1976

Neural Structures Mediating Water Intake To Cellular And Extracellular Thirst Stimuli

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NEURAL STRUCTURES MEDIATING WATER INTAKE

TO CELLULAR AND EXTRACELLULAR THIRST STIMULI

by

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Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Ontario

July, 1976

ABSTRACT

Previous work has shown that drinking behavior is an important regulatory response to depletion of the cellular or of the extracellular body fluid compartment. The purpose of the present study was to investigate which neural structures and pathways mediate water intake to cellular and extracellular stimuli of thirst.

Rats were tested for drinking following central or peripheral administration of various thirst challenges. Single neuron microelectrode recording and electrolytic lesioning techniques were then used to investigate which neural pathways subserved the elicited response.

Microinjection of angiotensin-II (a hormonal mediator of extracellular thirst) to the preoptic region elicited copious water intake and in subsequent acute recording experiments was found to influence the discharge rate of neurons in the midlateral hypothalamus and in the paramedial midbrain tegmentum. Small lesions of the midlateral zone of the lateral hypothalamus attenuated water intake induced by preoptic administration of angiotensin-II or by peripherally injected isoproterenol (a β-adrenergic agonist which causes increased angiotensin-II biosynthesis) or renin. Lesions of the midlateral hypothalamus or of the paramedial rostral midbrain had no effect on drinking

elicited by central or peripheral administration of cellular
stimuli of thirst (hypertonic NaCl and sucrose). Lesions
made further lateral in the hypothalamus, which destroyed
the ventromedial part of the internal capsule and globus
pallidus, produced a marked decrease in water intake
elicited by centrally or peripherally administered hyper-
tonic NaCl or sucrose without significantly attenuating
drinking to extracellular thirst stimuli. It is concluded
that separate lateral hypothalamic and midbrain pathways
mediate cellular and extracellular thirst.

Drinking behavior was also initiated by the micro-
injection of angiotensin-II into the subfornical organ.
In order to investigate whether water intake induced by
administration of angiotensin-II to subfornical organ and
preoptic region is mediated by the same neural pathways,
a series of lesion experiments was done. Lesions of the
subfornical organ attenuated, but did not abolish,
drinking elicited by the infusion of angiotensin-II through
a cannula chronically implanted in the jugular vein or
after peripheral injections of renin. Subsequently, it
was found that lesions of the midlateral hypothalamus
or of the paramedial rostral midbrain attenuated water
intake elicited in response to angiotensin-II administered
to the preoptic region but had no effect on drinking when
the hormone was microinjected into the subfornical organ.
The differential effects of these lesions suggest that

multiple neural pathways are involved in the dipsogenic
response to centrally administered angiotensin-II.

v

ACKNOWLEDGEMENTS

It is with pleasure and sincere gratitude that I acknowledge the many contributions of my supervisor and chief advisor, Dr. Gordon Mogenson, to this thesis and to the experimental work upon which it is based.

I wish also to thank Dr. Franco Calaresu, Dr. Dick Weick and Miss Blanche Box, who, importantly, taught me by example as well as by words.

Many other persons made this an enjoyable and valuable exercise in learning. I would like to thank:

- Drs. Vernon Brooks, David Cooke, Paul Mercer, and Mario Wiesendanger, who served ably and conscientiously, as members of my advisory committee.

- Vince Nicol, Wally Smyth, Bill Bottom, Becky Woodside, Marilyn Allen and the late Bill Luke for their friendship and assistance.

- Former and current members of Prof. Mogenson's laboratory for their comradeship, collaboration and advice.

- Jean Weick for so ably deciphering my writing and skillfully typing this thesis.

- Liz and Janet for caring enough to put up with me when I was selfish or obnoxious and for encouraging me when I was confused or unhappy. And for other things.

Finally, for the contribution of my parents and my brother for which no single expression of gratitude would ever suffice; nevertheless, I thank you.

TABLE OF CONTENTS

* * * * *

LIST OF TABLES

x

LIST OF FIGURES

To

my parents

and my teachers.

1.0 INTRODUCTION

Even though scientific interest in the physiology of thirst dates back to the early 1700's, only since the development of the stereotaxic technique in the 1930's has it become possible to study how the central nervous system participates in the control of water intake. It is now thought that drinking behavior is regulated by a complex neurological system which receives, transmits, and processes multiple neural and hormonal signals that arise in response to a loss of water from each of the major body fluid compartments. By acting to initiate water intake in response to body fluid depletions, the nervous system contributes to the preservation of homeostasis.

The early ablation and stimulation studies implicated the lateral hypothalamus as the neural "integrating center" for the body fluid deficit signals which evoke thirst, but more recently the attention has shifted to consider other brain structures and the chemo-specific neural pathways which link them together with the hypothalamus both anatomically and functionally. The present study is concerned with elucidating which lateral hypothalamic and limbic pathways mediate drinking behavior elicited by cellular and extracellular stimuli of thirst.

In the first section of this thesis the results of investigations which illustrate the importance of water intake to the maintenance of body fluid balance are

briefly reviewed. Section 1.2 considers studies which demonstrate that the hormone, ANG-II, is an important centrally acting mediator of extracellular thirst. A review of the anatomical and physiological studies of neural structures which mediate cellular and extracellular thirst is given in Section 1.4 and the Introduction is concluded with a general statement of the problem under investigation.

1.1 Water Intake as a Factor in Body Fluid Homeostasis

Body water, distributed between the cellular (ICF) and extracellular (ECF) fluid compartments is maintained within precise limits by a coordination of renal mechanisms of excretion and through water ingestion. Although the renal regulatory mechanisms are efficient in eliminating surfeits of body water and can promote water conservation under conditions of limited availability, only the ingestion of water will correct for fluid deficits. Moreover, when relying exclusively on renal systems, animals suffer from an accompanying loss of electrolytes and negative fluid and electrolyte balances are realized. On the other hand, the behavioral adjustments to fluid deficits are rapid and precise (cf. Fitzsimons, 1972).

Cellular Dehydration as a Stimulus for Thirst

The question of whether cellular dehydration or an increase in cellular osmotic pressure is a stimulus of thirst was in much dispute until 1937 when Gilman demon-

strated that dogs drank twice as much water following in-
fusions of hypertonic NaCl, which dehydrates cells, as they
did following infusions of equiosmotic urea, which does
not. Gilman's pioneering work was confirmed and clarified
by Holmes and Gregerson (1950) who found that drinking
activity was initiated only by solutions excluded from the
cell interior and not by changes in serum Na^+ or Cl^- levels.

The quantitative relationship between cellular dehy-
dration and water intake remained unclear until the early
1960's when Fitzsimons (1961) found that the volume of
water ingested by bilaterally nephrectomized rats given
injections of hypertonic solutions was precisely that
needed to attain body fluid isotonicity.

The mechanisms controlling urinary water losses via
antidiuretic hormone (ADH) from the neurohypophysis (Verney,
1947) and those regulating water intake operate syner-
gistically in most conditions to maintain cellular fluid
volumes. A 1-2% decrease in the volume of the ICF has been
reported to initiate drinking in dog and man (Wolf, 1950)
and in intact and nephrectomized rats (Fitzsimons, 1963)
and to elicit antidiuresis (Verney, 1947).

Extracellular Dehydration as a Stimulus for Thirst

There appear to be many bodily mechanisms for ensuring
the constancy of the ECF. The multiplicity of control
systems for this purpose is not surprising since the
volume and pressure of the intravascular compartment must be

maintained to sustain life. The mechanisms include those participating in the regulation of fluid exchange at the capillary involving the Starling forces. Controls intrinsic to the circulatory system such as the adjustment of cardiac output to venous return, the autoregulation of blood flow in peripheral tissues, and the direct effects of arterial pressure in glomerular filtration and urinary output appear to be important. There are also cardio-vascular reflexes involving the autonomic nervous system, adrenal medullary secretions, and other vasomotor controls. Finally, there are various endocrine influences on volume regulation which will be considered in Section 1.2.

Verney (1947) first demonstrated the influence of ADH on renal water loss and suggested that it was controlled by a central osmoregulatory system. However, additional controls for ADH secretion were sought because decreased urine output was also found to accompany Na^+ depletion and adrenal insufficiency, despite the loss of osmotic material (Holmes and Gregerson, 1950). Subsequently, specific volume regulatory mechanisms were implicated by reports of increased water intake and ADH levels following the isotonic depletion of plasma fluids (Fitzsimons, 1961; Stricker, 1966).

Experimentally, relative dehydration of ECF volume can be produced by injecting a hyperoncotic colloid such as polyethylene glycol into the peritoneal cavity (Fitz-simons, 1961b). This causes ECF to sequester in the

peritoneum by a Starling mechanism without any concurrent effect on body fluid osmolality. Stricker (1966, 1968) has demonstrated that intravascular hypovolemia and drinking are positively correlated with the concentration of colloid administered.

Endocrine mechanisms important in the control of renal function also have a direct influence on the intake of water and salt. It is the participation of one of these hormonal systems, renin-angiotensin, in mechanisms of extracellular thirst that is of particular interest to this study and which will be discussed next.

1.2 The Renin-Angiotensin System

Ever since the demonstration by Page and Helmer (1940) and Braun-Menendez et al. (1940) that renin exerts its biological actions by producing a smaller peptide, angiotensin-II (ANG-II), interest in the physiology and pharmacology of renin-angiotensin has grown considerably. A great impetus for further studies was the identification of the structure of angiotensin-I and ANG-II (Elliot and Peart, 1956; Lentz et al., 1956) and its synthesis by Bumpus et al. (1957). The availability, since then, of pure synthetic ANG-II (5-Valine-angiotensin-II; Hypertensin, CIBA) and more recently of naturally occurring rat ANG-II (5-Isoleucine angiotensin-II) has facilitated experimentation.

ANG-II is now known to exert an effect on many body tissues. Effects on smooth muscle have been reported for blood vessels (Khairallah *et al.*, 1966; Napodano *et al.*, 1962), gut (Bisset and Lewis, 1962; Regoli and Vane, 1964; Blair-West and McKenzie, 1966), uterus (Bumpus *et al.*, 1961; Khairallah *et al.*, 1962), and vas deferens (Benelli *et al.*, 1964). Effects on epithelial tissue have been seen in aldosterone secreting adrenal cortex (Davis, 1965 and 1971; Genest *et al.*, 1965) and on renal tubules to promote Na^+ reabsorption (Leyssac, 1965; Vander, 1967; Munday *et al.*, 1971). ANG-II also has several actions on the autonomic nervous system: (a) it inhibits the uptake of catecholamines by sympathetic nerve endings (Peach *et al.*, 1969); (b) it has a direct stimulatory effect on cholinergic ganglionic cells (Lewis and Reit, 1965); (c) it accelerates the rate of biosynthesis of norepinephrine in sympathetically innervated tissues (Boadle *et al.*, 1969); (d) it exerts a very marked pressor effect by a direct myotropic action (Regoli *et al.*, 1974) and indirectly by stimulating alpha adrenergic receptors (Smookler *et al.*, 1966; Buckley, 1972; Cryssanthou *et al.*, 1972). It also acts directly on the central nervous system to produce a generalized vasoconstriction (Hayden and Targett, 1971; Severs *et al.*, 1973). Finally, ANG-II exerts an extremely potent dipsogenic effect on the CNS, possibly by acting on chemosensitive receptors in the

brain (Fitzsimons, 1972). This effect on water intake will be discussed later in this part of the section.

Structure of ANG-II

A review of the pharmacological studies of ANG-II is beyond the scope of this section. There are several papers on this topic (Page and Bumpus, 1961; Page and McCubbin, 1968; Khairallah, 1971; Regoli *et al.*, 1974), one of which (Regoli *et al.*, 1974) gives a comprehensive summary of ANG-II analogues and their various actions.

ANG-II is an octapeptide which differs from one animal species to another in the composition of the fifth amino acid. For the rat, the amino acid sequence is: 1 - Asparagine -- 2 - Arginine -- 3 - Valine -- 4 - Tyrosine -- 5 - Isoleucine -- 6 - Histidine -- 7 - Proline -- 8 - Phenylalanine. Regoli *et al.* (1974) have shown that phenylalanine at the carboxyl position is required for the stimulatory effects of ANG-II. Thus replacing 8-phenylalanine by another amino acid produces a specific competitive antagonist (Regoli *et al.*, 1974). One such antagonist is 1-sarcosine-8-alanine angiotensin-II or P113. P113 has been found to block effectively the hypertensive effect of systemically administered ANG-II (Pals *et al.*, 1971), aldosterone secretion (Johnson and Davis, 1973), and peripheral catecholamine release (Regoli *et al.*, 1974). More recently, the antagonist has been used effectively to block water intake elicited by systemic

(Tang and Falk, 1974) and central (Cooling and Day, 1973; Epstein *et al.*, 1974) injections of ANG-II.

Biosynthesis of ANG-II

The *in vivo* biosynthesis of ANG-II is accomplished by the action of renin, a proteolytic enzyme secreted by specialized cells of the renal tubules, on its substrate angiotensinogen (Davis and Freeman, 1976). Page and Bumpus (1961) have shown that the plasma substrate is an $\alpha 2$ globulin of hepatic origin and that the initial product of its lysis by renin is a decapeptide, angiotensin-I. Angiotensin-I, in turn, acts as a substrate for a specific Cl-activated carboxypeptidase ("converting enzyme") which removes histidine and leucine from the carboxyl end to form ANG-II (Page and Bumpus, 1961). Although lung is believed to be the primary site for converting enzyme activity, it is also present in kidney, posterior pituitary, testis, plasma, brain, and other tissues (Erdös, 1975). ANG-II is enzymatically degraded by several plasma angiotensinases to biologically inactive short chain peptides (Lee, 1969).

The rate limiting event in the formation of ANG-II is believed to be the release of renin from the juxtaglomerular cells at the hilus of the afferent and efferent arterioles of the kidney (Vander, 1967). Most recent investigations of the factors controlling renal renin

release have been concerned with two possible mechanisms. These proposals have been labelled the baroreceptor and macula dense theories. Both theories propose that detection of the stimulus for renin release occurs in the kidney itself; however, neither theory can explain all of the experimental observations (Lee, 1969).

The baroreceptor theory states that the modified smooth muscle cells of the juxtaglomerular apparatus are sensitive to the degree of stretch in the wall of the afferent arteriole. Increased blood pressure stretches the cells and inhibits renin release, whereas decreased arteriolar pressure reduces cell distention and increases renin release (Vander, 1967). The baroreceptor theory adequately describes increased renin release under conditions of acute hemorrhagic hypotension (Brown et al., 1966; McKenzie et al., 1966) or acute systemic hypotension (Hamilton and Collins, 1942; Bunag et al., 1966). However, it fails to explain the elevated renin levels not accompanied by decreased arteriolar pressure in acutely or chronically salt-deprived animals (Gross et al., 1965; Page and McCubbin, 1968).

The macula densa theory (cf. Davis and Freeman, 1976) proposes that renal renin release is controlled by changes in the Na^+ load to the macula densa cells of the kidney. When Na^+ load is increased, renin release is decreased, and vice versa (Dirks et al., 1965; Gross et al., 1965; Vander, 1967; Davis, 1971; Davis and Freeman, 1976).

However, the macula densa theory does not appear to be able to account for increased renin levels produced by administration of potent natriuretic drugs such as chlorothiazide (Binion et al., 1965; Vander and Luciano, 1967), furosemide (Frazer et al., 1965) and ethacrynic acid (Meyer et al., 1966), since the macula densa Na^+ load is increased during the diuresis (Page and McCubbin, 1968). Renin has also been found in brain of dogs (Ganten et al., 1971a), rats (Fischer-Ferraro et al., 1971), in the pineal and pituitary glands of rats (Haulica et al., 1974), sub-maxillary gland of mice (Menzie et al., 1974), the uterus and placenta of rabbits (Gross et al., 1964), liver and adrenal gland of dogs (Ganten et al., 1970) and plasma and vascular beds of nephrectomized animals (cf. Laragh and Sealey, 1973). Haulica et al. (1974) have found that renin release in pineal and pituitary glands of rats was reduced following intravenous injection of hypertonic NaCl solution, suggesting that renin of cerebral origin may play a role in the regulation of Na^+ balance. Ganten, Boucher and Genest (1971) have observed a significant nega-tive correlation between brain iso-renin and brain tissue water, sodium, potassium, calcium and magnesium. They suggested that ANG-II formed locally may be involved in the regulation of water and electrolyte balance.

Reflex neural mechanisms may also influence renin release. Vander (1965,1967) has shown that increased sympa-

thetic activity in renal nerves can alter renin release in response to changes in blood volume. Hodge *et al.* (1966) have further found that the increase in plasma renin levels following an induced local hemorrhage can be prevented by anesthesia of the renal nerves. These results indicate that the sympathetic nervous system can influence renin release; the more important question is whether the renal nerves actually participate in the physiological regulation of renin release. It is known, for example, that renin can be produced by denervated kidneys (Fitzsimons, 1972).

There are other factors that may influence renin release. These include changes in potassium balance (Sealey *et al.*, 1970), hormonal feedback of ANG-II (Blair-West *et al.*, 1971; Shade *et al.*, 1973), changes in ADH levels (Vander, 1967; Shade *et al.*, 1973) and administration of certain prostaglandins (cf. Share and Claybaugh, 1972).

ANG-II and Extracellular Thirst

The involvement of the kidney in the control of water intake has been suspected for a long time. Renal diseases have been associated with irregularities in water intake (Brown *et al.*, 1969) and a "thirst hormone" of renal origin was postulated more than two decades ago (Linazasoro *et al.*, 1954).

More recently, a series of experiments by Fitzsimons (1961, 1964, 1966, 1969) has established the renin-angio-

tensin system as the endocrine link-between reduced ECF
volume and water intake. Fitzsimons (1969) observed that
ligation of the inferior vena cava distal to the liver is
a less effective stimulus to elicit drinking in a
nephrectomized rat than in the normal. Moreover, constric-
tion of the abdominal aorta above but not below the renal
arteries was found to induce drinking (Fitzsimons, 1964).
These findings suggested that the kidneys release renin
which may initiate water intake. Subsequent experiments
(cf. Fitzsimons, 1972) identified the factor mediating
drinking as ANG-II, and Fitzsimons and Simons (1969)
demonstrated that intravenous infusions of ANG-II induce
rats in normal water balance to seek out and ingest water.
The dipsogenic activity of systemically administered renin,
angiotensin-I and ANG-II was confirmed by studies in the
rat (Epstein, 1972; Hsiao and Epstein, 1973; Epstein and
Simpson, 1974; Tang and Falk, 1974), cat (Cooling and Day,
1974), and sheep (Abraham et al., 1975). Consistent with
the hypothesis of Fitzsimons are reports that the onset
of drinking elicited by the administration of beta-
mimetic drugs such as isoproterenol in the rat (Lehr et.
al., 1967), cat (Cooling and Day, 1975), and dog (Fitz-
simons and Szczepanska-Sadowska, 1973) is temporally
related to the increase in plasma renin activity (Meyer
et al. 1973) and is abolished by bilateral nephrectomy
(Meyer and Peskar, 1971; Blass et al., 1974).

Further support for the view that the renin-angiotensin system is involved in thirst is gained by the numerous reports that drinking is induced following intracranial microinjections of small doses of renin, renin substrate, angiotensin-I and ANG-II. Water intake elicited by central administration of components of renin-angiotensin has been reported in rats (Hendler and Blake, 1969; Epstein *et al.*, 1970; Swanson *et al.*, 1973a, b), cats (Cooling and Day, 1973, 1975), monkeys (Setler, 1971; Sharpe and Swanson, 1974) and other species (cf. Fitzsimons, 1972). The onset to drinking is less than one minute following intracranial microinjection of angiotensin-I and ANG-II and less than five minutes following renin administration (Fitzsimons, 1972). Rolls *et al.* (1972) and Krikstone and Leavitt (1974) have reported that intracranial microinjection of ANG-II in the rat elicits water intake comparable in volume to that following water deprivation. The dipsogenic effect of centrally administered ANG-II has also been shown to be additive to that of cellular or extracellular dehydration (Fitzsimons and Simons, 1969; Andersson and Westbye, 1970; Kozlowski *et al.*, 1972; Severs *et al.*, 1973).

Whether ANG-II acts on peripheral and/or central receptors or via some other mechanism to initiate water intake is not known. In this regard, Fitzsimons (1972) has suggested that the hormone may induce drinking (1) by

a direct action on central "thirst receptors"; (2) by sensitizing the central mechanisms to thirst stimuli arising from receptors elsewhere in the body; or (3) by a direct action on peripheral receptors. Haefeli and Peters (1971), on the other hand, have proposed drinking induced by ANG-II is due to ANG-II-induced reductions in plasma volume since it has been shown that the administration of renal extracts, renin or ANG-II induce necrotic vascular lesions and cause effusion of fluid into serous and pleural cavities of nephrectomized rats (Cuthbert and Peart, 1970). Abdelaal *et al.* (1974a, b) have found, however, that ANG-II infusions induce drinking without concomitant decreases in blood volume. Moreover, a loss of extracellular fluid induced by ANG-II administration does not seem to be able to account for the short latency drinking response following injection of ANG-II into the forebrain (Fitzsimons, 1972).

Attempts to identify the dipsogenically active component of the renin-angiotensin system have led to the use of specific pharmacological agents that block the biosynthetic chain at specific intermediate stages. Severs *et al.* (1973) and Summy-Long and Severs (1974) have shown that central pretreatment with angiotensin-I converting enzyme inhibitor attenuated water intake elicited by intracerebroventricular injection of angiotensin-I but had no effect on drinking elicited by ANG-II. Cooling and Day

(1973) have reported that intracerebroventricular admin-
istration of the enzyme inhibitor in cats reduced drinking
to intravenously administered angiotensin-I but not to
ANG-II. On the other hand, Swanson *et al.* (1973a) and
Lehr *et al.* (1973) in rats and Sharpe and Swanson (1974)
in monkeys found that intraventricular or intracranial
pretreatment with converting enzyme inhibitor had no
significant effect on water intake elicited by renin or
ANG-I. These latter data suggest that the central
"receptors" for ANG-II may be different from peripheral
ones (Swanson *et al.*, 1973a; Sharpe and Swanson, 1974) or
that angiotensin-I may induce drinking behavior without
being converted to ANG-II (Bryant and Falk, 1973; Lehr
et al., 1973).

1.3 The Double Depletion Hypothesis of Thirst

A major theme of this review has been that water in-
take is important for maintaining body fluid homeostasis
and that drinking is initiated as a regulatory response to
reductions of either the cellular or extracellular compart-
ments. A second major theme is that the cellular space
and the extracellular space each has its own intake
controlling mechanisms which can act independently of each
other, but which have an additive effect on drinking when
activated together. This concept of the dual nature of
the internal stimuli of thirst has been termed by Epstein
(1973) the "double depletion hypothesis"; its implications

for the present study are further discussed in subsequent sections.

While the double depletion hypothesis may be a good working model for homeostatic drinking, Fitzsimons (1972), among others (e.g., Kissileff, 1969; Oatley, 1973) has shown that under stable environmental conditions, most of the drinking which occurs is not due to deficits of body water. Studies have shown that under these normal, day-to-day conditions water intake is determined by other factors, including the nature of the diet (Kissileff, 1969; Chapman and Epstein, 1970; Ernits and Corbit, 1973), feeding habits (Dicker and Nunn, 1957; Fitzsimons and Le Magnen, 1969; Oatley, 1973), and by circadian influences (Fitzsimons and Le Magnen, 1969; Kissileff, 1969). Fitzsimons (1972) has suggested that food intake-associated drinking is biologically advantageous since feeding and drinking can take place within a relatively short period of time of each other, thereby lessening the time during which the animal is open to attack by its predators. The mechanisms of non-regulatory drinking are not known at present.

1.4 Neural Mechanisms Underlying Water Intake Initiated by Cellular and Extracellular Dehydration

Consistent with the foregoing discussion it is apparent that the concept of regulatory thirst is closely aligned with the more general idea of body fluid homeostasis. Water intake is regulated to maintain an ionic balance across the cell membrane and to ensure an adequate circulatory volume.

The introduction will now focus on a second fundamental issue which relates to the question of how the body, in fact, assesses the magnitude of its own regional and general volumes and ionic concentrations, or the deviations of these from normal. Studies will be reviewed which bear on the question of how depletions of the cellular and extracellular fluid compartments are detected by the central nervous system and how these "thirst signals" are transmitted and processed by the brain to initiate fluid intake.

Receptive Sites for Homeostatic Thirst Signals

Most of the hypotheses which have attempted to explain how water intake is initiated in response to cellular and extracellular fluid depletion have proposed that somewhere on the afferent side of a moderator reflex, there must be some sort of sensing or sensory cells which are capable of monitoring and responding to changes in the

composition and volume of the blood.

Largely as a result of the pioneering studies of Verney in the 1940's, the idea developed that there exist within the central nervous system "osmoreceptors", cells which are capable of shrinking or swelling in response to a change in the tonicity of their ambient fluid. Verney (1947) first proposed that these cells lay within tissue supplied by blood from the carotid artery and that they could act as stretch receptors for afferent nerves, impulses along which reflexly altered the rate of release of ADH from the neurohypophysis. In subsequent experiments Jewell and Verney (1957) determined that the osmosensitive region was located in the rostral hypothalamus. When some time later it was found that injections of a hypertonic NaCl solution into the anterior diencephalon induced drinking in a variety of animals, including the goat (Andersson and McCann, 1955), rat (Blass and Epstein, 1971), and rabbit (Peck and Novin, 1971), it was taken to indicate that "osmoreceptors", perhaps distinct from the cells controlling ADH release, could also initiate water ingestion. Recent electrophysiological work (e.g., Vincent et al., 1972; Malmo and Mundl, 1975; Malmo, 1976) has supported the view that the cells which are involved in initiating drinking to cellular fluid depletions are anatomically and functionally distinct from the neurosecretory cells that transport ADH to the posterior pituitary.

In spite of their postulated importance the exact location of the hypothesized "osmoreceptors" is not known. Although the results of electrophysiological studies (e.g., Malmo and Mundl, 1975; Malmo, 1976) have implicated the lateral preoptic region, the osmosensitive neurons demonstrated therein cannot be assumed to be "osmoreceptors" unless they continue to discharge in response to osmotic stimulation following complete surgical isolation of the area (Mogenson, 1975), and this experiment has not been done. Moreover, proponents of the view that osmoreceptors are located within the brain cannot yet say whether they are neurons, glial cells or modified ependymal tissue or whether they are normally stimulated from the outside or from the inside of the blood-brain barrier. Because of these difficulties, alternatives to central osmoreceptors have been proposed. For example, there is some evidence that peripheral osmoreceptors located in the pancreas (Inchina and Finkinshtein, 1965) or portal circulation (Haberich, 1968) may participate in the control of ADH release; whether they also have a role in water intake mechanisms has not been determined. Emmers (1973) has suggested that "osmoreceptors" are located in the oropharyngeal cavity and may be involved in drinking via inputs through the thalamus relay nucleus for taste, since bilateral ablation of this structure abolishes water intake in response to subcutaneous injections of hypertonic NaCl.

The finding that Na^+-depleted animals continue to drink and release ADH in spite of cellular hydration and that extracellular dehydration without concurrent change in osmolality also elicits water intake and ADH release, led to the suggestion that extracellular fluid volume is regulated by "volume receptors" (Stricker, 1966) rather than by osmoreceptors as was previously thought (cf. Fitzsimons, 1972). Volume receptors are believed to be distributed in the walls of the capacitance circulation of the thorax (pulmonary vessels, right heart and left atrium) and are innervated by the vagus (Epstein, 1973). Although there is no conclusive evidence for these receptors in thirst mechanisms (cf. Fitzsimons, 1972), thoracic hypovolemia inhibits the receptor discharge and blood loss of 10% or less causes ADH release, while both effects are abolished by vagotomy (Epstein, 1973).

As stated previously in this section, there is considerable experimental evidence for an endocrine influence on the regulation of water intake. It is thought that ANG-II is a dipsogenic hormone involved in extracellular thirst and that it initiates drinking behavior by acting on chemosensitive receptors in the brain. Unfortunately, there is as yet no consensus regarding the locus or loci of central receptor(s) mediating the dipsogenic activity of ANG-II. In the first studies in which ANG-II was infused into a number of areas of the brain, it was found

that the most sensitive sites for eliciting water intake
included the septum, anterior hypothalamus and the pre-
optic region (POA) (Epstein *et al.*, 1970). More recent
studies have shown that drinking may also be initiated by
injection of ANG-II into the cerebral ventricles (Swanson
and Sharpe, 1973; Hoffman and Phillips, 1975), the sub-
fornical organ (Simpson and Routtenberg, 1973), and the
mesencephalic central grey (Sharpe and Swanson, 1974).
Drinking behavior was not observed following the administra-
tion of ANG-II to the cerebellum, tegmentum of the midbrain,
posterior hypothalamus, caudate nucleus and the frontal
cortex (Epstein *et al.*, 1970). Injections of bradykinin,
ADH, oxytosin, adrenalin, and 0.9% NaCl into ANG-II
sensitive sites do not induce water intake (Fitzsimons,
1971a, c). Thus, although the early mapping studies pro-
vided evidence that the POA is a site of "receptors" for
ANG-II-induced drinking, more recent developments have
prompted a re-evaluation of these data. In this respect
the subfornical organ has received the most attention
(Epstein and Simpson, 1974).

The subfornical organ (SFO) is a small nodule of
neurogliovascular tissue situated in the midline on the
rostral wall of the third ventricle, near the point where
the choroid plexes of the lateral ventricles join to-
gether (Spiegel, 1918; Putnam, 1922; Akert *et al.*, 1961;
Akert, 1969). Since the SFO lies in contact with the CSF

(Andres, 1965; Akert and Steiner, 1970), is highly
vascular (Spoerri, 1963) and devoid of a blood-brain
barrier (Koella and Sutin, 1967), it has been suggested
that the SFO may be the locus of the central dipsogenic
receptive sites for ANG-II of renal origin (Simpson and
Routtenberg, 1973). Johnson et al. (1974), using auto-
radiographic techniques, have found that the highest
concentration of radioactive material following intra-
venous injection of ANG-II appears in the cerebrospinal
fluid and the SFO. Simpson and Routtenberg (1973) have
reported that drinking can be elicited with as little as
0.1 ng of ANG-II applied directly to the SFO. Analysis
of the time course of elicited water intake in their study
supports the view that the SFO may be a receptive site
for ANG-II since drinking behavior began less than 30 sec
following the administration of the hormone.

Central Integrative Mechanisms for Regulating Thirst:
Role of the Hypothalamus

The first evidence that the hypothalamus was involved
in the control of ingestive behaviors came from clinical
observations in which disruptions or normal feeding and
drinking were reported following hypothalamic damage
(cf. Fitzsimons, 1972). The introduction of the stereo-
taxic procedure in the 1930's facilitated experimentation
and demonstrated the role of the hypothalamus more clearly.

Heatherington and Ranson (1942) reported hyperphagia and
obesity after electrolytic lesions of the medial hypo-
thalamus in rats and Anand and Brobeck (1951), and others
(*e.g.*, Teitelbaum and Stellar, 1954; Teitelbaum and
Epstein, 1962), reported aphagia and adipsia induced by
ablation of the lateral hypothalamus (LH). Electrical
stimulation of the LH has been shown to elicit feeding and
drinking in goats (Andersson, 1953; Andersson and McCann,
1955) and rats (cf. Mogenson and Huang, 1973). It has
also been reported that drinking may be elicited by appli-
cation of carbachol, a cholinergic compound, to the LH
(Fisher and Coury, 1962; Grossman, 1962b), whereas feeding
is initiated by local administration of noradrenalin
(Grossman, 1962a). Considered together, these early
studies have led to the view (cf. Epstein, 1971; Hoebel,
1971; Fitzsimons, 1972; Mogenson and Huang, 1973) that the
hypothalamus, and the LH in particular, integrates food
and fluid deficits signals.

More recently, however, the concept of the "lateral
hypothalamic feeding and drinking center" (cf. Epstein and
Teitelbaum, 1964) has been challenged on the basis that
there may be multiple integrative mechanisms for the ini-
tiation and control of ingestive behaviors. For example,
rats "recovered" from adipsia following LH lesions show
prandial drinking (Kissileff, 1969; Epstein, 1971), but do
not respond to cellular or extracellular dehydration

(Epstein, 1971, 1973) which suggests that separate neural systems may mediate the different types of drinking behavior. The possibility of multiple integrative mechanisms for drinking is also suggested by reports that atropine blocks water intake elicited by central administration of carbachol (Grossman, 1962b) but has no effect on ANG-II-induced thirst (Setler, 1971).

The model of the LH integrative center has also been criticized because the experimental techniques used have in most cases been rather crude (Mogenson and Huang, 1973). The LH is nodal area in the brain where a vast number of chemo-specific pathways intermingle and it has been suggested that lesions and stimulation of this region of the hypothalamus may influence pathways concerned with several functions and produce multiple behavioral effects (Mogenson and Huang, 1973). For example, while large bilateral lesions of the LH often disrupt both feeding and drinking (Teitelbaum and Stellar, 1954; Teitelbaum and Epstein, 1962), smaller LH lesions have been reported to produce adipsia without aphagia (Montemurro and Stevenson, 1957) and aphagia (cf. Mogenson and Huang, 1973). Another case in point is that electrical stimulation of the LH with large electrodes elicits both food and water intake as well as other behaviors (Valenstein et al., 1968); drinking or feeding alone may be induced when smaller electrodes are used (Huang and Mogenson, 1974). It appears, therefore, that the multiple effects of LH lesions and stimu-

lation may be due to destruction or activation of
partially overlapping neural pathways. The proposal that
experimental manipulation of LH tissue may be producing
behavioral effects by influencing "fibers of passage" is
supported by the studies reviewed below, which have impli-
cated a number of extradiencephalic structures in the
control of drinking behavior.

Role of Limbic and Other Brain Structures

It is now known that drinking, feeding and
other behaviors that contribute to homeostasis are also
influenced by lesions and stimulation of limbic and other
brain structures. Harvey and Hunt (1965) and others
(*e.g.*, Huang and Mogenson, 1972) have reported that lesions
of the septum caused a 25 to 50% increase in daily water
intake in rats. Blass and Hanson (1970) and Blass *et al.*
(1974) have suggested that the basis of this polydipsia may
be an exaggerated reactivity to extracellular thirst
signals mediated by ANG-II. Electrical stimulation of the
septum has been demonstrated to reduce water intake in
rats previously subjected to water deprivation (Wishart
and Mogenson, 1970), suggesting that the septum has an
inhibitory influence on water intake. Recent electro-
physiological studies have shown, however, that the septum
may also have a facilitatory effect on drinking behavior
(Miller and Mogenson, 1971). Miller and Mogenson (1972)
have proposed that fibers of the precommissural fornix may

mediate facilitatory influences, whereas fibers of the
stria terminalis mediate inhibitory effects. It has also
been found that the pathway which has the dominant influ-
ence on hypothalamic neurons at any particular time depends
on the level of electrical activity of the LH (Miller and
Mogenson, 1971, 1972), suggesting that the septum may have
an important modulatory influence on hypothalamic
function (Mogenson and Huang, 1973; Mogenson, 1975).

The amygdala has also been implicated in the control
of ingestive behaviors (cf. Grossman, 1968; Mogenson and
Huang, 1973). Based on the available experimental evi-
dence, it appears that the amygdala, like the septum, is
concerned mainly with modulating hypothalamic mechanisms
for the initiation of feeding and drinking. The results
of studies by Olds *et al.* (1969) have suggested that the
amygdala may be involved in short-term satiety mechanisms,
perhaps by monitoring inputs related to chewing and
swallowing.

Other limbic structures including the hippocampus
(Olds *et al.*, 1969) and cingulate cortex (Fisher and
Coury, 1962; Robinson and Mishkin, 1968) have also been
shown to have both facilitatory and inhibitory influences
on ingestive behaviors. These results have led to the view
that the limbic forebrain is involved in the integration
of modulatory influences related to the ingestion of food
and water and that these influences are exerted onto hypo-
thalamic systems which are responsible, in turn, for

integrating food and fluid deficit signals (Mogenson, 1974).

Since lesions placed rostral to the proposed LH inte-
grative region have also been reported to cause adipsia
(Blass and Epstein, 1971), it has been proposed that
thirst signals pass caudally to the LH (Mogenson, 1974)
from osmosensitive cells in the forebrain (Blass and
Epstein, 1971; Peck and Novin, 1971) or from chemorecep-
tive sites for ANG-II in the preoptic region (Epstein *et
al.*, 1970). According to this model of the functional
neurology of the control of water intake, the critical
output for the initiation of drinking behavior from the LH
integrative sites appears to be to particular areas of the
limbic midbrain. While not as extensively studied as
limbic forebrain structures, the results of some investi-
gations have suggested that the limbic midbrain is in-
volved in ingestive behaviors. Lesions of the midbrain
tegmentum produce aphagia in rats (Parker and Feldman,
1967; Lyon *et al.*, 1968), whereas electrical stimulation
of this region elicits feeding (Wyriwicka and Doty, 1966)
and drinking (Robinson and Mishkin, 1968). Electrical
stimulation of the LH has been reported to produce evoked
potentials in the area of Tsai and in the midbrain teg-
mentum ventrolateral to the central grey (Wyrwicka and
Doty, 1966) and degenerating fibers have been traced from
sites where feeding and drinking were elicited in the LH
to the midbrain (Huang and Mogenson, 1972).

The precise role of the cerebral cortex in ingestive behaviors is not known, although cortical involvement is presumed at higher cognitive functions influencing intake (Mogenson, 1974).

Neural Pathways Underlying Limbic Forebrain-Hypothalamic-Limbic Midbrain Interactions in the Control of Ingestive Behavior

There has been considerable interest over the last 15 years or so in the anatomical relationships of various structures implicated in the control of ingestive responses. The present section will consider briefly some of the known connections between the limbic system and the hypothalamus which appear to be of particular relevance to this study. Detailed descriptions of the origins, trajectories and terminations of limbic-hypothalamic pathways are given in recent papers (*e.g.*, Swanson, 1976; Swanson and Cowan, 1976; Millhouse, 1969) and review articles (*e.g.*, Morgane, 1975).

A close relationship between the limbic forebrain and the hypothalamus is suggested by the morphological continuity of the LH with the preoptic and septal regions and by the many anatomical studies which have shown pathways linking forebrain structures with the diencephalon (cf. Morgane, 1975; Swanson, 1976; Swanson and Cowan, 1976). The fornix system, direct amygdalo-hypothalamic connections,

stria medullaris and the medial forebrain bundle are some of the major projections joining the two areas.

The pathways through which limbic forebrain structures may influence the midbrain have been extensively studied using histological and electrophysiological techniques. These investigations have demonstrated monosynaptic projections from the hippocampus (Nauta, 1958, 1960; Grantyn and Grantyn, 1972, 1973), cingulate cortex (Domesick, 1969) and lateral hypothalamus (Huang and Mogenson, 1972), to the midbrain reticular formation and central grey, and as well to the midbrain and pontine tegmentum (Nauta, 1958). There are also two major polysynaptic fiber systems interconnecting limbic forebrain and midbrain areas. The first group of fibers, originating in the septum pellucidum, olfactory tubercle and lateral preoptic region, enter the stria medullaris via the inferior thalamic peduncle, pass rostrocaudally to synapse at the lateral habenular nuclei and then continue ventrocaudally as diffuse and compact components of the fasciculus retroflexus to the interpeduncular nucleus, dorsal tegmental nucleus and caudal central grey (Nauta, 1958; Zyo et al., 1963; Ban, 1966; Millhouse, 1969; Morgane, 1975; Swanson, 1976; Swanson and Cowan, 1976).

The second major polysynaptic pathway from the limbic forebrain to the mesencephalon is the medial forebrain bundle (MFB). The descending MFB consists of finely

myelinated and unmyelinated fibers derived from many cyto-
archetonically distinct areas such as the hippocampus,
septum, nucleus of the diagonal band of Broca, frontal
cortex, nucleus accumbens, amygdala and pyriform cortex
which converge in the lateral preoptic region and pass
caudally through the LH to terminate near the ventrolateral
edge of the central grey or to distribute to various regions
near the midline encompassing the dorsal and ventral teg-
mental mesencephalic areas and their associated nuclei
(Nauta, 1958; Millhouse, 1969; Swanson, 1976). Millhouse
(1969) has found that the descending MFB contains long
fibers as well as numerous shorter axonal segments. MFB
fibers from the frontal pole, olfactory tubercle and
pyriform cortex pass without synapsing through the LH and
into the pontine tegmentum. Other MFB fibers, originating
in the septum and preoptic region, accompany the long
axons for varying distances through the LH, whereupon they
synapse or project collaterals to neurons along their
paths. These neurons are differentiated, in turn by the
amount of afferent input they receive from converging
fibers of different limbic structures (Millhouse, 1969).
Limbic forebrain pathways passing to the mesencephalon are
reciprocated by rostrally coursing fibers of the ascending
MFB and fasciculus retroflexus which project to the LH and
as well to many limbic forebrain structures (Nauta, 1958;
Grantyn and Grantyn, 1972, 1973; Morgane, 1975). These
prominent interconnections of the limbic system and hypo

thalamus via the MFB and the reports of a close anatomical correspondence of this fiber system with central sites effective for eliciting feeding and drinking (Huang and Mogenson, 1972) suggest that the MFB may be important in the control of motivated behaviors (Mogenson and Phillips, 1975).

The recent interest in chemical neuroanatomy based on mapping transmitter-specific pathways with histochemical and histofluorescence techniques has shifted emphasis from caudally to rostrally directed pathways associated with ingestive behaviors. These new neuroanatomical procedures have demonstrated amine specific pathways passing from the brainstem through the LH to terminate in thalamic, limbic forebrain and cortical regions (Dahlstrom and Fuxe, 1964; Ungerstedt, 1970, 1971). The findings are numerous and beyond the scope of this section; comprehensive reviews dealing with this work have recently been written by Mogenson and Phillips (1975) and Morgane (1975). What is of-particular interest and importance to the present study is that the histochemical and histofluorescence mapping studies have revealed the neurochemical organization of the lateral hypothalamic area for the first time, showing it to be both heterogeneous and complex. For example, the far-lateral hypothalamic area (FLH), which has usually been considered to encompass the most ventromedial aspects of the internal capsule, entopeduncular nucleus and globus pallidus, as well as the most lateral portion of the LH itself (cf. Mor-

gane, 1961, 1964), is dominated by dopaminergic-containing axons of the ascending nigro-striatal and meso-limbic pathways (Morgane, 1975). The midlateral hypothalamus (MLH), in contrast, is characterized by serotinergic and noradrenergic fibers of passage originating from cell bodies located in the midbrain and brainstem (Mogenson and Phillips, 1975). This new understanding of the chemical neuroanatomy together with the technique of selective lesions or stimulation of the MLH or FLH has therefore provided an important tool for establishing the identity of pathways that course through this area of the diencephalon, since manipulation of each region would produce specific neurochemical changes in brain areas to which these fibers of passage project (Morgane, 1975). Although Morgane (1961) suggested more than a decade ago that aphagia and adipsia resulting from hypothalamic lesions might be due to destruction of fiber pathways rather than integrative center, it is only with the increased awareness of the role of amine-specific pathways in the control of ingestive behaviors that his ideas have been taken seriously. Electrolytic damage of nigrostriatal and pallidofugal fibers passing through the FLH was described by Morgane (1961, 1964) as resulting in a "qualitatively different" type of disturbance of feeding and drinking than that seen when lesions were made in the lateral perifornical (MLH hypothalamic area. Typically, the MLH lesions caused a transitory disruption of ingestive behaviors that Morgane

(1961) termed "motivational inertia" since the animals
were found to have normal daily intakes of food and water
but would not cross an electrified grid or bar-press for
food or water reward. They lost weight during the
immediate postlesion period much like normal rats deprived
of food and water. Morgane (1961) showed that when the
lesions were made one mm more laterally (in the FLH) a
very different disruption resulted. FLH-lesioned rats did
not eat or drink, lost weight rapidly, and died still
within the period normal animals could withstand food and
water deprivation. Morgane's early work has been extended
to the entire limbic forebrain-limbic midbrain extent of
the MFB (cf. Morgane, 1975) and has shown that lesions
placed anywhere along the trajectory of this fiber system
produce disturbances of ingestive behaviors. Moreover,
interruption of the various component pathways comprising
the MFB at extrahypothalamic levels has demonstrated that
the severity of the disruption of feeding and drinking
in relation to the extent to which the lesions deplete the
forebrain of norepinephrine, serotonin and dopamine (cf.
Mogenson and Phillips, 1975; Morgane, 1975). These studies
indicate that in studying the LH we are dealing with
several ascending and descending transmitter-specific
pathways which can be followed anatomically and manipulated
both in the hypothalamus and at several other levels of the
neuraxis. The studies also suggest that distant neuro-
chemical effects of lesions placed in the LH may be very

important in determining the physiological and behavioral effects that are produced.

In summary, it is apparent from the foregoing introduction that the investigation of the neural mechanisms of ingestive behaviors which began in the 1950's has been pursued vigorously up to the present time. The earliest models of the control systems for regulatory thirst suggested that separate signals of cellular and extracellular dehydration acted on specific volume and osmosensitive receptive sites in the forebrain, were integrated in the LH and then transmitted to motor nuclei in the midbrain for the execution of drinking behavior. During the 1960's the scope of the studies expanded to include other brain sites, and limbic structures in particular were implicated as exerting modulatory influences on the LH. The involvement of the renin-angiotensin system in extracellular thirst mechanisms was proposed during this time and more recently experimental evidence has established ANG-II as a hormonal mediator of thirst and suggested that it may exert its dipsogenic effects by acting chemosensitive neurons in the POA or the SFO. This latter development has generated much interest in the neuroendocrinological mechanisms of thirst and has reinforced the idea (see p. 5) that there are multiple physiological mechanisms to preserve the volume and composition of the extracellular body fluid compartment. Finally, with the increased use of the new neurochemical mapping techniques referred to

above, a reassessment of the classical view of the neural
regulation of ingestive behaviors has been prompted. The
new outlook on how the central nervous system participates
in the behavioral regulation of body water constancy
emphasizes the importance of amine-specific ascending, as
well as descending, limbic forebrain, hypothalamic, and
midbrain pathways and of the complete neural circuitry of
which these pathways are a part.

1.5 The Present Study

On the basis of the studies reviewed thus far, it
appears that the central mechanisms subserving regulatory
drinking behavior involve a complex circuitry of specific
neural receptor sites and transmitter-specific pathways
interconnecting the LH with the limbic forebrain and mid-
brain. These studies have raised many interesting
questions concerning the functional neurology of cellular
and extracellular thirst, many of which can be examined in
the laboratory. The purpose of the present study was to
investigate whether specific neural pathways and integrative
structures in the LH and limbic midbrain transmit and
process the signals that initiate water intake in response
to cellular and extracellular stimuli of thirst. The
study is divided into four experimental series which appear
consecutively in Sections 3.1 to 3.4.

The first series of experiments used single unit
recording, lesioning and behavioral testing techniques to

try to determine if drinking behavior induced in response to the administration of the hormone, ANG-II, to the POA was mediated by pathways passing through the MLH or FLH. The major finding was that pathways traversing the MLH appeared to subserve drinking to ANG-II and other extracellular thirst stimuli, whereas pathways passing through the FLH-mediated water intake to cellular stimuli of thirst. A functional and anatomical separation of neural systems in the LH for the two types of regulatory thirst is consistent with the double depletion hypothesis as enunciated by Epstein (1973) and with Morgane's (1961, 1964, 1975) view that the MLH and FLH are concerned with different aspects of energy balance and body fluid homeostasis.

On the basis of the data it was decided, in the second series of experiments, to examine more closely the nature and specificity of the ingestive deficits following MLH and FLH lesions. The major conclusion of this part of the study was that the effects of MLH and FLH lesions were specific to extracellular and cellular thirst, respectively, and not to non-specific factors.

Recalling that the current view of the central control of ingestive behaviors (cf. Mogenson and Phillips, 1975; Morgane, 1975) postulates an interaction of limbic and and hypothalamic structures, the next series of experiments was designed to test whether drinking induced by cellular (hypertonic NaCl) and extracellular (ANG-II) stimuli of

thirst administered to the proposed forebrain receptive sites in the POA, was mediated by limbic midbrain structures previously implicated by Parker and Feldman (1967) and others (e.g., Wyrwicka and Doty, 1966; Robinson and Mishkin, 1968) in ingestive behaviors. Since it was found that lesions of the paramedial rostral midbrain disrupted drinking to ANG-II administered to the POA but not to hypertonic NaCl given to the same site, it was concluded that separate neural pathways mediate cellular and extra-cellular thirst at this level of the brain.

In the final part of this study, the focus of attention was shifted to the question of the location of "receptors" for ANG-II in the forebrain (see pp. 19-21) and whether there are multiple neural pathways mediating extracellular thirst. The results of the last series of experiments indicate that drinking initiated by administering ANG-II to the POA is mediated through neural pathways (passing through the MLH and paramedial rostral midbrain) distinct from those which subserve water intake when ANG-II is microinjected into the SFO. These results suggest that there are multiple receptive sites and neural pathways for mediating extracellular thirst.

2.0   GENERAL METHODS

Methods common to the experiments of this study are described in this section. Exceptions to these procedures and techniques specific to individual experiments are described in Sections 3.1 - 3.4 and in Appendix A.

2.1   Animals, Housing and Diets

A total of 483 male Wistar rats weighing between 200-425 g were used in the four parts of this study. The animals were obtained from Woodlyn Farms (Guelph, Ontario) or BioBreeding Laboratories (Ottawa, Ontario). They were housed individually in suspended wire-mesh cages (18 x 23 x 18 cm) in a temperature- and humidity-controlled room with a 12:12 day-night light cycle and were maintained on tap water and Purina rat chow *ad libitum*. A synthetic granular diet (high carbohydrate diet: 63.7% sucrose, 20% casein, 10% corn oil, 3.8% salt mix, 2.5% vitamin mix, by weight) was used in some of the chronic lesion experiments which required measurements of daily intakes of food.

2.2   Animal Preparations for Chronic Experiments

1. *Stereotaxic implantation of intracranial cannulae and lesioning electrodes*

Chronic intracranial cannulae (guide cannulae) were made by modifying a 23 or 26 gauge stainless steel needle.

The plastic hub of the needle was removed and the needle
ground to a length of 12 mm for SFO and cerebral ventricle
implantation or to 15 or 17 mm for POA implants. A
removable inner cannula (injection cannula) was made from a
length of 30 or 33 gauge stainless steel tubing (HTX-30 and
HTX-33, Small Parts Inc., Miami, Florida) which fitted
snugly into the 23 and 26 gauge guide cannulae, respec-
tively. The injection cannulae were bent into the shape
of an "L" with one arm precisely ground so that its length
was 0.5-1.5 mm greater than that of the guide cannula. The
other arm of the 30 gauge injection cannula was connected
to a length of polyethylene tubing of 0.011" inner diameter
(P.E..10; Becton, Dickinson and Co., Parsippany, New
Jersey). The 33 gauge injection cannula was connected to
teflon tubing of 0.008" inner diameter (#32 microliter
tubing, Small Parts Inc.). Obturators made from size 00
insect pins were inserted into the guide cannulae and
remained in place except during intracranial testing.

Monopolar lesioning electrodes were made from size 00
insect pins cut to a length of 12-20 mm and were coated
with an insulating lacquer (Epoxylite #6001-M Electrode
Insulation; Epoxylite Corp., Buffalo, New York). Three to
five coats of the lacquer were applied, allowing each to
dry before applying the next. Prior to implanting the
electrode the cross-sectional area at the tip was scraped
bare of insulation using a dissecting microscope and
electric sanding tool. Depending on the size of the lesion

to be made, the exposed cross-sectional area at the electrode tip was 0.2-1.0 mm in diameter. The electrical insulation of the electrode shaft was routinely tested under a dissecting microscope by passing a low voltage direct current while the electrode was immersed in saline.

The guide cannulae used for intracranial microinjection of the drugs and solutions described in Section 2.8 and the lesioning electrodes were individually implanted into designated areas of the brain using standard stereotaxic techniques. In the first series of experiments rats were pretreated (10 min) with Atropine Methyl Nitrate (Sigma Chemical, St. Louis, Missouri; 0.5 mg/kg body weight, i.p.) to reduce bronchial secretions, and were anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg body weight, i.p.). In later series of experiments the animals were anesthetized with ketamine hydrochloride (Ketaset; Rogar STP, London, 80 mg/kg body weight, i.p.) followed 5 min later by an i.p. injection of sodium pentobarbital (Abbot Laboratories, Montreal, 25 mg/kg body weight). The animals were placed in a stereotaxic frame (Kopf Model 500; David Kopf Instruments, Tujunga, California) with the head rigidly held by ear bars placed into the external auditory meatus and by an incision bar fixed at 5.0 mm above the level of the ear bars. A midline incision 10-20 mm in length was made through the scalp, the flaps of which were then retracted laterally and held with hemostats. The surface of the skull was cleared and dried and the stereotaxic coordinates of the sagital and coronal sutures

(bregma) were taken as zero reference. The cannula or
lesioning electrode was inserted to the appropriate depth
in the brain through a small hole drilled in the
calvarium and was fixed in place by applying cranioplastic
acrylic (L. D. Caulk Co., Millford, Ontario) to the
cannula or electrode and to stainless steel jewellery
screws which were inserted into small holes drilled in
the adjacent calvarium. In animal preparations intended
for subsequent single unit recording experiments an
additional jewellery screw was implanted in the calvarium
overlying bregma and the skull surface posterior to this
level was kept clear of cranioplastic acrylic so that
recordings could be made. The stereotaxic coordinates
used for cannula and lesioning electrode implants are
shown in Table 1.

2. *Cannulation of the jugular vein*

The right external jugular vein was permanently
cannulated using the procedure of Steffens (1969) with the
modifications employed by Abdelaal (1975). The cannulae
were made from 115 mm lengths of Silastic tubing (#602-155,
Dow Corning Corp.; Midland, Michigan). The end of the
tubing to be inserted into the jugular vein was cut at a
45° angle and a 3 mm ring, cut from larger diameter tubing,
was slid onto the cannula to a point 45 mm from the end to be
implanted. A 20 mm length of 20 gauge stainless steel tubing
was bent into the shape of an "L" and the distal end of the

TABLE 1

Stereotaxic Coordinates[1] Used for Intracranial Cannulae and Lesioning Electrode Implantations[*]

Intracranial Site	Angle from Perpendicular (measured towards midline)	mm from Bregma		
		Anterior-posterior	Lateral	Vertical[†]
POA	straight (parallel to midline)	+2.0 - +1.6	1.2 - 1.5	7.5 - 8.0
POA	angled (16°C)	+2.0 - +1.6	3.0 - 3.2	7.7 - 8.2
SFO	12°	+0.2 - -0.2	1.0	4.4 - 4.6
Lateral ventricle	straight	+0.2 - -0.2	1.5 - 2.0	2.5 - 3.0
Third ventricle	10°	+0.2 - -0.2	1.2	4.6 - 5.0
MLH.	straight	-0.4 - -0.6	1.5 - 1.7	8.5 - 9.0
FLH	straight	-0.4 - -0.6	2.0 - 2.8	8.0 - 8.5
Ventromedial hypothalamus	straight	-0.4 - -0.8	0.3 - 0.8	9.3 - 9.8
Thalamus	straight	-0.4 - -0.6	1.5 - 1.7	6.0 - 6.5
Caudate-putamen	straight	+2.4	2.5	5.5
Habenular nucleus	straight	-1.8	0.7	4.6
Cerebral cortex	straight	-0.4 - -0.6	1.5 - 1.7	1.8
Hippocampus	straight	-0.4 - -0.8	1.0	3.8
Midbrain	straight	-4.5 - -6.8	0.2 - 1.8	4.2 - 8.0

[*]The same coordinates were used in the microelectrode recording experiments described on p. 47, except where specifically noted.

[†]The vertical coordinate is the distance from the surface of the cortex to the most ventral position of the injection cannula or electrode.

[1]Modified from the atlas of Pellegrino and Cushman (1967).

cannula (cut at a right angle) was passed 7 mm over one arm of the "L" shaped tubing. The structurally completed cannula was filled with a solution of 50% (w/v) Polyvinyl Pyrolidone (PVP, MW 40,000; Nutrional Biochemicals, Cleveland, Ohio) in heparinized 0.9% NaCl. Penicillin (7,500 units/ml) was added to the PVP solution to prevent bacterial growth in the area of the cannula tip. The cannula was tapped clear of air bubbles and capped with a 10 mm length of polyethylene tubing (P.E. 60; Becton, Dickinson and Co.) heat-sealed at the distal end. At the time of surgery the implantation of the cannula into the jugular vein was done first after which the animal was placed in a Kopf 500 stereotaxic frame. The skull was exposed and forceps were guided subcutaneously around the neck to the jugular vein. The distal end of the cannula was held between the tips of the forceps and was guided up to the exposed skull surface. The end of the cannula was positioned centrally between four jewellery screws partially screwed into the adjacent calvarium and cranioplastic acrylic was applied to anchor the implant. Animal preparations intended for use in subsequent recording experiments were clear of cranioplastic acrylic on the skull surface posterior to bregma. A minimum of 5 days was allowed for postoperative recovery before the animals were used in any experiment.

2.3 Drinking Tests

1. *Intracranial administrations of test solutions*

The animal was removed from its home cage and lightly restrained by hand. The insect pin obturator was removed from the shaft of the guide cannula using forceps and was replaced by the injection cannula connected to a 20 μl capacity syringe (Hamilton Instruments, Reno, Nevada) or a 1 μl capacity microsyringe (Scientific Glass Engineering Ltd., Melbourne, Australia) via a length of P.E. 10 microlitre tubing filled with the test solution. A predetermined volume was injected over 5-10 sec, the injection cannula was withdrawn and replaced with the obturator, and the animal was returned to its home cage. At the end of a 15 or 30 min period following the injection, water intake (± 0.5 ml) was measured from calibrated 15 or 50 ml centrifuge tubes fitted with Richter tube spouts. Food (either high carbohydrate diet or Purina rat chow) was available during the drinking tests.

2. *Peripheral tests*

In some of the experiments the effects of cellular and extracellular dehydration on water intake in lesioned and control animals was studied. The animals were water-deprived for 12, 24 or 48 hr and water intake was measured for a 1-hr or 2-hr period following deprivation. Food was available throughout the deprivation periods but not during the test of water intake. To determine the effect of a

reduction in cellular fluid volume or water intake, animals were injected i.p. with hypertonic solutions of NaCl or sucrose or control isotonic solutions of equivalent volumes of same. Latency to drink following injection and 1-hr water intakes were determined. Food was not available during this period.

In order to study the effects of extracellular dehydration, polyethylene glycol, isoproterenol or renin was injected (s.c. or i.p.) and water intake was measured for 6 or 9 hr with no food available. As a control procedure equivalent volumes of the isotonic saline vehicle were administered.

In the experiments involving jugular vein infusions of angiotensin-II, the animals were transferred from their home cages to a plexiglass chamber (20 x 30 x 30 cm) two hours before the start of the test. The PVP was aspirated from the cannula implanted in the jugular vein and the cannula was connected to a 60 cm length of P.E. 60 tubing surrounded by a protective flexible lightweight spring. The test or control solution was infused at a constant rate for a 60 min period from a 2.0 ml syringe driven by an adjustable rate infusion pump (Model 255, Sage Instruments, New York, New York) after which the total water intake was measured from a 25 ml pipette fitted with a stainless steel drinking spout. The rate of infusion was adjusted to the body weight of the animal (2 ml/kg/hr) and

food was available throughout the test. Control infusions
of equivalent volumes of 0.9% NaCl were administered on the
day preceding the first test with angiotensin-II.

2.4 Extracellular Single Unit Recording

In 57 animals the effects of administering ANG-II or
hypertonic NaCl or sucrose through cannulae chronically
implanted into the POA on single unit activity in other
areas of the brain were investigated. Prior to the
recording experiment, each animal was tested for drinking
in response to the microinjection of ANG-II, hypertonic
NaCl or hypertonic sucrose according to procedures out-
lined in Section 2.3. Only those rats which drank follow-
ing administration of the test solution were used.

Animals were anesthetized with ethyl carbamate.
(urethan; 1.2-1.5 g/kg body weight, i.p.) and placed in a
stereotaxic instrument (Kopf, Model 1730) with the incisor
set at 5.0 mm above the level of the ear bars. The frontal
and parietal bones were exposed following midline incision
of the scalp, a 4 x 4 mm section of bone overlying the area
of brain under study was removed and the underlying dura
excised. The exposed cortex and surrounding wound edges
were covered with a layer of mineral oil or saline to
prevent drying. Rectal temperature was monitored with a
thermistor and telethermometer (Model 43TA; Yellow Springs
Instrument Co., Yellow Springs, Ohio) and was kept between

37° and 38°C using a hot-water bottle. Intracranial micro-injections were made over 5 sec through a 30 gauge injection cannula inserted to an appropriate depth into the implanted guide cannula and connected at its distal end to a short segment of P.E. 10 tubing to a 1 or 10 μl syringe filled with the test solution. No more than 10 intracranial microinjections were made during the recording session, and an interval of 40 min or more elapsed between two successive injections. Injection of equivalent volumes of the control vehicle solution were routinely administered.

Extracellular recordings of single neuron activity were made with stainless steel microelectrodes (1-5 μ tip diameter, 0.5-5.0 MΩ initial direct current resistance in saline) prepared from size 00 insect pins according to the method of Green (1958) and insulated with a lacquer (Type. E-33-N, Insl-x Co., Ossining, New York). The electrical insulation of the electrode shaft was tested by passing a low voltage direct current while the electrode was immersed in saline. Hydraulic reduction microdrives (Kopf, Model 1207B) were used to lower the microelectrodes into the brain. The stereotaxic coordinates used to position the recording electrodes were those shown in Table 1. The microelectrode was connected to one input of a differential AC preamplifier (Grass Model P14 or P15) while a stainless steel jewellery screw threaded partially into the skull was

connected to a second input of the preamplifier and served
as a reference electrode. The recorded activity was
filtered using a 30 Hz-30 KHz band pass and the filtered
electrical signals were led through an amplifier (Tektronix
Model 2A61; Tektronix Inc., Beaverton, Oregon) and then
into oscilloscopes (Tektronix Models 565, 549 and 5103N)
for continuous and single sweep display. Amplifier outputs
were connected to an audiomonitor (Grass Model AM4 or AM7)
and were also led to a magnetic tape recorder (Philips
Analog-7; N. V. Philips Inc., Eindoven, Netherlands) to
be stored for subsequent analysis. Photographs of neural
activity were made from the Tektronix 549 and 5103N
oscilloscope screens using a Polaroid camera (Tektronix
Model C-12).

Unit responses to PoA injections of the test
solutions were displayed by making frequency-time histograms
generated from a signal analyzer (Biomac 1000, Data
Instruments Ltd., London, England) which was triggered by
a stimulator (Grass Type SD9) through a 100 KΩ resistor.

In order to record arterial blood pressure, the
femoral artery was cannulated with polyethylene tubing
(P.E. 50) connected through a Statham 23Db pressure trans-
ducer to a low-level direct current preamplifier (Grass,
Type 7P122) and recorded on one channel of a polygraph

(Grass, Model 7B or 79C). Cortical EEG recordings were
made from jewellery screws threaded through small holes
drilled bilaterally into the skull overlying frontal
cortex. The EEG signals were led directly into a wide-
band AC preamplifier (band pass 0.6-15 Hz) mounted in the
same polygraph.

2.5 Lesions

In most experiments, lesions were made under ether
anesthesia by passing 1.0-2.0 mA anodal direct current
(Lesion Maker; C. H. Stoelting Co., Chicago, Illinois) for
15-40 sec between the implanted insect pin and an indif-
ferent electrode clipped to the ipsilateral ear. In some
of the experiments acute electrolytic lesions were placed
in animals anesthetized with sodium pentobarbital (40 mg/kg
body weight, i.p.) or ketamine hydrochloride (100 mg/kg
body weight, i.p.) by means of stereotaxically guided,
epoxylite-insulated insect pins similar in design to those
used for chronic implantation. Small burr holes were
drilled in the calvarium overlying the target structures,
the electrode was lowered, and an anodal direct current of
1.0-3.0 mA was passed for 10-30 sec with an indifferent
electrode placed on the exposed temporal muscles ipsilateral
to the side of the lesion. The electrode was withdrawn and
the incision was cleaned and sutured. Sham lesions were made
in one-fifth to one-fourth of the animals in each experi-

mental series. These rats were randomly selected and underwent all surgical manipulations except passage of current.

Following the placement of the lesion, the animals were allowed 6-14 days to recover before any post-lesion tests (except daily measurements of food and water intakes and body weights) were made. In some cases where large lesions caused adipsia and aphagia postoperatively, the animals received intragastric intubation of a solution of Metrecal high carbohydrate diet to facilitate recovery (Stevenson, Feleki, Szlavko and Beaton, 1964)..

2.6 Histology

Following completion of the experimental testing the rats were given an overdose of sodium pentobarbital (80 mg/kg body weight, i.p.) or ether and perfused intra-cardially with 60 ml 10% buffered formalin. The brains were removed and stored individually in numbered plastic or glass vials in 10% buffered formalin for a minimum of 48 hr after which they were sectioned on a freezing micro-tome at a thickness of 30-60 μ and every second section was mounted on gelatinized microscope slides. The sections were stained with thionin or cresyl violet and the location and size of the lesions were determined using the atlas of Pellegrino and Cushman (1967).

In recording experiments, localization of the tip of the stimulating electrodes was obtained by passing an anodal direct current (5 μA for 5 sec) through the inner lead of the bipolar electrodes with a reference electrode clipped to the exposed temporal muscles in order to deposit iron at the tip of the electrode. Marking of micro-electrode tips was made by passing anodal direct current of 8-10 μA for 20-25 sec. In most cases the marked sites of microelectrode recording were the most ventrally located in a specific penetration and the locations from which other recordings were made were determined by reference to this point. The animals were given an overdose of sodium pentobarbital and perfused intracardially with 30 ml of 0.9% NaCl solution followed by potassium-ferrocyanide-formalin (2 gm %) solution (30 ml) or by the ferrocyanide solution alone (50 ml). The brain was removed and stored in 10% buffered formalin solution for a minimum of 24 hr. Histological verification of the electrode sites was made by cutting 40 or 50 μ frozen sections and staining them with either thionin or cresyl violet. This fixation and staining procedure has been shown to result in minimal (< 8%) shrinkage of the tissue so that the locations of the electrode placements in the histological sections are relatively accurate indications of the actual sites of stimulation and recording (Konig and Klippel, 1963). Iron deposits appeared as bright blue spots against a violet

or light blue background. In some cases the current passed
was sufficient to produce a small lesion.

2.7 Data Analysis

Standard statistical methods were used
in evaluating the results of the experiments. Quantitative
results are expressed throughout as Mean ± standard error
of the mean (SEM) for each group. Differences between
means were evaluated using analyses of variance. For
comparisons between two means, Student's t-test was used.
For comparison of mean values obtained before and after
lesions within groups, a t-test for paired data was used.

2.8 Chemicals and Solutions Used

1. *Angiotensin-II*: 1-L-asparaginyl-5-L-isoleacyl
angiotensin was generously supplied by Dr. A. W. Castellion
of Norwich Pharmaceutical Company (Norwich, New York) in
its pure crystalline form. The quantity to be used in
each experiment was determined and measured out using a
lab electrobalance (Ventron Instruments, Los Angeles,
California) and transferred to a 5 ml glass vial. An
appropriate volume of sterile artificial CSF (see page 55)
was added; the solution was kept for no longer
than four weeks at 4°C. Angiotensin-II was used for
intracranial injections.

2. . *Synthetic Angiotensin-II:* l-L-asparaginyl-5-L-
valyl angiotensin-II amide (Hypertensin, CIBA) was obtained
in 6 ml factory-sealed glass vials. Each vial contained
2.5 mg angiotensin-II lyophilized in 47.4 mg mannitol and
0.1 mg thimerosal. For jugular vein infusions the powder
was reconstituted in the original vial by adding 5.0 ml
sterile 0.9% NaCl (Abbott, Chicago, Illinois). For intra-
cranial injections the powder was reconstituted by adding
5.0 ml sterile artificial CSF or sterile double distilled
water. The reconstituted stock solutions were kept at 4°C
for a maximum of four weeks, after which they were dis-
carded. Solutions of desired concentrations of angiotensin-
II were prepared as needed throughout the experiments by
adding appropriate volumes of the vehicle solvent.

3. *Angiotensin-II Competitive Antagonist:* l-sarcasyl-
8-alanyl angiotensin-II (P113) was provided by Norwich
Pharmaceutical Company in its pure crystalline form. Im-
mediately prior to use a small quantity of the drug was
dissolved in sterile artificial CSF to a concentration of
500 µg/ml. The solution was stored at 4°C for two weeks
and used for intracranial injection.

4. *Isoproterenol Hydrochloride (Winthrop, Aurora,
Ontario):* Fresh solutions of isoproterenol hydrochloride
(0.12-0.50 mg/ml in 0.9% NaCl) were prepared before each
series of injections. Isoproterenol was given subcutane-
ously in the area of the lower back or intraperitoneally

at doses of 0.07, 0.14, 0.15 and 0.21 mg/kg body weight.

5. *Polyethylene Glycol (P.G.) 20% in 0.9% Nacl:*
25 g polyethylene glycol 20,000 (J.T. Baker Chemicals Co.,
Phillipsburg, N.J.), and 1.13 g crystalline NaCl were
dissolved in 125 ml sterile distilled water and the solu-
tion was stored at 4°C in a sealed contained until used.
A small portion of the solution was warmed to room temper-
ature before injections. Injections were made at a dose
of 2.0 g/kg body weight subcutaneously to the area of the
lower back.

6. *NaCl Solutions:* 0.15-2.0 molar solutions (M)
were prepared by dissolving reagent grade sodium chloride
crystals in double distilled water. The solutions were
autoclaved and stored in small vials at 4°C. On the test
days, the solutions were warmed to room temperature and
injected either intracranially or intraperitoneally.

7. *Sucrose Solutions:* 0.15-4.0 molar
solutions were prepared by dissolving sucrose crystals
($C_{12}H_{22}O_{11}$; MW 342.00) in double distilled water. The
solutions were autoclaved and stored in small vials at
4°C. On the test days, the solutions were warmed to room
temperature and injected either intracranially or intra-
peritoneally.

8. *Renin:* Lyophilized hog renin (1 dog unit/mg;
General Biochemicals, Montreal, Quebec) was dissolved in

0.9% NaCl solution. The solution was
stored at 4°C in a 6 ml glass vial. On the days of
testing the solution was warmed to room temperature and
used for intracranial injections.

9. *Artificial CSF:* 1 liter of artificial CSF solu-
tion was prepared according to the formula given by Myers
(1974). Reagent grade salts were dissolved in distilled
water in the following amounts: NaCl (7.46 g), KCl (0.19 g),
$CaCl_2$ (0.14 g), $MgCl_2$ (0.19 g), $NaHCO_3$ (1.76 g), Na_2HPO_4
(0.18 g). The PO_4 and HCO_3 salts were dissolved
separately and added to 9/10 of the final solution.
Glucose ($C_6H_{12}O_6$; MW 180.16) was then added and the
solution was brought up to 1 liter. The stock solution
was autoclaved and then stored in a large flask at 4°C.
Small volumes were taken as needed for the intracranial
injections.

3.0 EXPERIMENTAL RESULTS AND DISCUSSIONS

The series of experiments which make up this study are described in the four parts of this section and the results obtained in each part are considered separately. One additional experiment is described in Appendix A. A general discussion and summary of the results are given in Section 4 and Section 5, respectively.

3.1 Lateral Hypothalamic Pathways Mediating Drinking in Response to Administration of ANG-II to the POA

Studies reviewed in Section 1 indicate that ANG-II is a dipsogenic hormone involved in extracellular thirst. A central locus for its dipsogenic action was suggested by the results of pioneering experiments in which copious drinking in water-replete rats was elicited when the hormone was microinjected into the region of the POA (Epstein *et al.*, 1970). Although these initial observations and the results of subsequent studies (Swanson and Sharpe, 1973) pointed to the POA and adjacent forebrain structures as having chemosensitive receptors for ANG-II, little data were available to indicate which neural pathways and integrative structures subserve the elicited drinking behavior.

The first series of experiments in this study was undertaken to investigate these questions. Since previous work showed that deficits in water intake initiated by cellular (Epstein and Teitelbaum, 1964; Epstein, 1971) and extracellular (Fitzsimons, 1966; Stricker and Wolf, 1967) stimuli of thirst resulted from bilateral lesions of the LH, it was decided to investigate whether pathways passing through the LH might be involved in mediating drinking behavior induced by the POA administration of ANG-II.

METHODS

1. Electrophysiological Recording Experiments

Initially, as a guide to the placement of lesions in the LH, it was decided to administer ANG-II through cannulae chronically implanted in the POA and to observe the effects of administering the hormone on the spontaneous discharge frequency of single neurons in the LH.

Eighteen rats were anesthetized with sodium pentobarbital (50 mg/kg body weight), preceded by atropine sulphate (5 mg), and implanted with a single 23 gauge stainless steel cannula into the POA using stereotaxic techniques described in Section 2.2. Following post-operative recovery, the rats were tested for the occurrence of drinking after the microinjection of 100 ng synthetic ANG-II dissolved in 1 µl distilled water through the indwelling POA cannula. Water intake (± 0.5 ml) was

then measured in the animal's home cage over the next 30 min.

Only those animals which drank more than 4 ml of water during each of the two preliminary tests with ANG-II were anesthetized with ethyl carbamate (1.2-1.5 g/kg body weight) and used in the recording experiments. In these animals (n = 13) ANG-II (100 ng in 1 μl distilled water) was microinjected into the POA through the implanted cannula and the effects of the hormone on the frequency of firing of neurons in the ipsilateral LH was measured. Action potentials of LH neurons were recorded extra-cellularly with stainless steel microelectrodes and frequency-time histograms were made. A maximum of 8 intracranial microinjections of ANG-II were made per animal, and an interval of 1 hr or more elapsed between two successive injections. Control injections of 1.0 μl distilled water were routinely made for every neuron from which recordings were made.

2. *Lesion Experiments*

In a second series of animals, lesions were made in the area of the LH in which unit responses to ANG-II had been recorded. The effects of these lesions and lesions in adjacent areas of the diencephalon on drinking elicited by POA administration of ANG-II were determined.

Forty-six rats were used. The animals were housed as described in Section 2.1 and were maintained on Purina

laboratory chow and tap water *ad libitum* except as noted below.

Under sodium pentobarbital (50 mg/kg body weight) anesthesia two 23-gauge stainless steel guide cannulae were implanted bilaterally into the preoptic region of each animal using stereotaxic techniques described in Section 2.2. Two monopolar electrodes made from size 00 insect pins and insulated except for the cross-sectional area at the tip were also implanted bilaterally into one of four areas in the diencephalon during the same operation. In 12 animals the electrodes were aimed at the MLH at the level of the dorsomedial nuclei. In a second group (n = 10) the electrodes were placed further lateral in the FLH in the area of the internal capsule and globus pallidus. The third group of rats (n = 12) were implanted with electrodes directed at the ventromedial nuclei of the hypothalamus and in the final group (n = 12) the electrodes were aimed at the dorsomedial thalamus.

On the ninth day after implantation of the cannulae and electrodes, food pellets and water bottles were removed from all animal cages and were replaced with food cups containing a measured weight of a high carbohydrate diet and 50 ml calibrated drinking tubes filled with tap water. For the next four days food and water intakes were determined daily. This procedure was repeated at selected four-day intervals throughout the experiment as indicated in Figure 1.
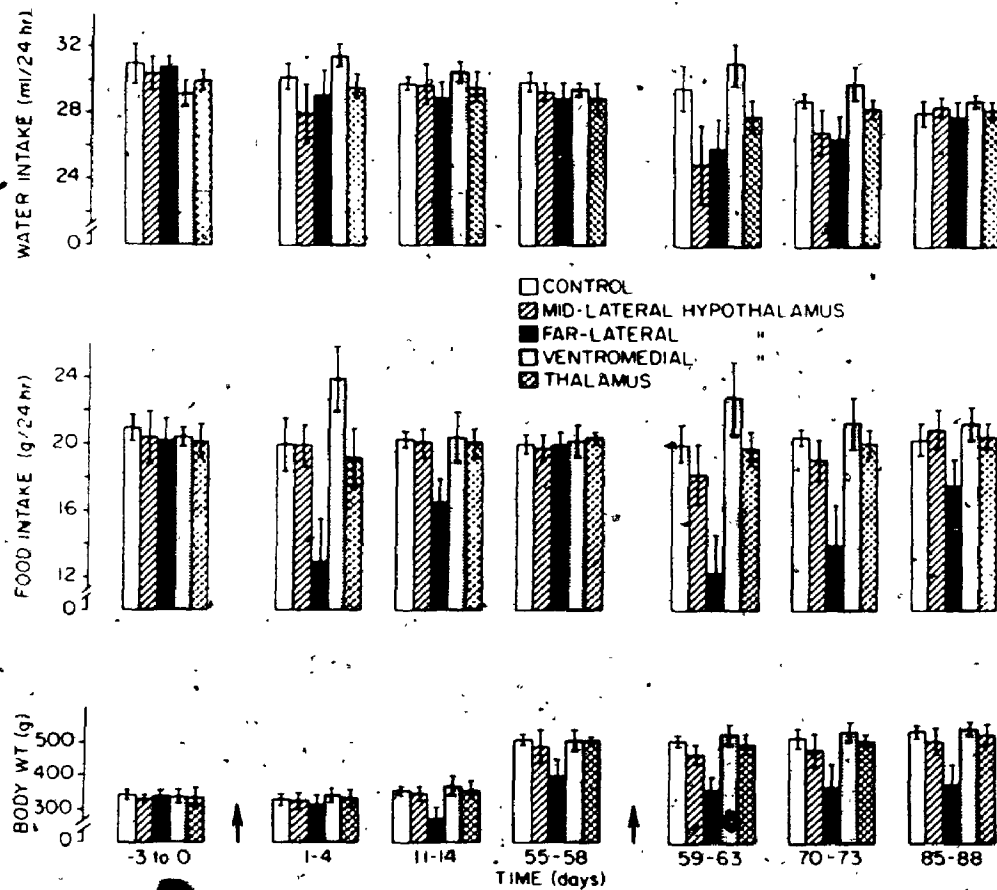
Figure 1

Mean daily intakes of water and food and body weights
for lesioned and sham-operated animals for 4 days pre-
ceding placement of first lesion (Days -3 to 0) (first
arrow) and for selected 4-day periods for Days 1-88 post-
operatively. A second lesion, contralateral to the first,
was made on Day 58 (second arrow). Vertical bars
represent ± SEM.

Beginning on the thirteenth postoperative day each animal was tested once daily for drinking following a unilateral microinjection of 100 ng synthetic ANG-II in 1 ul distilled water. Injections were made alternately through right and left cannulae. Water intakes (± 0.5 ml) were measured for the next 30 min in the animal's home cage with food freely available. Only those animals which drank more than 4 ml of water in each of four consecutive tests with ANG-II were retained for the lesion experiments.

Unilateral lesions were made in 32 rats under ether anesthesia by passing an anodal current of 2.5 mA for 15 sec between the implanted monopolar electrode and an indifferent electrode clipped to the ipsilateral ear. Testing with ANG-II was resumed one day after lesioning and was carried out daily for the next ten days, and at regular but less frequent intervals thereafter. A second lesion, contralateral to the first, was placed 58 days after the first lesion and testing with angiotensin was continued for an additional 34 days. At this time, a series of thirst challenges was carried out and at its completion on Day 138 a final intracranial test with ANG-II was made.

The first thirst challenge was produced by depriving rats of water for 24 hr and water intake was measured over the next 2 hr. Next, animals were induced to drink by a subcutaneous injection of isoproterenol (0.15 mg/kg

body weight) and the 2 hr water intake was measured. In the third test drinking induced by intraperitoneal injection of a 2 M NaCl solution (1% of body weight) was measured in a 1 hr test. Finally, the drinking response of rats to an intraperitoneal injection of renin (10 units hog renin) was measured over a 9-hr period. Control injections of equivalent volumes of 0.15 M NaCl were given for each test.

At the completion of the electrophysiological and lesion experiments, histological techniques as described in Section 2.6 were used to determine the locations of the electrodes and lesions. Data from six of the 32 animals receiving lesions were excluded from the results because tissue damage was not confined to the target structures.

Student's t-tests for correlated and uncorrelated samples were used to compare the responses of the different groups of animals in the lesion experiment.

RESULTS

1. Effect of POA Administration of ANG-II on LH Unit Activity

Extracellular recordings were obtained from 56 neurons in the LH of which 24 (43%) increased their rate of discharge in response to the administration of 100 ng synthetic ANG-II to the ipsilateral POA. Four neurons (7%) showed inhibition and the remaining 28 (50%) showed no change in their discharge frequency. Figure 2 shows the

location of iron deposits corresponding to the sites of
recording and Figure 3 shows a frequency-time histogram
for an MLH neuron which was facilitated by an injection
of ANG-II to the ipsilateral POA.

2. *Effects of the First Lesion on Drinking to POA*
   Administration of ANG-II

Unilateral lesions of the MLH caused a significant
decrease in water intake elicited by the microinjection of
ANG-II into the POA (t = 3.01, P < 0.01 in comparison with
sham-lesioned rats; t = 3.09, P < 0.01 in comparison with
pre-lesion intakes). As shown in Figure 4A the disruptive
effect of the lesion was significantly greater for the
administration of ANG-II on the side ipsilateral to
the lesion than for contralateral administration (t = 2.49,
P < 0.05). The deficit in elicited water intake also
persisted for a longer period of time on the ipsilateral
side, with recovery of the response occurring after
approximately four to five weeks; recovery to contra-
lateral administration was observed in all animals within
three weeks. In comparison to sham-lesioned animals, rats
with unilateral lesions of the MLH showed no significant
change in 24 hr food and water intakes or in the rate of
body weight increase with the exception of the immediate
postlesion period (Figure 1).

Unilateral lesions of the FLH also significantly
reduced water intake elicited by ANG-II, both in

Figure 2

Frontal sections through the LH redrawn from
the stereotaxic atlas of Pellegrino and Cushman
(1967) showing the sites of recordings and the
types of unit response observed following the uni-
lateral microinjection of 100 ng. ANG-II into the
ipsilateral POA. Solid triangles indicate sites
of facilitated units, solid squares indicate
sites of unit inhibition, and open circles mark
sites where no effect was obtained. Numbers beneath
each drawing are taken from the atlas and refer to the
number of mm anterior or posterior to bregma. Abbre-
viations: CC, corpus collosum; CL, internal capsule;
DMH, dorsomedial nucleus of hypothalamus; FX, fornix;
HPC, hippocampus; MT, mammillothalamic tract; OT, optic
tract; VMH, ventromedial nucleus of hypothalamus;
V, ventricle; ZI, zona incerta.
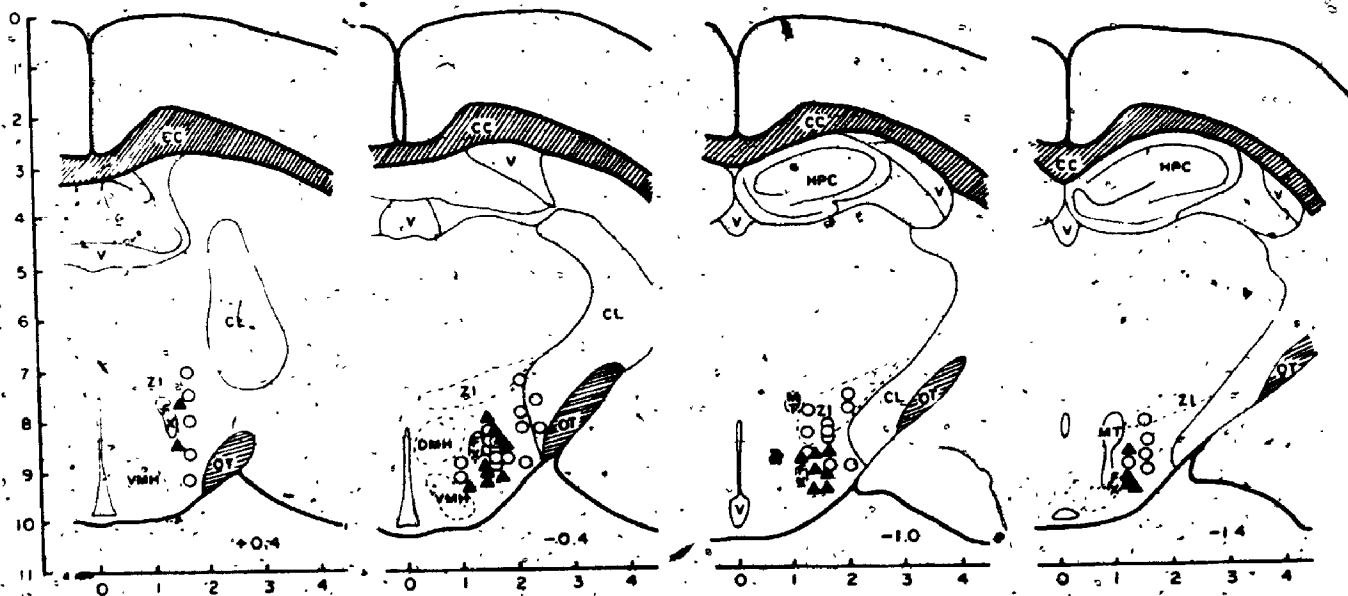
Figure 3

Frequency-time histogram showing the response
of a single neuron in the MLH to administration of
50 ng ANG-II to the ipsilateral POA.
The photomicrograph above the histogram shows the
location of the iron deposit corresponding to the site
of recording (arrow). Time of administration of
ANG-II is marked by the upward-pointing arrow which
appears beneath the histogram.

Figure 4.

Water intakes with (A) MLH and (B) FLH
lesions during the 30-min period following uni-
lateral microinjection of 100 ng ANG-II into
the POA. Results are expressed as mean ± S.E.M.
Designations "FIRST LESION" and "SECOND LESION"
refer to the first unilateral lesions of the MLH
and FLH and the second contralateral lesions of
the same structures, respectively. Experiments
on this series of animals were done concurrently
with those animal groups shown in Figure 5.

ANGIOTENSION INJECTIONS:
----- IPSILATERAL TO LESION
——— CONTRALATERAL TO LESION
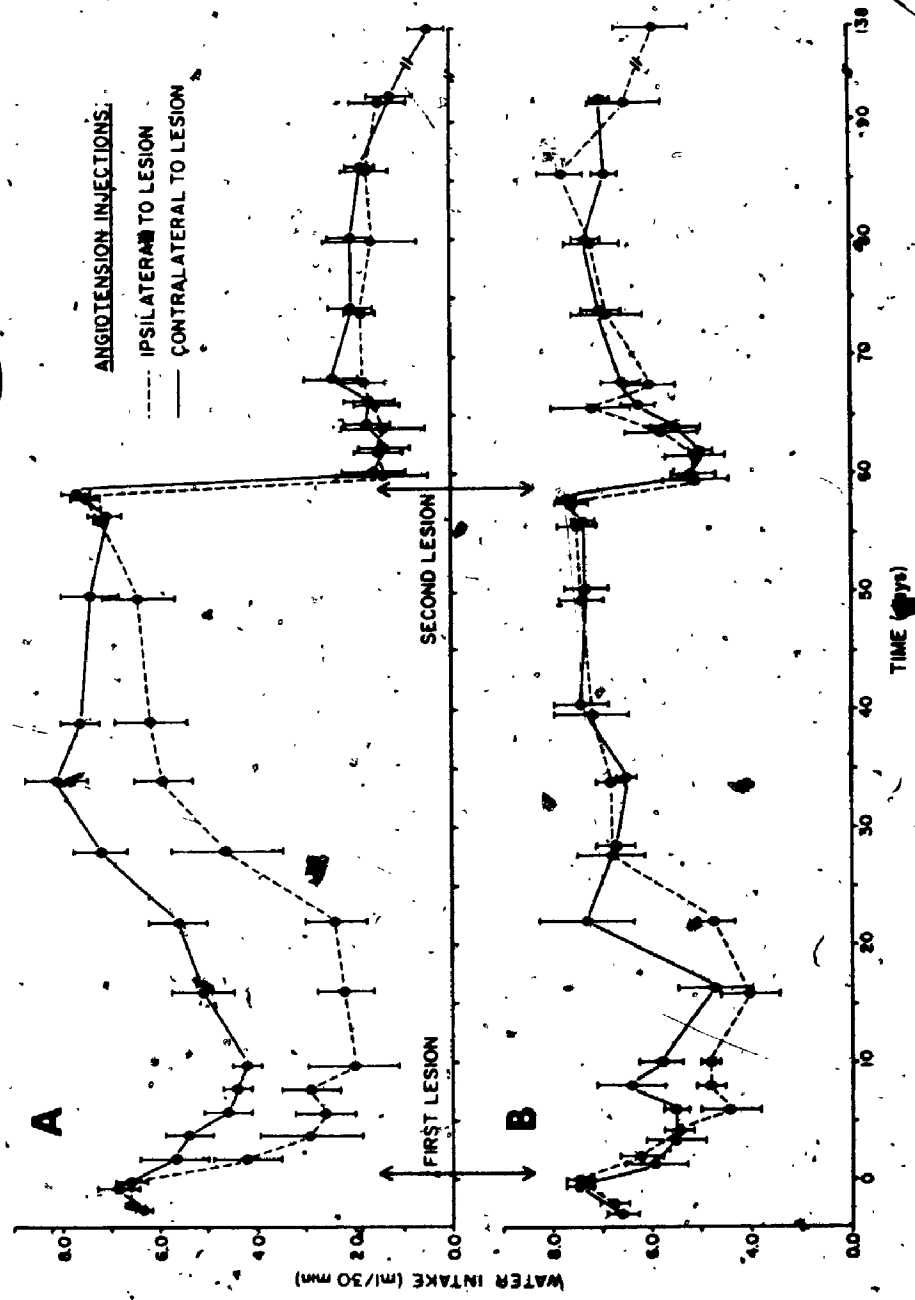
comparison with sham-lesioned animals (t = 2.42, $P < 0.05$) and with pre-lesion intakes (t = 2.39, $P < 0.05$). However, the attenuation of elicited drinking was significantly less severe (t = 2.26, $P < 0.05$) than in animals with lesions of the MLH. Daily water intakes did not change significantly after the first FLH lesion. In comparison with their 24 hr food intakes before the lesion, these animals ate significantly less (t = 2.28, $P < 0.05$) and showed a significantly reduced rate of body weight increase (t = 2.13, $P < 0.05$). Complete recovery of 24 hr food intakes was observed 9 to 17 days after lesioning although body weights remained significantly below those of sham-lesioned rats for the duration of the experiment.

Unilateral lesions of the ventromedial hypothalamus caused a significant increase in water intake elicited by ANG-II microinjected through either the contralateral or the ipsilateral POA cannula (t = 4.49, $P < 0.01$ in comparison with pre-lesion intakes; t = 4.32, $P < 0.01$ in comparison with sham-lesioned rats). The increased drinking response was observed in five of six animals on the first post-lesion test with ANG-II and continued for the duration of the experiments. As shown in Figure 1, rats with unilateral lesions of the ventromedial hypo-thalamus tended to show hyperphagia in the immediate post-lesion period but by the second week daily food intakes had returned to pre-lesion levels and were not signifi-

cantly different from sham-lesioned animals. The lesions also caused a mild hyperdipsia for four days following the lesion but thereafter daily water intakes returned to pre-lesion levels.

Unilateral lesions of the dorsomedial thalamus did not produce a significant change in the water intakes elicited by ANG-II (Figure 5) or in daily food and water intakes (Figure 1).

3. Effects of Second Lesion

Fifty-eight days after the first lesion was made, a contralateral lesion was placed in each animal through an indwelling electrode. The effects of the lesion on water intake elicited by ANG-II microinjected into the POA are shown in Figure 4 and Figure 5.

The second lesion of the MLH produced a severe and chronic decrease in water intake elicited by ANG-II administered through either POA cannula for the entire 34-day test period. During this time six of seven animals drank less than 15% of their pre-lesion intakes to ANG-II ($t = 3.60$, $P < 0.01$). In a final test with ANG-II on Day 138, four of six rats with MLH lesions failed to respond by drinking and the other two animals drank only 1.0 and 1.5 ml, respectively. The changes in daily food and water intakes after the second MLH lesion were similar to those observed after the first lesion. Within seven to ten days following placement of the lesion 24 hr water

Figure 5

Water intakes of rats with lesions of the
ventromedial hypothalamus (VMH) and thalamus and
of sham-operated controls during 30-min period
following unilateral microinjection of 100 ng
ANG-II. Results are expressed as means ± S.E.M.
of consecutive ipsilateral and contralateral
injections. The designations "FIRST LESION" and
"SECOND LESION" refer to the times at which the
first unilateral lesions and the second contra-
lateral lesions, respectively, were made.
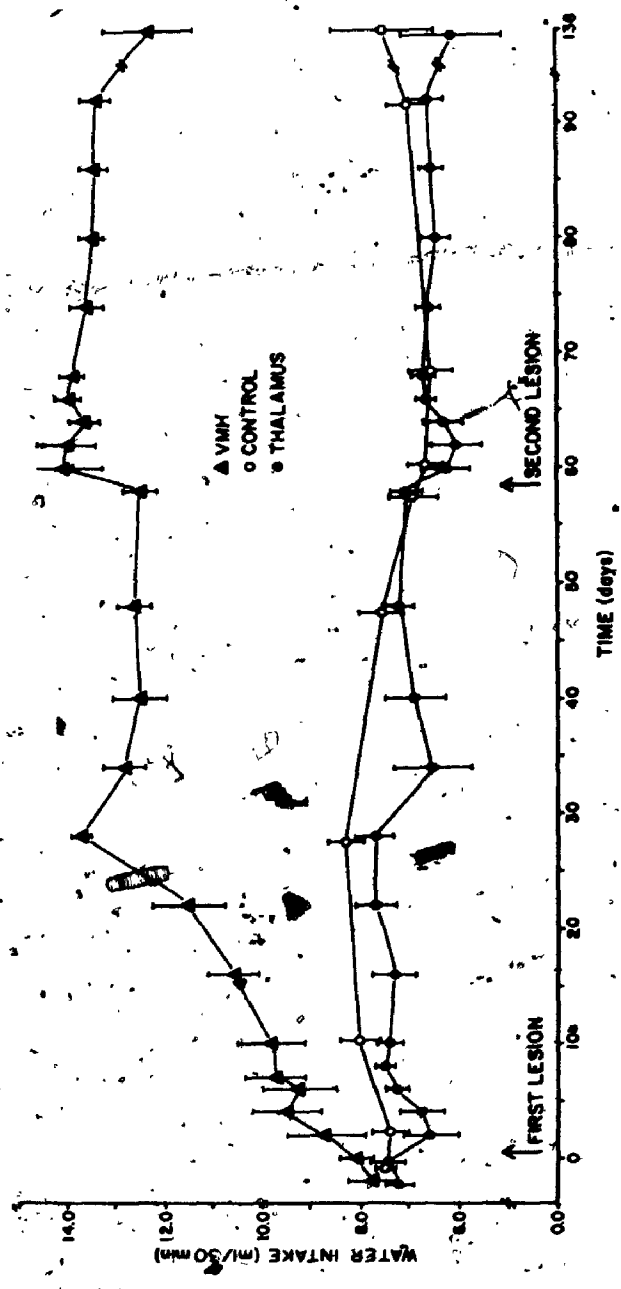
intakes had recovered and the rats began to gain in body weight. Daily food intakes were also lower (not significant) for the first week after the lesion was made.

As shown in Figure 4B the placement of a second lesion in the FLH caused a significant reduction in elicited water intake in the immediate post-lesion period ($t = 2.29$, $P < 0.05$ in comparison with elicited water intake of sham-lesioned rats for the same post-lesion period). The water intake elicited by ANG-II, however, was significantly greater ($t = 2.17$, $P < 0.05$) for these animals than for rats with MLH lesions. Recovery of the elicited response in rats with FLH lesions was complete within 14 days. In the final test with ANG-II, the mean water intake of these animals was not significantly different from sham-lesioned rats. In contrast to the relatively mild disruption of feeding produced by lesions of the MLH, animals with lesions placed further lateral in the hypothalamus exhibited a hypophagia of varying durations. Two rats in this group required two and three days of intragastric feeding on Days 71 and 72 and 71 through 73, respectively. In further contrast to the effects produced by MLH lesions, lesions placed in the FLH caused a lesser degree of hypodipsia in the period immediately following placement of the lesion.

The water intakes induced by ANG-II administered through either POA cannula were significantly increased

in all six animals given a second ventromedial hypothalamic
lesion (t = 3.37, $P < 0.01$ in comparison with sham-lesioned
rats; t = 3.01, $P < 0.01$ in comparison with pre-lesion
intakes). This effect was greatest during the immediate
post-lesion period and declined slowly thereafter to a
level 15% to 25% higher by the second week than for the
four-day period immediately before the second lesion. In
the final test with ANG-II ventromedial hypothalamic-
lesioned rats drank significantly more water (t = 2.40,
$P < 0.05$) than did sham-lesioned controls. As shown in
Figure 1 a second lesion of the ventromedial hypothalamus
caused an increase in 24 hr food and water intakes and an
accelerated rate of body weight increase; however, the
variability of these effects was great and the differ-
ences were not statistically significant.

A second, contralateral lesion of the dorsomedial
thalamus did not significantly change the response of
animals with such lesions to ANG-II, nor did the
lesions have any significant effects on daily food and
water intakes or on body weights.

4. *Effects of Lesions on Water Intakes Induced by
Cellular and Extracellular Thirst Stimuli*

Water depriving rats for 24 hr did not produce any
statistically significant differences between the groups
in a subsequent 1 hr drinking test, although on the
average, animals with ventromedial hypothalamic lesions

drank slightly more water than did sham-lesioned rats and
animals with MLH and FLH lesions drank slightly less
(Figure 6).

The effect of a single subcutaneous injection of the
β-adrenergic stimulant, isoproterenol, on water intake in
a 2-hr drinking test is shown in Figure 6. Animals with
lesions of the ventromedial hypothalamus drank signifi-
cantly more water than sham-lesioned rats (t = 3.46,
$P < 0.01$). In contrast, rats with lesions of the MLH
drank 30% as much water as sham-lesioned animals (t = 3.23,
$P < 0.01$) and 15% as much water as VMH lesioned rats
(t = 3.71, $P < 0.01$). Mean water intakes for rats with
FLH or with dorsomedial thalamic lesions did not differ
significantly from the sham-lesioned group following
isoproterenol.

As shown in Figure 6 lesions of the FLH significantly
attenuated water intake in response to a single peripheral
injection of 1 M NaCl (t = 2.37, $P < 0.05$, in comparison
with sham-lesioned rats). Lesions of the MLH, or ventro-
medial or dorsomedial thalamus had no significant effect
on the response to hypertonic NaCl during the 2-hr
drinking test.

The drinking responses of the different groups of
rats to a single peripheral injection of renin were
similar to those observed following isoproterenol.
Animals with lesions of the ventromedial hypothalamus

Figure 6

Means intakes of water of sham-operated and
lesioned groups following

A   24-hr water deprivation (2-hr intake)

B   single subcutaneous injection of
      isoproterenol (2-hr intake)

C   single intraperitoneal injection of
      hypertonic NaCl (1-hr intake)

D   single intraperitoneal injection of renin
      (9-hr intake)

Vertical bars represent ± S.E.M. Numbers
over columns refer to the number of animals in
that test.
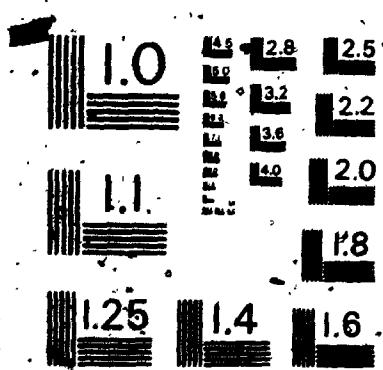
MICROCOPY RESOLUTION TEST CHART

NATIONAL BUREAU OF STANDARDS-1963-A

ARC-374
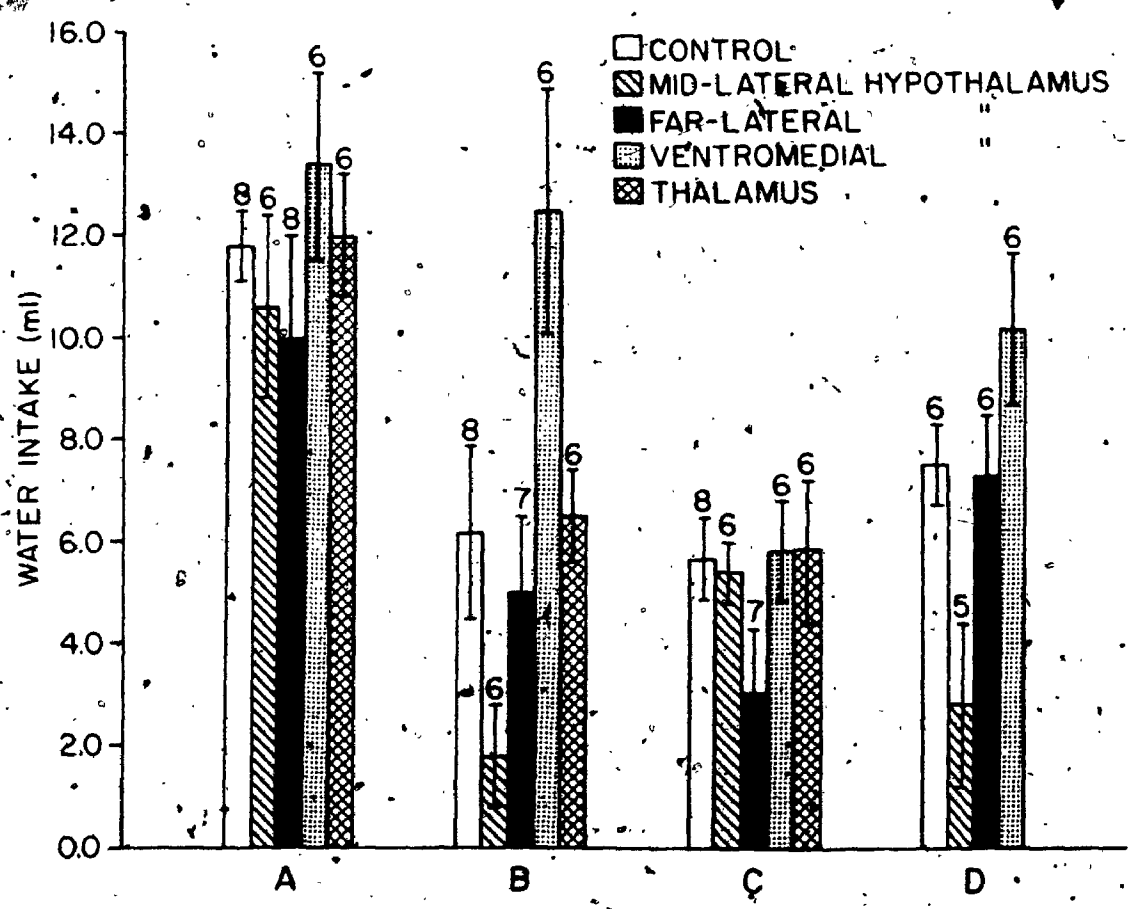
drank significantly more water than sham-lesioned rats
($t = 3.69$, $P < 0.05$), whereas animals with MLH lesions
drank significantly less ($t = 2.59$, $P < 0.05$). Mean
water intakes for FLH-lesioned rats following renin did
not differ significantly from sham-lesioned controls.

6. *Histology*

There was little variation in the location of the
cannulae in the POA (Figure 7). In each case the tip of
the cannula was in a region bounded dorsally by the
anterior commissure, ventrally by the optic chiasm and
extending rostrocaudally from the most rostral extent of
the third ventricle to the widest frontal extent of the
anterior commissure. In frontal sections the medial and
lateral edges of the POA defined the borders of the
cannula tip. Moderate damage to the cortex adjacent to
the walls of the longitudinal cerebral fissure and to
the corpus collosum was produced along the paths of the
cannulae. Incidental damage to other structures lying
near the POA was also observed in most cases. In about
50% of the brains the ventricles were enlarged, probably
a result of repeated injections of the ANG-II solution.
There was, however, no correlation between the disruption
of drinking induced by ANG-II and the degree of
ventricular damage.

The lesions produced through the chronic electrodes
extended 0.5-1.0 mm in the ventrodorsal plane, 0.3-1.0 mm

Figure 7

Representative photomicrographs showing
locus of cannulae in the preoptic region (POA)
and location of lesions in the midlateral (MLH)
and farlateral (FLH) hypothalamus (in each case
designated by arrows). Magnification 7 times.

FLH

MLH

POA

2 mm

laterally, and 0.5-1.5 mm rostrocaudally (Figure 7 and
Figure 8). However, it should be noted that lesions
produced in neural tissue contract greatly during pro-
longed postoperative periods (Greene, Stauff and Walters,
1972). In this study the rats were not sacrificed until
about six months after the first lesions were made, and
it is very likely that the initial area of destruction
was considerably larger than it appeared in histological
sections.

The most effective lesions for producing a disrup-
tion of drinking in response to ANG-II were located in
the lateral hypothalamic area, just dorsolateral to the
fornix and at the level of the dorsomedial nuclei. The
mammillothalamic tracts and the fornix were undamaged in
all brains and the MLH lesions were symmetrical and similar
in appearance in each case.

Lesions of FLH produced damage to the ventromedial
part of the internal capsule and the medial edge of the
globus pallidus. In seven of eight animals the lateral
aspect of the LH was also destroyed bilaterally. In the
remaining animal the lesions were asymmetrical, with only
partial ablation of the internal capsule, globus pallidus
and farlateral LH on one side.

The lesions produced through the electrodes aimed at
the ventromedial hypothalamus destroyed, in all cases, the
dorsomedial portion of the ventromedial hypothalamic

Figure 8

Location of lesions in the midlateral hypo-
thalamus (MLH); farlateral hypothalamus (FLH), and
ventromedial hypothalamus (VMH) marked on sections
of rat brain taken from the stereotaxic atlas of
Pellegrino and Cushman (1967). Portions shaded
in black indicate the area common to all lesions,
and black outline indicates largest extent of all
lesions. Abbreviations: CC, corpus collosum;
CI, internal capsule; DMH, dorsomedial hypothalamus;
FX, fornix; MT, mammillothalamic tract; OT, optic
tract; V, ventricle; ZI, zona incerta.

FLH

VMH
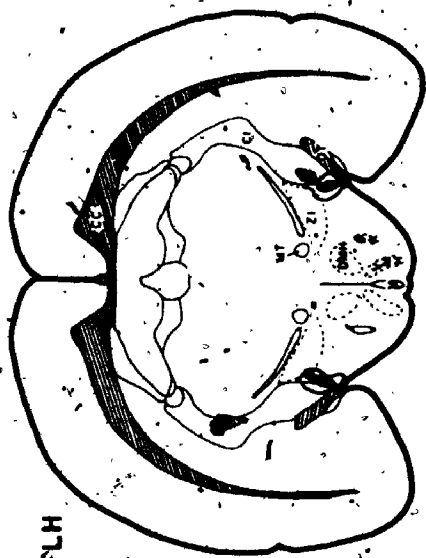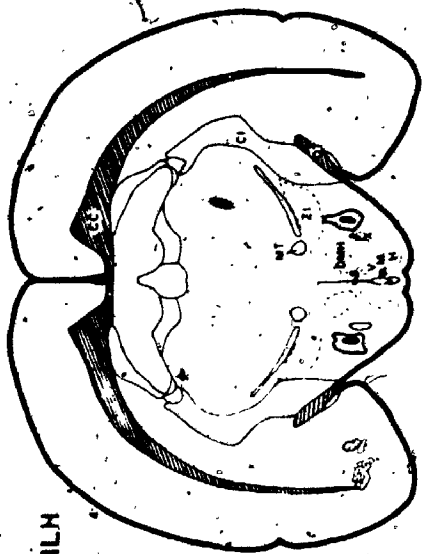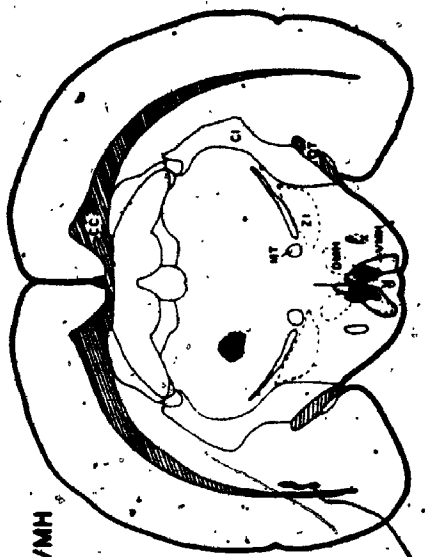
MLH

nuclei bilaterally. In three of the brains the baso-
lateral portion of the third ventricle was also damaged,
a consequence of angling electrodes into the basal hypo-
thalamus. There was, however, no correlation between the
disruption of elicited water intake and the degree of
ventricular damage in these rats.

DISCUSSION

The results of the experiments suggest that thirst
signals from ANG-II receptive sites in the POA are
directed to the MLH. Two sets of observations support
this proposal. First, microinjection of ANG-II through
cannulae chronically implanted into the POA of rats which
were previously tested for drinking behavior influenced
the activity of single neurons in the LH, and particularly
in the MLH. Second, lesions placed in the MLH signifi-
cantly attenuated water intake to POA administration of
ANG-II, whereas ablation of diencephalic structures
located ventral, dorsal and lateral to the MLH did not have
the same disruptive effect. Moreover, since the
attenuation of water intake after unilateral lesions of
the MLH was significantly greater to ipsilateral POA
administration of ANG-II than to contralateral POA injec-
tions, it may be suggested that the neural pathway
mediating the elicited drinking is directed primarily
ipsilateral to the receptive sites for ANG-II in the POA.

The results also demonstrate that ANG-II-induced drinking recovers from the disruption produced by a unilateral lesion of the LH. Whether this recovery is spontaneous or related to the continuance of testing with ANG-II, or some other factors, cannot be determined at the present time. It is interesting to note, however, that the recovery of the drinking response to ANG-II after a unilateral LH lesion is analogous to the recovery of daily food and water intake observed after bilateral ablation of the LH (Teitelbaum, Cheng and Rosin, 1969). Recently, it has been observed that the recovery process in the latter case may be accelerated by various procedures, including prelesion experience (DiCara, 1970; Glick and Greenstein, 1972a), pretreatment with α-methyl-p-tyrosine (Glick, Greenstein and Zimmerberg, 1972) and prior lesioning of the frontal cortex (Glick and Greenstein, 1972b). One possible explanation of these findings is that the recovery process is mediated by denervation supersensitivity (Glick, Greenstein and Zimmerberg, 1972). Alternatively, it may be due to the regrowth of neural connections, such as has been demonstrated by Moore, Bjorkland and Stenvi (1971) for lesions of the septal area. It should be noted that each of these interpretations of the basis for the observed recovery suggests that ascending neural pathways might be involved in the elicited response (Mogenson, 1974). This possibility is

considered further on p. 195.

Lesions of the MLH also produced an attenuation of water intake elicited by the peripheral injection of isoproterenol or renin but not of water intake to peripherally administered hypertonic NaCl. Ablation of the FLH, on the other hand, caused an attenuation of drinking to hypertonic NaCl but did not change water intake elicited by isoproterenol or renin. These observations are consistent with a recent report of Sclafani, Berner and Maul (1973) who showed that knife cuts in the medial region of the LH disrupted drinking to extracellular dehydration (administration of polyethylene glycol) without disrupting drinking to cellular dehydration (administration of hypertonic NaCl).

In contrast to the disruptive effects of MLH lesions on water intake induced by extracellular stimuli of thirst, animals with lesions of the ventromedial hypothalamus drank significantly more than control rats following a single peripheral injection of isoproterenol or renin or after the central administration of ANG-II. Grossman (1966) has proposed that ventromedial hypothalamic lesions produce a general disinhibition so that animals overrespond to a variety of stimuli. This hypothesis does not account for the findings obtained in the present study, however, since rats with lesions of the ventromedial hypothalamus did not drink any more than the other groups in

response to hypertonic NaCl and only slightly more follow-
ing 24-hr water deprivation. Furthermore, the changes in
daily food and water intakes in ventromedial hypothalamic-
lesioned rats were minor, although this may have been due,
in part, to the fact that the lesions were placed in two
stages (Greene et al., 1972).

The differential effects of MLH and FLH lesions on
ad libitum feeding also suggest that the lesions impinge
on different neural systems for the regulation of food
intake. As shown in Figure 1 rats with lesions of the FLH
showed a transient post-lesion aphagia while MLH lesions
had no effect. The possible neural mechanisms involved
are discussed in the next section on pp. 119-120.

In summary, the findings of the present experiments
suggest that at least two neural pathways participate in
the initiation of drinking to centrally-administered
ANG-II. One pathway projects caudally from the POA through
the MLH and appears to be involved in the facilitation of
drinking to ANG-II. When this pathway is disrupted by
lesions of the MLH the drinking to ANG-II is attenuated.
On the other hand, since lesions of the ventromedial hypo-
thalamus produce an increase in water intake to ANG-II,
the ventromedial hypothalamus appears to be part of a
neural system that inhibits drinking elicited by the
hormone. The ventromedial hypothalamus, in turn, could
interact with the LH directly (Morgane, 1975) or influence
water intake via caudal projections to the midbrain

(Mogenson, 1974). Finally, the results also suggest
that drinking to cellular thirst stimuli is mediated
through neural pathways passing through the FLH. This
possibility will be considered further in the next
section.

3.2   Specificity of the Disruption of Cellular and Extra-
cellular Thirst following Lesions of the MLH and FLH

The results of the experiments reported in Section
3.1 indicate that thirst signals from receptive sites for
ANG-II in the POA are transmitted to the MLH. This
possibility was suggested initially by the observation
that neurons in the MLH changed their rate of firing when
ANG-II was microinjected into the ipsilateral POA and was
subsequently confirmed by the finding that the elicited
drinking was significantly attenuated by MLH lesions. An
unexpected finding was that lesions of the MLH and FLH
had a differential effect on drinking to cellular and
extracellular thirst challenges. Lesions of the MLH
disrupted drinking to extracellular stimuli, whereas
lesions placed further lateral selectively impaired water
intake elicited by cellular thirst signals. From these
results it was postulated that cellular and extracellular
thirst signals are mediated by separate neural pathways
at the level of the LH.

This hypothesis was examined further in the series
of experiments presented in this section. In the first
experiment single unit recordings were made simultane-
ously in the MLH and FLH through two recording micro-
electrodes and the responses of the units to POA adminis-
tration of cellular (hypertonic NaCl and sucrose) and
extracellular (ANG-II) thirst stimuli were observed.

Subsequently, in a second series of animals, the question
of whether the disruption of elicited drinking following
LH lesions was due to interruption of neural pathways or
resulted from non-specific brain damage was investigated.
In order to see whether the deficits in thirst following
FLH lesions resulted from a greater susceptibility of the
animals to pain and stress following peripheral injections
of strongly hypertonic solutions (Marshall, Richardson and
Teitelbaum, 1974; Stricker, 1976), various doses of NaCl
and sucrose were given peripherally to FLH-lesioned rats
and the volumes of water consumed after each dose were
measured. A second series of FLH-lesioned rats was tested
for drinking following the administration of NaCl and
sucrose by intracranial microinjection, a method of
inducing thirst which is thought to be non-stressful
(Blass, 1973). Finally, the water intakes of a third
series of MLH- and FLH-lesioned animals to central admin-
istration of various doses of hypertonic NaCl and sucrose
and to ANG-II were compared.

METHODS

1. Electrophysiological Recording Experiments

Sixteen rats were anesthetized with sodium pento-
barbital (25 mg/kg) preceded by ketamine hydrochloride
(80 mg/kg) and implanted with a single 23-gauge cannula
into the POA using stereotaxic procedures described in

Section 2.2. Following postoperative recovery the rats were tested for the occurrence of drinking after the unilateral microinjection of ANG-II (5-Ileu angiotensin II, 50 ng dissolved in 0.2 μl artificial CSF) and hypertonic NaCl (1M) or sucrose (2M) (both 2 OSM in 0.2 μl distilled water) through the implanted cannula. Water intake (± 0.5 ml) was then measured in the animal's home cage over the next .15 min.

The animals which drank more than 4 ml of water in response to ANG-II and more than 2 ml of water in response to hypertonic NaCl and sucrose during each of two preliminary drinking tests were anesthetized with ethyl carbamate (1.2-1.5 g/kg) and used for microelectrode recording. In these animals (n = 12) ANG-II and hypertonic NaCl or sucrose was microinjected into the POA through the implanted cannula and the effects of the injection on single unit activity in the ipsilateral MLH and FLH were recorded using techniques described in Section 2.4. Control injections of equivalent volumes of artificial CSF or 0.15 M NaCl were made.

2. *Lesion Experiments*

a. *Peripheral administration of sucrose, NaCl and isoproterenol*. Bilateral lesions were made in two stages (with an intervening period of 5-7 days) in the FLH of 18 rats anesthetized with sodium pentobarbital (25 mg/kg body weight) preceded by ketamine hydrochloride

(80 mg/kg body weight). An anodal current of 1.5 mA for 15 sec was passed between a stainless steel monopolar electrode and an indifferent electrode clipped to the ipsilateral ear. Another group of 8 animals served as sham-operated controls.

Following postoperative recovery the animals were tested for drinking in response to single intraperitoneal (i.p.) injections of sucrose (doses of 0.3, 0.5, 1.0, 2.0, 4.0 M; 0.5% body weight), NaCl (0.15, 0.25, 0.5, 1.0, 2.0 M; 0.5% body weight), isoproterenol (0.07, 0.14, 0.21 mg/kg body weight) and to 24- and 48-hr water deprivation. Finally, drinking elicited in response to 24- and 48-hr water deprivation followed by an injection of 2.0 M NaCl (0.5% body weight, i.p.) was measured. Water intakes (± 0.5) were recorded in each test for a 60-min period following administration of the challenge in the animal's home cage. Tests were conducted between 9:00 and 11:00 a.m. on alternate days with the order of tests randomly determined.

b. *Central administration of sucrose, NaCl and*

angiotensin II. In a series of 36 rats bilateral 23-gauge cannulae were positioned into the preoptic region at an angle to by-pass the lateral ventricles (angled POA cannulae). In 10 additional rats cannulae were placed into the POA passing through the lateral ventricles (straight POA cannulae). The stereotaxic procedures have

been described previously in Section 2.2.

Beginning 5-12 days postoperatively, the animals were
tested daily for the occurrence of drinking following the
unilateral microinjection of ^{5}Ileu angiotensin II (doses of
0, 25, 50, 100, 500 ng, all dissolved in 0.2 µl artificial
CSF), sucrose (0.3, 0.5, 1.0, 2.0, 4.0 M, dissolved in
0.2 µl distilled water) or NaCl (0.15, 0.25, 0.5, 1.0,
2.0 M, dissolved in 0.2 µl distilled water) into the POA.
Water intakes (± 0.5 ml) were measured over a 30-min
period in the animal's home cage with food freely avail-
able.

At the completion of the pre-lesion tests, bilateral
lesions were made in two stages (with an intervening
period of 3-8 days) in the MLH (n = 30) or in the FLH
(n = 16). An anodal current (1.0-1.5 mA) was passed
through the stainless steel electrode for 10-25 sec using
an indifferent electrode clipped to the ipsilateral ear.
Drinking tests were resumed 3-8 days after the second
lesion.

3.   *Histology and Statistical Analysis*

Animals in both the microelectrode recording and
lesion experiments were sacrificed by an overdose of sodium
pentobarbital. Cannula and lesion placements and sites of
unit recordings were determined histologically according
to procedures described in Section 2.6.

Analyses of variance and *t*-tests were used to compare
the responses of the animals in the lesion experiments.

Data from 14 of the rats in the lesion experiments were not included in the analyses because the tissue damage produced by the lesions was not confined to the target structures (n = 10) or because the location of the tips of the cannulae could not be determined accurately (n = 4).

RESULTS

1. Dual MLH-FLH Single Unit Recordings

Since extracellular recordings were made for only 16 pairs of single neurons in the MLH and FLH for periods of time sufficiently long to test for the effects of administering ANG-II (n = 16), 1 M NaCl (n = 6) or 2 osM sucrose (n = 10) to the ipsilateral POA*, no conclusions may be drawn. Rather the data reported in Table 2 and in Figures 9 and 10 are included because the dual recording technique appears to be a potentially valuable technique for studying electrophysiologically whether different neural structures mediate cellular and extracellular thirst stimuli.

---

*As shown in Table 2, the number of MLH units which responded to POA microinjection of ANG-II (6) was higher than the number of FLH units (1). Conversely, more units in the FLH were influenced by POA administration of 1 M NaCl (4) or 2 M sucrose (6) than were MLH units (2 and 2, respectively). The results of one dual MLH-FLH recording experiment are shown in Figure 9. Figure 10 shows the sites of microelectrode recordings for the same animal.

TABLE 2

Numbers of Units in the MLH and FLH Facilitated, Inhibited or Showing No Effect following the Microinjection of ANG-II, NaCl, Sucrose and Artificial CSF[1]

Test Solution Administered[2] (dose and volume)	Pairs of Units Recorded	Sites of Recordings and Direction of Responses					
		MLH			FLH		
		Facili- tated	Inhib- ited	No Effect	Facili- tated	Inhib- ited	No Effect
ANG-II (50 ng/0.2 µl)	16	6	0	10	1	0	15
NaCl (1 M/0.2 µl)	6	0	2	4	3	1	2
Sucrose (2 M/0.2 µl)	10	1	1	8	4	2	4
Artificial CSF (0.2 µl)	5	1	0	4	0	1	4
NaCl (0.15 M/0.2 µl)	4	0	1	3	1	0	3

[1] Recordings of single unit activity in the MLH and FLH were made simultaneously.

[2] As is evident from the Table, not all "pairs" of units were tested with each of the five solutions. In each case, however, ANG-II and either 1 M NaCl or 2 M sucrose was administered.

Figure 9

Frequency-time histograms showing the responses
of single units in the MLH (left panel) and FLH
(right panel) to ipsilateral microinjection of ANG-II
(50 ng in 0.2 µl CSF), 1 M NaCl (0.2 µl distilled
water), 2 M sucrose (0.2 µl distilled water) and
artificial CSF (0.2 µl). Time of administrations is
indicated by arrows. Recordings were made simultane-
ously from sites in the MLH and FLH shown in Figure 10.

ANG II

NaCl

SUCROSE

CSF

AP/SEC

10

0

60 sec
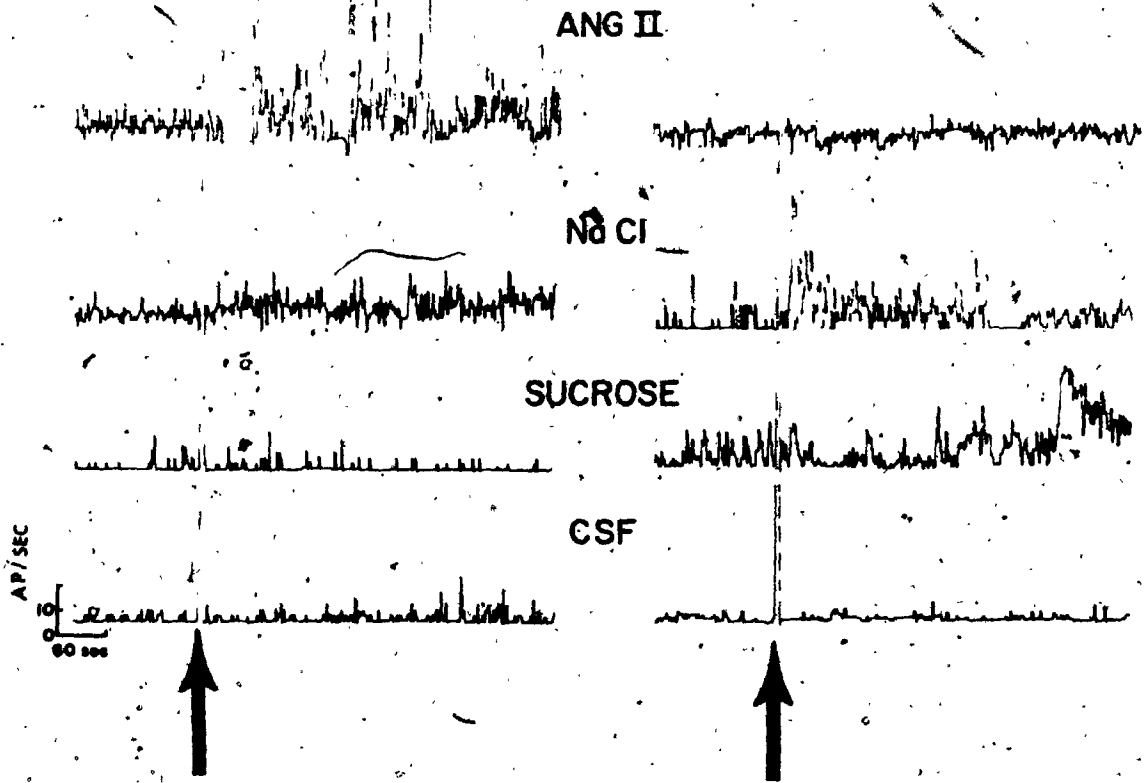
Figure 10

Photomicrograph showing the sites of unit
recordings (arrows) in the MLH (nearest midline)
and FLH from which the responses shown in
Figure 9 were obtained.

2. *Effect of Lesions of the FLH on Water Intakes Elicited.*
   by Cellular and/or Extracellular Stimuli of Thirst

a. <u>*Peripheral administration of thirst challenges*</u>.

The effects of FLH lesions on drinking elicited by the administration of sucrose and NaCl (i.p.) are shown in Figure 11A. In comparison with sham-operated controls, rats with lesions of the FLH drank significantly less water in response to sucrose or NaCl. Moreover, the intakes of water of lesioned animals were not dose-related whereas those of control rats increased significantly with the dose administered (sucrose: $F = 3.12$, df 4/28, $P < 0.05$; NaCl: $F = 15.0$, df 4/28, $P < 0.01$).

Following 24 and 48 hr water deprivation there were no significant differences between lesioned and control animals when water intake was measured for 60 min following the termination of the deprivation period (Figure 11B), although on the average the intakes of FLH-lesioned rats were less than controls following either 24 or 48 hr deprivation (mean intakes: 8.4 *vs*. 12.0 ml and 12.4 *vs*. 17.9, respectively). However, when the two periods of water deprivation were followed by a single i.p. injection of 2 M NaCl, FLH-lesioned animals drank significantly less water than controls given the same combined challenges.

The effects of single i.p. injections of the β-adrenergic agonist, isoproterenol, on water intakes in a subse-

Figure 11

A. Water intakes (ml/60 min) of sham-operated rats
   (n = 8) (open columns) and FLH-lesioned rats
   (n = 16) (diagonal-lined columns) following single
   intraperitoneal injections of sucrose and NaCl.
   Results are expressed as means ± SEM, $*P < 0.05$
   as compared with control values, $**P < 0.01$ as
   compared with control values.

B. Water intakes (ml/60 min) of same rats as in
   Figure 10A when subjected to 24 and 48 hr of water
   deprivation. Stippled and cross-hatched columns,
   respectively, represent additional water intakes
   when sham-operated and FLH-lesioned rats were
   challenged by an osmotic thirst stimulus, (NaCl,
   2 M   0.5% of body weight, i.p.) immediately
   following the water deprivation. Vertical bars
   represent ± SEM for the respective treatments,
   $*P < 0.05$ as compared with intakes of controls
   given the same challenges.

C. Water intakes (ml/60 min) of same rats as in
   Figure 11A following single i.p. injections of
   isoproterenol. Vertical bars represent ± SEM.
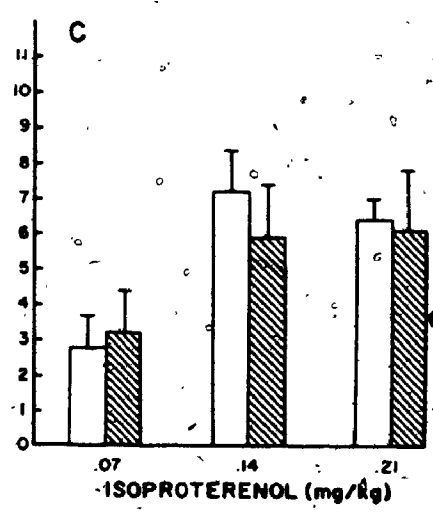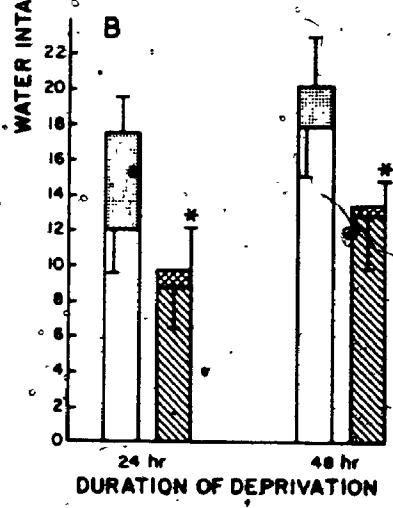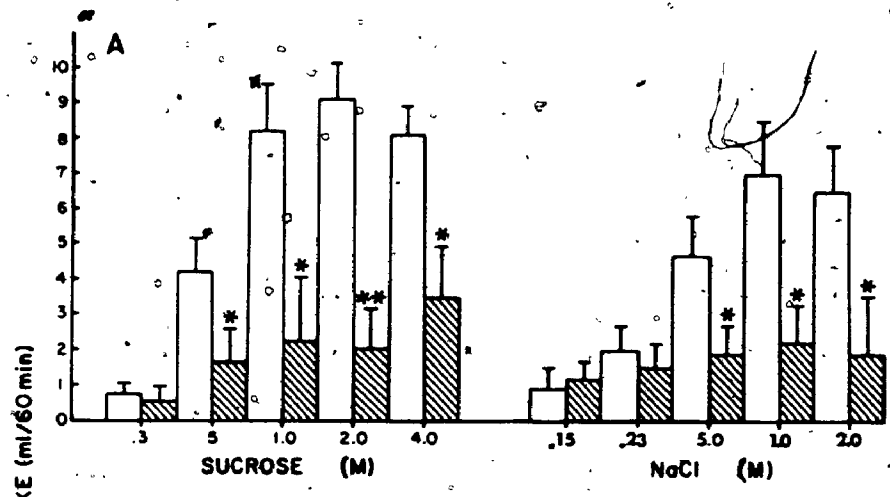
quent 60 min drinking test are presented in Figure 11C.
Mean water intakes of the FLH-lesioned and control
animals did not differ significantly for the three doses
of isoproteronol.

b. *Central administration of thirst challenges.*
Water intakes induced by the microinjection of ANG-II,
sucrose and NaCl through straight POA cannulae were not
significantly different from the mean intakes elicited
by POA administration through angled cannulae (see
Figures 12, 13 and 14, top panels). Therefore, the results
have been combined.

In comparison with prelesion values, lesions of the
FLH caused a significant decrease in water intakes
elicited by the microinjection of sucrose and NaCl into
the POA (Figure 12 and Figure 13). Furthermore, in com-
parison to prelesion water intakes, in which a significant
dose-response relationship was obtained, following FLH
lesions the intakes of rats in response to high doses
were not significantly different from low doses. Lesions
of the FLH did not cause any significant changes in the
mean volumes of water intakes elicited by the micro-
injection of ANG-II through straight or angled POA
cannulae when compared with prelesion values. Water
intakes elicited by ANG-II were significantly increased
with increasing doses of the hormone for both the prelesion

and postlesion periods (F = 60.0, df 4/36, $P < 0.01$;
F = 29.6, df 4/36, $P < 0.01$, respectively).

3.   *Comparison of the Effects of FLH and MLH Lesions on*
     Drinking Induced by Centrally Administered ANG-II,
     Sucrose and NaCl

In comparison to animals with lesions of the MLH,
rats in which the FLH was lesioned drank significantly
less water following microinjections of sucrose ($P < 0.05$
for all doses greater than 0.3 M)   and NaCl ($P < 0.05$ for
all doses greater than 0.25 M)   administered to the POA
(Figure 13 and Figure 14).   Lesions of the FLH did not
significantly affect drinking in response to centrally ad-
ministered ANG-II ($P > 0.10$ in comparison with MLH-lesioned
rats for all doses of ANG-II).   In contrast to the disrup-
tive effect of FLH lesions on drinking induced by osmotic
stimuli of thirst, ablation of the MLH caused a significant
attenuation of water intake elicited in response to micro-
injection of ANG-II into the POA ($P < 0.05$ in comparison
with FLH-lesioned animals for ANG-II at doses of 25, 50,
100 and 500 ng), but had little or no effect on water
intake to centrally administered sucrose or NaCl.

4.   *Histology*

The tips of the cannulae   used for central
administration of ANG-II, sucrose and NaCl were located in
an area bounded dorsally by the anterior commissure,

Figure 12

Water intakes (ml/30 min) of rats (n = 10) before
(top panel) and after (lower panel) FLH lesions in
response to single unilateral microinjections of
ANG-II, sucrose, or NaCl through POA cannulae traversing
the lateral cerebral ventricles (straight POA cannulae).
Results are expressed as Means ± S.E.M. for consecutive
ipsilateral and contralateral injections. *$P < 0.05$
as compared with prelesion intakes.

BEFORE

AFTER

WATER INTAKE (ml/30 min)

0   25 .50 400 500
ANG II (ng)

.3 .5 1.0 2.0 4.0
SUCROSE   (M)

.15 .23 .50 1.0 2.0
NaCl   (M)
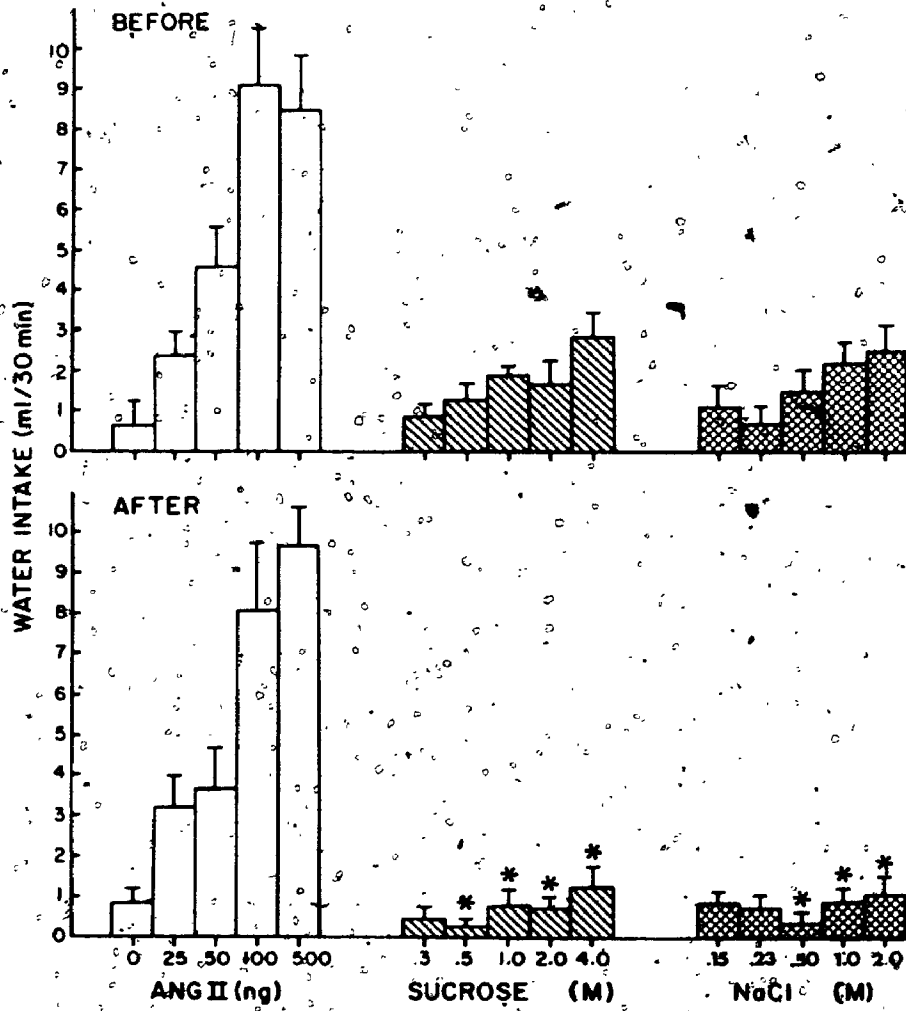
Figure 13

Water intakes (ml/30 min) of rats (n = 13) before
(top panel) and after (lower panel) FLH lesions in
response to single unilateral microinjections of
ANG-II, sucrose, or NaCl administered through POA
cannulae angled so as to bypass the lateral cerebral
ventricles (angled POA cannulae). Results are
expressed as Means ± S.E.M. of consecutive ipsilateral
and contralateral injections. $*P < 0.05$ as compared
to prelesion intakes.

BEFORE

AFTER

WATER INTAKE (ml/30 min)

ANG II (ng)   SUCROSE (M)   NaCl (M)
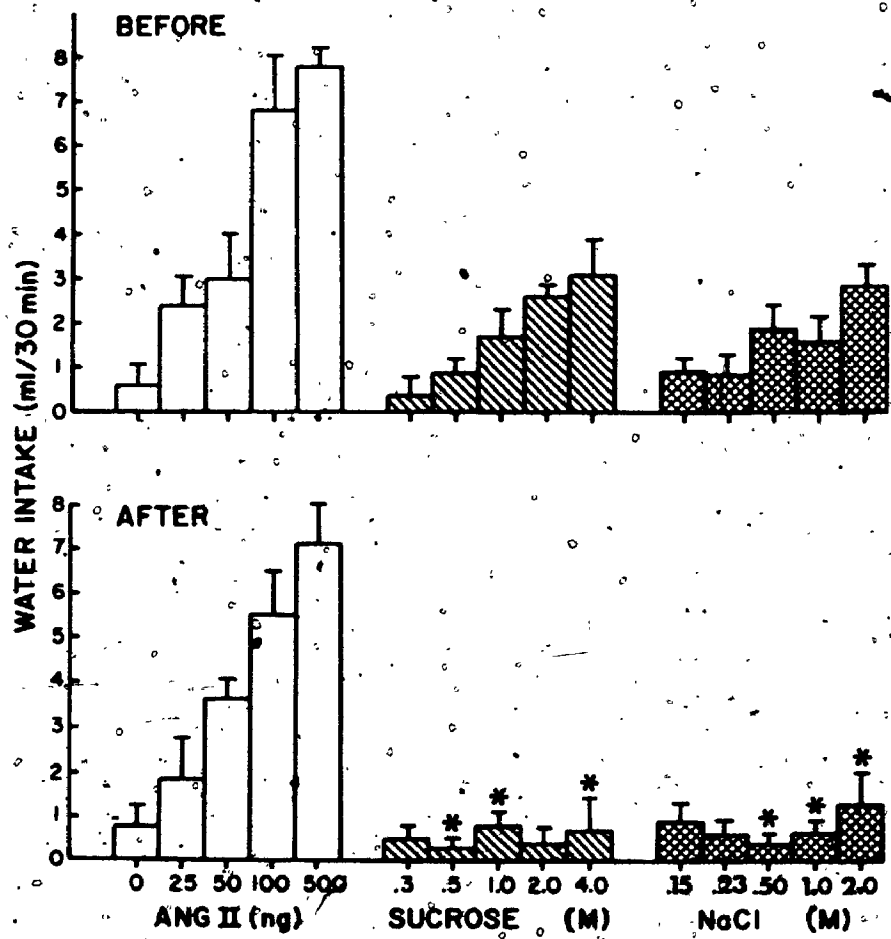
Figure 14

Water intakes (ml/30 min) of rats (n = 17) before
(top panel) and after (lower panel) MLH lesions in
response to single unilateral microinjections of ANG-II,
sucrose, or NaCl administered through POA cannulae
angled so as to bypass the lateral cerebral ventricles
(angled POA cannulae). Results are expressed as
Means ± S.E.M. of consecutive ipsilateral and contra-
lateral injections. *$P < 0.05$ as compared with pre-
lesion intakes; **$P < 0.01$ as compared with prelesion
intakes.

BEFORE

AFTER

WATER INTAKE (ml/30min)

ANG II (ng)   SUCROSE   (M)   NaCl   (M)
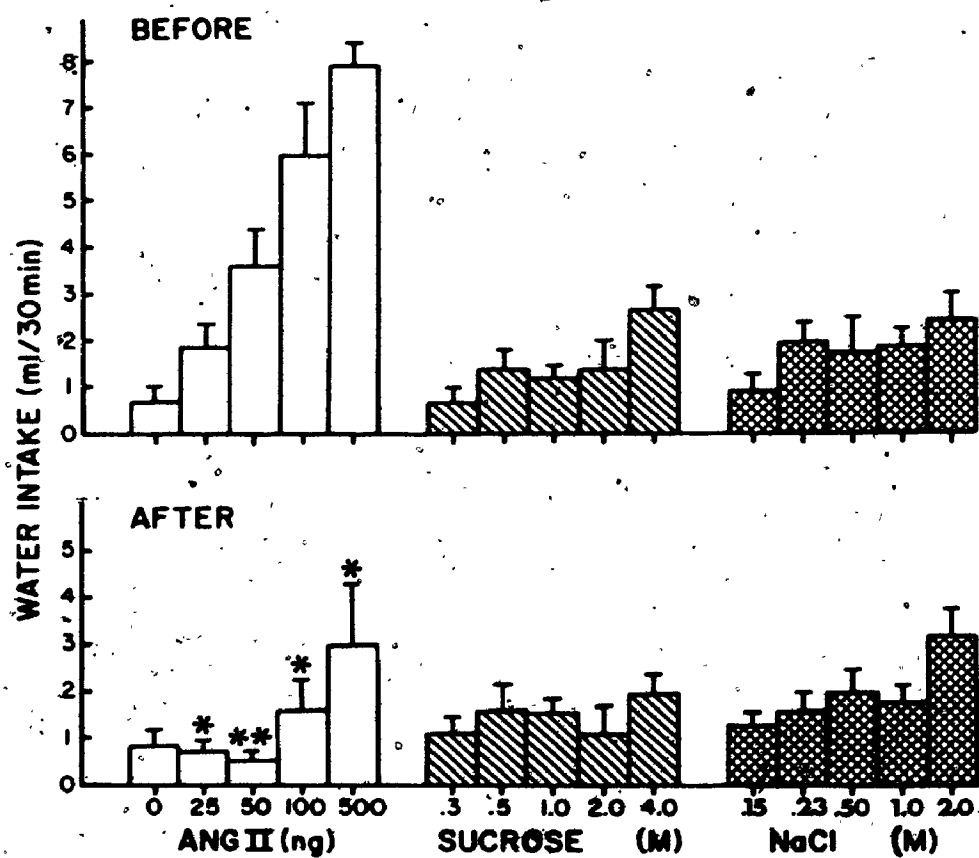
ventrally by the optic chiasm, and extended rostro-
caudally from the most anterior extent of the ventricle
to the widest frontal extent of the anterior commissure
(Figure 15). In about one-third of the brains into which
straight POA cannulae were implanted, some enlargement of
the ventricles was observed. Enlarged ventricles were
not seen with angled POA cannulae.

As shown in Figure 16, the MLH lesions were located
in the midlateral hypothalamic area, just lateral to the
fornix, and at the rostrocaudal level of the largest
frontal extent of the dorsomedial and ventromedial hypo-
thalamic nuclei. The lesions extended 0.5-1.2 mm in the
ventro-dorsal plane, 0.2-0.5 mm in the lateral plane and
0.5-1.2 mm rostrocaudally. In all but one case the for-
nices were undamaged and the lesions were bilaterally sym-
metrical and similar in appearance.

The FLH lesions were typically larger than those in
the MLH, extending 0.7-1.5 mm ventrodorsally, 0.2-1.2 mm
laterally, and 0.7-1.5 mm rostrocaudally. The largest FLH
lesions destroyed the ventromedial part of the internal
capsule, medial edge of the globus pallidus, and the far-
lateral aspects of the LH. In 3 animals there was also
damage to the optic tract and zona incerta. With the
smaller FLH lesions tissue damage was confined to the far-
lateral hypothalamus and medial internal capsule.

Figure 15

Representative photomicrographs of coronal
brain sections showing the locus of cannulae in the
POA (arrows).

A   tips of cannulae which penetrated the
     lateral cerebral ventricles (straight
     POA cannulae)

B   tips of cannulae which were angled so as
     to bypass the lateral cerebral ventricles
     (angled POA cannulae)

Note that the ventricles are enlarged in (A).
Horizontal bar equals 2 mm.

B

A

2 mm

Figure 16

Representative photomicrographs of coronal brain
sections showing the locus of lesions in the MLH and
FLH Hypothalamus (in each case designated by arrows).

A   smaller MLH lesion

B   smaller FLH lesion

C   largest MLH lesion

D   largest FLH lesion

A- and C-type lesions were equally "effective" in
disrupting drinking induced by POA administration of
ANG-II. Neither lesion had any effect on water
intakes elicited by centrally administered sucrose
or NaCl. B- and D-type lesions equally disrupted
water intake to sucrose and NaCl without attenuating
thirst induced by ANG-II. Horizontal bar equals 2 mm.

B

D

A

C
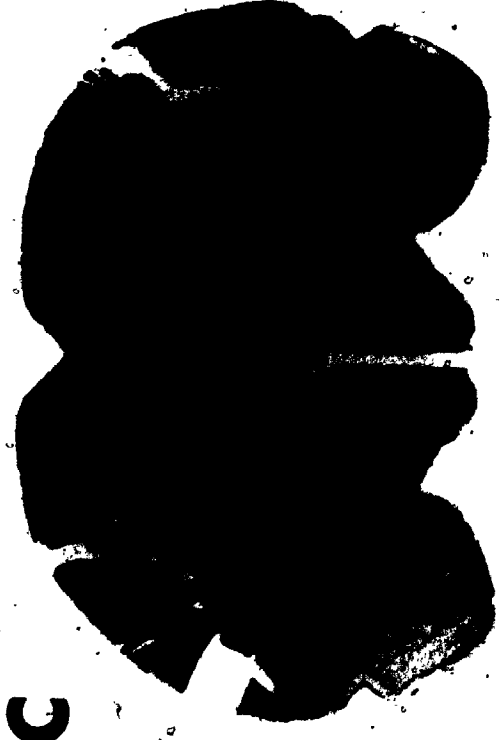
2 mm

Figure 17

Location of lesions in the MLH and FLH marked
on coronal diagrams of rat brain modified from the
stereotaxic atlas of Pellegrino and Cushman (1967).
Portions shaded in black indicate area common to
all lesions, and black outline denotes the largest
extent of all lesions.

DMH   dorsomedial hypothalamic nucleus

FX   fornix

MT   mammillothalamic tract

OT   optic tract

VMH   ventromedial hypothalamic nucleus

ZI   zona incerta

MLH
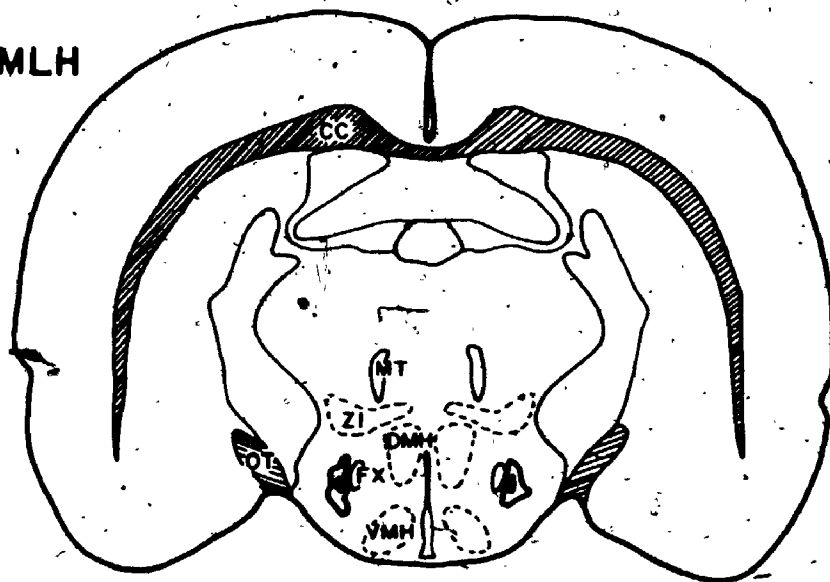
FLH
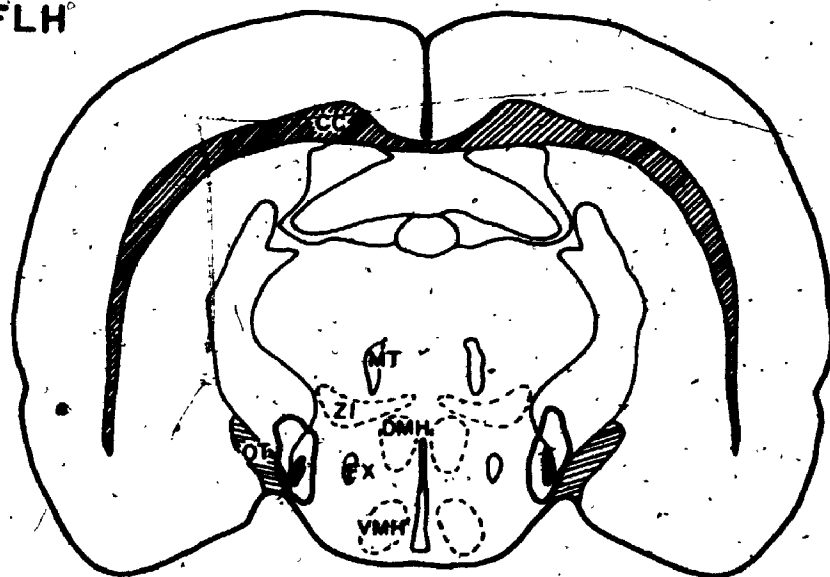
DISCUSSION

The results of the experiments described in this section support the hypothesis that cellular and extra-cellular stimuli of thirst are mediated by separate lateral hypothalamic pathways. Initial support for this proposal was obtained from the dual recording experiments in which differential responses to POA administration of hypertonic NaCl and sucrose and to ANG-II were recorded from single neurons in the FLH and MLH, respectively. In subsequent experiments it was shown that FLH lesions, which did not alter drinking to peripherally-administered isoproterenol or centrally administered ANG-II, disrupted water intake elicited in response to peripheral or central injections of sucrose or NaCl or to 24- and 48-hr water deprivation followed by a peripheral injection of NaCl. In contrast, MLH lesions attenuated drinking elicited by the microinjection of ANG-II into the POA but had no effect on water intake induced by administering hypertonic sucrose or NaCl to the POA.

In addition to their differential effects on cellular and extra-cellular thirst, lesions of the FLH also caused a short-term attenuation of ad *libitum* feeding and minor losses of body weight, whereas these effects were not observed with MLH lesions. These observations are consis- tent with the results reported in Section 3.1 and with previous studies (*e.g.*, Gold, 1967) and suggest that FLH

and MLH lesions may impinge on different neural systems
for the regulation of ingestive behaviors. As suggested
by Morgane (1961) the impairments of food and water intake
following FLH ablation may be at least partially attribu-
table to destruction of pallidofugal fibers, since lesions
of this pathway along its trajectory in the entopeduncular
nucleus (Gold, 1967) and the midbrain reticular formation
(Parker and Feldman, 1967; Lyon, Halpern and Mintz, 1968)
cause disruptions in ingestive behaviors. Another possi-
bility, since some of the animals with larger FLH lesions
were hypoactive during the immediate postlesion period, is
that the lesions may have impinged on fibers of the nigro-
striatal bundle. Previously, Ungerstedt (1970, 1971) and
other workers (Oltmans and Harvey, 1972; Breese, Smith,
Cooper and Grant, 1973; Fibiger, Zis and McGeer, 1973;
Zigmond and Stricker, 1974) found that destruction of this
pathway by intracerebral 6-hydroxydopamine injections or
electrolytic lesions produces aphagia and adipsia, senso-
rimotor disturbances (Levine and Schwartzbaum, 1973;
Turner, 1973; Marshall, Richardson and Teitelbaum, 1974)
and other disabilities (Levine and Schwartzbaum, 1973).
On the other hand, it should be noted that although the
larger FLH lesions produced deficits which were similar
in some respects to those reported after pallidal or
nigrostriatal lesions, the impairments were considerably
less severe and complete recovery had occurred prior to

to the resumption of testing. Moreover, small lesions of
the FLH, which had little or no effect on feeding and did
not cause gross sensorimotor deficits, were as effective
as the large lesions in disrupting drinking to sucrose and
NaCl. Finally, the disruption of elicited water intake
after FLH lesions was specific to cellular stimuli of
thirst; water intake elicited by ANG-II was not affected.
These observations, and especially the latter one, suggest
that although pallidofugal and/or nigrostriatal pathways
may have been interrupted, other fiber systems specific to
cellular thirst mechanisms were also destroyed.

Another important consideration when interpreting the
lesion data is whether the disruption of cellular thirst
following FLH lesions reflects changes in neural thirst
systems or is secondary to other more general deficits
resulting from non-specific brain damage. It has been
suggested, for example, that FLH-lesioned rats may fail to
respond to regulatory thirst challenges (i.e., i.p. injec-
tions of hypertonic solutions) because they are more
easily incapacitated by the pain and stress produced by
the injections (Marshall, Richardson and Teitelbaum, 1974;
Stricker, 1973, 1976). If the failure of FLH animals
to drink to cellular dehydration was due to a general
impairment of behavior during stressful conditions, then
it would be expected that the rats would drink more
readily at low or moderate doses of sucrose or NaCl
given i.p. than at high doses, since administration of

less concentrated solutions should be less incapacitating (Marshall *et al.*, 1974). For similar reasons, FLH-lesioned animals would also be expected to drink when thirst was induced by central administration of hypertonic sucrose or NaCl (Blass, 1973). However, in the present study rats with lesions of the FLH failed to drink to low as well as high doses of sucrose or NaCl, whether given i.p. or following intracranial microinjection. Second, and more importantly, these same animals showed no deficits in water intake following central administration of ANG-II, even though this procedure has been shown to cause marked increases in systemic blood pressure (Hoffman and Phillips, 1975) and could therefore also be presumed to be stressful to the animals. These findings suggest that the attenuation of elicited water intake following FLH lesions reflects a disruption of neural pathways mediating drinking in response to osmoregulatory challenges, rather than a non-specific impairment of motivated behaviors.

With regard to the MLH pathways, ablation of which caused a selective disruption of drinking in response to centrally administered ANG-II, the results of the lesion experiments indicate that the disruption may have resulted from destruction of the descending MFB which passes through the LH just lateral to the fornix (Nanta, 1958; Millhouse, 1969). This interpretation is also supported by the finding reported in Section 3.1, that single neurons in the MLH change their rate of discharge following

the microinjection of ANG-II into the POA.

In summary, the results of the present experiments indicate that a separation between neural systems subserving cellular and extracellular thirst is preserved within the brain, in distinct pathways through the LH. One pathway projects caudally from the POA through the MLH and is involved in the initiation of drinking to ANG-II, whereas water intake to cellular thirst stimuli is mediated through pathways passing through the FLH.

3.3 Separate Pathways Mediating Cellular and Extracellular Thirst in the Midbrain

The experimental data reported in Section 3.1 suggested the possibility that cellular and extracellular thirst signals are mediated by separate neural pathways in the LH. The findings described in Section 3.2 supported this hypothesis by showing that single neurons in the MLH and FLH responded differentially to POA administration of ANG-II and hypertonic NaCl and sucrose. Furthermore, the lesion experiments reported in Section 3.2 suggested that the effects of MLH and FLH ablation were likely due to interruption of specific neural pathways subserving cellular and extracellular thirst signals, respectively, and not to non-specific deficits.

In the present series of experiments, it was decided to investigate whether the separation between the neural pathways mediating cellular and extracellular thirst is preserved at more caudal levels of the neuraxis or whether the pathways converge onto common structures. Since previous studies reviewed in Section 1 have shown that lesions of the midbrain tegmentum and central grey cause adipsia and aphagia (Lyon, 1966; Blatt and Lyon, 1968; Lyon, Halpern and Mintz, 1968) and electrical stimulation of the ventral tegmentum elicits drinking (Wyrwicka and Doty, 1966; Robinson and Mishkin, 1968), the signals associated with water balance deficits may be

transmitted to these regions of the mesencephalon. This possibility was investigated using electrophysiological and lesion techniques.

METHODS

Initially, as a guide to the placement of lesions in the midbrain, it was decided to administer ANG-II through chronic cannulae implanted in the POA and to observe the effects of ANG-II on the spontaneous discharge frequency of single neurons in the midbrain and upper brainstem in subsequent acute recording experiments.

1. Microelectrode Recording Experiments with ANG-II

Animals (n = 38) were anesthetized with sodium pento-barbital (50 mg/kg), preceded by atropine sulphate (5 mg), and implanted with a single 23-gauge stainless steel cannula into the POA using stereotaxic techniques des-cribed in Section 2.2. Following postoperative recovery, rats were tested for the occurrence of drinking after the microinjection of 100 ng of synthetic ANG-II dissolved in 1 µl distilled water through the indwelling POA cannula. Water intake (± 0.5 ml) was then measured in the animal's home cage over the next 30 min.

Only those animals which drank more than 5 ml of water during each of two preliminary tests with ANG-II were used in the recording experiments. In these animals (n = 27) ANG-II (100 ng in 1 µl distilled water) was micro-

injected into the POA through the implanted cannula and
the effects of ANG-II on the frequency of firing of neurons
in the midbrain and upper brainstem was measured by means
of frequency-time histograms. In addition, every fifth
unit was routinely tested with distilled water. In 7
animals, the femoral artery was cannulated with a poly-
ethylene catheter in order to record the arterial blood
pressure. For the same group of animals (n = 7) cortical
EEG recordings were obtained through 3 nichrome wires
implanted in the skull overlying the occipital cortex.

In selected control experiments, ANG-II was micro-
injected into the POA and unit recordings were obtained
from sites in the cerebral cortex, hippocampus, thalamus
and the basal forebrain rostral to the POA. In other
experiments, ANG-II was administered through a cannula
implanted in the cerebral cortex and the response of
units in the midbrain and brainstem was measured. Finally,
the frequency of firing of units in the midbrain and
brainstem were recorded following the microinjection of
25, 50, 100, and 200 ng ANG-II (all dissolved in 1 µl
distilled water) through an indwelling POA cannula.

2.   *Lesion Experiments*

In order to determine whether circumscribed critical
areas for the drinking response to intracranial ANG-II
might be found within the larger area of the midbrain in
which unit responses to ANG-II were obtained, the effects

of unilateral and bilateral mesencephalic lesions on drinking elicited by ANG-II were determined.

Animals (n = 44) were individually housed and maintained on Purina laboratory chow and tap water *ad libitum*, except as noted. Two 23-gauge stainless steel cannulae were implanted bilaterally into the POA of each animal using the standard stereotaxic techniques and barbiturate anesthesia. During the same operation two monopolar electrodes made from size 00 insect pins and insulated except for 0.5 mm at the tip were also implanted bilaterally into various locations in the mesencephalon.

About one week after implantation of the cannulae and electrodes, food pellets were removed from the cages and replaced with food cups containing a measured weight of a high-carbohydrate diet. For the next 6 days, food and water intakes and body weights were determined daily. This procedure was repeated at selected 6-day intervals to ensure that the animals were healthy and gaining in body weight.

Beginning on the tenth postoperative day, each animal was tested once daily for drinking following the unilateral administration of ANG-II (100 ng in 1 µl distilled water) to the POA. Water intakes (± 0.5 ml) were measured for the next 30 min in the animal's home cage with food freely available. Only those animals which drank more than 5 ml of water in each of 4 consecutive tests with ANG-II were used in the lesion experiments.

Unilateral lesions were made in 36 rats under ether anesthesia by passing 1.5-3.0 mA direct current for 15-40 sec between the implanted monopolar electrode and an indifferent electrode clipped to the ipsilateral ear. Testing with ANG-II was resumed following complete post-operative recovery (2-14 days postlesion) (see Figure 23). A second lesion, contralateral to the first, was made in 17 of the animals 24 to 31 days after the first lesion and testing with ANG-II was continued for an additional 50 to 58 days.

In another series of animals (n = 12), the effects of one-stage bilateral lesions of the paramedial rostral mid-brain (RMB) on water intake elicited by microinjection of ANG-II (50 ng in 0.2 µl artificial CSF) and NaCl (2 M in 0.2 µl) into the POA (straight POA cannula) and lateral ventricle were determined using surgical procedures just described. Two injections of ANG-II and NaCl were made into each site before and after the lesions were made.

At the end of the experiments histological techniques as described in Section 2.6 were used to determine the sites of the recordings and of the lesions. Student's t-test for correlated samples was used to compare respon-ses of the different groups of animals to ANG-II.

RESULTS

1. Effect of POA Administration of ANG-II on Midbrain Unit Activity

Extracellular recordings were obtained from 147 neurons in the midbrain and upper brainstem of which 52 (35%) increased their rate of discharge in response to the administration of 100 ng ANG-II to the ipsilateral POA. Two units (1%) showed inhibition and 93 (64%) others showed no change in their discharge frequency. Table 3 summarizes the numbers of units and the patterns of responses and Figure 18 shows the location of iron deposits corresponding to the sites of recording. Figure 19 shows the location of the cannula in the POA and the site of recording in the midbrain of a representative animal.

When 100 ng ANG-II was administered to the POA, activation (n = 18) or inhibition (n = 2) of unit activity occurred concomitantly with desynchronization of cortical EEG and an increase in arterial blood pressure (mean change = +5 mm Hg, range = +3 ⥵ +15 mm Hg). A typical response is shown in Figure 20A. With the lowest dose of ANG-II (25 ng) cortical EEG and blood pressure changes were not observed although 2 of 14 units increased their rate of discharge (Figure 20B). Frequency-time histograms showing the response of a single neuron in the midbrain tegmentum to the administration of 25, 50, 100, and 200 ng ANG-II to the POA are presented in Figure 21.

TABLE 3

Numbers of Units in Various Midbrain Structures
and the Patterns of Response following the Administration
of 100 ng Angiotensin to the Ipsilateral Preoptic Region

Sites of Recording	Total Numbers of Units Recorded	Numbers of Units Responding	Patterns of Response Activated	Inhibited
Periventricular grey substance	16	4	4	0
Bed nucleus posterior commissure	19	10	10	0
Reticular formation	75	17	15	2
Ventral tegmental nucleus Tsai	3	3	3	0
Interpeduncular nucleus	14	10	10	0
Nucleus Darkschewitz	5	5	5	0
Central tegmental nucleus	2	1	1	0
Red nucleus	3	0	0	0
Substantia nigra	5	0	0	0
Interstitial nucleus of Cajal	5	4	4	0
Totals	147	54	52	2
% of Totals	100	37	35	1

Figure 18

Diagrams of frontal sections through the mesencephalon redrawn from the atlas of Pellegrino and Cushman (1967), showing the sites of recordings and the types of unit responses observed following the unilateral microinjection of 100 ng ANG-II into the ipsilateral POA. Solid triangles indicate sites of facilitated units and open circles mark sites where no effect was obtained. Numbers at the lower left of each figure are taken from the atlas and refer to the distance (mm) behind bregma. Abbreviations: A, cerebral aqueduct; CP, posterior commissure; CS, superior colliculus; CT, central tegmental nucleus; D, nucleus of Darkschewitz; FLD, dorsal longitudinal bundle; IP, interpeduncular nucleus; LM, medial lemniscus; NCP, bed nucleus of the posterior commissure; NR, red nucleus; P, pons; PC, cerebral peduncle; PVG, central grey; RF, reticular formation; V, ventricle; VTN, ventral tegmental nucleus; III, third ventricle.
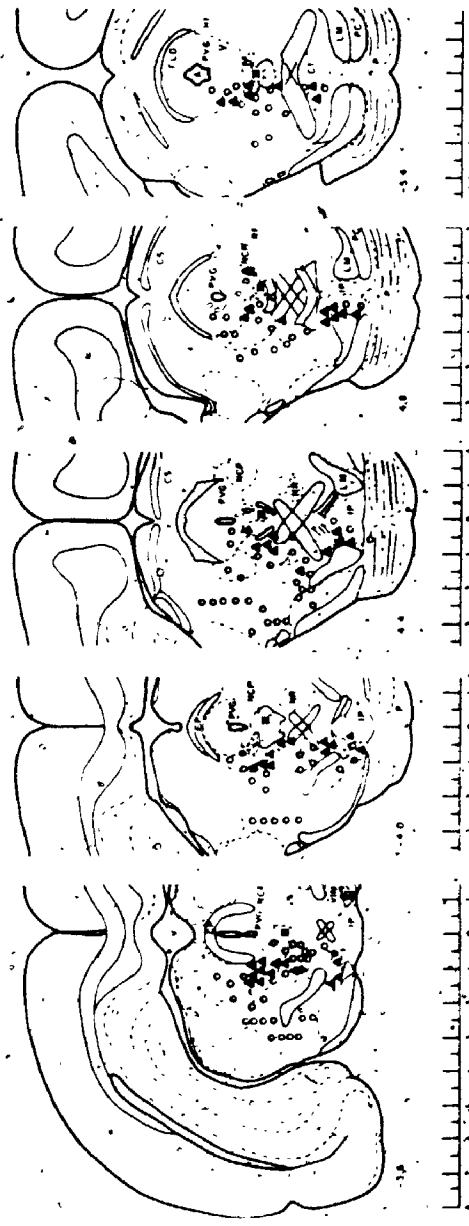
Figure 19

Photomicrographs of brain sections from an
animal in the first series showing the location
of a unilateral cannula implant in the POA (a)
and the microelectrode recording site in the
ventral tegmental area of the midbrain (b) (in
each case designated by arrows). Magnification
7 times.

2mm

Figure 20

Continuous polygraph tracings showing:

A.   Changes in cortical EEG (top tracing), arterial
      blood pressure (middle tracing), and spike dis-
      charge of a neuron in the midbrain following the
      unilateral administration of 100 ng ANG-II to
      the ipsilateral POA. Note that the blood pressure
      (trace 580) and EEG (trace 583) changes preceded
      the increase in unit discharge frequency
      (trace 583).

B   Cortical EEG, arterial blood pressure and dis-
      charge of another unit in the midbrain following
      the administration of 25 ng ANG-II. Note that
      blood pressure and EEG did not change although
      the neuron increased its rate of discharge
      (trace 444-445).

The time at which ANG-II was administered is indicated
by arrows.

CORTICAL
EEG

ARTERIAL
BP

UNIT
DISCHARGE

TIME

CORTICAL
EEG

ARTERIAL
BP

UNIT
DISCHARGE
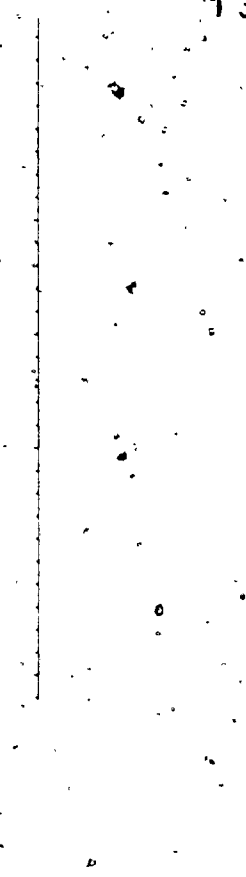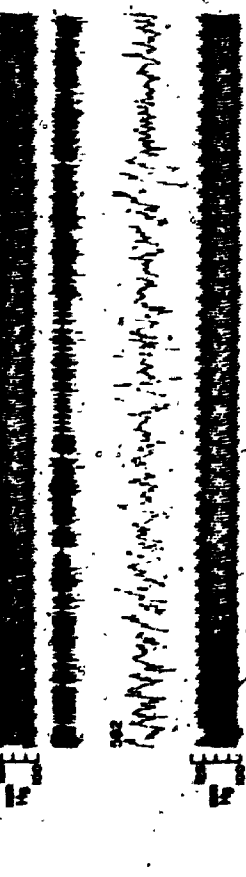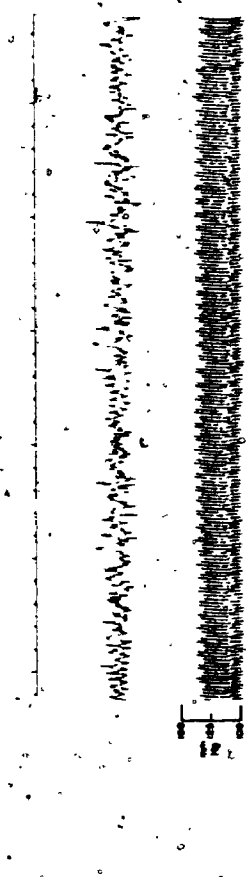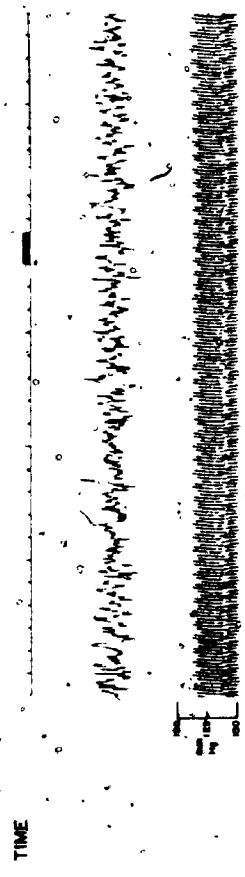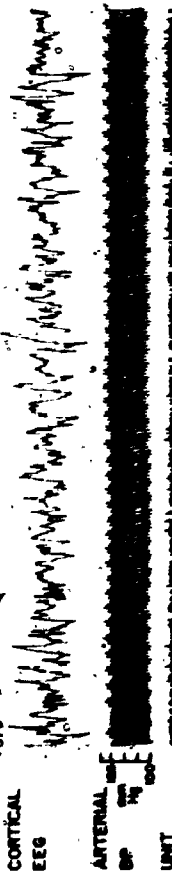
TIME

Figure 21

    Frequency-time histograms showing the response
of a single unit in the midbrain tegmentum to the
unilateral administration of different doses of
ANG-II to the ipsilateral POA.

    A   25 ng   B   50 ng

    C   100 ng   D   200 ng

Time of administrations is indicated by arrows. No
less than 40 min elapsed between successive
injections. The order in which doses were administered
was: 100 ng, 50 ng, 25 ng, 200 ng.

A

B

C

D

No. of AP's/sec

5

0

60 sec
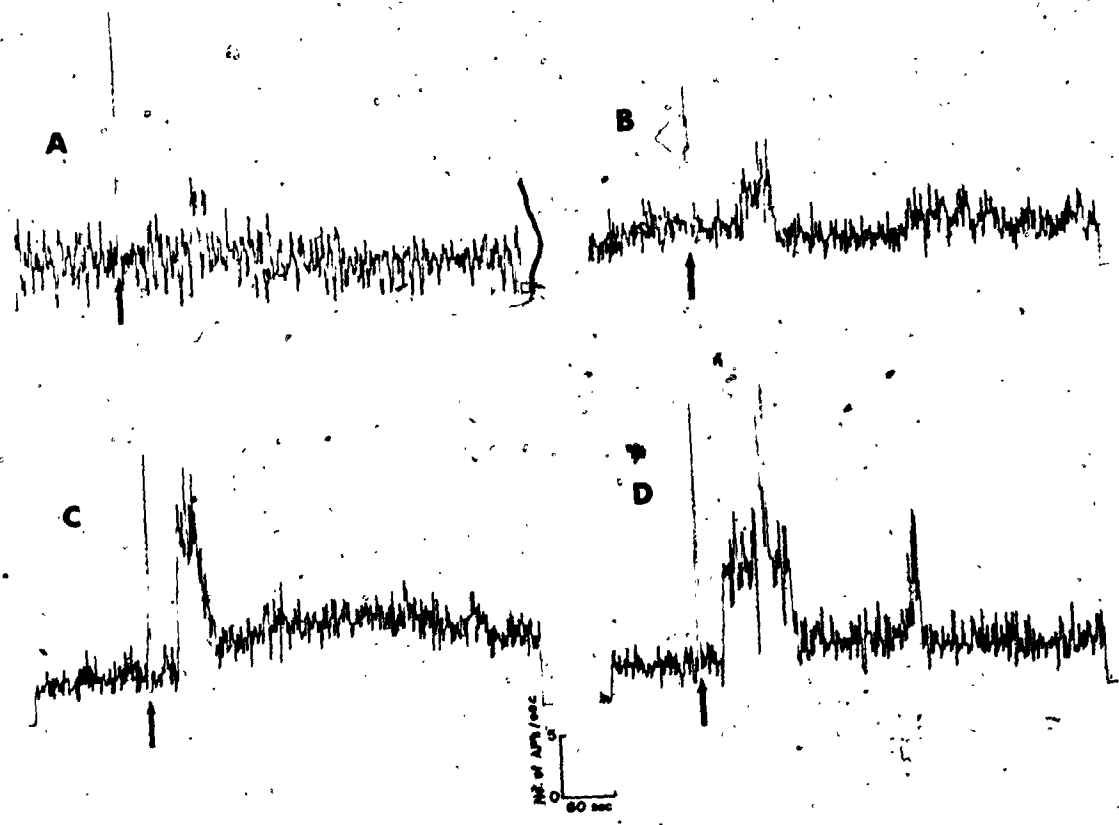
The effects of microinjecting 100 ng ANG-II into the POA on the rate of discharge of neurons in the cerebral cortex, thalamus, basal forebrain rostral to the POA and hippocampus were investigated in 5 additional urethane-anesthetized animals. Of the 17 neurons in the cerebral cortex for which unit recordings were obtained, 2 increased their firing rate, 1 unit showed inhibition and the remaining 14 units showed no change in response to ANG-II. For the other areas under study, the corresponding number of units which showed activation, inhibition or no effect in response to ANG-II were as follows: thalamus, 0, 0, 8; caudate nucleus, 0, 0, 7; hippocampus, 2, 0, 8. In two other animals, microinjection of 100 ng ANG-II into the cerebral cortex did not affect the baseline discharge rate of 12 units in the midbrain for which extracellular recordings were made.

2. *Effects of Unilateral Lesions of the Midbrain on Drinking to ANG-II Administered to the POA*

The lesions, illustrated in Figures 22 and 23, were located within an area bounded rostrally by the mammillary peduncle and caudally by the anterior border of the inferior colliculus and between the midline and the lateral lemniscus (coordinates: 0.1-4.0 mm ventrodorsal, 0.4-2.0 lateral, 0.5-2.0 rostro-caudal). Three groups were formed on the basis of the locus of the lesions.

Figure 22

Reconstructions of the individual lesions in
the midbrain:

A   Unilateral lesions which did not affect
        drinking to ANG-II.

B   Bilateral lesions which were not effective in
        disrupting water intake elicited by ANG-II.

The lesions are super-imposed on frontal sections from
a rat stereotaxic atlas (Pellegrino and Cushman, 1967).
Numbers at the lower left of each figure are taken
from the atlas and refer to the distance behind bregma.
The numbers inside the lesions shown in B refer to the
individual animals in which the lesions were made.
Abbreviations are the same as those given for Figure 18.
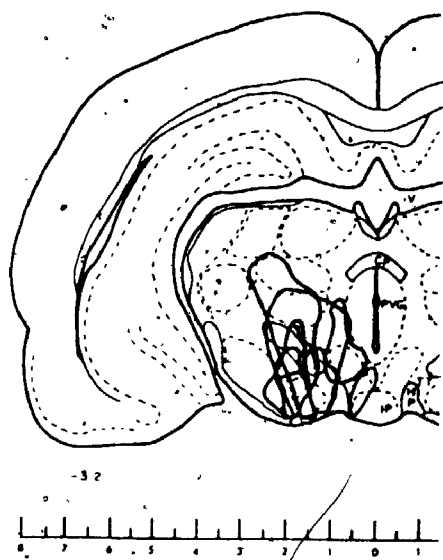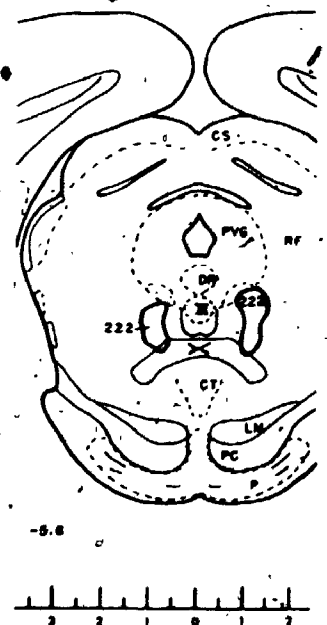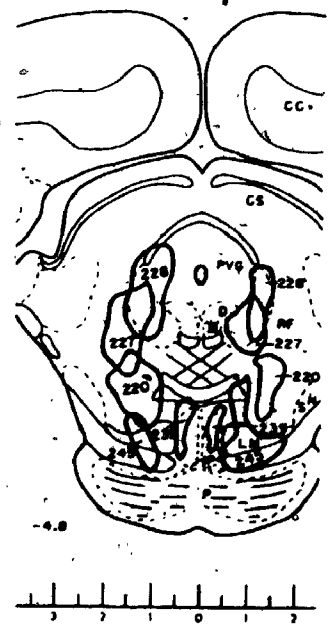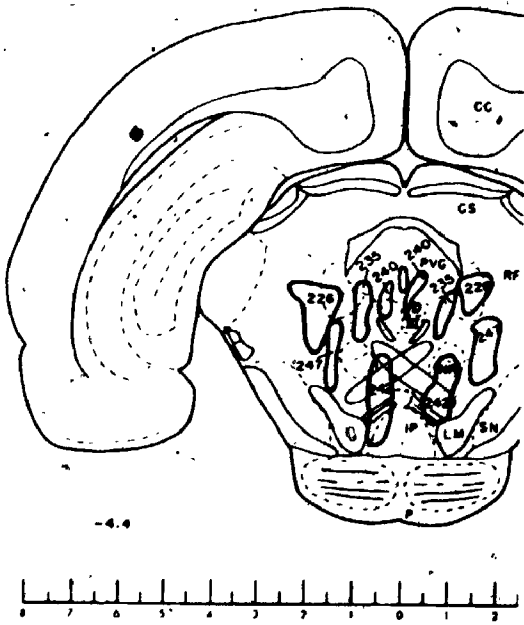
The lesions in the first group (n = 13) were located in the region of the ventral tegmentum. The nuclear structures which were damaged or destroyed unilaterally by this type of lesion included the interpeduncular nucleus, the ventral tegmental nucleus of Tsai, the central tegmental nucleus, the red nucleus and the medial aspects of the substantia nigra. Fiber tracts which appeared to have been transected unilaterally by the ventral tegmental lesions included the habenulo-interpeduncular tract, the mammillo-tegmental tract and the central tegmental tract. The medial lemniscus also received moderate to extensive damage. Unilateral ventral tegmental lesions had little or no effect on drinking elicited by ANG-II administered to the POA (Figure 24). There was an indication of a slight attenuation of elicited water intake when the lesion encroached on the neural tissue adjacent to the interpeduncular nucleus (n = 5); however, the variability of this effect was great and the differences were not statistically significant. Seven of the animals with unilateral lesions in the ventral tegmental area showed varying durations of aphagia postoperatively (mean number of days of aphagia = 2.8, range of 1-4 days). Of these seven rats, 5 required intragastric feeding for from 2 to 4 days. The lesions also typically caused a period of mild hypodipsia for 1-3 days postlesion, but thereafter daily water intakes returned to prelesion levels.

A second group of lesions (n = 15) was located in the
reticular formation ventrolateral to the central grey.   In
8 of the animals the lesions extended unilaterally into
the central grey and produced damage to the bed nucleus of
the posterior commissure, the nucleus of Darkschewitz and
the interstitial nucleus.   In the remaining animals the
lesions unilaterally damaged or destroyed the lateral
tegmental nucleus, the substantia nigra, large areas of
the midbrain reticular formation, the medial lemniscus
and the habenulo-interpeduncular tract.   None of the uni-
lateral lesions in the second group had a significant
effect on drinking elicited by ANG-II administered to the
POA (Figure 24).   Four of the lesions which were located
dorsal and lateral to the red nucleus (n = 9) produced
1-5 days of aphagia and 3-9 days of hypodipsia post-
operatively.   Most of the animals with lesions of the
reticular formation ventrolateral to the central grey were
hyperactive, lost body weight in the immediate post-lesion
period and were difficult to handle.   Testing with ANG-II
was not resumed until 5-14 days postoperatively.

In a third group the lesions (n = 8) destroyed the
mammillary nuclei and the ventral tegmental nucleus of
Tsai unilaterally, transected the medial lemniscus and the
medial forebrain bundle and partially ablated the rostral
part of the midbrain reticular formation (Figure 23).   The
common area of destruction produced by these lesions was
dorsal and lateral to the mammillary peduncle, in the area

of passage of fibers of the MFB. These unilateral lesions of the rostral midbrain (RMB) caused a significant decrease of water intake elicited by ANG-II administered through either preoptic cannula in the immediate post-lesion period in 7 of 8 animals (t = 2.44, P_o < 0.05 in comparison with pre-lesion intakes). The attenuation of elicited drinking was transient, however, and recovery was observed in all 7 animals by the sixth postoperative day (Figure 24). In contrast to the effects of lesions located more caudally in the midbrain ventral tegmentum or in the area ventrolateral to the central grey, rats with lesions of the rostral midbrain showed no significant change in 24 hr food and water intakes or in the rate of body weight increase.

3. *Effects of Bilateral Lesions of the Midbrain on Drinking to ANG-II Administered to the POA*

Seventeen of the animals that received unilateral lesions were given a second, contralateral lesion, through an indwelling electrode 26 to 32 days following the first lesion.

As shown in Figure 22, the second lesion of the midbrain ventral tegmentum (n = 4) or of the area ventrolateral to the central grey (n = 6) did not significantly affect drinking elicited by ANG-II. In the majority of cases, animals that received lesions of either of these two sites were hypophagic and hypodipsic in the immediate

post-lesion period and could not be tested with ANG-II for 7-10 days postoperatively. Three of the rats with lesions of the midbrain ventral tegmentum required 2-4 days of intragastric feeding. Two of these animals and 2 rats with extensive lesions in the reticular formation dorso-lateral to red nucleus showed signs of spasticity of the hind limbs, loss of balance and rotational movements for 2-5 days post-lesion.

All seven animals in which a second lesion was made in the rostral midbrain showed a significant (t = 2.31, $P < 0.05$ in comparison with prelesion intakes) and chronic decrease in water intake elicited by ANG-II for the entire 58-day period following the second lesion. During this time the animals drank less than 30% of their pre-lesion intakes to ANG-II.

In the final test with ANG-II on Day 58 the animals with rostral midbrain lesions drank significantly less than during the pre-lesion period (t = 2.23, $P < 0.05$). The changes in daily food and water intakes after the second lesion were minor, although 2 of 7 animals were hypodipsic for 7 and 11 days post-lesion, respectively. All rats had regained their pre-lesion body weights by the fourth postoperative day.

Figure 23

A.   Location of lesions in the ventromedial region

of the rostral midbrain (RMB) in the area of

passage of the MFB marked on a drawing taken

from the atlas of Pellegrino and Cushman (1967).

The number in the lower left hand corner

adjacent to the drawing is taken from the

atlas and indicates the number of mm posterior

to bregma.   These lesions produced a signifi-

cant attenuation of the drinking response

elicited by ANG-II (see text).   Abbreviations:

CC, corpus collosum; HPC, hippocampus; LM,

medial lemniscus; MP, mammillary peduncle;

PC, cerebral peduncle; PVG, central grey;

SN, substantia nigra; SUM, supramammillary

nucleus; V, ventricle; ZI, zona incerta.

-2.4
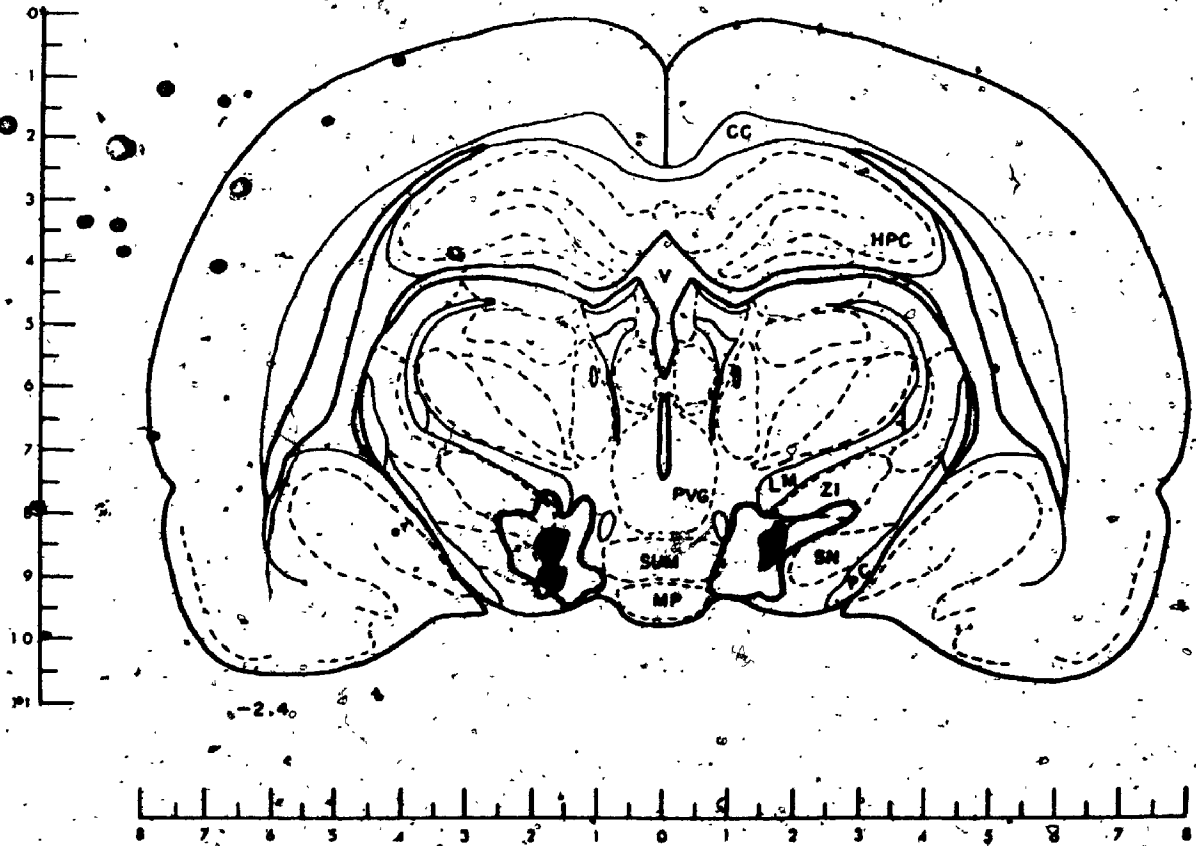
Figure 23 (Cont.)

B   Representative photomicrograph showing the locus
of the RMB lesions (arrows). Magnification
12 times.

2mm

Figure 24

Water intakes of rats following lesions of the
midbrain ventrolateral to the central grey (open
circles), of the paramedial dorsal and ventral teg-
mentum (solid circles) and of the ventromedial area
of the rostral midbrain (RMB) (triangles) during the
30-min period following the uunilateral microinjection
of 100 ng ANG-II into the ipsilateral POA.

A   following unilateral lesions

B   following a second, contralateral lesion

Results are expressed as Mean ± S.E.M. of consecutive
ipsilateral and contralateral injections. Time of
the lesions is indicated by arrows.
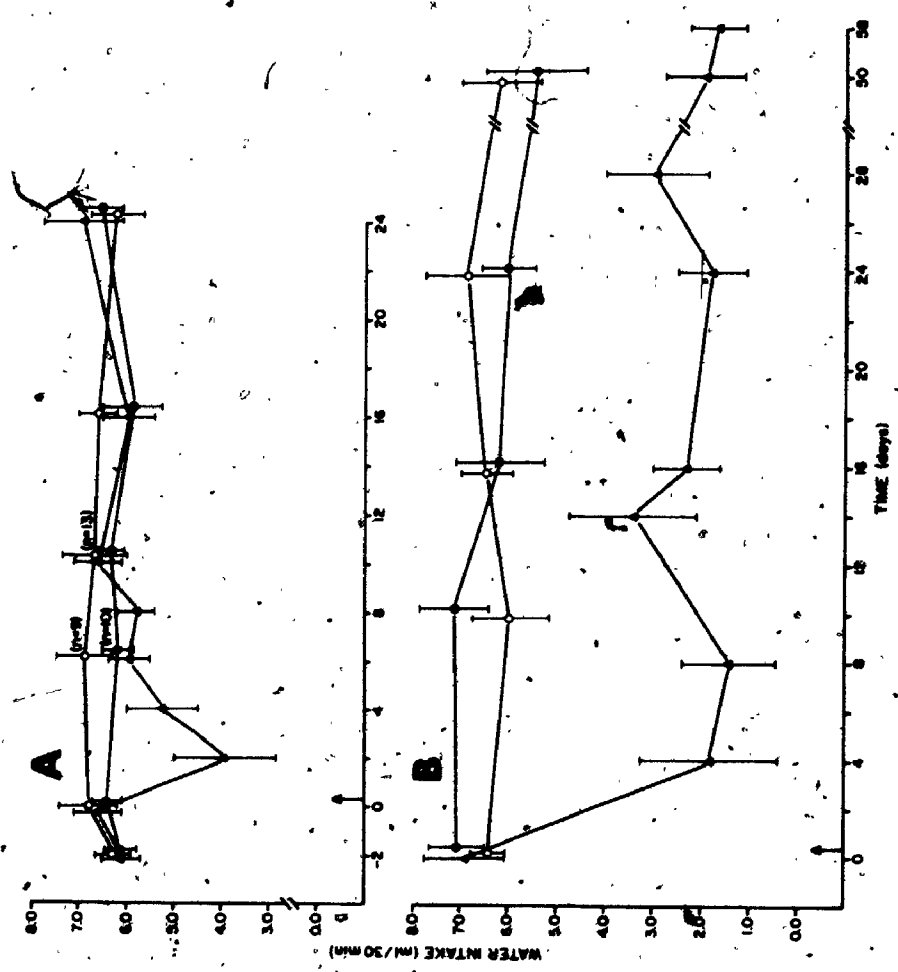
4. *Effect of Bilateral RMB Lesions on Water Intakes*
    Elicited by POA or Ventricular Microinjection of
    Ang-II and Hypertonic NaCl.

As shown in Fig. 25 lesions placed in the RMB in the area of passage of fibers of the MFB caused a significant disruption of drinking in response to ANG-II administered to the POA (t = 2.56, $P < 0.05$) but had no effect when ANG-II was administered to the lateral ventricles. The RMB lesions had no effect on drinking elicited by microinjection of 2 M NaCl to either the POA or to the lateral ventricles. The lesions placed in the rostral mesencephalon in this series of animals were similar in location, size and extent to those shown diagrammatically in Fig. 23.

DISCUSSION

The results of the first two experiments reported in this section indicate that signals from receptive sites for ANG-II in the forebrain are transmitted to the RMB. This interpretation was suggested initially by the electrophysiological results, in which it was found that microinjection of ANG-II into the POA influenced the discharge rate of single neurons in the midbrain, and was subsequently confirmed by the lesion experiments in which ablation of the RMB disrupted drinking to ANG-II.

Figure 25

Mean intakes of water of rats before (open
columns) and after (diagonal-lined columns) RMB
lesions in response to microinjection to 50 ng
ANG-II or 1 M NaCl into the POA and lateral
cerebral ventricles. Vertical bars represent ±
SEM of consecutive ipsilateral and contralateral
injections. Number of animals per test is
indicated above column.

WATER INTAKE (ml/15 min)
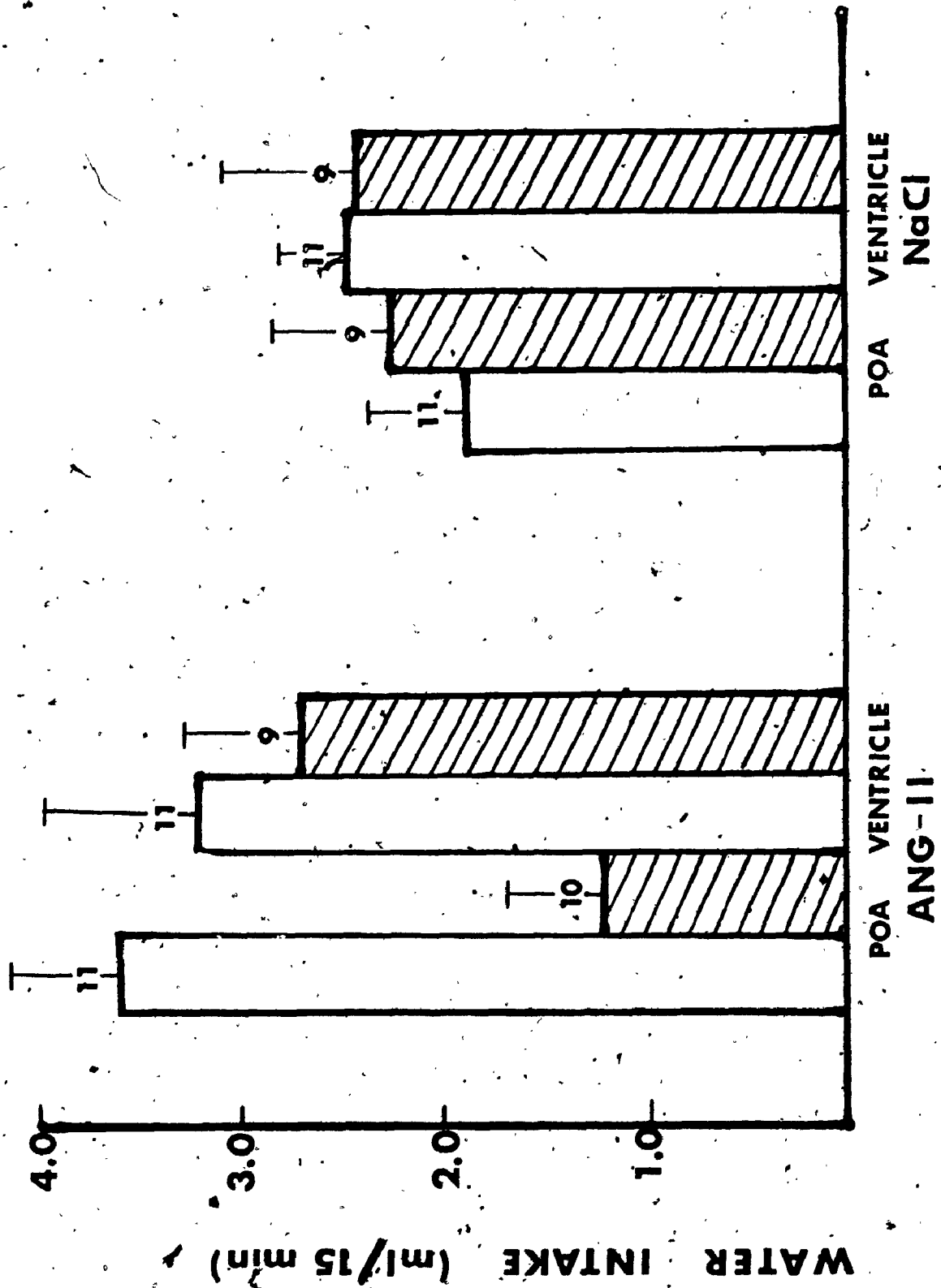
Most of the units which responded to ANG-II were located in an area just ventrolateral to the central grey, or in the paramedial dorsal or ventral tegmentum. Since lesions placed in either of these areas had little or no effect on drinking induced by ANG-II, the changes in mid-brain unit discharge frequency could be interpreted as a non-specific effect of ANG-II. Alternatively, it is possible that the lesions placed posterior to the meso-diencephalic junction were not large enough to destroy a significant portion of the projection area in the midbrain fibers carrying information from ANG-II receptive sites in the forebrain. This suggestion is supported by the results of anatomical studies which have shown fibers of the major limbic input into the midbrain, viz. the MFB, demonstrate considerable ipsilateral divergence and crossing-over at the level of the supra-mammillary decussation (Nauta, 1958, 1961; Ban, 1966; Millhouse, 1969) and as well by the observations that lesions of the more compact aspects of the MFB at the level of the midlateral hypothalamus (Sections 3.1 and 3.2) or in the rostral midbrain disrupt drinking induced by the microinjection of ANG-II into the POA.

A second important consideration when interpreting the electrophysiological data is whether the changes in the discharge of midbrain neurons reflect changes in mid-brain thirst systems activated by ANG-II or are secondary to blood pressure or EEG changes or to other factors.

The issue of the specificity of the unit responses is further complicated because desynchronization of EEG or increased discharge-frequency of neurons is difficult to interpret in terms of specific behavioral arousal when the animal is anesthetized. The recorded latencies for the unit effects were generally the same or longer than those observed for the elicitation of drinking in the behavioral experiments and typically occurred with approximately the same latency as the EEG and blood pressure changes. Similar pressor responses have been reported by Hoffman and Phillips (1975, 1976) and Severs and Daniels-Severs (1973) following intraventricular injections of ANG-II. Hoffman and Phillips (1976) have suggested that the elicited changes in blood pressure may be mediated by ADH release and increase sympathetic discharge. The present results could therefore be interpreted as a non-specific effect of ANG-II, since it has been shown that slight increases in arterial pressure can cause marked changes in the spontaneous activity of central neurons (Hilton and Spyer, 1968). However, two sets of observations suggest that the changes in neuronal firing frequency may have been mediated by a direct action of ANG-II. First, at the lowest dose of ANG-II (25 ng), facilitation of two neurons in the ventral tegmentum occurred in the absence of any detectable changes in EEG or blood pressure. Second, since Findlay (1972) has shown that POA neurons respond with a latency of 25 sec or more following

local application of ANG-II by microiontophoresis, it is possible that the long latency responses of midbrain neurons merely reflects slow binding of ANG-II at its receptor sites. In any case, it is quite difficult to satisfy the criterion of functional specificity when using electrophysiological techniques to investigate the neural systems mediating thirst (Malmo, 1976). The problems associated with interpreting single-unit recording data will be considered further in Section 4.0.

The results of the third experiment reported in this section indicate that separate pathways mediate drinking to cellular and extracellular thirst signals in the midbrain. Lesions of the RMB disrupted drinking to ANG-II microinjected into the POA but had no effect on water intake elicited by the administration of 4 OSM NaCl to the POA or lateral cerebral ventricle. Although the locus of the "osmotic pathways" in the midbrain was not revealed by the results of this experiment, the data strongly suggest that cellular signals must be transmitted along pathways which are distinct from those subserving extracellular thirst. Recently, Malmo (1976) has reported that cells in the midbrain dorsolateral to the periaqueductal grey substance respond to intracarotid administration of hypertonic NaCl in rats. This finding suggests that cellular signals may project to this region of the midbrain, a possibility that was not tested in this study.

Finally, the results of the third experiment demonstrate that lesions of the RMB, which disrupted thirst induced by POA injection of ANG-II, were entirely without effect if the hormone was administered to the lateral ventricle. This observation suggested the possibility that more than one central receptive site might be involved in ANG-II-induced thirst and thereby prompted the series of experiments reported in Section 3.4.

3.4 Multiple Neural Receptive Sites and Pathways for Extracellular Thirst

The results of the electrophysiological and lesion experiments reported in Section 3.3 suggest that signals from ANG-II receptive sites in the POA are transmitted to the mesencephalon. Considered together with results described in Section 3.1 and 3.2 the data suggest that the POA, MLH and RMB are connected by a neural pathway through which signals induced by extracellular stimuli of thirst are directed. It should be noted, however, that a definite interpretation of the results presumes that the POA is a locus of receptive sites for the hormone, and this point remains controversial.

As stated previously (p. 21), the dipsogenic effects of ANG-II were initially attributed to its action in the region of the POA and adjacent forebrain structures because of observations that drinking could be reliably induced when the polypeptide was administered in ng doses to these structures (Epstein *et al.*, 1970). More recently, however, a report by Simpson and Routtenberg (1973) has implicated the SFO as a receptive site for ANG-II. Simpson and Routtenberg (1973) have further suggested that water intake elicited by administering ANG-II to the POA by means of chronic cannulae is due to diffusion of the hormone to the SFO via the CSF of the cerebral ventricles.

Since cannulae aimed at the POA invariably penetrate the lateral cerebral ventricles (see Fig. 7 and Fig. 31), the proposal of Simpson and Routtenberg is reasonable. On the other hand, their suggestion that the SFO is the exclusive central receptive site for ANG-II is inconsistent with some findings of the lesion experiments reported in Sections 3.1 and 3.2. These experiments, it may be recalled, showed that unilateral lesions of the MLH disrupted drinking elicited by the unilateral microinjection of ANG-II significantly more to ipsilateral administration than to contralateral injections. It is difficult to account for these differential effects if the SFO, a midline structure, is the exclusive site of receptors for ANG-II.

The experiments reported in the present section were done to test the possibility that there are multiple receptive sites in the brain mediating drinking to ANG-II. In the first experiment the effects of electrolytic lesions of the SFO on drinking induced by infusion of ANG-II into the jugular vein or following peripheral administration of cellular and extracellular thirst challenges were investigated to see if SFO ablation would abolish the elicited response.

A second experiment, in which the competitive antagonist of ANG-II (P113) was microinjected into the POA prior to administering ANG-II to the same site, was done to test

whether drinking following POA administration of ANG-II
results from diffusion of the hormone to the cerebral ven-
tricles or to the SFO. Finally, in a third experiment, the
possibility of there being multiple neural pathways mediat-
ing drinking to centrally administered ANG-II was investi-
gated by making lesions in the MLH, RMB, habenular nucleus
and interpeduncular nucleus and observing the effects of
the lesions on water intake elicited by microinjecting
ANG-II into the POA, SFO and anterior third ventricle.

METHODS

In the first experiment two series of animals were used.
Twenty-one rats were implanted with cannulae into their
jugular veins using procedures described in Section 2.2.
Following postoperative recovery the animals were tested
for drinking in response to synthetic ANG-II infused
through the implanted cannula at a continuous rate of
0.38 ± 0.018 µg/min per kilogram of body weight for a
60-min period, after which the total water intake
(± 0.1 ml) of each rat was measured. Lesions aimed at the
SFO were made in 15 rats which drank at least 3.5 ml of
water in two consecutive behavioral tests using procedures
described in Section 2.5 and testing with ANG-II was
resumed on the sixth postoperative day. At the completion
of the last test the rats were sacrificed and the sites of
lesions determined according to procedures described in
Section 2.6. Only those rats in which the tissue damage

produced by the lesions was confined to and completely destroyed the SFO were considered experimental animals for the purpose of statistical comparisons. Rats in which the SFO was not completely ablated comprised a second ("partial-lesion") group. A third group consisted of unoperated, control animals.

In a second series of 12 rats lesions were made in the SFO at 3 sites along its longitudinal extent using a stainless steel monopolar electrode and an anodal direct current of 1.0 mA/10 sec for each lesion. Beginning on the 7th postoperative day, a series of tests was begun to determine the effects of the lesions on water intake elicited by various cellular and extracellular stimuli of thirst. An unoperated group of 6 animals was used as controls. The first thirst challenge was produced by depriving the rats of water for 24 hr and water intake was measured for the first 2 hr following the termination of the deprivation. In the next test, animals were induced to drink by an i.p. injection of 2.0 M NaCl (0.5% of body weight) and 1-hr intake was measured. In the third test drinking induced by i.p. injection of 12 dog units of renin was measured after 6 hr. Next, water intake to an i.p. injection of isoproterenol (0.15 mg/kg body weight) was measured after 2 hr. Finally, the drinking response of the animals to a s.c. injection of 20% polyethylene glycol (1.5% of body weight) was measured after 6 hr. Control injections of appropriate volumes of 0.15 M NaCl

were given for each test. The injections were made on alternate days over a period of three weeks.

In the second experiment 8 rats were used. The animals were implanted with 23-gauge cannulae bilaterally into homologous sites in the POA at an angle of 16° from the vertical (angled POA cannulae) to avoid penetrating the lateral cerebral ventricles. Beginning on the fifth day postoperatively, 30 min water intakes elicited in response to POA administration of ANG-II (50 ng in 0.2 or 1.0 µl artificial CSF) preceded by 5 min by an injection of P113 (500 ng in 0.2 or 1.0 µl artificial CSF) were determined. Injections were made on alternate days to the right and left POA cannulae and only one injection was administered on any single day. Control injections of ANG-II (50 ng in 0.2 or 1.0 µl artificial CSF), P113 (500 ng in 1.0 µl artificial CSF) and artificial CSF (1.2 µl) were also made.

In the third experiment, 58 rats were implanted bilaterally with 23-gauge cannulae into homologous sites of the POA and a single 23-gauge cannula was implanted into either the SFO (n = 42) or the anterior third ventricle (n. = 16). During the same operation monopolar insect pin electrodes were implanted bilaterally into either the MLH (n = 16), the RMB (n = 16), the habenular nucleus (n = 13) or the interpeduncular nucleus (n = 13).

Beginning 5-11 days postoperatively, each of these animals was tested once daily for drinking in response to

a single microinjection of synthetic ANG-II into either the POA (50 ng ANG-II in 0.2 µl artificial CSF), the SFO (10 ng ANG-II in 0.2 µl artificial CSF) or anterior third ventricle (50 ng ANG-II in 0.2 µl artificial CSF). Only one microinjection was made in any animal per day. Each rat received two injections of ANG-II into each of the intracranial loci with the order of administration occurring randomly. Water intakes (± 0.1 ml) were measured for 15 min after the injection in the animal's home cage with food also available.

Fifty rats in this series were lightly anesthetized with ether and bilateral lesions were made by passing an anodal current of 1.0-2.5 mA for 10-25 sec between the implanted monopolar electrode and an indifferent electrode clipped to the ipsilateral ear. Testing with ANG-II was resumed on the fifth day post-lesion and each animal received two tests at each intracranial site before sacrifice. Cannula and lesion placements were determined according to the histological procedures described in Section 2.6. Tests of significance were made by an overall analysis of variance and means were compared by Newman-Keuls tests or Student's t-test for each of the experiments.

RESULTS

1. *Effect of lesions of the SFO on water intakes elicited by the infusion of ANG-II into the jugular vein and by peripherally administered cellular and extracellular stimuli of thirst.*

The average net intake of water during the two pre-lesion tests with ANG-II infused into the jugular vein was 4.2 ± 0.5 ml. The average latency to elicit water intake was 20.0 ± 1.7 min. Little or no drinking was observed following the control infusion of isotonic saline.

All nine rats in which the lesions completely destroyed the SFO showed a significant attenuation of drinking in response to jugular administration of ANG-II (Fig. 26; t = 3.30, $P < 0.01$ in comparison with pre-lesion intakes; t = 2.91, $P < 0.05$ in comparison with controls). Water intake was reduced from 4.8 ± 0.6 ml to 0.5 ± 0.4 ml in these animals. In each case, the SFO was completely destroyed. A photomicrograph showing the site of the lesion in a representative animal is presented in Fig. 27.

Lesions that partially ablated the SFO (Fig. 27) also significantly reduced water intake elicited by the jugular infusion of ANG-II (t = 2.98, $P < 0.05$ in comparison with controls). However, the attenuation of elicited drinking was significantly less (t = 2.42, $P < 0.05$) than in animals in which the SFO was completely destroyed.

Figure 26

The effect of ablating the subfornical organ
(SFO) on drinking elicited by a 60-minute constant
rate infusion of ANG-II through a jugular vein cannula.
The amount drunk during the ANG-II infusion minus the
amount drunk during a control saline infusion is
designated net water intake. In 9 rats the lesion
completely destroyed the SFO. In 6 rats the SFO was
only partially destroyed. The results for the non-
operated control group (n = 6) is a comparison of the
water intake during the first two infusions (open
columns) and the last two infusions (cross-hatched
columns). Values are mean ± S.E.M. Open and diagonal-
band-marked columns, respectively, denote elicited
water intakes before and after the complete and
partial SFO lesions.
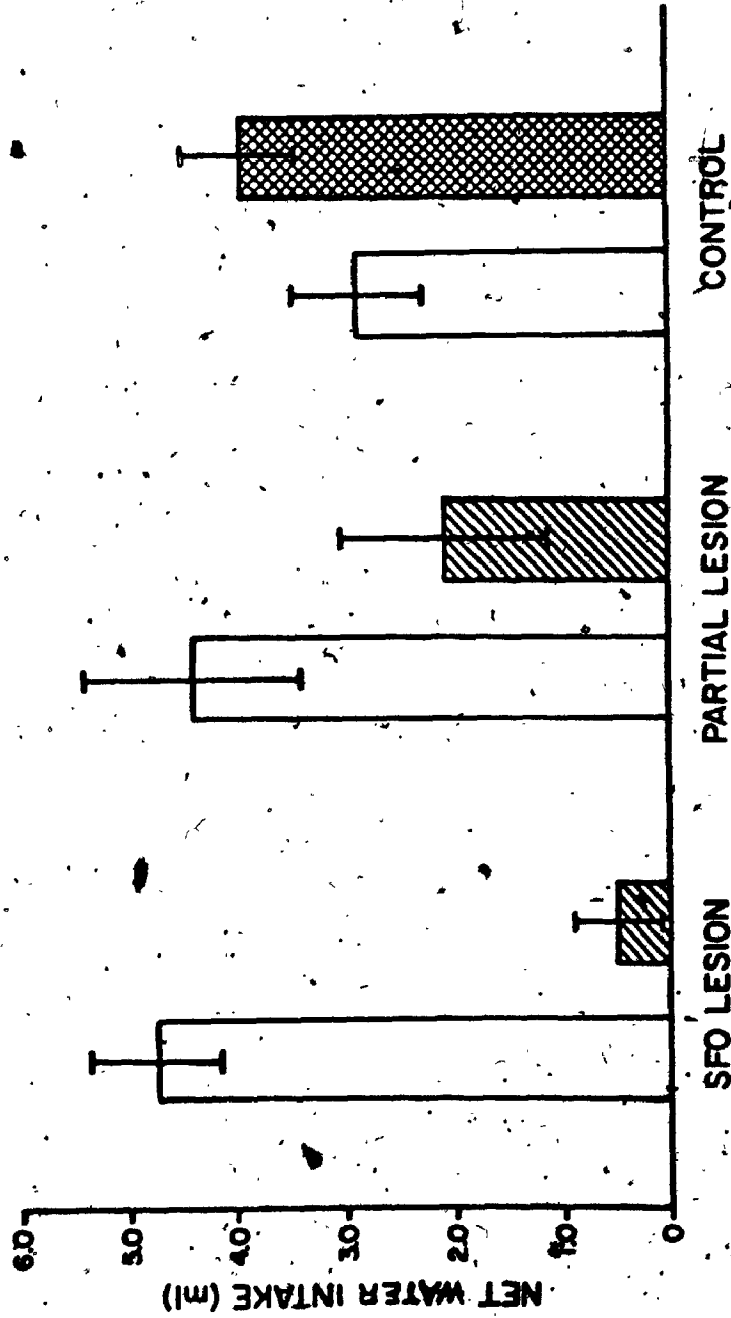
Figure 27

Photomicrographs of brain sections from two
representative animals showing

A   the locus of a lesion in which the SFO was
     completely destroyed (arrow)

B   the locus of a lesion in which damage to
     the SFO was partial (arrow).

A

B

2mm

As shown in Figure 28, in the second group of animals with lesions of the SFO (n = 10), water intakes elicited by a single peripheral injection of isoproterenol or renin were significantly less than for control animals (t = 5.39, $P < 0.01$ and t = 3.29, $P < 0.01$, respectively). The mean elicited water intakes for SFO-lesioned and control animals did not differ significantly following the administration of polyethylene glycol (t = 0.35, $P > 0.05$), hypertonic saline (t = 1.95, $P > 0.05$) or after 24 hr water deprivation (t = 0.53, $P > 0.05$). A representative photomicrograph showing the location of lesions in the SFO is given in Figure 29.

2. *Effect of varying the volumes of ANG-II or the relative volumes of P113 and ANG-II administered to the POA on water intake.*

As shown in Figure 30, ANG-II microinjected alone through angled POA cannulae elicited significant drinking. However, the water intake was significantly greater if the hormone (50 ng in both cases) was in a 1.0 µl volume as compared to a 0.2 µl volume (6.2 ml vs. 3.85 ml, t = 2.28, $P < 0.05$). Pretreating the POA with P113 significantly attenuated water intake elicited by microinjecting ANG-II into the same site. However, the degree of attenuation depended on the relative volumes of antagonist and ANG-II. When the volume of ANG-II was 0.2 µl, drinking was completely blocked by pretreatment of the POA with 1.0 µl of

Figure 28

Mean intakes of water of SFO-lesioned and
control animals following:

A   24 hr water deprivation (2 hr intake)

B   single intraperitoneal injection of hyper-
     tonic saline (1 hr intake)·

C   single intraperitoneal injection of renin
     (6 hr intake)

D   single intraperitoneal injection of iso-
     proterenol (1 hr intake)

E   single subcutaneous injection of polyethylene
     glycol (6 hr intake)

Vertical bars represent ± S.E.M. Number of
animals per test is indicated above columns.
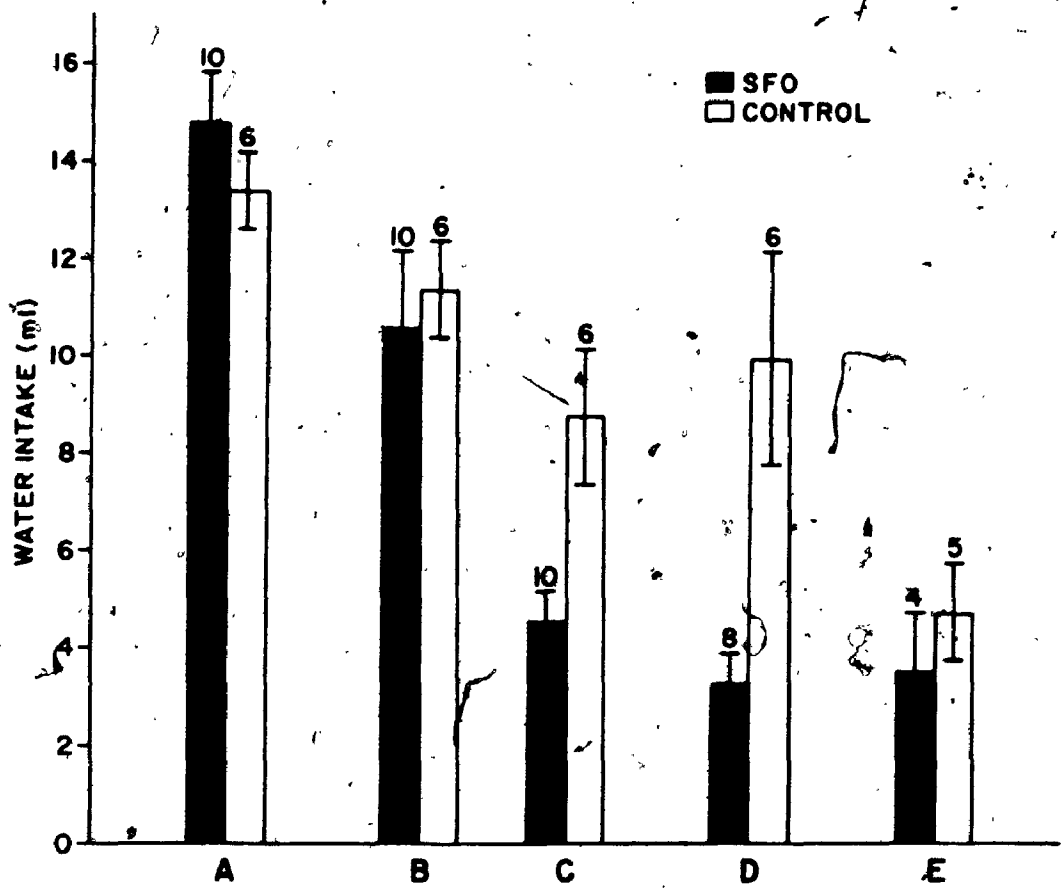
Figure 29

Photomicrographs of lesions of the SFO.
A and B are representative sections from the same
animal and show complete destruction of the SFO
in the (A) anterior and (B) posterior aspects.
The section in C shows the site of ineffective
lesion adjacent to the SFO. In each case the
sites of lesions are designated by arrows.

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Figure 30

Effect of varying the volumes of ANG-II or
the relative volumes of the competitive antagonist
(P113) and ANG-II on water intake during a 30 min
period. All injections were made through a
cannula into the POA. The volumes infused are
indicated in the various columns. Vertical bars
represent Means ± S.E.M. of consecutive ipsilateral
and contralateral injections.
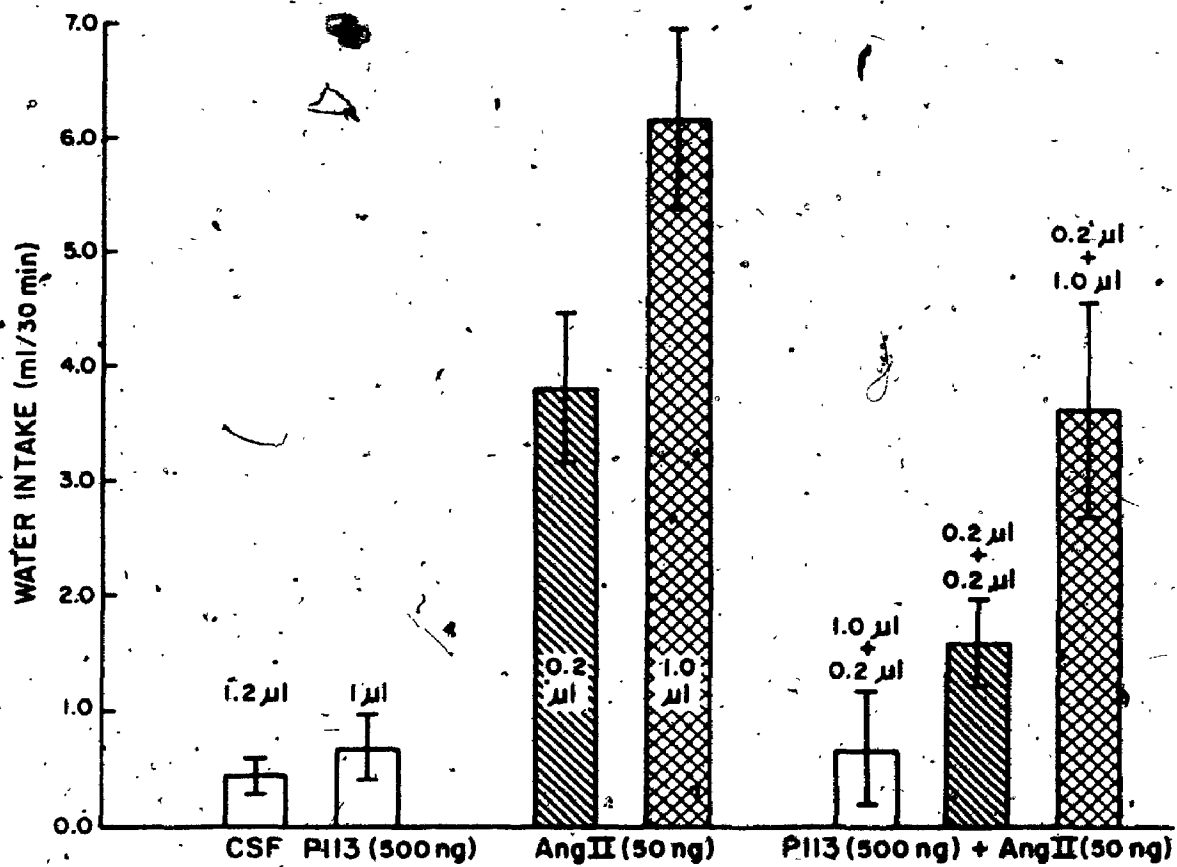
the antagonist. When the volume of the antagonist was reduced to 0.2 μl, water intake was still significantly attenuated (t = 2.31, $P < 0.05$) but not completely blocked. Finally, a volume of 0.2 μl P113 significantly attenuated drinking initiated by 1.0 μl ANG-II (from 6.2 to 3.65 ml, t = 2.46, $P < 0.05$) but the water intake was not significantly different from that observed when ANG-II (50 ng) was administered alone in an 0.2 μl volume. A photomicrograph showing the locus of the cannulae in the POA is given in Figure 31.

3.   *Effect of lesions of the MLH, RMB, habenular nucleus and interpeduncular nucleus on drinking induced by POA administration of ANG-II.*

As shown in Figure 32, during the prelesion tests the animals consumed significant volumes of water in response to control administration of ANG-II (mean intake of 4.56 ml for all sites tested). There were no significant differences in elicited water intakes due to structure stimulated at the doses used ($F_{2,35}$ = 1.13, $P > 0.05$). Photomicrographs showing the location of the cannulae in the POA and SFO are presented in Figure 33.

Lesions of the MLH or MB caused a significant decrease in water intake elicited by microinjection of ANG-II into the POA ($F_{3,35}$ = 3.62, $P < 0.05$) without attenuating drinking to ANG-II applied to the SFO or anterior third ventricle (Figure 32). Habenular lesions had no effect on

Figure 31

Coronal photomicrograph of brain from a
representative animal showing the location of
angled cannulae in POA (arrows) used to inject
ANG-II and P113. Magnification 12 times.
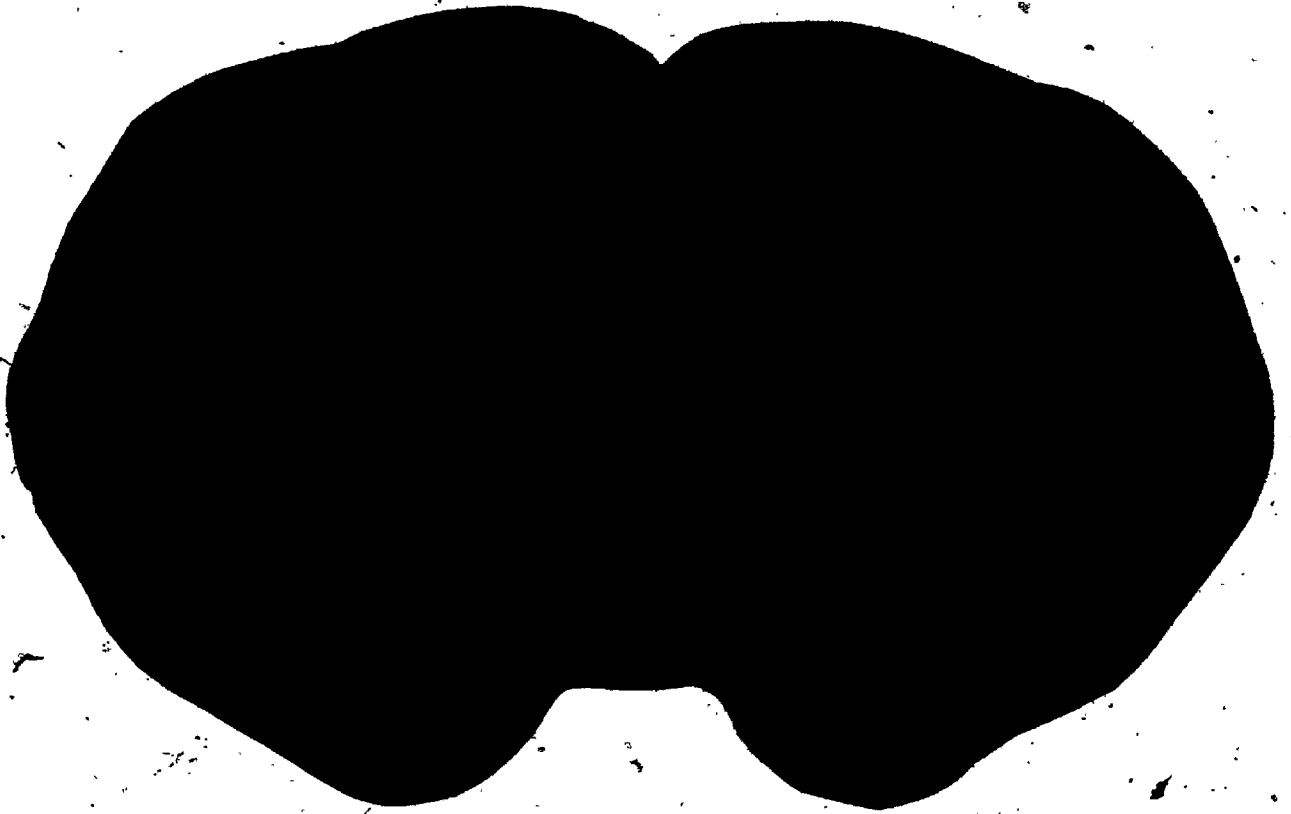
Figure 32

Effects of lesions of the MLH, RMB, habenular
nucleus (HB), and interpeduncular nucleus (IP) on
water intake during the 15 min following the
administration of ANG-II to the POA, SFO or
anterior third ventricle (V). Results are
expressed as means and vertical bars represent
± S.E.M. Number of animals per test is indicated
above columns. Although the drinking response to
ANG-II microinjected into the V was significantly
attenuated by IP lesions, the number of rats
(n = 2) in this group is too small to allow any
conclusions to be made.
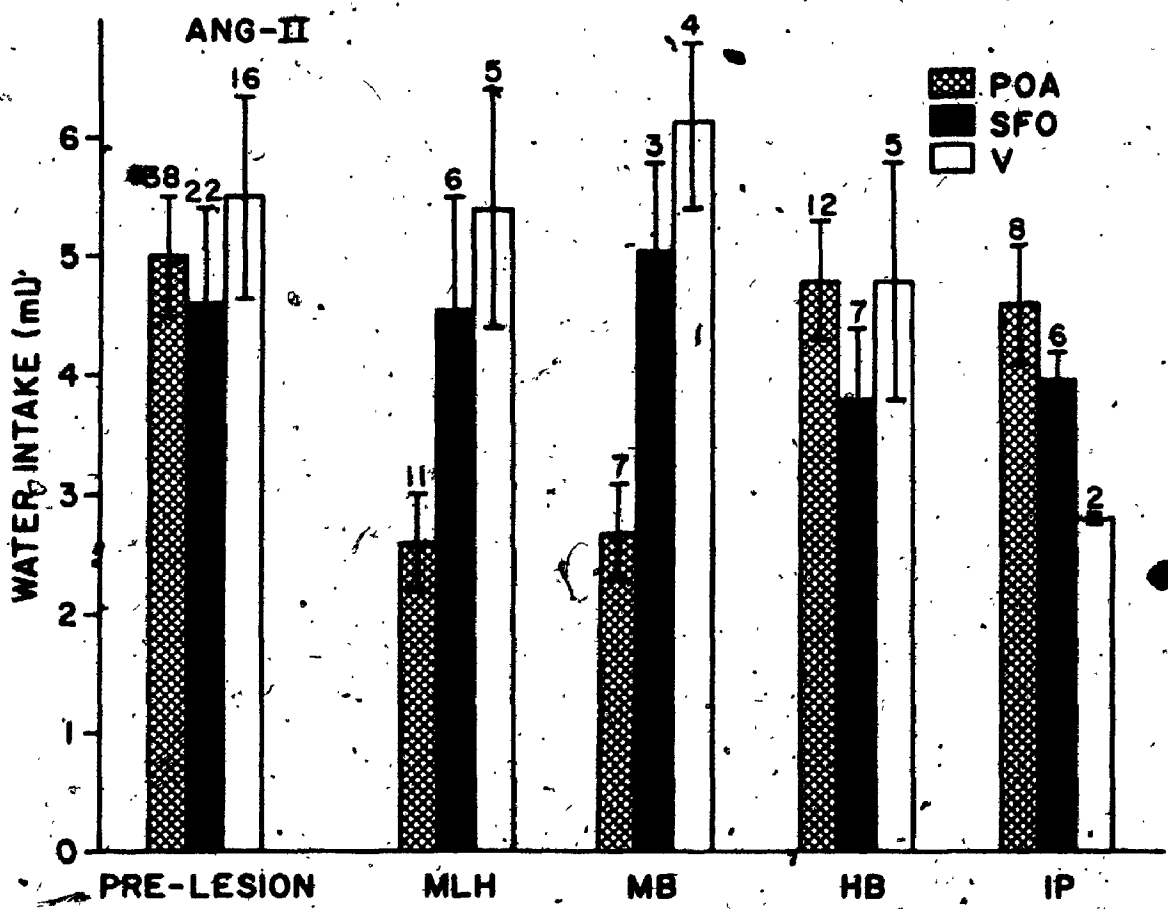
Figure 33

Photomicrographs showing the locus of the
cannulae (arrows) used for intracranial injections
in

A   the POA

B   the SFO

Magnification 7 times.

B

A

2mm

Figure 34

Photomicrographs showing the location of the
bilateral lesions (in each case designated by
arrows) in

    A   the MLH

    B   RMB (effective large lesions)

    C   habenular nucleus

    D   RMB (small, ineffective lesions)

    E   lateral aspects of the interpeduncular
        nucleus

Magnification for A to D, inclusive, is 5 times;
for panel E, 7 times.

A

B

C

D

E

2mm

water intake elicited by administering ANG-II to any of the 3 sites ($F_{6,70} = 1.06$, $P > 0.05$). In the two animals given interpeduncular lesions, the drinking response to ANG-II microinjected into the anterior third ventricle was attenuated.

The MLH lesions which were effective in disrupting drinking to ANG-II administered to the POA were located in the midlateral hypothalamus, just dorsolateral to the fornix and at the level of the dorsomedial nuclei. The effective RMB lesions were dorsal and lateral to the mammillary peduncle in the area of passage of the MFB. The interpeduncular lesions destroyed the lateral aspects of the interpeduncular nucleus bilaterally. The locations of the MLH, RMB, habenular nucleus and interpeduncular nucleus lesions are shown in Figure 34. MLH and RMB lesions, which disrupted drinking elicited by administering ANG-II to the POA but not drinking elicited by micro-injecting ANG-II to the SFO or anterior third ventricle, did not produce noticeable ventricular damage in any of the animals.

DISCUSSION

The results of the experiments reported in this section indicate that the central mechanisms which mediate drinking to ANG-II may involve multiple receptive sites.

The results of the first experiment support the view that the SFO is a receptive site for ANG-II. Lesions that

completely destroyed the SFO significantly reduced water intake in response to ANG-II infused through a cannula chronically implanted in the jugular vein or following peripheral administration of renin or isoproterenol. Since SFO ablation did not completely abolish elicited drinking in any of the animals, however, it may be suggested that receptive sites for the hormone are present at other neural loci. The results of the second experiment indicate that the POA is one of the receptive sites for ANG-II. The results of this experiment further suggest that the "receptors" are diffusely represented in the anterior forebrain since it was found that drinking to equal doses of ANG-II is increased as the volume administered to the POA is increased and by the observation that the degree of attenuating of ANG-II induced water intake by P113 pretreatment depends, at least in part, on the relative volumes of the antagonist and ANG-II.

The results of the third experiment suggest that there are also multiple pathways subserving drinking initiated by ANG-II. This conclusion is based on the observation that the effects of lesions of the MLH and RMB on elicited drinking depend on the site of administration of ANG-II. Lesions of the MLH and RMB significantly attenuated drinking to microinjection of ANG-II into the POA but had no effect on water intake initiated by the administration of ANG-II into the SFO or anterior third ventricle. Although the neural pathways which subserve water intake

initiated by microinjecting ANG-II into the SFO or into the ventricles are not known at present, the results indicate that they are not the same pathways which mediate thirst elicited by administering ANG-II to the POA. Therefore, it is necessary to consider the possibility that there are both multiple receptive sites and pathways for the dipsogenic effects of ANG-II. The MLH and RMB appear to be part of the pathway which transmits signals from the POA but not from SFO or ventricular "receptors".

4.0   GENERAL DISCUSSION

Body fluid balance depends on drinking behavior to replace obligatory losses of water.   The purpose of the present study was to investigate which neural pathways and integrative structures transmit and process the signals that initiate water intake in response to cellular and extracellular dehydration. The major findings may be summarized as follows:

(1). Drinking behavior induced by administration of the hormone, ANG-II, to the POA is mediated by a neural pathway which passes through the MLH and RMB.

(2) Multiple neural receptive sites and pathways subserve drinking elicited by extracellular stimuli of thirst.

(3) Cellular and extracellular thirst signals are mediated by separate neural pathways in the LH and midbrain.

Each of these findings will now be considered in terms of the objectives of this study.

1.   Neural Pathways Mediating Drinking Behavior to POA
      Administration of ANG-II

The results of the electrophysiological experiments reported in Sections 3.1 and 3.3 suggested that the MLH and paramedial dorsal and ventral midbrain are involved

in the initiation of water intake in response to POA admin-
istration of ANG-II. It was difficult to be certain that
the activated neurons were specifically involved in the
drinking response because desynchronization of the EEG and
increases in arterial blood pressure were also observed to
occur (p. 136). Moreover, although facilitation of two
midbrain neurons was observed at low doses of ANG-II in the
absence of concurrent changes in EEG and arterial blood
pressure (p. 136) the possibility that the unit effects
resulted from local disruptions in cerebral hemodynamics
cannot be excluded. In this regard, it should be noted
again that ANG-II is an extremely potent vasoactive pressor
agent (Page and McCubbin, 1968).

Other factors also make it difficult to establish the
functional specificity of the changes in neuronal activity
solely on the basis of electrophysiological results. For
one thing, the acute experiments were done under ethyl
carbamate, an anesthetic agent known to influence the re-
lease of epinephrine (Aub, Bright and Foreman, 1922) and
adrenocorticotrophic hormone (Spriggs and Stockham, 1964),
and which might therefore be expected to disrupt normal
neural, and especially hypothalamic, excitability (Cross
and Dyer, 1971). Komisaruk, McDonald, Whitmoyer
and Sawyer (1967), and others (e.g., Lincoln,
1969) have shown that some hypothalamic units in the ethyl
carbamate-anesthetized rat show acceleration and decelera-
tion of firing rate associated with changes in EEG wave

pattern and amplitude. These fluctuations in unit discharge activity further complicate assessments of unit responsiveness to ANG-II.

Another requirement for determining the functional specificity of the observed changes in unit firing frequency is that the "ANG-II receptors" be stimulated discretely. In this regard, the proposal of Simpson and Routtenberg (1973) that drinking elicited by administration of ANG-II to the POA results from diffusion of the hormone to the SFO must be considered carefully. This issue is further dealt with on pp. 197 to 202. A final objection to concluding that the increased LH and midbrain neuronal discharge is specific to thirst mechanisms is that these changes may in fact be associated with motor, attentional or associative processes (Mogenson, 1975). For these reasons the main contribution of the electrophysiological recordings was to implicate several neural structures in the initiation of drinking behavior. It was chiefly to meet the objections of functional specificity that it was important that the involvement of the MLH and midbrain be confirmed in the subsequent lesion studies.

The effective hypothalamic lesions for producing a disruption of drinking in response to administration of ANG-II to the POA were located in the LH, just lateral or dorsolateral to the fornix, and at the level of the dorsomedial hypothalamic nuclei. Since anatomical studies (reviewed in Section 1.4) have shown that fibers of the

descending MFB project from the lateral preoptic region
through the MLH to the midbrain, it may be suggested that
destruction of these MFB pathways disrupted the elicited
drinking. This proposal is supported by the finding that
lesions of the MFB at the level of the RMB had a similar
disruptive effect.

As shown in Figure 35 the MFB lesions which disrupted
elicited drinking at the level of the rostral mesencephalon
(effective MB) were much larger than lesions placed in the
MLH (effective MLH). Lesions comparable in size to those
made in the MLH were without effect when placed in the
rostral midbrain (ineffective MB). This observation is in
agreement with work by Millhouse (1969), Swanson (1976),
and others (e.g., Nauta, 1958; Zyo, Oki and Ban, 1963;
and Ban, 1966) who have shown that the descending MFB
diverges at the rostral mesencephalic level by decussating
as well as by diverging on the ipsilateral side. The
divergence of MFB at the level of the RMB may also explain
why unilateral and bilateral lesions placed more caudally
in the mesencephalon (see Figure 35) were not effective in
disrupting drinking to POA administration of ANG-II despite
the fact that they may have interrupted connections at loci
of single units activated by ANG-II (see Figures 18 and 22).

Although a rostro-caudally directed pathway subserving
ANG-II-induced drinking is suggested by the results of this
study, other neural systems may also be involved. For
example, it has been shown that in addition to the descend-

Figure 35

Figure 35 is a composite of photomicrographs
presented previously in Figs. 16, 23, and 34,
showing that the size of lesions required to disrupt
drinking at the level of the LH ("effective MLH") is
much smaller than those required to produce similar
effects when placed more caudally in the midbrain.
These data are in agreement with anatomical studies
(see text) and support the hypothesis that the MFB
may mediate drinking to POA administration of ANG-II.
Arrows, in each case, point to the lesions.

EFFECTIVE MLH
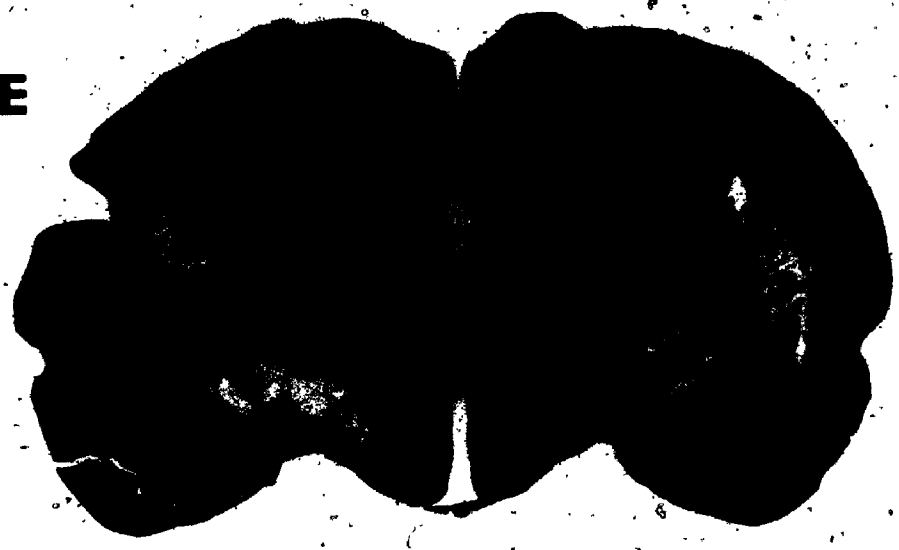
INEFFECTIVE MB

EFFECTIVE MB

2mm

ing MFB limbic structures project caudally by a more dorsal route which passes through the habenular nucleus and then continues ventrocaudally to the interpeduncular nucleus, dorsal and ventral tegmental nuclei and caudal central grey (Nauta, 1958; Zyo, Oki and Ban, 1963; Ban, 1966; Millhouse, 1969). Although the present results indicate that the MFB is the major pathway for transmitting signals from ANG-II receptive sites in the POA to the mesencephalon, the observation that the elicited drinking was not completely abolished following lesions of the MLH or RMB suggests that the dorsal pathway also may play a role.

It is also possible that water intake induced by POA administration of ANG-II may be mediated by rostral pathways since limbic forebrain projections to the mesencephalon are reciprocated by ascending parallel fibers in the MFB which terminate in, or send collaterals to, the LH, POA, septum and the rhinencephalon (Millhouse, 1969; Grantyn and Grantyn, 1972, 1973). Morgane (1964, 1975) and others (Nauta, 1960; Grossman, 1968) have suggested that these rostral connections may be important in ingestive behaviors. Another alternative is that other limbic and hypothalamic structures may play an important modulatory role in the initiation of drinking by ANG-II by exerting descending influences directly, or indirectly, on to the midbrain systems. This possibility is suggested by anatomical data which have shown that the path neurons of the descending MFB, especially those in the LH, demon-

strate highly developed dendritic fields that intermingle
extensively with adjacent neural populations so that
activity in widespread forebrain and diencephalic fields
may be conveyed to midbrain structures (Millhouse, 1969;
Swanson, 1976). The finding reported in Section 3.1 that
lesions of the ventromedial nucleus of the hypothalamus
cause an increase in water intake elicited by the micro-
injection of ANG-II into the POA is in agreement with this
view.

Consistent with the foregoing discussion, the possi-
bility of a forebrain-hypothalamic-midbrain neural circuit
underlying ANG-II thirst presents an attractive working
model. Previous studies have implicated limbic forebrain-
midbrain interactions in other important behavioral and
physiological functions such as the neural control of
agonistic (Carli, Malliani, and Zanchetti, 1963; Berguist,
1970) and emotional (Grastyn, Karmos, Vereczkey and
Kellenyi, 1966; Komisaruk and Olds, 1968; Routtenberg, 1968;
Molnar, 1973) behaviors, in the neural processing of
sensory information (Redding, 1967; Parmeggiani and
Rapisarda, 1969) and in sleep (Parmeggiani and Rapisarda,
1969; Linberry and Siegel, 1971). For agonistic and
ingestive behaviors, it has been proposed that the primary
role of the limbic forebrain structures and the hypothala-
mus is to integrate the various incoming endocrine and
autonomic signals whereas the midbrain may be more involved
in coordinating the motor patterns required to perform the

appropriate behavior (Mogenson and Huang, 1973).

According to such a view, signals from ANG-II receptors in the POA are integrated and processed at forebrain and diencephalic levels and could then activate appropriate mesencephalic systems to initiate water intake.

2.   Multiple Neural Pathways for Extracellular Thirst

In the first part of this section the discussion was focused in the possibility that a neural pathway linking the POA with the MLH and RMB mediates drinking initiated by POA administration of ANG-II. However, to be certain that the MLH and MB have a specific role in the elicited response depends on knowing whether the hormone is acting at receptive sites in the POA, or elsewhere in the brain. This requirement relates once again to the problem of establishing functional specificity, a major issue not only in the field of regulatory physiology but in many areas of neurophysiology as well (Mogenson, 1975). As Mogenson (1975) has stated (p. 262) ". . . the critical (neural) pathways that influence and initiate drinking can only be determined when the appropriate receptors are stimulated."

Consistent with the foregoing, two closely related issues will now be considered: (1) Do the present data support the hypothesis that the SFO is the exclusive site of "receptors" for the dipsogenic action of ANG-II? (2) Do the results support the view that the POA is also a receptive site?

The considerable emphasis recently given the SFO as a receptive site for ANG-II (*e.g.*, Simpson and Routtenberg, 1973, 1975; Felix and Akert, 1974; Johnson and Epstein, 1975) appears, in view of the present results, to be justified. The SFO was consistently the most sensitive site for the initiation of drinking activity following direct administration of ANG-II and lesions of this structure produced/a significant reduction of water intake in response to systemic infusion of ANG-II or to peripheral administration of renin or isoproterenol. The results also agree in part with the proposal of Simpson and Routtenberg (1973) that drinking initiated by the administration of ANG-II to the POA through chronic cannulae (that pass through the lateral central ventricle) is due to diffusion of the hormone to the SFO, since water intakes elicited with straight cannulae typically were greater than those observed with cannulae that bypassed the lateral central ventricle. The findings of Assaf and Mogenson (1975) also support the diffusion hypothesis. They showed that pretreating the lateral central ventricle with an ANG-II competitive antagonist (P113) attenuated by 25% water intake elicited by POA administration of ANG-II through cannulae that passed through the lateral central ventricle, whereas no effect on drinking resulted if the cannulae were angled.

On the other hand, several observations indicate that diffusion of ANG-II from its site of administration in the

region of the POA to the SFO cannot account entirely for the copious drinking that is elicited. For one thing, although electrolytic ablation of the SFO attenuated, drinking to peripheral administration of renin or isoproterenol or following jugular infusion of ANG-II, the response was not completely abolished. Simpson and Epstein (1974) have reported a comparable decrement of water intake elicited by systemic infusion of ANG-II following SFO lesions, while Hoffman and Phillips (1975) found that lesions of this structure had little or no effect on drinking after peripheral administration of renin. A second finding that cannot be readily explained by assuming that the SFO, a midline structure protruding into the third ventricle, is the exclusive site of "receptors", is that following unilateral MLH lesions, the degree of disruption of elicited drinking depends on whether ANG-II is administered ipsilateral or contralateral to the side of the lesion. Indeed, this latter result suggests that with unilateral POA injections of ANG-II only ipsilateral receptive sites are stimulated.

In addition to its possible spread via the ventricular CSF, there are two other routes by which ANG-II might be transported to the SFO. Johnson and Epstein (1975) have suggested that the cerebral circulation may be one such avenue. However, since Assaf and Mogenson (1975) have demonstrated that the attenuation of water intake when P113 was administered to the SFO was no greater than when

the antagonist was given into the lateral central ventricle, diffusion of the hormone from the POA through the ventricles is a more likely possibility. Transport by the cerebral vasculature is also unlikely because it has been found that ANG-II penetrates the blood-brain-barrier very slowly, if at all (Volicer and Loew, 1971). Finally, since the ANG-II was administered in a solution, the possibility that it may diffuse through neural tissue itself cannot be overlooked. Johnson and Epstein (1975) have reported that following injection of 1.0 μl of tritiated ANG-II into the POA (through straight cannulae) the density of labelling falls off sharply within 0.5 mm of the cannula tip, but reappears in the ventricular walls. Their finding suggests that a greater proportion of the injectate may be transported up the sides of the cannula and into the CSF of the ventricles than through the cerebral circulation or neural tissue. Myers (1974) has also shown that 0.1-1.0 μl (the volumes used in this study) of solutions infused into brain remain localized to the vicinity of the injection site. Considered together, the results suggest that there are two components of drinking initiated by administering ANG-II to the preoptic region -- one component due to the diffusion of ANG-II to the SFO (or perhaps to "receptors" in the walls of the ventricles) and a second component due to ANG-II acting on the POA. The results of subsequent experiments which support this view will be considered next.

In one experiment (see Figure 30) the POA was pre-

treated with P113 competitive antagonist prior to micro-injecting ANG-II into the same site by means of angled cannulae. There were two salient observations. First, the degree of attenuation by P113 pretreatment of water intake induced by ANG-II depended directly on the relative volumes of the antagonist and ANG-II. The larger was the volume of the antagonist over that of ANG-II, the greater was the attenuation. Second, drinking to equal doses of ANG-II was found to increase as the volume administered was increased. From these results it may be concluded that the POA is receptive to the dipsogenic effects of ANG-II, but in addition it may be suggested that the "receptors" are diffusely represented in the anterior forebrain.

Additional support for the POA as a site of receptors for the dipsogenic action of ANG-II has been gained by the recent work of Sharpe and Swanson (1974). They reported that low doses of ANG-II microinjected into forebrain structures, and especially the POA, of the monkey reliably elicited drinking. Perhaps more importantly, Sharpe and Swanson (1974) found no correlation between the distance of injection sites from the ventricles and their sensitivity to ANG-II, which would be expected if the ventricles or a circumventricular structure such as the SFO were the exclusive site of receptors. Sharpe and Swanson (1974) also observed negative drinking sites, *e.g.*, midline thalamic nuclei, despite the fact that the cannulae used to deliver the solutions to these structures

passed directly through the lateral cerebral ventricle. Preliminary electrophysiological data supporting the POA as an ANG-II sensitive drinking site have been obtained by Findlay (1972) and Wayner (1973) who have reported changes in the rate of discharge of POA neurons following micro-iontophoretic application of ANG-II.

Studies in which the effects of lesions of the preoptic region on water intake elicited by ANG-II have been investigated have not been conclusive. In some experiments POA ablation has been reported to attenuate ANG-II-induced drinking (Peck and Novin, 1971; Almli and Wiess, 1974), whereas in others no change was observed (Blass and Epstein, 1971; Simpson and Routtenberg, 1975). In any case, lesioning the POA is clearly not the best method of studying the role of this structure in body fluid homeo-stasis since POA lesions may impinge on the septal area, ablation of which has been reported to result in a poly-dipsia which is specific to ANG-II (Blass et al., 1974) as well as a more general polydipsia (Wishart and Mogenson, 1970). Lesions of the POA may also result in a poly-dipsia secondary to a reduction of ADH release (Gemert, Miller, Carey and Moses, 1975). Using competitive antagonists of ANG-II such as P113 may therefore be a more definitive way to investigate the role of specific neural structures in ANG-II-mediated drinking behavior. The suitability of this analog is indicated by the results of a recent study which showed that P113 applied iontophor-

etically to the SFO of cats inhibited the activity of SFO neurons activated by local application of ANG-II (Phillips and Felix, 1975).

The already complex issue of the locus of central receptors for ANG-II is further complicated by reports of a complete renin-angiotensin system in brain (Roth, Weitzman and Piquilloud, 1969; Fischer-Ferraro, Nahmod, Goldstein and Finkielman, 1971; Ganten, Boucher and Genest, 1971; Daul, Heath and Garey, 1975). Although the role of the cerebral renin-angiotensin system and its relationship to renal or submaxillary (Menzie, Michelakis and Yoshida, 1974) mechanisms for renin release are unknown, it is of considerable potential significance that the polypeptide may be synthetized within brain. There is at least some evidence for a neurotransmitter function for ANG-II (Severs and Daniels-Severs, 1973), and although octa-peptide or precursor storage in particular areas of the brain has not been established, a recent study by Roth, Heath and Ward (1975) is suggestive. They demonstrated that within human brain angiotensin converting enzyme is localized to the caudate nucleus and is present there in high concentrations. In view of the dipsogenic potency of ANG-II administered to the anterior forebrain (Epstein et al., 1970; Swanson and Sharpe, 1973), it is possible that cerebral ANG-II may normally be formed in the region of the caudate and subsequently exert its physiological effects locally, on tissue in the POA and other adjacent

structures. Peripheral, ANG-II may, on the other hand, be transported via the systemic circulation to the SFO since this structure is highly vascularized (Spoerri, 1963; Akert, 1969; Phillips, Bachhorn, Leavitt and Hoffman, 1974) and devoid of a blood-brain-barrier (Koella and Sutin, 1967; Phillips et al., 1974). Although it is largely speculative, this interpretation would account for the findings that both the POA and SFO appear to be capable of mobilizing drinking behavior when stimulated by direct application of exogenous ANG-II and that lesions of the SFO do not completely disrupt ANG-II induced drinking. Moreover, the proposal would appear to circumvent the problem of low permeability of the blood-brain-barrier to haematogenic ANG-II.

From the foregoing discussion it is reasonable to postulate that more than one central receptive site mediates ANG-II elicited water intake. This proposal is consistent with the results of the lesion experiments reported in Section 3.4 which showed that ablation of the MLH and RMB produced a significant reduction in water intake to ANG-II microinjected into the POA, but did not affect drinking following administration of ANG-II to the SFO or anterior third ventricle.

3. Separate Neural Pathways for Cellular and Extracellular Thirst

Up to this point the emphasis of the discussion has been on neural pathways which subserve extracellular thirst. It was proposed that a pathway passing through the MLH and RMB mediated drinking to POA administration of ANG-II and to peripheral injections of renin and isoproterenol. In the final part of this section the results that bear directly on the question of whether separate neural pathways are involved in water intake initiated by cellular and extracellular thirst stimuli will be considered. Before discussing the present data, however, a brief perspective how the "double depletion hypothesis of thirst" relates to this study will be offered.

The dramatic disruption of water ingestion in response to cellular (Epstein and Teitelbaum, 1964; Epstein, 1971) and extracellular (Fitzsimons, 1966; Stricker and Wolf, 1967) thirst challenges following bilateral lesions of the LH has long been interpreted to mean that this area is an integrative center in the central control of regulatory thirst (Teitelbaum and Stellar, 1954; Montemurro and Stevenson, 1957; Epstein, 1971). However, as pointed out by Morgane (1975), to ascribe as the locus of complex and physiologically vital functions an extensive area in the brain destroyed by large lesions is of doubtful validity, except for the most molar types of behavioral analyses.

It is likely, as Morgane (1975) has argued, that "bull-dozer approaches such as large electrolytic lesions simply do not have adequate resolving power to define the relative roles of the maze of chemo-specific pathways criss-crossing in the lateral hypothalamus" (p. 28). Indeed, anatomical studies (*i.e.*, Millhouse, 1969; Morgane, 1975) have shown clearly that the LH is a nodal point in a vast and heterogeneous field of overlapping multi-synaptic neural connections linking the limbic forebrain structures reciprocally with the mesencephalon. Viewed in this manner, it is clear that re-examination of the concept of the "lateral hypothalamic thirst center" using discrete lesions is warranted.

In the present study rats were implanted with intracranial cannulae aimed at sites of putative receptors for cellular and extracellular thirst signals in the forebrain. A series of tests was given following recovery in order to study whether the pathways mediating the two types of homeostatic thirst signals converge onto common structures in the LH. There were two findings of note. First, small lesions placed in the MLH-perifornical area disrupted water intake to each extracellular thirst challenge that was given, whether centrally to the POA or peripherally via s.c. and i.p. administration. These lesions were uniformly without effect on drinking to cellular challenges, irrespective of the method of administration. In contrast, lesions placed just 1 mm further

lateral in the LH produced a qualitatively different effect. Lesions of the FLH disrupted water intake elicited in response to cellular thirst stimuli but had no effect on drinking elicited by extracellular challenges. The differential effects of MLH and FLH ablation suggest that cellular and extracellular signals are mediated by anatomically proximal yet functionally separate pathways in the LH. Considered in the context of earlier interpretations of the "lateral hypothalamic syndrome" (Epstein, 1971), the present results suggest that the deficits in drinking to both types of homeostatic thirst signals after large bilateral LH lesions may be due to interruption of both MLH and FLH pathways.

Although they are not conclusive, the results of the dual MLH-FLH single unit recording experiments are consistent with the lesion data. In this regard, it may be pointed out that the dual recording technique has some advantages over the single microelectrode recordings done in other parts of this study, in that it is possible to characterize the neurons on the basis of any differential responses after the administration of cellular and extracellular thirst stimuli. However, the limitations of interpreting these results must still be recognized, since these cells may be interneurons which receive numerous fiber projections and respond to a variety of sensory signals (Mogenson, 1975). Moreover, since relatively high concentrations of NaCl and sucrose (2 osM) were used it

cannot be concluded that the FLH neurons were responding
to a physiologically relevant stimulus (Mogenson, 1975).
The same reservation applies to interpreting the responses
of MLH neurons to ANG-II, since the dose used (50 ng)
was shown earlier in this thesis (p. 136) to elicit blood
pressure and cortical EEG changes.

The results of lesion experiments reported in Section
3.3 and Section 3.4 indicate that the separation of neural
pathways for cellular and extracellular thirst is pre-
served in the mesencephalon. Lesions of the RMB disrupted
drinking to POA administration of ANG-II and were without
effect when hypertonic NaCl or sucrose was given into the
same cannula. Recently Malmo (1976) found that single
neurons in the midbrain dorsolateral to the periaqueductal
grey respond to intracarotid infusion of hypertonic NaCl
solution suggesting that the pathways mediating drinking
in response to cellular dehydration may project to this
region of the midbrain.

Previously, the studies of Blass and co-workers had
shown that experimental separation of the neurological sub-
strates underlying cellular and extracellular thirst may
also be accomplished at rostral levels of the neuraxis.
Blass and Hanson (1970) found that rats made hyperdipsic
by septal lesions drank no more than controls to cellular
dehydration but were overresponsive to intravascular hypo-
volemia caused by i.p. injections of hyperoncotic colloid
and to ANG-II (Blass, Nussbaum and Hanson, 1975). Rats

with lesions of the frontal pole area, on the other hand, drank substantially less or not at all to cellular dehydration, yet were not deficient in their response to intravascular depletions (Blass, 1968). It appears, therefore, that the neuroanatomical substrates for cellular and extracellular signals may be separable at the level of receptors in the forebrain and in the respective neural circuitries in the diencephalon and midbrain.

A final issue of major importance to this discussion concerns the nature of the deficits of water intake following MLH and FLH lesions. Most of the early studies (c.f. Epstein, 1971) reporting the loss of feeding and drinking activity following large bilateral lesions were evaluated solely on the basis of comparing pre- and post-lesion consummatory-response scores. Moreover, "deficits", if any, accrued subsequent to lesioning, were usually attributed to a "loss in motivation to eat or drink" (Teitelbaum and Epstein, 1962). Clearly, however, even the most careful analyses of complex behaviors such as feeding and drinking were incomplete and remain speculative if the consequences of experimentally induced brain damage to normal sensorimotor function as well as to motivation are not considered (Stricker, 1973, 1976; Marshall et al., 1974).

That these processes are intimately related and difficult to separate at a behavioral level is well illustrated by several recent studies. Marshall and Teitelbaum

(1974) and Marshall *et al.* (1974) have shown that in addition to impairments in responding to hydrational challenges, rats with bilateral LH lesions have taste-preference abnormalities and sensorimotor deficits (ataxia, akinesis and occasionally spasticity) which may play an important role in the central control of regulatory thirst. Impairments in the ability to lick have also been reported following LH damage (Levine and Schwartzbaum, 1973). The experiments of Ungerstedt (1970, 1971) first suggested that destruction of dopamine-containing neurons traversing the farlateral region of the LH en route to the corpus striatum (Anden, Dahlström, Fuxe, Larsson, Olson and Ungerstedt, 1966) may be responsible for the initial aphagia and adipsia, progressive recovery of function and residual ingestive deficits. Subsequent work (Marshall *et al.*, 1971, 1974; Marshall and Teitelbaum, 1974) has demonstrated that rats with nigrostriatal destruction might fail to eat or drink because of simple motor impairments in licking, chewing or swallowing or because of prolonged sensory inattention which may render them incapable of localizing food in the cage. In support of this hypothesis numerous other studies have demonstrated that substantial depletions of striatal dopamine in rats following lesions of the lateral hypothalamic area, extrahypothalamic damage of structures along the nigrostriatal pathway, or specific destruction of central catecholamine-containing neurons by intracerebral injection of 6-hydroxy-

dopamine (Oltmans and Harvey, 1972; Breese, Smith, Cooper
and Grant, 1973; Fibiger, Zis and McGeer, 1973; Marshall
et al., 1974; Stricker and Zigmond, 1976) produced similar
effects. Although the two-stage bilateral FLH lesions
made in the present investigation undoubtedly produced
some damage to nigrostriatal pathways, the postlesion
effects observed did not generally parallel those reported
following nigrostriatal bundle ablation. In contrast to
nigrostriatal bundle-lesioned animals, rats with FLH
lesions did not show gross motor impairments or somnolence
(Wampler, 1970) (although they were typically hypoactive
in the immediate postlesion period), nor did they show any
finickiness towards the food given them as evidenced by
their relatively normal 24-hr intakes on both the dry
laboratory chow as well as with the more moist synthetic
carbohydrate diet. It would seem likely, therefore, that
the small FLH lesions made in this study only partially
interrupted the nigrostriatal pathway.

The reinterpretation of the classical view of neural
mechanisms underlying LH-lesion-induced deficits in thirst
by Stricker (1976) and Marshall et al. (1974) must also be
considered. They have suggested that LH-lesioned animals
have a general or "activational" impairment of behavioral
arousal and are more susceptible to being incapacitated by
stress than normal rats. In support of this proposal,
Stricker (1973, 1976) has shown that rats recovered from
LH lesions which fail to drink in response to hypertonic

NaCl in a 1-hr test of water intake, nevertheless ingest substantial volumes of water when the test period is extended to 24 hr. That the standard peripheral regulatory challenges are indeed stressful is borne out by reports that at 750 mg/kg, 2-deoxy-D-glucose produces ataxia and stupor (Marshall *et al.*, 1974), isoproterenol given i.p. at 0.21 mg/kg produces a marked hypotension (Lehr *et al.*, 1967), and ANG-II administered centrally at a dose of 100 ng causes an increase in arterial blood pressure (p. 136; Hoffman and Phillips, 1976). Intraperitoneal injections of hypertonic NaCl are quite painful as evidenced by the squealing of the animals at the time of administration and for several minutes afterwards.

However much the foregoing considerations may be important in explaining the effects of LH lesions, an increased susceptibility to stress and/or generalized behavioral unresponsiveness cannot account for the observed deficits in water intake for the following reason: The wide range of doses of hypertonic NaCl and sucrose and of ANG-II and isoproterenol used in this study were selected to elicit maximal drinking without producing debilitating side effects. If as Marshall *et al.* (1974) have reasoned, ". . . for the incapacitated rat . . . as the conditions for responding become less difficult, the animal's likelihood of responding should improve. . . .," then as the doses (and hence the intensities of stress produced) of drugs were varied, one would expect some signs of a

reversal of the deficit. Such was clearly not the case, for FLH-lesioned failed to respond by drinking to any dose of NaCl or sucrose, whether given peripherally or by intracranial administration, but drank as much water as controls to all central and peripheral injections of extracellular thirst stimuli. In direct contrast to the specific deficits in responding to cellular stimuli following FLH ablation, rats with MLH lesions showed specific impairments in responding to extracellular thirst challenges. Thus, although sensorimotor dysfunction and/or general behavioral unresponsiveness cannot be completely excluded, neither would seem to be the basis of the deficits following MLH and FLH ablation. It is concluded, therefore, that the differential effects of MLH and FLH lesions are due to interruption of specific neural pathways mediating drinking behavior in response to cellular and extracellular regulatory challenges, respectively. As Morgane (1975) has so succinctly and decisively stated (p. 25) in his analysis of the functional interrelationships between different brain areas, in this case . . . the pathway is the message."

5.0 SUMMARY

1. Considerable experimental data indicate that the body
    can detect depletions of the cellular and extracellular
    body fluid compartments and that drinking behavior is
    elicited by body fluid deficit signals. The present
    study was done to investigate which neural pathways
    and integrative structures transmit and process the
    signals that initiate water intake in response to
    cellular and extracellular dehydration.

2. A combination of behavioral testing, electrolytic
    lesioning, chemical stimulation and extracellular
    single unit recording, and histological techniques
    were used in 483 male Wistar rats.

3. Since there was already evidence that angiotensin-II
    (ANG-II) is a dipsogenic hormone involved in extra-
    cellular thirst which exerts its effects on drinking
    behavior by acting on receptive sites in the preoptic
    region (POA), the study was first focused on the
    question of which neural pathways mediate this
    drinking response.

4. Single unit recordings were made in ethyl carbamate-
    anesthetized rats in which drinking had been elicited
    by administration of ANG-II through chronically-
    implanted POA cannulae in previous behavioral tests.

214

5. When ANG-II was administered to the POA a number of neurons in the midlateral hypothalamus (MLH) changed their spontaneous rate of discharge. These observations suggested the possibility that signals from ANG-II receptive sites in the POA are transmitted to the MLH.

6. In subsequent lesion experiments it was found that electrolytic ablation of the MLH markedly attenuated water intake elicited by microinjecting ANG-II into the POA or in response to peripheral administration of renin or isoproterenol. MLH lesions had no effect on drinking induced by cellular thirst stimuli (hypertonic NaCl or sucrose), given centrally or peripherally.

7. Lesions placed further lateral in the lateral hypothalamus (FLH), on the other hand, reduced water intake initiated by central or peripheral administration of hypertonic NaCl or sucrose, without attenuating drinking to centrally or peripherally administered extracellular stimuli of thirst (ANG-II, renin, isoproterenol).

8. Although the FLH lesions caused motor impairments and some loss of body weight, these changes were minor and restricted to the immediate postlesion period; deficits to drinking induced by cellular thirst challenges persisted for the entire duration of the experiment (138 days).

9. The differential effects of MLH and FLH lesions indicated that the neural pathways which mediate extracellular and cellular thirst signals are separate at the level of the lateral hypothalamus (LH), passing through the MLH and FLH, respectively.

10. Since the results described in 4-8 above suggested that separate neural pathways subserved extracellular and cellular thirst in the diencephalon, it was decided to see next if this separation was preserved at the level of the midbrain.

11. As a guide to placing lesions, an electrophysiological experiment was done first in which ANG-II was administered through cannulae chronically implanted in the POA and the effects of the hormone on the spontaneous discharge activity of single midbrain neurons was recorded.

12. It was shown that microinjection of ANG-II into the POA initiated drinking and influenced the firing frequency of single neurons in the ipsilateral midbrain in subsequent acute recording experiments.

13. In another series of animals, lesions of the rostral paramedial midbrain (RMB) attenuated drinking in response to POA administration of ANG-II but had no effect on water intake elicited by administering hypertonic NaCl through the same chronically implanted cannulae.

14. These results suggested that separate neural pathways mediate extracellular and cellular thirst signals in the midbrain.

15. While these experiments were in progress, several reports by other investigators implicated the subfornical organ (SFO) as the exclusive site of receptors for the dipsogenic effects of ANG-II and, suggested that ANG-II administered through chronic cannulae to the POA diffuses into the CSF of the ventricles and thereby reaches the SFO to initiate drinking behavior. In order to test these possibilities a series of five experiments was done.

16. In preliminary behavioral tests it was found that copious drinking could be elicited in water-sated rats in response to microinjection of ng doses of ANG-II into the POA, SFO and anterior third ventricle. These observations supported the view that the SFO was responsive to the application of low doses of ANG-II but they also suggested the possibility of there being more than one receptive site for ANG-II.

17. Additional support for the proposal that the SFO is not the only site of "receptors" for ANG-II was gained by the results of a lesion study in which it was found that lesioning the SFO attenuated but did not completely abolish drinking in response to jugular

administration of ANG-II or after peripheral injections of other extracellular stimuli of thirst.

18. In order to confirm that the POA itself was a receptive site for ANG-II, an experiment was done in which a competitive antagonist of ANG-II (P113) was microinjected into the POA prior to administering ANG-II through the same cannula. It was found that the degree of attenuation of elicited water intake by P113 pretreatment depended on the relative volumes of the antagonist and ANG-II. Moreover, when equal doses of ANG-II alone were microinjected into the POA, water intake increased with the volume of solution administered. These observations indicated that the POA is a receptive site for the dipsogenic effects of ANG-II but also suggested that the "receptors" are diffusely represented in this region of the brain.

19. Since these results suggested that there are multiple receptive sites for ANG-II, a lesion experiment was done to determine if there are also multiple neural pathways mediating ANG-II-induced thirst.

20. Lesions of the MLH and RMB were found to attenuate drinking significantly to ANG-II administered to the POA (thereby supporting the results reported in 4-13 above), but had no effect on water intake when ANG-II was microinjected into the SFO or anterior third

ventricle. Although the neural pathways which sub-
serve water intake initiated by microinjecting ANG-II
into the SFO or anterior third ventricle are not
known at present, the results indicate that they are
not the same as those which mediate drinking induced
by POA administration of ANG-II.

21. The results therefore suggest that there are both
multiple receptive sites and pathways for the
dipsogenic effects of ANG-II.

22. In conclusion, the results of this study support the
following proposals: The neural mechanisms for
initiating drinking behavior in response to body
fluid deficit signals include specific neural
receptive sites and pathways. For the case of extra-
cellular dehydration, a hormonal signal, increased
plasma ANG-II, stimulates "receptors" in the POA,
SFO and perhaps the ventricles. Signals for drinking
from receptive sites in the region of the ROA are
transmitted along a neural pathway which traverses
the MLH and are communicated to midbrain structures
receiving inputs from fibers of the descending medial
forebrain bundle. The locus of the "osmotic
receptors"-remains uncertain, although the behavioral
and electrophysiological data suggest that they may
be found in the vicinity of the POA or in the walls
of the cerebral ventricles, or both: Wherever

their location, it appears that cellular thirst
signals are transmitted through the FLH along
pathways separate from those mediating extracellular
thirst.

Effect of Administration of ANG-II to the POA

on Single Unit Activity in the Lateral Hypothalamus

of Unanesthetized, Freely-moving Rats.

Introduction

Drinking behavior in the water-replete rat can be
initiated by administration of ng doses of angiotensin-II
(ANG-II) to the preoptic region (POA) and it has been sug-
gested that the POA may contain receptors for the hormone
(Epstein, Fitzsimons and Rolls, 1970; Swanson and Sharpe,
1973). Since lesions of the lateral hypothalamus (LH) have
been shown to abolish or delay drinking in response to
plasma volume depletion (Fitzsimons, 1966; Stricker and
Wolf, 1967; Stricker, 1973) and ligation of the
inferior vena cava   (Stricker, 1973), it is possible
that thirst signals from POA receptors for ANG-II are
transmitted along neural pathways passing, through the LH.
The experiment reported here supports this hypothesis by
showing that the spontaneous discharge activity of LH
neurons in unanesthetized, freely-moving rats is influenced
by administration of ANG-II to the POA.

Materials and Methods

Eleven male albino rats (300-350 g in body weight)
were used in the experiment. Under sodium pentobarbital

(Nembutal; 50 mg/kg body weight, intraperitoneally)
anesthesia, two 23-gauge stainless-steel guide cannulae were
bilaterally implanted into homologous sites in the PQA.
Four to eight microelectrodes made from 62.5 μ diameter
nichrome wire (Driver Harris Co., N.J.) were implanted
into the LH for bipolar recording with a single 125 μ
diameter nichrome wire implanted into parietal cortex.
The microelectrodes were electroetched to a tip diameter
of 5-10 μ using the method of Green (1958), insulated with
epoxylite, and then soldered to the pins of a plastic
recording socket (Amphenol Hexagonal 7 or Strip Recording
Socket). The pins and solder were then insulated with
epoxylite. After the epoxylite dried the electrodes were
cut to the appropriate length and collectively coated 2-3
times with a solution of dextrose (heated to 145°C and
then cooled to 120°C before dipping) to form a unit stiff
enough to penetrate brain tissue (Chorover and DeLuca,
1972). The electrode portion of the recording assembly
was then implanted into the LH through a large burr hole
with the Amphenol portion remaining externalized and
anchored to the skull by cranioplastic acrylic and
jewellery screws. Cortical EEG was recorded from jewel-
lery screws threaded through small holes drilled bilater-
ally into the skull surface overlying frontal cortex; the
ground electrode was a screw over the nasal sinus. The
screws were soldered to one end of 3 cm lengths of 125 μ
diameter nichrome wire from which the formvar finish had

been scraped clean. The soldered point was then reinsu-
lated by applying two coats of epoxylite. The distal
end of the wire was soldered to pins on the Amphenol
assembly.

Two hours prior to an experimental session the
animals were transferred to a plexiglass chamber (20 x 30
x 30 cm) that contained only a graduated 50 ml tube with a
glass drinking spout. The animals were connected to the
recording apparatus after a 1 hr period of adaptation via
a matching male Amphenol plug connected by two separately
shielded sections of low noise cable (Amphenol, Type
to a Grass P14 AC preamplifier (gain 1000, band pass 300-
3000 Hz). The electrical signal originating at the
recording microelectrodes was also led to a Grass 7P3B
AC preamplifier and integrator with its vertical signal
output half-wave rectified and integrated using an RC
network and to a Tektronix 549 oscilloscope. The resulting
DC signal was recorded with a Philips Analog magnetic
tape recorder or displayed on a Grass 7B polygraph. The
DC signal provided a quantitative record of activity
versus time. An elevation in integrated single-unit
activity reflected an increased firing rate of the neuron
under observation, whereas a depression indicated a
decrease in unit discharge activity. EEG was recorded
through a separate Amphenol cable connected to the
assembly mounted on the animal's head and was led directly
into a wide-band AC preamplifier (Grass Type 7P5B, band

pass 0.6-15 Hz) and displayed on a separate channel of the polygraph.

A stable record of unit activity for about 30 min was established as the prerequisite to the administration of ANG-II (100 or 500 ng dissolved in 1.0 µl distilled water) through P.E. 10 tubing into the implanted POA cannula. Administration of equivalent volumes of distilled water one hr preceding or one hr following ANG-II microinjection controlled for changes attributable to the injection procedure. Only changes in single unit activity that returned to preinjection levels were regarded as being ANG-II induced. Also, since hypothalamic neurons have been shown to have different rates of spontaneous activity in sleep and arousal (Ramirez, Komisarik, Whitmoyer and Sawyer, 1967), only those single unit recordings made during the same pre-injection EEG state (rest) were evaluated in the results.

Water intakes elicited in response to POA administration of ANG-II and control vehicle were measured to the nearest 0.5 ml and food was not available during the tests. A maximum of 3 injections of ANG-II (and 3 of distilled water) was made in any animal on a given day. Most of the animals were tested every third or fourth day for a period of 2-3 weeks.

At the completion of each series of recording experiments iron was deposited at the tip of the microelectrodes by passing 10 µA anodal DC for 25 sec and the animals were

perfused intracardially with 30 ml potassium ferri-
cyanide-formalin (1 g %) and 20 ml potassium-ferrocyanide-
formalin (2 g %). Frozen sections were cut at 50 μ thick-
ness and stained with thionin. The sites of micro-
electrode recordings were identified by the Prussian blue
reaction.

Results

Nine of 22 single neurons in the LH from which extra-
cellular recordings were made showed an increase in their
rate of firing following the administration of ANG-II to
the POA. The spike discharge frequency of the remaining
13 units was not altered by the hormone. One of the nine
neurons which responded to ANG-II also responded by activa-
tion to injection of distilled water to the POA. The
response of 7 of the 9 neurons facilitated by ANG-II fell
into two populations: an initial short latency (range of
5-27 sec)-short duration (range of 6-20 sec) effect
followed by a longer latency (range of 1 min 20 sec-3 min
45 sec)-long duration (range of 2 min-5 min 15 sec) period
of increased discharge. This pattern of activation was not
observed for the neuron facilitated by distilled water.

For each of the nine LH units which showed facilita-
tion, the initial period of activation appeared to occur
independently of changes in cortical EEG and in each case
facilitation preceded EEG changes as well as the onset of
drinking behavior. The longer latency response, on the

other hand, generally occurred concomitantly with desyn-
chronization of the EEG and at approximately the same time
as the onset of drinking. Of interest also was the observa-
tion that the longer latency component of the unit responses
was closely related in its duration with the observed dura-
tion of drinking behavior. That is, a return of unit
discharge activity to pre-injection levels in most cases
coincided with the cessation of drinking. The changes in
EEG were not as closely correlated with changes in unit
activity or with drinking, although in each case EEG
desynchronization preceded the onset of drinking but this
change was of longer latency than the short latency com-
ponent of the brief response. There did not appear to be
any easily discernible correlation between the magnitude,
or latency-duration patterns of the unit responses, the
EEG changes, and the volumes of water ingested by the
animals.

Figure 1 shows the polygraph record from an experi-
ment in which the activity of an LH neuron increased follow-
ing the administration of ANG-II to the POA and illustrates
some of the relationships between the single unit responses,
EEG changes and drinking behavior. Note that the increase
in firing rate of the neuron (center of trace 464) pre-
ceded both the change in EEG (beginning of trace 465) and
the onset of drinking (trace 565). It should be noted also
that the firing rate of the neuron returned to near pre-
injection levels following the initial burst of increased

activity and that a second increase in firing rate (trace 465) occurred with approximately the same latency as the onset of drinking and desynchronization of the EEG.

As shown in Figure 2, 8 of the 9 LH units which were facilitated by POA microinjection of ANG-II were located in the region of the LH immediately lateral or dorso-lateral to the fornix. The remaining unit which showed activation in response to the hormone was located more ventrally, adjacent to the ventromedial nucleus.

Discussion

The results of this study suggest that thirst signals from POA receptive sites for ANG-II are transmitted to the LH. This proposal is based on the observation that 9 neurons in the LH increased their rate of discharge following the administration of ANG-II at a dose which caused rats in normal water balance to engage in copious drinking activity. Since 8 of the 9 units activated by ANG-II were located in the midlateral hypothalamus (MLH)-peri-fornical area, it may be suggested that the signals to initiate water intake are transmitted through this area of the diencephalon. Further support for this proposal is gained from the results of the lesion experiments reported in Sections 3.1 and 3.2 which demonstrated that ablation of the MLH disrupted drinking elicited in response to POA administration of ANG-II.

Figure 1

Continuous polygraph tracings showing the changes in cortical EEG (top tracing), integrated single unit activity (middle tracing), and spike discharge of a single neuron in the MLH following the unilateral administration of 100 ng ANG-II to the ipsilateral POA (arrow). Beginning on tracing 465 the upward-pointing solid blocks (▲) marked on the time tracing beneath the EEG record indicate the onset of drinking, and downward-pointing blocks (▼) mark the time at which drinking was observed to stop.
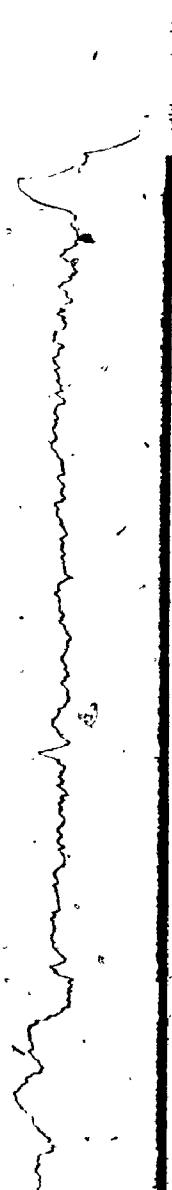
Figure 2

Schematic of a frontal section through
the lateral hypothalamus redrawn from the stereo-
taxic atlas of Pellegrino and Cushman (1967),
showing the sites of recordings and the types of
unit responses observed following the unilateral
microinjection of 100 ng ANG-II into the ipsi-
lateral POA. Solid triangles indicate sites of
facilitated units and open circles mark sites
where no effect was obtained.
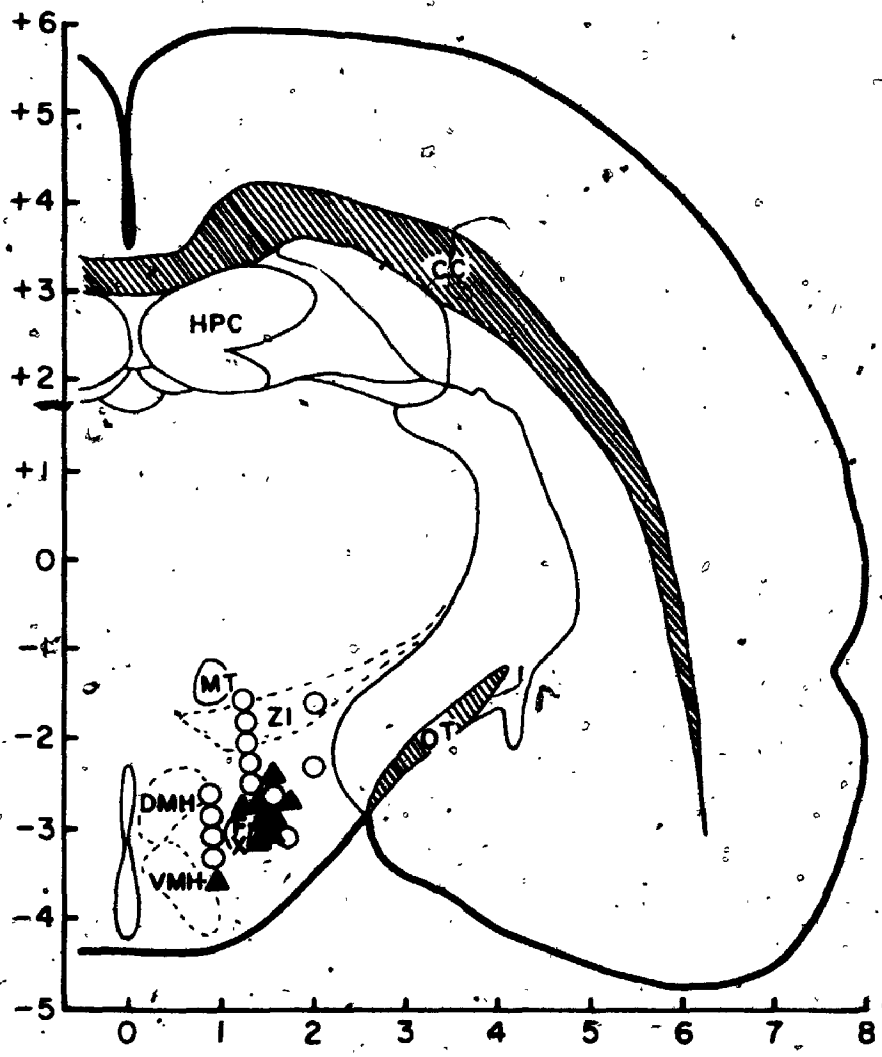
It is difficult to be certain of the significance of the increases in unit discharge rate and of the two components of the unit responses in terms of what role these changes may play in the functioning of behaviorally relevant neural mechanisms. On the one hand, since the short latency-short duration component preceded both the EEG changes as well as the onset of drinking behavior, it is possible that this initial burst of increased unit activity reflects excitation of LH neurons synaptically driven by inputs from POA receptive sites for ANG-II. However, because the doses of ANG-II (100 and 500 ng) were high and the volume of the injectate (1 µl) was large, it cannot be concluded that the observed changes in LH neuronal were specific to ANG-II.

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