SIMULTANEOUS LIMBIC-HYPOTHALAMIC STIMULATION: EFFECTS ON ELECTRICALLY-INDUCED FEEDING BEHAVIOR, AND ON THE DEVELOPMENT OF SEIZURE ACTIVITY

Richard J. Friend

A THESIS

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements for
the Degree of Master of Arts at
Sir George Williams University
Montreal, Canada

August, 1972

Abstract

Richard J. Friend

SIMULTANEOUS LIMBIC-HYPOTHALAMIC STIMULATION: EFFECTS ON ELECTRICALLY-INDUCED FEEDING BEHAVIOR, AND ON THE DEVELOPMENT OF SEIZURE ACTIVITY

During a preliminary investigation of the effects of simultaneous limbic cholinergic stimulation and lateral hypothalamic (LH) electrical stimulation on LH induced drinking, five of seven male Wistar rats exhibited seizure behavior. To test for the reliability of seizure evocation, and to determine if cholinergic circuitry was involved in seizure development (Experiment 1), twenty-three male Wistar rats were studied under the following conditions: limbic cholinergic stimulation (crystalline carbachol) plus LH electrical stimulation (CARES); limbic noradrenergic stimulation (noradrenaline) plus LH electrical stimulation (NORES); LH electrical stimulation (ES); and, limbic cholinergic stimulation (CAR). Group CARES developed seizures before groups NORES ($U=4$; $n=7,5$; $p<.016$; 1-tail); ES ($U=7$; $n=7,6$; $p<.027$; 1-tail); or CAR ($U=2.5$; $n=7,5$; $p<.007$; 1-tail). Four additional animals were tested using a different electrical stimulation procedure (Experiment 2). Results were similar to those of Experiment 1 in that CARES animals seized faster than ES animals.

Acknowledgements

This research was supported by the National Research Council of Canada, Grant No. APA0362, awarded to Dr. Roy Wise.

The author is indebted to Dr. Roy Wise and Dr. Nancy Taylor for their critical appraisal of the manuscript.

Table of Contents

The studies reported here represent the initial stages of research aimed at investigating the nature of the neural interactions between the hypothalamus and the limbic system. Two behavioral indices of neural function were used: feeding (eating and drinking), and seizure behavior. Thus the literature from two distinct areas of research are relevant to the thesis; the literature on the neural circuitry involved in eating and drinking is reviewed first.

Hypothalamic Eating and Drinking Centers

Hetherington and Ranson (1939, 1940) were the first to demonstrate that hypothalamic factors alone could cause hyperphagia and subsequent obesity in the rat. Bilateral lesions in or close to the ventromedial nucleus of the hypothalamus (VMH) were the most effective in causing hyperphagia (Hetherington, 1941, 1944). Other investigators, using rats, cats, and monkeys, have confirmed these results (Brobeck, Tepperman, & Long, 1943; Brooks, Lambert, & Bard, 1942; Ruch, Patton, & Brobeck, 1942; Wheatley, 1944). In addition, Anand and Brobeck (1951a, 1951b) found that bilateral lesions of the area adjacent to the VMH, the lateral hypothalamus (LH), resulted in the opposite effect, since they caused aphagia in rats and cats. Anand and Brobeck concluded that the LH and VMH were responsible for the regulation of hunger, and that the "feeding center," the LH, was inhibited by activation of the "satiety center," the

VMH. The suggestion that the LH was a "feeding center" was further supported by evidence showing that electrical stimulation of LH areas can increase food consumption (Delgado & Anand, 1953).

Histological verification of reciprocal pathways between the two structures was slow to appear. Nauta (1958) had shown that fibers existed which extended from the LH to the VMH, but had not proven the reverse to be true. More recently, however, Arees and Mayer (1967), using a refinement of Nauta's technique (see Fink & Heimer, 1967), have found fibers extending from the VMH to the medial portion of the LH. When the fibers between these two areas were severed without damaging either structure, hyperphagia occurred which was similar to the hyperphagic syndrome described by Teitelbaum (1955) after VMH lesioning (Albert & Storlien, 1969; Gold, 1970; Grossman and Grossman, 1971; Sclafani & Grossman, 1969). Further evidence provided by neurophysiological studies substantiated the idea of LH-VMH reciprocity. Spontaneous unit discharges in the cat VMH were shown to decrease when the LH was stimulated electrically, and a similar decrease occurred in the LH when the VMH was stimulated (Oomura, Kimura, Ooyama, Maeno, Iki, & Kuniyoshi, 1964; Oomura, Ooyama, Yamamoto, & Naka, 1967; Oomura, Ooyama, Yamamoto, Naka, Kobayashi, & Ono, 1967). Such evidence established the concept of reciprocally connected hypothalamic centers of hunger and satiety.

Although it has been reported that lesioning of the LH caused simultaneous occurrence of aphagia and adipsia (Morrison, Barrnett, & Mayer, 1958; Teitelbaum & Stellar, 1954), there is evidence from lesion and stimulation studies which suggested that a separate system operating in the LH was responsible for the regulation of thirst. Greer (1955) demonstrated that electrical stimulation of the LH in rats could induce drinking, while Andersson and McCann (1955) showed that similar results could be achieved by stimulating the hypothalamus in goats. Moreover, electrolytic lesioning of the rat LH has been shown to cause adipsia without aphagia (Montemurro & Stevenson, 1957), with similar results occurring when the anterior hypothalamic region of the dog and chicken, and the preoptic area of the goat, are lesioned (Andersson, Gale, & Sundsten, 1964; Andersson & McCann, 1956; Lepkovsky & Yasuda, 1967; Witt, Keller, Batsel, & Lynch, 1952).

More direct evidence indicating independent thirst and hunger mechanisms, came from a study that showed that a hypertonic saline solution injected into the medial hypothalamic region of goats could induce a rapid increase in water ingestion (Andersson, 1953). This was followed by Grossman's (1960, 1962a) demonstration of chemically coded, independent neural mechanisms involved in eating and drinking in the rat. He found that a cholinergic substance (acetyl-

choline) inserted into the perifornical region of the rat brain induced drinking, while a noradrenergic substance (noradrenaline) inserted at the same site induced eating. Further support for chemical specificity came from the demonstration that cholinergic blocking agents decreased cholinergically-induced drinking as well as deprivation-induced drinking, while noradrenergic blocking agents suppressed eating but not drinking (Grossman, 1962b).

Limbic Thirst Circuitry

The perifornical region of the rat brain is only one of many areas at which application of noradrenergic (noradrenaline) and cholinergic (acetylcholine or carbachol) substances can induce eating and drinking (Coury, 1967; Fisher & Coury, 1962). In the case of drinking, Fisher and Coury (1962, 1964) have provided evidence in support of the hypothesis that the neural mechanism for drinking involves multiple limbic structures connected by cholinergic circuitry; the suggested circuitry corresponds essentially to the circuit espoused by Papez (1937) for the regulation of emotion. Some of the main sites that are implicated are the hippocampal and septal regions, the lateral hypothalamic-medial forebrain bundle region, the mamillary-interpeduncular regions, and the cingulate cortex.

An alternate hypothesis to that of widespread cholinergic

thirst circuits, suggested that chemicals, when directly applied to sites within the limbic system that lie close to a ventricle, have their influence by diffusing into the ventricle and stimulating receptors which line its walls, rather than by activating neural elements which are functionally related and synaptically interconnected (Baxter, 1967; Routtenberg, 1967). Fisher and Levitt acknowledge the possibility that ventricular diffusion might influence cholinergically-induced drinking, since the cholinergic blocking agent atropine, which attenuates carbachol-induced drinking if applied centrally (Levitt & Fisher, 1966), exerts similar effects on cholinergically-induced drinking when infused directly into the ventricular spaces (Fisher & Levitt, 1967). In support of the notion of a chemically specific neural circuitry which passes throughout the limbic system, Fisher and Levitt have argued that cholinergic drinking can be induced at sites distant from a ventricle, while it cannot be induced at a number of closer sites to the ventricle. Levitt, White, and Sander (1970) stimulated many widely separated sites of the limbic system with several different concentrations of carbachol; drinking latencies did not differ between sites, nor did the volume ingested. Drug threshold dosages were comparable for each site and also did not differ as a function of distance from the closest ventricle. More-

over, Mountford (1969) has shown that chemical stimulation of a limbic site (hippocampus) led to a significant increase in water ingestion when compared to a prestimulation baseline, whereas stimulation of the immediately adjacent ventricle did not. Also, carbachol inserted directly into the ventricle does not appear to alter water intake appreciably in rats (Khavari, Heebink, & Traupman, 1968; Myers & Cicero, 1968). Thus, the exact role the ventricles play in carbachol-induced drinking has yet to be determined (Lovett & Singer, 1971), but the currently accepted view is one that postulates a cholinergically specific neural circuitry with many complex interconnected pathways interwoven throughout the limbic system (Fisher & Coury, 1962, 1964).

Limbic-Hypothalamic Interactions

The idea that diffuse limbic circuitry is responsible for specific behavioral effects has contributed to a general decrease in the tendency to label portions of the hypothalamus as functional "centers"; instead, the hypothalamus is now being considered by many researchers as playing an intricate role in more widely dispersed systems. Morgane (1964), for example, has suggested that limbic forebrain-limbic midbrain circuitry acts as an integrative system which mediates the regulation of an organism's response to its internal or external milieu. Recent evidence has added further strength

to this position. A close analysis of knife cuts through the fiber pathways entering or leaving the LH along its lateral border has shown that aphagia or adipsia may occur (Grossman & Grossman, 1971; Sclafani & Grossman, 1969). This condition is similar to the LH lesion syndrome described by Teitelbaum and Stellar (1954), and Teitelbaum and Epstein (1962). Grossman and his colleagues have interpreted these results as support for Morgane's (1961) hypothesis that afferent or efferent fibers which terminate or leave the hypothalamus by the lateral area of the LH are important in the regulation of eating and drinking behavior. Thus the limbic forebrain system may influence such behaviors as eating or drinking, by altering the neural input to, or outflow from, the hypothalamic area, either directly or indirectly.

Functional evidence. The amygdala, septum, and hippocampus are three major limbic structures which have been shown to be intricately involved in the mediation of ingestive behaviors. On the basis of lesion and electrical stimulation studies, the amygdala has been implicated in the mediation of both eating and drinking in a number of species (Fuller, Rosvold, & Pribram, 1957; Green, Clemente, & de Groot, 1957; Grossman & Grossman, 1963; Morgane & Kosman, 1957a, 1957b; Robinson & Mishkin, 1962; Wood, 1958). The exact function of the

amygdala is not clear, although the general concensus has been that like the VMH, it acts as part of a satiety or inhibitory system. Grossman (1964a), however, has suggested the possibility of the existence of a subtle relation between the amygdala and hypothalamus. He has found that water-deprived rats, when stimulated with a cholinergic substance in the ventral amygdala near the cortical nuclei, drink more water than when water deprived, but not stimulated. Similarly, when rats were food deprived, noradrenergic stimulation resulted in an increase in food consumption. Noradrenergic or cholinergic stimulation when the rats were satiated, failed to produce any substantial increase in food or water consumption. These results led Grossman to propose that the amygdala might modulate ongoing hypothalamic activity during periods when the eating or drinking system at the hypothalamic level was activated. Activation of the amygdala would either have an inhibitory effect on a satiety system, possibly at the VMH, or an excitatory influence on an eating or drinking system, which was probably located at the LH. Recently, Russell, Singer, Flanagan, Stone, and Russell (1968) have confirmed Grossman's results with respect to cholinergic stimulation of the amygdala. They agreed with his conclusions and suggested that the degree of amygdaloid modulation of hypothalamic function is dependent

upon drug dosage levels, since they had found that an increase in cholinergic drug dosage resulted in an increase in overall water consumption. White and Fisher (1969) have provided additional evidence in support of amygdalo-hypothalamic interaction. They found that electrical stimulation of the cortico-medial piriform transition zone of the amygdala in food-deprived rats resulted in a suppression of food intake. If the VMH or the stria terminalis, a pathway connecting the amygdala and the VMH, was lesioned, then electrical stimulation of the amygdala did not suppress eating. They concluded that excitation of the amygdala resulted in an increase in VMH activity, which in turn suppressed activity in the LH, and hence resulted in a suppression of eating. Thus, both excitatory and inhibitory influences of amygdala on hypothalamus have been suggested.

The septum, like the amygdala, seems involved in some sort of control of drinking. Electrical stimulation of the septal area results in a suppression or decrease in water consumption in sated or water-deprived rats (Mabry & Peeler, 1968; Wishart & Mogenson, 1970), while cholinergic stimulation (Coury, 1967; Fisher & Coury, 1962, 1964; Grossman, 1964b) or lesioning (Donovick & Burright, 1968; Harvey & Hunt, 1965; Harvey, Lints, Jacobson, & Hunt, 1965; Lubar,

Schaefer, & Wells, 1969; Pizzi & Lorens, 1967; Wolfe, Lubar, & Ison, 1967) results in an increase in drinking. Evidence provided by self-stimulation studies indicates that the inhibitory action of the septum may be an indirect one; that is, the septum may affect behavior by influencing hypothalamic structures. Keesey and Powley (1968) have shown that lesions of the septum cause an increase in responding for LH stimulation. This effect is similar to that noted for increased LH responding when the VMH has been lesioned (Hoebel, 1965) or temporarily inactivated (Hoebel & Teitelbaum, 1962). Keesey and Powley further note that this inhibitory release due to septal lesioning is evident not only in an increase in self-stimulation responding, but also in a decrease of the current intensity necessary to elicit the self-stimulation behavior.

Although very little direct evidence is available, the hippocampus seems to be involved to some extent in the mediation of eating and drinking behavior. Electrical stimulation of the hippocampus does not directly elicit eating or drinking. Milgram (1969), however, has shown that the hippocampus may have an indirect influence on eating. He has found that immediately following the cessation of electrical stimulation, rats begin to eat. Milgram argues that activation of the hippocampus inhibits activity in

another structure, possibly the LH. With cessation of hippocampal stimulation, a rebound excitation occurs in this structure which causes eating. Although Milgram was not able to obtain similar results for drinking, other studies have shown the involvement of the hippocampus in this behavior. Kimble and Coover (1966) for example, have demonstrated that rats with hippocampal lesions will consume more water than they did before lesioning. Similarly, other studies have indicated that cholinergic stimulation of the hippocampus will elicit drinking behavior (Coury, 1967; Fisher & Coury, 1962, 1964; Levitt, White, & Sander, 1970; MacPhail, 1968; Mountford, 1969).

Evidence strongly points to the importance of limbic-hypothalamic interactions in the mediation of behaviors such as eating and drinking. It is not known, however, if this influence is of an inhibitory or excitatory nature, or both. Electrical stimulation studies indicate an inhibitory influence, while chemical stimulation studies suggest an excitatory one. Perhaps both inhibitory and excitatory influences occur, depending upon the synaptology of the neural circuitry involved.

Anatomical, histochemical, and neurophysiological evidence. Evidence provided by anatomical, histochemical, and neurophysiological studies indicates that a number of major

fiber pathways provide the means, either by direct or indirect transmission, for the amygdaloid, septal, hippocampal and hypothalamic regions to influence one another.

The amygdala in the rat contains seven main nuclei and occupies an extensive portion of the ventral part of the cerebral hemispheres. It extends from diagonal band nuclei to the most caudal regions of the hemispheres (Brodal, 1947; Cowan, Raisman, & Powell, 1965). The main afferent fibers projecting to the amygdala originate primarily from diencephalic structures, the principal sites being within the hypothalamic region. Cowan et al. (1965) have shown that these fibers reach the same nuclei in the amygdala by two different pathways, the stria terminalis, and the more diffuse dorsal and ventral amygdalopetal pathways. Similarly, efferent fibers projecting from the amygdala, have been found to terminate primarily in diencephalic structures, including septal (Raisman, 1966) and hypothalamic nuclei, by way of the stria terminalis and the larger and more diffuse ventral amygdalofugal pathway. The stria terminalis, which originates from corticomedial and baso-lateral nuclei, can be divided into two segments, postcommissural and supracommissural. The postcommissural pathway extends diffusely throughout the rostral hypothalamic region, and may partially interact with medial forebrain

bundle (MFB) fibers (Cowan et al., 1965; Heimer & Nauta, 1969), while the supracommissural pathway terminates in the region surrounding the VMH. This area is rich in dendrites extending from the VMH as well as from arcuate and dorsomedial nuclei, and it is probable that the fibers from the stria terminalis synapse here (Heimer & Nauta, 1969). Heimer and Nauta have also found that fibers from the ventral amygdalofugal pathway terminate within the LH region.

The MFB, which is scattered diffusely throughout the LH region, is one of the more important projection systems involved in limbic-hypothalamic interactions. It consists of a large number of loosely interwoven fibers lying ventral and lateral to the postcommissural fornix. These fibers course in both directions, and hence the MFB has reciprocal connections with most of the structures it innervates (Guillery, 1957; Raisman, 1966). Guillery (1957) has shown that the ascending MFB is divided primarily into two segments, one of them, which is referred to as the medial bundle, originates in the midbrain or the pons, and terminates in the medial septal nucleus. The other segment, the lateral bundle, originating from the premammillary nucleus of the hypothalamus, passes through the LH and preoptic regions before turning in a medial direction to terminate in the lateral septal nucleus. Also, it has been

shown that in rats, and similarly in cats, fibers from the septum descend ventrally by way of the MFB, to terminate in hypothalamic, thalamic, and tegmental regions (Nauta, 1958; Powell, 1963). Thus it can be seen that due to the influence of the MFB, the septal region has extensive reciprocal relationships with many diencephalic structures.

In a similar way, the fornix and fimbria create a reciprocal relationship between the septum and the hippocampus (Raisman, 1966; Raisman, Cowan, & Powell, 1965). The dorsal fornix, which originates from the anterodorsal part of the hippocampus, and the fimbria, are the major efferent and afferent pathways of the hippocampus. While precommissural fibers of the fornix terminate in septal nuclei, nucleus accumbens, and the MFB, the postcommissural fornix distributes fibers to the anteroventral and anteromedial nucleus of the thalamus, preoptic and rostral hypothalamic nuclei, as well as mammillary nuclei. In addition, fibers also extend throughout the midbrain (Raisman et al., 1965). The fimbria, however, are not as extensive, and project primarily to septal nuclei and rostral hypothalamic areas (Powell & Cowan, 1955).

Histochemical studies provide evidence for the existence of chemically specific noradrenergic and cholinergic pathways. By use of histochemical fluorescence and autoradiographic techniques, monoaminergic pathways have been mapped through-

out the rat brain (Fuxe, 1965; Reivich & Glowinski, 1967; Ungerstedt, 1971). These studies show the existence of heavy concentrations of catecholamine terminals throughout limbic forebrain structures, such as the hippocampus and septum, as well as hypothalamic, mammillary and preoptic regions. Most of the catecholamine fibers which innervate these structures, were shown to originate from, or pass through, the MFB. In addition, these studies demonstrate the existence of other catecholamine fiber systems which include the fornix, stria terminalis, or stria medullaris.

Other histochemical studies provide evidence for the existence of cholinergic pathways (Lewis & Shute, 1967; Shute & Lewis, 1967). Shute and Lewis (1967) have traced an ascending reticular cholinergic fiber system, called the ventral tegmental pathway, which originates from anterior mesencephalic regions, and, as part of the MFB, passes through the zona incerta, supramammillary and LH regions, rostrally to forebrain structures. The amygdala is heavily innervated by cholinergic fibers which extend from the bed nucleus of the stria terminalis and lateral preoptic areas. Fibers of the stria terminalis extend to cortical and medial amygdaloid nuclei which give rise to cholinergic fibers which Brodal (1947) and Heimer and Nauta (1969) have shown project from the amygdala to preoptic and hypothalamic regions. Fibers originating from the medial septal nucleus and the

diagonal band nucleus, which project to the hippocampus via the dorsal fornix and fimbria, were found to be cholinergic, and lie in the path of the ascending ventral tegmental pathway. Hippocampal efferents, although not primarily cholinergic, extend via the fornix to innervate cholinergic neurons in the hippocampal commissure, anterior thalamus, habenular and interpeduncular nuclei, as well as the midbrain tegmentum. These neurons innervate cortical nuclei as well as nuclei of the ascending cholinergic system.

Electrophysiological studies provide further evidence for limbic-hypothalamic interactions. The administration of electric shock to the amygdala causes evoked potentials to occur in a number of structures, including septum, preoptic and hypothalamic regions (Gloor, 1955), while amygdaloid stimulation has been shown to alter the level of neural activity within the hypothalamus (Murphy, Dreifuss, & Gloor, 1968; Oomura, Ooyama, Yamamoto, Naka, Kobayashi, & Ono, 1967; Oomura, Ooyama, Yamamoto, Ono, & Kobayashi, 1969; Oomura, Ono, & Ooyama, 1970; Stuart, Porter, & Adey, 1964; Tsubokawa & Sutin, 1963; Wendt & Adey, 1960). Recently, Gloor, Murphy, and Dreifuss (1969) have found that hypothalamic cells in the cat which respond either in an excitatory or inhibitory manner when the amygdala is stimulated, will also respond when the septum, dorsal hippocampus, or midbrain tegmentum is stimulated. This stimulation, which has a direct effect on

hypothalamic cells, affects septal nuclei as well. Thus, Gloor et al. suggest that the septum may play a modulatory role between the amygdala and the hypothalamus.

Similarly, it has been suggested that the septum may modulate or control hippocampal activity, since it has been found that bursts of neural activity in medial septal nuclei closely correlate with theta rhythm in the hippocampus (Petsche, Stumpf, & Gogolak, 1962). Petsche et al. therefore suggest that initiation of neural activity in the septum could possibly result in the occurrence of theta rhythm in the hippocampus.

In summary therefore, it is evident that neural pathways of a specific noradrenergic or cholinergic nature, exist and extend on similar courses throughout midbrain and forebrain regions. These pathways make it possible for example, for any specific limbic structure to influence any other limbic structure, as well as most regions of the hypothalamus.

Preliminary Investigation

The preceding review leads to the suggestion that structures within the limbic system interact with hypothalamic systems to alter neural activity. Chemical stimulation studies suggest that this interaction leads to a facilitation of behavior, since noradrenergic and cholinergic stimulation

at various sites throughout the limbic system results in the initiation or continuation of eating or drinking. On the other hand, electrical stimulation studies suggest the opposite, since electrical stimulation of sites within limbic structures has been shown to suppress drinking behavior, while termination of limbic stimulation excites eating. Thus, chemical limbic stimulation, either cholinergic or noradrenergic in nature, would seem to result in a facilitation of behavior which is not evident when the same structures are stimulated electrically. Why this is so is not clear.

The suggestion, however, that limbic systems interact with hypothalamic systems to suppress feeding behavior is not necessarily inconsistent with the finding that chemical stimulation of limbic areas facilitates eating and drinking. Wise (1971) has recently shown that carbachol can have two opposite effects on eating: an inhibitory effect, followed by a facilitatory one. He found that as Grossman (1960, 1962) had suggested, the main effect of carbachol was to induce drinking and inhibit eating. This initial drinking period, however, was followed by a period of induced eating and minimal drinking. Thus, in the case of eating, the response to carbachol was biphasic, and involved both inhibition and excitation. The same may be true for drinking.

To further complicate matters, it is possible that there is yet an earlier effect of the drug; perhaps the principal effect occurs during the latency period which precedes the elicitation of drinking. This would be consistent with the notion of Milgram (1969) that limbic influences are inhibitory. Perhaps during the latency period, carbachol actively inhibits drinking, and the subsequent drinking which occurs is a rebound from this inhibition. This hypothesis therefore suggests another phase of response in addition to the two seen by Wise. Although this line of discussion is speculative, it points out the need to investigate the entire time course of the cyclic effects of carbachol.

The purpose of the preliminary study, therefore, was to determine the sequence of the cyclic effects of cholinergically-induced drinking on a moment to moment basis. In this way, it was thought that it might be possible to determine on a continuous basis, whether carbachol has an excitatory or inhibitory effect on drinking or eating, and whether the effects change predictably as a function of time. Since electrical stimulation of the hypothalamus can be used to produce states of drive or satiety alternately (Mendelson, 1966), this technique was used in conjunction with the simultaneous application of carbachol to one of a number of

limbic sites.

Twenty adult male Wistar rats, weighing 250 to 300g, were each implanted with a cannula in one of a number of limbic areas, as well as a unipolar electrode in the LH region. Table 1 lists the coordinates for the various electrode and cannula placements. After recovery from

---

Insert Table 1 about here

---

surgery, the animals were tested for electrically-induced eating or drinking (Wise, 1968). Following the emergence of one of the specified behaviors, and, depending upon which behavior was elicited, the goal object which had elicited the initial response was removed, and the subjects (Ss) were tested for the emergence of the other behavior. Upon completing this procedure, the seven Ss that reliably ate and drank during stimulation, were tested once a day for 1/2 hour with electrical stimulation until eating or drinking thresholds were stabilized. The duration of each stimulation interval was 10 sec., and each stimulation interval was immediately followed by a 10-sec. interval during which the Ss were not stimulated.

Next, simultaneous cholinergic and electrical stimulation (CARES) sessions were begun. At the beginning of

each session, the Ss were tested with electrical stimulation alone, only long enough to estimate the current threshold intensity for eating or drinking for that session. The S was then taken from the test box, its inner cannula removed and cleaned, and 3 to 5 ug of crystalline carbachol was tamped into the tip. The inner cannula was then screwed back into the outer cannula, the S placed back in the test box, and electrical stimulation was commenced again. Stimulation-induced eating or drinking, as well as spontaneous eating or drinking, was noted. Electrical stimulation threshold intensities were also obtained. Electrical stimulation only (ES), and CARES sessions, were run on alternate days for a number of days. Following the completion of the experiment, the Ss were tested with carbachol alone for several sessions; they were then sacrificed and their brains removed and stored in 10% formalin for a minimum of one week. Next, the brains were frozen and sectioned, and electrode and cannula locations were verified.

The predominant effect of CARES sessions was the initiation of seizures which lasted from 1 to 3 min., and obscured any systematic changes in eating or drinking thresholds. Five of the seven Ss exhibited some form of seizure behavior; 10 of the 21 CARES sessions had to be terminated for this reason. An interesting finding, however,

was that seizures only occurred during CARES sessions. They were neither evident during ES sessions, nor, when after the completion of the experiment, the Ss were tested with carbachol alone.

Although electrical stimulation alone, as well as carbachol alone, can result in the initiation of seizure activity (Goddard, 1969; Miller, Gottesman, & Emery, 1964), it appeared that it was the interaction of the two methods which caused the seizures. If electrical stimulation alone were responsible for the elicitation of seizure activity, then seizures should also have occurred on alternate days during ES sessions. Moreover, if the seizures had been induced by carbachol, the pattern of seizure activity should have been different from those which did occur. Seizures induced by even a single dose of carbachol tend to persist and multiple seizure activity may continue over a number of hours (Belluzzi & Grossman, 1969).

When seizure activity did not occur during CARES sessions, the performance of the Ss in relation to eating and drinking behavior was extremely variable. Hence, evidence for the facilitatory or inhibitory effects of carbachol was inconclusive. One reason for the high degree of performance variability might be the difficulty of controlling drug concentration and dose level precisely when using intra-

cranial application of the drug. That dose and concentration levels of carbachol are important factors in the initiation of motivated behaviors has been firmly established (Miller et al., 1964; Russell et al., 1968).

The data suggested that the simultaneous intracranial administration of cholinergic and electrical stimulation resulted in a summation effect, the consequence of which was the elicitation of seizure behavior. The occurrence of these seizures, which might be attributed to the interaction of limbic and hypothalamic circuits, suggests that the investigation of the seizure phenomenon itself might be of value in studying the effects of limbic-hypothalamic influences. In order to do so, however, the reliability of seizure evocation must first be determined. The primary purpose of the subsequent studies therefore, was to establish the reliability and generality of the seizure phenomenon.

Experiment 1

Seizure development. Recently, Goddard and Morrell (1969), and Racine (1972a, 1972b), have shown that repeated electrical stimulation of non-motor subcortical regions, will, over a number of days, result in permanent neuronal changes in the brain tissue at the tip of the stimulating electrode. Although the repeated stimulation treatment did

not have any initial effect on neural activity at the site of stimulation, the eventual result was the evocation of afterdischarge (AD) activity, consisting of neuronal discharges of various frequencies and high amplitude spikings. Both Goddard and Morrell (1969), and Racine (1972a, 1972b), attributed the development of this region of aberrant neuronal discharges, that is, the development of an epileptic focus, directly to the repeated low intensity electrical stimulation.

In a similar manner, these researchers found that repeated stimulation of this first or primary focus, would result in neuronal changes that established secondary foci in other structures. Early subcortical chemical stimulation studies had demonstrated that a second focus, referred to as a mirror focus, could be formed in the contralateral hemisphere at the contralateral locus of the chemically stimulated structure (Morrell, 1961). More recently, Goddard and Morrell (1969), and Racine (1972a, 1972b), expanding on this theme, found that secondary foci could be developed not only in the contralateral hemisphere, but in ipsilateral regions as well. The repeated low intensity electrical stimulation, which they had shown would eventually result in the formation of a primary focus, would, if continued, result in the subsequent formation of secondary foci in

both hemispheres. Once these foci were firmly developed, AD activity could be recorded from these sites when the primary focus was stimulated.

The development of secondary foci would appear to be a direct consequence of aberrant neuronal bombardment from the primary focus. Morrell's early chemical stimulation studies demonstrated that the mirror focus was initially driven by continuous aberrant discharge activity originating from the primary region of stimulation (Morrell, 1961). Moreover, Racine (1972b) has shown that AD's originating from the primary focus when this region was electrically stimulated, eventually resulted in the development of secondary foci. Thus, these findings suggested that the development of secondary foci was not only a consequence of local neuronal changes occurring at these foci, but of neuronal changes outside these regions which resulted in an increase in sensitivity, or a strengthening of connections between structures. Although it is not known why or how these changes occur, it is evident that they do occur and do play an important role in the development of seizure activity (Racine, 1972b).

Originally, the development of overt seizure behavior was attributed to repeated electrical stimulation treatment per se, since it had been found that repeated low intensity

electrical stimulation would result in the eventual evocation of motor convulsions (Goddard, 1967; Goddard, McIntyre, & Leech, 1969). Racine (1972b) however, has qualified this conclusion. He stimulated rats subcortically at stimulation intensities which either evoked AD activity, or did not do so. Animals in which AD's were regularly evoked, developed motor convulsions, while the non-AD animals showed no signs of seizure behavior. Racine thus concluded that the necessary requisite for the development of overt seizure behavior was repeated stimulation treatment in which AD's were evoked, and not simply electrical stimulation treatment per se.

Interlimbic connections would also appear to play a crucial role in the development of motor convulsions. Simultaneous electrical stimulation of both amygdalae in rats was found to produce motor convulsions quicker than when animals were either stimulated unilaterally, or simultaneously, but with lesions severing the important pathways between the two areas (Racine, Okujava, & Chipashvili, 1972). It would seem, therefore, that seizures evoked by stimulation of non-motor subcortical regions result from propagation of AD's from local limbic regions to other limbic structures and then to motor areas, although the exact relationship between limbic and motor structures has not been clearly

determined (Racine et al., 1972). The important point that has been established, however, is that limbic circuitry can be involved in the development and evocation of seizure activity. Thus, on the basis of this work, it might be predicted that the seizures observed in the Preliminary Investigation were propagated by some of the same neural circuits which mediate eating and drinking behavior.

Cholinergic Specificity. It seems that at least some forms of seizure activity are propagated by a cholinergic system. Carbachol applied subcortically, has been shown to result in abnormal and persistent neuronal activity (Belluzzi & Grossman, 1969), as well as evoking overt seizure attacks (Goddard, 1969; Grossman, 1963). In addition, cortical acetylcholine release is enhanced by the administration of convulsants (Beleslin, Polak, & Sproull, 1965; Celesia & Jasper, 1966), and reduced by systemic application of anticonvulsants (Giarman & Pepeu, 1964). It has also been demonstrated that the systemic application of the cholinergic blocking agent atropine, can retard the development of electrically induced limbic seizures (Albright, 1971).

Although small dosages of subcortically applied carbachol, or electrical stimulation of the LH, have been shown to induce motivated behaviors such as eating or drinking, the results of the Preliminary Investigation

suggested that the simultaneous combination of the two stimulation procedures caused an interaction which resulted in the evocation of overt motor seizures. It may be possible therefore, by use of the repeated electrical stimulation procedure outlined by Goddard et al. (1969), in conjunction with chemical limbic stimulation (Preliminary Investigation), to show an interaction between limbic and hypothalamic regions , as well as to demonstrate a chemical specificity of action. If cholinergic stimulation of the septum interacts with LH electrical stimulation to produce seizure activity, then combined electrical stimulation and carbachol treatments might be expected to result in faster seizure development than with either of the two stimulation treatments alone, or with noradrenergic septal stimulation combined with LH electrical stimulation.

Method

Subjects. The Ss were 23 male Wistar rats, obtained from the Canadian Breeding Farm and Laboratories Limited, St. Constant, Quebec. Immediately prior to surgery, all Ss weighed between 250 and 300 g. They were housed individually, and had unlimited access to food and water.

Apparatus. Intracranial electrical stimulation, set at a constant current intensity level of 70 uA, was administered by a 60 hz sine wave generator. Stimulation on and off

intervals were regulated automatically by a Lafayette timer. The Ss were tested in plywood boxes 10 in. wide, 16 in. high, and 12 in. long. A 4 in. by 12 in. section of the front side of each box was constructed of plexiglas in order to provide an unobstructed view of the Ss behavior.

Surgery. Following the procedure of Wise (1968), a unipolar electrode and a cannula, were implanted in the LH and lateral septal regions respectively. Target coordinates were as described in Table 1.

Procedure. The Ss were divided into four groups: two groups received simultaneous chemical and electrical stimulation treatment, with one group of 7 Ss receiving carbachol plus electrical stimulation (CARES), and the other group, consisting of 5 Ss, receiving noradrenaline plus electrical stimulation (NORES); the third group (ES), consisting of 6 Ss, was tested with electrical stimulation alone; while the fourth group (CAR), consisting of 5 Ss, was tested with carbachol alone. Electrical stimulation treatment consisted of 30 10 sec. intervals, during which the Ss received electrical stimulation. Each stimulation interval was immediately followed by a 10 sec. rest interval during which electrical stimulation was not administered. Five minutes prior to the commencement of electrical stimulation, approximately 5 ug of crystalline chemical was tamped into the inner cannula

of each S in groups CARES and NORES; Ss in group ES simply had their inner cannulas unscrewed and tightened. Ss in group CAR were given carbachol centrally and then left in the test boxes for 1/2 hour. All Ss were tested once a day for 21 days unless seizure behavior developed. Once an S exhibited seizure behavior he was not tested on subsequent days. When motor seizures did occur, they were classified into one of the five stages described by Racine (1972b) : (1) mouth and facial movements; (2) head nodding; (3) forelimb contractions; (4) rearing; (5) rearing and falling. The measure used in this experiment was the number of days necessary to elicit a number four or five motor convulsion.

At the end of the experiment, the Ss were sacrificed and perfused with physiological saline, followed by 10% formalin. The brains were then removed and stored in formalin for a minimum of one week. Following this, they were frozen and cut into 40 micra sections. Electrode and cannula tip locations were verified with the aid of an atlas for the rat brain (Pellegrino & Cushman, 1967).

Results

Ss in group CARES exhibited seizure behavior after fewer stimulation sessions than Ss in groups ES (Mann-Whitney U Test: $U=7$; $n=7,6$; $p < .027$; 1-tail); CAR ($U=2.5$;

n=7,5; $p < .007$; 1-tail); or NORES (U=4; n=7,5; $p < .016$; 1-tail). Table 2 lists the median number of sessions to first seizure. All seizures observed were classified as

---

Insert Table 2 about here

---

being within stages four or five, with the majority being within stage five. Extreme physical stress and discomfort was evident in many of the animals undergoing the multiple stimulation treatment. In some instances, injuries occurred when the animals attempted to escape from the stimulation.

Discussion

Since the development of motor convulsions was significantly facilitated by the simultaneous administration of cholinergic and electrical stimulation, the results indicated that limbic cholinergic stimulation and hypothalamic electrical stimulation interact, facilitating one another in the development of motor seizures. An important finding here was that the facilitatory influence of the interaction appeared to be a cholinergic one. The combination of noradrenergic stimulation and electrical stimulation did not result in a facilitatory effect on the development of seizures. Neither, however, did it result in retarding their development, as might be expected if there was a reciprocal

inhibitory relationship between noradrenergic and cholinergic systems (Grossman, 1960, 1962a, 1962b).

Experiment 2

The electrical stimulation parameters used in Experiment 1 were not typical of those employed by other researchers working in the area of seizure development (Goddard, 1967; Goddard et al., 1969; Racine, 1972a, 1972b); rather, Experiment 1 involved the same stimulation parameters as were used in the feeding study (Preliminary Investigation). The purpose of Experiment 2 was to establish the reliability of the seizure phenomenon using stimulation parameters less aversive to the animals and more typical of traditional seizure studies.

Method

The Ss were four male Wistar rats weighing between 250 and 300 g before surgery. They were each implanted with a hypothalamic unipolar electrode and a septal cannula. The surgical procedure used was identical to that described previously.

Procedure. Two of the Ss received simultaneous cholinergic and electrical stimulation treatment, while the remaining two Ss were treated with electrical stimulation alone. The testing procedure was identical to that used in Experiment 1,

except that there was only one electrical stimulation interval per day. This interval lasted for 1 sec., and the current intensity was set at 100 uA.

Results

The two Ss receiving simultaneous stimulation treatment, exhibited seizure behavior after 34 and 48 days respectively. Neither of the two Ss receiving electrical stimulation alone had seized after 86 days.

Discussion

The results were in agreement with those obtained in Experiment 1. Seizure activity occurred first in animals that received simultaneous cholinergic and electrical stimulation treatment. The major difference between the results of the two experiments was that Ss in the second experiment required up to three times as many days to establish seizure behavior as did those in the first experiment. This difference in rate of seizure development would be expected since much less stimulation was given in Experiment 2. The difference in rate was not directly proportional to the difference in total amounts of stimulation, which is consistent with Racine's (1972a, 1972b) finding that the number of stimulation sessions is more important than the total amount of current administered in each session.

General Discussion

The experiments reported here support the following conclusions: that cholinergic limbic stimulation and LH electrical stimulation interact and result in the emergence of motor convulsions quicker than with either of the two stimulation procedures alone; and, that some of the neural elements in the involved circuits are cholinergic. No conclusions can be made in terms of the directionality of the interaction; that is, whether the septum acts upon the LH, or conversely, whether the LH influences the septum. Neither can conclusions be made in relation to the nature of reciprocal cholinergic-noradrenergic influences upon the development of seizure behavior, although it would appear that cholinergic, as opposed to noradrenergic influences, play a more important role in seizure development, since simultaneous limbic cholinergic stimulation and LH electrical stimulation resulted in a facilitation of seizure evocation. Conclusions here, in regards to the extent of the involvement of cholinergic circuitry, must be tentative, since only one limbic structure, the lateral septal region, was chemically stimulated during the seizure experiments.

The finding that simultaneous limbic cholinergic stimulation and LH electrical stimulation resulted in the development of overt seizure activity quicker than with

either of the two stimulation treatments alone, indicates that this method might be of use in evaluating the effects of limbic-hypothalamic interactions, as well as providing a means for indicating the chemical specificity of the involved neuronal circuitry. This procedure, however, is of limited use if neurophysiological recording techniques are not implemented as well. If recording techniques are utilized, it might be possible to obtain information in relation to the nature and directionality of neuronal interactions.

In addition to utilizing neurophysiological recording data, the use of multiple electrical stimulation treatment (Experiment 1) should be discontinued, and replaced with the stimulation procedure employed in Experiment 2. The shorter and less frequent electrical stimulation intervals used in Experiment 2 did not appear to have any observable aversive effects on the animals, and yet, similar results to Experiment 1 were obtained in relation to the evocation of seizure behavior.

To summarize, some of the cholinergic limbic-hypothalamic circuitry thought to be involved in the mediation of eating and drinking behavior, also appear to be important for the development of seizure activity. Moreover, the dual stimulation procedure used in the experiments reported in

this thesis, in conjunction with neurophysiological recording techniques, could be of use in evaluating the effects of limbic-hypothalamic influences on seizure development and evocation, as well as aiding in determining the chemical specificity of the involved neural circuitry.

References

Albert, D. J., & Storlien, L. H. Hyperphagia in rats with cuts between the ventromedial and lateral hypothalamus. Science, 1969, 165, 599-600.

Albright, P. S. The role of cholinergic transmission in seizure development. Unpublished honours thesis, Sir George Williams University, 1971.

Anand, B. K., & Brobeck, J. R. Hypothalamic control of food intake in rats and cats. Yale Journal of Biology and Medicine, 1951, 24, 123-140. (a)

Anand, B. K., & Brobeck, J. R. Localization of a "feeding center" in the hypothalamus of the rat. Proceedings of the Society for Experimental Biology and Medicine, 1951, 77, 323-324. (b)

Andersson, B. The effect of injections of hypertonic NaCl-solutions into different parts of the hypothalamus of goats. Acta Physiologica Scandinavica, 1953, 28, 188-201.

Andersson, B., Gale, C., & Sundsten, J. W. Preoptic influences on water intake. In M. J. Wayner (Ed.), Thirst. New York: Pergamon Press, 1964. Pp. 361-377.

Andersson, B., & McCann, S. M. A further study of polydipsia evoked by hypothalamic stimulation in the goat. Acta Physiologica Scandinavica, 1955, 33, 333-346.

Andersson, B., & McCann, S. M. The effect of hypothalamic

lesions on the water intake of the dog. Acta Physiologica Scandinavica, 1956, 35, 312-320.

Arees, E. A., & Mayer, J. Anatomical connections between medial and lateral regions of the hypothalamus concerned with food intake. Science, 1967, 157, 1574-1575.

Baxter, B. L. Comparison of the behavioral effects of electrical or chemical stimulation applied at the same brain loci. Experimental Neurology, 1967, 19, 412-432.

Beleslin, D., Polak, R. L., & Sproull, D. H. The effect of leptazol and strychnine on the acetylcholine release from the cat brain. Journal of Physiology, 1965, 181, 308-316.

Belluzzi, J. D., & Grossman, S. P. Avoidance learning: Long-lasting deficits after temporal lobe seizure. Science, 1969, 166, 1435-1437.

Brobeck, J. R., Tepperman, J., & Long, C. N. H. Experimental hypothalamic hyperphagia in the albino rat. Yale Journal of Biology and Medicine, 1943, 15, 831-853.

Brodal, A. The amygdaloid nucleus in the rat. Journal of Comparative Neurology, 1947, 87, 1-16.

Brooks, C. McC., Lambert, E. F., & Bard, P. Experimental production of obesity in the monkey (Macaca mulatta). Federation Proceedings, 1942, 1, 11.

Celesia, G. G., & Jasper, H. H. Acetylcholine released from

cerebral cortex in relation to state of activation. Neurology, 1966, 16, 1053-1063.

Coury, J. N. Neural correlates of food and water intake. Science, 1967, 156, 1763-1764.

Cowan, W. M., Raisman, G., & Powell, T. P. S. The connexions of the amygdala. Journal of Neurology, Neurosurgery and Psychiatry, 1965, 28, 137-151.

Delgado, J. M. R., & Anand, B. K. Increase of food intake induced by electrical stimulation of the lateral hypothalamus. American Journal of Physiology, 1953, 172, 162-168.

Donovick, P. J., & Burright, R. G. Water consumption of rats with septal lesions following two days of water deprivation. Physiology and Behavior, 1968, 3, 285-288.

Fink, R. P., & Heimer, L. Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Research, 1967, 4, 369-374.

Fisher, A. E., & Coury, J. N. Cholinergic tracing of a central neural circuit underlying the thirst drive. Science, 1962, 138, 691-693.

Fisher, A. E., & Coury, J. N. Chemical tracing of neural pathways mediating the thirst drive. In M. J. Wayner (Ed.), Thirst. New York: Pergamon Press, 1964. Pp. 515-529.

Fisher, A. E., & Levitt, R. A. Drinking induced by carbachol: Thirst circuit or ventricular modification? Science, 1967, 157, 839-841.

Fuller, J. L., Rosvold, H. E., & Pribram, K. H. The effect of affective and cognitive behavior in the dog of lesions of the pyriform-amygdala-hippocampal complex. Journal of Comparative and Physiological Psychology, 1957, 50, 89-96.

Fuxe, K. The distribution of monoamine terminals in the central nervous system. Acta Physiologica Scandinavica, 1965, 64, Supplementum 244, 37-85.

Giarman, N. J., & Pepeu, G. The influence of centrally acting cholinolytic drugs on brain acetylcholine levels. British Journal of Pharmacology, 1964, 23, 123-130.

Gloor, P. Electrophysiological studies on the connections of the amygdaloid nucleus in the cat. I. The neuronal organization of the amygdaloid projection system. Electroencephalography and Clinical Neurophysiology, 1955, 7, 223-242.

Gloor, P., Murphy, J. T., & Dreifuss, J. J. Electrophysiological studies of amygdalo-hypothalamic connections. In Neural regulation of food and water intake. Annals of the New York Academy of Sciences, 1969, 157 (Art. 2), 629-640.

Goddard, G. V. Development of epileptic seizures through brain

stimulation at low intensity. Nature, 1967, 214, 1020-1021.

Goddard, G. V. Analysis of avoidance conditioning following cholinergic stimulation of amygdala in rats. Journal of Comparative and Physiological Psychology, 1969, Monograph 68 (2, Part 2), 1-18.

Goddard, G. V., McIntyre, D. C., & Leech, C. K. A permanent change in brain function resulting from daily electrical stimulation. Experimental Neurology, 1969, 25, 295-330.

Goddard, G. V., & Morrell, F. Unpublished research, 1969. Cited by F. Morrell, Physiology and histochemistry of the mirror focus. In H. H. Jasper, A. A. Ward, & A. Pope (Eds.), Basic mechanisms of the epilepsies. Boston: Little, Brown and Company, 1969. Pp. 357-370.

Gold, R. M. Hypothalamic hyperphagia produced by parasaggital knife cuts. Physiology and Behavior, 1970, 5, 23-25.

Green, J. D., Clemente, C. D., & de Groot, J. Rhinencephalic lesions and behavior in cats. Journal of Comparative Neurology, 1957, 108, 505-545.

Greer, M. A. Suggestive evidence of a primary "drinking center" in hypothalamus of the rat. Proceedings of the Society for Experimental Biology and Medicine, 1955, 89, 59-62.

Grossman, S. P. Eating or drinking elicited by direct

adrenergic or cholinergic stimulation of hypothalamus. Science, 1960, 132, 301-302.

Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. American Journal of Physiology, 1962, 202, 872-882. (a)

Grossman, S. P. Effects of adrenergic and cholinergic blocking agents on hypothalamic mechanisms. American Journal of Physiology, 1962, 202, 1230-1236. (b)

Grossman, S. P. Chemically induced epileptiform seizures in the cat. Science, 1963, 142, 409-411.

Grossman, S. P. Behavioral effects of chemical stimulation of the ventral amygdala. Journal of Comparative and Physiological Psychology, 1964, 1, 29-36. (a)

Grossman, S. P. Effects of chemical stimulation of the septal area on motivation. Journal of Comparative and Physiological Psychology, 1964, 58, 194-200. (b)

Grossman, S. P., & Grossman, L. Food and water intake following lesions or electrical stimulation of the amygdala. American Journal of Physiology, 1963, 205, 761-765.

Grossman, S. P., & Grossman, L. Food and water intake in rats with parasaggital knife cuts medial or lateral to the lateral hypothalamus. Journal of Comparative and Physiological Psychology, 1971, 74, 148-156.

Guillery, R. W. Degeneration in the hypothalamic connexions

of the albino rat. Journal of Anatomy, 1957, 91, 91-115.

Harvey, J. A., & Hunt, H. F. Effect of septal lesions on thirst in the rat as indicated by water consumption and operant responding for water reward. Journal of Comparative and Physiological Psychology, 1965, 59, 49-56.

Harvey, J. A., Lints, C. E., Jacobson, L. E., & Hunt, H.F. Effects of lesions in the septal area on conditioned fear and discriminated instrumental punishment in the albino rat. Journal of Comparative and Physiological Psychology, 1965, 59, 37-48.

Heimer, L., & Nauta, W. J. H. Hypothalamic distribution of the stria terminalis in the rat. Brain Research, 1969, 13, 284-297.

Hetherington, A. W. The relation of various hypothalamic lesions to adiposity and other phenomena in the rat. American Journal of Physiology, 1941, 133, 326-327.

Hetherington, A. W. Non-production of hypothalamic obesity in the rat by lesions rostral or dorsal to the ventromedial hypothalamic nuclei. Journal of Comparative Neurology, 1944, 80, 33-45.

Hetherington, A. W., & Ranson, S. W. Experimental hypothalamico-hypophyseal obesity in the rat. Proceedings of the Society for Experimental Biology and Medicine,

1939, 41, 456-466.

Hetherington, A. W., & Ranson, S. W. Hypothalamic lesions and adiposity in the rat. Anatomical Record, 1940, 78, 149-172.

Hoebel, B. G. Hypothalamic lesions by electrocauterization: Disinhibition of feeding and self-stimulation. Science, 1965, 149, 452-453.

Hoebel, B. G., & Teitelbaum, P. Hypothalamic control of feeding and self-stimulation. Science, 1962, 135, 375-377.

Keesey, R. E., & Powley, T. L. Enhanced lateral hypothalamic reward sensitivity following septal lesions in the rat. Physiology and Behavior, 1968, 3, 557-562.

Khavari, K. A., Heebink, P., & Traupman, J. Effects of intraventricular carbachol and eserine on drinking. Psychonomic Science, 1968, 11, 93-94.

Kimble, D. P., & Coover, G. D. Effects of hippocampal lesions on food and water consumption in rats. Psychonomic Science, 1966, 4, 91-92.

Lepkovsky, S., & Yasuda, M. Adipsia in chickens. Physiology and Behavior, 1967, 2, 45-47.

Levitt, R. A., & Fisher, A. E. Anticholinergic blockade of centrally induced thirst. Science, 1966, 154,

520-522.

Levitt, R. A., White, C. S., & Sander, D. M. Dose-response analysis of carbachol-elicited drinking in the rat limbic system. Journal of Comparative and Physiological Psychology, 1970, 72, 345-350.

Lewis, P. R., & Shute, C. C. D. The cholinergic limbic system: Projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the sub-fornical organ and supra-optic crest. Brain, 1967, 90, 521-540.

Lovett, D., & Singer, G. Ventricular modification of drinking and eating behavior. Physiology and Behavior, 1971, 6, 23-26.

Lubar, J. F., Schaefer, C. F., & Wells, D. G. The role of the septal area in the regulation of water intake and associated motivational behavior. In Neural regulation of food and water intake. Annals of the New York Academy of Sciences, 1969, 157 (Art. 2), 875-891.

Mabry, P. D., & Peeler, D. F. Response rate for food and water in the rat as a function of noncontingent reinforcing septal stimulation. Psychonomic Science, 1968, 13, 51-52.

MacPhail, E. M. Effects of intracranial cholinergic stimulation in rats on drinking, EEG, and heart rate.

Journal of Comparative and Physiological Psychology, 1968, 65, 42-49.

Mendelson, J. Role of hunger in T-maze learning for food by rats. Journal of Comparative and Physiological Psychology, 1966, 62, 341-349.

Milgram, N. W. Effect of hippocampal stimulation on feeding in the rat. Physiology and Behavior, 1969, 4, 665-670.

Miller, N. E., Gottesman, K. S., & Emery, N. Dose response to carbachol and norepinephrine in rat hypothalamus. American Journal of Physiology, 1964, 206, 1384-1388.

Montemurro, D. G., & Stevenson, J. A. F. Adipsia produced by hypothalamic lesions in the rat. Canadian Journal of Biochemistry and Physiology, 1957, 35, 31-37.

Morgane, P. J. Alterations in feeding and drinking behavior of rats with lesions in globi pallidi. American Journal of Physiology, 1961, 201, 420-428.

Morgane, P. J. Limbic-hypothalamic-midbrain interaction in thirst and thirst motivated behavior. In M. J. Wayner (Ed.), Thirst. New York: Pergamon Press, 1964. Pp. 429-453.

Morgane, P. J., & Kosman, A. J. Alterations in feline behavior following bilateral amygdalectomy. Nature, 1957, 180, 598-600. (a)

Morgane, P. J., & Kosman, A. J. A rhinenecephalic feeding

center in the cat. American Journal of Physiology, 1957, 197, 158-162. (b)

Morrell, F. Lasting changes in synaptic organization produced by continuous neural bombardment. In J. F. Delafresnaye (Ed.), Brain mechanisms and learning. Toronto: Ryerson Press, 1961.

Morrison, S. D., Barrnett, R. J., & Mayer, J. Localization of lesions in the lateral hypothalamus of rats with induced adipsia and aphagia. American Journal of Physiology, 1958, 193, 230-234.

Mountford, D. Drinking following carbachol stimulation of hippocampal formation or lateral ventricles. Psychonomic Science, 1969, 16, 124-125.

Murphy, J. T., Dreifuss, J. J., & Gloor, P. Topographical differences in the responses of single hypothalamic neurons to limbic stimulation. American Journal of Physiology, 1968, 214, 1443-1453.

Myers, R. D., & Cicero, T. J. Are the cerebral ventricles involved in thirst produced by a cholinergic substance? Psychonomic Science, 1968, 10, 93-94.

Nauta, W. J. H. Hippocampal projections and related neural pathways to the mid-brain in the cat. Brain, 1958, 81, 319-340.

Oomura, Y., Kimura, K., Ooyama, H., Maeno, T., Iki, M., &

Kuniyoshi, M. Reciprocal activities of the ventromedial and lateral hypothalamic areas of cats. Science, 1964, 143, 484-485.

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

Oomura, Y., Ooyama, H., Yamamoto, T., Naka, F., Kobayashi, N., & Ono, T. Neuronal mechanisms of feeding. In W. R. Adey, & T. Tokizane (Eds.), Progress in Brain Research, 1967, 27, 1-33.

Oomura, Y., Ooyama, H., Yamamoto, T., Ono, T., & Kobayashi, N. Behavior of hypothalamic unit activity during electrophoretic application of drugs. In Neural regulation of food and water intake. Annals of the New York Academy of Sciences, 1969, 157 (Art. 2), 642-665.

Oomura, Y., Ono, T., & Ooyama, H. Inhibitory action of the amygdala on the lateral hypothalamic area in rats. Nature, 1970, 228, 1108-1110.

Papez, J. W. A proposed mechanism of emotion. Archives of Neurology and Psychiatry, 1937, 38, 725-743.

Pellegrino, L. , & Cushman, A. A stereotaxic atlas of the rat brain. New York: Appleton-Century-Crofts, 1967.

Petsche, H., Stumpf, C., & Gogolak, G. The significance of

the rabbit's septum as a relay station between the midbrain and the hippocampus. I. The control of hippocampus arousal activity by the septum cells. Electroencephalography and Clinical Neurophysiology, 1962, 14, 202-211.

Pizzi, W. J., & Lorens, S. A. Effects of lesions in the amygdalo-hippocampo-septal system on food and water intake in the rat. Psychonomic Science, 1967, 7, 49-55.

Powell, E. W. Septal efferents revealed by axonal degeneration in the rat. Experimental Neurology, 1963, 8, 406-422.

Powell, T. P. S., & Cowan, W. M. An experimental study of the efferent connexions of the hippocampus. Brain, 1955, 78, 115-132.

Racine, R. J. Modification of seizure activity by electrical stimulation: I. After-discharge threshold. Electroencephalography and Clinical Neurophysiology, 1972, 32, 269-279.

Racine, R. J. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalography and Clinical Neurophysiology, 1972, 32, 281-294.

Racine, R. J., Okujava, V., & Chipashvili, S. Modification of seizure activity by electrical stimulation: III.

Mechanisms. Electroencephalography and Clinical Neurophysiology, 1972, 32, 295-299.

Raisman, G. The connexions of the septum. Brain, 1966, 89, 317-348.

Raisman, G., Cowan, W. M., & Powell, T. P. S. The extrinsic afferent, commissural and association fibres of the hippocampus. Brain, 1965, 88, 963-996.

Reivich, M., & Glowinski, J. An autoradiographic study of the distribution of C^{14} - norepinephrine in the brain of the rat. Brain, 1967, 90, 633-646.

Robinson, B. W., & Mishkin, M. Alimentary responses evoked from forebrain structures in macacca mullata. Science, 1962, 136, 260-262.

Routtenberg, A. Drinking induced by carbachol: Thirst circuit or ventricular modification? Science, 1967, 157, 838-839.

Ruch, T. C., Patton, H. D., & Brobeck, J. R. Hyperphagia and adiposity in relation to disturbances of taste. Federation Proceedings, 1942, 1, 76.

Russell, R. W., Singer, G., Flanagan, F., Stone, M., & Russell, J. W. Quantitative relations in amygdaloid modulation of drinking. Physiology and Behavior, 1968, 3, 871-875.

Sclafani, A., & Grossman, S. P. Hyperphagia produced by

knife cuts between the medial and lateral hypothalamus in the rat. Physiology and Behavior, 1969, 4, 533-538.

Shute, C. C. D., & Lewis, P. R. The ascending cholinergic reticular system: Neocortical, olfactory and subcortical projections. Brain, 1967, 90, 497-520.

Stuart, O. G., Porter, R. W., & Adey, W. R. Hypothalamic unit activity. II. Central and peripheral influences. Electroencephalography and Clinical Neurophysiology, 1964, 16, 248-258.

Teitelbaum, P. Sensory control of hypothalamic hyperphagia. Journal of Comparative and Physiological Psychology, 1955, 48, 156-163.

Teitelbaum, P., & Epstein, A. N. The lateral hypothalamic syndrome: Recovery from feeding and drinking after lateral hypothalamic lesions. Psychological Review, 1962, 69, 74-90.

Teitelbaum, P., & Stellar, E. Recovery from the failure to eat produced by hypothalamic lesions. Science, 1954, 120, 894-895.

Tsubokawa, T., & Sutin, J. Mesencephalic influence upon the hypothalamic ventromedial nucleus. Electroencephalography and Clinical Neurophysiology, 1963, 15, 804-810.

Ungerstedt, U. I. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiologica Scandinavica,

1971, Supplementum 367, 1-48.

Wendt, R. H., & Adey, W. R. A study of evoked unit activity in the hypothalamus. Anatomical Record, 1960, 136, 301. (Abstract)

Wheatley, M. D. The hypothalamus and affective behavior in cats. Archives of Neurology and Psychiatry, 1944, 52, 296-316.

White, N. M., & Fisher, A. E. Relationship between amygdala and hypothalamus in the control of eating behavior. Physiology and Behavior, 1969, 4, 199-205.

Wise, R. A. Organization of eating and drinking sites in the lateral hypothalamus. Unpublished doctoral dissertation, McGill University, 1968.

Wise, R. A. Eating induced by cholinergic stimulation in the sated rat. A paper presented at the Eastern Psychological Association, 42nd Annual Meeting, New York City, April, 1971.

Wishart, T. B., & Mogenson, G. J. Reduction of water intake by electrical stimulation of the septal region of the rat brain. Physiology and Behavior, 1970, 5, 1399-1404.

Witt, D. M., Keller, A. D., Batsel, H. L., & Lynch, J. R. Absence of thirst and resultant syndrome associated with anterior hypothalamectomy in the dog. American Journal of Physiology, 1952, 171, 780. (Abstract)

Wolfe, J. W., Lubar, J. F., & Ison, J. R. Effects of medial cortical lesions on appetitive instrumental conditioning. Physiology and Behavior, 1967, 2, 239-244.

Wood, C. D. Behavioral changes following discrete lesions of temporal lobe structures. Neurology, 1958, 8, 215-220.

TABLE 1

Cannula and Electrode Coordinates

Cannula Location	Cannula Coordinates (mm)			Electrode Coordinates (mm)		
	AP	ML	DV[a]	AP	ML	DV[a]
Hippocampus	-3.0	1.7	3.0	-0.8	1.5	8.2
Septum	1.6 2.0	0.6	5.5			
Amygdala	-0.6 -1.0	5.0 5.5	9.0			
Mamillary Bodies	-2.1	0.5	9.7			

[a] DV coordinate was measured from surface of skull to target site.

TABLE 2

Median Number of Experimental Sessions to First Seizure

Group	N	N who seized	Median	Range
CARES	7	6	2.5	5
NORES	5	3	15.0	10
CAR	5	0	>21[a]	-
ES	6	3	13.0	12

[a]Ss were not tested beyond 21 sessions.