BEHAVIORAL EFFECTS OF INTRACRANIAL ADRENERGIC AND CHOLINERGIC CHEMICAL STIMULATION IN THE CAT

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CHAPTER I

INTRODUCTION

Even during the early study of the morphology and physiology of the nervous system, His (1886, 1889), Forel (1887) and Cajal (1888, 1890) opposed the reticular theory of neurohistologists such as Gerlach (1871) and Golgi (1885) by proposing that each nerve cell is an independent unit whose branches are not in continuity but, rather, are in close contact. Waldeyer, in 1891, introduced the term "nervenheiten" (neuronen) to describe these nerve cells as single units separate from but intermingled with other nerve fibers. Sherrington (1897) assigned the name "synapsis" to the point of contact between these proposed individual nerve fibers, and Waldeyer (1891) suggested that the theory of the independence of nerve cells be known as the "neurone theory" in contrast to the reticular theory. Subsequent to these early developments, various explanations of the nature of transmission across neural junctions have been presented.

According to Eccles (1964) and De Roberts (1964), Du Bois Reymond, in 1877, was the first experimenter to suggest that neuronal transmission at the synapse may be either chemical or electrical. Although Reymond apparently preferred the concept of chemical transmission, other early investigators, such as Kuhne (1888), thought that junctional transmission was probably due to the current of the nerve impulse of one neuron directly exciting other neurons. These theories of chemical

and electrical transmission, which suggest two basically different mechanisms of synaptic transmission, were the basis for many years of subsequent debate. Since the early 1950's, however, there has been accumulating evidence that synaptic transmission is generally chemical in nature.

Chemical Transmission Theory

Chemical transmission implies that a chemical mechanism is involved in neural transmission between fibers at the synapse. Further, it assumes that there is at least one or possible several chemical substances acting as transmitters which are synthesized and stored in neuronal terminals and which are released by nerve impulses to produce a specific bio-electrical charge in postsynaptic fibers. The concept that neuronal activity is mediated by the release of some specific chemical agent from the ending of the stimulated nerve was initially postulated by Elliott (1904, 1905). He demonstrated that there was a similarity between sympathetic nerve stimulation and the administration of epinephrine. In order to explain his findings, Elliott suggested that the sympathetic nerve impulse actually causes secretion of epinephrine at the peripheral nerve ending. Presumably, this secretion served as an agent for mediating between the neuronal fibers. Several years later, Dixon (1906, 1907) argued that, although there was no chemical substance known at the time which could mimic their action, parasympathetic nerves acted by releasing a muscarine-like substance. Dale (1914), in a speculative paper, provided additional support for the chemical transmitter hypothesis when he suggested that a choline-ester, probably acetylcholine, was the parasympathetic transmitter.

This suggestion by Dale was later supported by Loewi (1921) who demonstrated that the vagus nerve inhibits action of the heart through the release of a specific chemical substance, "vagusstoff", which later proved to be acetylcholine. After Loewi's discovery, there followed a series of investigations (reviewed by McLennan, 1963), which extended the chemical transmitter hypothesis to sympathetic ganglia and to neuromuscular junctions. It is now generally accepted that synaptic transmission in the postganglionic parasympathetic nervous system is cholinergic, with acetylcholine as the naturally occurring agent involved (McLennan, 1963). In the postganglionic sympathetic nervous system, the mode of action is identified as adrenergic, with norepinephine as the primary transmitting agent (Eccles, 1964).

With respect to the central nervous system, Adrian (1924) proposed that inhibitory synaptic action might result from the release of a chemical transmitter analogous to the discharge of acetylcholine which Loewi had shown to be responsible for vagal inhibition of the heart. In the year following Adrian's proposal, Sherrington (1925) suggested that the concept of neurohumoral inhibition and excitation of peripheral nerves might be extended to all forms of inhibition and excitation in the nervous system. A decade later, Dale (1935) reiterated Sherrington's suggestion by proposing that the chemical transmitter hypothesis be extended to the neural junctions of the central nervous system.

Despite all these theoretical efforts, as a review by Feldberg (1945) reveals, early empirical attempts to establish the chemical transmitter hypothesis applicability to the central nervous system were not as successful as attempts to apply the hypothesis to the peripheral

neural junctions. It was not until the advent of the electron microscope in the early 1950's, the employment of intracellular recording, and the development of histochemical fluorscopic techniques that the chemical hypothesis of junctional transmission in the central nervous system began to receive empirical support.

Central Nervous System Neurochemistry

Catecholamines, which are possible neurohumoral agents, were observed first in the cerebral cortex by Raab (1943). His initial finding was supported by v. Euler (1946) and Holtz (1950) who observed the presence of both epinephrine and norepinephrine in mammalian brains. Later, Vogt (1954, 1957) analyzed the distribution of norepinephrine in the cortex of the dog and cat and reported that in both species the highest concentrations were found in the hypothalamus. Amin, et al. (1954) conducted one of the first investigations of the quantitive distribution of 5-hydroxytryptamine (5-HT) in various parts of the nervous system of a mammal and found that the hypothalamus, portions of the mesencephalon, the central gray matter and the area postrema were among the centers containing relatively high levels of 5-HT. In the white matter, cerebellum, peripheral nerve and autonomic ganglia, however, concentrations of 5-HT were very low or even lacking. Subsequent studies on the dog, other mammals and lower vertebrates are in general agreement with these initial findings (Bogdanski, et al., 1957; Passonen, et al., 1958; Welsh, 1964). In contrast to the reports of high concentrations of biogenic amines in the hypothalamus, dopamine, a catecholamine precursor, has been found to have its greatest concentration in the corpus striatum in several species, including man (Bertler and

Rosengren, 1959; Sano, et al., 1959; Ehringer and Hornykiewicz, 1960; Bertler, 1961). Other chemicals which have been observed to have unequal distributions in the cortex are acetylcholine (Shute and Lewis, 1966), gamma aminobutyric acid (GABA) (Roberts, 1956; Sisken, et al., 1960), and histamine (Adam, 1961).

Although the reports of unique distributions of chemical substances in the brain are highly provocative, these data provide only suggestive evidence of possible central neural transmitting substances. As McLennan (1963) noted, identification to that time of substances released at various central nervous system junctions was, in almost all instances, based on indirect biological tests and not on direct chemical identification of the materials. Direct morphological evidence for aminergic systems within the central nervous system was lacking until recently.

Although originally introduced for the study of catecholamines in the adrenal medulla (Eranko, 1955), the histochemical technique was developed by Falck and his associates (Falck, 1962; Falck, et al., 1962; Falck and Owman, 1965; Corrodi and Jonsson, 1967) to investigate monoamine distribution at the tissue level in specific cells or fibers in the central nervous system. Introduction of this and related histochemical techniques (Angelakos and King, 1965, 1967; Juhlin and Shelley, 1966) has provided powerful new tools for study of potential central nervous system transmitting agents. Previous methods (i.e., color and staining techniques) for detection of amine containing cells were relatively non-specific or lacked sensitivity; while, in contrast, chemical reactions which are dependent on the formation of fluorescent products have been found to have a high degree of specificity which is not found

in any other previously available method (Angelakos and King, 1968).

Using a fluorescent histochemical technique in which individual tissue sections were illuminated with ultraviolet light in a fluoscence microscope, various investigators have differentiated biogenic amines on the basis of their specific fluorescent characteristics. Epinphrine, norepinephrine, and 5-HT have been found to be uniquely dispersed in various neurons and neuron terminals in the central nervous system (Carlsson, et al., 1962; Dahlstrom and Fuxe, 1964a, 1964b, 1965; Fuxe, 1964, 1965a, 1965b). It should be noted, however, that this technique does have limitations. Histochemical analysis does not attain the degree of specificity obtained in direct chemical analysis, and this method does not allow the investigator to determine if the condensing agent involves predominantly "bound" or "free" amines in the neural tissue.

Employing less sophisticated bioassay techniques, Florey (1960a, 1960b) has extracted from the brain and spinal cord a variety of inhibitory substances (i.e., gamma-aminobutyric acid). There is a paucity of evidence, however, relating to the distribution and mode of action of these and other inhibitory agents at the synapses in any of the vertebrate central nervous systems (Eccles, 1964). Finally, there are compounds such as adenosine triphosphate and some unidentified substances whose possible role in neuro-junctional transmission in the central nervous system is less evident (De Roberts, 1964).

Although the foregoing survey of CNS neurochemistry literature has indicated support for the contention that synaptic transmission in the central nervous system is chemical in nature, it has proven difficult for experimenters to demonstrate conclusively that any specific

chemical serves as a natural junctional transmitter. In addition to limitations due to technique, there is the problem that even a small section of brain tissue contains vast numbers of neuronal junctions which may or may not be similar with respect to transmission characteristics. Nevertheless, investigators have often suggested that one chemical, acetylcholine, was the main central nervous system neurhumoral agent. The literature suggests, however, that its action is not universal. As previously noted, several noncholinergic agents such as epinephrine, norpinephrine, and 5-hydroxytryptamine have been found in the central nervous system with preferential localization at specific brain loci. Thus, until more conclusive data are reported, these adrenergic chemicals and their precursors, in addition to acetylcholine, must be considered as possible central nervous system neurohumoral transmitters.

In conclusion, while data on chemical transmission in the central nervous system provides a strong indication for the existance of both adrenergic and cholinergic synapses, the specific chemical action of these substances remains obscure. In addition, a demonstration of the functional significance of the various adrenergic and cholinergic agents and their possible neurochemical systems has not yet been achieved. Nevertheless, the non-uniform distribution of these chemicals throughout the central nervous system neural tissue provides some support for speculation that these agents may function as specialized modulators or synaptic transmitters in the control and integration of specific responses such as feeding, drinking, temperature, and emotionally.

The foregoing sections have presented a brief review of chemical

transmitter theory and of possible central nervous system transmitters. The information in this initial chapter provides the basic theoretical and empirical framework from which the present investigation of the effects of intracranial adrenergic and cholinergic chemical stimulation of central neuronal-junctions developed.

CHAPTER II

REVIEW OF THE LITERATURE

Application of chemical transmission theory to the central nervous system, identification of possible central nervous system neurohumoral agents, and development of new techniques, such as intracranial chemical stimulation, have given rise to burgeoning research and contrasting views with respect to the nature of neural regulation of food and water intake. A decade ago, the theory of how the brain controls ingestive behavior was relatively simple. Within the hypothalamus opposing excitatory and inhibitory systems were believed to control feeding and drinking by monitoring events in the blood and/or by receiving inputs from afferent pathways (Miller, 1958; Brobeck, 1960). Recent research, however, indicates that food and water intake is probably regulated by a much more diffuse and widely distributed system than has been previously supposed. Further, data from the employment of experimental animals other than the laboratory rat are presenting a challenge to the formerly accepted concept that phylogenetically primitive cortical formations, such as mechanisms in the diencephalon, function similarly across all species (Myers and Yaksh, 1968; Sharpe, 1969).

Because the literature indicates that central control of feeding and drinking behavior is not exclusively hypothalamic, the present review will include both a sketch of the major hypothalamic regulation centers and a survey of the role played by various extrahypothalamic

mechanisms in the control of food and water intake.

Lateral and Ventromedial Hypothalamic Mechanisms

Feeding Centers

Although there were earlier reports of hypothalamic damage changing feeding patterns (Bailey and Bremer, 1921; Smith, 1931), Hetherington and Ranson (1940), according to Ehrlich (1964), were the first investigators to provide a conclusive demonstration that bilateral hypothalamic lesions in or near the lateral edge of the ventromedial nuclei can cause obesity in the laboratory rat. The initial finding was replicated by other investigators (Brobeck, et al., 1943), and bilateral ventromental hypothalamic damage has been observed to cause hyperphagia and obesity in other species (Wheatley, 1944; Anand, et al., 1955). Hetherington initially suggested that an enormous decrease in activity appeared to be responsible for obesity produced by bilateral ventromedial lesions. Later, however, feeding experiments with measurements of food intake, metabolic rate and activity level have indicated that changes in both metabolism and activity are small in comparison to huge increases in food intake (Ehrlich, 1964).

Electrical stimulation in the ventromedial area of the hypothalamus has been found to decrease or stop food intake (Anand, et al., 1955; Wyrwicka and Dobrzecka, 1960), whereas, cessation of ventromedial stimulation causes feeding to increase (Morgane, 1961). When hypertonic saline solutions were applied to the ventromedial region, an inhibition of feeding was produced which was similar to that found in electrical stimulation of the same region (Epstein, 1960).

The existence of a feeding center in the lateral region of the

hypothalamus was first suggested by Brugger (1943) who observed that electrical stimulation of this area caused feeding in satiated cats. More recently, electrical stimulation of the lateral hypothalamus in rats and cats (Anand and Brobeck, 1951a), in cats (Delgado and Anand, 1953), in rats (Smith, 1956) and in goats (Larsson, 1957; Wyrwicka, et al., 1959) has been reported to cause increases in food intake which is similar to that observed in normal hungry animals. With food available, hypertonic saline solutions applied to the lateral hypothalamic region increase food intake (Epstein, 1960), and animals which have been operantly conditioned to obtain food when hungry have been observed to perform the same task when satiated after lateral hypothalamic stimulation (Robinson and Mishkin, 1962; Miller, 1957; Wyrwicka, et al., 1960; Wyrwicka, 1964; Wyrwicka and Doty, 1966). In contrast, lateral hypothalamic damage produces opposite effects. Lesions in the lateral region cause aphagia which in some cases may be fatal (Anand and Brobeck, 1951a, 1951b; Anand et al., 1955).

Direct chemical stimulation of the lateral hypothalamus in rats with adrenergic drugs increases food and water intake (Grossman, 1960, 1962a; Miller, 1965; Hutchinson and Renfrew, 1967), while injections of adrenergic blocking agents inhibit feeding due to intrahypothalamic chemical stimulation (Grossman, 1962b). Miller, et al. (1964) found a dose-response relationship when they injected different concentrations of an adrenergic substance into the lateral hypothalamus. They observed that increasing doses of norepinephrine resulted in successive decreases in food intake, but, cholinergic substances had slight effect on feeding behavior in the rat. Booth (1968) has indicated that the chemical activity in the adrenergic feeding mechanism in the rat

involves only alpha transmitting agents. He demonstrated that while propranol, an adrenergic beta blocking substance, affected no blocking action on adrenergic feeding, both phentolamine and phenoxybenzamine, which are adrenergic alpha blocking substances, inhibited feeding induced by injections of norepinephrine. In contrast, investigators using the rat (Myers, 1961, 1964a; Hernandez-Peon, et al., 1963) have reported neither adrenergic nor cholinergic chemicals have any effect on food intake in the cat, although others (Sommer, et al., 1967) have found a cholinergic substance, carbachol, injected into the lateral hypothalamus of a rabbit produces a significant increase in food intake.

In some respects, ingestive responses to chemical stimulation in the monkey appear to be different than those found in the rat (Myers, 1968a; Myers and Sharpe, 1968a, 1968b). While in the satiated monkey, adrenergic stimulation of the lateral hypothalamus elicited a dose-dependent eating response, cholinergic substances injected into the lateral hypothalamus of the deprived monkey inhibited food intake, and atropine sulfate injected at the same loci reversed the blocking effect so that the hungry monkey began to eat normally. Although these data are somewhat puzzling with respect to the chemical coding hypothesis, they still provide further support for the contention that the lateral hypothalamus plays a major role in the regulation of feeding behavior.

Other studies (i.e., Epstein, 1960; Hoebel and Teitelbaum, 1962) have reported that lateral hypothalamic injections of procaine inhibit feeding behavior and ventromedial hypothalamic injections of this anaesthetic increase food intake, indicating a reciprocal relationship. Intraventricular injections of anaesthetic have been found to increase food intake in both the cat and dog prior to ataxia (Feldburg, 1963).

Feldburg suggested that the hyperphagia is the result of the anaesthetic's removal of ventromedial hypothalamic inhibitory control over lateral hypothalamic feeding behavior.

Additionally, the degree of hypothalamic electrogram activity has been correlated with amount of body glucose utilization and with level of hunger or satiety in animals (Brobeck, et al., 1956; Chain, et al., 1960; Sharma, et al., 1961). That is, an increase in the ventromedial hypothalamic electrogram pattern with a concurrent decrease in lateral activity is generally observed when glucose utilization is increased. In the deprived animal, a decrease in arterio-venous glucose difference concurrent with a decrease in ventromedial electrogram activity and an increase in the lateral activity has been observed. In the recently satiated animal ventromedial electrogram activity has been found to increase while a slight drop occurs in the level of lateral activity. In general, investigations employing single unit recordings also indicate that a reciprocal relationship exists between neural activity of the lateral and ventromedial hypothalamus (Sawa, et al., 1959; Anand, et al., 1962; Barraclough and Cross, 1963; Tsubokawa and Sutin, 1963; Anand, et al., 1964; Oomura, et al., 1964; Wagner and Oomura, 1968).

Drinking Centers

Delineation of the cortical mechanisms for thirst began several decades ago. In 1921, Bailey and Bremer reported that destruction of the basal hypothalamus in dogs resulted in polydipsia and polyuria. It remained unclear, however, as to which response was primary. That is, polyuria might be a secondary effect of increased water intake or polydipsia might result from a polyuria caused directly by the hypothalamic

lesion. Subsequently, the issue was answered by Bellows and Van Wagenen (1938, 1939). They separated water intake from body water by using esophageal fistules and observed that lesions in the tuber cinereum caused dogs to drink enormous amounts of water which passed out of their bodies through the fistulas. The authors concluded that since uninary excretion remained normal, the lesions were producing a primary polydipsia. Although further evidence demonstrating primary polydipsia with the lesion technique is lacking, Stevenson, et al., (1950) reported that bilateral destruction of the ventromedial hypothalamic nuclei in rats produces a pronounced decrease in water intake despite hyperphagia, and Andersson and McCann (1956) have reported that restricted lesions in the medial hypothalamus of goats produced adipsia, even though feeding continued.

As previously noted, lesions in the lateral hypothalamus have been shown to cause both aphagia and adipsia (Teitelbaum and Stellar, 1954). Although additional observations of aphagia and adipsia after lateral hypothalamic lesions have been reported (Williams and Teitelbaum, 1959; Teitelbaum and Epstein, 1962), it has often been difficult when using lesions, to separate the neural mechanisms controlling feeding from mechanisms controlling drinking. Montemurro and Stevenson (1955), however, have found that rats continued to eat normally but became adipsic following small discrete lesions placed from 0.5 to 0.75 mm caudal to the lesions most effective for producing aphagia in the lateral hypothalamus. Other investigators (Anand and Dua, 1958; Smith and McCann, 1962) have also been able to produce this apparent independence of neural systems which regulate food and water intake.

Teitelbaum and his associates have analyzed recovery patterns from

adipsia and aphagia in rats following lateral hypothalamic lesions (Teitelbaum and Stellar, 1954; Williams and Teitelbaum, 1959; Epstein and Teitelbaum, 1960; Teitelbaum, 1961; Teitelbaum and Epstein, 1962; Epstein and Teitelbaum, 1964; Teitelbaum, 1967). Essentially, the four stages involved in recovery are as follows: (1) no drinking or feeding; (2) no drinking, but eating highly palatable foods; (3) no drinking; but feeding normal; (4) drinking and feeding normal. Teitelbaum and his associates note that adipsia always outlasts aphagia and that the drinking in the final stage of recovery is largely prandial in response to dry food. The authors describe this final stage drinking as a secondary type because the animals demonstrate great reluctance to drink in response to intraperitoneal injections of hypertonic saline solutions, to water deprivation, or to heat exposure.

Recently, Teitelbaum, et al. (1969) have presented a rather provocative theoretical explanation as to the nature of recovery from the lateral hypothalamic syndrome in rats. They report that the four stages of recovery observed in these animals is an exact parallel to the developmental stages of feeding behavior in the maturing rat pup. Thus, they conclude that recovery of feeding following hypothalamic damage recapitulates ontogeny. That is, they suggest that recovery from the lateral hypothalamic syndrome is essentially a process of re-encephalization paralleling the process of encephalization of function which occures after birth. It should be noted, however, that this view of restoration of function after damage to the lateral hypothalamic area is somewhat contrary to the old concept of circumscribed centers which exercise exclusive control over specific behaviors such as feeding and drinking.

In studies employing stimulation techniques, Andersson (1952, 1953) observed immediate drinking in goats when a hypotonic sodium chloride solution was injected into the hypothalamus near the descending column of the fornix. Further, Andersson found that injections of saline solutions into the third ventricle of goats resulted in very reproductible drinking, although the amount of water consumed was smaller than the amounts consumed when saline was injected directly into the hypothalamus.

Because it was difficult to obtain repeated drinking behavior after intrahypothalamic injections of saline solutions, Andersson extended his work to include electrical stimulation of hypothalamic areas (Andersson and McCann, 1955; Andersson, Larsson and Persson, 1960). He and his associates observed the effect of electrical stimulation on drinking behavior with suitably positioned electrodes was very dramatic. Goats would start to drink shortly after onset of electrical stimulation, and drinking would continue, usually uninterrupted, as long as stimulation was maintained. This type of stimulus bound drinking was highly repeatable, and, as Andersson notes, it often produced enormous overhydration (up to 40 percent of body weight) with marked haemodilution and haemolysis. The primary region of the hypothalamus eliciting polydipsia in the goat is located chiefly between the mamillothalamic tracts and the descending columns of the fornix, ranging from the dorsal to the ventral hypothalamus.

Drinking behavior induced by electrical stimulation has been reported in other species. Greer (1955) found rats showed stimulus bound drinking when stimulated through electrodes located in the lateral region of the dorso-medial nucleus just anterior to the paraventricular

nucleus. Andersson (1959) reported hypothalamic stimulation via electrodes inserted in the sphenoid causes excessive drinking in dogs.

More recently, Mogenson and Stevenson (1966, 1967) observed polydipsia in rats during electrical stimulation in the same lateral hypothalamic area in which lesions led to adipsia without aphagia. It should be noted that this region, which is located in the medial portion of the medial forebrain bundle just dorso-lateral to the fornix at the level of the dorso-medial nucleus, has also been shown to be an effective reward center (Olds, et al., 1960; Olds, 1962).

Intracranial microinjections of cholinergic compounds in the lateral hypothalamus of the rat have been observed to increase water intakes (Grossman, 1960, 1962a; Miller, 1965; Hutchinson and Renfrew, 1967), while pretreating rats with cholinergic blocking agents inhibits cholinergic drinking (Grossman, 1962b). After adrenergic stimulation little or no effect on drinking behavior was reported. In addition, Miller, et al. (1964) found a dose-response relationship when a cholinergic chemical was injected into the lateral hypothalamus. That is, while lower test concentrations of carbachol caused an increase in water intake, higher test concentrations resulted in a relative decrease in water consumption. Additional support for a cholinergic mechanism mediating water intake has been provided by Miller (1965). He reported that following cholinergic stimulation of the lateral hypothalamus in rats an increase in osmolarity and decrease in volume of urine could be observed. This cholinergic involvement in the release of antidiuretic hormone would be expended if a cholinergic system was assumed normally to mediate water intake.

Further, there are data which indicate that the cholinergic

drinking mechanism is muscarinic in its mode of action. For example, hypothalamic injections of muscarine, but not nicotine, have been found to increase water intake in rats, whereas, hypothalamic injections of atropine sulfate, which is a muscarinic blocking agent, have been observed to inhibit cholinergically facilitated drinking (Grossman, 1960, 1962a, 1962b; Stein and Seifter, 1962; Miller, 1965; Levitt and Fisher, 1966).

Although the foregoing review indicates that drinking behavior can be drastically modified by lesions or stimulation in certain hypothalamic regions, Fitzsimmons (1966) states that unequivocal proof for the existance of a hypothalamic drinking center is still lacking. Grossman (1964) notes that areas thus far located in the hypothalamus which control drinking behavior do not function as autonomously as had been previously assumed. He suggests that the hypothalamus may contain several centers which participate in the regulation of water intake and that certain extrahypothalamic areas contribute importantly to the control of drinking behavior.

Qualification of Lateral and Ventromedial Center Concept

Although these data remain inconclusive, the previously reviewed studies suggest that, in addition to a medial satiety center, the hypothalamus contains a lateral feeding center. This indicates that the brain may be uniquely designed in such a fashion that regulation of food and water intake is modulated by discrete hypothalamic centers. Such a conclusion, however, is an oversimplification of the role played by these hypothalamic areas. The concept of both a lateral feeding center and a ventromedial satiety center needs clarification on several

points.

With regard to the lateral feeding center, there is the question of whether or not the primary effects of lesions in the lateral hypothalamic area is aphagia or adipsia. Anand and Brobeck (1951a, 1951b) reported that bilateral lesions in the lateral hypothalamus caused rats and cats to become aphagic. In a more extensive study, Teitelbaum and Stellar (1954) found that lateral hypothalamic lesions in rats resulted in both aphagia and adipsia, but that following prolonged tube feeding animals recovered from both deficits. More recently, investigators (Montemurro and Stevenson, 1955; Anand and Dua, 1958; Smith and McCann, 1962) have reported small lesions caudal to the most effective site for producing aphagia in the lateral region cause rats to become adipsic without changing their patterns of food intake. In contrast, others (Williams and Teitelbaum, 1959; Teitelbaum and Epstein, 1962) have found that it is difficult to separate neural feeding and drinking mechanisms using this technique. Further, Teitelbaum and Epstein (1962) have observed that while recovery of feeding usually occurs in animals after lateral hypothalamic damage, adipsia in some subjects is permanent, and in "recovered" rats drinking appears to be largely prandial in response to consumption of dry food (Epstein and Teitelbaum, 1964; Kissileff, 1969). Currently, although adipsia appears to be more permanent, lesioning studies do not provide a clear answer as to which response is primary in the lateral hypothalamus.

An alternate proposal suggests that both food and water mechanisms may be found at the same anatomical loci. The possibility was first empirically supported by Grossman (1960, 1962a, and 1962b). Substantiation of the differential feeding and drinking effects due to

adrenergic and cholinergic stimulation, respectively, of the lateral hypothalamus of the rat have been reported by various investigators (Wagner and de Groot, 1963; Miller, et al., 1964; Miller, 1965; Hutchinson and Renfrew, 1967). Other studies, however, have found different species such as the cat (Myers, 1961, 1964a; Hernandez-Peon, et al., 1963), the rabbit (Sommer, et al., 1967), and the monkey (Myers, 1968a; Myers and Sharpe, 1968a; Sharpe, 1969) do not show differential effects to chemical stimulation for feeding and drinking as observed in the rat. Thus, the exact role of the lateral hypothalamus in the regulation of ingective behavior remains to be delineated.

A second question about the lateral hypothalamic feeding center is whether lesions and chemical blockage in this region cause a motivational deficit or simply a motor failure. Morgane (1961) concluded mid-lateral lesions disrupted a motivational system, whereas, far-lateral lesions involve disturbance of a basic feeding system. Teitelbaum and Epstein (1962), however, have reported lateral lesions produce degeneration in the pallidofugal fiber system which they believe to be involved in metabolic control. On the other hand, Baillie and Morrison (1963) have concluded rats with lateral hypothalamic aphagia have a motor rather than a motivational deficit. In another report. Rogers, Epstein, and Teitelbaum (1965) have rejected Baillie and Morrison's conclusion. Rogers, et al. (1965) have found after lateral hypothalamic lesions, rats begin feeding before regaining the ability to bar press. Finally, Grossman (1967) has recently reiterated the view that the effects of lateral hypothalamic lesions involve a motivational factor mediated by the medial forebrain bundle. Thus, the issue of what conditions actually underlie hypothalamic aphagia remains unresolved.

The concept of a ventromedial satiety center also needs some qualification. Although ablation of this region does cause hyperphagia, after a passage of time food intake returns to normal, and obesity enters a static or equilibrium phase during which body weight is maintained with no further weight increase. In addition, ventromedial obesity appears to be a graded effect. That is, the degree of electrolytic lesions and damage in the ventromedial hypothalamus is directly proportional to the extent of obesity (Mayer and Barrnett, 1955; Graff and Stellar, 1962; McBurney, et al., 1965; Liebelt and Perry, 1967). Similarly, the dosage of intrahypothalamically injected goldthioglucose, a lesion producing compound, is proportional to the resulting weight gain (Gray and Liebelt, 1961). As a result of these data, Kennedy (1950, 1953), and more recently Teitelbaum (1961), has suggested the primary regulation carried out by the ventromedial nucleus involves stabilization of fat stores. Possible, then, hyperphagia following ventromedial lesions is merely a means to the end of reaching a new stabilization of lipostatic control.

Further, Brobeck, et al. (1950) have reported ventromedial activity increases after injections of amphetamine and the anorepic effects of the drug have their origin in the ventromedial center. Behavioral tests indicate, however, that amphetamine inhibits ingestive behavior in both normal and hyperphagic animals (Stevenson, et al., 1956; Stowe and Miller, 1957; Epstein, 1959; Reynolds, 1959; Sharpe, et al., 1962). In review of these studies, Grossman (1967) has suggested that either a satiety mechanism exists elsewhere in the cortex or that the anorexigenic effects result because of other indirect actions of the drug, such as peripheral or side effects.

Further, experimental data on the role of the ventromedial hypothalamus in appetitive behavior do not confirm predictions from the glucostatic theory of food regulation. While glucostatic theory (Mayer, 1953; Mayer and Marshall, 1956) indicates the hypothalamus is the control center for ingestive behavior based on feedback from blood sugar levels, Lewinska (1963, 1964) reported ventromedial damage in rabbits resulted in no change in the animal's cyclic rise and fall of blood sugar level. Also, behavioral tests showed that systemic injections of glucose in hypothalamic, hyperphagic cats (Russek and Morgane, 1963), rats (Reynolds and Kimm, 1965), and rabbits (Balinska, 1965) resulted in a marked depression of food intake. Thus, in addition to the failure of the glucostatic theory of generate verifiable predictions with respect to the ventromedial area, there is evidence that the ventromedial center is not the sole area involved in the control of satiation.

Preoptic and Anterior Feeding and Drinking Mechanisms

Even though a comprehensive understanding of the role of the preoptic and anterior areas in the regulation of feeding and drinking behavior has not been achieved, there is evidence that both the preoptic and anterior areas are involved in the control of food and water intake. Further, the preoptic and anterior nuclei apparently participate in a complicated pattern of interaction with other parts of the central nervous system which play a role in the regulation of ingestive behavior. The preoptic-anterior complex has been considered an area of possible central control of food and water intake since Magoun, et al. (1938) indicated that the preoptic-anterior hypothalamic region was probably a site for central "warm detectors". Investigators, such as

Brobeck (1948), have proposed that an animal's energy equation may be kept in balance by a central temperature regulatory mechanism, which possbility exists in the hypothalamus, and that food and water consumption are controlled as a part of the normal regulation of the animal's body temperature.

Empirical support for Brobeck's thermoregulatory theory of hunger has been provided by Andersson and Larsson (1961), who have suggested the preoptic area plays a role in the regulation of food and water intake. They found that local cooling of the preoptic area inhibits drinking in thirsty goats and induces feeding in spite of continuing dehydration. Local warming of the preoptic area produced the opposite effects. Goats which were hungry stopped eating and began consumming large quantities of water. Grossman and Rechtschaffen (1966), however, have pointed out the temperature of the preoptic area of the cat often varies less than 1.0°C. These results are in contrast to the 10.0°C rise or fall required to change food and water intake in the goat (Andersson and Larsson, 1961). Further, Grossman and Rechtschaffen reported that temperature in the preoptic region was not found to covary with food or liquid intake, food deprivation, or satiety.

In a recent paper, Spector, et al. (1968) provide some clarification of the role of the preoptic area, especially with respect to its involvement in temperature regulation. They state that, although feeding and drinking behavior is clearly related to both internal and ambient temperatures, there does not appear to be any single point in the body which uniquely governs the rate of food and water intake. Consequently, a complex interaction of both central and peripheral temperature centers must be considered in the regulation of feeding and

drinking behavior.

As previously mentioned, studies involving electrical stimulation of the hypothalamus indicate stimulus bound drinking can be elicited at sites within the medial and midlateral hypothalamus anterior and posterior to the descending column of the fornix (Andersson and McCann, 1955; Andersson, Larsson and Persson, 1960). Although different areas within this drinking region have been shown to cause polydipsia, Andersson, et al. (1956) reported that an attempt to induce stimulus bound drinking through electrical stimulation of the preoptic area, which causes peripheral vasodilatation and vigorous panting, was not successful. In another study, however, Andersson, et al. (1964) reported electrical stimulation of the medial section of the preoptic area causes activation of the physical heat loss mechanism accompanied by a marked increase in water intake.

The importance of the preoptic-anterior region in the central control of feeding and drinking behavior has also been demonstrated in studies employing lesion and ablation techniques. Witt, et al. (1952) reported electrocoagulation of the ventral portion of the anterior hypothalamus in the dog causes a well defined and sustained polydipsia. However, when coagulation is extended dorsally and laterally to obtain complete destruction of the anterior hypothalamus, the post-operative effects are reversed in that there is adipsia which may be temporary or permanent. Using a lesioning technique, Andersson and Larsson (1961) found that complete destruction of the preoptic area in the goat caused permanent adipsia, even though the area of the hypothalamus where electrical stimulation elicits stimulus bound drinking was undisturbed by the lesion.

Feldburg and Myers (1963, 1964a, 1964b, 1965) observed intraventricular and intrahypothalamic injections of biogenic amines in both anaesthetized and unanaesthetized cats cause temperature changes. Although the authors concluded the effects result from the action of epinephrine, norepinephrine and 5-HT on the anterior hypothalamus, they did not report any changes in ingestive behavior. In contrast, Myers and Yaksh (1968) have found consistent evocation of feeding following injections of norepinephrine into the lateral ventricles of satiated rats. After intraventricular injections of cholino-mimetic substances, other investigators have reported no changes in either feeding or drinking behavior in the cat (Kavari, et al., 1968; Myers and Cicero, 1968) and in rat (Myers and Yaksh, 1968). Fisher and Coury (1964), however, have reported that cholinergic stimulation of the preoptic area will significantly increase water intake in the satiated rat. Further, Hutchinson and Renfrew (1967), who studied the behavioral effects of adrenergic and cholinergic chemical stimulation in both the preoptic and the anterior hypothalamic areas in the rat, have found injections of norepinephrine and carbachol appear principally to affect water intake. In the satiated rat, norepinephrine was observed to inhibit drinking, while carbachol increased water intake. During food deprivation, injections of norepinephrine in the preoptic region produced an increase in drinking, whereas, in water deprived rats, injections of carbachol in the anterior hypothalamus caused a decrease in water intake. More recently, however, Sharpe (1969) has reported that, of all compounds tested, an adrenergic chemical, norepinephrine, injected in the preoptic area was the most effective agent in stimulating feeding and drinking in the satiated monkey. In contrast, cholinergic

substances injected in the same region caused inhibition of both food and water intake in the deprived monkey.

Finally, a relation between the preoptic and the lateral hypothalamus in the rat has been observed by Wolf and Miller (1964). They demonstrated that the drinking response to cholinergic stimulation of the preoptic area in the rat could be eliminated by bilateral lesions in the lateral hypothalamus. The result of these lesions was immediate aphagia and adipsia. Although water intake gradually returned to normal levels, the aphagia was permanent and the effects of preoptic cholinergic stimulation did not reappear. Conversely, lesions in the preoptic region did not change drinking behavior and failed to modify the drinking response to cholinergic stimulation of the lateral hypothalamus. Thus, the preoptic effects appeared to be secondary to the effects of the lateral hypothalamus. As is the case with other hypothalamic areas, the problem of differential effects consequent to adrenergic and cholinergic drug stimulation in the preoptic-anterior complex of various species remains to be solved. Nevertheless, these chemical stimulation data generally support the findings of studies employing other techniques which indicate preoptic and anterior hypothalamic involvement in the regulation of ingestive behavior.

Extrahypothalamic Feeding and Drinking Areas

The wide distribution of feeding and drinking areas in the hypothalamus indicates food and water intake may be controlled by even more diffuse and widely distributed mechanisms than have been previously supposed. The following survey of some of the recent literature supports this view.

Many studies have implicated structures on the medio-basal aspect of the temporal lobe as participating in some manner in the neural regulation of ingestive behavior. In an early report, Fuller (1951) found that lesions which destroyed only the amydaloid complex caused constant nibbling and extended feeding in monkeys. Green, Clemente and de Groot (1957) studied the effects of stereotaxically placed lesions in the amygdala of cats. These authors reported selective lesions in the basal and lateral nuclei caused aphagia. Morgane and Kosman (1957a, 1957b) found bilateral lesions and ablation of the amygdala in the cat produced hyperphagia, but they did not report aphagia. While the overeating effects were related to the close anatomical relationship between the amygdala and the hypothalamic systems, the authors suggested the amygdaloid complex in the cat normally functions as a satiety center. In contrast, other authors (Anand and Brobeck, 1952) report that lesions in the amygdaloid complex of both cats and monkeys produce aphagia which is only temporary. Wood (1958), however, has reported that stereotaxic lesions placed in the central and medial nuclei of the amygdala caused increased feeding in four of nine experimental cats. Although he reported daily food intake quadrupled in some animals, there were no other quantatitive data presented. Fuller, Rosvold and Pribram (1957) studied the effects of open ablation of the pyriform-amygdala-hippocampal complex in dogs. They observed that during the initial 14 post-operative days there was a doubling of mean daily food intake. This early feeding increase, however, was only temporary, for food intake returned to normal during the second post-operative month.

The foregoing studies which report that dogs, cats and monkeys can recover normal feeding patterns after amygdaloid lesions or ablation

suggest that, while the amygdala is involved in food regulation, its action is possibly not primary in nature. In this connection, Grossman and Grossman (1963) have found small bilateral lesions in the posterior-ventral amygdala of rats produce a temporary increase in both food and water consumption. Further, larger lesions in the posterior amygdala result in permanent hyperphagia and hyperdipsia, while damage to the anterior amygdala results in increased food and water consumption. The exact nature of amygdaloid involvement in feeding behavior, however, remains unclear.

Ehrlich (1964) reported destruction of tissue in the region of the fornix and hippocampal commissure in rats produced a significant increase in food and water intake. By contrast, lesions restricted to the midbrain reticular formation produced no change in ingestive behavior. Ehrlich suggested that the increase in feeding and drinking was due to a motivational increment. More recently, Kimble and Coover (1966) have confirmed Ehrlich's findings that lesions in the hippocampal area will increase both feeding and drinking in laboratory animals.

Studies on the response of extrahypothalamic areas to chemical stimulation indicate the effects on drinking behavior often equal or exceed the effects of direct lateral hypothalamic electrical stimulation. Grossman (1964b) observed that carbachol injected into the medial part of the septal region elicited intensive and extended water intake in satiated rats. In addition, drinking behavior of rats which had been water deprived for 24-hours could be inhibited by injections of atropine into the medial septal area. Neither cholinergic or adrenergic stimulation or blockage, however, affected the food intake of the animals.

In another study (1964a), Grossman chemically stimulated the amygdala region in the rat brain and found some interesting results. In the satiated animal, chemostimulation of the ventral amygdala caused no changes in feeding and drinking patterns. Deprived rats, however, ate more food and drank less water following stimulation of the amygdala with norepinephrine. Cholinergic stimulation of the same locus produced opposite effects. That is, feeding decreased and drinking increased.

Fisher and Coury (1964) reported that cholinergic stimulation of the anterior thalamic nuclei, mammillary region and medial midbrain produced, in each case, a significant increase in water intake. Further, they observed that injections of cholinergic substances into the dorso-medial hippocampus, dentate gyrus, midline thalamus and cingulate cortex caused a strong and prolonged drinking response which was greater than the drinking behavior associated with electrical stimulation of the hypothalamus.

The apparent relationship between the limbic structures and the hypothalamus which has been suggested by various authors such as Morgane and Kosman (1959a, 1959b) and Grossman (1964b), was recently studied by Pizzi and Lorens (1967). They reported that bilateral damage to the stria medullaris, stria terminalis and post commissural fornix of the rat resulted in a significant decrease in water intake, although food consumption was only transiently affected. Thus, efferent systems of the septum, amygdala and hippocampus, which connect with the preoptic and anterior hypothalamus are implicated in the control of water intake.

In conclusion, the literature indicates that extra-hypothalamic

mechanisms, such as the amygdala, may play important roles in the neural control of feeding and drinking behavior. At present, however, there is a lack of data about the specific nature of the contributions made by each component involved in the regulation of ingestive behavior. Thus, a complete hypothesis regarding their interaction is difficult to formulate. To achieve a complete understanding of the central regulation of thirst and hunger, the specific characteristics and complex relations of both hypothalamic and extrahypothalamic areas must be delineated.

Peripheral Mechanisms

As investigators have abandoned Cannon's local theories of hunger and thirst (Cannon and Washburn, 1912; Cannon, 1918, 1934) increasing emphasis has been placed on the role of central mechanisms in the regulation of ingestive behavior. It should be pointed out, however, that an understanding of the food and water control system must include a consideration of peripheral influences. Code (1967) has compiled a comprehensive review of various peripheral contributors. For example, some of the factors which have been studied recently include--general metabolism (Fabry, 1967); general hydration (Adolph, 1967); gustation (Towbin, 1967); prefeeding reactions (Wyrwicka, 1967); temperature (Hamilton, 1967); oropharyngeal factors (Epstein, 1967; Young, 1967); and gastrointestinal factors (Jawowitz, 1967; Sharma, 1967).

In regulation of hunger, an animal's metabolic processes, which involve integration of fat, protein and carbohydrate metabolism, mediate the relationship between caloric intake and expenditure. Although the literature indicates that various hypothalamic and limbic structures interact to regulate energy intake and expenditure, the

information upon which this control is based is received from the blood and afferent neural pathways which signal facilitation or inhibition of ingestive behavior. The mechanisms underlying this afferent signaling system are not well understood. However, several hypotheses as to the nature of peripheral activity have been developed. These explanations include: the thermostatic hypothesis concerning the dynamic action of food (Brobeck, 1946, 1948, 1957; Strominger and Brobeck, 1953); the shift in water concentration among body compartments (Adolph, 1947); the lipostatic hypothesis concerning the amount of free fat metabolites (Kennedy, 1950, Mayer, 1955); the concentrations of serum amino acids (Mellinkoff, et al., 1956); the glucostatic theory concerning the availability and utilization of carbohydrates in the blood (Mayer, 1953, 1955; Mayer and Thomas, 1967); the sensations from the digestive tract (Quigley, 1955); and the palatability and aroma of foodstuffs (Epstein, 1967; Young, 1967).

As in hunger regulation, thirst regulation appears to involve a complicated series of interrelationships between central and peripheral mechanisms. The drive to drink is usually the result of body dehydration or hyperosmotic body loadings. As viewed by Adolph (1967), thirst satiation is effected initially by oropharyngeal muscular activity and by stomach distension which is later followed by cellular hydration. These various water satiation effects are continually mediated by central homeostatic mechanisms, and any deviation from the norm of either volume or concentration of these tissue fluids causes neural activation of central regulatory mechanisms until water balance is restored.

McClymont (1967) suggests that peripheral mechanisms may be considered as signaling systems which contribute to the excitation or

inhibition of the central nervous system which regulates ingestive behavior. Theoretically, these afferent signals can summate so as to influence the threshold for initation and cessation of ingestive behavior. Thus, ingestion occurs when total facilitatory stimuli exceed summated inhibitory stimuli, while no ingestive behavior occurs when inhibitory input is greater than facilitatory stimulation.

Centers or Circuits

On both empirical and rational grounds, Morgane (1964, 1966, 1969) has opposed the hypothalamic center theories of Anand and Brobeck. In the rat, Morgane (1961a, 1961b, 1961c, 1961d), using electrical and chemical stimulation and lesion techniques found that the lateral feeding center could be experimentally divided into two functional anatomical systems. Medial forebrain bundle lesions or electrical stimulation rostral and caudal to the level of the ventromedial hypothalamus resulted in changes in motivation patterns, but such lesions and stimulation did not alter ingestive behavior (Morgane, 1961c; 1961d). Electrical stimulation of far-lateral hypothalamic regions caused satiated rats to perform tasks for food, but lesions in the medial forebrain bundle abolished motivation to do the work (Morgane, 1961d). When the globi pallidi was lesioned, Morgane (1961b) reports the occurrence of more drastic effects, which include the failure of animals to recover normal feeding patterns following prolonged maintenance by intubation. Morgane (1962) called the condition of these animals "metabolic decay" because the rats showed a very rapid body weight loss, which was much greater than normal weight loss due to starvation. Although some investigators (Teitelbaum and Epstein, 1962; Ehrlich, 1964;

Robinson and Mishkin, 1968) suggest that the results are due to disturbances other than disruption of metabolic processes, Morgane has concluded that midlateral and periformical portions of the hypothalamus essentially conscribe a hunger motivational system; whereas, far-lateral hypothalamic regions involve a more primary metabolic feeding system and are highly dependent on activation from pallidofugal fiber trajectories of the globus pallidus. Thus, Morgane (1961b) suggests that the feeding and drinking centers are only convergence sites for critical fiber systems which are destroyed by far-lateral lesions.

Investigations of the functional significance of various anatomical areas in the hypothalamus and analysis of the interaction of these hypothalamic areas with structures in the limbic system convinced Morgane (1961c) that extrahypothalamic regions participate in the neural regulations of food and water intake and that other behaviors are controlled by the so-called hypothalamic ingestive centers. For example, Morgane noted that ventromedial hypothalamic stimulation causes decreases in lateral hypothalamic electrical activity, inhibits area stimulation from other limbic structures, depresses feeding behavior in a deprived rat, and may cause a hungry animal to fall asleep. Other investigators have reported that electrical stimulation of the ventromedial necleus in hungry animals may cause a general disruption of behavior which may not represent a primary effect on hunger (Krasne, 1962; Turner, et al., 1967). Valenstein, et al. (1968) have suggested that activities of lateral hypothalamic neclei may be nonspecific. They state that with lateral stimulation a learning component appears to be related to evoked behaviors such as feeding and drinking. These investigators, therefore, question the assumption that stimulation,

electrical or chemical, activates fixed neural circuits which mediate natural motivational sequences.

Subsequently, Morgane (1966, 1969) has concluded that the hypothalamic "centers" should be conceived of as only a portion of a complex integrated circuitry which includes ascending and descending trojectory systems. These systems function in a reciprocating fashion to balance complex excitatory and inhibitory interactions between other mechanisms in the central nervous system, including the amygdala, septal region, hippocampus, temporal neocortex and other basal telencephalic regions. In promulgating this sophisticated conceptualization of the neural circuitry regulating feeding and drinking behavior, Morgane does not deny the functional significance of various hypothalamic neuclei. He merely states that hypothalamic mechanisms should be placed in their proper role in the total scheme of neural organization. Finally, Morgane (1961c, 1964, 1966, 1969) suggests that the "best hope" of unraveling the complex organization of neural components and circuitry controlling ingestive behavior lies in the employment of various combinations of experimental strategies such as lesioning, electrical and chemical stimulation, and electrophysiological, morphological, and behavioral methodologies.

CHAPTER III

STATEMENT OF THE PROBLEM

As indicated in the foregoing review of literature, various hypothalamic and extrahypothalamic regions have been found to play an important role in the integration and regulation of food and water behavior. Further, apart from identification of a lateral feeding area and a ventromedial satiety area, it has been demonstrated in a variety of studies employing cats (Magoun, et al., 1938; Nakayama, et al., 1961), dogs (Hemingway, et al., 1941), monkeys (Beaton, et al., 1941), oxen (Ingram and Whitton, 1962), and goats (Andersson and Larsson, 1961; Andersson, et al., 1962; Andersson, et al., 1963) that the preoptic-anterior region of the hypothalamus is involved in some manner in the neural regulation of body heat loss and heat maintenance. Additionally, other investigations (Strom, 1950a, 1950b, 1950c) have indicated that certain neural fibers in the preoptic-anterior region are differently sensitive to temperature changes in certain ranges, while other neural units are sensitive to temperature variations in other ranges. Therefore, these data show functional involvement of the preoptic-anterior complex in body heat regulation. The work of Andersson and his associates (Andersson and Larsson, 1961; Andersson, et al., 1962; Andersson, et al., 1963) relates increases in feeding and drinking in the goat to local temperature changes in the preoptic-anterior region. Thus, there is an impressive body of data indicating that food and water

intake are related to heat-loss and heat-maintenance functioning and are, therefore, regulated to some degree by the preoptic-anterior complex. Consequently, several possibilities must be considered as to the exact nature of preoptic and anterior hypothalamic involvement in the regulation of feeding and drinking behavior. For example, the complex may control ingestive behavior as an aspect of primary regulation of body temperature. Another possibility is that the structure may be a specific locus for cells which directly regulate appetitive behavior in addition to body heat. Finally, the site may be simply a neural pathway carrying efferent limbic system fibers which are involved in the control of food and water intake.

With respect to extrahypothalamic regions, the area along the medio-basal portion of the temporal lobe (amygdala) has been one of the loci most widely implicated as a participant in the neural regulation of appetitive behavior. With regard to cats, various authors, such as Green, et al. (1957), Morgane and Kosman (1957, 1959) and Wood (1958), have reported that lesions and ablation in the amygdaloid complex cause changes in feeding patterns. Although these findings are unclear as to what specific changes in feeding are affected by damage to different nuclei in the complex, these data do indicate that the amygdala participates in some way in the neural regulation of feeding behavior.

While it is apparent that the evidence strongly suggests preoptic-anterior hypothalamic and amygdaloid involvement in the central control of feeding and drinking behavior, most of the data remain inconclusive because of the limited usefulness of the techniques employed to investigate the relationship between a given brain structure and ingestive behavior. For example, lesions in the preoptic-anterior complex

interfere with an unknown number of nerve tracts passing through the area. Some of the tracts may be passing from other loci which may be involved in regulation of appetitive behavior. Further, electrical stimulation provides an advantage over lesion and ablation techniques in that evoked responses are often reversed by simply turning off the electrical current. Electrical stimulation effects, however, are not limited to neural junctions but also affect fibers of passage, causing conduction in both dromic and antidromic directions. Thus, some of the behavioral effects following both lesions and electrical stimulation may be due to interference with neural pathways or stimulation of axions of passage rather than to direct action on a regulating center. Additionally, as pointed out by Andersson, et al. (1964), the data from studies which employ heating and cooling of the preoptic-anterior region are possibly confounded. That is, local warming of the preoptic-anterior area may serve as a nonspecific stimulus, while local cooling may serve to inhibit all neuronal activity in the general vicinity of the thermode. Therefore, although the heating and cooling data indicate support for a preoptic-anterior thermostatic mechanisms which controls feeding and drinking, one cannot exclude the possibility that the effects of preoptic-anterior warming and cooling may be generally nonspecific rather than solely due to stimulation or inhibition of neurons explicitly sensitive to temperature.

In recent investigations of the functions of various central nervous system structures, intracranial chemical stimulation has proven to be an effective alternate method of studying a structure's functional relationship to behavior. Stimulation with biochemical substances has made it possible to determine if a structure merely contains pathways

involved in some function under study or if it is an initiating locus. However, as previously indicated in the review of literature, only several attempts have been made to stimulate chemically the preoptic-anterior region, and data from these studies are inconclusive. In one such study, Fisher and Coury (1964) reported that cholinergic stimulation of the preoptic area caused a significant increase in water intake in sated rats. Also, Hutchinson and Renfrew (1967) studied the effects of adrenergic and cholinergic injections in the preoptic and anterior areas. They found that norepinephrine inhibited water intake, while carbachol increased drinking in both areas in satiated rat. After food deprivation, however, norepinephrine injected in the preoptic area increased drinking, whereas, after water deprivation, carbachol injected in the anterior region caused a decrease in drinking.

While the predominant effects of chemical stimulation in the preoptic-anterior region in rats appear to be on water intake, Sommer, et al. (1967) have found somewhat different results following cholinergic stimulation at several brain sites in the satiated rabbit. They noted that, as carbachol concentrations increased, food intake increased after injections in the lateral preoptic area and in the area dorsal and anterior to the medial preoptic area. Drinking was facilitated by injections of lower doses of carbachol, while water intake decreased as drug concentrations increased. The region low between the medial and lateral preoptic areas near the supraoptic region was the most effective site for eliciting drinking. In contrast, Sharpe (1969) has reported that of all chemicals tested, an adrenergic chemical, norepinephrine, was the most effective in stimulating both food and water intake in the preoptic-anterior region of satiated monkeys; whereas,

cholinergic substances injected into this region produced inhibition of both feeding and drinking in the deprived monkey.

The issues are further confounded when the chemostimulation data on cats are considered. Various authors (Myers, 1961, 1964a; Hernandez-Peon, et al., 1963; Macphail and Miller, 1968) who chemically stimulated in the lateral and ventromedial hypothalamus reported that in each instance no change was produced in feeding and drinking behavior. Additionally, one study (Myers, 1964) reported that 5 cholinergic and 3 adrenergic injections in the anterior and 2 cholinergic injections in the preoptic resulted in no changes in ingestive patterns. Instead, in all hypothalamic areas stimulated, a number of emotional and autonomic reactions were observed.

Thus, while adrenergic and cholinergic drug stimulation has produced some type of change in the patterns of food and water intake in most species tested, intracranial chemical stimulation in the cat has never been reported to cause changes in ingestive behavior. It should be noted, however, that most of the previously reported investigations employing cats dealt primarily with the lateral and ventromedial hypothalamic areas when they searched for possible chemically responsive ingestive centers. Subsequently, the present study was designed to investigate further the behavioral effects of adrenergic and cholinergic chemical stimulation of brain areas in the cat. On the basis of previously reviewed studies which employed other techniques such as lesions and ablations, the preoptic and anterior hypothalamus were selected as sites likely to be involved in feeding and drinking. In this likelihood, chemical stimulation of these sites may evoke changes in ingestive behavior. In addition, again on the basis of previous research,

an extrahypothalamic area, the amygdaloid complex, was chosen as a brain site which might also regulate appetitive behavior and similarly show responsiveness to chemical stimulation.

The specific objectives of the present investigation were as follows:

1. To investigate changes in ingestive patterns and other responses following adrenergic and cholinergic stimulation in the preoptic and anterior hypothalamic areas and the amygdala in unrestrained, satiated cats.
2. To observe the neuropharmacological properties of the brain areas under study and to obtain dose-response relationships.
3. To determine if the preoptic and anterior hypothalamic areas are merely pathways for fibers connecting other brain sites which control ingestive behavior or if these regions themselves are possible regulatory centers.
4. To analyze further the suggestion by Morgane and Kosman (1957) that the amygdaloid complex in the cat is a feeding "satiety center."
5. To search for evidence indicating possible neurochemical coding of feeding and drinking behavior which has been demonstrated in the laboratory rat (Grossman, 1960, 1962a, 1962b).

CHAPTER IV

MATERIALS AND PROCEDURE

Subjects

Sixteen adult, male and female, mongrel and Siamese cats, which had an initial mean body weight of 3.1 SD 0.6 kilograms, served as experimental subjects. After acquisition and before surgery, all animals were given pneumonitis and feline distemper vaccine and dystricillan injections and were placed in cages in an isolation room. After recovery from surgery, each animal was individually housed and tested in stainless steel cages measuring 77 x 45 x 61 cm. which were located in an animal room in the neurophysiology laboratory of the Department of Psychology, University of Houston, Houston, Texas. Throughout the study, animals were provided Purina Dry Cat Food and water ad libitum.

Experimental Apparatus

Cannula

A light-weight double-walled cannula assembly, shown in Figure 1a was constructed in the following manner. The guide cannula was made from a 63-mm, 20-guage stainless hyperchrome hypodermic needle, a piece of teflon tubing, and internal brass fixture of a Harris-type #211 banana plug. The guide cannula length was fixed by cutting and grinding the syringe needle to 41-mm. Next, approximately 6-mm of teflon tubing was glued to one end of the syringe needle with Eastman 910

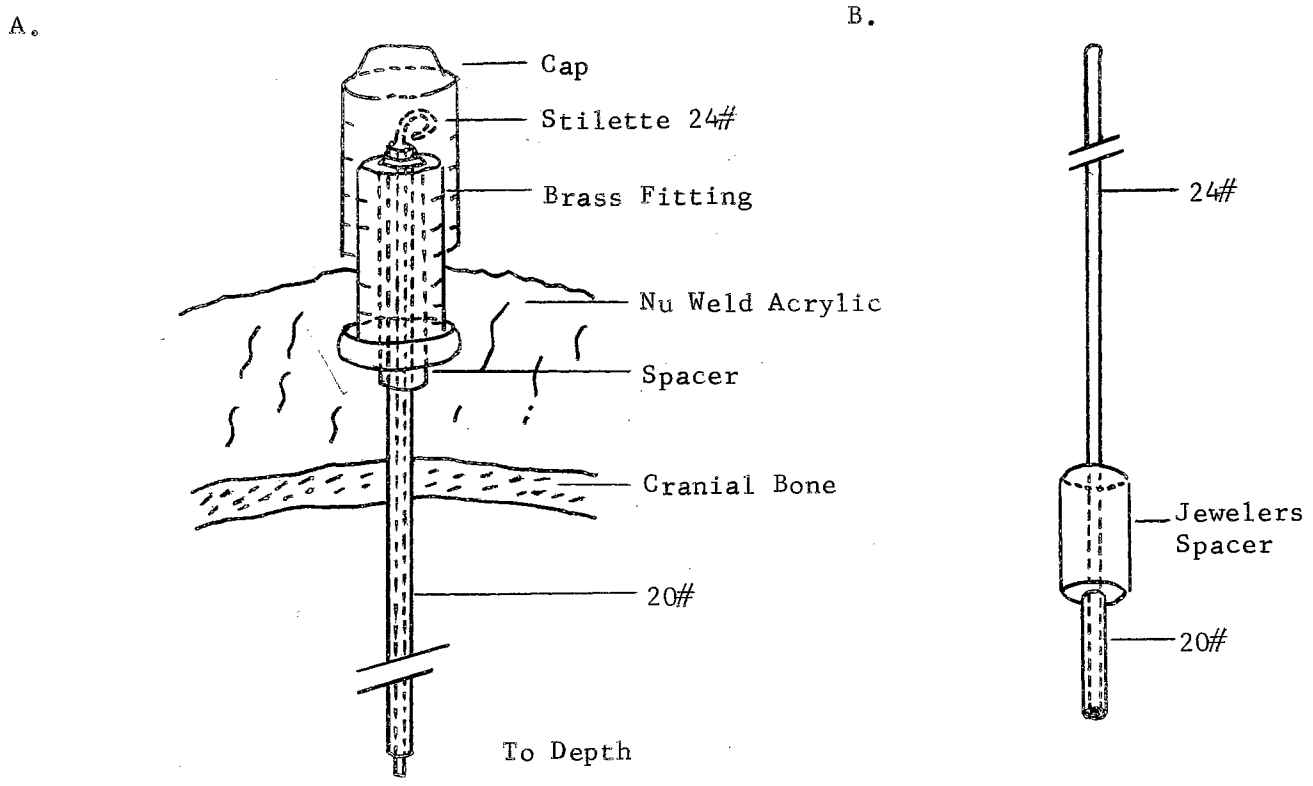

Figure 1. A. Diagram of Stainless Steel Guide Cannula and Indwelling Stilette

B. Diagram of Stainless Steel Internal Injection Cannula

adhesive. The teflon tubing served as a spacer between the syringe needle and the internal brass fitting from the banana plug. After the tubing was in place, the syringe needle and tubing were inserted into the brass fitting and glued.

A 43-mm indwelling stilette (dummy injection cannula) was made from a piece of 24-gauge stainless steel wire. The stilette remained in place in the guide cannula except during insertion of the internal injection cannula. The presence of the stilette served to keep the guide cannula open.

The internal injection cannula, as shown in Figure 1b, was constructed from a 24-gauge stainless steel hypodermic syringe needle, a 6-mm long brass jeweler's spacer, and a 10-mm section of 20-gauge stainless steel tubing. First, the jeweler's spacer was glued to the the 24 gauge stainless steel syringe at a point 43-mm from one end. The spacer served as a stop so that the internal injection cannula would extend only 2-mm beyond the internal tip of the guide cannula. The 10-mm section of 20-gauge tubing was then slipped over and glued to the 24-gauge injection cannula above the jeweler's spacer. The 20-gauge tubing was employed to insure a snug fit between the internal injection cannula and polyethylene tubing, which attached the injection cannula to a microsyringe.

Microsyringe

All chemical microinjections were made using a Hamilton 100 microliter syringe #710 made by Hamilton Company, Whittier, California and a vernier calibrated micrometer manipulator made by C. H. Stoelting Company, Chicago, Illinois. Both the microliter syringe and the

micrometer manipulator were mounted on a metal stand which permitted measured quantities as small as 1 µl to be injected. A piece of polyethylene tubing, approximately 48-cm in length, attached the microsyringe to the injection cannula. After each day of chemical injections, the microsyringe, the internal injection cannula, and the polyethylene tubing were rinsed in purified acetone (99.5 percent) to ensure a clean and open system for the next series of injections.

Chemicals

Adrenergic and cholinergic stimulation was provided by microinjections of L-norepinephrine bitartrate hydrate and carbaminocholine (K and K Laboratories, Plainview, New York). The various drug doses were obtained by dissolving each chemical in 0.9 percent sodium chloride. Although test concentrations for each drug were determined on the basis of previous research (Myers, 1961, 1964a; Macphail and Miller, 1968; Nance, 1969), an adjustment was made during the initial stages of the experiment to extend the range of carbachol in the direction of its lower limit of measurable effectiveness. Both chemicals were mixed on a weight-volume basis (one gram of drug in 100 ml of solvent equals a 1.0 percent solution). The drug concentrations employed in the present experiment were as follows: 2.0%, 4.0%, and 8.0% norepinephrine; and .005%, .025%, .050%, and .100% carbachol. In addition to test injections of various drug concentrations, 0.9 percent sodium chloride was injected into all brain areas as a control. The various concentrations in terms of micrograms of chemical per microliter of test solution were as follows: 20µg, 40µg, and 80µg norepinephrine per µl; and, .05µg, .25µg, .50µg, and 1.0µg carbachol per µl. Carbachol solutions were mixed and stored in clear plastic bottles, and

norepinephrine solutions were stored in light shielded bottles and refrigerated.

Surgical Technique

Prior to surgery, all surgical instruments and stereotaxic components were washed in Phisohex antiseptic soap and were placed in a solution consisting of 0.13% Zephiran chloride and a rust preventive. The general anesthetic employed in the present study was veterinary pentobarbitol sodium purchased from Pioneer Veterinary Supply, Houston, Texas. The pentobarbitol sodium was injected into the liver or peritoneal cavity of the cat in the amount of 1cc per 2.2 Kg of body weight.

Following injection of anesthetic, the scalp of the cat was shaved with electric animal clippers and cleaned with Phisohex and alcohol. A local anesthetic, 1 cc of 2.5% solution of procaine hydrochloride (Bio-Ceutic Laboratories, St. Joseph, Missouri), and 1 cc of adrenalin chloride solution (Park, Davis and Company, Detroit, Michigan), was infiltrated under the scalp.

After anesthetization and preparation of the scalp, the cat was placed in a Johnson cat stereotaxic instrument. A 30-mm medial-sagittal incision exposed the underlying cranial bone. When necessary, gauze sponges were placed subcutaneously on each side of the exposed cranium to retract the superior temporal musculature and to inhibit bleeding. In addition to gauze, hemostats were used to hold back laterally both muscular and epidermal layers. The periosteum was scraped laterally and the cranium was washed in normal saline and dried with gauze.

Once the cranium was exposed and cleaned, stereotaxic placements

were marked on the cranial bone with vegetable dye. Next, the skull was trephined using a dental burr with two placements in each hemisphere. The brain loci and stereotaxic coordinates, as determined from a stereotaxic atlas of the diencephalon of the cat by Jasper and Ajmone-Marsan (1954), were as follows: Preoptic hypothalamus (right hemisphere), 16.0 mm anterior-posterior, 2.0 mm lateral, -2.0 mm horizontal; anterior hypothalamus (left hemisphere), 14.0 mm anterior-posterior, 3.0 mm lateral, -2.0 mm horizontal; basal-lateral amygdala (right hemisphere), 11.0 mm anterior-posterior, 10.0 mm lateral, -5.0 mm horizontal; and cortio-medial amygdala (left hemisphere), 12.0 mm anterior-posterior, 6.5 mm lateral, -4.5 mm horizontal. In the foregoing coordinate system, two points of reference determine the basal plane used by the stereotaxic instrument. The first point of reference is the inter-aural line which connects the center of each external auditory meatus. The second point of reference is the center of each inferior orbital ridge. The basal horizontal plane then passes through the inter-aural line and the inferior orbital ridges. Although these reference points determine the basal plane, it is not the zero horizontal plane used by Jasper and Ajmone-Marsan (1954). In Jasper and Ajmone-Marsan's (1954) atlas, the zero horizontal plane is arbitrarily taken to be 1-cm above the inter-aural basal plane.

Two additional holes were trephined anterior and posterior to the four cannula placements. These holes were tapped and stainless steel anchor bolts were inserted in the cranium. Subsequent to placement of each cannula, dental acrylic was used to bond the cannula to the skull. After all cannula and anchor bolts were in position, a permanent plug was constructed by applying layer upon layer of dental acrylic.

Following completion of the plug, all gauze was removed, and the wound was cleansed with saline. The incision was then sutured anteriorly and posteriorly to the acrylic plug. Postoperatively, Pelizone, an ointment which stimulates epithelial growth and prevents surface bacterial infection, was applied to the wound, and an injection of 900,000 units of veterinary dystricillan was administered intramuscularly. Coagulen and Bemegride, a barbiturate antagonist, were administered to facilitate recovery.

Testing Procedure

After a two week recovery period following surgery, behavioral responses to a placebo and to the experimental drugs were observed following injections at each brain site. Initially, 2 μl of 0.9 percent sodium chloride was injected into each brain area as a control. Next, norepinephrine and cabachol were injected into each brain region. The technique for injections was as follows. On injection days, the indwelling stilette was removed from the implanted guide cannula and the internal injection cannula was inserted. Injections were made manually by slowly injecting a 2 microliter volume of test solution into the brain area over a 30 second period. Before insertion and after removal of the injection cannula, 1 microliter of test solution was injected to the tip of the injection cannula to ensure an open system both before and after each injection. All brain areas and chemicals at all concentrations were chosen randomly for each test with the restriction that no replication occur. Each animal was tested in his home cage, and each test was separated from the next by at least 48 hours. On each test day, food and water intakes, corrected for

spillage, were recorded for a one-hour pre-injection baseline period and for a one-hour post-injection test period at 1:00 and 2:00 p.m., respectively.

Verification of Cannula Placements

Perfusion Technique

At the conclusion of the testing period, all subjects were perfused in preparation for subsequent histological examination. The perfusion procedure was as follows. Using veterinary pentobaritol sodium as a general anesthetic, a large longitudinal incision was made along the medial portion of the cat's chest. The sternum was then split with scissors and spread open with a self-retaining retractor. After the abdominal aorta was clamped with a hemostat, the pericardium was exposed and cut away. With the heart fully in view, a perfusion needle was inserted in the left ventricle and saline (0.9 per cent) was allowed to flow into the heart. Immediately, the right atrium was cut open widely, and the saline was allowed to circulate until the return from the atrium was clear of blood. After perfusion with saline, stereotaxic bars were placed in the interaural cannals, and the cat's mouth was blocked open so that the animal could be put back into the stereotaxic instrument. The cat was perfused with 10 per cent formalin until its ears and jaw were stiff. Each animal was decapitated, and its head skinned and placed in a 10 per cent formalin solution for at least 24 hours.

Tissue Blocking Procedure

After perfusion, the animal's head was placed in a Johnson cat stereotaxic instrument. The cranium and dura surrounding the brain

were carefully removed. After the brain was fully exposed, the extent of the desired block in the anterior-posterior plane needed for sectioning was determined. Using the stereotaxic instrument with a blade attached, the block was then cut in the anterior-posterior plane exactly perpendicular to the horizontal plane. A notch was cut in the upper left side of the block for later identification. Notation was made of both the anterior-posterior coordinates and of all punctures on the brain grid. The block of brain tissue was then removed from the remaining cranium base and placed in a jar containing 10 per cent formalin solution for at least 24 hours.

Histology Procedure

After completion of perfusion and blocking of brain tissue, the block was removed from the 10 per cent formalin solution and placed in a container of distilled water for 5 minutes. The block of tissue was then frozen on a CO_2 freezing microtome stage. After freezing, tissue slices of approximately 50 microns were made. Each slice that contained a cannula placement was floated in distilled water and placed on a glass slide. The tissue section was coated with glycerin, and a cover slide was attached. Next, the sections were numbered and allowed to dry. Finally, the anatomical placements of the cannula tips were determined, and Polaroid photographs were taken of the tissue sections.

CHAPTER V

RESULTS

Statistical Model and Test

In the selection of a statistical test, measurement and model requirements must be considered. Although data obtained in the present study exceed minimum measurement requirements (interval scaling) necessary for employment of a parametric test, analysis of the underlying statistical model indicates that any parametric evaluation would be inappropriate. A statistical model is based on the characteristics of the parent populations and on the nature of sampling. In the present investigation, the variance of the samples indicate that the assumption of homoscedasticity could not be made. Further, the sample sizes are relatively small, and the exact nature of the parent population distributions are not known. Under such conditions Siegel (1956) states that "... there is no alternative to using a nonparametric statistical test. . . ." Empirically, apart from sample size limitations, the major difficulty with the present data lies in the fact that various drug doses produced large variations in food and water intakes. Apparently, variability was inherent in the study consequent to such factors as day-to-day environmental changes, individual differences among subjects, daily changes within animals, differential sensitivity of brain sites to drugs and fluctuations in cortical neural interactions among responsive and non-responsive sites.

In view of the statistical model requirements, the Wilcoxen matched-pairs signed-ranks test was selected because it utilized information about both the direction of change and the relative magnitude of change within pairs. Additionally, the Wilcoxen test is one of the few nonparametric procedures which allows for the use of each subject as his own control.

Effects of Adrenergic and Cholinergic Stimulation on Ingestive Behavior

Preoptic Region

With respect to effects on drinking behavior, Figure 2 indicates that, except for a slight elevation in water intake after 2 injections of 0.9 per cent sodium chloride, all concentrations of both adrenergic and cholinergic drugs caused a depression in water intake. With both norepinephrine and carbachol, as concentrations increased, the amount of water intake decreased. While the blockage of drinking behavior showed only trend effects ($p > .05$) at all concentrations of carbachol, significant depression effects ($p < .01$) were observed at the two highest concentrations, 4% and 8%, of norepinephrine. At the 4% concentration three of the seven experimental animals showed no water intake during the test period, whereas, at the 8% level, five of seven subjects showed no drinking.

Figure 3 indicates that the effects of preoptic adrenergic stimulation with norepinephrine on feeding behavior were similar to the effects of norepinephrine on drinking behavior. That is, as concentrations of the drug increase, the amount of food consumed decreases. Again, depression of ingestive behavior was significant ($p \leq .01$) at

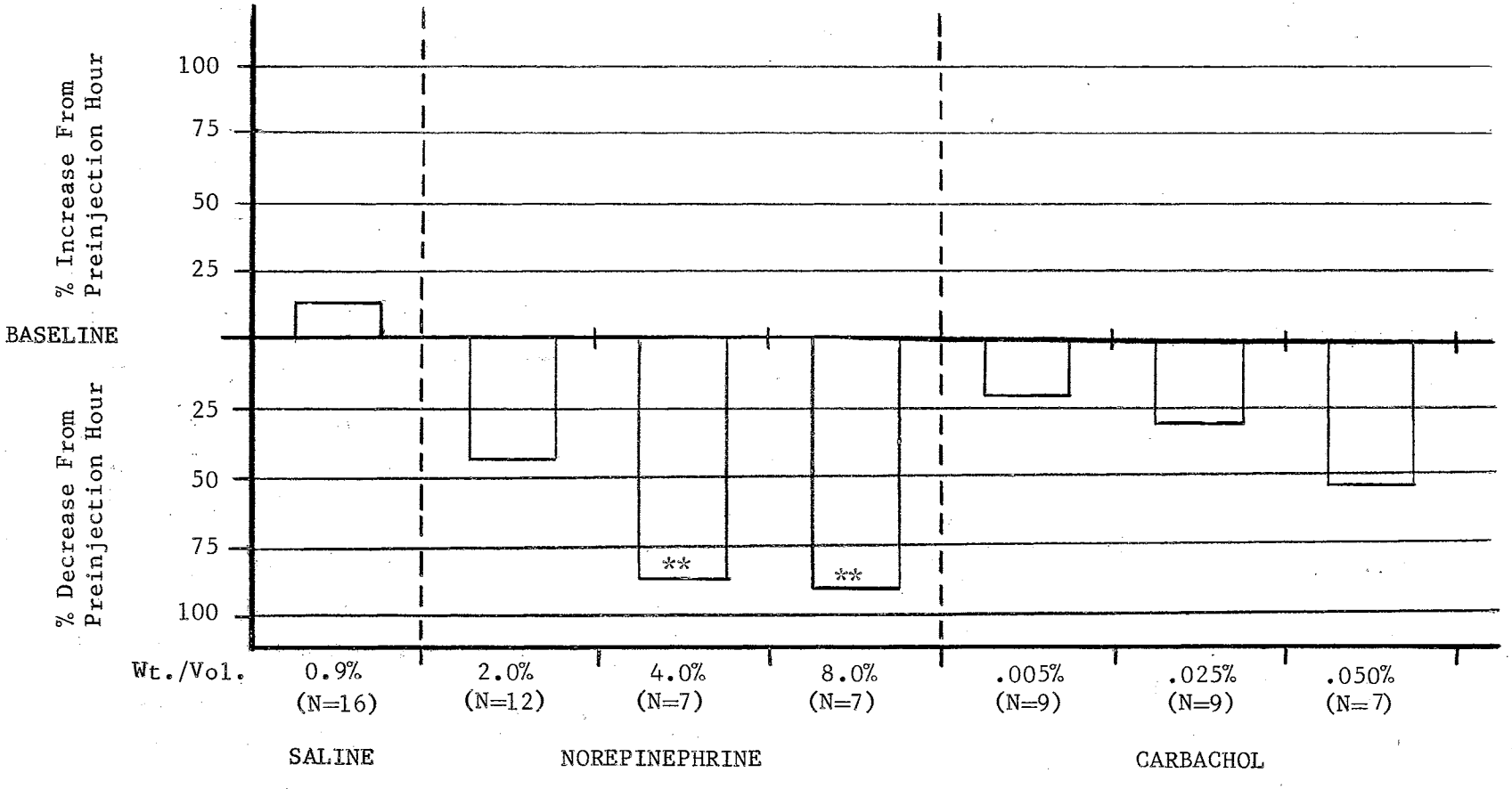

Figure 2. Effects of 2 µl Intra-Preoptic Adrenergic and Cholinergic Drug Injections on Water Intake in the Cat

**Significant at the .01 level

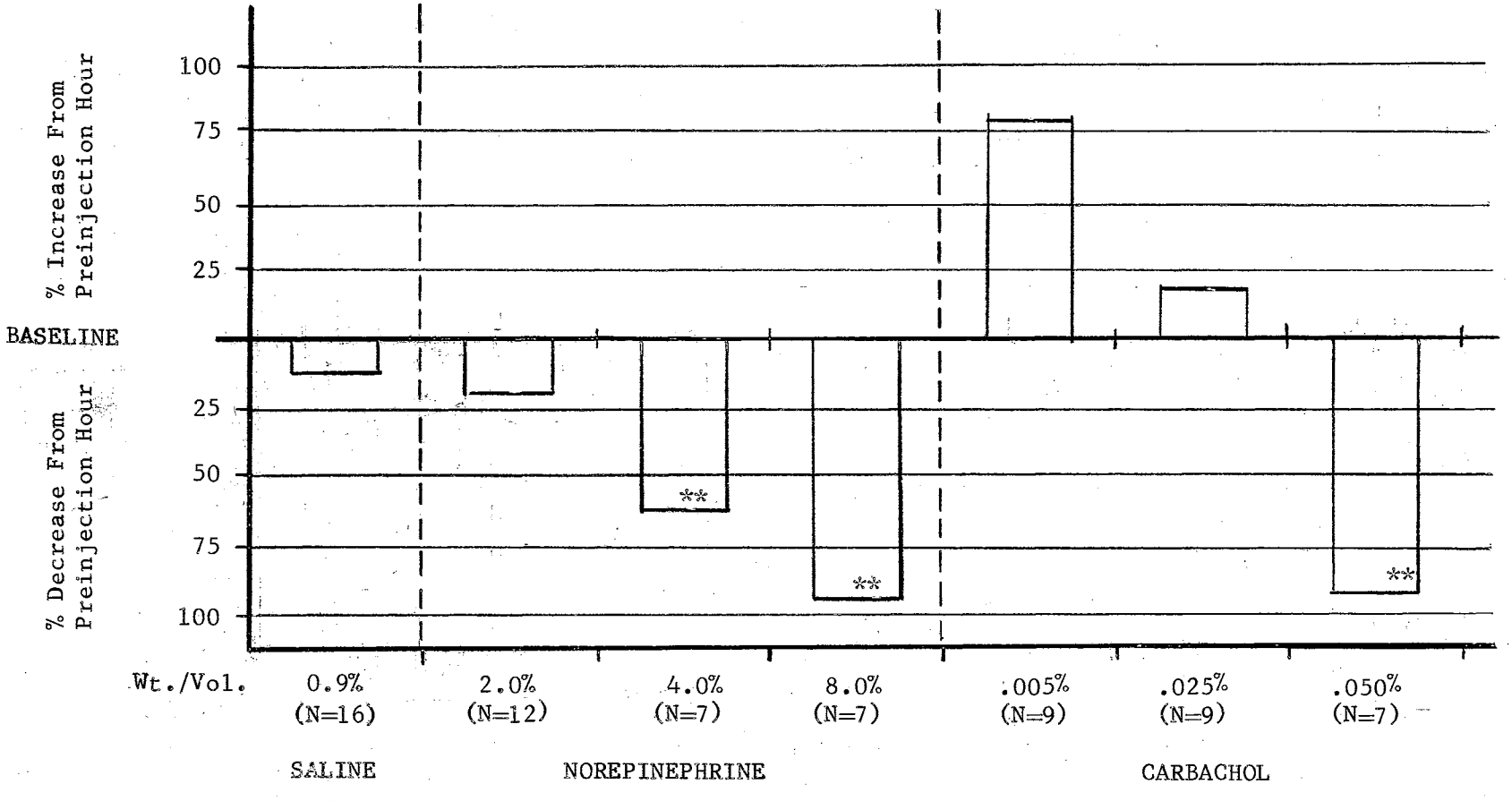

Figure 3. Effects of 2 μl Intra-Preoptic Adrenergic and Cholinergic Drug Injections on Food Intake in the Cat

**Significant at the .01 level

the two highest concentrations with two of seven animals showing complete blockage of food intake at the 4% level and five of seven cats showing total inhibition of feeding at the 8% concentration. With injections of carbachol into the preoptic region, food intake was observed to increase after stimulation with .005% and .025% concentrations. These increases in food intake, however, were not significant ($p > .05$). In contrast, at the highest concentration of carbachol, .05%, food intake was observed to be blocked significantly ($p < .01$), with four of seven animals showing no feeding during the test period.

Anterior Hypothalamus

Figure 4 shows that with both adrenergic and cholinergic chemical stimulation of the anterior hypothalamus no systematic drug dose effects on drinking behavior were observed. Although water intake was found to decrease after injections at all concentrations of norepinephrine, the only significant blockage effects ($p < .05$) occurred at the highest concentration of norepinephrine. In this instance drinking was completely blocked in three of eight subjects. Carbachol showed variable dose dependent effects in which significant blockage ($p < .01$) of drinking behavior occurring only after injections at the .025% level. Total inhibition of drinking behavior was observed in only one of seven animals.

Possible aminergic evocation of feeding behavior after stimulation with the two lowest concentrations of norepinephrine is shown in Figure 5. The effects of both 2% and 4% concentrations, however, were found to be insignificant ($p > .05$). Again, as in the preoptic region, the highest test concentration of norepinephrine was found significantly ($p < .01$) to inhibit food intakes. Complete blockage was observed in

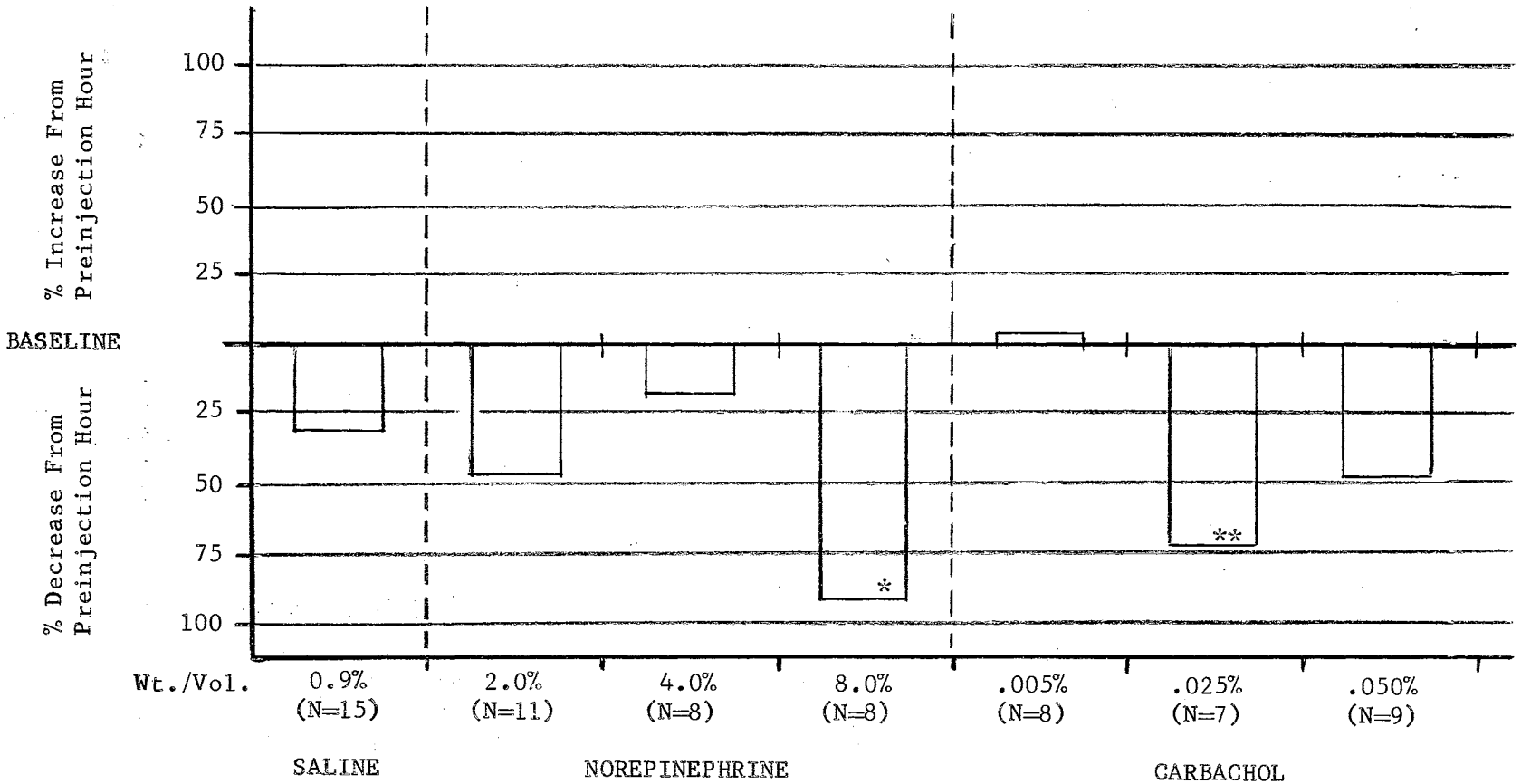

Figure 4. Effects of 2 µl Intra-Anterior Adrenergic and Cholinergic Drug Injections on Water Intake in the Cat

*Significant at the .05 level
**Significant at the .01 level

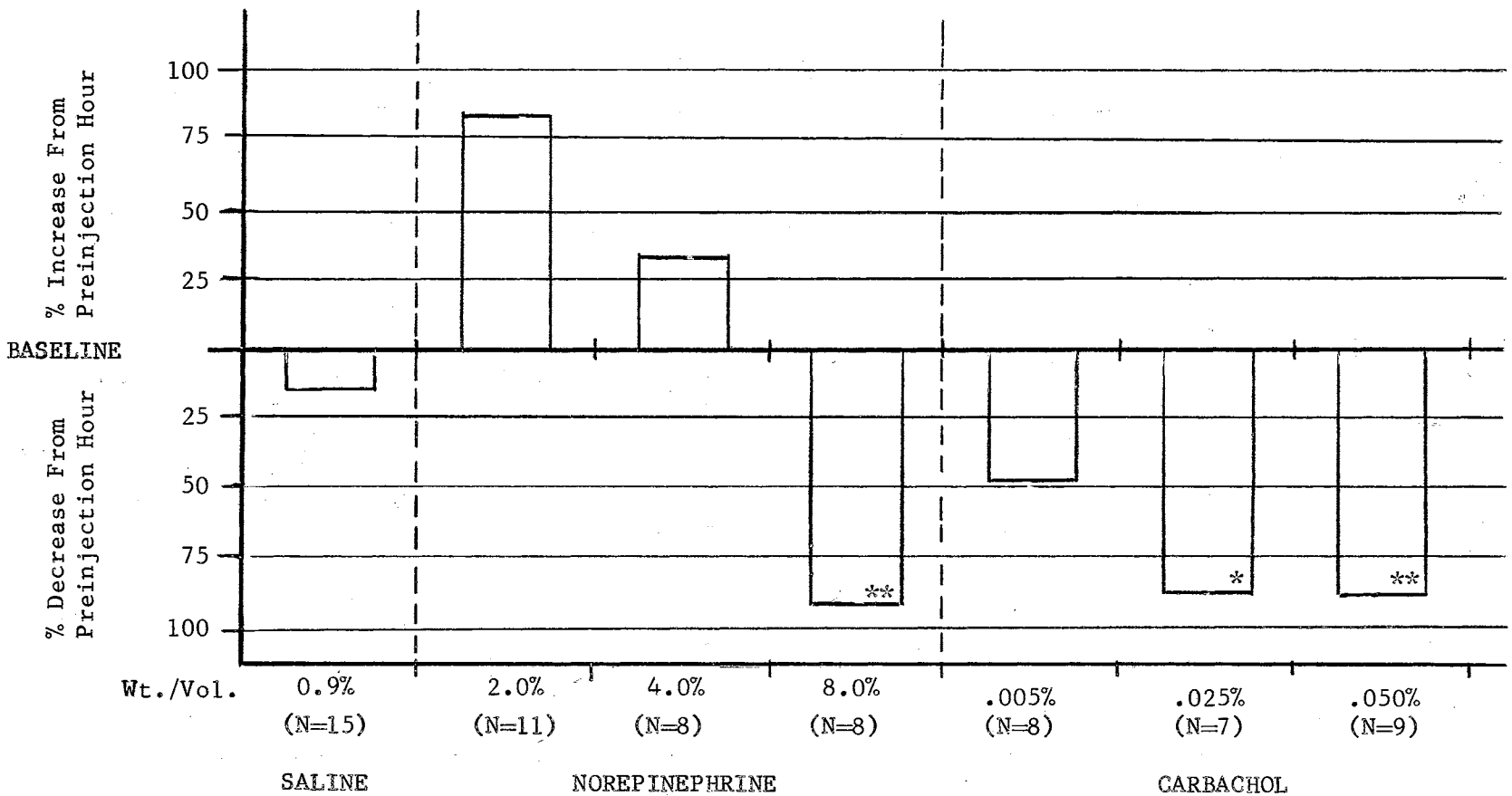

Figure 5. Effects of 2 µl Intra-Anterior Adrenergic and Cholinergic Drug Injections on Food Intake in the Cat

*Significant at the .05 level
**Significant at the .01 level

six of the eight experimental subjects. In contrast, carbachol showed depression of feeding behavior at each level tested, and there was increasing inhibition of food intake as concentrations were increased. While only trend effects were observed at the .005% level, both the .025% ($p < .05$) and the .05% ($p < .01$) carbachol concentrations were found to be significant in their depressions of food intake. At the .025% level, five of seven animals showed no food intake; whereas, at the .05% level seven of nine cats tested showed complete blockage of feeding behavior.

Amygdaloid Complex

The effects of adrenergic stimulation in the baso-lateral amygdala on water intake are shown in Figure 6. While only trend effects were observed at the two lower norepinephrine concentrations, a significant ($p < .01$) blockage of drinking was observed after the 8% drug concentration was injected. At 8% concentration, total inhibition of water intake was observed in 3 of 8 experimental animals.

Figure 7 shows that significant ($p < .05$) aminergic blockage of food intake was observed at the two highest test concentrations (4.0% and 8.0%) of norepinephrine following injections in the baso-lateral amygdala. At the 4.0% level, three of eight animals showed complete blockage of food intake; whereas, at the 8.0% concentration five of eight cats showed total blockage of feeding behavior. Figure 6 shows that effects were variable following injections of carbachol and were, apparently, dose dependent. While trend blockage effects were observed at the lowest (.005%) and at the highest (.050%) test concentrations, significant ($p < .05$) blockage effects were observed at the middle (.025%) carbachol concentration. At the .025% level, complete

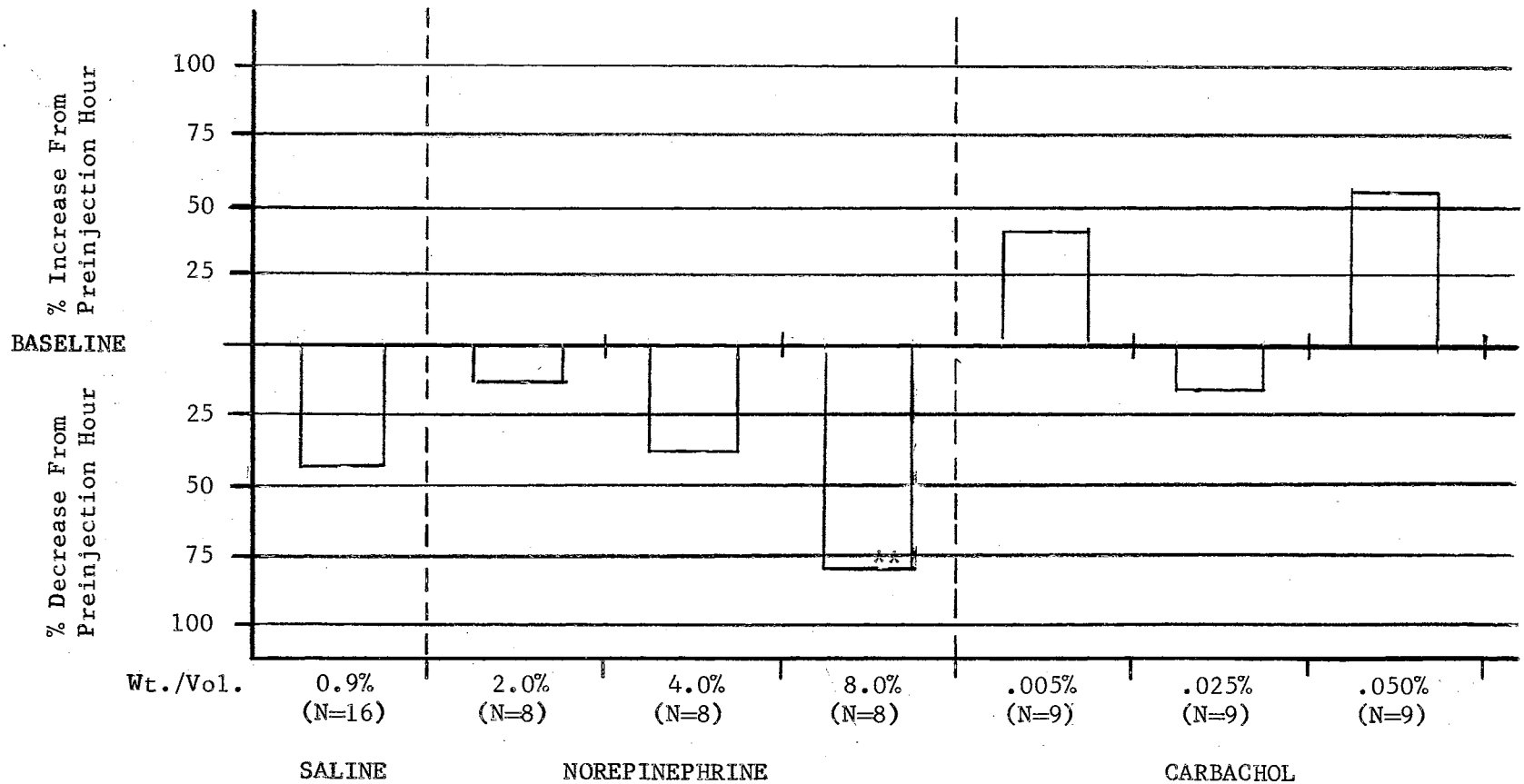

Figure 6. Effects of 2 μl Intra-Amygdala Adrenergic and Cholinergic Drug Injections on Water Intake in the Cat

**Significant at the .01 level

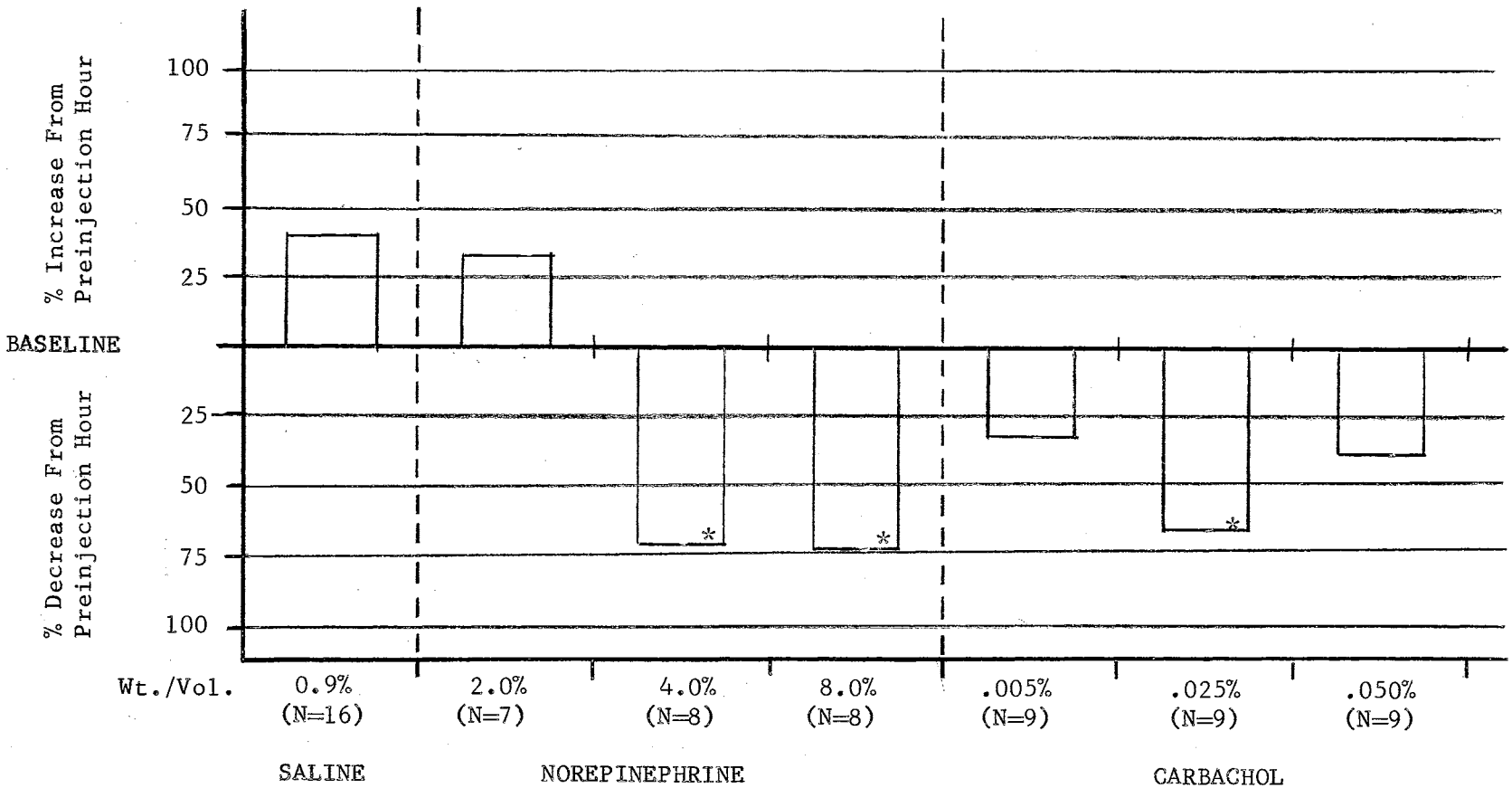

Figure 7. Effects of 2 μl Intra-Amygdala Adrenergic and Cholinergic Drug Injections on Food Intake in the Cat

*Significant at the .01 level

inhibition of feeding behavior was observed in five of nine animals in the baso-lateral amygdala. Data on the cortico-medial amygdala are not presented because histological investigation indicated a paucity of appropriate cannula placements.

Emotional and Autonomic Responses

Although no continuous records of drug-evoked emotional and autonomic responses were made, notations of various behavioral effects additional to changes in ingestive behavior were made during each testing period. Summaries of evoked responses shown in Table I and Table II indicates that, although evoked responses were apparently chemically specific, there was a surprising lack of localization of both adrenergic and cholinergic effects to any single brain region tested. In general, injections of norepinephrine altered autonomic activities causing changes in respiratory rates and possibly body temperature. Although all the test concentrations of norepinephrine injected into the hypothalamic areas produced a stupor or drowsy-like state, sleep was never observed. The most dramatic change found after norepinephrine injections was that most animals exhibited a pronounced lack of any emotional behavior.

In contrast, carbachol stimulation, at each concentration except the lowest (.005%), produced, in varying dose dependent degrees, generalized emotional and sympathomimetric responses. Typically, pupillary dilation was exaggerated, and the emotional state of the animal was sometimes characterized by fear-like behavior which included withdrawal responses, crouching and meowing. More often, however, at the higher concentrations, a rage-like condition accompanied by hissing, spitting,

TABLE I

OBSERVABLE EMOTIONAL AND AUTONOMIC EFFECTS EVOKED BY 2 μl INTRACRANICAL ADRENERGIC CHEMICAL INJECTIONS

Brain Area	Saline 0.9%	2.0%	Norepinephrine 4.0%	8.0%
Preoptic	no effect	slight hyperventilation	hyperventilation lethargic stupor proneness	excessive hyperventilation lethargic, stupor proneness, motionless emotional behavior absence temperature up(?)
Anterior	no effect	lethargic	some hyperventilation lethargic stupor proneness	hyperventilation stupor, meowing proneness, motionless emotional behavior absence temperature up(?)
Amygdala	no effect	no effect	some hyperventilation	excessive hyperventilation motionless, stupor emotional behavior absence

TABLE II

OBSERVABLE EMOTIONAL AND AUTONOMIC EFFECTS EVOKED BY 2 μl INTRACRANICAL CHOLINERGIC CHEMICAL INJECTIONS

Brain Area	Saline 0.9%	Norepinephrine .005%	.025%	.050%	.100%
Preoptic	no effect	no effect	alert, active pupillary dilation	fear-like response pupillary dilation growling, hissing crying, meowing piloerection crouching	fear-like response or sham rage pupillary dilation growling, hissing piloerection crouching
Anterior	no effect	no effect	alert, very active pupillary dilation some growling, meowing	fear-like response pupillary dilation growling, meowing	no injection
Amygdala	no effect	no effect	alert, motionless or very active growling hissing	motionless or very active, alert pupillary dilation growling, meowing fear-like response or sham rage	sham rage, active contralateral circling pupillary dilation hissing, licking retching, vomiting growling, meowing death (1)

*dose discontinued because of death

and growling was observed. This rage-like behavior may have been of a non-directed variety because no direct attacks were made on the experimenter. At the .10% level of carbachol, evoked effects were extremely exaggerated. Convulsions, vomiting, contralateral circling and loud vocalizations were common. In one animal, after a 2 μl injection of carbachol into the amygdala, evoked responses were so severe as to result in death within 12 hours. Subsequently, injections of .10% carbachol were discontinued.

CHAPTER VI

DISCUSSION

General Comments

Comparison of Cat Chemostimulation Data

Results from the present study indicate that direct intracranial adrenergic and cholinergic chemical stimulation of the preoptic and anterior hypothalamus and the amygdaloid complex can produce significant changes in patterns of feeding and drinking behavior in satiated cats. These results contrast with cat chemical stimulation data reported by other investigators (Myers, 1961, 1964a; Hernandez-Peon, et al., 1963; Macphail and Miller, 1968). Myers (1961, 1964a) found no changes in food and water intake following 1-50 μq injections of epinephrine, norepinephrine, acetylcholine and carbachol in the preoptic, anterior, posterior, dorsomedial and ventromedial portions of the hypothalamus. Also, Hernandez-Peon, et al. (1963) reported no instances of evoked feeding or drinking behavior with either adrenergic or cholinergic stimulation of different sites in the cat's brain. More recently, Macphail and Miller (1968) confirmed these earlier findings. They reported .14-3.9 μq of carbachol injected in 43 different brain sites in the cat produced no changes in ingestive behavior.

Following inspection of the literature, however, there appears to be several factors which may have contributed to divergence between present findings and data reported by other studies. For example,

Myers' (1961, 1964a) conclusions on the effects of chemostimulation in the preoptic and anterior hypothalamus are based on extremely small samples. He made only 5 cholinergic and 3 adrenergic chemical injections in the anterior, and 2 cholinergic but no adrenergic drug injections in the preoptic. In addition, there is very little overlap in the chemical concentrations tested in the present study and in Myers' investigation. The importance of considering differential drug concentrations has been pointed out by Miller, et al. (1964). They demonstrated that laboratory rats which increase feeding and drinking in response to adrenergic and cholinergic stimulation, respectively, show inhibition of ingestive behavior as drug concentrations increase. It is interesting to note that at the one level where concentrations did overlap, at the lowest dose (40 μg) of norepinephrine in the present study and at the highest dose (50 μg) of norepinephrine in Myers' study, the results of both investigations agree. That is, in this range of drug concentration, no significant changes in feeding and drinking behavior were observed in either study. The investigation by Hernandez-Peon, et al. (1963) is difficult to evaluate because quantitative measurements of the amounts of drug injected were not made, although the authors do state that the amounts were ". . . certainly in the order of micrograms". Also, there is some question as to the accuracy of the various cannula placements. In the Macphail and Miller (1961) study, where 43 brain loci were stimulated, the drug doses compare well with those used in the present investigation. However, only one chemical (carbachol) was injected into the various brain sites and, again, small samples were employed. Finally, in addition to difficulties caused by limited sample sizes and differential drug

concentrations, it should be pointed out that in all these studies the authors appeared to be anticipating facilitation of ingestive behavior, rather than blockage, and, in most instances, the authors were studying other behaviors such as sleep or emotionality. Given such orientation, depression effects upon appetitive behavior may have been overlooked.

Amygdala "Satiety Center" Hypothesis

The present data offer general support for the hypothesis (Morgane and Kosman, 1957) which states that the amygdaloid complex in the cat is concerned with feeding behavior and functions normally as a "satiety center". Following both adrenergic and cholinergic chemical stimulation in the amygdala, feeding decreased. In most cases, strongest blockage of food intake occurred after injections of the two highest test concentrations of norepinephrine. It should be noted, however, that blockage effects were highly variable between animals following injections in the amygdala. Other authors have reported differential responses are mediated by various subdivisions in the amygdala (MacLean and Delgado, 1953). Subsequently, it has been purposed that the medial portion of the basal nucleus is associated predominantly with sympathetic effects while the lateral portion of the basal nucleus and the cortical and medial nuclei are primarily involved in parasympathetic functions (Koikegami, et al., 1954). Thus, in the present study, slight variation in amygdala cannula placements might have produced the between subjects differences in blockage effects. Gloor (1960), however, has stated that "Functional representation in the amygdala . . . seems to be of a . . . global and topographically undifferentiated type". He is impressed by the wide overlap of loci evoking a variety of similar responses and believes that no topographical organization of functions

can be deduced from the data presently in the literature. Thus, the issue of whether the amygdala does or does not contain nuclei which mediate functionally different responses remains to be answered. Also, the possibility that stimulation of various loci in the amygdala might produce variation in ingestive patterns remains speculative.

Emotional and Autonomic Responses

Observations in the present study with respect to chemically evoked autonomic and emotional responses which are apparently chemically specific, yet lack localization to any single brain site, generally agree with the findings of other investigations. Myers (1964a) reported that adrenergic stimulation of the anterior hypothalamus produced slight pupil constriction, ataxia, stupor and slight hypalgesia at low doses (5-10 μg) and pupillary constriction, marked ataxia, compulsive sleeping and hypalgesia at high doses (25-50 μg). Cholinergic stimulation of the same area produced pupillary dilation, changes in heart and respiratory rates, meowing, fear-like behavior at low doses and pupillary dilation, nystagmus, piloerection, twitching, growling, spitting and attack behavior at high doses. Similar effects were obtained by Myers after adrenergic and cholinergic in the lateral and posterior hypothalamus. In the present study, only hypalgesia and compulsive sleeping following adrenergic stimulation were not found, and these differences may be due to variations in experimental procedures. For example, in the present study, hypalgesia may have been present even though not measured, while compulsive sleep may not have been observed because of drug concentration levels. Other studies, however, are not in agreement with Myers (1964a) on the issue concerning adrenergic drug-induced sleep. In contrast with Myers' findings,

Hernandez-Peon, et al. (1963) reported that, although alertness and hyperactivity were found at some cholinergic injection sites, cholinergic stimulation often produced light to deep sleep in cats. Although adrenergic stimulation also resulted in increased alertness, there were no reports of sleep. Baxter (1967), who implanted various adrenergic and cholinergic crystalline chemical compounds in the hypothalamus, amygdala and hippocampus of cats, observed cholinergically evoked emotional responses similar to those described by Myers (1964a), but Baxter failed to find any instances of either adrenergic or cholinergic drug-induced sleep. In another study, Macphail and Miller (1968) also reproduced the general emotional and antonomic responses reported by Myers (1964a), but they failed to find any occurrence of chemically evoked sleep. Thus, while the present study is in agreement with the literature with respect to the general nature of chemically elicited emotional and autonomic responses, the present data along with that of Baxter (1967) and Macphail and Miller (1968) present a serious challenge to earlier reports of either adrenergic (Myers, 1964a) or cholinergic (Hernandez-Peon, et al., 1963) drug induced sleep.

Comparison with Other Species

The present data on the effects of intrahypothalamic adrenergic and cholinergic drug stimulation in cats show drastic differences from chemical stimulation data on laboratory rats. While the present investigation indicates no significant facilitation of ingestive behavior, other investigators (Grossman, 1960, 1962a, 1962b; Miller, et al., 1964; Miller, 1965; Hutchinson and Renfrew, 1967) have reported adrenergically induced feeding and cholinergically induced drinking at hypothalamic sites in the rat. It appears that the only chemical

stimulation data the cat and the rat have in common is that the deprived rat, like the satiated cat, shows reduced water intake with adrenergic stimulation and reduced food intake with cholinergic stimulation. However, unlike the rat, no reciprocal inhibition is involved because norepinephrine and carbachol injected in the satiated cat also inhibit feeding and drinking, respectively.

In the satiated rabbit, Sommer, et al. (1967) injected .06-17.20 µg of carbachol into various brain sites. As concentrations increased, food intake was found to increase following injections in the lateral hypothalamus, lateral preoptic area and the region dorsal and anterior to the medial preoptic area. Drinking was facilitated by lower concentrations of carbachol, but decreased to baseline at the higher test doses. The most effective area for eliciting drinking behavior, which was independent of feeding, was in an area low between the medial and lateral preoptic areas near the supraoptic region. At similar concentrations of carbachol, therefore, rabbits show a significant increase in drinking behavior, whereas, the present data indicate that cats show a significant decrease in both feeding and drinking.

The present findings for cats also contrast with chemostimulation data for rhesus monkeys in that norepinephrine which significantly inhibited feeding and drinking in all areas tested in the cat was found to be most potent in the facilitation of feeding when injected into the preoptic region and lateral hypothalamus of satiated monkeys (Shape, 1969). With injections of carbachol, however, data obtained on the cat and monkey are more similar. The present study indicated that carbachol (0.1-2.0 µg) injected unilaterally into the preoptic region and anterior hypothalamus of the satiated cat exercises inhibitory effects

on feeding and drinking behavior. In an extension of the work by Myers (1968a), Sharpe (1969) has shown that injections of carbachol (0.5-25.0 µg) into the preoptic region, anterior hypothalamus, lateral hypothalamus and ventromedial hypothalamus of the hungry and/or thirsty monkey blocks feeding and drinking. Additionally, in both the cat and the monkey, injections of carbachol into the amygdala were relatively less effective in blocking ingestive behavior.

Chemical Coding Hypothesis

A principle question to be answered with regard to reported effects following intracranial chemical stimulation concerns the nature of neural and chemical activities responsible for eliciting the behavioral changes. In view of evidence that both adrenergic and cholinergic drugs are possible neurohumoral transmitters in the central nervous system, it is tempting to conclude observed effects after chemical injections are due to a transmitting action produced by the drugs. Before this possibility can be established, however, several problems and alternate possibilities must be considered.

In the few studies employing adrenergic and cholinergic drug injections in the brain, there has not been strict control over diffusion. In fact, there are no specific data on the diffusion rates of adrenergic and cholinergic chemicals in the CNS of the cat. Thus, only gross localization has been achieved. Nevertheless, the accuracy of chemical stimulation is probably greater than that achieved by electrical stimulation. On the basis of MacLean's (1957) work on the diffusion rates of dyes and radiophosphorus in cats, Miller, et al. (1964) has estimated one micro-liter of a adrenergic or cholinergic chemical will diffuse over a sphere of tissue from 1 to 2 mm in radius and involves from 5 to 40 mg of brain tissue. In chemical stimulation work, dose size is

also important because extremely high doses relative to endogenous chemical levels have produced abnormal EEG activity in laboratory rats (Grossman, 1962a). A rough estimate of mean endogenous chemical levels has been reported for several animals. Giarman and Schmidt (1963) reported 2.76 μg/g for the acetylcholine content of the rat brain stem, and Kuntzman, et al. (1961) have reported 2.00 μg/g for the content of norepinephrine in the brain of the white mouse. If one can extrapoate from the rat and mouse, one would estimate the mean endogenous levels for the spheres of stimulated tissue in the present study to range from .028 to .224 μg and from .02 to .16 μg for acetylcholine and norepinephrine, respectively. Thus, while lower test doses of carbachol overlap with the higher estimated endogenous levels of acetlcholine, test concentrations of norepinephrine were many times greater. It should be noted, however, that the estimates of endogenous contents calculated above are only averages, and the chemical concentrations at an activated loci must be considerably greater. Thus, it is possible that even norepinephrine test doses may be in the general order of magnitude of endogenous levels during activation. This possibility is further supported if it is assumed that a large number of neurons have to be activated to evoke a reliable behavioral response which is in competition with other responses in the free-moving animal.

Ever if observed behavioral effects are due to excitation or inhibition of neuronal elements within a limited distance from the cannula tip, the contention remains to be supported that activation is due to the neurohumoral properties of the adrenergic and cholinergic chemical rather than to one or more side effects. In an attempt to demonstrate observed behaviors are due to neurohumoral properties of the

drugs, Grossman (1962a) has conducted a series of control experiments. He concluded that possible side effects of adrenergic and cholinergic stimulation such as nonspecific activation, osmotic stimulation, vasomotor effects, and local changes in the acid-base composition of the stimulated region do not contribute to elicitation of the observed responses. Additionally, other experiments (e.g. Grossman, 1962b) employing specific adrenergic and cholinergic antagonists which block elicited behaviors, further supplement the view that chemical agents produce activation which is due to their neurohumoral properties.

In the present study, the hypothesis of adrenergic and cholinergic chemical coding of functional neural systems regulating ingestion and satiety, respectively, which has been demonstrated by Grossman (1960, 1962a, 1962b) in the laboratory rat, was not supported with cats. With respect to emotional and autonomic responses, however, there appeared to be chemically specific (i.e. chemically coded) responses in the cat. There was, however, a surprising lack of localization of these responses to any of the brain areas tested. The inability to localize the responses in specific loci in the brain may be due, in part, to the fact that the preoptic-anterior hypothalamic regions have close antonomical ties with the amygdaloid complex. Hence, if other brain regions lacking close functional antonomical relationships were selected as test loci, they might not yield such similar behavioral responses.

Blockage of Ingestive Behavior - Primary or Secondary Effect

Another issue in the present investigation is how norepinephrine and carbachol exert their inhibitory effects on feeding and drinking behavior in the satiated cat. That is, do the drugs act specifically

to effect satiation or do the chemicals evoke other reactions which are antagonistic to ingestive behavior? Although a final answer has to await additional study, the latter alternative may well be the process involved in the ingestive blockage phenomena currently reported. Several of the present findings support this contention. First, the data indicate that blockage of feeding and drinking following hypothalamic injections increased as drug doses increased. Similarly, as drug concentrations increased, general emotional and autonomic arousal increased. Doses of norepinephrine, the most effective blocking agent of ingestive behavior, evoked a stupor, drowsy-like state after injections at all test concentrations. It is possible, therefore, that this chemically evoked emotional state produced a quantative decrease in food and water intake as merely as secondary effect. Finally, carbachol, especially at the higher test concentrations, produced either a fear or rage-like state with several instances of retching and vomiting. Under these conditions, even though the animal may remain active, the emotional and autonomic effects appear to be antatonistic to feeding and drinking behavior. Injections of carbachol which do not produce more severe overt effects might still be antagonistic to ingestive behavior because, as Sharpe (1969) has suggested, low doses of carbachol probably make animals nauseated.

Turning to a more molecular level, some thought should be given to the type of activities which may occur intraneuronally when one is considering the nature of ingestive blockage effects. The probability of facilitation or inhibition of a specific response mediated by a chemically coded neural system stimulated at a given brain site would depend on several factors. That is, EPSP and IPSP vary from moment to moment

depending on conditions such as concentration of various extracellular chemical components, levels of endogenous neurochemical transmitting agents and effects of excitatory of inhibitory impulses from other neural systems. Thus, if the activation threshold for a given functional system, such as feeding, is lower than the threshold for any other behavior controlled by the same system or an overlapping system, then changes in patterns of feeding behavior would be evoked by chemical stimulation. If, however, another excitatory threshold for some other functional system is lower than that for feeding, one would expect that another response would be preponent. In this view, no neural pathway is necessarily monopolized by any one behavioral or physiological event. This position is supported by Morgane (1966, 1969) who has stated that the conception of neural circuits in which each is chemically coded for only one response would be "circuit phrenology" and is ". . . just as fatal in thinking as nuclear or cortical phrenology." Thus, it appears possible for antonomic, emotional and ingestive effects to each be primary in that chemical stimulation effects could result from excitation of a system involved in the regulation of multiple responses. Contrarywise, they could result from stimulation of overlapping functional systems. It remains to be determined, therefore, if ingestive changes in the present study were the result of secondary reactions in functionally specific emotional and autonomic pathways or if the multiple autonomic and control changes exhibited were produced by stimulation of concurrent or overlapping functional systems.

The foregoing description of neural activity might also help explain some of the differences both between and within species which have been reported in the literature. It is obvious that in the

various chemical stimulation studies if any differences occur in drugs, chemical concentration or brain site one might expect variation in evoked responses. Even when these latter three criteria are met, however, dynamic moment to moment changes within the neural systems of an animal might cause differences in neural excitation levels at a given site and produce differential results within species. Further, considering the probable nature of underlying neural physiology, one would not always expect agreement in results from studies employing different techniques. While chemostimulation may be fairly specific to one or several functional neural systems and activate the system with the lowest threshold, other methods are less subtle. Electrical stimulation might activate not only any system controlled at a given site, but also any system that might be passing through or located near the site of stimulation. Lesions and ablations are probably even less selective with respect to affecting change in any particular neural system. These procedures may merely halt any facilitatory or inhibitory neural activities, either primary or secondary, which are mediated by the loci under study. In addition, electrical stimulation and damage of neural fibers may in some manner alter the neural chemistry in both the area under study and in distal neural regions. Thus, before any comparison of behavior data from different studies is made, careful consideration of the experimental procedures employed is necessarily warranted.

Species Differences

Differential, and sometimes opposing, evoked responses among different species as a result of intracranial injections of the same chemical agent at similar concentrations remains a troublesome and puzzling issue. In fact, data on different species are so divergent that Myers

and Sharpe (1968) have concluded each species may possess its own unique neural coding system for chemically elicited behavior. Also, as a consequence, each species may require a volume of its own for delineation of its specific neural chemical coding systems. In contrast to this view, Miller (1965) has suggested many of the apparent behavioral differences among species may be explained in terms of the relative dominance of systems in various animals. For example, the laboratory rat, which has been widely used in chemostimulation work, is largely rhinal and, subsequently, much of its activity is involved in sniffing, feeding and drinking. Therefore, electrical or chemical stimulation of the hypothalamic or limbic regions usually evokes ingestive responses. Conversely, the cat, which might be described as a more viual animal, displays a broad repertorie of emotional behavior from purring and social contact to rage and vicious attack reactions which include responses such as growling, hissing, piloerection, flattening of the ears, arching of the back, scratching and biting. Unlike the rat, stimulation of the hypothalamus or the amygdala in the cat might be expected to evoke predominantly autonomic and emotional responses like those found in the present study. It may be that different species which display different types of apparently preprogrammed behavioral patterns or survive in vastly different ecological ranges may each demonstrate species specific patterns of behavior when centrally stimulated. Thus, even though comparative neuroanatomical data indicate various species show remarkable phylogentic similarities in subcortical morphology, this does not necessarily preclude functional similarities in the mediation of behavioral or physiological responses by hypothalamic and limbic neural systems. At present, therefore, it remains to be

determined whether or not a general model which describes neurochemical coding mechanisms of behavior can be developed on the basis of experiments with a few species, such as the laboratory rat or cat.

CHAPTER VII

SUMMARY

While intracranial adrenergic and cholinergic drug stimulation has produced some type of change in the patterns of food and water intake in most species tested, chemical stimulation in the cat has never been reported to cause changes in ingestive behavior (Myers, 1961, 1964a; Hernandez-Peon, et al., 1963; Macphail and Miller, 1968). This finding is unique among all mammals thus far tested. Thus, since most of the previously reported investigations employing cats dealt primarily with the lateral and ventromedial hypothalamic areas in the search for chemically responsive ingestive centers, the present study was designed to investigate further the behavioral effects of adrenergic and cholinergic chemical stimulation of brain areas in the cat. On the basis of previous research, the preoptic area, the anterior hypothalamus and the amygdaloid complex were selected as sites likely to be involved in feeding and drinking, and subsequently, to show changes in ingestive behavior following chemical stimulation. Specifically, the objectives of the present investigation were as follows: to investigate changes in ingestive patterns and other responses following adrenergic and cholinergic chemical stimulation in the preoptic and anterior hypothalamus and the amygdala in the unrestrained, satiated cat; to observe the neuropharmacological properties of the brain sites under study and to obtain dose-response relationships; to determine if the preoptic and

anterior hypothalamic areas are merely pathways for fibers connecting other brain loci which control ingestive behavior or if these regions themselves are possible regulatory centers; to analyze further the suggestion by Morgane and Kosman (1957) that the amygdaloid complex in the cat is a feeding "satiety center"; and to search for evidence indicating possible neurochemical coding of feeding and drinking behavior which has been demonstrated in the laboratory rat (Grossman, 1960, 1962a, 1962b).

Sixteen adult, male and female, mongrel and Siamese cats served as experimental subjects. Light-weight, double-walled, stainless steel cannula were chronically implanted unilaterally in the preoptic area, the anterior hypothalamus, and the amygdala using a Johnson cat stereotaxic instrument. Brain loci and stereotaxic coordinates were determined from a stereotaxic atlas of the diencephalon of the cat by Jasper and Ajmone-Marsan (1954). Adrenergic and cholinergic chemical stimulation was provided by injections of L-norepinephrine bitartrate hydrate and carbaminocholine (carbachol), respectively. Various drug concentrations were obtained by dissolving each chemical in normal saline (0.9 per cent) on a weight-volume basis. The drug concentrations employed in the present study were as follows--2.0%, 4.0% and 8.0% norepinephrine; and .005%, .025%, .050% and .100% carbachol. After a two week recovery period following surgery, behavioral responses to a placebo (0.9 per cent saline) and to the experimental drugs were observed after injections at each brain site. All brain areas and each chemical at all concentrations were chosen randomly for each test with the restriction that no replication occur. Food and water intakes, corrected for spillage, were recorded for a one-hour pre-injection baseline

period and for a one-hour post-injection test period. At the conclusion of the testing period, all surviving animals were profused and all cannula placements were histologically verified.

Data from the present study indicate that direct intracranial adrenergic and cholinergic chemical stimulation of the preoptic and anterior hypothalamus and the amygdaloid complex can elicit drug and dose-dependent blockage of ingestive behavior in the satiated cat. Norepinephrine was the most potent drug in producing inhibition of both feeding and drinking behavior in each of the areas tested. In contrast, while carbachol was effective in producing blockage of feeding in each of the sites tested, blockage of drinking occurred only in the anterior hypothalamus. Observations were also made of chemically evoked autonomic and emotional responses which are apparently drug specific and lacked localization to any specific brain site. While this latter finding generally agreed with reports of other investigators, the results on ingestive behavior contrasted with other cat chemical stimulation data. It was suggested that differences between the findings of the present study and other studies might be due, in part, to variation in experimental procedures. With respect to the exact nature of the drugs' inhibitory effects on feeding and drinking behavior, two explanations were presented. Either test chemicals acted specially on satiation or they evoked other reactions (i.e., emotional and autonomic) which were antagonistic to ingestive behavior.

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A P P E N D I X

TABLE III

PERCENTAGE CHANGE FROM BASELINE IN FOOD AND WATER INTAKE FOLLOWING 2 μl INTRACRANIAL ADRENERGIC AND CHOLINERGIC DRUG INJECTIONS IN THE CAT

Brain Area		Saline 0.9%	Norepinephrine 2.0%	Norepinephrine 4.0%	Norepinephrine 8.0%	Carbachol .005%	Carbachol .025%	Carbachol .050%
Preoptic	Food	-10 (16)	-18 (12)	-60 (7)**	-92 (7)**	+78 (9)	+8 (9)	-89 (7)**
	Water	+13 (16)	-45 (12)	-89 (7)**	-92 (7)**	-21 (9)	-29 (9)	-51 (7)
Anterior	Food	+12 (15)	+84 (11)	+34 (8)	-91 (8)**	-49 (8)	-87 (7)*	-87 (9)*
	Water	-30 (15)	-47 (11)	-17 (8)	-89 (8)*	+1 (8)	-72 (7)**	-48 (9)
Amygdala	Food	+41 (16)	+33 (8)	-70 (8)*	-72 (8)*	-33 (9)	-66 (9)*	-37 (9)
	Water	+46 (16)	-11 (8)	-36 (8)	-79 (8)**	+40 (9)	-15 (9)	+55 (9)

( ) Numbers in parenthesis denote sample size

* Significant at the .05 level

** Significant at the .01 level

VITA

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