M.A. PSYCHOLOGY

Helmut Krebs

EFFECTS OF ANOREXIGENIC AGENTS ON NEURONAL ACTIVITY

Amphetamine inhibits eating. In a search for the mechanism of this anorexigenic effect, the effects of d-amphetamine sulphate and ether on the spontaneous firing rate of single neuronal units in the ventromedial and lateral hypothalamic areas were studied in curarized rats. Amphetamine increased and ether decreased the firing rate of units in the ventromedial nuclei. Various lateral hypothalamic units were affected differently by these agents; amphetamine produced no change in some units, and a decrease or an increase in others; ether produced no change in some units and a decrease in others. These results are discussed in relation to two views, first that amphetamine anorexia arises from increased neural activity in the ventromedial hypothalamus, and second that it arises from a decrease in the lateral hypothalamic activity. While the evidence is not decisive, it clearly establishes the second view as a defensible alternative to the first.

M.A. PSYCHOLOGY

Helmut Krebs

EFFECTS OF ANOREXIGENIC AGENTS ON NEURONAL ACTIVITY

Amphetamine inhibits eating. In a search for the mechanism of this anorexigenic effect, the effects of d-amphetamine sulphate and ether on the spontaneous firing rate of single neuronal units in the ventromedial and lateral hypothalamic areas were studied in curarized rats. Amphetamine increased and ether decreased the firing rate of units in the ventromedial nuclei. Various lateral hypothalamic units were affected differently by these agents; amphetamine produced no change in some units, and a decrease or an increase in others; ether produced no change in some units and a decrease in others. These results are discussed in relation to two views, first that amphetamine anorexia arises from increased neural activity in the ventromedial hypothalamus, and second that it arises from a decrease in the lateral hypothalamic activity. While the evidence is not decisive, it clearly establishes the second view as a defensible alternative to the first.

EFFECTS OF ANOREXIGENIC AGENTS ON NEURONAL ACTIVITY

by

Helmut Krebs, B.A.

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Arts.

Department of Psychology
McGill University
Montreal

July, 1968

ACKNOWLEDGMENTS

The writer is greatly indebted to Dr. Dalbir Bindra for critical reading of the manuscript; and to Dr. James F. Campbell for his technical advice and assistance rendered in the establishment of the unit recording facilities.

This research was supported by the United States Public Health Service (Grant No. MH 03238-08) and by the National Research Council of Canada (Grant No. APB-74). The major portion of the work reported here was undertaken while the writer was a Woodrow Wilson Fellow; completion of the manuscript took place while he held a National Research Council Postgraduate Scholarship.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	5
RESULTS	15
DISCUSSION	27
SUMMARY	29
REFERENCES	30
APPENDIX A	

INTRODUCTION

Amphetamine has been observed to depress food consumption in the absence of peripheral bodily changes (e.g., Nathanson, 1937) and following surgical interruption of gastric hunger contractions (Harris, Ivy, & Searle, 1947). Hence, this anorexigenic effect appears to be mediated primarily by sites in the central nervous system. The search for the exact central nervous system sites that mediate amphetamine's anorexigenic effect has naturally been focused on the hypothalamus, and within the hypothalamus, on the ventromedial hypothalamic nuclei which have been shown to have an inhibitory effect on eating behavior ("satiety centre"), and on the lateral hypothalamic area, whose stimulation facilitates food consumption ("eating centre") (Anand & Brobeck, 1951; Anand & Dua, 1955). Lesions in the ventromedial hypothalamic nuclei and in the mid-lateral and far-lateral hypothalamic area have been shown to affect the anorexigenic effect of amphetamine (e.g., Stowe & Miller, 1957; Carlisle, 1964), emphasizing the importance of these central sites in mediating the drug's effect on food consumption.

An increase in electrical activity (increase in EEG amplitude and frequency) has been observed in the ventromedial hypothalamic nuclei following the administration of amphetamine derivatives to anaesthetized cats by Brobeck, Larsson, and Reyes (1956). This finding and the related hypothesis proposed by them that amphetamine has its anorexigenic effect by selectively stimulating the "satiety centre" has prompted a number of experimental investigations. However, subsequent studies have produced results that are not fully consistent with Brobeck et al.'s hypothesis. For example, when the ventromedial nuclei of the hypothalamus are elect-

rolytically destroyed, an exaggeration rather than an elimination of the amphetamine-induced anorexia is noted (Stowe & Miller, 1957; Epstein, 1959; Reynolds, 1959). This indicates that the hypothalamic ventromedial nuclei are not the primary receptor site for amphetamine, although it is somehow implicated since its destruction results in an increase of susceptibility to amphetamine's anorexigenic action. Some support for the general notion of Brobeck is to be found in the work of Sharp, Neilson, and Porter (1962), who found that the anorexigenic effect of amphetamine attenuated following hypothalamic ventromedial nuclei lesions. Clearly, the evidence is equivocal and further work is required.

Recent studies of the role of other brain sites in amphetamine anorexia indicate that the part played by central nervous system sites in the mediation of amphetamine effects is rather more complex than was assumed by Brobeck et al. (1956). For example, Carlisle (1964) found that lateral hypothalamic area ("eating centre") lesions, while they reduce eating, also reliably attenuate amphetamine anorexia. Cole and Hudspeth (1964) and Cole (1966) reported that anterior hypothalamic lesions exaggerate the depressant effects of amphetamine on eating. And Carlisle and Reynolds (1961) have demonstrated an exaggerated anorexigenic effect of the drug following lesions of the area postrema, indicating that not only the diencephalon, but also the mesencephalon is involved.

The above findings point to the need for an examination of the roles of both the ventromedial and the lateral areas of the hypothalamus in amphetamine anorexia. In particular, any interaction between the ventromedial hypothalamic nuclei and the lateral hypothalamic area in the

regulation of food intake deserves attention. The work of Brobeck et al. (1956) would suggest that amphetamine acts by stimulating medial hypothalamus; their data showed no change in the electrical activity of the lateral area following amphetamine. But Carlisle (1964) in his detailed lesioning and behavioral study suggests the effect is due to inhibition of the lateral hypothalamus. Considering possible interaction between the two areas, Brooks (1959) found no reciprocal relation between the electrical activity of the medial and lateral hypothalamic areas. The work of Anand, Dua, and Singh (1961) indicates, however, that there may be some interaction between medial and lateral hypothalamus. They found that electrical activity increased medially and decreased laterally when hyperglycemia was induced by peripheral glucose injections. Hypoglycemia induced by peripheral insulin injection reduced electrical activity medially and produced an occasional slight increase in activity laterally. In a single cell study Oomura et al. (1964, 1967) report reciprocal activity in the ventromedial hypothalamic nuclei and the lateral hypothalamic areas in cats immobilized with gallamine triethiodide (Flaxedil) following intravenous infusion of glucose and saline and following the administration of ether. These interactions suggest that the complex picture emerging from studies of the amphetamine-induced anorexia may be clarified by a study of the effects of amphetamine on electrical activity in both ventromedial hypothalamic nuclei and lateral hypothalamic areas. This was the primary purpose of the present investigation.

The inconsistent and sometimes divergent views of some investigators concerning amphetamine-induced anorexia may be generated by different

procedures employed. One feature of the procedure that appears particularly important concerns the use of anaesthetic agents. Brooks (1959) found that the ventromedial hypothalamic nuclei were exceedingly sensitive to the level of anaesthesia; the electrical activity from this region decreased as the level of general anaesthesia increased. This suggests that amphetamine might have attenuated the effect of the anaesthetic agent (pentobarbital sodium) in the Brobeck study, and thereby produced an apparent increase in the electrical activity of the ventromedial hypothalamic nucleus. Oomura et al. (1967) have also commented on this specific susceptibility to ether anaesthesia in the region of the ventromedial hypothalamic nuclei. Whereas some attention has been given already to factors influencing the spontaneous activity of hypothalamic neurons during general anaesthesia (i.e., level and plane of anaesthesia) (Hoagland et al., 1939; Green & Morin, 1953), no work has been done to establish the effect of amphetamine or its combination with the effect of an anaesthetic (e.g., ether). A secondary purpose of the present investigation was to study possible interactions between the neural effects of an anaesthetic agent (ether) and amphetamine.

Some incidental observations made by Campbell, Krebs, and Bindra (1968), seem to support the notion of a specific amphetamine effect on hypothalamic ventromedial cells. Working with acute, curarized cat preparations, a relative increase in firing frequency of units in the ventromedial hypothalamic nuclei was discovered after slow systemic infusion or single loading via the femoral vein of d-amphetamine sulphate. They also found decrease in firing rate or inhibition of ventromedial hypothalamic units following induction of light ether anaesthesia.

Following these incidental observations, the present study was aimed at systematically investigating the effects of amphetamine and ether anaesthesia on the spontaneous firing of neural units in the ventromedial and lateral hypothalamic areas in the curarized rat.

MATERIALS AND METHODS

Subjects

Seventy-two hooded rats, half of them female, 290-320 gm. in body weight, were used as subjects in the formal study reported here. The animals were maintained on ad libitum food and water until the time of surgery.

Surgical Procedure

For surgical operations each rat was lightly anaesthetized with ether and secured in the stereotaxic instrument. A 20 mm longitudinal incision was made over the anterior portion of the throat in order to dissect free the underlying trachea by blunt dissection. Ligatures were placed around the trachea and an incision was made in the wall for intubation by polyethylene tubing. Ligatures were tied and the animal was made ready for artificial respiration by connecting the tubing to an air pump adjusted to 47 strokes per minute and an approximate volume of 10 cc. Before artificial respiration commenced, the animal was maintained on light ether anaesthesia via the tracheal tube for the duration of the remainder of surgery.

Next, either the right or left carotid artery was cannulated at the locus of the tracheotomy, and a thin (0.024-in. outer diameter)

polyethylene tube was introduced rostrally. The tube aperture lay near the bifurcation of the internal and external branches of the artery. This artery cannulation was chosen, rather than an intubation of the femoral vein, in order to have a direct route to the brain and initially to curtail the peripheral effects. Necessary ligatures were placed and a test injection of 0.1 cc isotonic Ringer's solution was made to ascertain free passage. The incision site (i.e., wound margins) and pressure points of the stereotaxic frame were then infiltrated with procaine hydrochloride and, in addition, topical 2% xylocaine hydrochloride was applied to the wound. The wound edges were held lightly together by Michell clips.

For the placement of microelectrodes, the scalp was incised and craniectomy was performed over bregma in order to allow bilateral placements. Using a dental drill, a special chafing technique was employed for removing the cranium to reduce hemorrhage which seems particularly evident when ether is in use. Once the bony structure was reduced to a thin film, thus exposing the mid-sagittal sinus and other blood vessels, drilling was discontinued and the remainder removed with sharp forceps; approximately an area of 3 X 4 mm was removed. The dura was carefully incised with eye scissors and cut transversely reflecting the flaps as far as possible towards the midline. Gelfoam absorbable gelatin sponge, previously immersed in warm physiological saline, was then used to cover the cortex in order to reduce hemorrhage and prevent evaporation of lymphatic fluids.

The animal was then given gallamine triethiodide (Flaxedil) i.c. to effect respiratory collapse, at which point artificial respiration was

commenced. After routine infiltration of scalp incision site with procaine, the animal was ready for first microelectrode placement.

During the subsequent period of the experiment no further surgical manipulation of the animal was required, and it could be maintained satisfactorily for 4-6 hr. with periodic administration of the local anaesthetic. The animal in the stereotaxic instrument was transported from the operative field to an electrically shielded copper wire cage. For the whole of the observational period the rats were kept warm by means of thick layers of cotton wool which enveloped the body. Temperature was routinely checked via a rectal probe; additional heat did not have to be provided as ether anaesthesia does not seem to interfere greatly with temperature regulation.

Electrocardiographic Recording

Heart rate was monitored throughout the experimental session. Two 21-gauge hypodermic needles were placed subdermally over the pectoral region. Signals from these were fed into a d.c. differential preamplifier (Argonaut, Model LRA 045) and a write-out was obtained on one channel of an oscillograph (Oscillo-riter). Changes in heart rate proved to be a reliable indicator of the state of the preparation. Over- or under-ventilation and gross temperature deviations invariably led to departures from the baseline heart rate. Furthermore, changes in heart rate also served as valuable guides for assuring the continuing sufficiency of local anaesthesia in preventing unnecessary discomfort to the animal. The primary purpose of observing heart rate changes was, of course, to study sympatheminetic effects of amphetamine and for determining the

depth of ether anaesthesia when it was induced as a part of the experimental schedule. Blood pressure changes were inferred from accompanying amplitude changes of the electrocardiograph.

Microelectrodes and Recording Equipment

Stainless steel and tungsten wire microelectrodes were used. Recordings were made initially with steel microelectrodes according to the technique of Green (1958), but in later preparations tungsten electrodes prepared following Hubel's (1965) procedure were used. All electrodes were electrolytically etched to a tip diameter of either one micron or less, or of 1-3 micra, and a diameter of approximately 30 micra at a distance of 150 micra from the tip. The electrodes were dipped in lacquer (Insl-X), turned up and allowed to dry and harden for at least 24 hr. before use. The tip resistance of the microelectrodes was 0.5-2.0 MΩ.

Each electrode was mounted in an electrode carrier and could be manipulated by the use of a micromanipulator. The electrode was a.c.-coupled to the input stage of a conventional preamplifier (Grass, Model P8) with a gain of 100, an input impedance of 10 MΩ, and a passband of 0.5 cyc./sec. to 30 kc. The recording was single-ended with the grounded input connected to scalp and stereotaxic frame. The preamplifier was plugged directly into the input socket of a differential amplifier (Tektronix, Model 2A61). Unit responses were processed by a Schmidt trigger and gating unit (Ferch, Model 119) for write-out as square wave pulses on an oscillograph (Oscillo-riter) and for analysis by computer. Extracellular action potentials from neurons were recorded for periods of 5-60 min. In all experiments loudspeaker monitoring of unit activity

was employed. In addition, a solid-state binary counter and timing unit was used to determine the firing frequency over ten second periods. Permanent records of all important test procedures were made on magnetic tape using a two-channel tape recorder (Concord, Model R-1100); unit activity was recorded on one channel and spoken commentary on the other. In order to obtain a visual reproduction of the unit discharges, the appropriate tape record was sometimes played back into the oscilloscope and polaroid pictures were taken of wave form and spike distribution, but usually the unit discharges were written out on paper at the time of the experiment by means of the oscillograph. The Schmidt trigger gate was set in such a way as to cause a deflection of the pen with each action-potential.

Placement of Microelectrodes

In order to orient the microelectrodes in the tuberal region of the rat's diencephalon, the stereotaxic coordinate systems of de Groot (1959) was used, as well as a simple modification of the de Groot system proposed by Pellegrino and Cushman (personal communication; for details see Note 1, Appendix A). An accurate micrometer reading for the horizontal plane of the surface of the cerebral cortex was first obtained by slowly lowering the microelectrode with the micromanipulator until the characteristically abrupt change in the noise level was heard on the loudspeaker. After depressing the electrcde a little deeper into the cortex, the hole in the skull was sealed by applying warm agar solution which quickly solidified around the microelectrode, thus reducing to a minimum the repiratory or vascular pulsations of the brain and thereby preventing tissue movement around the electrode tip.

As a general procedure the microelectrodes were oriented through the brain in a sagittal plane about 0.5-1.0 mm. from the midline near bregma, so as to bring the tip of the electrode into the central portion of the ventromedial hypothalamic nucleus at about 9.0 mm. from the surface of the cortex, and for mid-lateral lateral hypothalamic area placements medial-lateral distance was about 1.5 mm. so as to ensure placement at a depth of 7.8-8.5 mm. in the vicinity of the fornix (columna) or peri-fornical area and the medial forebrain bundle (fasciculus medialis telencephali); far-lateral lateral hypothalamic placements were reached by medial-lateral distance of 2.0-2.4 mm. and a depth of 8.5-9.0 mm. thus coming very close laterally and ventrally to the optic tract (Fig. 1; Fig. 2). Other far-lateral placements in the lateral hypothalamic area were made somewhat more posterior, A 4.9, and parasagittal planes 1.7-2.5, and were taken to a depth of 7.5-9.0. This distinction of mid-lateral and far-lateral areas of the lateral hypothalamus was made in accordance with the findings of Morgane (1961), who described the lateral hypothalamus as organized in a complex of a more medial "hunger motivational system" and a far-lateral "metabolic system." As Barraclough and Cross (1963) noted, more anterior regions of the hypothalamus appear to yield few neurons suitable for continued unit recording and placements were not attempted beyond A 6.2 in the later stages of the experiment.

As the microelectrode tip penetrated the cerebral cortex, action potentials were readily picked out if the microelectrode was adequately insulated and had not deteriorated since production. It was soon discovered that if the tip was not etched concentrically, constant injury of units was sustained and any one cell could be studied for only 1-2 min.

Figure 1. Coronal brain section of the rat's diencephalon at coordinates A 5.8 (de Groot, 1959) and bregma 0.0 (Pellegrino & Cushman, 1966). Placements which were most frequently made are indicated for the ventromedial hypothalamic nuclei (●) and the lateral hypothalamic area (⊙). Abbreviations of fibre tracts and cell groups (according to de Groot) for the hypothalamus and bordering regions:

ARH Nucleus arcuatus hypothalami

CI Capsula interna

DMH Nucleus dorsomedialis

EP Nucleus entepeduncularis

FX Fornix (columna)

MFB Fasciculus medialis telencephali

OT Tractus opticus

RE Nucleus reuniens thalami

RT Nucleus reticularis thalami

V Ventriculus cerebri

VMH Nucleus ventromedialis hypothalami

ZI Zona incerta

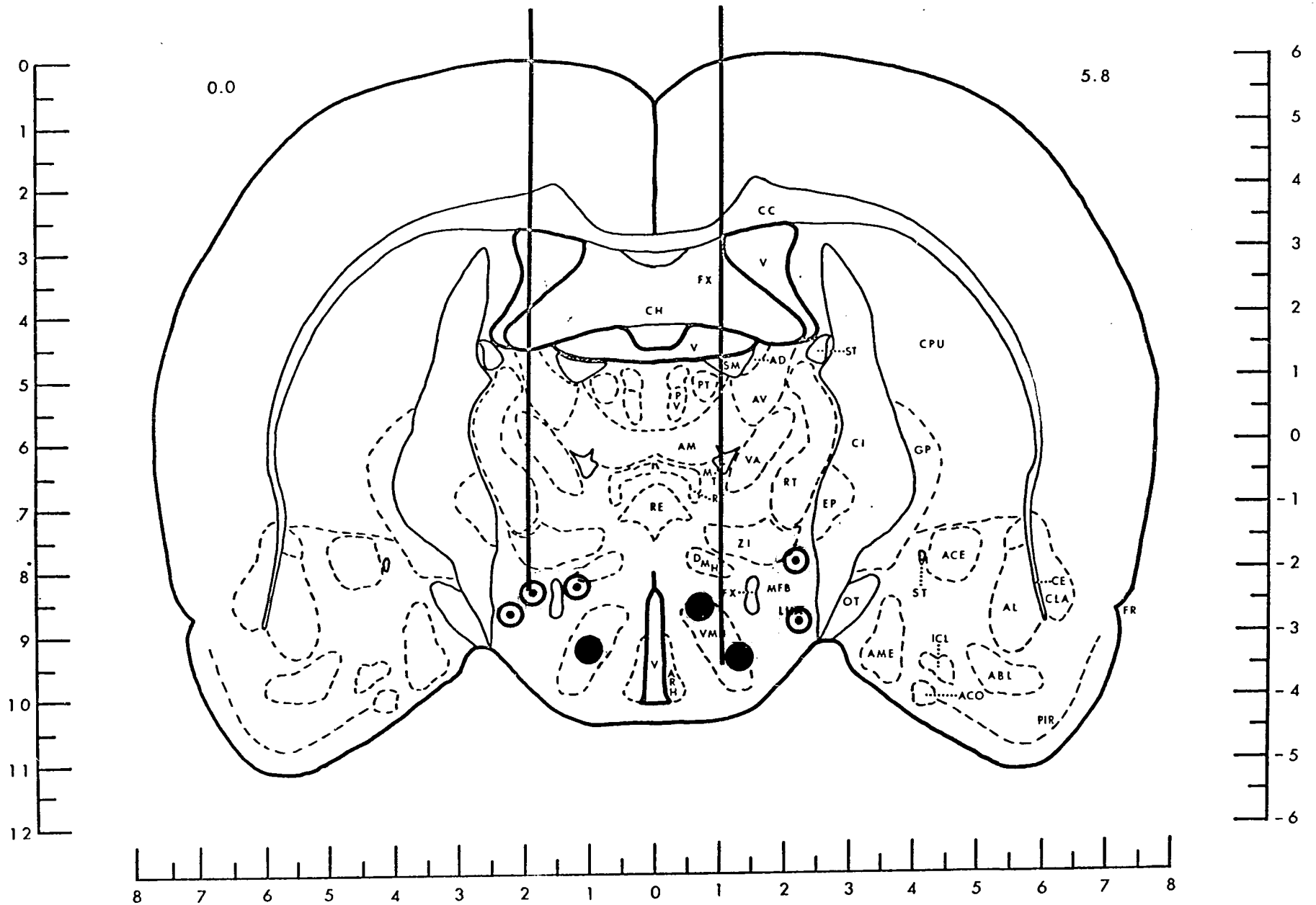
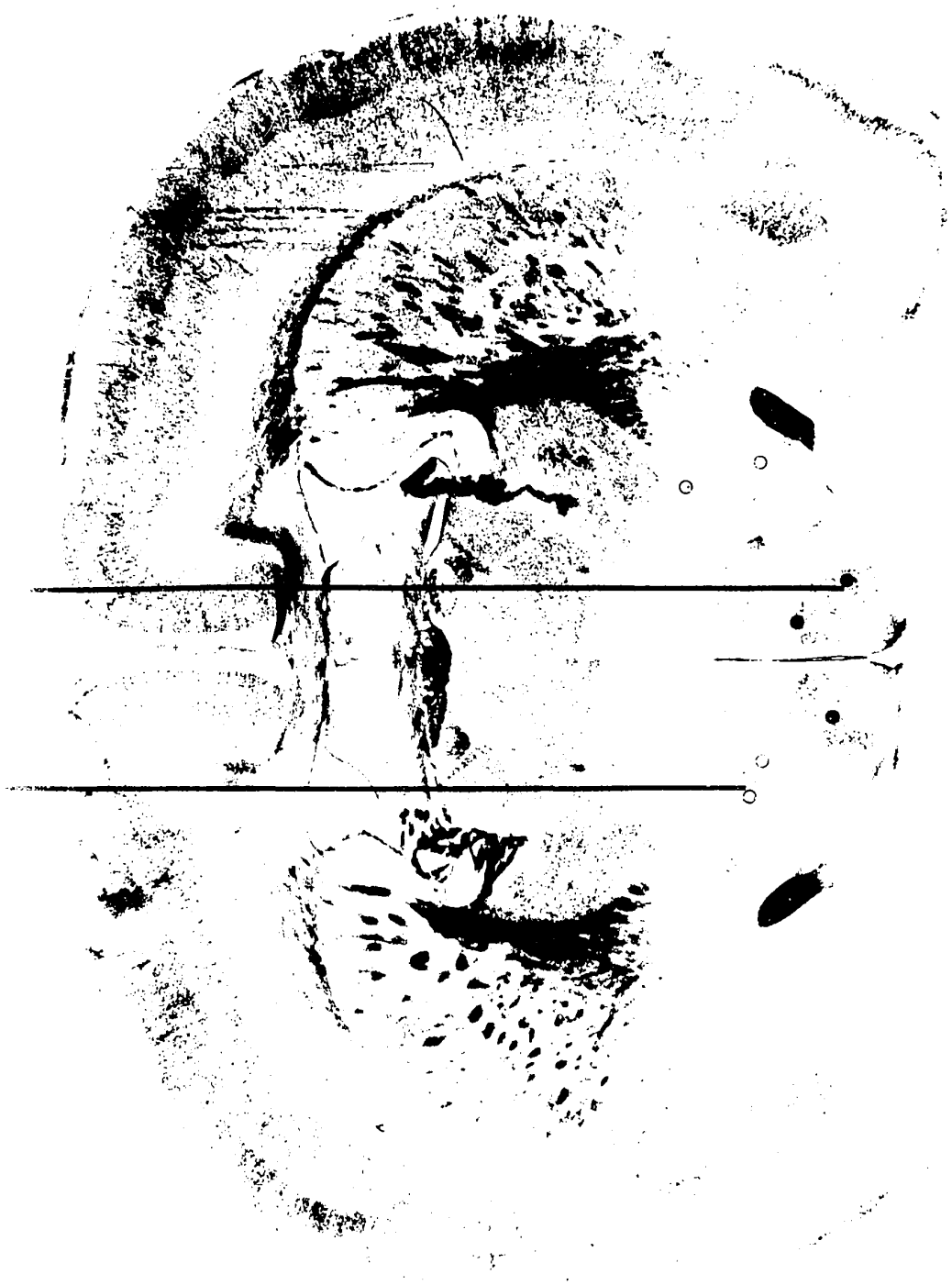

0.0
5.8
CC
FX
V
CH
CPU
ST
AD
SM
PT
PV
AV
AM
VA
CI
GP
MT
RT
RE
EP
ZI
DMH
FX
MFB
OT
ACE
CE
CLA
AL
FR
VM
V
ARH
ICL
AME
ABL
ACO
PIR
0 1 2 3 4 5 6 7 8 9 10 11 12
8 7 6 5 4 3 2 1 0 1 2 3 4 5 6 7 8
6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6

Figure 2. Microphotograph (12X) of coronal brain section of the rat's diencephalon at coordinates A 5.8 (de Groot, 1959) and bregma 0.0 (Pellegrino & Cushman, 1966). Placements which were most frequently made are indicated for the ventromedial hypothalamic nuclei (●) and the lateral hypothalamic area (○).



Such microelectrodes were readily recognized as "killer" electrodes and eventually could be eliminated after a test at the cortical level with each new electrode. With good electrodes an abundance of units could generally be found in the cortex and deeper in the thalamus (anterodorsal, antereventral, ventralis anterior, mediodorsal, ventromedial, and reticular). These units were usually large and distinct with signal-to-noise ratio as great as 20:1. As the hypothalamic region was reached, it became more difficult to detect activity if the tip was by then still intact; considering a traversal of over 8 mm. of brain tissue by such a delicate structure as a 1 micron tungsten tip (even if distortion resistance is much greater than in steel electrodes of the same specifications), it is small wonder that out of ten electrodes on the average only 3-5 reached target intact, (i.e., did not bend while penetrating larger vessels, or the tip did not become blunted while traversing ventricle fascia).

In the hypothalamus, the units yielded action potentials of about 0.5-1.0 mV in amplitude and 1.5 msec. in duration. All units studied were positive/negative and most showed a prespike potential which sometimes occurred without an accompanying main spike potential (Fig. 3). Microelectrodes with tips larger than 1 micron rendered multispike signals; records containing two clearly discriminable spike amplitudes were considered acceptable, the larger of the amplitudes being treated in the data evaluation as a unit.

Experimental Plan

The microelectrode was lowered into the hypothalamic area until a spike was observed on the scope. Any spike encountered was studied if it

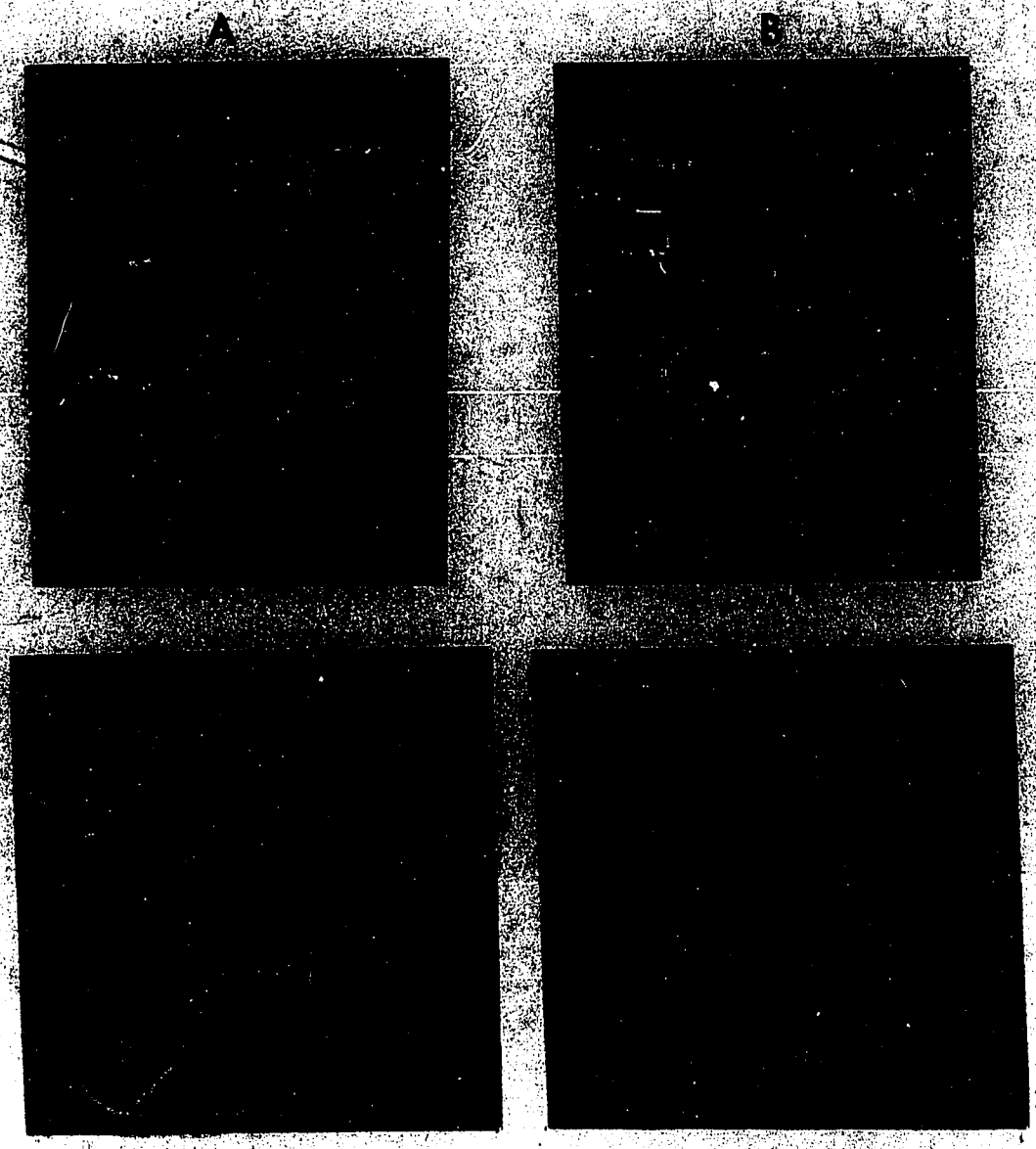
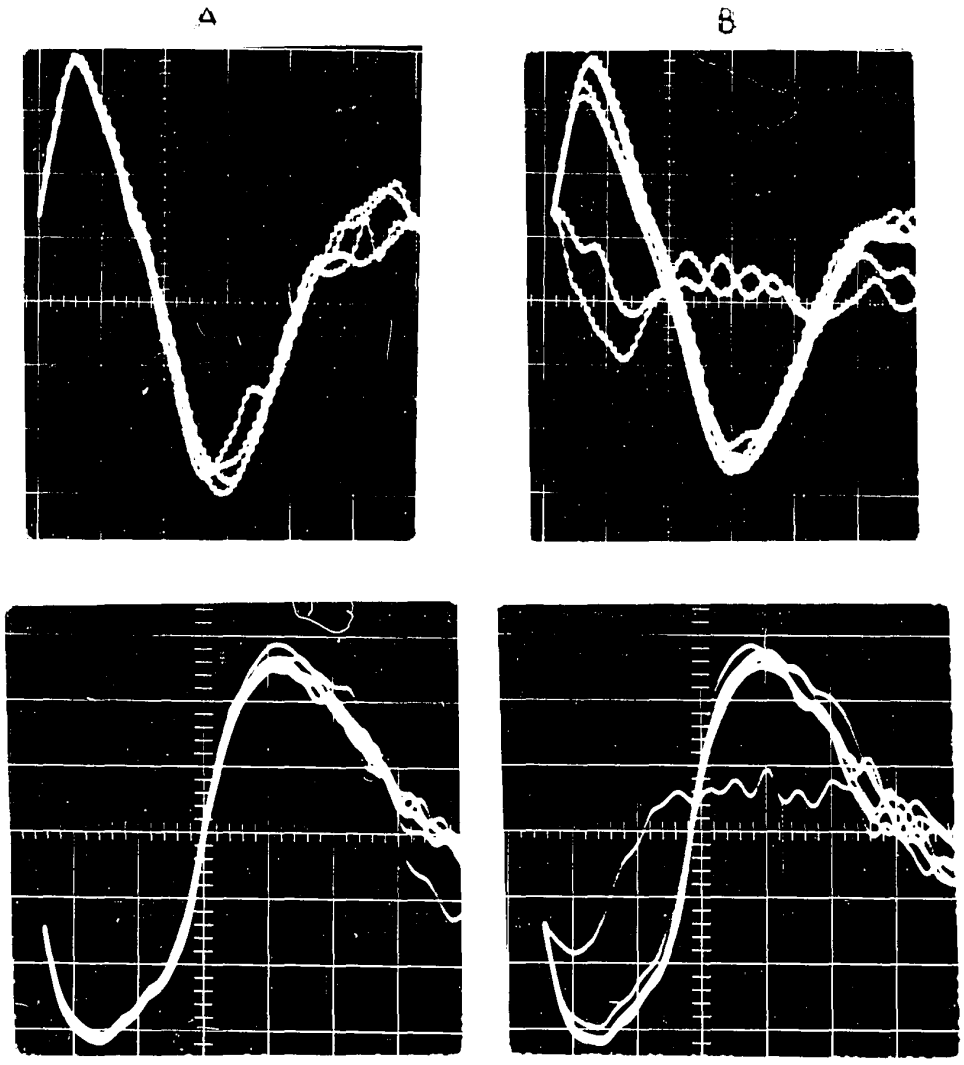


Figure 3. Photographs of superimposed oscilloscope sweeps to show the waveform (upward deflection positive in Sample 1; upward deflection negative in Sample 2) of two neurons (Sample A) recorded in the ventromedial (Sample 2) and lateral (Sample 1) hypothalamic areas. The figure '8' appearance (Sample B) was due to a prespike potential which sometimes fired separately and was observed in both units studied.


A
B

met the criterion of good discrimination from background noise and other cells and could be reliably triggered by the normal sweep trigger of the discriminator circuit to permit display of waveform on the scope. Upon location of a unit, the stability of its spontaneous firing was determined by its relative constant maintenance of spike potential amplitude and waveform over a period of at least 2, and usually 5, minutes. If the unit was stable, a 0.2 cc saline (98° F) control infusion over a 5 sec. period was made. Apart from serving as a control for later amphetamine infusions, this test would indicate instability of the unit through possible tissue movement caused by blood pressure changes. In view of the rich vascularization of the hypothalamus (Daniel, 1966), controls for pressure effects are important. If the unit remained stable following the control infusion, the unit was selected for the standard testing procedure. For individual units the recording periods varied from 10 to 60 minutes (the average was about 25 min.). Before commencing the standard tests of the responsiveness of a unit to ether and amphetamine, its spontaneous firing frequency was determined over 10 sec. intervals with the binary counter. After this, three initial tests were given with ether and amphetamine in the following order: ether, amphetamine, ether.

In a previous study (Campbell et al., 1968) it was noted that ether invariably inhibited unit activity in the ventromedial hypothalamic nucleus, but the effect was easily reversible within minutes by simple discontinuation of the gas. In contrast, a dose of 2.5-5.0 mg/kg d-amphetamine took anywhere from 4 to 6 hr. to be eliminated from the blood and eventually rendered ineffective (Goodman & Gilman, 1955; Carlisle, 1964). Hence, amphetamine tests were always preceded by ether tests.

As the stability of a cell could not be taken for granted, all initial tests (ether-amphetamine-ether) were made quickly within the first 4-10 min. of recording, in order to insure some comparability.

For the ether test, the ether was contained as fluid and gas in a special gas bottle which could be easily connected to the input valve of the respirator. The animal inhaled ether evaporating at 68-72° F. The bottle remained connected from 20-90 sec., whether a change in unit response to ether was noted or not; this test was repeated at least once. In some ether retests the duration of the test was extended to 3 min., but this was done only after at least one amphetamine test had already been given.

From 5 to 10 minutes following the initial ether tests, either a single infusion of 1.0 cc d-amphetamine sulphate over a 10 sec. period was made or a repeat quick-loading of 1.0 cc was given in 0.1 cc amounts, waiting after each loading for 60 sec. Each 1.0 cc of the infused solution contained an adequate amount of d-amphetamine to serve as a dose of either 2.5 mg/kg or 5.0 mg/kg. If a change in unit firing rate was noted, loading stopped. After the amphetamine test, the ether test procedure was repeated to ascertain repeatability of previously noted effects and to observe interaction of the agents; for example, whether a cell that was previously inhibited by ether would still show inhibition when ether is administered following facilitation of the cell by amphetamine.

Histological Reconstruction

During the experiment, note was kept of the depth from cortex surface of each unit studied and also of the horizontal coordinates and

final depth of all electrode tracks. Small Prussian blue spots were made at strategic sites in steel microelectrode tracks to aid later histological reconstruction. An anodal d.c. current of less than 5μA. was passed through the steel electrode for 2-3 sec. in order to deposit iron particles in the tissue. These rats were sacrificed with i. c. pentobarbital sodium and the heads were intracardially perfused, using gravity flow technique, with a weak solution of potassium ferrecyanide (2%) in normal saline to develop the Pressian blue reaction. Subsequently the brains were fixed by perfusion of 40 cc 10% formol saline while the animals remained in the stereotaxic instrument. Tungsten electrodes were left in situ and these animals were perfused routinely with saline followed by 10% formol saline. The brains were blocked stereotaxically and carefully removed from the calvarium after perfusion was completed. Finally, the brains were stored in 10% formalin for a minimum of 72 hours, taking care to change the formalin every 24 hours. The brains were sectioned on a cryotome (Lipshaw) at a thickness of approximately 40 micra; the mounting of the brains on the object disc of the microtome was such as to render sections in the same plane as those used in the de Groot (1959) atlas. Every section throughout the tuberal region of the brain from A 6.6 to 4.8 was stained with luxol fast-blue (modified Klüver & Barrera technique, 1953) for myelinated fibres and counterstained with either neutral red or cresyl violet for cell groups. The neutral red was used to give greater contrast for the Prussian blue spots. These sections were then photographed at 6 1/2 X. The upper portions of the electrode tracks in the plane of section were readily identified, but the finely tapered tips often left little trace in the sections, so that the Prussian blue spots

in the case of steel electrodes or green staining blood particles around the tungsten electrode insertion, most critically along the finely tapered portions of the electrode shaft, were of some help. Shrinkage usually was not more than 1/8 and the site of all recorded neurons could be estimated by their distance either from cortex or a Prussian blue spot and from the formation of green stained tissue.

RESULTS

Preliminary Observations

Preliminary observations, made before the formal procedures described in the last section, established that under the procedure specified for ether and amphetamine administration and the doses used, no significant or consistent changes in unit firing were seen in such control areas as parietal cortex, various thalamic sites including zona incerta, the reticular formation of the mesencephalon and the thalamic reticular formation (nucleus reticularis). Apparently larger doses of amphetamine are required to demonstrate increases in electrical activity of the reticular formation (Rothballer, 1959). The dorsomedial, paraventricular, anterior, and posterior hypothalamic areas were also briefly sampled and again no consistent effects were noted following ether or amphetamine. The anterior hypothalamic area neurons showed an increase following amphetamine in some instances, but it was very difficult to record from single neurons for any length of time.

In traversing the brain from cortex to target, several ether tests were carried out on some rats. Cortical cells were usually affected at high evaporation rates of ether and upon induction of deep anaesthesia

(plane 3, stage 3). Thalamic units did not seem to show significant changes in over-all rate of firing, but showed an increase in incidence of multi-spike bursting; some units actually showed a long-term increase in firing rate (i.e., acceleration of firing was maintained outlasting the facilitating effect of ether by several minutes before returning to base line).

Furthermore, preliminary control observations were made on agents other than ether anaesthetic and amphetamine. Chloroform, another anaesthetic administered as a gas had no effect on firing rates of cells in the cortex, thalamus, or hypothalamus. Atropine sulphate (1.2%) was given to effect heart rate increase and adrenaline (epinephrine, 2µg/kg) to induce blood pressure changes in order to observe possible cardiovascular influences upon changes in unit activity of hypothalamic nucleus cells. Intra-arterial infusion of epinephrine may have some direct effects not associated with cardiovascular or baroceptive factors (Brobeck, 1956; Van Orden & Sutin, 1963). No changes were seen in spontaneous activity of ventromedial and lateral hypothalamic units after intra-arterial infusion of these substances; although with reference to epinephrine the results were equivocal. For hypothalamic unit response to arterial boood pressure effects note also the work done by Frazier et al., 1965. (See Appendix A, Note 2.)

Spontaneous General Characteristics of Unit Activity in Hypothalamic Areas

As briefly noted earlier, in the hypothalamus the units yielded action potentials of about 0.2-1.0 mV in amplitude and 1.5 msec. in duration. All units studied were of the positive/negative type and the majority of them showed a prespike potential which sometimes occurred without an accompanying main spike potential (cf., Fig. 3). Variations were of course noted from one unit to another, but for individual units one waveform persisted

for long periods indicating relative stability. It was easy to ascertain that the waveform observed depends in part on the location of the microelectrode tip in relation to the neuron, as movement in micron steps with the micromanipulator in either direction changed the waveform characteristics. In relatively silent areas, unit activity could still be detected accurately if moved away in this fashion up to about 100 micra. The prespike seemed to assume predominance in this instance if it had been observed initially. The type of waveform shown in Fig. 3 was regarded as the action potential recorded from the immediate vicinity of a nerve cell body. Small negative spikes of less than 1 msec. duration appeared in the lateral hypothalamic area recordings as well as in the ventro-lateral portions of the ventromedial hypothalamic nucleus. These could be interpreted as nerve fibre action potentials.

Firing rates varied considerably, from less than one impulse/10 sec. to over 100 impulses/sec. Ventromedial hypothalamic neurons were generally slow, firing at frequencies in the range 1-8/sec. The firing pattern displayed almost always a regular rate, (i.e., equal interspike intervals). This held also for lateral hypothalamic area units but not for signals described tentatively as taking their origin from other than main cell body components. However, occasionally a cyclical pattern was observed in or near the ventromedial hypothalamic nuclei; every three or four minutes an acceleration of firing rate was noted which lasted for 10-60 sec. A similar phenomenon has been described as intermittent bursts of spikes or rhythmic changes (Cross & Green, 1959), but was reported as being irregularly distributed while being superimposed on spikes of regular firing rate.

Effects of Ether and Amphetamine

Quantitative data. In all 39 electrodes were placed in the ventromedial hypothalamic nuclei and 33 in the lateral hypothalamic area. The total number of units studied with these electrodes was 160. With these units 50 tests were carried out with amphetamine and 71 with ether in the ventromedial hypothalamic nuclei, and 38 tests were made with amphetamine and 49 with ether in the lateral hypothalamic area. Two doses (2.5 mg/kg and 5.0 mg/kg) of amphetamine were used, but only one dose was used with any one animal; the larger dose was used in the earlier experiments. No difference was noted in the effects of the two doses on spontaneous unit activity with the exception of some cells; hence, no distinction will be made between the dosage level employed.

For the purposes of quantitative comparisons only those tests that complied strictly with the formal experimental procedures outlined earlier will be considered; 72 units met this criterion. Test results were specified as no change (0), facilitation or increase (+), and inhibition or decrease (-) in the spontaneous firing rate. This decision was made easily with visual inspection of the oscillographic write-out; only those changes in firing rate that lasted for at least 15 sec. were considered. The latter stipulation is necessary as latency of onset of change in the dominant response to the agent tested was important in the case of some units. For example, the predominant effect of ether, inhibition of spontaneous firing, on ventromedial hypothalamic nucleus units sometimes appeared after an initial 3 sec. increase. This transitory increase was followed by the long term deceleration of firing which continued until ether administration was discontinued. Similarly,

after amphetamine infusion, of 1.0 cc 5.0 mg/kg solution, 7 ventromedial hypothalamic nuclei units showed a 2-10 sec. deceleration before the prolonged increase in firing rate that extended from 5-40 minutes.

The number of units showing different types of changes in firing rate (increase, decrease, or no change) following the administrations of ether and amphetamine are shown in Table I. Consider the 39 ventromedial hypothalamic nuclei units first. No change following amphetamine was observed in only 3 units; no units showed a deceleration. All of the remaining 36 units demonstrated an increase in firing rate. In response to ether, no facilitation was noted; 5 units did not change spontaneous firing rate, and the predominant inhibitory effect of ether on spontaneous activity was demonstrated in 34 units. The saline control infusion had no effect in any of the 39 ventromedial hypothalamic nuclei units. (For details of data see Table IA, Appendix B.)

In the lateral hypothalamic area the 33 units responded in the following fashion to the administration of amphetamine: 12 units showed no change, 12 units showed deceleration followed in 7 by long-term inhibition or complete cessation of firing, and 9 units clearly demonstrated facilitation. Ether had a similarly varied effect on spontaneous firing rates; no change was evident in 23 of the units tested; however, 9 units showed inhibition and one unit actually displayed a significant increase of activity which was maintained for some time after termination of anaesthesia. Saline did not produce any changes in any of the units. (For details of data, see Table IA, Appendix B.)

Two χ^2 statistic tests were carried out to ascertain formally whether amphetamine and ether affect the two hypothalamic areas differen-

Table I.

Experimental condition	Ventromedial hypothalamic placements N=39			Lateral hypothalamic placements N=33		
	No change	Inhibition	Facilitation	No change	Inhibition	Facilitation
d-amphetamine sulphate	3	0	36	12	12	9
Ether	5	34	0	23	9	1
Saline	39	0	0	33	0	0

tially. The effects of each of the two agents were found to be significantly different ($p < .001$) in the two hypothalamic areas studied; This means that the distribution of the different types of units (i.e., with different responses to the agents) is not the same in the two areas, though some units of every kind are apparently present in both areas.

Histological reconstructions were plotted on charts of the relevant de Groot atlas (1959) sections. Refer to Fig. 2 for the most common microelectrode placements. Primary target areas in the ventromedial hypothalamic nucleus were a dense cell-body grouping in the dorso-medial aspects, a central portion of less densely packed cells, and a lateral region bordering on lateral hypothalamic area, medial forebrain bundle, and in posterior sections (A 5.0) on the ventral premammillary nucleus. Lateral hypothalamic area placements were more varied. Here units sampled tended to congregate around the following loci; perifornical and medial forebrain bundle (including the area traversed by paraventriculo-supraoptic fibres, ansa lenticularis, and pallido-hypothalamic fibres), also a more dorso-lateral area identified mainly by fasciculus lenticularis of Forel, and a ventral lateral hypothalamic area somewhat dorsal and medial to dorsal supraoptic commissure, pars ventralis (Meynert).

As far as can be determined from such reconstructions of recording sites, 9 units of the total 12 in the lateral hypothalamic area that were inhibited following amphetamine proved to be distributed in the ventral area bordered dorso-medially by the fornix (Fig. 4). Other units sampled showed no close correlation between effect observed and their anatomical location.

Figure 4. Microphotograph (6X) of coronal section through rat's brain at approximately A 5.6 (de Groot, 1959), showing microelectrode placement (O) in the lateral hypothalamic area. The fornix (columna) appears as darkly stained circular structure dorsal to the placement.

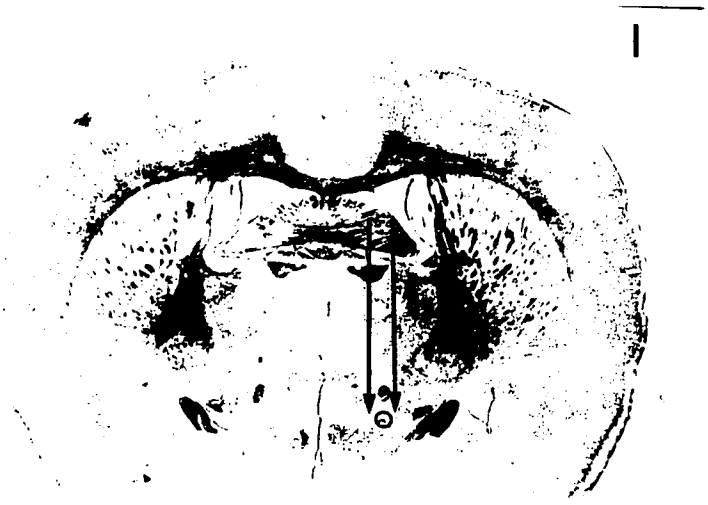


Figure 4. Microphotograph (6X) of coronal section through rat's brain at approximately A 5.6 (de Groot, 1959), showing microelectrode placement (O) in the lateral hypothalamic area. The fornix (columna) appears as darkly stained circular structure dorsal to the placement.

Qualitative data. In the following report detailed descriptive data will be presented on one of the units studied and a general commentary on further 7 units. Of these 8 units, 4 were located in the lateral hypothalamic area and 4 in the ventromedial hypothalamic nuclei. For each a brief report and one or two figures are supplied, showing portions of continuous oscillograph write-outs covering considerable periods of observation. The selection of these representative units of lateral and ventromedial hypothalamic areas was made on the basis of representativeness of all different types of effects seen. The effects of the drugs are described by two symbols, the first symbol describing the drug, amphetamine (A) or ether (E), and the second symbol describing the direction of the effect, no change (0), deceleration (-), and facilitation (+). Thus, A(+) means amphetamine increased the spontaneous firing rate.

The lateral hypothalamic area unit sample displayed five types of effects: A(0), A(-), A(+), E(0), and E(-). The ventromedial hypothalamic nuclei unit sample displayed two main effects: A(+) and E(-); of the 4 ventromedial hypothalamic nuclei units chosen, two had extremely slow spontaneous firing rates (about 1/sec. and 1/10 sec.). The observations on each of the 8 units will now be described.

Unit 1: MA-29. Lateral hypothalamic area; perifornical placement, left track, Fig. 5; observed effects: E(-), A(-), E(-). A recording was obtained for this relatively slow firing unit for 13 min. and 48 sec. The present discussion covers a period of 10 min., of which 3 min. are presented in six 30-sec. time samples (Fig. 6). The first 30-sec. sample (Sample 1) represents the baseline rate of firing for this unit with a rate of 9/sec. Note the fairly evenly spaced interspike intervals.

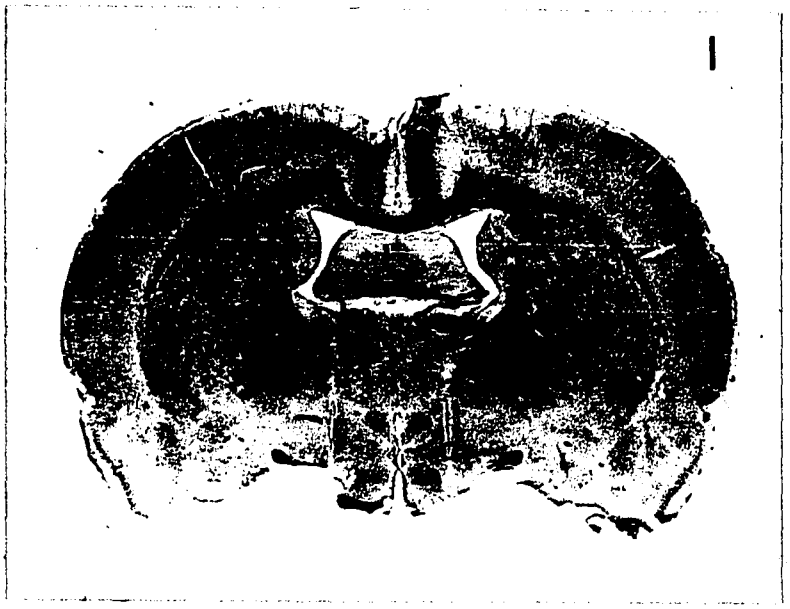


Figure 5. MA-29. Lateral hypothalamic area at the A 6.0 de Groot plane; unit recording was made from the left track shown to be oriented in the perifornical area.

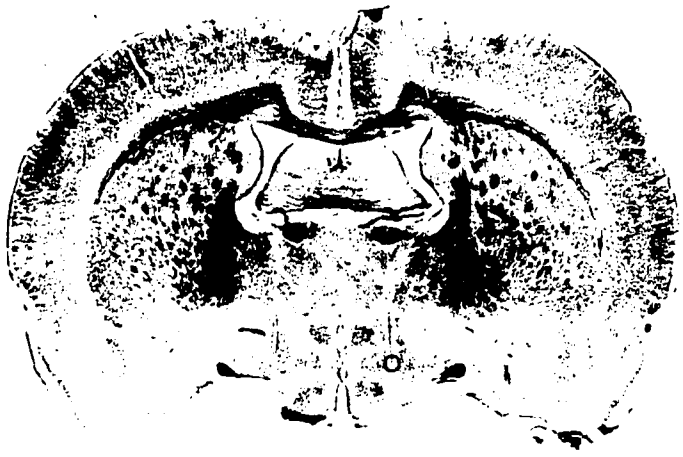
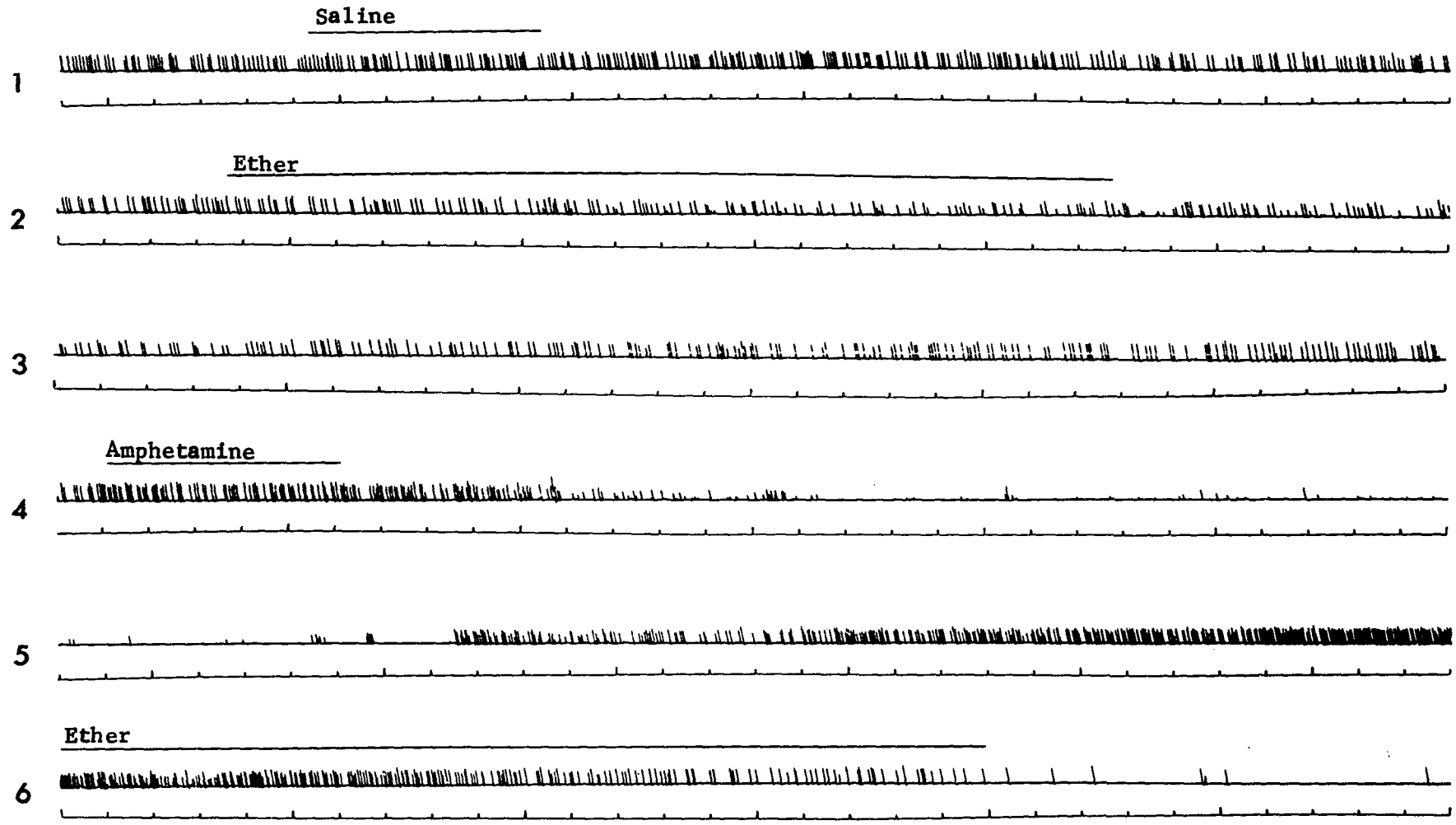


Figure 5. MA-29. Lateral hypothalamic area at the A 6.0 de Groot plane; unit recording was made from the left track shown to be oriented in the perifornical area.

Figure 6. Unit 1: MA-29. Effects of ether and amphetamine on spontaneous unit activity of lateral hypothalamic area. The middle trace represents the unit activity, 1/sec., time marker is on bottom trace, and the timing of tests is shown at top of unit activity.


Saline
1
Ether
2
3
Amphetamine
4
5
Ether
6

Saline control injection was given after the first five seconds (horizontal line above the first record) and no change in unit activity was noted. The next 30-sec. sample is continuous with the first record; an ether test was administered during this period. After 6 sec., frequency of spike was reduced (about 5/sec.) and a low amplitude spike became predominant; this signal may have originated from some other point of the same unit under observation, or may be another unit, but the change does not indicate cell movement (instability) as the high amplitude unit returned spontaneously after recovery from ether. The third sample is not continuous with the preceding one; 2 1/2 min. intervened between Samples 2 and 3. Note the recovery of firing rate and the elimination of the low amplitude activity; during the last 4 sec., rate had returned almost to the original base line (7/sec.). The following 3 min. of recording are not presented; during this time another ether test was given with results similar to those mentioned above. The fourth shows the effect on firing rate following infusion of amphetamine (2.5 mg/kg). Firing incidence of high amplitude unit decreased 4 sec. after termination of infusion and again (for 5 sec. only) low amplitude activity was seen. All activity stopped approximately 10 sec. following the infusion termination. Over the next 13 sec. period only 3 high amplitude spikes were seen. This inhibition lasted for 98 sec. and recovery during the last 8 sec. of this period is shown in the fifth sample. Over the remaining 22 sec., firing rate increased to the baseline of 9-10/sec. and for about 8 sec. reached a high rate of over 20/sec. The last sample is continuous with the fifth and shows a deceleration to 15/sec. during the first 6 sec. while an ether retest was given. During the 10 sec.

following ether administration the unit fired only 6 times; the low amplitude component was absent, however.

Unit 2: MA-19. Lateral hypothalamic area; perifornical placement; observed effects: E(-), E(-), A(-), E(-). Sample 1 demonstrates again changes following ether administration; note also, the 360/min. heart rate decreased during this test to 300/min. with an accompanying amplitude decrease. Throughout the recording, bursts of unit firing were seen; bursting denotes unit firing in rapid succession. Towards the end of Sample 4, firing rate of unit had returned to 8-10/sec. Sample 5 shows the amphetamine infusion (5 mg/kg); heart rate decreased to 240/min. and amplitude increased slightly. Within 1 sec. of infusion, some rapid firing was noticeable, but after 7 sec. a sharp decline in firing rate was clearly indicated by the long interspike intervals. The last sample follows the preceding one by about 8 1/2 min. Firing rate was 7/sec. Ether induced some reduction in firing of the unit, despite the amphetamine primed conditioned of the animal. The total inhibition shown after the ether test preceding amphetamine was abolished, however.

Unit 3: MA-13. Lateral hypothalamic area; ventral-lateral hypothalamic area dorsal and medial to dorsal supraoptic commissure, pars ventralis; observed effects: E(0), A(0). Sample 1 shows a typical hypothalamic unit with its slow but regular firing rate of 5/sec. Note the distribution of the interspike intervals. Ether administration did not change the firing pattern. Sample 3 is following the ether test within 3 1/2 min.; amphetamine was given (5 mg/kg). The change in heart rate from 360-300/min. and the amplitude variations out-lasted the infusion. Again no significant change was noticeable. Sample 4 shows

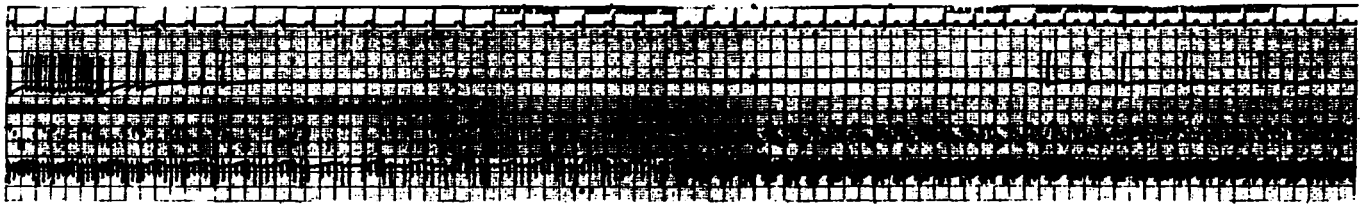
Figure 7. Unit 2: MA-19. Effects of ether and amphetamine on spontaneous unit activity of lateral hypothalamic area: a) 1/sec. time marker, b) unit activity oscillograph write-out, c) on-off test time, d) electrocardiograph.

1

Ether

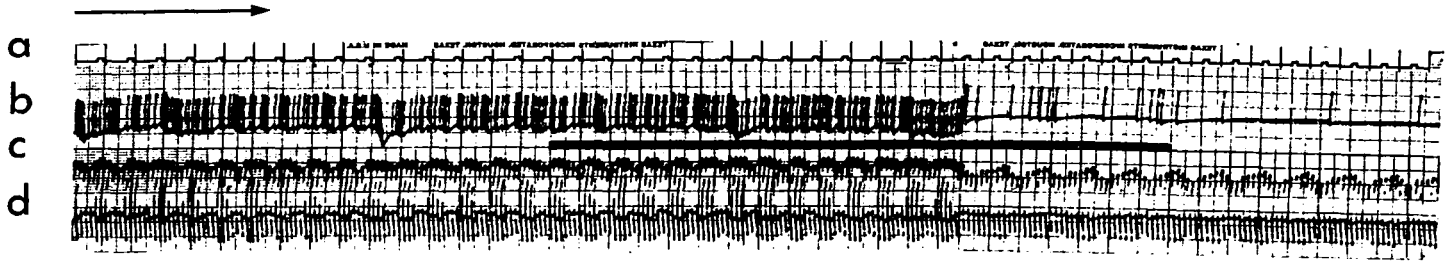
2

3



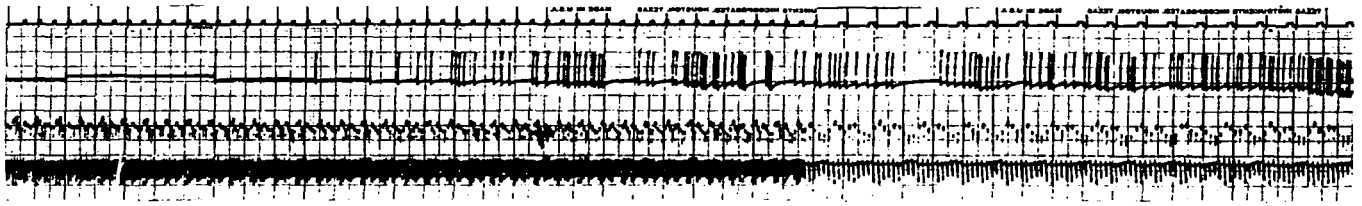
Ether

1

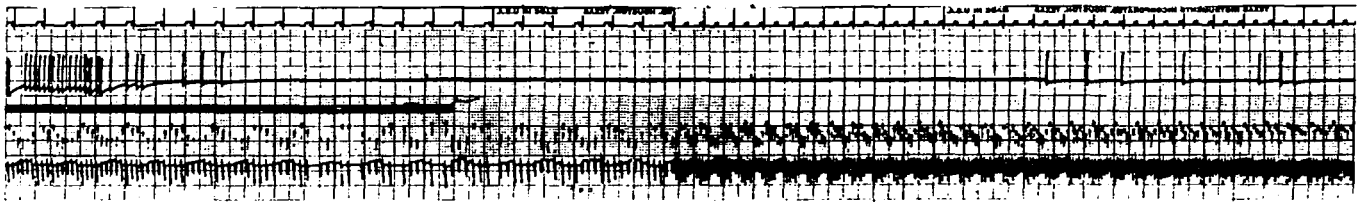


Ether

2

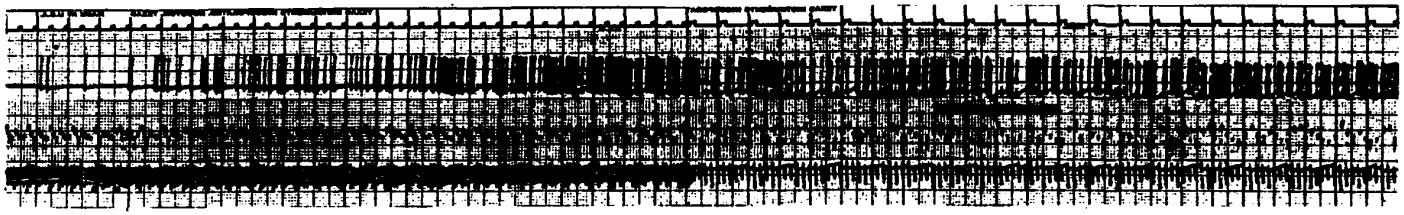


3



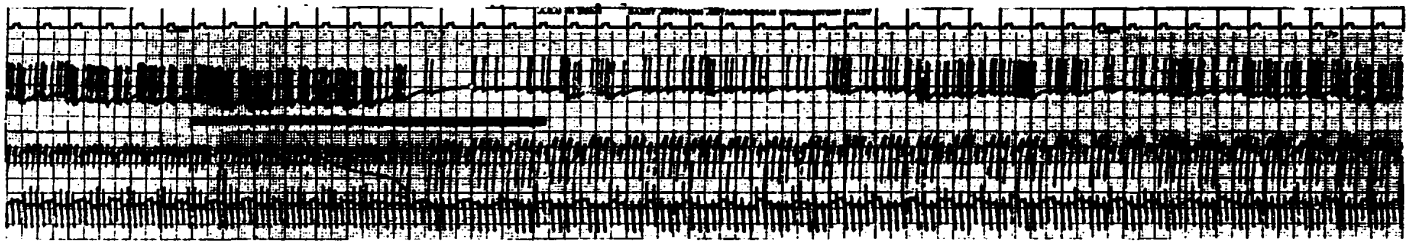
Ether

4



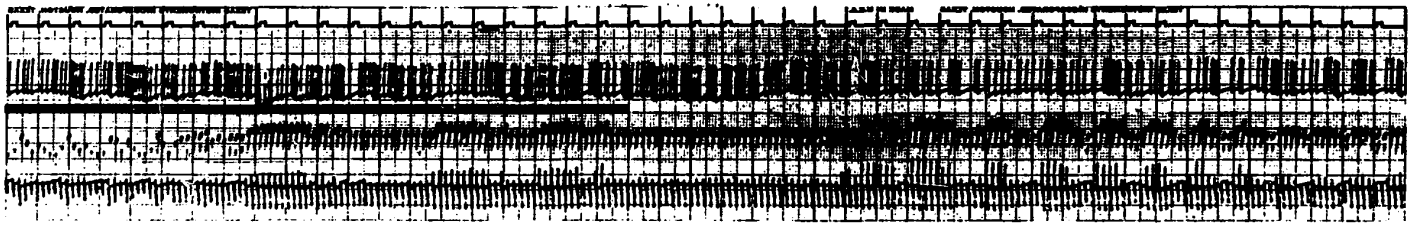
Saline

5



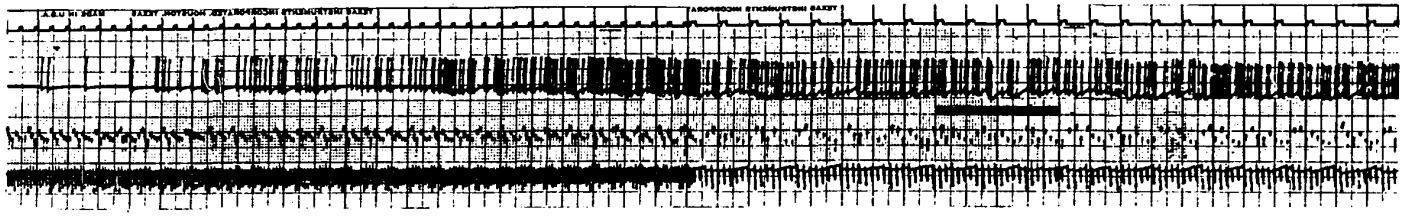
Amphetamine

6



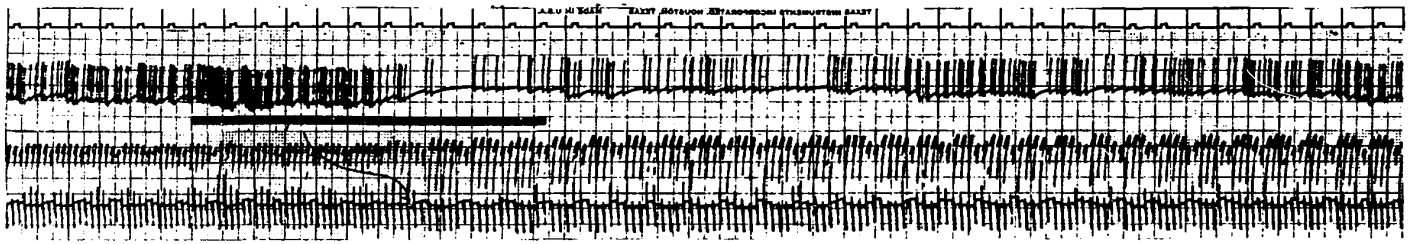
Ether

4



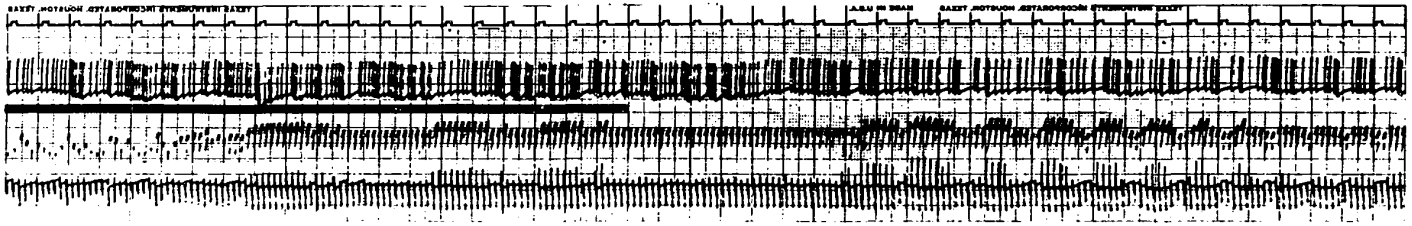
Saline

5



Amphetamine

6

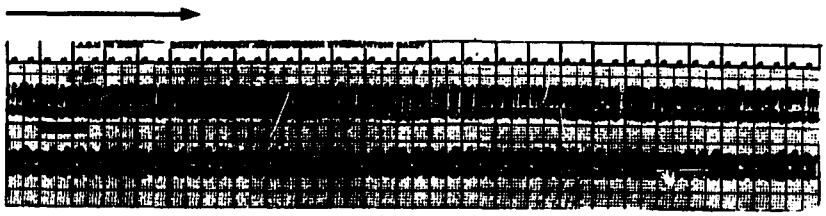


Ether

Figure 8. Unit 3: MA-13. Effects of ether and amphetamine on spontaneous unit activity of lateral hypothalamic area: a) 1/sec. time marker, b) unit activity, c) on-off test time, d) electrocardiograph.

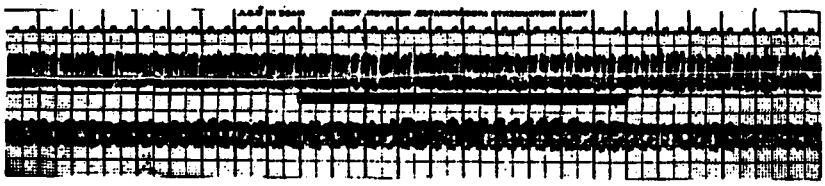
1

a
b
c
d



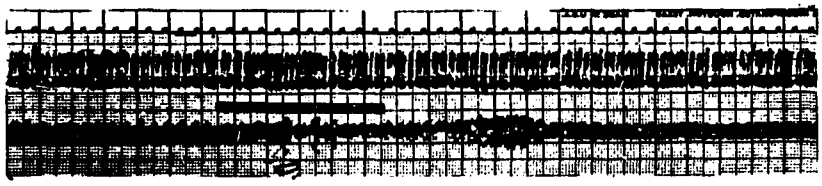
Saline

2



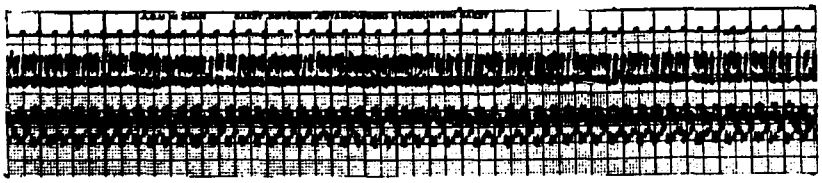
Ether

3



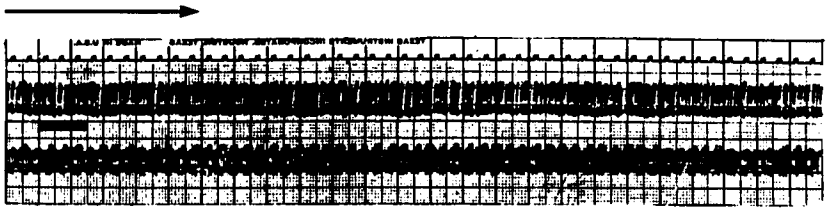
Amphetamine

4



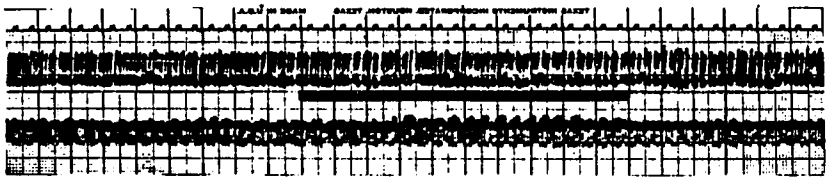
1

a
b
c
d



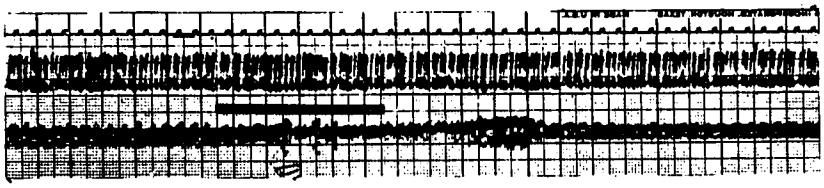
Saline

2



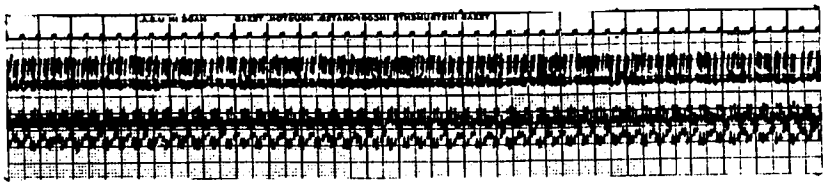
Ether

3



Amphetamine

4

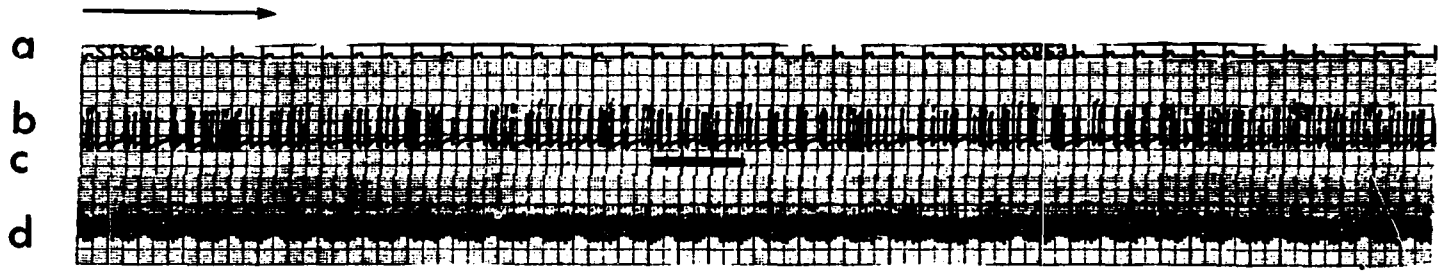


recovery of heart rate; this is 10 min. after amphetamine infusion and firing rate remained unchanged (5/sec.).

Unit 4: MA-33. Lateral hypothalamic area; dorso-lateral area identified by fasciculus lenticularis of Forel; observed effects: E(-), A(+). Sample 1 indicates a baseline firing rate of approximately 5/sec.; note interspersed bursting activity. Heart rate was above the normal 360/min. with its high rate of 420/min. Sample 2 is a recording taken 38 sec. after an ether test was administered; deceleration is shown clearly. Heart rate remained at 420/min., however, but amplitude had increased. Wave crests riding on the top and bottom of the heart rate write-out were reflecting the 47/min. respiratory rate maintained by the air pump. Samples 3 and 4 give the amphetamine test effect (2.5 mg/kg). No evident change in firing pattern was seen for the first 35 sec. after infusion. After a 58 sec. latency period, Sample 4 shows the increase in firing rate to 10/sec. and short trains of bursts were seen. Note changes in heart rate and amplitude of electrocardiograph.

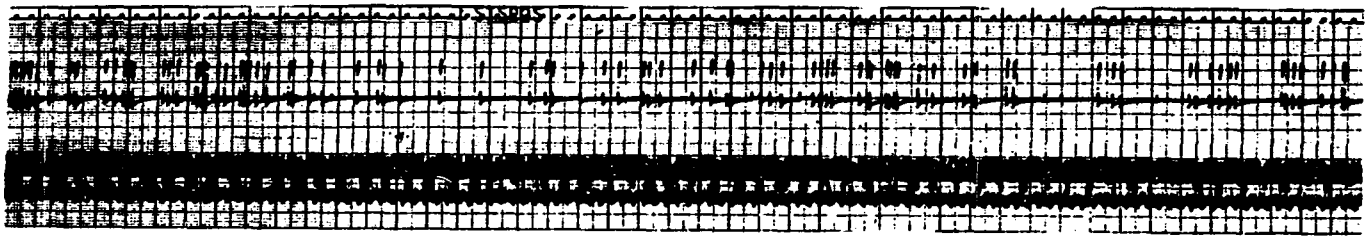
Unit 5: R-19. Ventromedial hypothalamic nuclei; densely packed cell-body grouping in the dorso-medial aspects of the nucleus; observed effects: E(-), E(-), A(+), E(-). These time samples give a good demonstration of the ether and amphetamine effect upon spontaneous ventromedial hypothalamic nucleus unit activity. This was a slow firing unit (5/sec.) with only occasional bursting. After the ether test this unit remained silent for about one min. Part of the recovery of firing (4/sec.) is shown for the first 15 sec. of Sample 4. Suddenly, however, bursting commenced. Note two-spike bursts following each other in equal intervals; (cf., Fig. 3 for pre-spike or two-spike potential wave form.).

Figure 9. Unit 4: MA-33. Effects of ether and amphetamine on spontaneous unit activity of lateral hypothalamic area: a) 1/sec. time marker, b) unit activity, c) on-off test time, d) electrocardiograph.



1

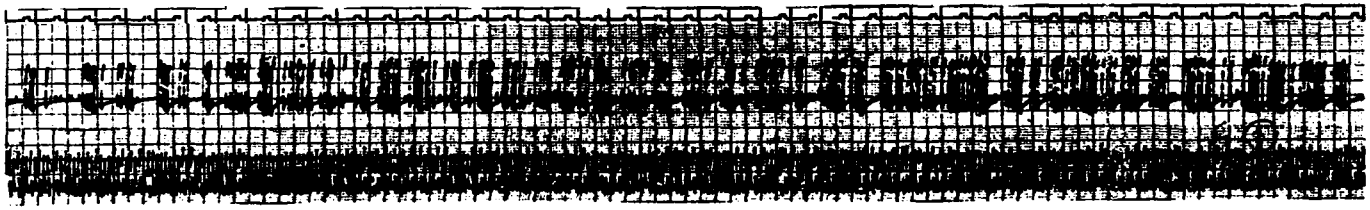
Saline



2

3

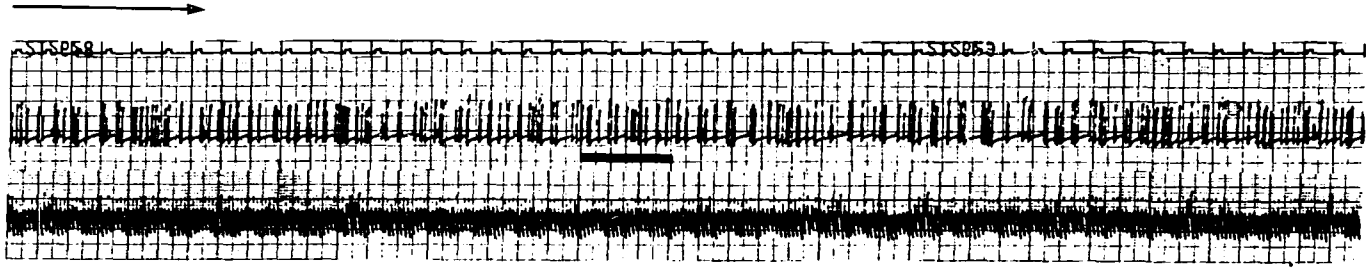
Amphetamine



4

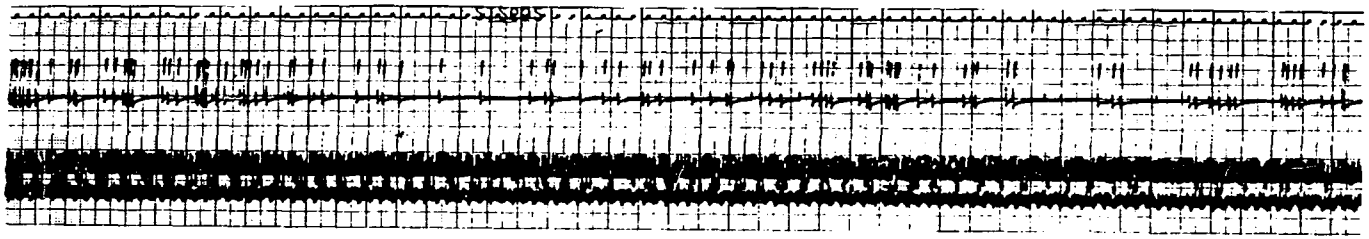
1

a
b
c
d

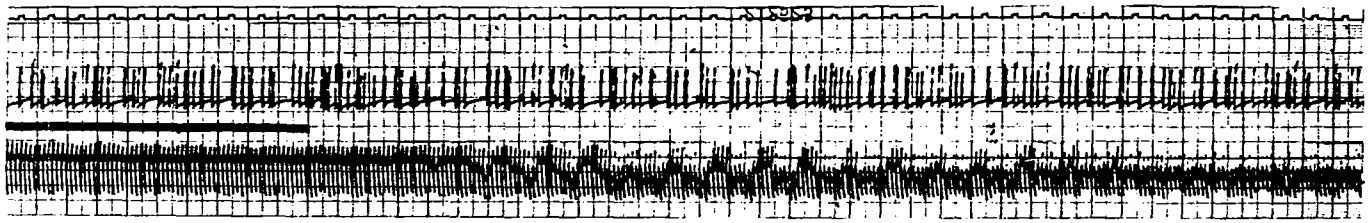


Saline

2



3



Amphetamine

4

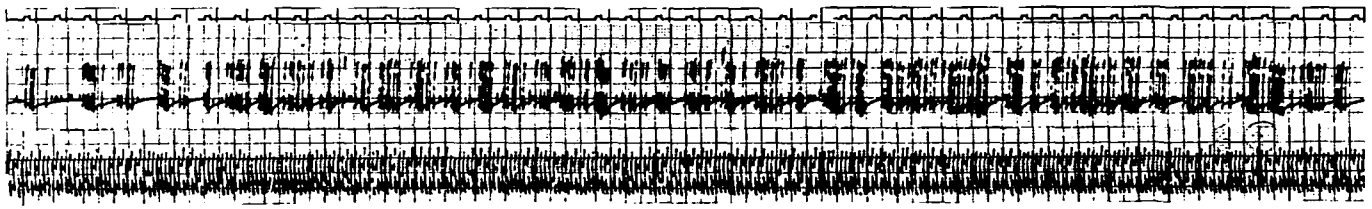


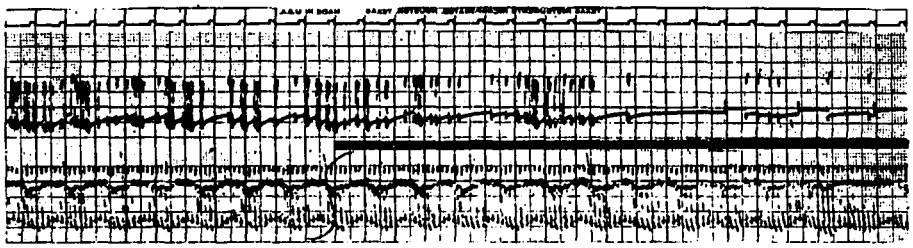
Figure 10. Unit 5: R-19. Effects of ether and amphetamine on spontaneous unit activity of ventromedial hypothalamic nuclei: a) 1/sec. time marker, b) unit activity, c) on-off test time, d) electrocardiograph.

1

a
b
c
d

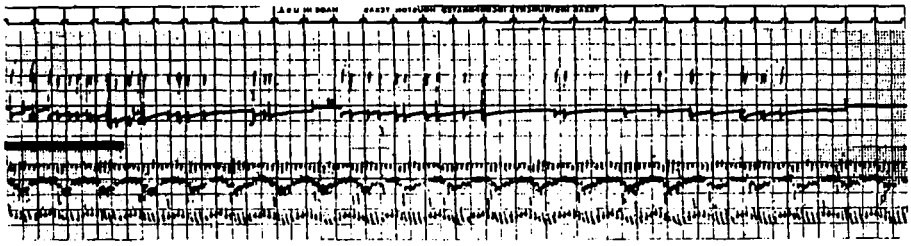
Saline

2

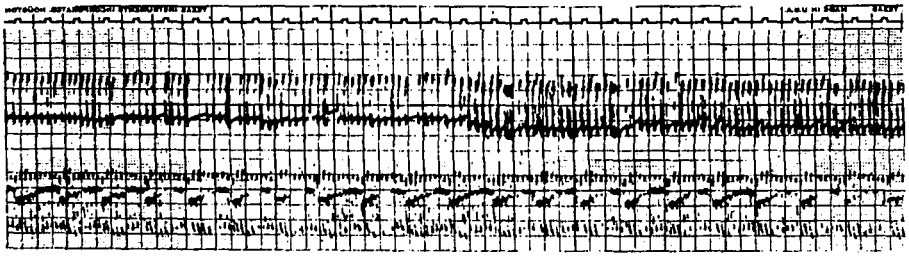


Ether

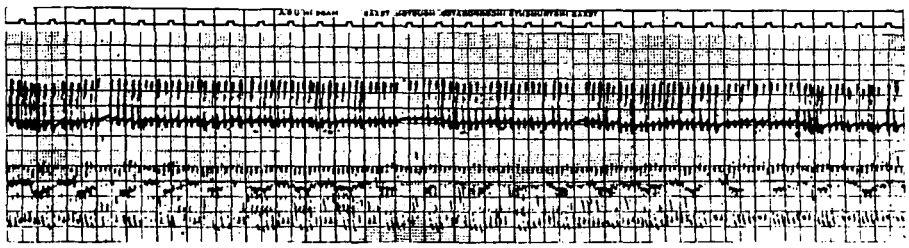
3



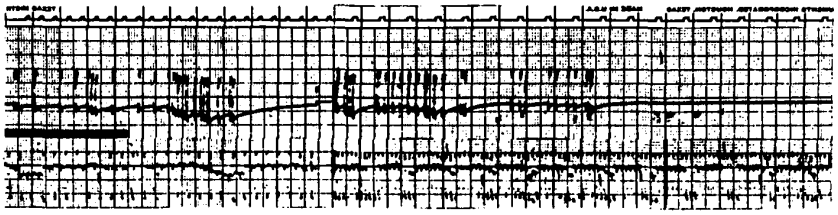
4



5

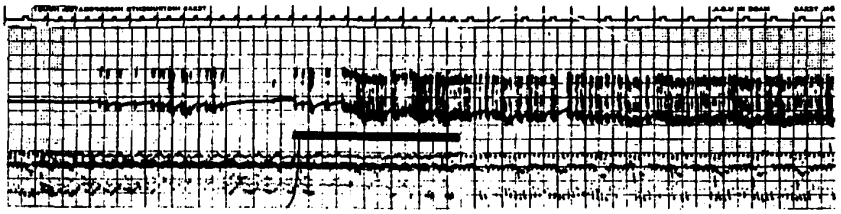


6



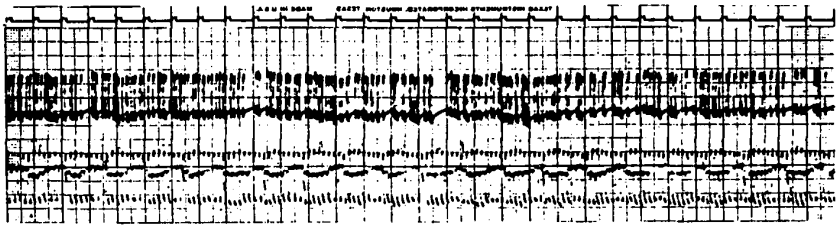
Ether

7

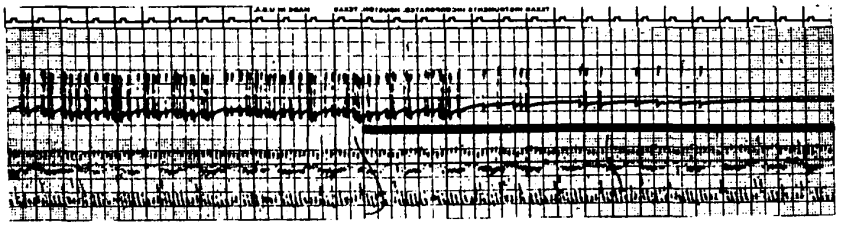


Amphetamine

8

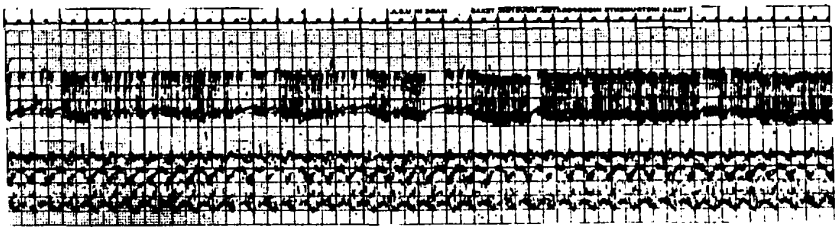


9



Ether

10



The fifth sample gives a good idea of regularly firing units in the hypothalamic areas under investigation. Sample 7 impressively demonstrates an amphetamine effect; the unit had not fully recovered from the inhibitory influence of ether when only 4 sec. after infusion of 2.5 mg/kg amphetamine led to a sharp increase in activity (7-10/sec.). Apparently ether was much more effective than in tests preceding amphetamine infusion; firing decelerated over a 9 sec. period and then was inhibited completely for about 4 min.

It might be mentioned again that only 5 mg/kg amphetamine given as 1.0 cc infusion resulted in transitory deceleration (7 units) before inducing the typical ventromedial hypothalamic nuclei unit firing rate increase (cf., R-34), which was observed to last from 5-40 min. Usually, however, as in this unit (also R-16), following a 2.5 mg/kg dose of amphetamine, short latency increase in unit activity was observed.

Unit 6: R-34. Ventromedial hypothalamic nuclei; densely structured cell-body grouping in the dorso-central aspects of the nucleus. Left track, Fig. 11; observed effects: E(-), A(+), E(-). This was a unit which fired irregularly at a rate of 16/sec. with bursts of 5-8 spikes. Sample 2 shows deceleration and brief cessation of firing following ether. Samples 5 and 6 show effects of amphetamine infusion upon spontaneous firing and cardiac output; note drastic amplitude changes in electrocardiograph. Following amphetamine (5 mg/kg) there was a 3 sec. inhibitory period after which unit firing rate accelerated to a bursting pattern (25/sec.). Following the repeated ether test (Sample 7), decrease in unit activity was noted but no clear inhibition for prolonged periods resulted.

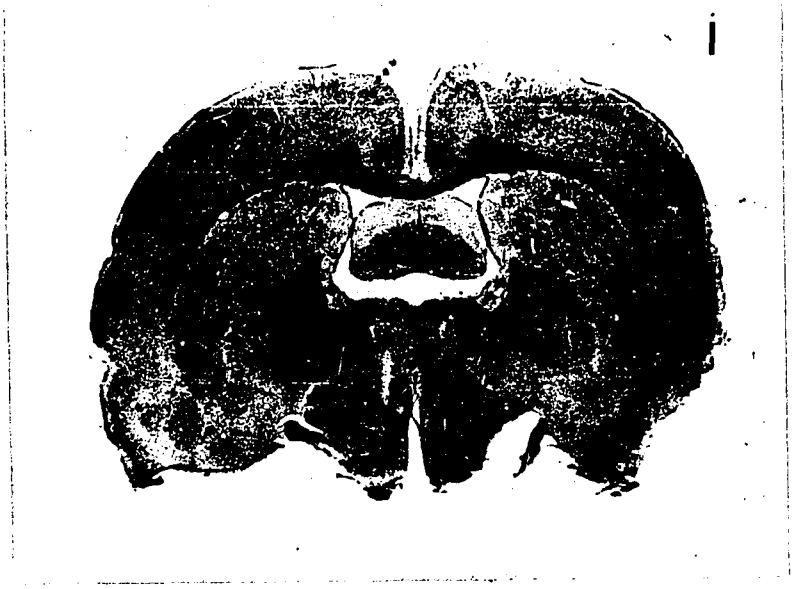


Figure 11. R-34. Ventromedial hypothalamic nuclei at the A 6.2 de Groot plane; unit recording was made from the left track ending in the dorsal-central portion of the densely structured nucleus.

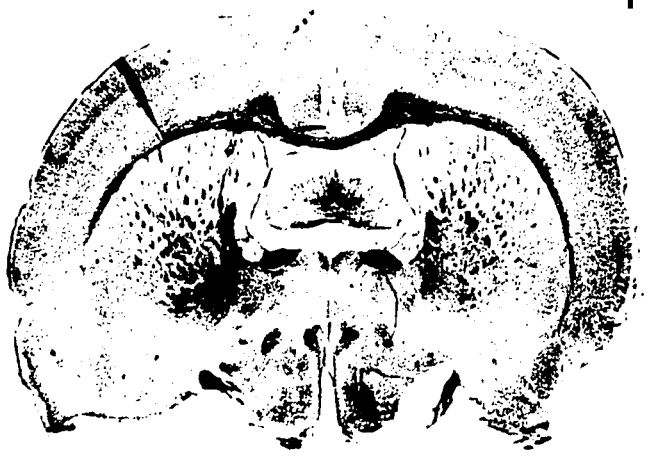
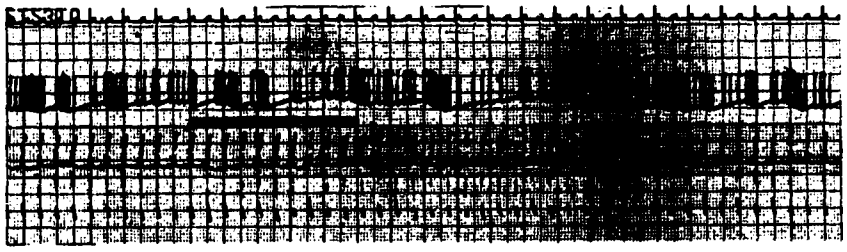


Figure 11. R-34. Ventromedial hypothalamic nuclei at the A 6.2 de Groot plane; unit recording was made from the left track ending in the dorsal-central portion of the densely structured nucleus.

Figure 12. Unit 6: R-34. Effects of ether and amphetamine on spontaneous unit activity of ventromedial hypothalamic nuclei: a) 6/sec. time marker, b) unit activity, c) on-off test time, d) electrocardiograph.

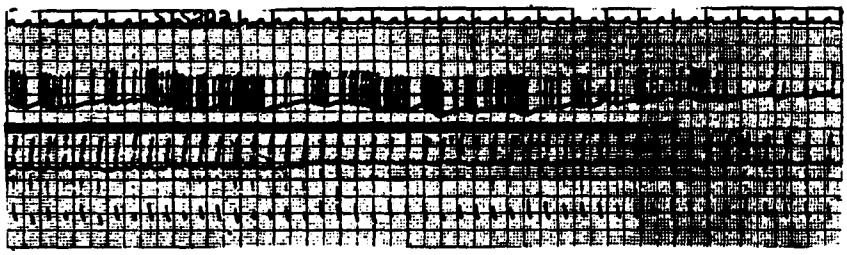
1

a
b
c
d



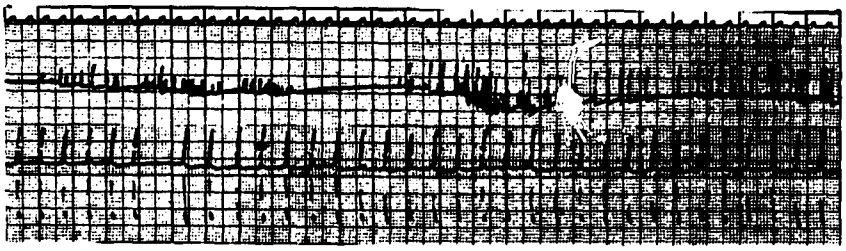
Saline

2

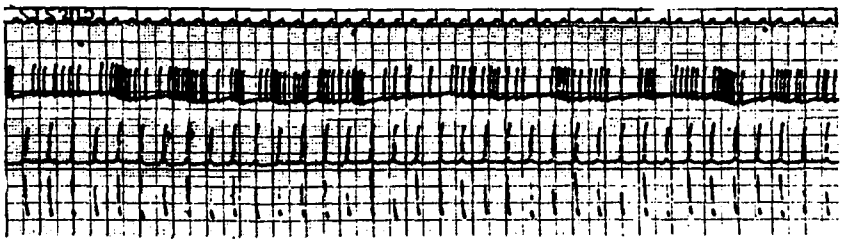


Ether

3



4



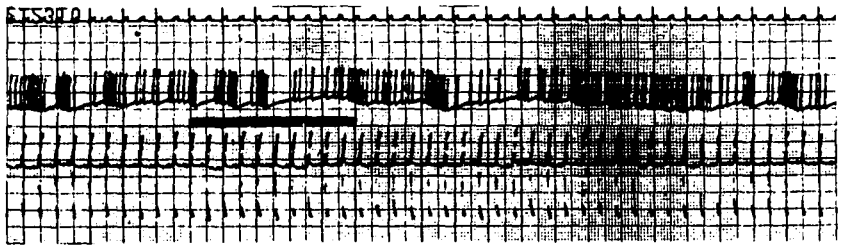
a

b

c

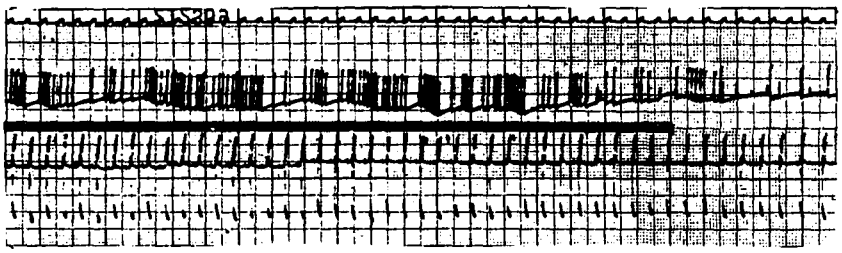
d

1



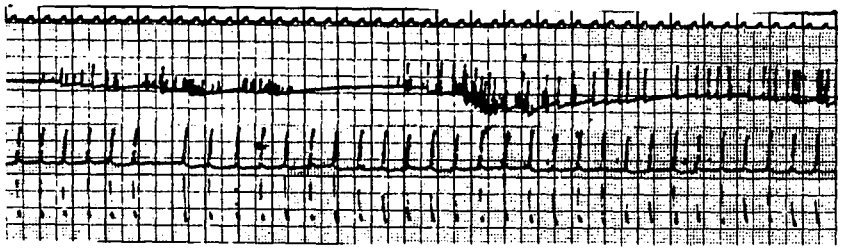
Saline

2

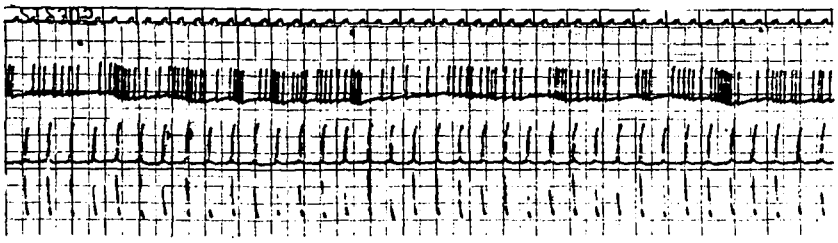


Ether

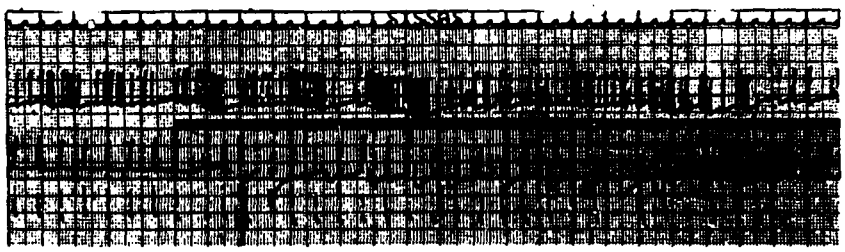
3



4

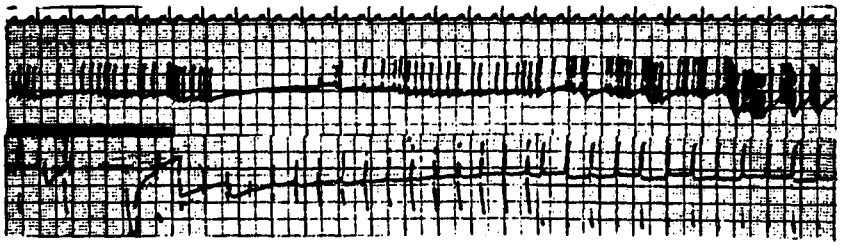


5

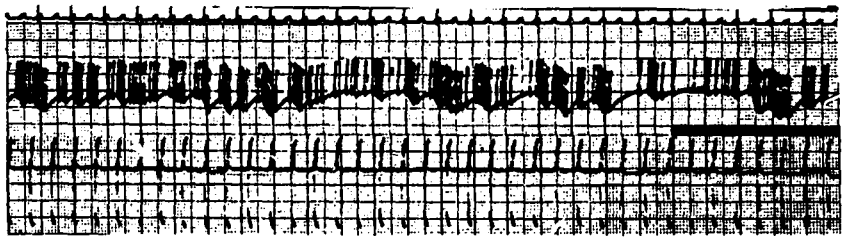


Amphetamine

6

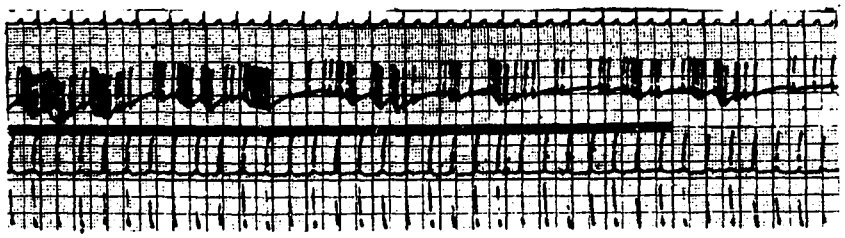


7

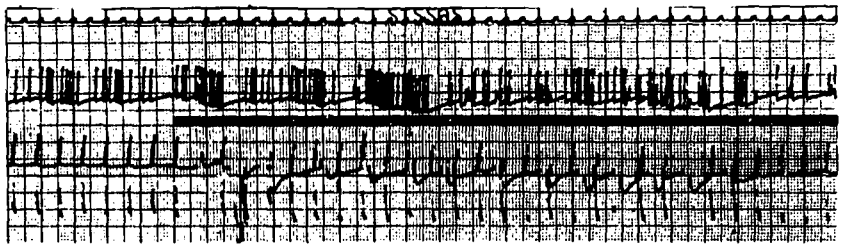


Ether

8

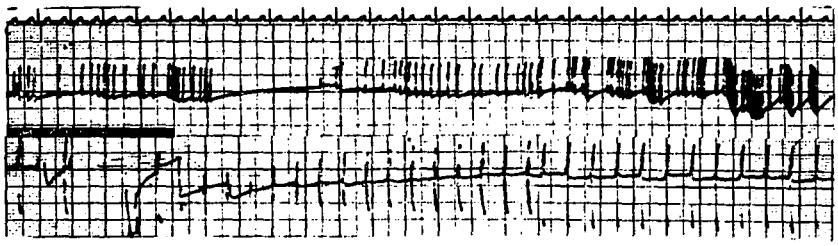


5

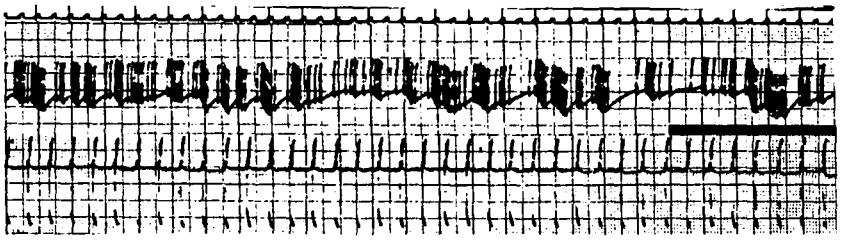


Amphetamine

6

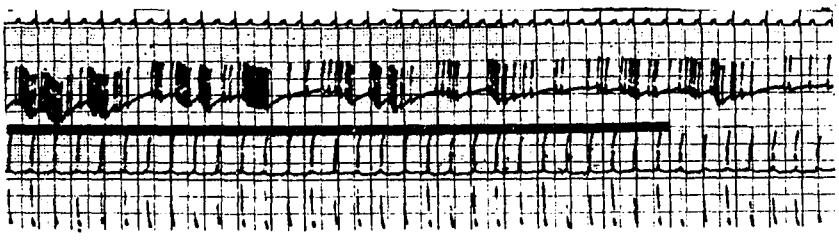


7



Ether

8



Unit 7: R-16. Ventromedial hypothalamic nuclei; central portion of nucleus; observed effects: E(-), A(+), E(-). Even very slow firing units in the hypothalamus can be shown to change firing characteristics following either ether or amphetamine or both. A continuous record of 213 sec. is provided for this unit firing 1/sec. to 1/2 sec. Following ether (Sample 2), the unit fired only 6 times during 60 sec. The third sample showed an increase in firing and the appearance of a high amplitude spike following a 0.1 cc loading of amphetamine (2.5 mg/kg); the remainder of the drug was given and the firing frequency increased enormously. The large spike disappeared and the previously observed spike stabilized at a rate of 20/sec. (Sample 5). Following ether, the unit remained silent for 68 sec.; as with R-19, ether became more effective after amphetamine infusion, leading now to termination of firing.

Unit 8: R-39. Ventromedial hypothalamic nuclei; lateral region bordering on lateral hypothalamic area; observed effects: E(-), A(+), E(-). This is a continuous recording of unit activity over a 7 min. period. This is a further, even if less dramatic, demonstration of an amphetamine effect on a slow firing unit [which was accidentally detected during the period preceding search for units; the microelectrode was left stationary in order to let tissue settle around the tip after more rapid traversal of hypothalamus]. Ether was given and Samples 1 and 2 show the recovery; the small spike is not the unit under consideration. Amphetamine (2.5 mg/kg) was given and at offset of infusion firing of 2/sec. ensued for 6 sec.; firing remained about 1/2 sec. and in Sample 5 increased to 1/sec. Some artifacts caused by muscle potential (encircled areas) were noted as the animal needed more Flaxedil

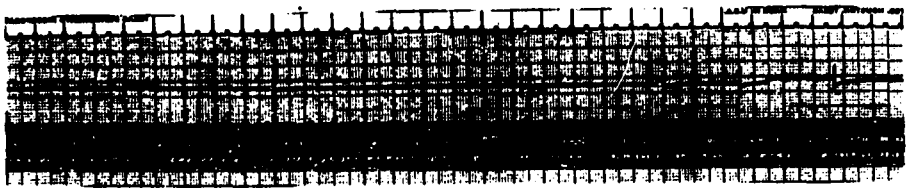
Figure 13. Unit 7: R-16. Effects of ether and amphetamine on spontaneous unit activity of ventromedial hypothalamic nuclei: a) 1/sec. and 6/sec. (for Samples 5 and 6) time marker, b) unit activity, c) on-off test time, d) electrocardiograph.

1

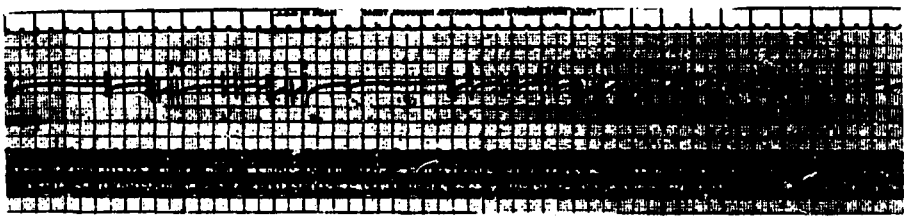
a
b
c
d

Ether

2



3

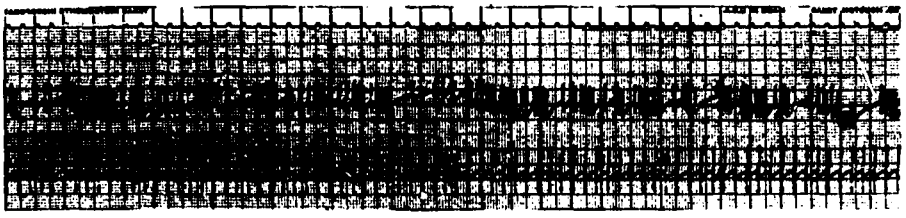


Amphetamine

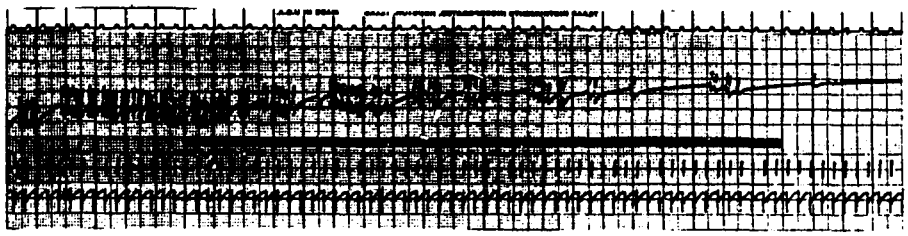
4

Amphetamine

5

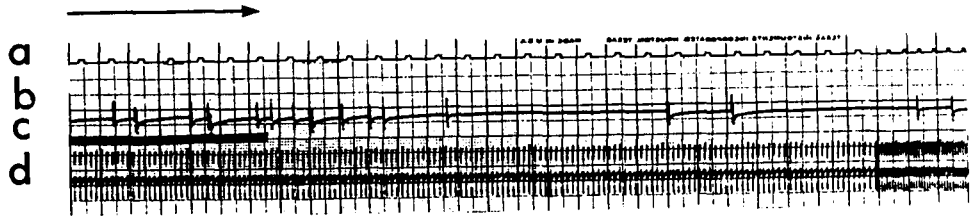


6



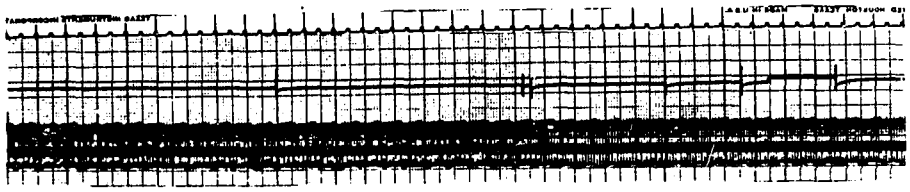
Ether

1

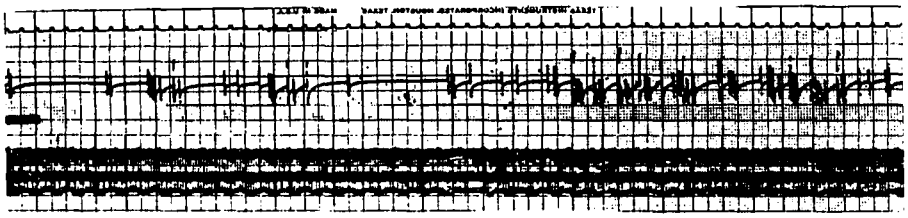


Ether

2

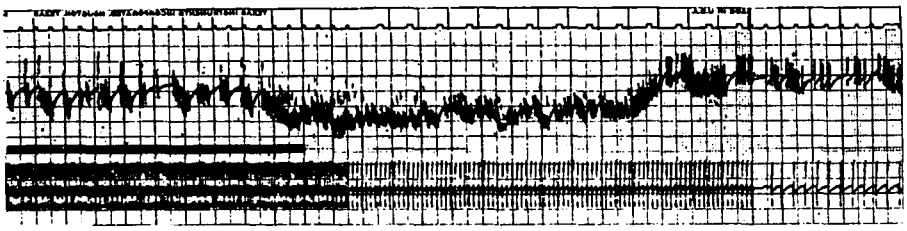


3



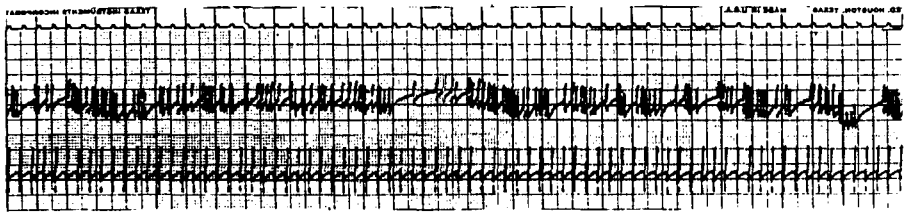
Amphetamine

4

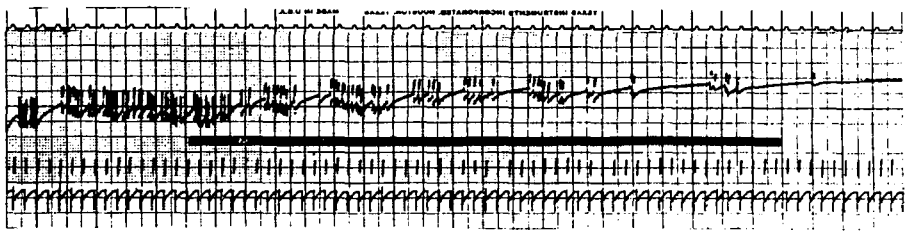


Amphetamine

5



6



Ether

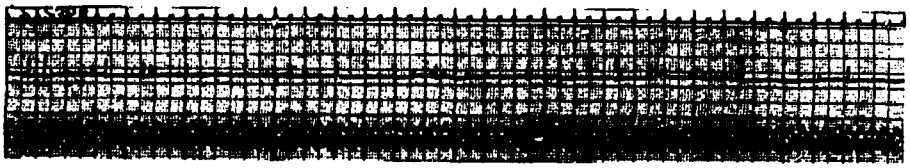
Figure 14. Unit 8: R-39. Effects of ether and amphetamine on spontaneous unit activity of ventromedial hypothalamic nuclei: a) 1/sec. time marker, b) unit activity, c) on-off test time, d) electrocardiograph.

1

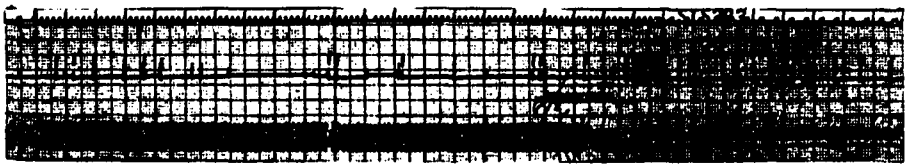
a
b
c
d

Ether

2



3

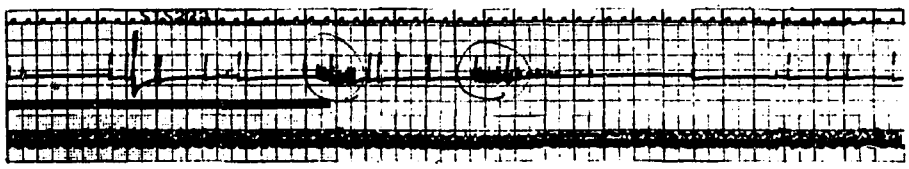


Amphetamine

4

5

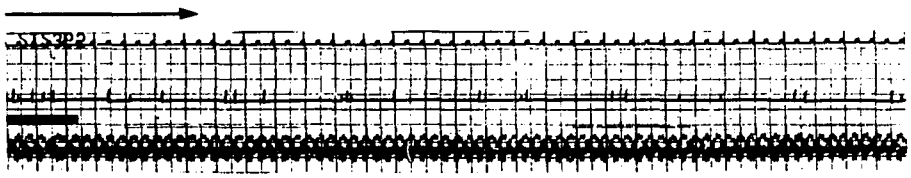
6



Ether

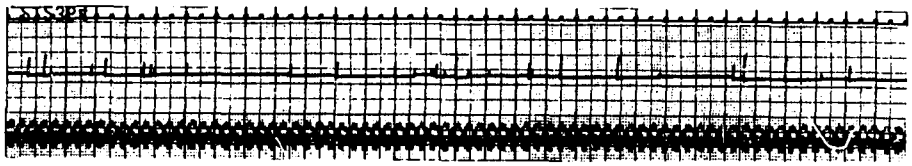
1

a
b
c
d

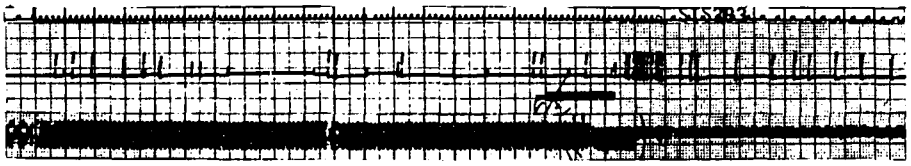


Ether

2

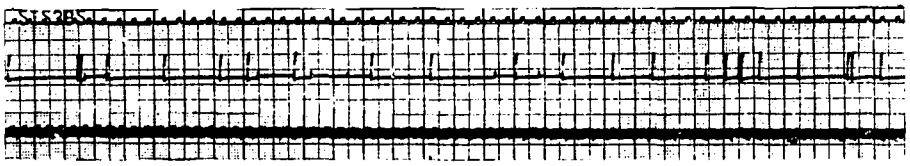


3

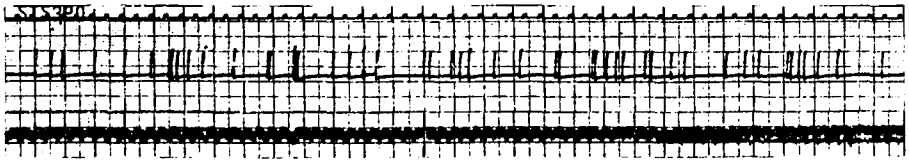


Amphetamine

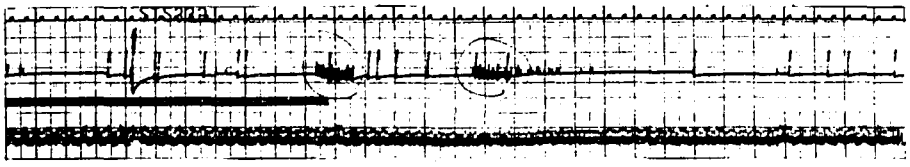
4



5



6



Ether

and ether caused reflexive neck musculature movement. The spike is still clearly seen, and, although the firing rate was reduced again (1/2 sec.), the unit was not inhibited altogether.

DISCUSSION

This investigation was concerned with the neural mechanisms by which amphetamine produces its anorexigenic effect. One of the views with which the present findings are consistent is that amphetamine produces its anorexigenic effect by enhancing neural activity in the nuclei of the ventromedial hypothalamus (Brobeck et al., 1956); the spontaneous firing rate of units in the ventromedial nuclei increased following administration of amphetamine. However, the present findings are also consistent with the view that the anorexigenic effects are produced by amphetamine's inhibitory action on the neural activity in the lateral hypothalamic area (Carlisle, 1964); the firing rate of many units in the lateral hypothalamus decreased following amphetamine infusion. The present investigation provides the first clear evidence that lateral hypothalamic area units can be inhibited by amphetamine.

Several further questions arise now. For example, is the observed inhibitory effect of amphetamine on the units of the lateral hypothalamic area mediated through the ventromedial hypothalamic nuclei? In other words, it could be that the primary effect of amphetamine is on the nuclei of the ventromedial hypothalamus, and that this increased neural activity then increases the normal inhibitory effect that ventromedial activity is presumed to have on the lateral hypothalamus. Two types of evidence are against such an interpretation. First, lesions in the

ventromedial hypothalamic nuclei do not disrupt the anorexigenic action of amphetamine. Second, no clear evidence of fibres projecting from the ventromedial nuclei to the lateral hypothalamic area exists at the present. However, these considerations cannot be decisive, and further work is clearly needed concerning the much debated interaction of these hypothalamic sites and their opposing effects on hunger-motivated behavior (Anand et al., 1961; Oomura et al., 1967). Further studies of this question could profitably use such techniques as the local placement of amphetamine by microelectrophoresis, and the simultaneous recording of unit activity from both ventromedial and lateral hypothalamic areas.

Another question raised by the present findings is this: are the observed effects of amphetamine on spontaneous unit activity of the ventromedial hypothalamic nuclei and the lateral hypothalamic area direct effects or mediated by still other structures? Incidental observations made in the course of the present investigation showed little effect of amphetamine on units in other cerebral structures, including some closely related hypothalamic structures. Therefore, it appears that other areas probably play a rather subordinate role, if any, in the mediation of the anorexigenic effect. However, more systematic investigation of the extent or exact distribution of cells that respond to amphetamine are needed. The fact noted in the present experiment that, in the lateral hypothalamic area, some units are inhibited whereas others are facilitated by amphetamine, and that they tend to lie in separate areas may provide a good starting point for such an investigation.

Ether was shown to inhibit the rate of firing of units in both the ventromedial and lateral hypothalamic areas. At the dose used, this

inhibitory effect was not seen in all the units in the lateral hypothalamus but was clearly evident in all units of the ventromedial nuclei. This suggests that the ventromedial units may be exceptionally sensitive to light anaesthesia. If this is so, it may be that this region of the hypothalamus may serve a general function in determining responsiveness to environmental stimuli and may not be as directly and uniquely implicated in eating behavior as is believed at present. Another point to note in connection with the effects of ether on unit activity is that it may or may not attenuate the facilitatory effects of amphetamine; hence, the use of anaesthetics appears to be an important variable in the preparation used in electrical recording studies.

SUMMARY

Amphetamine inhibits eating. In a search for the mechanism of this anorexigenic effect, the effects of d-amphetamine sulphate and ether on the spontaneous firing rate of single neuronal units in the ventromedial and lateral hypothalamic areas were studied in curarized rats. Amphetamine increased and ether decreased the firing rate of units in the ventromedial nuclei. Various lateral hypothalamic units were affected differently by these agents; amphetamine produced no change in some units, and a decrease or an increase in others; ether produced no change in some units and a decrease in others. These results are discussed in relation to two views, first that amphetamine anorexia arises from increased neural activity in the ventromedial hypothalamus, and second that it arises from a decrease in the lateral hypothalamic activity. While the evidence is not decisive, it clearly establishes the second view as a defensible alternative to the first.

Anand, B.K., & Brobeck, J.R. Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med., 1951, 24, 123-140.

Anand, B.K., & Dua, S. Feeding responses induced by electrical stimulation of the hypothalamus in the cat. Indian J. Med. Res., 1955, 43, 113-122.

Anand, B.K., Dua, S., & Singh, B. Electrical activity of the hypothalamic "feeding centres" under the effect of changes in blood chemistry. EEG clin. Neurophysiol., 1961, 13, 54-59.

Barraclough, C.A., & Cross, B.A. Unitary activity in the hypothalamus of the cyclic female rat: effect of genital stimuli and progesterone. J. Endocrin., 1963, 26, 339-359.

Brobeck, J.R., Larsson,S., & Reyes, E. A study of the electrical activity of the hypothalamic feeding mechanism. J. Physiol., London, 1956, 132, 358-364.

Brooks, D.C. Electrical activity of the ventromedial nucleus of the hypothalamus. Amer. J. Physiol., 1959, 197, 829-834.

Campbell, J.F., Krebs, H., & Bindra, D. Sensory responses of the hypothalamic ventromedial nucleus. Paper presented at the meeting of the Eastern Psychological Association, Washington, April, 1968.

Carlisle, H.J. Differential effects of amphetamine on food and water intake in rats with lateral hypothalamic lesions. J. comp. physiol. Psychol., 1964, 58, 47-54.

Carlisle, H.J., & Reynolds, R.W. Effect of amphetamine on food intake in rats with brain-stem lesions. Amer. J. Physiol., 1961, 201, 965-967.

Cole, S.O. Increased suppression of food intake by amphetamine in rats with anterior hypothalamic lesions. J. comp. physiol. Psychol., 1966, 61, 302-305.

Cole, S.O., & Hudspeth, W.J. Hypothalamic mediation of amphetamine anorexia. Paper presented at the meeting of the Western Psychological Association, Portland, April, 1964.

Cross, B.A., & Green, J.D. Activity of single neurons in the hypothalamus: effect of osmotic and other stimuli. J. Physiol., 1959, 148, 554-569.

Daniel, P.M. Blood supply of hypothalamus and pituitary gland. British Med. Bull., 1966, 22, 202-208.

de Groot, J. The rat forebrain in stereotaxic coordinates. Verh. Kon. Ned. Akad. Wet. B. Naturkunde, 1959, 2, 1-40.

Epstein, A.N. Suppression of eating and drinking by amphetamine and other drugs in normal and hyperphagic rats. J. comp. physiol. Psychol., 1959, 52, 37-45.

Frazier, D.T., Taquiny, C., Byarsski, L.L., & Wilson, M.F. Hypothalamic unit response to increases in arterial blood pressure. Proc. soc. exper. biol. and med., 1965, 120, 450-454.

Goodman, L.S. & Gilman, A. The pharmacological basis of therapeutics. (2nd ed.) New York: MacMillan, 1955.

Green, J.D. A simple microelectrode for recording from the central nervous system. Nature, Lond., 1958, 182, 962.

Green, J.D. & Morin, F. Hypothalamic electrical activity and hypothalamo-cortical relationships. Am. J. Physiol., 1953, 172, 175-186.

Gylys, J.A., Hart, JJ.D., & Warren, M.R. Chlorphentermine, a new anorectic agent. Fed. Proc., 1961, 20, 328.

Harris, S.C., Ivy, A.C., & Searle, L.M. The mechanism of amphetamine loss of weight. J. Amer. Med. Assoc., 1947, 134, 1468-1475.

Hoagland, H., Himwich, H.E., Campbell, E., Fazeka, J.F., & Hadidian, Z. Effects of hypoglycemia and pentobarbital sodium on electrical activity of cerebral cortex and hypothalamus (dogs). J. Neurophysiol., 1939, 2, 276-288.

Hubel, D.H. Tungsten microelectrode for recording from single units. Science, 1957, 125, 549-550.

Klüver, H. & Barrera, E.A. Method for the combined staining of cells and fibres in the nervous system. J. Neuropath. exp. Neurol., 1953, 12, 400-403.

Morgane, P.J. Evidence of a 'hunger motivational' system in the lateral hypothalamus of the rat. Nature, 1961, 191, 672-674.

Nathanson, M.H. The central action of benzedrine. JAMA, 1937, 108, 528-531.

Oomura, Y., Kimura, K., Ooyama, H., Maeno, T., Iki, M., & Kuniyoshi, M. Reciprocal activities in the ventromedial and lateral hypothalamic areas in cats. Science, 1964, 143, 484-485.

Oomura, Y., Ooyama, H., Yamamoto, T., & Naka, F. Reciprocal relationship of the lateral and ventromedial hypothalamus in the regulation of food intake. Physiol. Behav., 1967, 2, 97-115.

Pellegrino, L.J., & Cushman, A.J. A stereotaxic atlas of the hooded rat brain. Allan Memorial Institute, McGill University, 1966.

Reynolds, R.W. The effect of amphetamine on food intake in normal and hypothalamic hyperphagic rats. J. comp. physiol. Psychol., 1959, 52, 682-684.

Rothballer, A.B. The effects of catecholamines on the cerebral nervous system. Pharmacol. Rev., 1959, 11, 494-547.

Sharp, J.C., Nielson, H.C., & Porter, P.B. The effect of amphetamine upon cats with lesions in the ventromedial hypothalamus. J. comp. physiol. Psychol., 1962, 55, 198-200.

Stowe, F.R., Jr., & Miller, A.T., Jr. The effect of amphetamine on food intake in rats with hypothalamic hyperphagia. Experientia, 1957, 13, 114-115.

Van Orden, L.S., & Sutin, J. Differential effects of norepinephrine on responses evoked in the hypothalamic ventromedial nucleus. EEG clin. Neurophysiol., 1963, 15, 796-803.

Warembourg, H., & Jaillard, J. Experimentation clinique du S. 62-2. Lille Medical, 3e Serie, Anné 1964, Tome IX, 962-967.

APPENDIX A

Note 1

In the modified version of the de Groot system (Pellegrino & Cushman, 1966), the rostral-caudal zero reference point is the skull landmark at which the coronal suture crosses the sagittal suture (bregma). Thus at A 5.8 on the de Groot system, which would allow a ventromedial hypothalamic target placement in the largest portion of the nucleus (cf., Fig. 1), the modified system reference point (bregma) would be exactly 0.0. Hence, if the skull showed variations as to the coincidence of bregma and the A 5.8 coordinate, the latter would be disregarded. In turn, the depth of the microelectrode placements was measured both from the cortex and from the stereotaxic zero. Preference was given to cortical identification after it was established that cortex to ventromedial hypothalamic nucleus (dorsal aspects of nucleus) and cortex to lateral hypothalamic area distance was approximately 9.0 mm. and 7.5 mm., respectively. The mid-sagittal sinus was taken as the medial-lateral zero point.

Note 2

With some animals, methylphenidate (Ritalin) and chlorphentermine hydrochloride (Pre-Sate) (e.g., Gylys et al., 1965, Warembourg & Jaillard, 1964) were tried out. Methylphenidate given to the animal in high doses (usually double the amphetamine dosage normally given) produced results comparable with those of amphetamine which will be reported in the section headed Spontaneous General Characteristics of Unit Activity in Hypothalamic Areas (page 16). Chlorphentermine, which was reported to have only an anorexigenic effect without accompanying central nervous

system stimulation (Gylys et al., 1965), showed an inconsistent effect, but only four units in the ventromedial nucleus were sampled. These drugs were not systematically explored to any extent, however.

Table IA

MA-Series

Subject	Strain	Sex	Histology	Total Cells	Amphetamine		Ether		Saline
					Response	Cells	Response	Cells	
1	G	F	LHA	2	(-)	1	(0)	1	(0)
2	G	F	LHA	2	(-)	1	(0)	1	(0)
3	G	F	HVM	1	(0)	1	(0)	1	(0)
4	G	F	LHA	3	(0)	1	(0)	3	(0)
5	G	F	HVM	2	(+)	1	(-)	2	(0)
6	G	F	LHA	2	(-)	1	(0)	2	(0)
7	G	F	LHA	2	(0)	1	(-)	1	(0)
8	G	F	HVM	2	(0)	1	(0)	1	(0)
9	G	F	HVM	1	(+)	1	(-)	1	(0)
10	G	F	HVM	1	(+)	1	(-)	1	(0)
11	G	F	LHA	1	(+)	1	(-)	1	(0)
12	G	M	LHA	1	(-)	1	(0)	1	(0)
13	G	M	LHA	6	(0)	1	(0)	4	(0)
14	G	M	LHA	4	(-)	2	(-)	4	(0)
15	G	M	LHA	6	(+)	2	(0)	4	(0)
16	G	F	LHA	2	(+)	1	(-)	2	(0)
17	G	F	HVM	1	(0)	1	(-)	1	(0)
18	B	M	HVM	5	(+)	4	(-)	5	(0)
19	G	F	LHA	2	(-)	1	(-)	1	(0)
20	B	M	LHA	1	(+)	1	(0)	1	(0)

Table IA

MA-Series (Cont'd.)

Subject	Strain	Sex	Histology	Total Cells	Amphetamine		Ether		Saline
					Response	Cells	Response	Cells	
21	G	F	LHA	1	(-)	1	(0)	1	(0)
22	B	M	LHA	1	(-)	1	(0)	1	(0)
23	B	M	LHA	1	(0)	1	(0)	1	(0)
24	G	F	HVM	1	(+)	1	(-)	1	(0)
25	G	F	HVM	1	(+)	1	(-)	1	(0)
26	B	F	HVM	1	(+)	1	(-)	1	(0)
27	B	F	HVM	1	(+)	1	(-)	1	(0)
28	B	F	LHA	1	(0)	1	(-)	1	(0)
29	B	F	LHA	1	(-)	1	(-)	1	(0)
30	G	M	LHA	2	(+)	2	(0)	2	(0)
31	G	M	LHA	2	(-)	1	(+)	1	(0)
32	G	M	LHA	1	(0)	1	(0)	1	(0)
33	B	F	LHA	1	(+)	1	(-)	1	(0)
34	B	F	LHA	1	(0)	1	(0)	1	(0)
35	B	F	LHA	1	(+)	1	(-)	1	(0)
36	B	M	LHA	1	(0)	1	(0)	1	(0)
					R-Series				
11	B	F	LHA	1	(+)	1	(0)	1	(0)
12	G	M	HVM	4	(+)	1	(-)	3	(0)
13	G	M	LHA	2	(0)	1	(0)	1	(0)

Table IA

R-Series (Cont'd.)

Subject	Strain	Sex	Histology	Totai Cells	Amphetemine Response	Amphetemine Cells	Ether Response	Ether Cells	Saline
14	B	M	HVM	2	(+)	1	(-)	1	(0)
15	B	M	LHA	3	(-)	2	(0)	1	(0)
16	B	F	HVM	4	(+)	1	(-)	3	(0)
17	G	M	HVM	8	(+)	1	(-)	8	(0)
18	B	M	HVM	5	(+)	2	(-)	2	(0)
19	G	M	HVM	1	(+)	1	(-)	1	(0)
20	B	F	HVM	4	(+)	2	(-)	3	(0)
21	B	M	HVM	3	(+)	2	(-)	1	(0)
22	G	F	HVM	1	(+)	1	(0)	1	(0)
23	G	M	HVM	2	(+)	1	(0)	1	(0)
24	G	M	HVM	5	(+)	3	(-)	2	(0)
25	G	M	HVM	4	(+)	1	(-)	3	(0)
26	G	M	HVM	5	(+)	2	(-)	2	(0)
27	B	F	HVM	8	(+)	1	(0)	4	(0)
28	B	F	HVM	4	(+)	2	(-)	2	(0)
29	G	M	HVM	4	(+)	1	(-)	2	(0)
30	B	M	HVM	1	(+)	1	(-)	1	(0)
31	B	M	LHA	2	(0)	2	(0)	2	(0)
32	B	M	LHA	1	(-)	1	(0)	1	(0)
33	B	M	LHA	3	(+)	1	(0)	2	(0)

Table IA

R-Series (Cont'd.)

Subject	Strain	Sex	Histology	Total Cells	Amphetamine		Ether		Saline
					Response	Cells	Response	Cells	
34	G	M	HVM	2	(+)	1	(-)	2	(0)
35	G	M	HVM	2	(+)	1	(-)	1	(0)
36	G	M	HVM	3	(+)	2	(-)	3	(0)
37	B	M	HVM	2	(+)	1	(-)	2	(0)
38	B	F	HVM	1	(+)	1	(-)	1	(0)
39	B	F	HVM	1	(+)	1	(-)	1	(0)
40	B	F	HVM	1	(+)	1	(-)	1	(0)
41	B	M	HVM	1	(+)	1	(-)	1	(0)
42	B	M	HVM	1	(+)	1	(-)	1	(0)
43	B	M	HVM	1	(+)	1	(-)	1	(0)
44	B	M	LHA	1	(0)	1	(0)	1	(0)
45	B	F	HVM	1	(+)	1	(-)	1	(0)
46	B	F	LHA	1	(0)	1	(0)	1	(0)