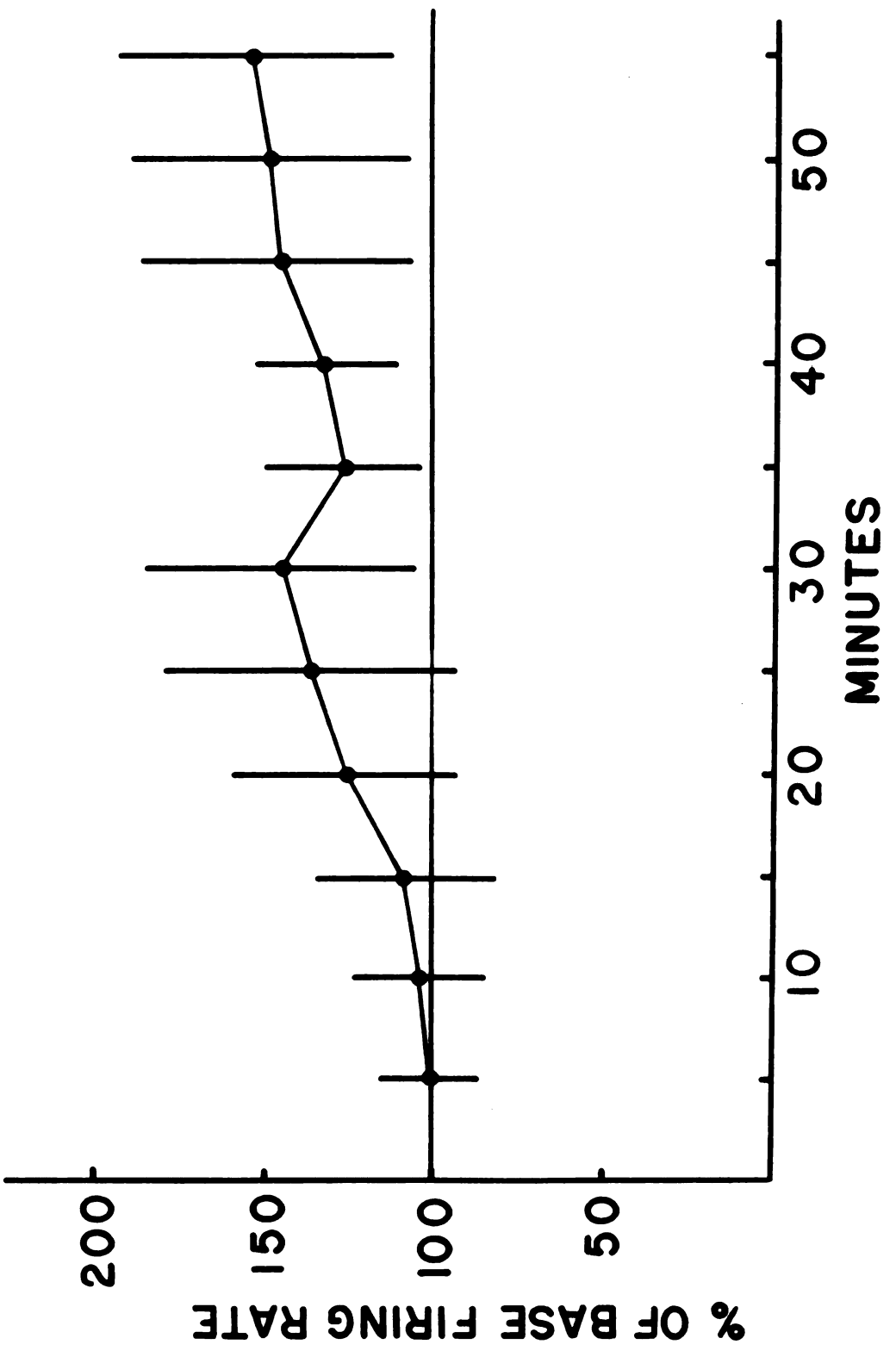

% OF BASE FIRING RATE
200
150
100
50
10
20
30
40
50
MINUTES

Figure 3.--Indicated are mean (± S.E.) percent changes from base rate during and after a 5.0 cm^3 extraction of blood from the femoral vein. N = 6.

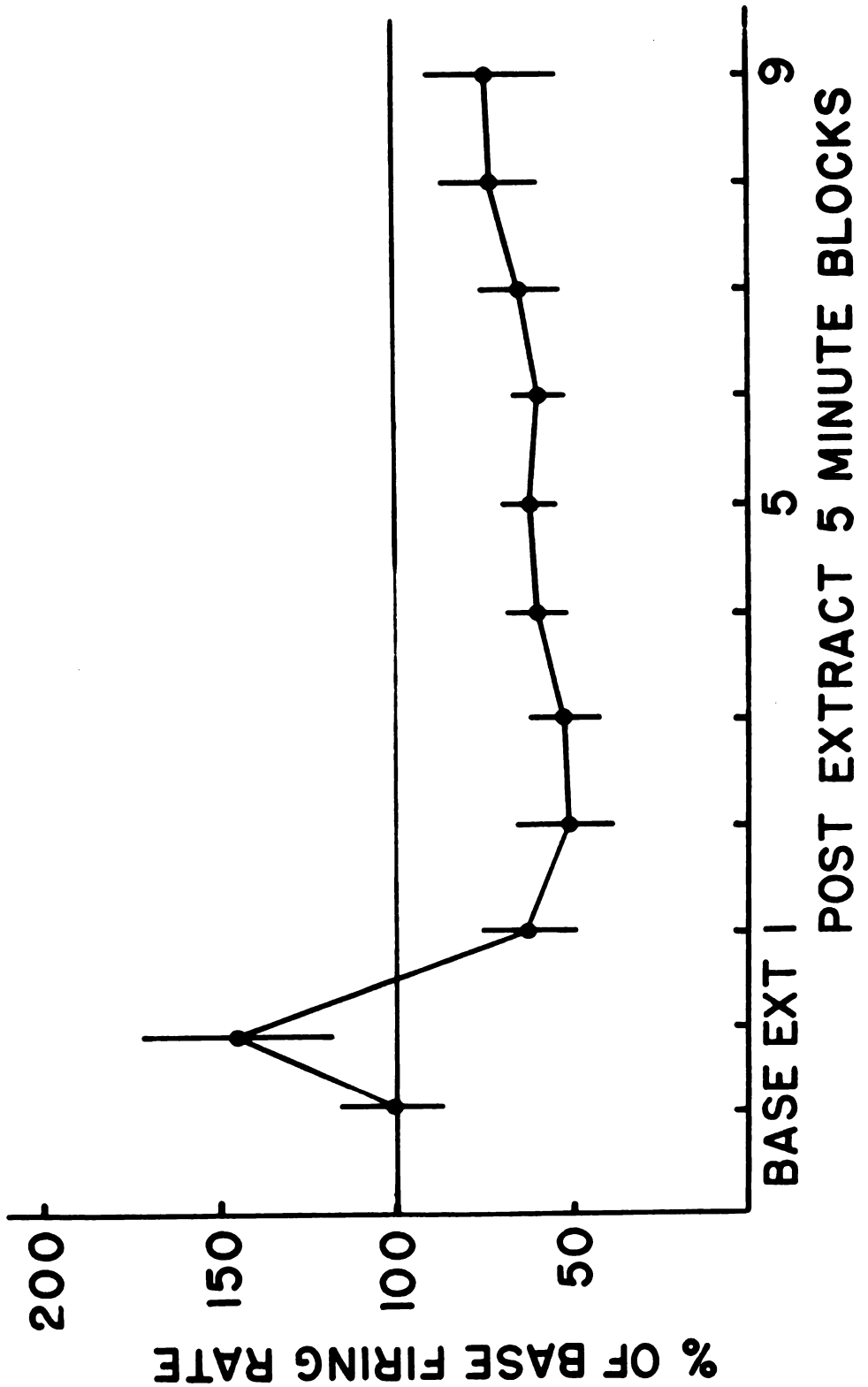
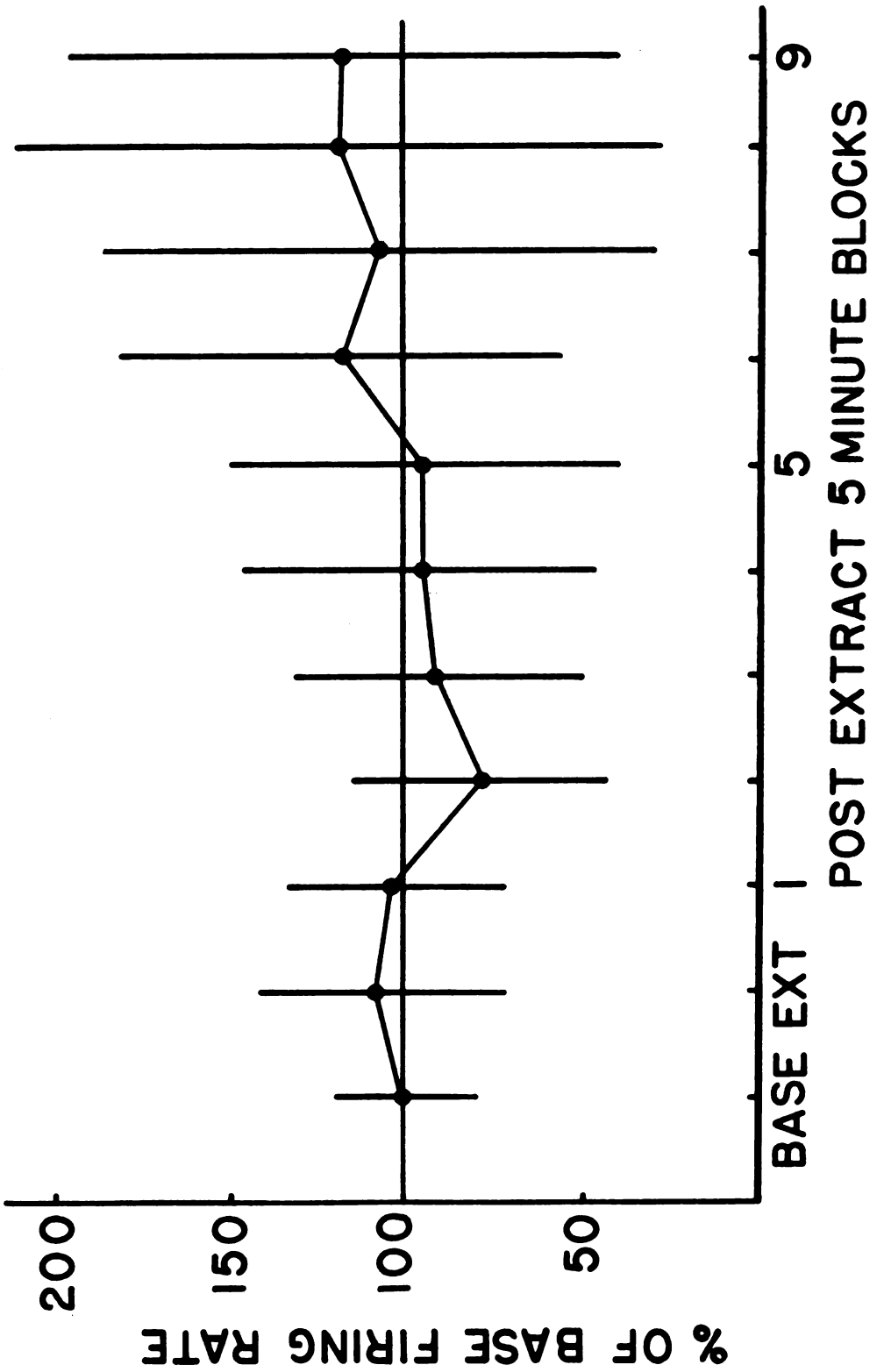

% OF BASE FIRING RATE
200
150
100
50
BASE EXT 1
5
9
POST EXTRACT 5 MINUTE BLOCKS

Figure 4.--Indicated are mean (± S.E.) percent changes from base rate during and after a 2.0 cm^3 or 3.5 cm^3 extraction of blood from the femoral vein. N = 6 (two 3.5 cm^3 and four 2.0 cm^3 extractions).


% OF BASE FIRING RATE
200
150
100
50
BASE EXT
1
5
9
POST EXTRACT 5 MINUTE BLOCKS

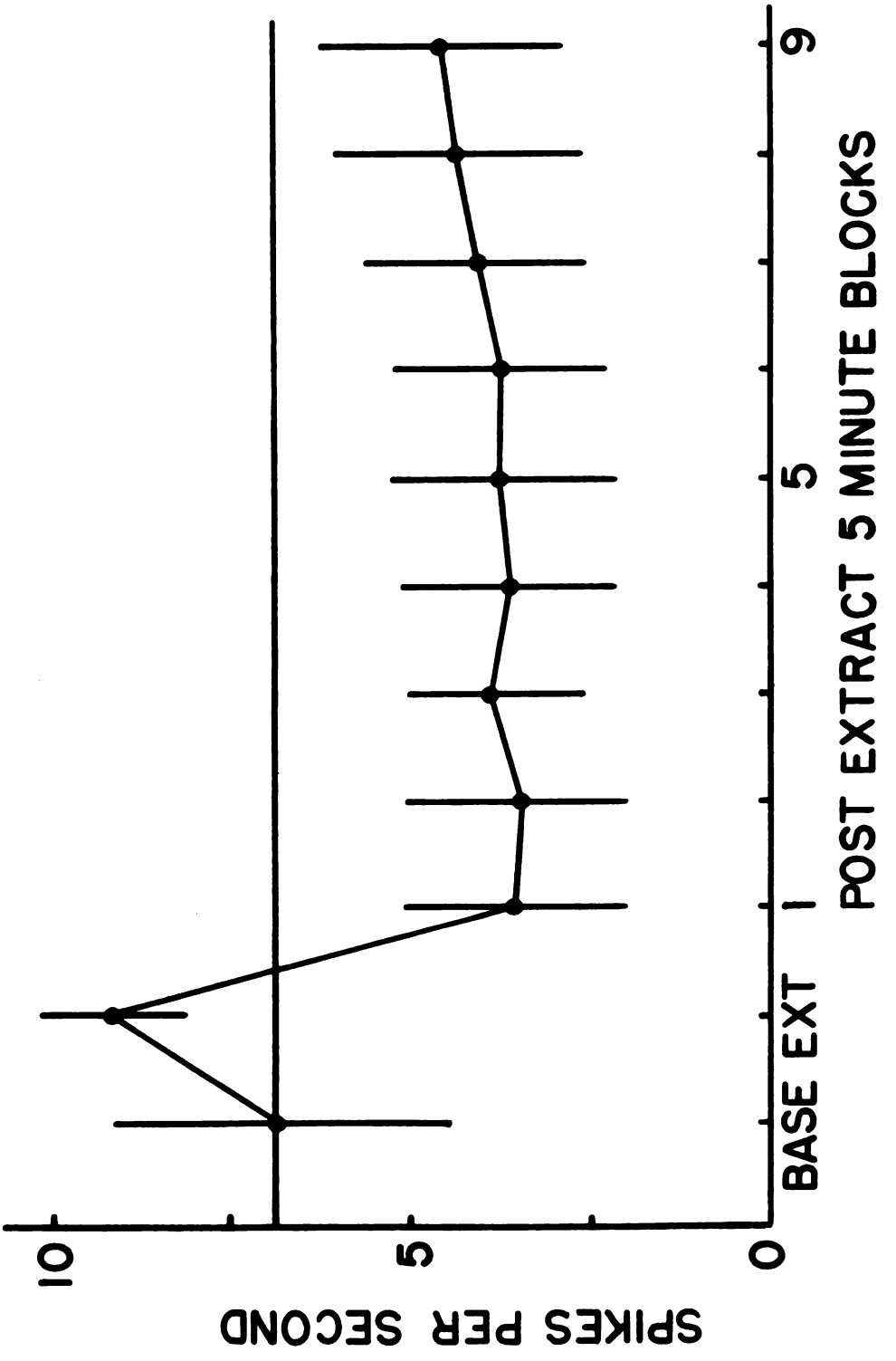
been observed if the sessions had been extended for longer periods. During the extraction of blood producing hypovolemia, the 5 cm^3 extract group (Figure 3) showed a tendency to increase, and this was followed by a decrease during the post-extract period. The 5 cm^3 extract group did not differ from the controls during the extract, but each post-extract mean was significantly ($p < .05$) lower than the control group mean. Extracts of intermediate sizes (2.0 cm^3, N = 4, and 3.5 cm^3, N = 2) showed mean responses intermediate to those of the control and the 5.0 cm^3 groups (Figure 4). However, individual records of the intermediate size extracts reveal that rather than discharging in an intermediate pattern, these cells characteristically respond in a pattern similar to either the control group or the 5.0 cm^3 group. Of the four cells with 2.0 cm^3 extractions of blood, two were similar to the control group and two were similar to the 5.0 cm^3 group; likewise, the 3.5 cm^3 group was evenly divided. This accounts for the large amount of variance in the intermediate group.

While a measure of percent of base rate offers a good index of the effects of the manipulation on single units, it may be misleading in assessing the overall

changes in septal activity, since a percent change measure would give slower firing cells a disproportionately greater weight than faster firing units. To obtain an index of overall discharge rate changes (and to reveal how closely the percent change measure reflects the overall change measure), the absolute number of discharges per second minus the mean base discharge rate was calculated for each cell during the extract and post-extract periods, and this mean absolute change for the 5.0 cm^3 group is shown in Figure 5.

Confirmation of the recording sites revealed that in the control group 4 of the 6 cells were in the lateral septal area. Of the 6 cells in the 5 cm^3 extract group all were in the medial septal region, and in the 2 3.5 cm^3 extract group 3 of 6 cells were in the medial septum. No differential effects between the lateral and medial septal nuclei were observed, nor were any differential effects apparent along anterior-posterior, dorsal-ventral, or medial-lateral dimensions. For anatomical sites of individual cells, see Appendix B.

Figure 5.--Indicated are discharge rates before, during, and after a 5.0 cm^3 extraction of blood from the femoral vein. N = 6.


SPIKES PER SECOND
10
5
0
BASE EXT
1
5
9
POST EXTRACT 5 MINUTE BLOCKS

EXPERIMENT 2

This experiment determined single unit firing rates during water deprivation and satiation, stomach loads of water, and subcutaneous injections of hypertonic saline solutions. With this design, two perspectives were available: a) discharge rates of septal cells during naturally occurring states of hydration and dehydration were measured when the animals were recorded under deprived and satiated conditions, and b) unit discharges in response to relative changes in hydration were measured under experimentally controlled conditions during and after the stomach load and hypertonic injection recording periods.

Method

Subjects

Sixteen naive, male albino rats of the Holtzman strain 350-425 g and 101-110 days old on the recording date were housed in individual cages under constant light.

For a minimum of five days preceding the recording date, each subject was adapted to a 23.5 hour water deprivation schedule; food (to Appendix) was available ad libitum.

Procedure

Recording procedure was the same as described in experiment one. Subjects were assigned to either a deprived (DEP) group or a satiated (SAT) group; in the DEP group subjects were removed from their home cages at the end of the 23.5 hour water deprivation period, weighed, and injected with 1.0 g/kg Dial-urethane (mixed in our laboratory). When the rat was sufficiently anesthetized, a polyethylene tube was inserted into the stomach. A heart lead was affixed and the animal was stereotaxically positioned as in the hypovolemia experiment. Once a cell was isolated, a ten minute deprived base was recorded. Then, a 10.0 cm^3 stomach load of room temperature tap water was injected via the polyethylene tube at approximately 0.25 cm^3/15 seconds (10.0 cm^3 in 10.0 minutes) with a 20 cm^3 plastic syringe. A post-stomach load period (P-SL) of 20 minutes followed in which no manipulation was made. (The stomach load tube was left in the animal

throughout the experiment.) At the end of the P-SL period--40 minutes into the recording session--a 1.0 cm^3 16% NaCl solution was injected subcutaneously into the hip area. This injection always took less than 10 seconds, and the 10-second time bin in which it occurred was not included in the data analysis. Finally, a post-injection period (P-INJ) of 20 minutes was recorded. Following the recording session a blood sample was extracted by heart puncture, centrifuged at 3300 rpm, the plasma analyzed for protein with a refractometer and frozen for later osmometry.

Subjects in the SAT group were removed from the home cage immediately after the 0.5 hour drink period of the 23.5 hour deprivation schedule. As with the DEP group, once the animal was anesthetized a stomach tube was inserted, a heart lead was affixed to the chest, and the rat was stereotaxically positioned. The order of manipulations in the SAT group differed from that for the DEP group in that the hypertonic saline injection preceded the stomach load of water in the SAT animals. After a 10-minute base rate recording, the SAT animals were injected with 1.0 cm^3 of NaCl, and a 20-minute P-INJ period was recorded. The recording session continued through a 10-minute stomach load period (10.0 cm^3 at 0.25 cm^3/15 seconds) and a 20 minute

P-SL period. Blood sampling and perfusion procedures were identical to those described for the DEP group.

Results

By comparing the 10 minute base rates of the DEP and SAT groups it was determined that, under these operationally defined conditions of water deprivation and satiation, firing rates of septal cells in dehydrated rats do not differ significantly from firing rates of cells in relatively hydrated rats (DEP $\bar{X} \pm$ S.E. = 0.594 ± 0.067; SAT $\bar{X} \pm$ S.E. = 0.0501 ± 0.255). The firing rates of septal cells in response to DEP and SAT group manipulations are summarized in Figures 6 and 7. In general, cells tended to increase discharge rates in response to hypertonic injections and decrease discharge rates in response to stomach loads of water. When the mean firing rate during the stomach load and P-SL period was compared to the mean firing rate of the P-INJ period, 13 of the 16 units (8 DEP and 8 SAT) discharged faster in response to the hypertonic injection than to the stomach loads ($p < .02$). Osmotic pressure of the DEP group was higher than that of the SAT group (306.2 ± 1.7 and 297.3 ± 3.8 mOs, $p < .01$), but the

Figure 6.--Indicated are mean (± S.E.) percent of base firing rate during a 10 minute stomach load period (S.L.) in which 10.0 cm^3 of tap water were loaded at 1.0 cm^3/minute, a 20 minute post-stomach load period (Post-S.L.), and a 20 minute post-injection period (Post-inj.). The base (Dep base) rate was recorded from 23.5 hour deprived rats and the injection was 1.0 cm^3 16% NaCl administered subcutaneously in the hip area. N = 8.

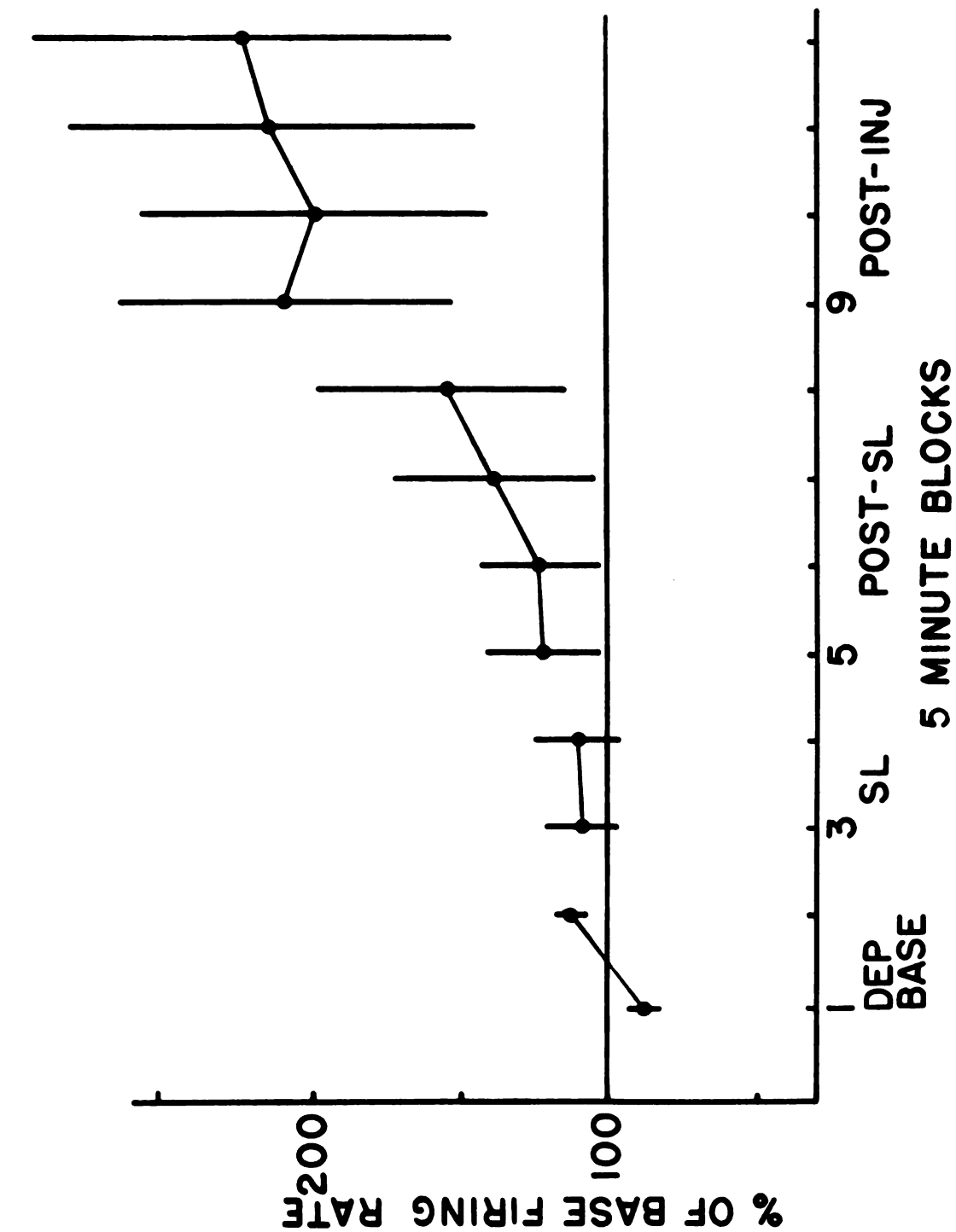
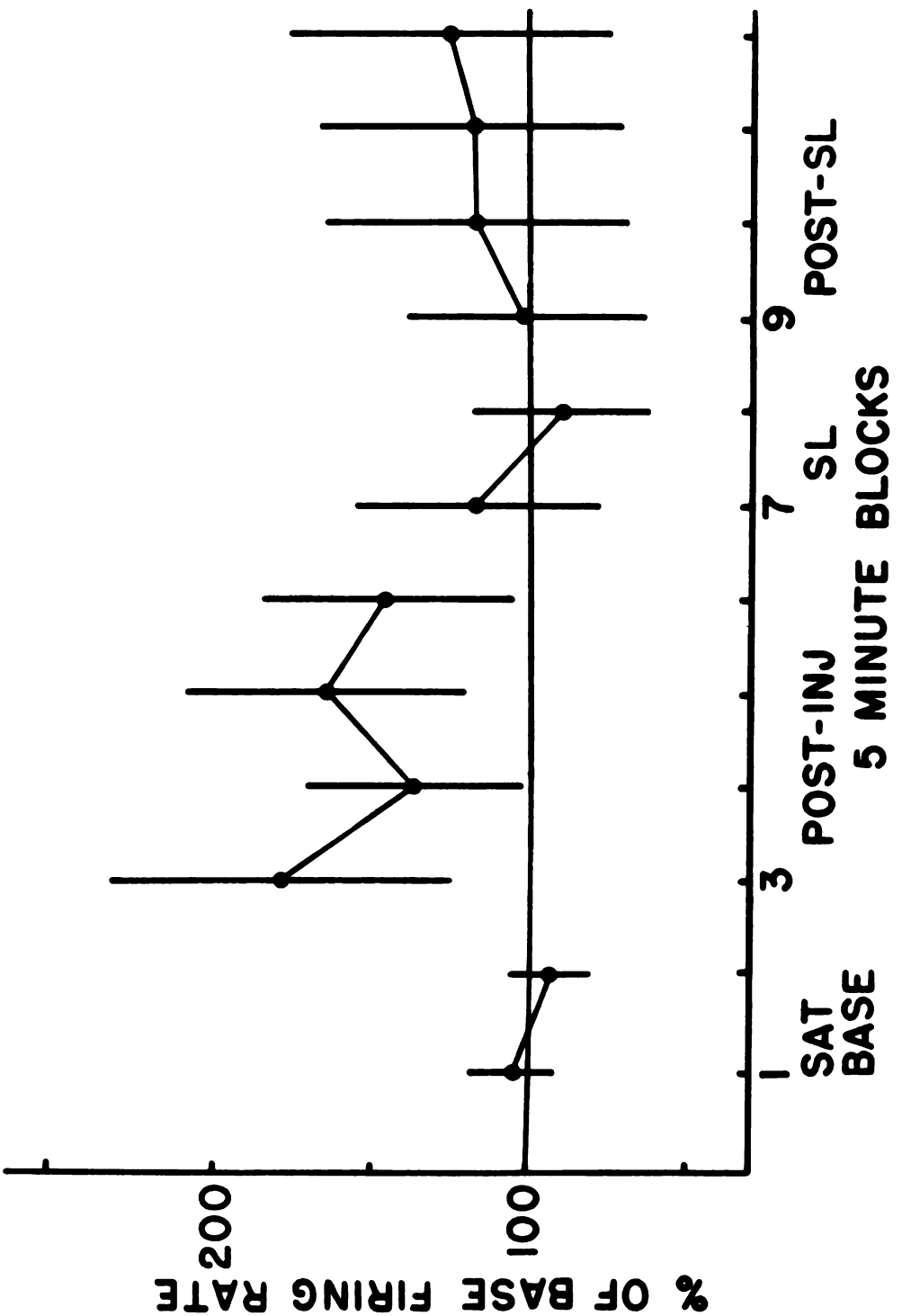

% OF BASE FIRING RATE
200
100
1
DEP
BASE
3
SL
5
POST-SL
9
POST-INJ
5 MINUTE BLOCKS

Figure 7.--Indicated are mean (± S.E.) percent of base firing rate during a post-injection period (post-inj) of 20 minutes, a 10 minute stomach load period (post-S.E.) in which 10.0 cm^3 of tap water were loaded at 1.0 cm^3/minute and a 20 minute post-injection period (post-inj). The base (SAT base) rate was recorded from rats after the 0.5 hour drink period of a 23.5 hour water deprivation schedule, and the injection was 1.0 cm^3 16% NaCl administered in the hip area. N = 8.


% OF BASE FIRING RATE
200
100
1
SAT
BASE
3
POST-INJ
5 MINUTE BLOCKS
7
SL
9
POST-SL

protein concentrations of the DEP group (5.1 ± 0.2 g/100 ml) and the SAT group (5.1 ± 0.1 g/100 ml) were nearly identical. Heart rates of the DEP and SAT groups are analyzed in Figures 8 and 9.

In both the SAT and DEP groups 7 of 8 cells were in the lateral septum. For individual recording sites see Appendix B.

Figure 8.--Indicated are mean (± S.E.) percent changes in heart rate for six animals chosen at random from the DEP group. Change in heart rate for each rat was calculated from its respective mean baseline rates. N = 6.

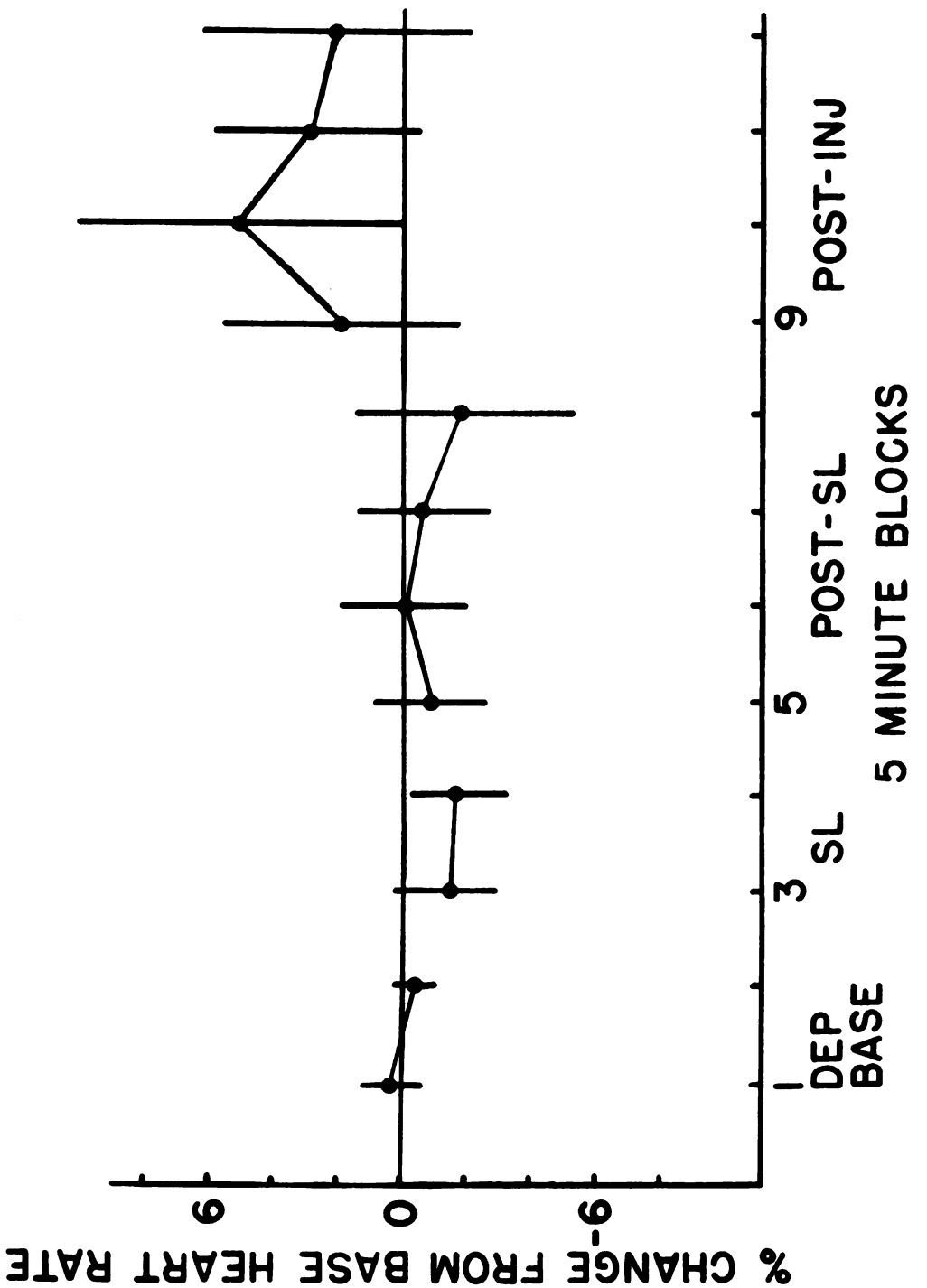
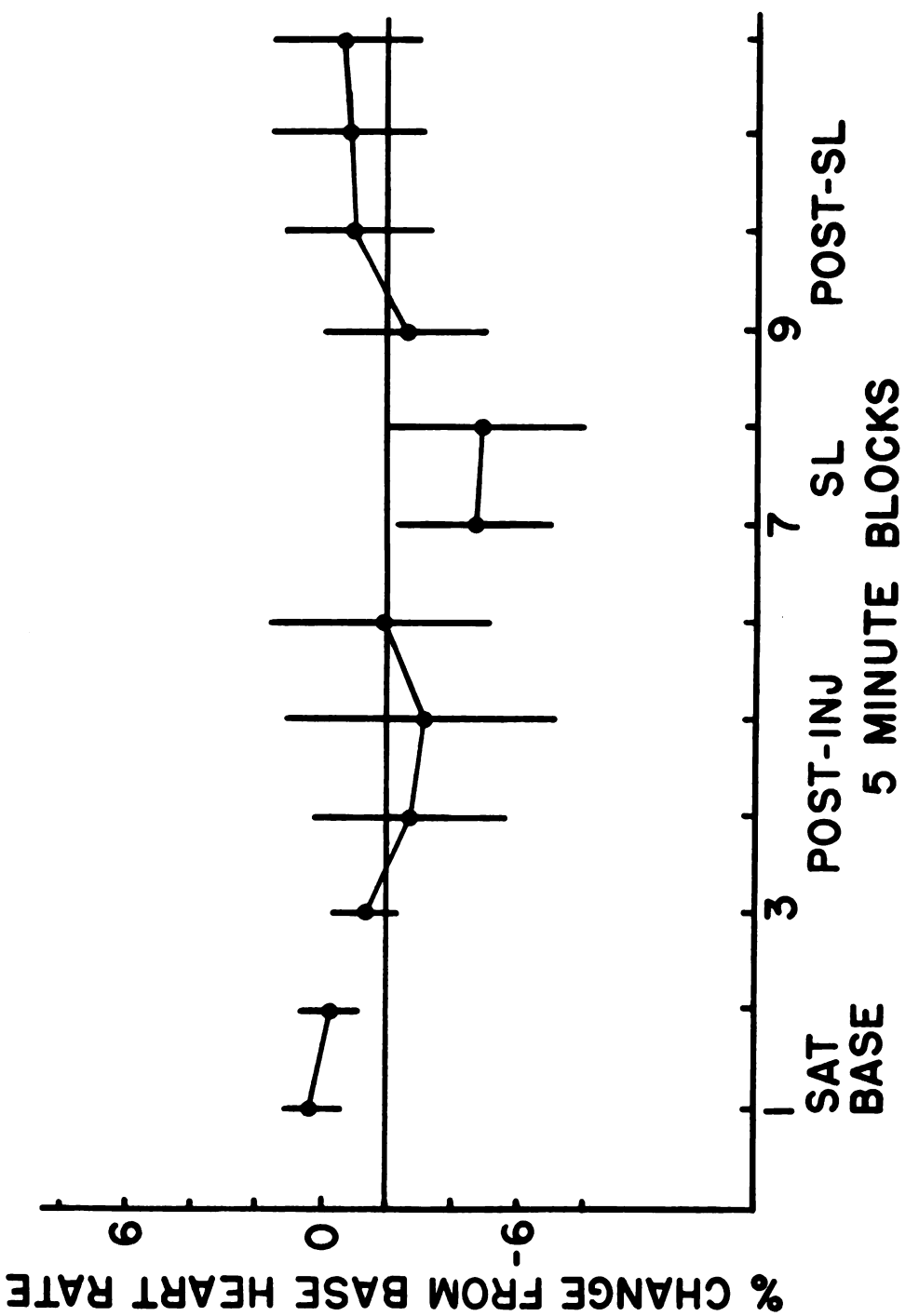

% CHANGE FROM BASE HEART RATE
9
0
-9
1
DEP
BASE
3
SL
5
POST-SL
9
POST-INJ
5 MINUTE BLOCKS

Figure 9.--Indicated are mean (± S.E.) percent changes in heart rate for six animals chosen at random from the SAT group. Changes in heart rate for each rat was calculated from its respective mean baseline rates. N = 6.


% CHANGE FROM BASE HEART RATE
9
0
-9
1
SAT
BASE
3
POST-INJ
7
SL
9
POST-SL
5 MINUTE BLOCKS

EXPERIMENT 3

To determine the effects of changes of blood tonicity on firing rates of septal cells, solutions of hypotonic, isotonic, and hypertonic saline were injected into the left common carotid artery. Since no injection is without a volume component, the isotonic injections were necessary to reflect the effects of the volume increase. Firing rates during and after injections of hypertonic and hypotonic solutions are then compared to both the original base firing rate and the firing rate of the isotonic injections to reflect changes due to the alterations in osmotic pressure.

Method

Subjects

Five naive, male albino rats of the Holtzman strain, 350-425 g and 101-116 days old on the recording date were housed in individual cages under constant light.

For a minimum of five days preceding the recording date, food and water was available ad libitum.

Procedure

Subjects were anesthetized as were those in the first two experiments, although supplementary doses were more frequently needed during the surgery for the present experiment. Once the rat was anesthetized, the left common carotid artery was exposed by cutting the skin along the throat, separating the left sternomastoideus muscle from the sternothyroideus muscle, cutting the tissue away from the artery with a scalpal, lifting the artery with a pair of small tweezers, and slipping a piece of plastic tubing behind it. A 26 ga needle was slipped through the left branch of the sternothyroideus muscle and into the carotid artery. Prior to the experiment a small (0.03 cm^3) chamber made by a shortened neck of a 1.0 cm^3 plastic syringe had been inserted into the needle head and sealed with dental acrylic. From this chamber two 25 cm lengths of plastic tubing (0.012 I.D. x 0.025 O.D.) led to two 1.0 cm^3 plastic syringes that held the solutions of varying tonicity to be injected into the carotid artery.

Once the needle was in the artery, the plastic tube behind the artery was removed and the incision closed with wound clips. With the wound clips placed closely together so that only the two plastic tubes could emerge from the skin (and not the bulbous dental acrylic portion), the apparatus was held in place at two points: the skin and the left sternomastoideus muscle. So that the needle would not slip out of the artery while the rat was prepared for recording, a small towel was rolled from each side toward the middle and taped along the abdomen of the rat from head to tail; when the rat was turned over to be stereotaxically positioned, the injection apparatus protruded between the two rolls of the towel, and these rolls supported the rat. Skull surgery and isolation of a unit was as described in experiment one, except that the heating coil was folded along the back of the rat rather than wrapped around it.

Prior to the experiment the syringes were loaded with solutions of the desired tonicity. When an injection of one tonicity was followed by one of a different tonicity, it was necessary to flush the chamber between the two injections so that the chamber would contain a solution whose salinity approximated that of the next injection.

Pilot data indicated that this was best done by a small (0.05 cm^3) flush injection, depressing the plunger of the syringe containing the solution to be injected next. When three different solutions were to be injected during the same session, it was necessary to replace one of the original syringes with a syringe containing the third solution. When this occurred, it was necessary to flush not only the chamber, but also the plastic tube between the syringe and the chamger; this was accomplished by four successive flush injections of 0.05 cm^3.

Once a septal unit was isolated, a two minute base rate was recorded. Injections lasted for 10 seconds; a 0.2 cm^3 injection, for example, was injected at 0.02 cm^3/ second. By using the auditory cue of the click of the on-line printer at the beginning of a 10 second time bin and a watch with a second hand, an injection could be contained in a single 10 second bin. Because no best concentration, volume, or sequence of injections had been determined, a variety of combinations was used, and each of the five recording sessions was a unique procedure. Only in sessions three and four--sessions in which two cells were isolated simultaneously--were two units subjected to identical manipulations. Because of the procedural differences

among sessions no data grouping was done, and effects of the injections on the firing rate of each cell was examined individually.

Results

The volume, tonicity, and sequence of each injection, and the effects of the injection on the discharge rate of each cell are shown in Figures 10 through 16. Since effects of an injection were not confined to the 10 second bin in which it was administered, the next two 10 second bins are included in the means representing an injection; each non-injection mean represents six 10 second bins.

Hypertonic injections increased firing rates more consistently and to a greater extent than did isotonic or hypotonic injections. There appeared to be a strong tendency of the septal cells to habituate to these injection stimuli: the first injection usually had the greatest effect regardless of the tonicity, and if a second large reaction occurred it usually came later in the recording session rather than shortly after the first injection. Flush injections had little or no effect on discharge rates.

ADDENDUM

In figures 10-16 (Experiment III), F = flush injection, A = hypertonic injection, B = isotonic injection, and C = hypotonic injection. For concentration and size of these injections, see Appendix B.

Figure 10.--Indicated are mean (± S.E.) discharge rates during base rate periods, hypotonic, and hypertonic NaCl injections into the left carotid artery. Base means are six 10 second time bins; injection means are three 10 second time bins.

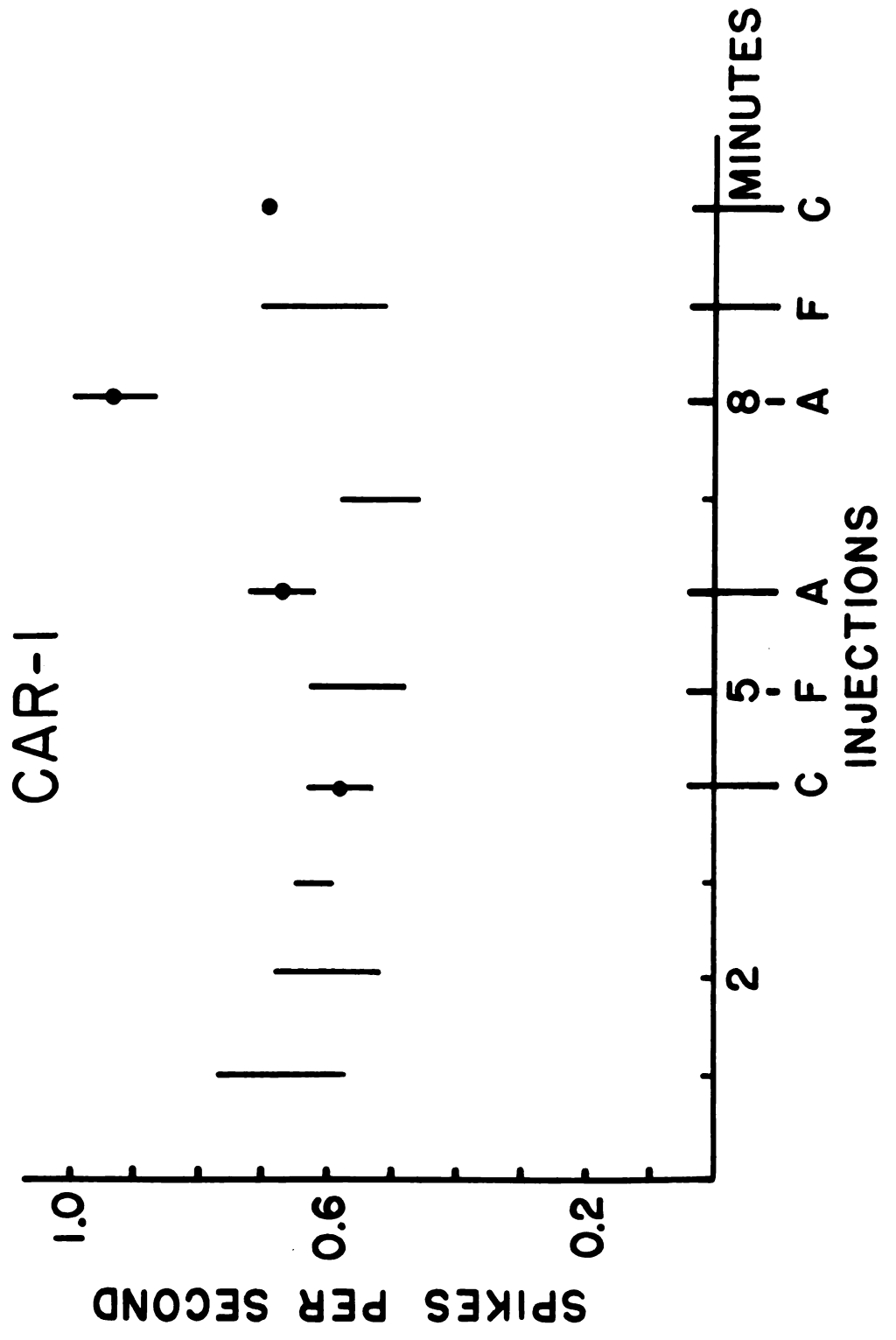

CAR-1
SPIKES PER SECOND
1.0
0.6
0.2
2
5
8
MINUTES
C
F
A
C
F
A
INJECTIONS

Figure 11.--Indicated are mean (± S.E.) discharge rates during base rate periods, hypertonic, and hypotonic NaCl injections into the left carotid artery. Base means are six 10 second time bins; injection means are three 10 second time bins.

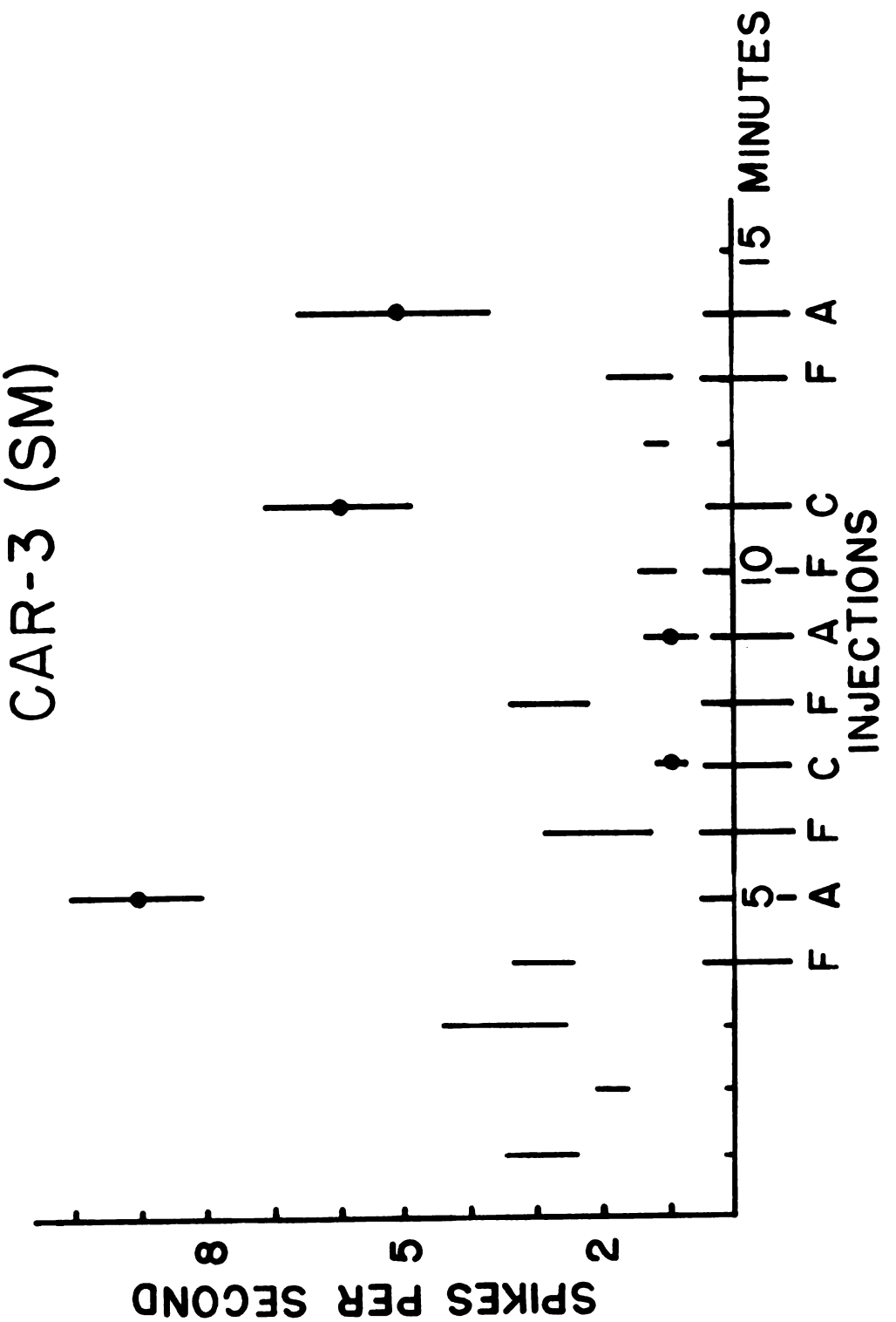

CAR-3 (SM)
SPIKES PER SECOND
8
5
2
INJECTIONS
F A F C F A F C F A
5
10
15 MINUTES

Figure 12.--Indicated are mean (± S.E.) discharge rates during base rate periods, hypertonic, and hypotonic NaCl injections into the left carotid artery. Base means are six 10 second time bins, injection means are three 10 second time bins.

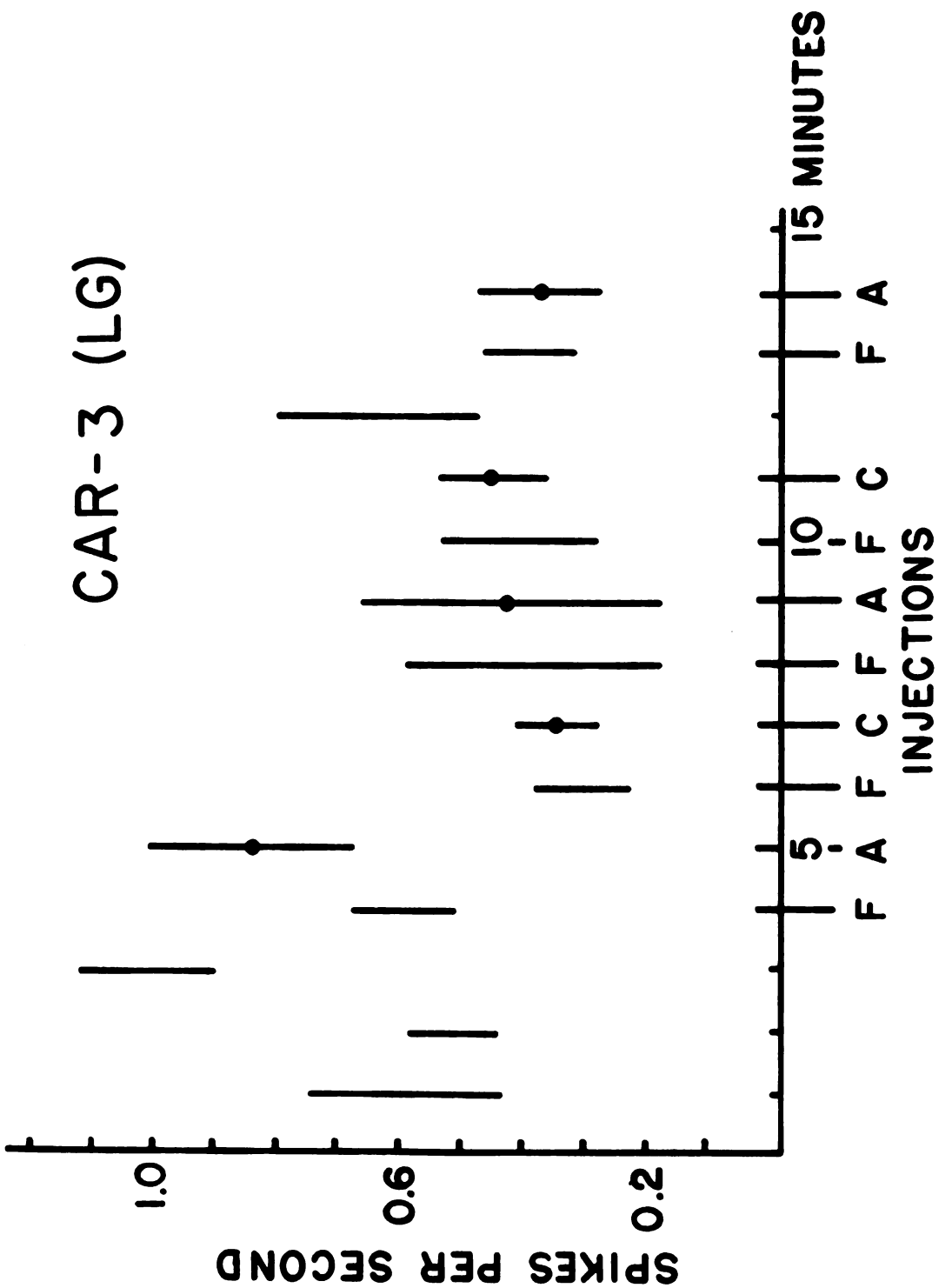

CAR-3 (LG)
SPIKES PER SECOND
1.0
0.6
0.2
5 -
10 -
15 MINUTES
F A F C F A F C F A
INJECTIONS

Figure 13.--Indicated are mean (± S.E.) discharge rates during base rate periods, isotonic, hypertonic, and hypotonic NaCl injections into the left carotid artery. Base means are six 10 second time bins; injection means are three 10 second time bins.

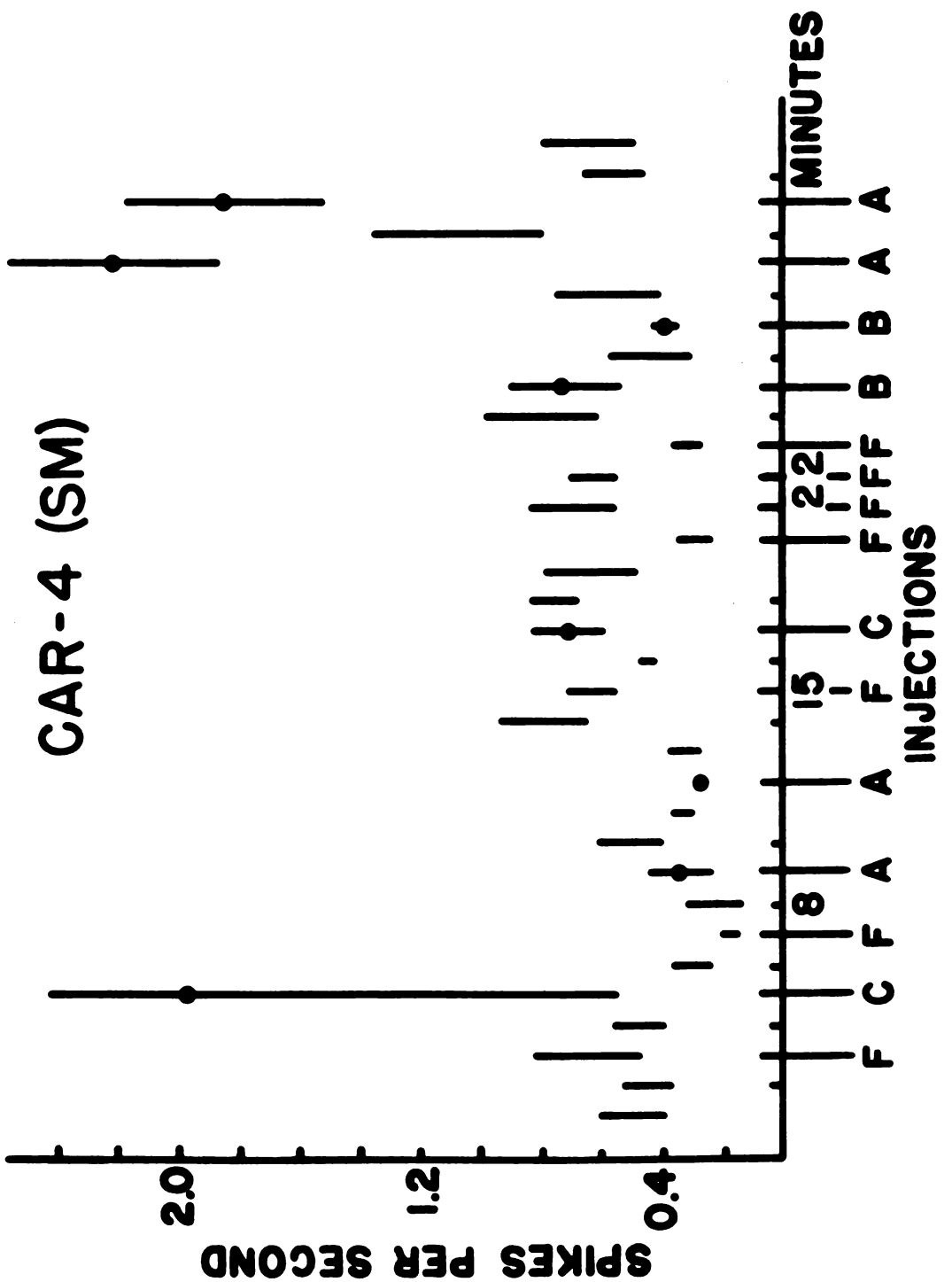

CAR-4 (SM)
SPIKES PER SECOND
2.0
1.2
0.4
INJECTIONS
F C F A A F C F F F B B A A
8
15
22
MINUTES

Figure 14.--Indicated are mean (± S.E.) discharge rates during base rate periods, isotonic, hypertonic, and hypotonic NaCl injections into the left carotid artery. Base means are six 10 second time bins; injection means are three 10 second time bins.

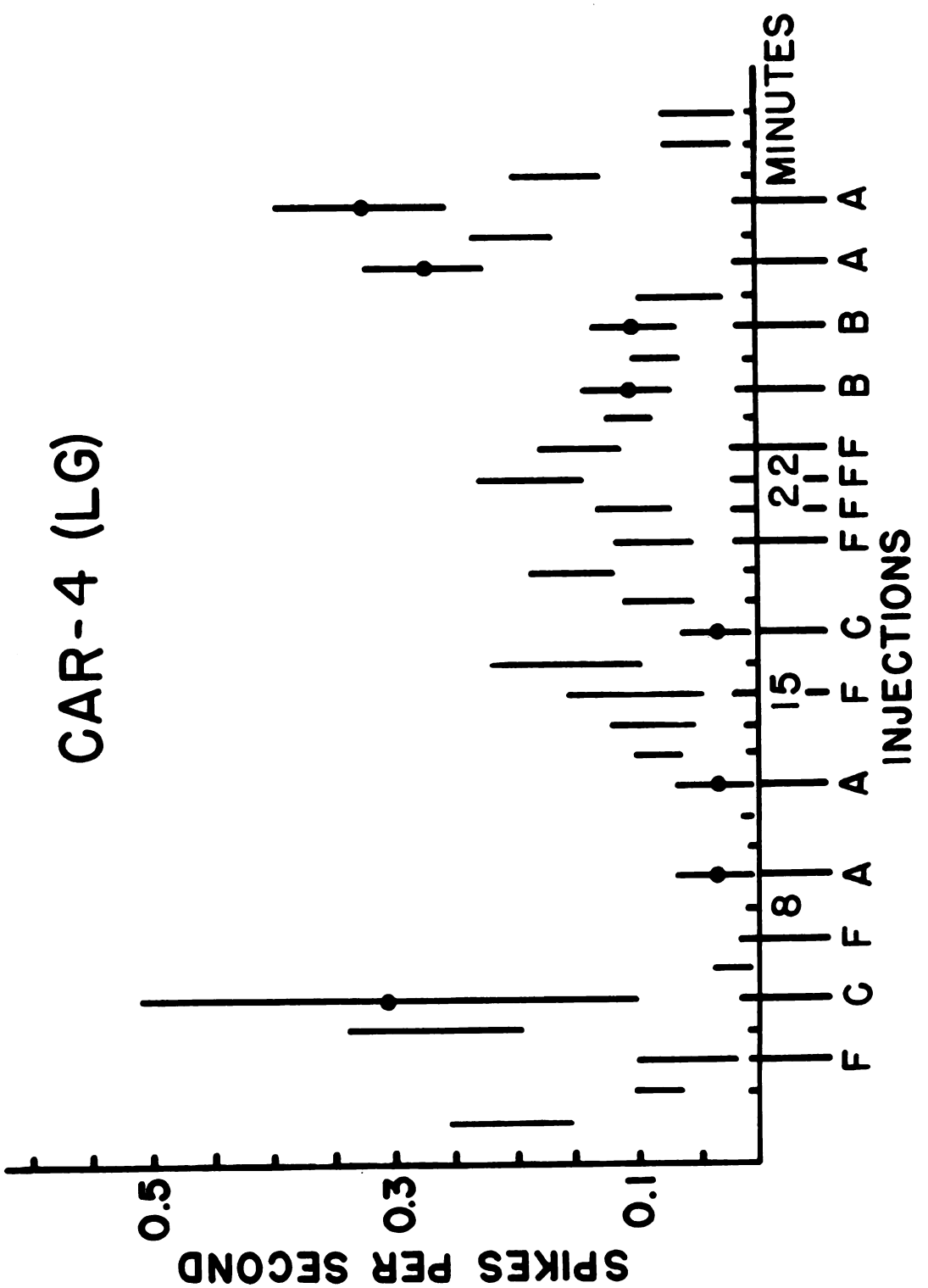

CAR-4 (LG)
SPIKES PER SECOND
0.5
0.3
0.1
MINUTES
INJECTIONS
F C F A A F C F F F B B A A
8
15
22

Figure 15.--Indicated are mean (± S.E.) discharge rates during base rate periods, hypertonic, and hypotonic NaCl injections into the left carotid artery. Base means are six 10 second time bins; injection means are three 10 second time bins.

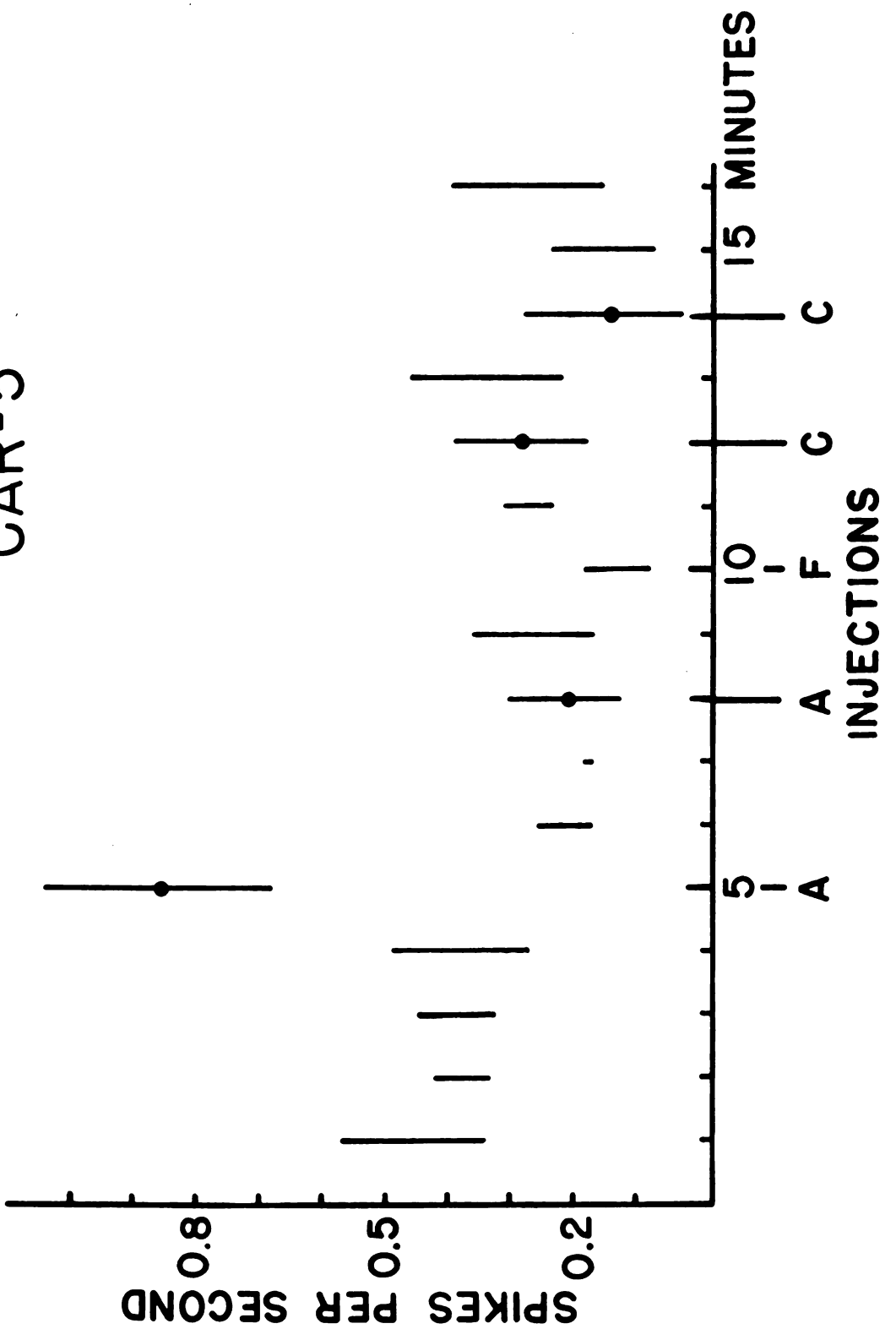
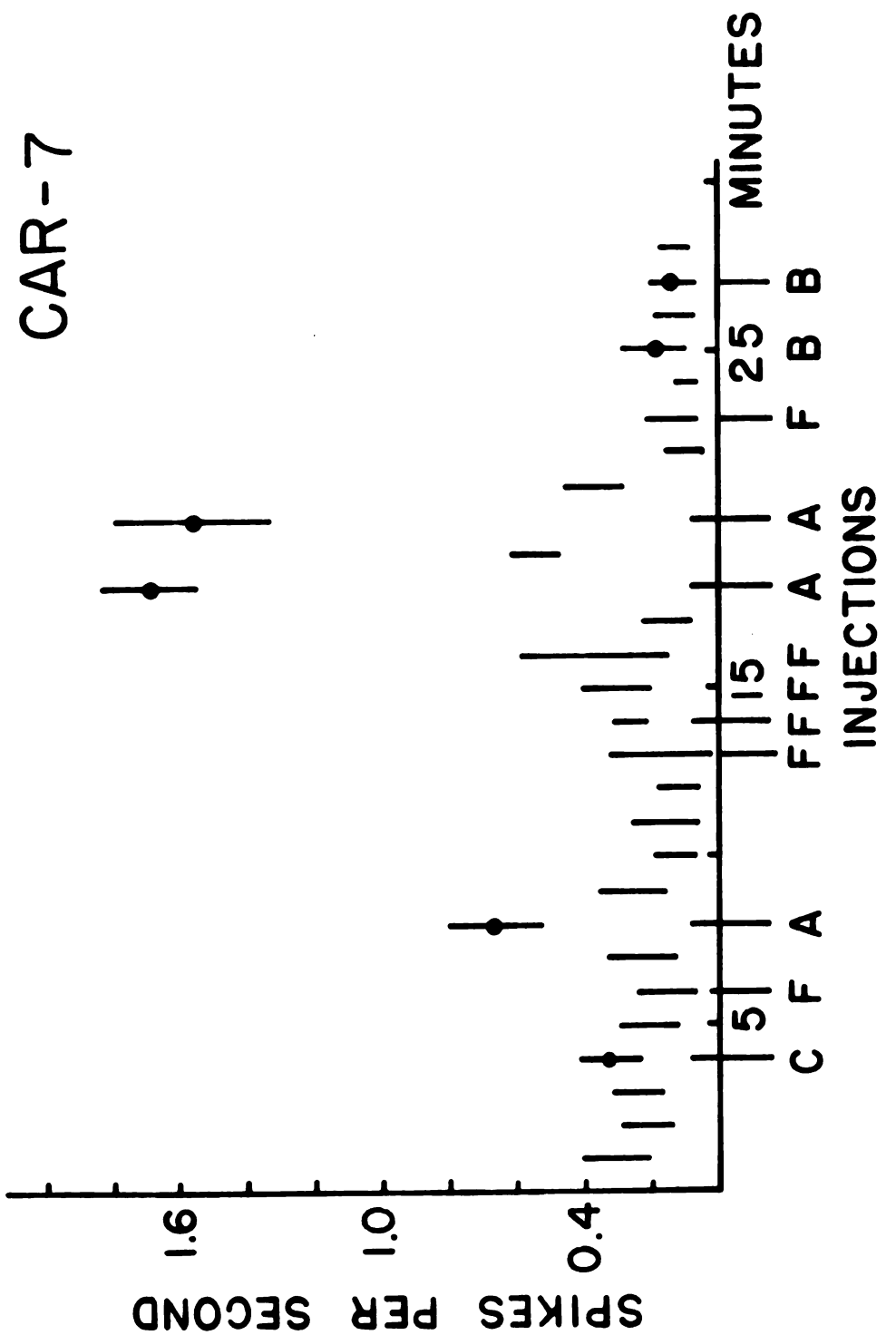

CAR-5
SPIKES PER SECOND
0.8
0.5
0.2
5
A
10
F
15 MINUTES
A
C
C
INJECTIONS

Figure 16.--Indicated are mean (± S.E.) discharge rates during base rate periods, isotonic, hypotonic, and hypertonic NaCl injections into the left carotid artery. Base means are six 10 second time bins; injection means are three 10 second time bins.


CAR-7
SPIKES PER SECOND
1.6
1.0
0.4
MINUTES
5
15
25
INJECTIONS
C F A
F F F A A
F B B

After two of the sessions (an unreported pilot session and session five) blood was extracted from the carotid artery into the chamber and syringe barrel. Small increases in discharge rates were observed following both these extractions.

All cells but 1 in experiment 3 were in the lateral septal nucleus. For individual recording sites, see Appendix B.

DISCUSSION

The results obtained in experiments 1, 2, and 3 did not display any differential effects along the anterior-posterior, lateral-medial, or dorsal-ventral dimensions. This, however, does not contradict the report of Lubar, et al. (1969) that lesions in the anterior portion of the posterior third of the septum cause the greatest increment in water intake, while lesions in the anterior septum cause the least. In the present study such a great proportion of the recording sites were in the middle third of the septum that no sound inference can be made as to differential effects along the anterior-posterior dimension. In fact, Lubar, et al. reported moderate increments in water intake following middle septal lesions; the moderate effects found in the present experiments may have been greater had the recording sites been in a more posterior location. These effects were considered "moderate" in a number of respects. For example, the 2-3.5 cm^3 group in experiment 1 (Figure 4) did not differ from base rates

or discharge rates of control cells. In the 5 cm^3 extract group the 5 minute means of the extract and post-extract periods differed significantly when measured by percent of base rate (Figure 3), but not when measured as an absolute firing rate (Figure 5). Also, the septal cells of 23.5 hour water deprived rats did not discharge significantly faster than cells in water satiated rats (experiment 2). Bennett (1971) found that anterior hypothalamic (AH) cells in 23.5 hour water deprived rats fire approximately 50% faster than AH cells in ad lib rats, and supraoptic (SON) cells in 23.5 hour water deprived rats discharge 400% faster than cells in ad lib rats. Although Bennett compared discharge rates of cells in 23.5 hour water deprived rats to those in ad lib rats while the present study compared cells in deprived and satiated rats, AH and SON cells apparently are more responsive to extended (23.5 hour) periods of deprivation than are septal cells. Bennett also applied the deprived base--stomach load--hypertonic saline injection series of manipulations (identical to the DEP group of experiment 2) to rats while recording from SON and AH cells. Interestingly, under these conditions of rapid alterations of hydration states sept and AH cells appeared less responsive to the stimuli than

did the septal cells of the present experiment. (See page for discussion of response of septal cells to novel stimuli.)

Comparisons among the present experiments must be made with caution, since distribution of recording sites was not the same for each experiment. For example, 13 of 18 cells in the first experiment were in the medial septum, 14 of 16 cells in the second experiment were in the lateral septum, and 6 of 7 cells in the third experiment were lateral septal cell. In addition to being more in the lateral septum, cells in the second and third experiments were slightly more dorsal than were those in the first experiment. When the control cells for all groups in experiment 1 (during the first five minutes of base rates) were divided into lateral or medial cells, the ad lib firing rates per second were:

lateral septum: 2.63 ± 0.82, N = 6
medial septum: 5.82 ± 1.35, N = 11

These values were considerably greater than firing rates during water deprived (0.68 ± 0.14 spikes per second, N = 8) and water satiated (0.51 ± 0.23 spikes per second, N = 8) conditions found in experiment 2. It is difficult to believe that cells in deprived and satiated states can

discharge at similar rates while ad lib cells fire significantly faster. While the differential sampling of the ad lib group (mostly medial) and the satiated group (mostly lateral) can account for most of the greater firing rate of the ad lib cells, three other sources of this discrepancy are possible. First, different electrodes were used for the hypovolemia group (from which the ad lib samples were drawn) than were used for the SAT and DEP groups. How different electrodes can effect firing rates or bias sampling is not known. Apparently, when an electrode is introduced to the immediate vicinity of a cell (or actually touching the cell) the cell can be stimulated, causing it to discharge faster. Another possible alteration of discharge rates by the introduction of the electrode could be a result of separating a cell from synapses on that cell. Because a different population of electrodes was used for each experiment, inconsistencies in physical properties (size, shape, conductivity, etc.) of electrodes among groups was realized. It is possible, then, that a differential effect of the introduction of the electrode has occurred across groups (although neither the direction of this effect nor the underlying principle is understood). The second possible source of this discrepancy is that the

CIBA-supplied urethane used in the hypovolemia experiment was twice the concentration of the urethane mixed in our laboratory and used in the SAT and DEP experiments. However, instead of requiring twice as great a dosage, these latter rats required only about 150% as much as the HV rats, and therefore had only approximately 75% as much urethane anesthetic. Third, the SAT and DEP rats had stomach load tubes inserted prior to recording.

Another major inconsistency cannot be resolved anatomically. Hypovolemia, a characteristic of the water deprived state, caused cells to decrease in firing rates in experiment 1. But experiments 2 and 3 indicated that septal cells fire faster during the hypertonic state: the post-injection period is characteristically more active than the SL and the P-SL periods, and hypertonic carotid injections increase firing rates considerably more than carotid injections of hypotonic saline. Why would one component of deprivation (hypovolemia) decrease firing rates, while another (hypertonicity) increase the discharge rate?

All of the 5 cm^3 extract cells were located in the medial septum: if the consistent decrease in discharge rate characteristic of this group were due to the

anatomical location rather than the size of the extraction, the HV group with the intermediate size extracts (2.0 and 3.5 cm^3) should show responses indicating that the medial septal cells responded as the 5.0 cm^3 group did, and the lateral cells as the control group did. The data did not support this. Two of the four lateral septal cells and one of the two medial septal cells increased, while half of both lateral and medial septal cells decreased in firing rate, indicating that the decrease was not related to the anatomical location of the cell.

A 5.0 cm^3 loss of blood--especially following loss of approximately 0.5-1.0 cm^3 in femoral vein surgery and 1.0-2.0 cm^3 in the skull surgery--is considerable. Ganong (1967) described hypovolemia of 5-15 ml/kg (about 2-6 cm^3 for a 400 g rat) as a "moderate" stress. One of the hypotheses entertained to explain why the septal cells respond differently to hypovolemia and hypertonicity was that the decrease in discharge rate during hypovolemia was due to hemorrhagic shock. The heart rates did not support this hypothesis. No subject showed the rapid pulse that Ganong (1967) and Wright (1965) regard as symptomatic of hemorrhagic shock.

Cells in the carotid injection experiment whose firing rates did change almost always responded to the injection by increasing discharge rates. Although greater and more frequent changes were elicited by hypertonic injections, even hypotonic injections often were accompanied by increased firing rates. If this is interpreted as a response to the volume component of the injection stimulus, then the carotid injection experiment corroborates the hypovolemia study--an injection slightly distends the artery, momentarily stimulating hypervolemia, and increases the discharge rate, the expected reaction if hypovolemia decreases the discharge rate.

Almli (1970) noted that a 2.2% increase in osmolality follows hemorrhagic hypovolemia (5.0 cm^3 from a tail cut) when measured at initiation of drinking (approximately 53 minutes after the hemorrhage). In the present experiment, post-recording blood samples did not differ significantly in plasma osmolality from blood extracted during the recording session. However, two minor hemorrhages preceded recording (femoral vein surgery approximately 30 minutes prior to recording and skull surgery about 15 minutes before recording), and the recording

extract and the post recording sample were separated by only 50-52 minutes.

The only significant difference in osmotic pressure found was between samples taken after the DEP (306.2 ± 1.7 mOsm) and SAT (297.3 ± 3.8 mOsm) recordings, $p < .01$. Osmotic shifts concomitant with the manipulations of the SAT and DEP groups are of interest. In a free intake period of 30 minutes, Hatton and Thornton (1968) found that rats drink 9-10 ml in response to a 1.0 cm^3 subcutaneous injection of 16% NaCl. In the present experiment, the injection and the stomach load, then, approximately offset each other. The total exogenous solution administered was approximately 3% body weight of 1.5% NaCl (1.0 cm^3 16% NaCl injection plus 10 cm^3 water). This was not a severe challenge: rats are able to regulate internally (i.e., without drinking) up to 2.0% NaCl solutions (2.0% body weight, stomach loaded: Hatton and O'Kelly, 1966).

A 1.0 cm^3 injection of 16% NaCl is sufficient to initiate drinking at approximately six minutes post-injection (Hatton and Thornton, 1968). Following a 23.5 hour water deprivation period rats stop drinking after 9-10 minutes, with osmotic decreases and volume increases occurring at approximately 8 and 10 minutes, respectively

(Hatton and Bennett, 1970). Examination of individual firing rates (see Appendix B) indicates that only a few cells responded in temporal patterns closely coincidental to these behavioral and physiological indices.

Cells in the DEP group tended to increase firing rates as a function of time, although from Figure 2 it is apparent that even control cells tend to increase discharge rates throughout the recording session. Throughout the deprived base rate, stomach load, and post-stomach load periods, this pattern closely resembles the pattern of the control cells (see Figure 2); not until the post-injection period did the DEP group cells begin to fire faster than the control cells.

The paradoxical result of the hypovolemic manipulation decreasing discharge rates (experiment 1) while hypertonic manipulation increased discharge rates (experiments 2 and 3) has not been resolved by the present experiment. An examination of individual records in experiment one shows that of the three cells that increased during the post extract period (two 2.0 cc and one 3.5 cc extracts), two were quite similar to the control group while the other kept increasing far above the mean control rate. These data are consistent with the hypothesis that

there is a threshold of hypovolemia (unattained in the recording sessions of the two intermediate extracts that fired in control-like patterns) necessary to increase septal single unit firing rates (this threshold was attained by the one cell that fired considerably faster than control cells); and a second threshold--a stress component not registered by the heart rate monitor--which decreases septal unit activity (this threshold was attained by all the 5.0 cc extract animals and those in the intermediate group that decreased in discharge rate). This interpretation would render all three experiments consistent and corroborate the theory offered in the introduction: that septal cells are more active during deprivation, and stimulate the supraoptic-hypophysial system to increase ADH release. However, with only one cell responding at an increased rate, this interpretation is highly speculative. But this possibility strongly suggests new research: a replication of the present HV experiment with small (perhaps 1.0-1.5 cm^3) blood extracts. A chronic preparation would eliminate effects of the femoral vein surgery. The large extracts (up to 5.0 cm^3) of the present experiment were used to facilitate a comparison with the results of the Almli study (1970) in which he used 5.0 cm^3 extract,

and to avoid masking such changes by the hypovolemia (1.0-2.0 cm^3) of the surgery.

Evidence that septal cells increase firing rates in response to blood hypertonicity comes from another, unquantifiable source: during three recording sessions great increases in background activity in response to hypertonic injections could be heard via the auditory monitor. This occurred during the first injection for the CAR-5 cell and the first injection of an unreported pilot cell, and during the first and second hypertonic injections for CAR-3. This suggests that in addition to the microelectrode approach, a gross electrode study could be useful in detecting changes in the activity of the septum.

Some interesting comparisons were found between the Cross and Green (1959) study and the present one. Cross and Green applied a number of stimuli (pinch, visual, auditory) before they applied the osmotic stimulus. They recorded from the septum, but they declined to infuse the hypertonic stimulus when a very slow cell was isolated, because of the difficulty in statistical interpretation and because many slow-firing neurones were passed by the electrode tip while the cells were inactive. For these reasons, Cross and Green did not apply the hypertonic

stimulus when a septal cell was isolated, but they did apply the other stimuli frequently enough to observe that the lateral septum was quite responsive to pinch stimuli and the medial septum sensitive to light stimuli. They also reported a strong tendency for septal cells to habituate to repeated stimuli. This corresponds to the interpretations of the reactions of some of the cells in experiment 3. The septal response to novel stimuli and habituation to repeated stimuli may be important septal characteristics.

The effects of stomach loads of water and injections of 16% NaCl on heart rates (Figures 8 and 9) are interesting in comparison to two previous experiments. Bennett (1971) found heart rate changes similar to those for the DEP group. However, O'Kelly, Hatton, Tucker, and Westall (1965) found that stomach loads of water increase heart rates during and after the load, while stomach loads of hypertonic saline increase heart rates during the load but decrease heart rates after the load.

Indications by Blass and Hanson in 1970 that the septal area of rats are receptive to volemic but not osmotic stimuli was not supported by the present study. Experiment 3 indicated there are septal cells that, in

addition to responding to volemic stimuli, also react to osmotic stimuli.

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APPENDIX A

Apparatus and Suppliers

Equipment and suppliers for microelectrode construction.

1. Stainless steel tubing, 26 gauge, Superior Tube Co., Norristown, Pennsylvania.

2. Tungsten wire, 0.005", Sylvania, New York, N.Y.

3. Glass tubing, Corning Glass, Corning, New York.

4. Micrometer eyepiece, x8, Zeiss, Matheson Scientific, 1600 Howard St., Detroit, Michigan.

5. Stereozoom dissecting microscope, Nikon, E.H. Sargent & Co., 8560 W. Chicago Ave., Detroit, Michigan.

6. Microscope, Unitron BMLU No. 42024, E. H. Sargent & Co., 8560 W. Chicago Ave., Detroit, Michigan.

Equipment and suppliers for blood analysis, extractions, and infusions.

1. Centrifuge, International Equipment Company.

2. Refractometer, American Optical Co., Instrument Division, Buffalo, N.Y.

3. Freezing point osmometer, Precision Systems, Inc., 44 Rumford Ave., Waltham, Mass.

4. Plastic syringes, Plastipak, Apothecary Shop, Lansing, Mich.

5. Catheters, Dow Chemical, Midland, Mich.

Equipment and suppliers for bioelectrical data recording and analysis.

1. Stereotaxic instrument, Stoelting Co., 424 N. Homan Ave., Chicago, Ill.

2. Preamplifier, 122, Tektronix, Inc., P. O. Box 500, Beaverton, Oregon.

3. Power supply, 125, Tektronix, Inc., P. O. Box 500, Beaverton, Oregon.

4. Oscilloscope, 502A, Tektronix, Inc., P. O. Box 500, Beaverton, Oregon.

5. Tape recorder, 1028, Magnecord, Main Electronics, 5558 S. Pennsylvania Ave., Lansing, Mich.

APPENDIX B

Raw Data

EXPERIMENT I

Unit Activity, 5 cm^3 extract group, spikes/second.

Cell No.	5 minute means Base	Ext.	1	2	3	4	5	6	7	8	9	10
HV27	3.33	4.50	1.58	2.80	1.80	2.60	2.80	2.60	3.20	3.60	4.20	5.00
HV50	8.60	9.50	1.74	4.08	5.10	5.18	4.88	4.90	4.98	5.26	5.30	5.37
HV21	17.03	23.77	7.58	6.20	8.28	6.76	6.70	6.90	6.86	6.22	6.74	6.73
HV28	5.66	5.42	6.08	5.94	5.50	5.06	4.76	4.78	4.56	4.78	4.76	4.83
HV49	5.31	5.70	3.50	1.82	2.02	1.82	2.66	2.96	4.56	4.76	6.24	6.30
HV31	1.00	2.71	0.88	0.11	0.26	0.56	0.58	0.34	0.23	0.31	0.06	0.23

Unit Activity, 2 - 3.5 cm^3 extract group, spikes/second.

Cell No.	5 minute means Base	Ext.	1	2	3	4	5	6	7	8	9	10
HV17	2.75	2.89	3.25	2.12	1.87	1.79	0.72	0.88	1.73	2.01	2.31	---
HV29	2.50	6.09	5.55	5.55	7.22	2.22	10.5	13.9	11.1	13.3	9.24	---
HV41	0.16	0.18	0.09	0.13	0.07	0.06	0.05	0.05	0.06	0.05	0.03	0.07
HV51	3.24	2.85	2.20	2.72	3.30	3.47	3.37	4.12	3.92	3.47	3.92	4.05
HV55	0.83	0.82	0.58	0.20	0.43	0.48	0.48	0.53	0.53	0.53	0.56	0.50
HV20	8.40	0.50	7.48	0.42	0.08	0.34	0.42	0.50	0.50	0.50	0.59	0.59

EXPERIMENT I

Unit Activity, 0 cm^3 extract group, spikes/second.

Cell No.	5 minute means Base	Ext.	1	2	3	4	5	6	7	8	9	10
HV37	2.86	1.38	0.60	0.46	0.34	0.43	1.60	1.60	0.37	0.29	0.29	0.29
HV46	12.1	11.0	11.4	12.1	11.7	12.2	12.9	12.8	13.4	14.0	14.0	14.2
R-30	4.94	9.63	10.6	13.1	15.9	14.6	11.1	10.7	15.1	15.7	15.6	15.6
SR-2	0.50	0.52	0.68	0.72	0.84	1.04	0.56	0.68	0.86	0.82	0.90	0.96
HV58	0.48	0.43	0.40	0.54	0.46	0.52	0.62	0.60	0.65	0.64	0.65	0.70
HV63	1.24	1.19	1.25	1.43	1.48	1.68	1.84	1.75	1.76	1.78	1.95	2.14

EXPERIMENT I

Heart Rate, beats/second.

Cell No.	5 minute means 1	2	3	4	5	6	7	8	9	10	11	12
HV29	5.95	5.65	5.29	5.32	5.31	5.25	5.23	5.02	4.99	4.85	4.81	4.67
HV41	6.06	5.97	5.81	5.87	5.92	5.86	5.87	5.86	5.91	5.90	5.89	5.84
HV49	6.67	---	5.78	4.98	5.02	5.45	5.61	5.68	6.45	6.58	6.24	6.20
HV21	5.01	4.80	5.23	5.27	5.49	5.35	5.34	5.33	5.23	5.20	5.21	5.17
HV50	5.83	---	5.27	5.34	5.46	5.41	5.46	5.42	5.49	5.48	5.49	5.47
HV31	5.89	5.35	5.33	5.24	5.42	5.37	4.95	5.09	5.05	5.03	5.00	4.89
HV37	7.37	7.12	7.15	6.98	6.97	6.88	6.72	6.60	6.50	6.41	6.34	6.21
HV63	5.98	5.97	6.04	6.15	5.94	5.80	5.92	5.94	5.96	5.97	5.99	6.00
HV46	6.70	---	6.99	7.11	7.02	6.54	6.30	6.13	6.39	6.35	6.13	6.14
R-30	6.62	6.59	6.55	6.54	6.52	6.50	6.42	6.37	6.17	6.04	5.91	6.00
HV58	7.00	6.95	6.86	6.78	6.71	6.66	6.56	6.47	6.40	6.35	6.30	6.29

EXPERIMENT II

Unit Activity, DEP group, spikes/second.

Cell No.	5 minute means 1	2	3	4	5	6	7	8	9	10	11	12
DEP10	0.52	1.03	0.90	1.17	1.73	1.72	2.02	2.46	4.14	4.18	4.93	5.13
DEP9	0.67	1.14	1.17	1.01	1.19	1.18	1.39	1.62	1.05	0.44	0.49	0.78
DEP8	0.49	0.60	0.82	0.77	0.79	0.88	1.52	1.68	1.76	1.84	1.76	1.85
DEP4	0.38	0.49	0.58	0.50	0.55	0.63	0.74	0.77	1.21	0.75	0.78	0.77
DEP6	0.52	0.53	0.22	0.23	0.19	0.24	0.20	0.17	0.71	0.43	0.53	0.39
DEP14	0.08	0.09	0.09	0.08	0.07	0.06	0.08	0.02	0.08	0.08	0.08	0.07
DEP13	0.50	0.47	0.44	0.41	0.47	0.59	0.52	0.60	0.74	0.68	0.74	0.67
DEP11	0.60	0.73	0.73	0.96	0.88	0.55	0.44	0.42	0.47	1.18	1.06	1.00

EXPERIMENT II

Unit Activity, SAT group, spikes/second.

Cell No.	5 minute means 1	2	3	4	5	6	7	8	9	10	11	12
SAT10L	0.03	0.01	0.05	0.05	0.05	0.05	0.02	0.01	0.01	0.02	0.02	0.03
SAT8L	0.08	0.12	0.01	0.01	0.03	0.00	0.01	0.01	0.01	0.01	0.01	0.00
SAT10S	0.15	0.05	0.14	0.27	0.36	0.34	0.32	0.20	0.29	0.39	0.40	0.43
SAT8S	0.44	0.52	0.88	0.41	0.98	0.37	0.14	0.18	0.19	0.22	0.17	0.22
SAT5	0.72	0.84	1.18	1.06	1.10	1.45	1.54	1.49	1.80	1.79	1.70	1.60
SAT7S	0.17	0.18	0.15	0.16	0.19	0.26	0.25	0.22	0.19	0.15	0.14	0.14
SAT4	1.88	2.48	2.28	2.25	1.86	2.08	1.68	1.55	1.10	1.42	1.10	1.38
SAT7L	0.15	0.20	0.15	0.16	0.13	0.10	0.04	0.03	0.03	0.01	0.00	0.01

EXPERIMENT II

Heart rate, DEP group, beats/second.

Cell No.	5 minute means 1	2	3	4	5	6	7	8	9	10	11	12
DEP11	6.52	6.60	6.50	6.31	6.34	6.34	6.15	6.02	6.24	6.54	6.58	6.63
DEP10	5.49	5.12	4.95	5.29	5.53	5.71	5.75	5.95	5.96	5.91	5.85	5.70
DEP9	6.13	6.08	5.88	5.89	6.00	6.03	6.04	5.70	5.57	5.47	5.61	5.27
DEP14	6.14	6.14	6.06	5.99	5.97	5.98	6.03	5.95	6.95	7.73	8.25	8.25
DEP3	6.65	6.67	6.99	6.99	6.97	6.91	6.47	6.08	6.40	6.69	6.89	7.02
DEP2	5.25	5.29	5.27	4.97	4.88	5.01	5.22	5.41	5.36	5.42	5.71	5.80

Heart rate, SAT group, beats/second.

Cell No.	5 minute means 1	2	3	4	5	6	7	8	9	10	11	12
SAT10	5.78	5.85	5.73	5.89	5.85	5.76	5.25	5.35	5.55	5.75	5.83	5.81
SAT8	6.14	5.76	4.98	5.14	5.08	5.31	5.40	5.63	5.89	6.04	6.10	6.14
SAT6	7.28	7.26	7.17	6.93	6.56	6.82	6.94	6.96	6.93	6.85	6.77	6.75
SAT5	6.49	6.40	6.20	6.36	6.39	6.37	6.19	5.60	5.87	6.06	6.10	6.17
SAT2	5.74	5.99	5.93	6.09	6.39	6.43	6.09	6.16	6.22	6.18	6.16	6.12
SAT1	6.24	6.10	5.11	6.06	6.31	6.42	6.53	6.59	6.64	6.67	6.66	6.67

EXPERIMENT II

Plasma protein and osmolality.

Cell No.	Protein concentration, g/100ml	Plasma osmolality, mOsm/kg
DEP11	5.7	304
DEP2	5.4	313
DEP4	4.9	309
DEP6	5.3	306
DEP8	4.7	303
DEP10	4.7	302
SAT1	5.2	306
SAT5	5.0	295
SAT8	4.9	300
SAT10	5.1	288
SAT2	5.2	288
SAT6	4.9	298

EXPERIMENT III

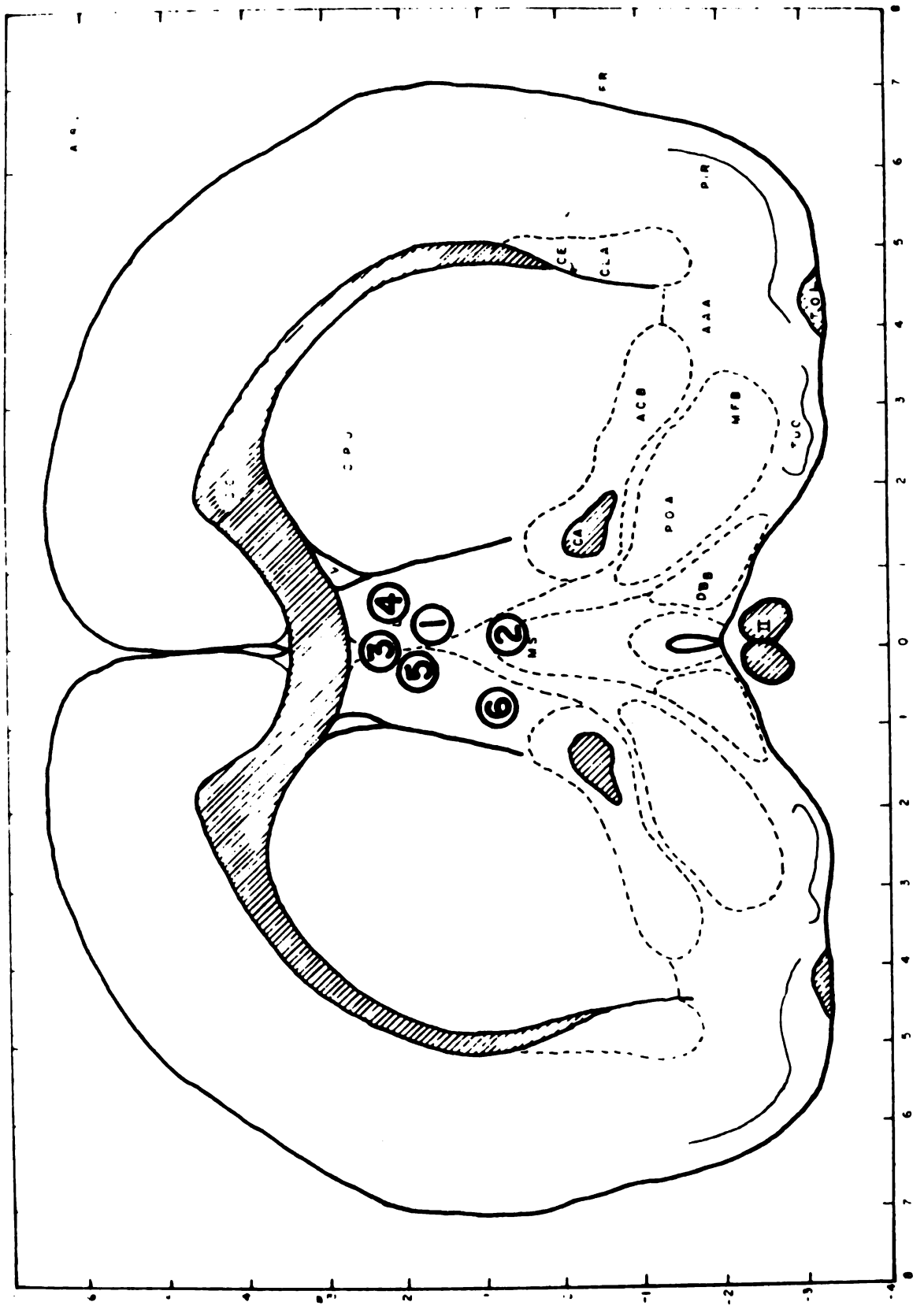
Size* and concentration of carotid infusions.

Cell No.	Inj no.	NaCl conc. (%)	Size in cm^3
CAR-1	1	0.5	0.2
	2	2.0	0.2
	3	2.0	0.5
	4	0.5	0.5
CAR-3	1	2.0	0.2
	2	0.2	0.2
	3	2.0	0.2
	4	0.2	0.2
	5	2.0	0.2
CAR-4	1	0.1	0.2
	2	1.7	0.2
	3	1.7	0.2
	4	0.1	0.2
	5	0.9	0.15
	6	0.9	0.15
	7	2.0	0.15
	8	2.0	0.15
CAR-5	1	1.7	0.2
	2	1.7	0.2
	3	0.1	0.2
	4	0.1	0.2
CAR-7	1	0.5	0.2
	2	1.7	0.2
	3	4.0	0.1
	4	4.0	0.1
	5	0.9	0.1
	6	0.9	0.1

* All injections took 10 seconds.

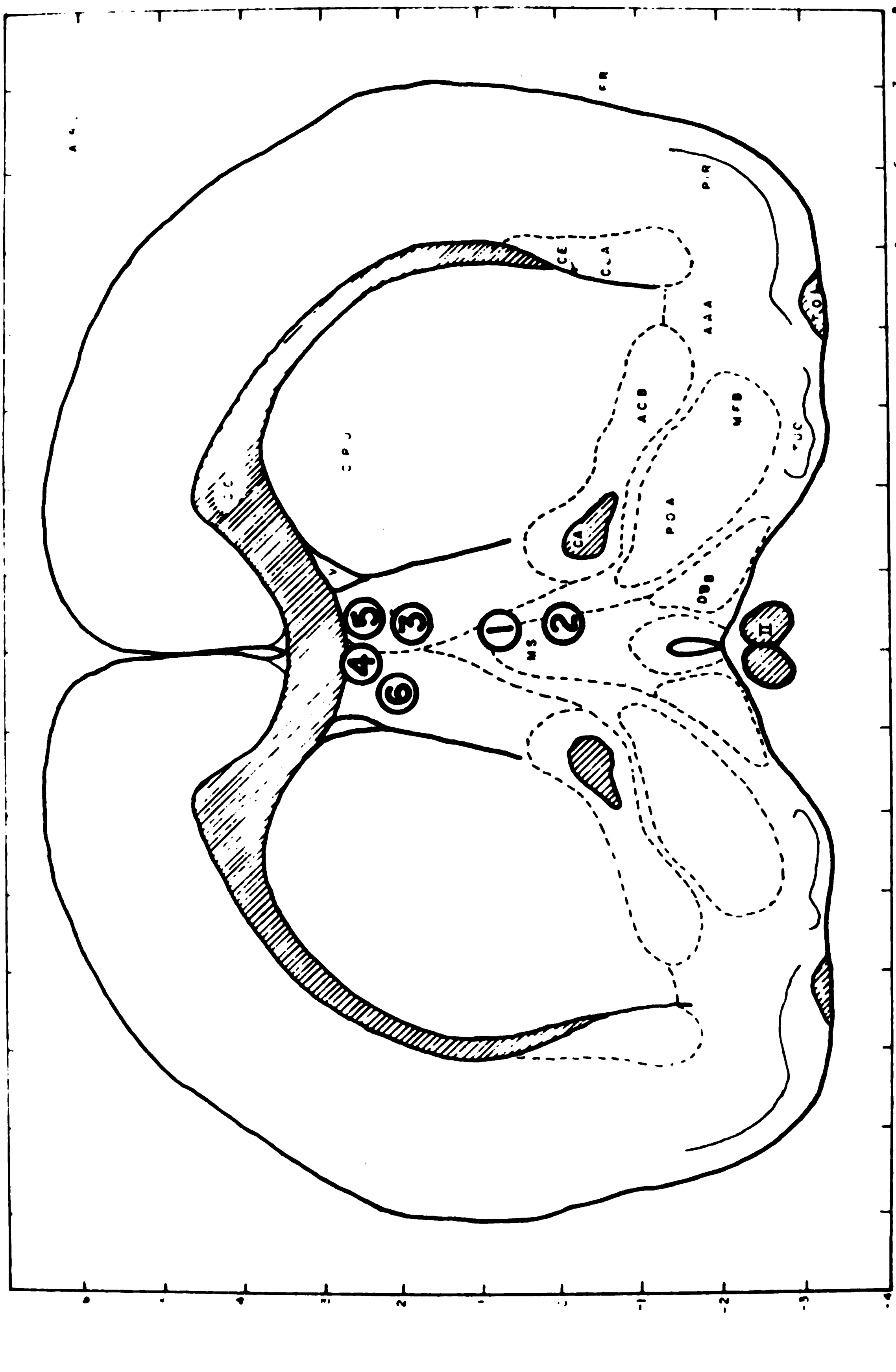
Indicated on the facing section are the following confirmed recording sites:

Site	Cell No.	Experiment No.
1	HV46	1
2	HV58	1
3	HV37	1
4	HV60	1
5	HV21	1
6	HV51	1


CPU
CE
CLA
ACB
AAA
PIR
MFB
TUC
VOT
CA
POA
DBB
MS
H
1
2
3
4
5
6

Indicated on the facing section are the following confirmed recording sites:

Site	Cell No.	Experiment No.
1	HV28	1
2	HV50	1
3	HV55	1
4	HV29	1
5	DEP4	2
6	DEP8	2

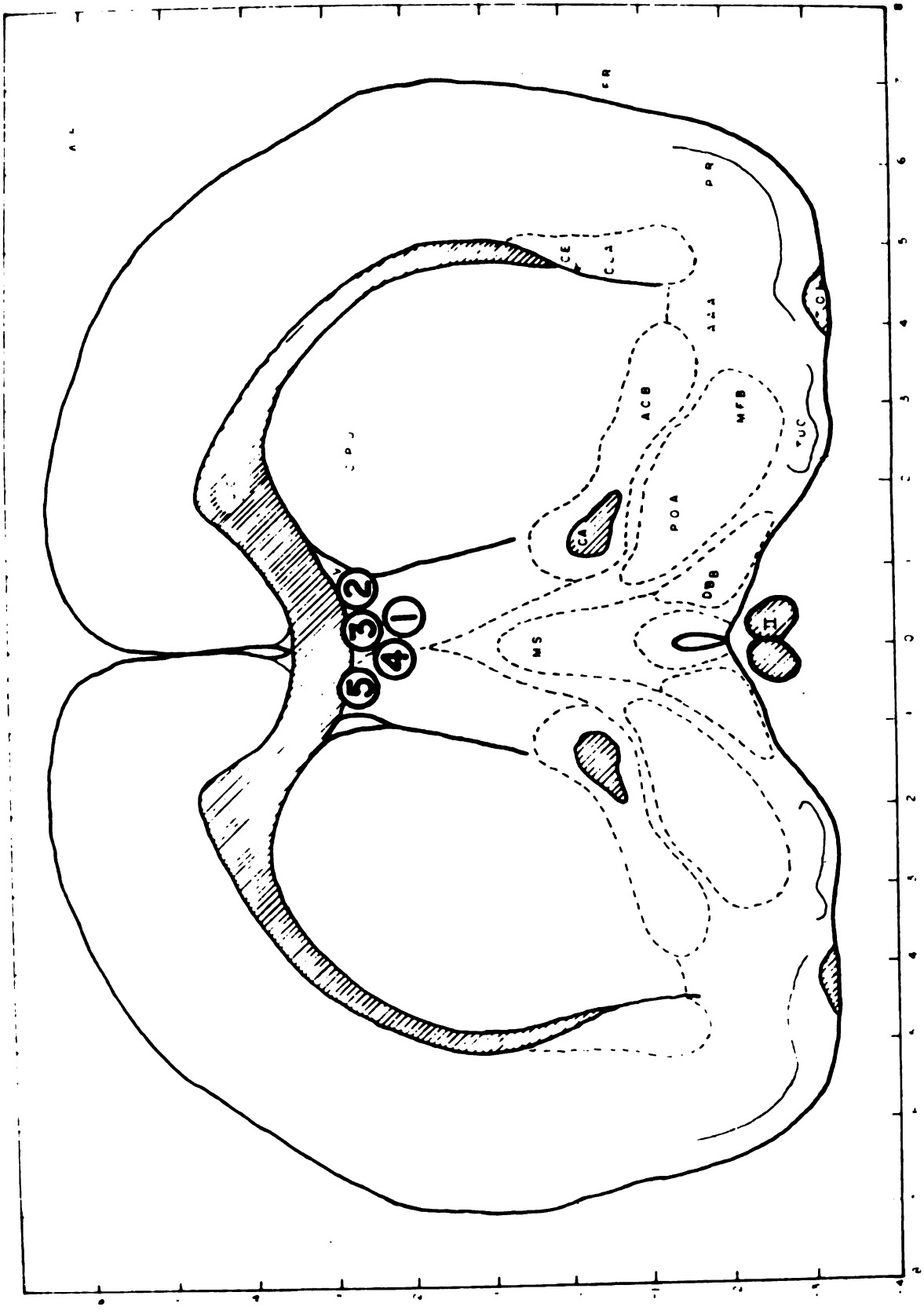

FR
CE
AAA
ACB
MFB
POA
DBB
MS
CA
II
1
2
3
4
5
6

Indicated on the facing section are the following confirmed recording sites:

Site	Cell No.	Experiment No.
1	DEP9	2
2	DEP10	2
3	DEP11	2
4	DEP13	2
5	DEP14	2
6	SAT5	2

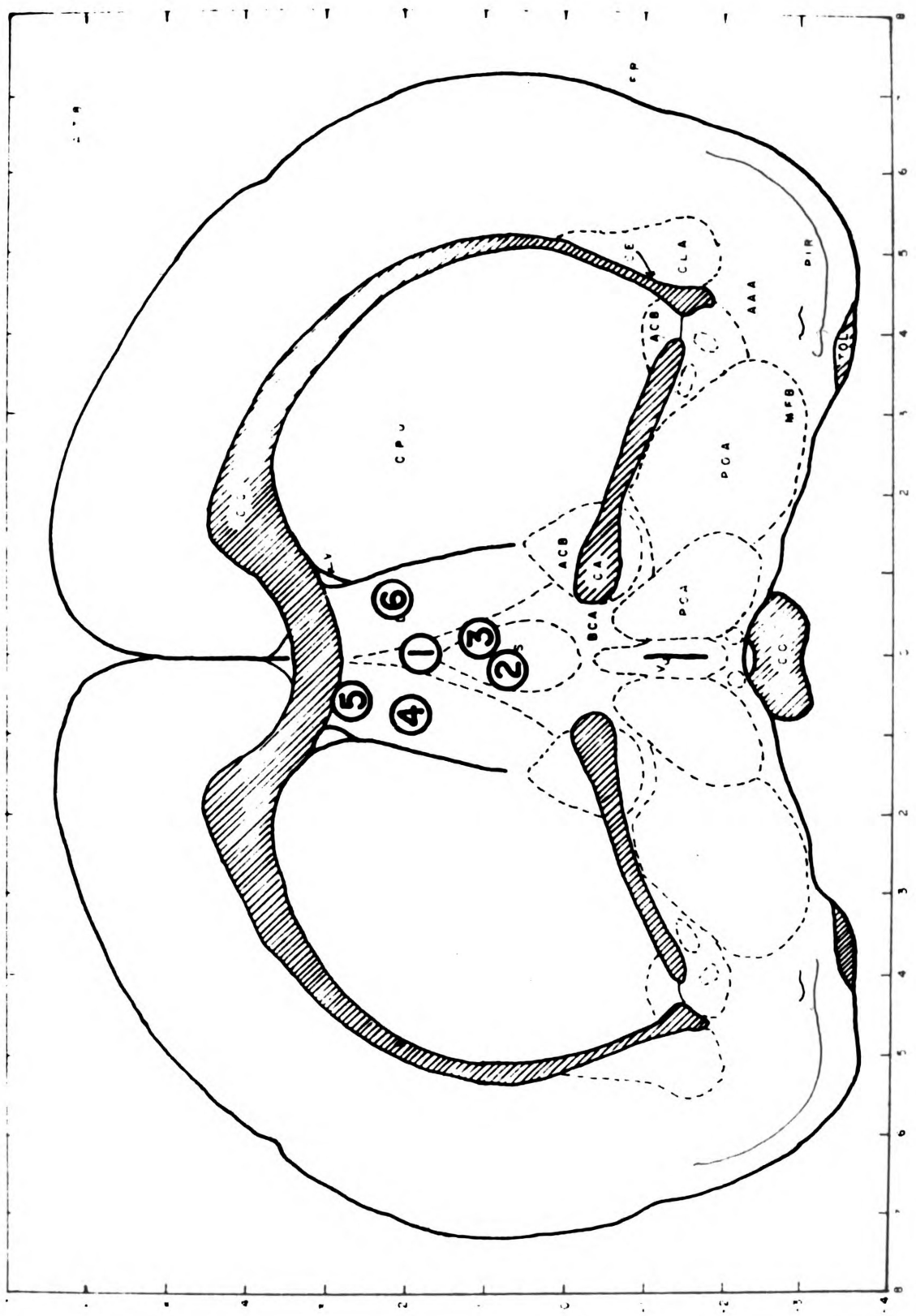
Indicated on the facing section are the following confirmed recording sites:

Site	Cell No.	Experiment No.
1	SAT4	2
2	SAT8	2
3	CAR-3	3
4	CAR-4	3
5	CAR-5	3


AL
CR
CPU
CCE
CLA
PIR
ACB
AAA
MFB
TUC
OT
CA
POA
DBB
MS
1
2
3
4
5

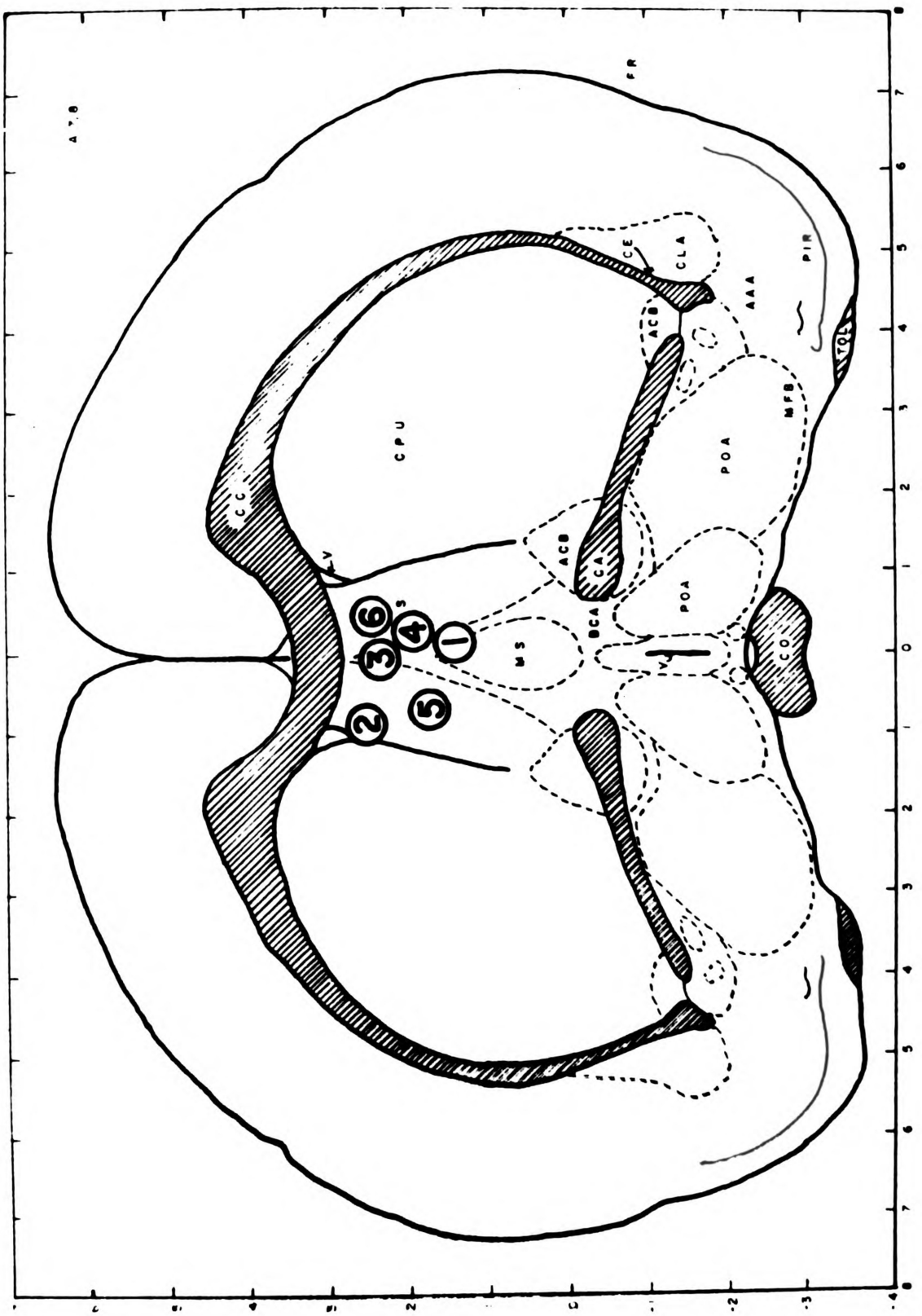
Indicated on the facing section are the following confirmed recording sites:

Site	Cell No.	Experiment No.
1	HV21	1
2	SR-2	1
3	HV31	1
4	HV41	1
5	SAT10	2
6	CAR-1	3


CPU
ACB
CLA
AAA
PIR
PGA
MFB
BCA
CA
CC
6
1
3
2
5
4

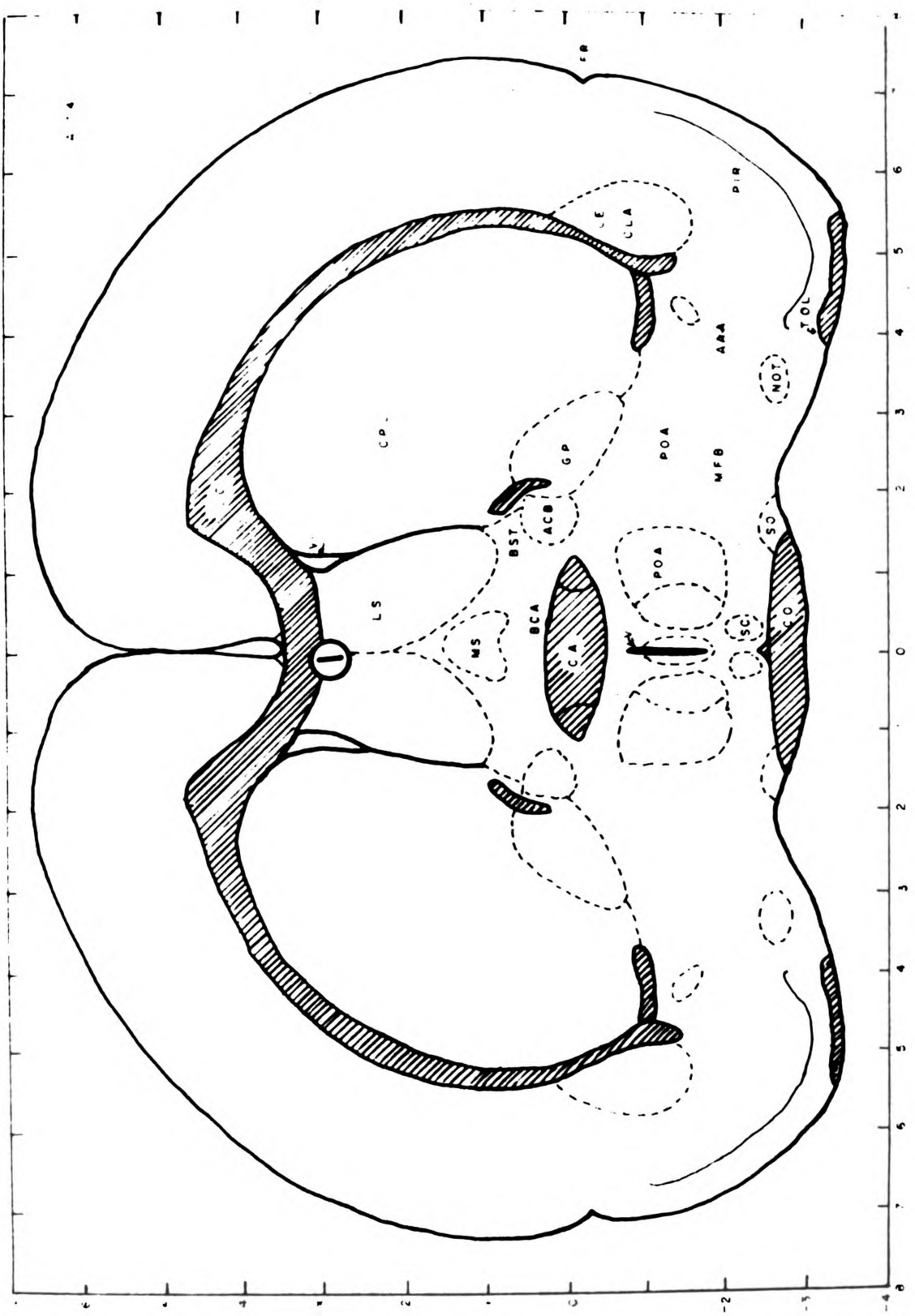
Indicated on the facing section are the following confirmed recording sites:

Site	Cell No.	Experiment No.
1	HV49	1
2	HV17	1
3	HV20	1
4	DEP6	2
5	SAT7	2
6	CAR-7	3


A 7.6
FR
CC
LV
CPU
ACB
CA
BCA
MS
POA
VL
CO
CE
CLA
AAA
PIR
TOL
MFB
1
2
3
4
5
6

Indicated on the facing section is the following confirmed recording site:

Site	Cell No.	Experiment No.
1	R-30	1


FP
CLE
CLA
PIR
CP
GP
POA
AAA
MFB
TOL
(NOT)
ACB
BST
SO
LS
MS
BCA
CA
SC
FC
6
5
4
3
2
1
0
-1
-2
-3
-4