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CENTRAL NEURAL MECHANISMS MEDIATING FEEDING AND ANOREXIA INDUCED BY MONOAMINERGIC DRUGS

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

CENTRAL NEURAL MECHANISMS MEDIATING FEEDING AND ANOREXIA INDUCED BY MONOAMINERGIC DRUGS

by

Joseph T. McCabe

Adviser: Professor Thomas E. Frumkes

Studies which report central injections of norepinephrine and clonidine stimulate feeding, and administration of dopamine, amphetamine, and fenfluramine suppress feeding suggest these drugs may be acting via the hypothalamus. The present series of experiments has attempted to determine hypothalamic regions where these drug effects are mediated, and has attempted to delineate the course of neural fiber systems that mediate these drug effects. Norepinephrine, clonidine, amphetamine, dopamine, and fenfluramine were administered to selected groups of rats that had sustained electrolytic lesions in the hypothalamus, or coronal wire knife cuts (KCs) either at the level of the posterior hypothalamus, midbrain, or pons.

Electrolytic lesions of the paraventricular nucleus attenuated feeding induced by intraperitoneal administration of clonidine. Lesions of the perifornical region abolished anorexia induced by amphetamine. Caudal, midlateral hypothalamic KCs attenuated anorexia induced by central and peripheral administration of amphetamine. Coronal KCs at the

midbrain level that severed tissue dorsal to the medial lemniscus attenuated anorexia induced by central and peripheral injections of amphetamine. Dorsal pontine KCs, rostral or caudal to the level of the locus coeruleus, attenuated feeding elicited by paraventricular injections of norepinephrine and intraperitoneal injections of clonidine. Ventral pontine cuts, dorsal to the nucleus of the facial nerve, significantly decreased anorectic response to amphetamine.

It is suggested that an efferent fiber system(s) originating in the paraventricular nucleus mediates feeding induced by norepinephrine and clonidine. These paraventricular nucleus fibers that mediate norepinephrine and clonidine eating project caudally from the paraventricular nucleus through the periventricular region. At the level of the rostral pons, fibers move lateral and then farther caudal to perhaps the dorsal vagal complex. Amphetamine-induced anorexia was disrupted by presumably severing afferents to the perifornical region that mediate drug response by releasing endogenous catecholamines. Catecholamine fibers that mediate amphetamine-induced anorexia arise from medullary cell groups and ascend through the ventral half of the pons. At the midbrain level, these fibers are joined by midbrain dopamine fibers and these continue rostrad through the midbrain and hypothalamus and terminate in the perifornical region.

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Central Neural Mechanisms Mediating Feeding and Anorexia Induced by Monoaminergic Drugs

General Introduction

Feeding behavior provides living organisms with nutrients for energy and somatic maintenance. This response is certainly tantamount to many other adaptive behaviors. The present series of experiments concern a specific aspect of the matter of food intake, namely, the role of central monoaminergic systems in drug-induced feeding stimulation and inhibition. These studies will relate the effects of various electrolytic lesions and wire knife cuts in the brain to eating induced by clonidine, and anorexia resulting from administration of amphetamine and fenfluramine. Before outlining specific experiments, a brief overview of central mechanisms of food intake is presented to give the reader some background to these studies.

A. Administration of Neurotransmitter Substances and Feeding Behavior.

1. Norepinephrine and Epinephrine.

a. Pioneering studies using central injections. According to Hoebel (1977), the major impetus to the study of neuropharmacology of feeding was the discovery by Grossman (1962a,b) that norepinephrine (NE) injections directly to the hypothalamus elicited feeding. Grossman used a double-walled cannula that was cemented to the rat's skull and enabled a single animal to be repeatedly injected with various drugs to the same brain site. When NE crystals (1-5 μg) were implanted into the lateral hypothalamus, a fairly robust feeding response began within a few minutes after drug implantation and repeated administrations of NE over

several days increased body weight. Centrally applied equimolar doses of epinephrine (EPI) elicited a feeding response but it was not as effective as NE. Subsequent experiments using NE dissolved in saline elicited feeding at doses as low as 0.8 μg (Miller, Gottesman & Emery, 1964). For extensive reviews of early studies of neurotransmitter stimulation of feeding, see Hoebel (1977) and Myers (1969; 1974).

b. Noradrenergic/Adrenergic receptor mechanisms and endogenous neurotransmitter effects. NE-induced feeding appears to be differentially mediated in terms of alpha- and beta-adrenergic receptor subtypes (Lehr, 1969). This schema, based on peripheral bioassays of sympathetic nervous system function (Ahlquist, 1948), denotes alpha-adrenergic receptors as sites exhibiting sensitivity to stimulation in the rank order NE>EPI>isoproterenol. In contrast, beta-adrenergic receptors in tissue show sensitivity in the order isoproterenol>EPI>NE. Beta-receptors were further subdivided into two subtypes based upon their responsiveness to beta-receptor stimulants and blockers. $Beta_1$-receptors are the primary beta-receptor in the heart and small intestine, while $beta_2$-receptors predominate in the bronchi, uterus, and vascular beds (Innes & Nickerson, 1975; Lands, Arnold, McAuliff, Luduena, & Brown, 1967). Alpha-adrenergic receptors are also subdivided to at least two subtypes (Berthelsen & Pettinger, 1977; Langer, 1979). $Alpha_1$-adrenergic blockers antagonize the stimulatory action of NE and EPI in smooth muscle tissue. $Alpha_2$ sites, which show approximately an eight-fold affinity for clonidine and para-aminoclonidine, compared to NE, are antagonized by $alpha_2$-adrenergic blockers such as yohimbine.

While alpha-adrenergic agonists such as metaraminol, clonidine, and

oxymetazoline, facilitated feeding (Booth, 1968; Leibowitz, 1975b; Ritter & Epstein, 1975; Slangen & Miller, 1969), the $beta_2$-adrenergic agonists, dl-salbutamol, l-isoproterenol, and dl-terbutaline, suppress feeding (Goldman, Lehr, & Friedman, 1971; Leibowitz, 1970; Leibowitz & Rossakis, 1978b). In addition, feeding elicited by NE could be antagonized by alpha-adrenergic blockers such as phentolamine, phenoxybenzamine, and tolazoline, and feeding suppression resulting from injections of EPI could be blocked by pretreatment with the beta-adrenergic blockers l-propranolol, l-aprenolol, and dl-butoxamine (see review by Leibowitz, 1980).

Endogenous NE neurotransmitter levels and turnover rates have also been correlated with feeding behavior. Food deprivation decreases hypothalamic NE levels and increases NE turnover (Friedman, Starr, & Gershon, 1973; Glick, Waters, & Milloy, 1973: 22 and 48 hr food deprivation periods, respectively). Studies examining subregions of the hypothalamus report that food deprivation decreases NE levels in the ventromedial and arcuate nuclei, but has little effect on levels in the lateral hypothalamic area, dorsomedial nucleus, medial preoptic area, or diagonal band (Stachowiak, Bialowas, & Jurkowski, 1978; 48 hr food deprivation period). The paraventricular nucleus (PVN) is reported to show an increase in NE turnover and decreased binding for the alpha-adrenergic antagonist, WB-4101, while the perifornical region exhibits increased WB-4101 binding after food deprivation periods of 12-48 hours (Jhanwar-Uniyal, Fleisher, Levin, & Leibowitz, 1982).

Insulin-induced glucoprivation has also been used to study hypothalamic NE function. Ritter, Bellin, & Pelzer (1981) found insulin

administration will increase turnover of hypothalamic NE and that subsequent feeding will normalize NE levels. The changes in NE levels in the hypothalamus does not seem to mediate glucoprivic feeding, however, since glucose infusions after insulin will normalize NE function but not inhibit post-insulin feeding (Bellin & Ritter, 1981). Since their studies did not examine discrete nuclei of the hypothalamus, however, these data cannot completely rule out the possibility that NE neurons in a subregion of the hypothalamus mediate feeding (Ritter, et. al., 1981).

c. Anatomical localization. Initial work investigating specific brain sites subserving NE feeding suggested the most responsive brain region was near the *fornix* in the anterior hypothalamus (Booth, 1967). However, more extensive mapping studies suggest that NE injections to the paraventricular nucleus of the hypothalamus produce the greatest feeding response in terms of amount consumed (Davis & Keesey, 1971; Matthews, Booth, & Stolerman, 1978; Leibowitz, 1978). Cannula placements 0.5 mm away from the paraventricular nucleus in any plane significantly reduce the magnitude of the eating response (Leibowitz, 1978).

2. Dopamine.

a. Feeding behavior. Dopamine (DA) can either inhibit or stimulate feeding depending upon experimental procedure. Central injections of DA suppress food intake in hungry rats (Hansen & Whishaw, 1973; Kruk, 1973; Leibowitz & Rossakis, 1978a). Similarly, central and peripheral administration of the DA precursor l-dopa will suppress feeding (Baez, 1974; Heffner, Zigmond, & Stricker, 1977; Leibowitz & Rossakis, 1979a; Sanghvi, Singer, Friedman, & Gershon, 1975). Indirect experimental approaches, using amphetamine injections in conjunction with a

catecholamine inhibitor, also suggest a role for DA in feeding suppression. Pretreatment with alpha-methyl-p-tyrosine, an agent that decreases DA stores by inhibiting production of l-dopa from tyrosine, attenuates amphetamine's ability to suppress feeding (Baez, 1974; Frey & Schulz, 1973; Holtzman & Jewett, 1971; Leibowitz, 1975a; Weissman, Koe, & Tenen, 1966). But when l-dopa is given in addition to the alpha-methyl-p-tyrosine pretreatment, the anorectic response to amphetamine is restored (Baez, 1974).

Other studies examining the consequences of electrolytic and neurotoxic lesions suggest central DA systems do not merely inhibit feeding but also play a facilitatory role. Depletion of almost all brain DA by administration of 6-hydroxydopamine will produce severe deficits in feeding behavior (see Ungerstedt, 1979; Stricker & Zigmond, 1976). Ungerstedt (1971) used fluorescence histochemistry in conjunction with his behavioral analyses and concluded the aphagia and adipsia seen after 6-hydroxydopamine treatment was primarily the result of damage to the dopamine-containing nigrostriatal system. Detailed observation of animals with damage to either the nigrostriatal bundle or lateral hypothalamus indicated these animals exhibit attention and arousal deficits as well as motor impairments (Balagura, Wilcox, & Coscina, 1969; Marshall, 1978; Marshall & Teitelbaum, 1974; 1977; Stricker & Zigmond, 1974; Wolgin, Cytawa, & Teitelbaum, 1976; Ungerstedt, 1971). Lateral hypothalamic lesioned rats also suffer gastric disturbances (Schallert, Whishaw, & Flanigan, 1977).

Evidence of a stimulatory role for DA in feeding includes the observation that administration of apomorphine, a DA receptor agonist,

will reestablish feeding in DA-depleted, aphagic rats (Ljungberg & Ungerstedt, 1976). Likewise, amphetamine (apparently releasing endogenous DA from residual neurons) can elicit feeding in aphagic rats (Stricker & Zigmond, 1976) and cats (Wolgin & Teitelbaum, 1978). Also, feeding can be elicited by administering the DA releaser, gamma-butyrate (Redgrave, Taha, White, and Dean, 1982).

Several authors have hypothesized that evidence supporting both a stimulatory and inhibitory feeding, demonstrates the modulatory function mediated by this neutrotransmitter in behavioral functions. There may be an optimal level of brain DA that is necessary for normal food intake behavior. Procedures that drastically increase or decrease DA levels beyond this optimal region disrupt feeding (Heffner, et. al., 1977). Also, perhaps more than one system of DA receptors mediate feeding and these systems may operate at anatomically distinct sites (Leibowitz, 1980). Aphagia results from depleting the dopaminergic nigrostriatal system that normally mediates arousal, attentional, and sensorimotor function. Non-nigrostriatal DA receptors localized to the perifornical hypothalamus modulate feeding inhibition (see below).

b. Receptor and neurotransmitter mechanisms. The specific receptor mediating DA's anorexigenic action has not been determined. However, based upon the relative potencies of neuroleptic DA blocking agents in disrupting DA anorexia (Leibowitz & Rossakis, 1979c, Table 1), and recent classification of receptor subtypes (Seeman, 1981, Figure 2), suggests DA anorexia may operate via D_2-type receptors.

Changes in endogenous DA levels and turnover have been related to food deprivation states. Increased hypothalamic DA levels are observed

after 22 hr of food deprivation (Friedman, Starr, & Gershon, 1973) and DA metabolism increases after hungry animals feed (Heffner & Seiden, 1980). Similarly, feeding and bar pressing for food releases DA (Martin and Myers, 1976). Some studies, however, have not observed any consistent increase or decrease in hypothalamic DA level after 24 h food deprived rats were allowed to feed. (van der Guten and Slangen, 1977).

c. Anatomical localization. Leibowitz and Rossakis (1979b) determined the brain region where central DA (as well as EPI) injection most effectively suppressed feeding. Using a total of 299 animals to examine 24 brain sites, injections of DA into the perifornical region of the hypothalamus, from the caudal level of the paraventricular nucleus to the caudal region of the ventromedial nucleus, maximally suppress feeding. DA injections to the striatum and nucleus accumbens, in contrast, were relatively ineffective.

3. Serotonin.

a. Feeding behavior. Generally, central administration of serotonin suppresses feeding. Intraventricular administration of serotonin suppressed feeding, and PVN and lateral hypothalamic administration of serotonin and its precursor 5-hydroxytryptophan (5HTP) reduce food intake (Kruk, 1973; Lehr & Goldman, 1973; Leibowitz & Papadakos, 1978; Singer & Kelly, 1972). Two studies, however, report serotonin injections to either the medial or lateral hypothalamus, or lateral ventricle, do not influence feeding (Baile, 1974; Blundell, 1977). Peripheral administration of the serotonin precursors, 5HTP and tryptophan, increase brain serotonin levels and suppress feeding in a dose dependent manner (Blundell & Leshem, 1975; Fernstrom & Wurtman, 1972; Joyce &

Mrosovsky, 1964).

b. Serotonergic receptors and neurotransmitter mechanisms. Pharmacological treatments that stimulate serotonin release generally decrease food intake. For example, peripheral administration of chlorimipramine, a drug that inhibits serotonin reuptake and thereby increases concentration of endogenous serotonin, decreases food intake (Blundell, 1977). Fluoxetine, a drug that inhibits the serotonin membrane pump (Fuller, Perry, Snoddy, & Molloy, 1974), also suppresses food intake (Goudie, Thorton & Wheeler, 1976). Likewise, central injections of these drugs, as well as the serotonin agonist quipazine, all decrease food intake (Leibowitz & Papadakos, 1978). As outlined below, fenfluramine, a drug that releases and/or blocks the re-uptake of serotonin, also decreases food intake. Biochemical studies report food deprivation increases turnover and synthesis of endogenous sertonin (Curzon, Joseph, & Knott, 1972; Kantak, Wayner, & Stein, 1978a,b; Perez-Cruet, Tagliamonte, Tagliamonte, & Gessa, 1972).

c. Anatomical localization. To date, no laboratory has performed an extensive study to localize the anatomical region where central injections of serotonin can most effectively inhibit feeding. Injections to the medial and lateral hypothalamus were slightly effective (Baile, 1974; Blundell, 1977), and injections to the perifornical region of the hypothalamus (Lehr & Goldman, 1973) and paraventricular nucleus (Leibowitz & Papadakos, 1978) suppressed food intake. Some question exists regarding the specificity of the findings of the latter two studies since these investigators report the beta-blocker, propranolol, could antagonize serotonin's anorexigenic effect. Central injection of

fenfluramine to the lateral hypothalamus (Blundell & Leshem, 1973) and norfenfluramine to the interstitial nucleus of the stria terminalis or neostriatum (Broekkamp, Weemaes, & van Rossum, 1975) induce anorexia. Leibowitz (1980) has suggested that the most effective site for serotonergic anorexia may be the paraventricular nucleus since NE-induced feeding could be antagonized by a comparatively low 0.5 μg dose of norfenfluramine.

It is not clear that serotonin plays a specific role in feeding behavior. Hoebel (1977) and others (Blundell, 1977; Leibowitz, 1980; Myers, 1974) have pointed out that peripheral administration of serotonin affects the general health of the animal and central injection of serotonin affects arousal, REM sleep, temperature regulation, and aggressiveness. Therefore, in spite of continuing reports showing that serotonin depletion leads to chronic obesity (Breisch, Zemlan, & Hoebel, 1976; Saller & Stricker, 1976; Waldbillig, Bartness, & Stanley, 1981), careful experimental work is required to fully understand serotonin's role in the mediation of single feeding episodes.

B. Administration of the Drugs Clonidine, Amphetamine, and Fenfluramine and their Effects on Feeding Behavior.

The preceding section has briefly reviewed what is currently known about how central monoamine systems participate in feeding. As will be outlined in the sections below, information regarding specific brain sites and fiber pathways that mediate stimulation and inhibition feeding is limited. One means for studying monoamine systems would be to examine how specific lesions and wire knife cuts (KCs) that disrupt crucial aspects of neural feeding circuits affect drug induced feeding. Three

compounds, clonidine, an alpha-adrenergic agonist, and the anorectic agents amphetamine, a catecholamine releaser, and fenfluramine, a serotonergic releaser, were selected as tools to investigate neural mechanisms of feeding behavior.

These three drugs are now briefly reviewed. In Section C, specific experiments that will be conducted are outlined. These studies attempt to answer questions about anatomical sites of action of these drugs and this may enable us to further understand the roles played by NE, EPI, DA, and serotonin systems in feeding.

1. Clonidine.

Peripheral administration of clonidine elicits feeding in rats, mice, and monkeys (Atkinson, Kirchertz, & Peters-Haefeli, 1978; Beales, Callahan, & Oltmans, 1982; Mauron, Wurtman, & Wurtman, 1980; Sanger, 1983; Schlemmer, Casper, Narasimhachari, & Davis, 1979). This hyperphagic response to clonidine may be mediated via an $alpha_2$-adrenergic receptor mechanism since the feeding response to clonidine was blocked by injections of the $alpha_2$-antagonist, yohimbine, but not by administration of an $alpha_1$-adrenergic antagonist, prazosin (Sanger, 1983; Schlemmer, Casper, Narasimhachari, & Davis, 1979).

Clonidine can also induce feeding in satiated rats when centrally injected. Intracerebroventricular injections of clonidine as well as administration to the anterolateral hypothalamus at the level of the stria terminalis elicits feeding (Broekkamp & van Rossum, 1972; Holman, Shillito, & Vogt, 1971; Ritter, Wise, & Stein, 1975). To date, however, no studies have attempted to delineate a primary anatomical locus for

clonidine's effect on food intake.

2. Amphetamine.

Amphetamine is the most widely studied anorectic agent (see Cole, 1978; Garratini & Samanin, 1978). Among its many biochemical effects, amphetamine releases NE and inhibits NE and DA uptake (see Biel & Bopp, 1978; Cooper, Bloom, & Roth, 1978; Copeland, Aulakh, Bhattacharyya, & Pradhan, 1980; Glowinski, 1970; Roffman, Cassens, & Schildkraut, 1978; Ziance & Rutledge, 1972; Ziegler, Lake, & Ebert, 1979), and releases EPI from the hypothalamus (Burgess & Tessel, 1980). Amphetamine is able to also release serotonin from the midbrain, but this effect requires higher dose levels (Holmes & Rutledge, 1976). Feeding inhibition from amphetamine administration is antagonized by pretreatment with dopamine blockers (Abdallah, Roby, Boekler, & Riley, 1976; Barzaghi, Gropetti, Mantegazza, & Muller, 1973; Clineschmidt, et. al., 1974; Frey & Schulz, 1973; Heffner, et. al., 1977; Kruk, 1973; Kruk, Smith, & Zarrindast, 1976; Leibowitz, 1978; Zis & Fibiger, 1975). Antagonism of amphetamine anorexia by such adrenergic blockers as propranolol, a beta-adrenergic antagonist, or phentolamine, an alpha-adrenergic antagonist, has been inconsistent with some showing the effect (Frey & Schultz, 1973; Sanghvi, Singer, Friedman, & Gershon, 1975), and others failing to do so (Dobrzanski & Doggett, 1979; Frey & Schulz, 1973; Kruk, 1973; Kruk, et. al., 1976; Lehr & Goldman, 1973; Sanghvi, et. al., 1975).

The site of action for amphetamine's anorexigenic effect may be the lateral hypothalamus since lesions to this region abolish or greatly decrease feeding inhibition (Blundell & Leshem, 1974; Carlisle, 1964; Fibiger, Zis, & McGeer, 1973; Russek, Rodriquez-Zendejas, & Teitelbaum,

1973; Sanger, 1983). While Booth (1968) initially demonstrated central injections to the lateral hypothalamus inhibit feeding, Leibowitz demonstrated that the perifornical hypothalamus at the level of the ventromedial nucleus is a maximally sensitive region to elicit amphetamine's effect on feeding. (Leibowitz, 1975a; Leibowitz & Rossakis, 1978a). At this hypothalamic site, doses as low as 0.8 μg suppressed food intake in 20 hr food-deprived rats.

3. Fenfluramine.

Anorexia following administration of fenfluramine is believed to result from its effect on either endogenous serotonin levels or on serotonin receptors (see Blundell & Burridge, 1979). Fenfluramine releases serotonin and increases turnover (Costa, Groppetti, Revuelta, 1971), and can release NE but to a far less extent than compounds such as amphetamine (Ziance & Rutledge, 1972). Feeding suppression from fenfluramine is antagonized by serotonin blockers, such as, methergoline, methylsergide, & AHR-3009, (Blundell & Latham, 1980; Blundell, Latham, & Lesham, 1973; Clineschmidt, McGuffin, & Werner, 1974; Funderbunk, Hazelwood, Ruckart, & Ward, 1971; Jespersen & Scheel-Kruger, 1973). Depletion of central serotonergic systems by either the administration of the serotonergic neurotoxin, 5, 7-dihydroxytryptamine, or by lesions of the raphe antagonize fenfluramine's anorectic action (Clineschmidt, McGuffin, & Werner, 1974; Garattini, Borroni, Mennini, & Saminin, 1978; Samanin, Ghezzi, Valzelli, & Garattini, 1972), but the precise mechanism(s) by which fenfluramine induces anorexia is not known. For example, various 'serotonergic' anorectics fail to show complete cross tolerance (Rowland, Antelman, & Kocan, 1982), and when administered peripherally, fen-

fluramine is actively metabolized to norfenfluramine, and these compounds release serotonin via different neuronal mechanisms (Mennini, Borroni, Samanin, & Garattini, 1981).

Blundell, Latham, and Leshem (1976) have shown amphetamine and fenfluramine produce decreases in food intake by acting upon different aspects of feeding. Continuously monitoring food intake over 24 h periods, they found amphetamine delayed the onset of eating, while fenfluramine produced no delay in the initiation of eating but resulted in the earlier termination of a meal. Both drugs produced opposite effects on rate of eating: amphetamine increased while fenfluramine decreased rate of eating, and these drug effects were respectively antagonized by the DA blocker, pimozide, and serotonin receptor blocker, methergoline (Blundell & Latham, 1980).

C. Overview of the Dissertation: Neurotransmitter Fibers Systems and Drug-Mediated Feeding.

Studies which report central injections of clonidine elicit feeding and amphetamine and fenfluramine suppress feeding suggest these effects may be mediated by the hypothalamus. Initial experiments, therefore, will place lesions in selected subregions of the hypothalamus to localize possible sites where drug responses may be mediated. Once the primary hypothalamic site has been identified in which clonidine, amphetamine, and fenfluramine influence feeding, it seems possible that one can trace the pathways of neurons involved in these drug responses. Using neuroanatomical studies that have delineated efferent and afferent systems of the hypothalamus as guides, coronal knife cuts (KCs) were placed at selected points along the neuraxis. Severing fibers crucial to drug

induced feeding or anorexia will suggest the anatomical routes of neurons that coordinate food intake responses.

Figure 1 provides the basic nomenclature and a simplified depiction of all brain manipulations that will be examined. In this sagittal view of the rat brain, an approximate location of all brain manipulations are represented: electrolytic lesions are denoted by circles, and coronal KCs as vertical lines. (Detailed anatomical descriptions of these brain manipulations are provided later.) At the hypothalamic level, the effects of lesions placed in either the PVN, dorsomedial nucleus, or perifornical area will be examined. Also two different coronal KCs will be made just caudal to the level of the ventromedial nucleus to sever fibers as they pass through the caudal hypothalamus. The "midline KC" extends from midline to approximately 0.7-0.9 mm lateral to the midline on each side of the brain. The "perifornical KC", produced in other animals, extends from approximately 0.7 mm from midline to 1.2 mm lateral. At the midbrain level, three separate KCs will be made, in different groups of rats, just caudal to the level of the red nucleus. A "Far-Ventral midbrain KC" extends from the base of the brain to just under the level of the medial lemniscus that courses ventral to the red nucleus at this level of the brain. The "Ventral midbrain KC" severs tissue around the level of the medial lemniscus and just slightly dorsal. Finally, the "Dorsal midbrain KC" extends from behind the level of the red nucleus and 3 mm dorsal. At the pontine level, KCs will be made in other groups of rats either rostral to the level of the locus coeruleus (rostral pontine KC) or behind the level of the locus coeruleus (caudal pontine KC).

After sustaining one of the above outlined lesions or knife cuts, animals will be administered clonidine, amphetamine, and fenfluramine intraperitoneally. Some animals receiving midline, perifornical, or pontine KCs will also receive PVN or perifornical cannula implants. PVN cannulated animals will then receive injections of NE and clonidine, while rats implanted with a perifornical cannula will be administered amphetamine and dopamine.

These studies will be performed in order to answer the following questions:

1. Clonidine Induced Feeding: Where are the Central Sites of Action and Fiber Projections Mediating this Response?

The present group of experiments were initiated to examine the following questions regarding clonidine's action.

a. Clonidine: Hypothalamic Lesions. Since clonidine is an alpha-adrenergic agonist and is known to elicit feeding, then possibly the response produced by this drug results from stimulating the noradrenergic feeding circuit which operates via alpha-adrenergic receptors in the PVN. Animals sustaining electrolytic lesions in this brain site should fail to exhibit a reliable feeding response to peripherally injected clonidine.

b. Clonidine: Hypothalamic KCs. If clonidine stimulates feeding by acting through the PVN, coronal KCs in the caudal hypothalamus may damage this noradrenergic feeding circuit and disrupt drug-stimulated feeding. Likewise, by severing possible brainstem fiber projections of the noradrenergic feeding circuit, it may be possible to disrupt feeding

stimulated by central injection of NE and clonidine.

c. Clonidine: Midbrain and Pontine KCs. Clonidine and NE stimulated eating may depend also upon PVN-brainstem fiber projections at midbrain and pontine levels. KCs will be introduced in these brain regions caudal to the hypothalamus in order to determine how these manipulations affect feeding induced by clonidine and NE.

2. Determination of the Course of Catecholamine Fibers Mediating Amphetamine Anorexia.

The second half of this dissertation attempts to delineate the specific anatomical routes taken by ascending catecholamine- containing fibers that are involved in amphetamine anorexia. In order to more fully discuss the implications of these experiments, findings related to studies involving hypothalamic manipulations will be reported in a separate chapter from experiments dealing with hindbrain mechanisms of amphetamine anorexia.

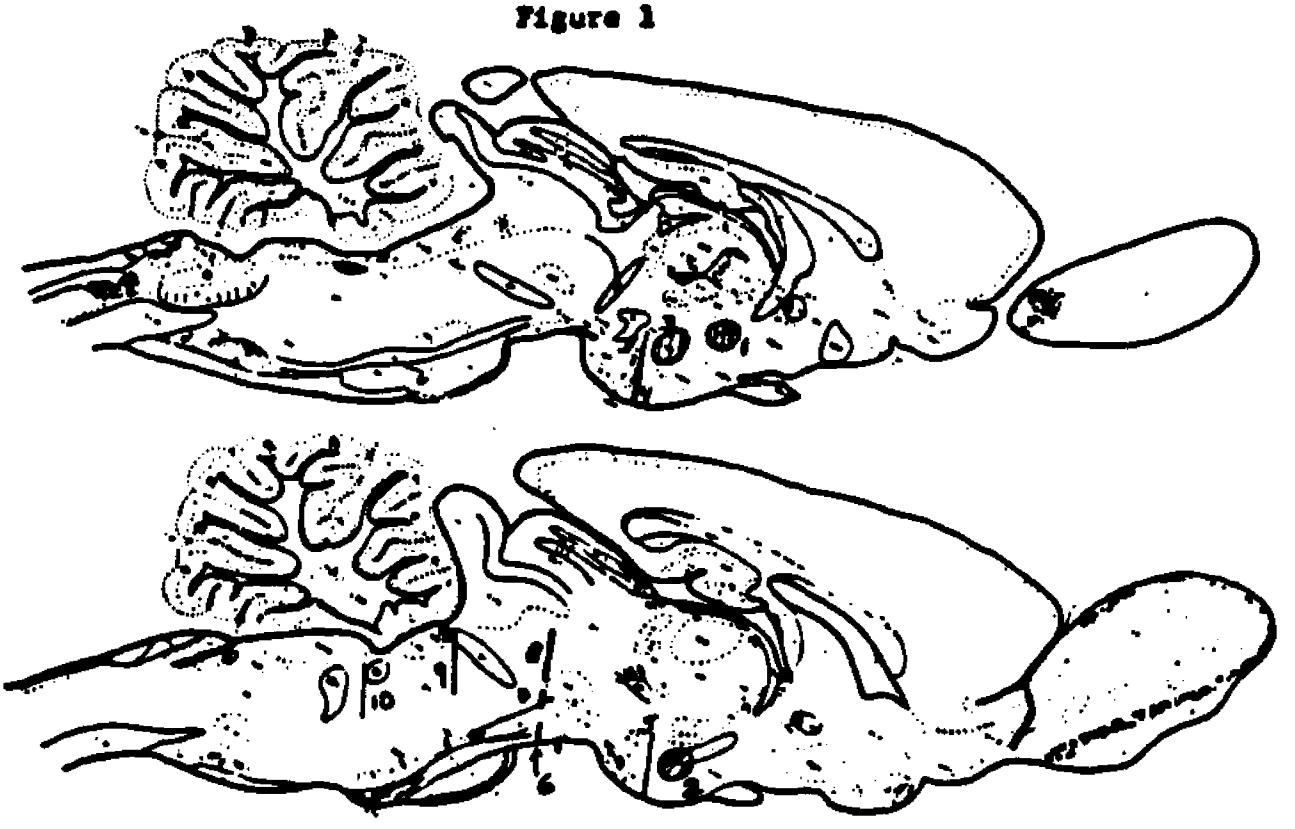
a. Amphetamine and Fenfluramine: Hypothalamic Lesions. The first experiments will investigate the anatomical site of action of amphetamine within the hypothalamus. Since the lateral perifornical region of the hypothalamus has been suggested to mediate feeding suppression from central injection of amphetamine, then electrolytic lesions to this brain region should disrupt the usual anorectic response that follows amphetamine administration. To determine the specificity of the effect of perifornical lesions on amphetamine anorexia, these animals will also be tested to examine their response to the anorectic drug, fenfluramine. To date there is no evidence that fenfluramine-induced anorexia is medi-

ated via perifornical hypothalamic mechanisms. It is therefore predicted that these perifornical lesion animals will show an attenuated response to amphetamine, but not to fenfluramine.

b. Amphetamine and Fenfluramine: Hypothalamic KCs. A second question related to hypothalamic mediation of amphetamine anorexia is whether one can determine the precise course taken by ascending catecholamine fibers that mediate this response. To determine this, coronal KCs will be made at different levels in the caudal hypothalamus to disrupt catecholamine fibers involved in amphetamine anorexia. Since amphetamine's anorectic effect depends upon the release of catecholamines from relevant fibers, KCs at a specific medial-lateral level of the caudal hypothalamus will disrupt amphetamine feeding suppression when fibers mediating this response are severed. This will indicate the specific course of fibers crucial for this drug response. The effect of these KCs on fenfluramine anorexia will also be examined.

c. Amphetamine and Fenfluramine: Midbrain and Pontine KCs. The final chapter will report findings dealing with disruption of brain tissue caudal to the hypothalamus. KCs will be placed in the midbrain and in the pontine tegmentum in order to examine how amphetamine anorexia is affected by disruption of catecholamine fibers at midbrain and pontine levels of the neuraxis. The results of these investigations will provide information regarding the precise anatomical routes, at the caudal level of the brain, taken by fibers mediating amphetamine anorexia. The feeding response to fenfluramine will also be tested in these animals.

Figure 1



Sagittal brain drawings above depict all brain manipulations that will be examined. Electrolytic lesions are denoted by circles, and the adjacent number refers to label of lesion in chart below. Similarly, coronal KCs are drawn as single lines and adjecent number refers to label used to designate each cut.

Hypothalamic Level
1. Paraventricular Hypothalamic Lesion
2. Perifornical Hypothalamic Lesion
3. Dorsomedial Hypothalamic Lesion
4. Midline Hypothalamic KC
5. Perifornical Hypothalamic KC

Midbrain Level
6. Far Ventral Midbrain KC
7. Ventral Midbrain KC
8. Dorsal Midbrain KC

Pontine Level
9. Rostral Pontine KC
10. Caudal Pontine KC

Chapter 2

General Method

A. Subjects.

Male Sprague-Dawley rats (350 g: Charles River Labs) were initially group-housed, but were transferred to individual cages shortly before testing began. A sweetened milk mash diet (25 g Purina powdered chow: 20 g granulated sugar: 25 g Carnation brand evaporated milk) was provided daily in a dish placed in the corner of the cage. Water was available ad libitum. Animals were maintained on a 12:12 hr light (0700 hr): dark (1900 hr) cycle.

B. Surgeries.

1. Cannula Implants.

In all central injection experiments, each rat was anesthetized with 60 mg/kg (approximately 20 mg in 0.4 cc saline) sodium pentobarbital (Nembutal; Abbott Laboratories) and implanted stereotaxically (Kopf) with a stainless steel 23-gauge guide cannula. With the incisor bar set at 3.1 mm (Kreig) above interaural line, the cannula tip was aimed at either the dorsolateral edge of the paraventricular nucleus (PVN) (0.0 mm AP/0.4 mm LAT/ -8.2 mm DV) or the perifornical region at the level of the ventromedial nucleus (-1.5 mm AP/ 1.5 mm LAT/ -8.7 mm DV). The cannula was affixed with acrylic cement to two stainless steel hooks which were inserted into the skull along the lateral edge. The incision was then sutured and cannula made patent with a cap and an inserted stylette. At least three days were allowed for recovery from surgery.

2. Electrolytic Lesions.

Bilateral electrolytic lesions were made to the PVN, the dorsomedial nuclei, or the perifornical region in other groups of anesthetized rats that did not receive cannula implants. Anodal lesions were made using stainless steel insect pins (size 00) that were coated with epoxylite except for 0.5 mm at the tips. All lesions were made by passing approximately 1 mA anodal current for 12-15 sec and the incisor bar was always set 3.1 mm (Kreig) above interaural line. With the electrode tilted 4^{o} in the lateral direction, PVN lesions were made using the coordinates: 0.7 mm rostral to bregma suture/ 0.8 mm LAT/ -8.7 mm DV. For dorsomedial nucleus lesions, the same procedure was used, except that the electrode was aimed slightly more posterior to 0.3 mm behind bregma suture. Perifornical lesions were made with the electrode lowered perpendicular to the skull using the coordinates: -0.6 mm behind bregma suture/ 1.2 mm LAT/ 8.8-9.2 mm DV. Sham animals received similar treatment except that the electrode was only lowered to within 3 mm of the targeted sites, and no current was passed through the electrode.

3. Coronal Knife Cuts.

The knife cut surgical procedure utilized a wire encephalotome (Sclafani, 1971) made of a stainless steel wire (0.175 mm) inserted into 26-gauge stainless steel tubing. The tip of the tubing was slightly bent so that when the wire knife was pushed out of the tubing, it protruded in a ventrolateral direction. The pitch and length of the wire knife varied, depending upon the desired shape and size of the cut. The "pitch" varied between 1.2 and 1.5 mm in the ventral direction and the "length" varied between 1.2-2.5 mm in the lateral direction. Cuts were

made by lowering the guide into the brain, extending the wire knife from the guide tubing, and then raising and lowering the entire encephalotome three times. The pontine cuts involved a two-stage cutting procedure. A more ventral cut was first made and the knife retracted, followed by a dorsal cut. After the cutting procedure, the wire was retracted into the guide and the guide was removed from the cranium. The same procedure was then repeated for the other side of the brain. For sham animals, an identical surgical procedure was followed, except that the guide was lowered to a point 3 mm above where the cuts were made in experimental animals, and the knife was not extended. Table 1 presents stereotaxic coordinates for each KC.

C. Test procedures.

Animals underwent slightly different testing procedures that depended upon their cannula placement, what drug was being used, and whether the drug was administered centrally or peripherally. All animals were weighed at weekly intervals and were tested every other day. In every group of animals tested, unless specifically noted, saline and drug (or drug doses) were administered in a counterbalanced manner. Noncannulated rats usually received tests on four drugs, after lesion or knife surgery, in the sequence: clonidine (1-3 weeks), chlorpromazine (3-5 weeks), amphetamine (4-6 weeks), and fenfluramine (6-8 weeks). (The results from the chlorpromazine trials will not be reported.)

1. Peripheral Injection of Clonidine.

a. Dose Response Study. Experiments with clonidine utilized a

procedure where animals were pretreated for one hour with fresh mash before testing began. To reduce spontaneous meals initiated by handling, animals were handled when given the fresh mash and also 30 min later in the middle of the pretreatment period. After pretreatment, animals in the dose response study received an intraperitoneal injection of either normal saline (1 ml/kg) or 1.25, 3.125, 12,50, 50.0, or 100.0 µg/kg clonidine hydrochloride (Boehringer-Ingelheim) dissolved in saline (1 ml/kg). Mash dishes were weighed at time of injection and two hr after injection. All animals received at least two tests at each dosage level and from these scores a mean feeding response for each animal was calculated. At least three different dose levels were tested on any specific day. Due to the consistency of the mash, spillage was negligible and was not measured.

b. Lesion and Knife Cut Studies. Noncannulated rats, after sustaining bilateral electrolytic lesion, knife cut, or sham surgery were first tested for level of food intake to clonidine (50.0 µg/kg/1 ml saline) and saline (1 ml/kg) using the satiation feeding test outlined for the clonidine dose response study. The 50 µg/kg dose of clonidine was used since it produced the strongest feeding response, in terms of amount of mash consumed, of all dosages tested in the dose response study. After 2-3 weeks of clonidine testing, these animals were then tested for response to chlorpromazine using the satiation feeding test (these results will not be reported). Following the chlorpromazine tests and one or two four-hour food deprivation pilot test procedures, animals were then administered amphetamine, fenfluramine,and saline trials as outlined below.

2. Central Injection of Norepinephrine and Clonidine.

Animals with PVN cannulas were tested for three weeks for level of food intake response to NE (l-norepinephrine bitartrate; Sigma), clonidine, and saline before (pre-KC) receiving either sham surgery, midline hypothalamic, or rostral or caudal pontine KCs. Identical tests were then undertaken for three weeks after surgery (post-KC). During a typical test session, fresh mash was placed in the test cage. At this time, each animal was handled in order to stimulate feeding and then was handled again 30 min later. At the end of the one hr satiation treatment, mash dishes were removed and weighed. Each animal then received a 0.5 µl injection of sterile saline through a 10 µl syringe (Hamilton) affixed with a needle that, when inserted in the guide cannula, reached but did not extend beyond the tip of the cannula. Forty-five min after the initial injection, mash dishes were again removed and weighed to determine food intake, and each rat received a second central injection of either sterile normal saline, NE at a dose of 40 nM (12.8 µg/0.5 µl sterile saline), or 7.5 nM clonidine hydrochloride (2.0 µg/0.5 µl sterile saline). Forty-five min later mash dishes were again removed and weighed to determine consumption. The three drug treatments that every animal received were administered once in the first pre-KC week, and then again (but in different orders of presentation) to each animal during the second and third pre-KC weeks. At this point each animals level of response to each drug was averaged over the three weeks. An animals mean feeding score to NE and clonidine were determined by subtracting the average food intake following saline injection from the average food intake to NE and clonidine. Any animal that did not exhibit a feeding score of greater than 3.0 g to either NE or clonidine was

eliminated from the study. Animals were matched in pairs for level of feeding response to NE and clonidine and one animal of the pair was assigned to the sham group while the other rat received a midline hypothalamic or a pontine KC. By matching animals on feeding scores to NE and clonidine the KC and sham groups initially exhibited approximately equivalent pre-KC group feeding scores.

3. Peripheral Injection of Amphetamine and Fenfluramine.

a. Dose Response Study. Separate groups of animals were used to determine dose responses to peripherally injected d-amphetamine sulfate (Smith, Kline, & French) and fenfluramine hydrochloride (A.H. Robins). Rats were food deprived for four hr, and then fifteen min before fresh preweighed mash was presented, animals in the amphetamine study received 0.0, 0.25, 0.50, 1.0, or 2.0 mg/kg injections of amphetamine dissolved in saline (1 cc/kg). In the fenfluramine study, four hr food-deprived animals received 0.0, 0.22, 0.66, 2.0, 6.0, or 12.0 mg/kg injections of fenfluramine dissolved in saline (1 cc/kg). Food intake measures were made 60 min later. Each dose level was given to every animal 2-4 times, and an animals' average response at each dose level was calculated.

b. Lesion and Knife Cut Studies. As noted earlier, noncannulated rats were also tested for level of response to amphetamine and fenfluramine after trials involving clonidine and chlorpromazine administrations. Therefore, after one or two 4 hr food deprivation test procedures, these rats received amphetamine and fenfluramine tests identical to those used in the dose response study. Animals were administered amphetamine (0.5 mg/kg) and saline tests (1 cc/kg) during weeks 1-3 after chlorpromazine testing, and then fenfluramine (2.0 mg/kg) and

saline trials (1 cc/kg) during weeks 3-6 after chlorpromazine tests.

4. Central Injection of Dopamine and Amphetamine.

Each animal implanted with a perifornical cannula received either a perifornical, midbrain, or pontine KC or sham surgery at the time of cannula implantation. On testing days, animals were food deprived for four hr, and 15 min before food presentation were centrally injected with 0.5 µl sterile saline, a 150 nM dose (18.96 µg/0.5 µl sterile saline) of dopamine hydrochloride (Sigma), or a 150 nM dose (5.5 µg/0.5 µl sterile saline) of d-amphetamine sulfate. On days when DA was administered, animals received a 15 mg/kg peripheral injection of pargyline hydrochloride (Saber), a monoamine oxidase inhibitor. Control tests involving pargyline administration with central injection of saline were also conducted. Some of these animals, after central tests, also received trials using peripherally injected (0.5 mg/kg) amphetamine and saline (1 cc/kg). Animals were tested 2-3 times on each of these drugs. Identical with the procedure used in the amphetamine and fenfluramine dose response studies, food intake was measured 60 min postinjection.

D. Daily Food Intake.

Daily food intake measures were taken on a portion of the rats used in this experiment, during the first three weeks after sham, lesion, or KC surgery.

E. Histology.

After completion of all experiments, animals were anesthetized with a 60 mg/kg dose (approximately 25 mg in 0.5 cc saline) of pentobarbital

(Nembutal) and transcardially perfused with 10% buffered formalin. The brains were removed from the calvarium and stored for at least three days in a 30% sucrose-buffered formalin solution before they were sectioned (50 μ), mounted, and stained with cresyl violet. The brains of animals that sustained electrolytic lesions were sectioned in the coronal plane, while the brains of animals in the KC studies were sectioned parasaggitally. Sections were compared to the stereotaxic atlases of Koenig and Klippel (1963/1974), Pellegrino, Pellegrino, and Cushman (1979), and Palkovits and Jacobowitz (1974).

F. Data Analysis.

Results were evaluated by analysis of variance (BMDP Biomedical Computer Program P-2V: Dixon & Brown, 1977). For the clonidine experiments, significant differences in level of feeding responses, when comparing either lesioned or KC rats to sham rats, were recognized only when the interaction effect (group x drug) reached statistical significance. Tests on main effects and simple main effects were used to determine differences in drug responses across groups. Within group comparisons of feeding responses to saline and drug utilized tests on main effects, while comparisons between groups in the clonidine experiments were made by calculating differences scores (drug - saline feeding score), and a subsequent analysis of variance. In the amphetamine and fenfluramine experiments, raw data (saline and drug) were converted to a percent suppression measure since the data from over one-third of the experiments were heterogenous. Analysis of variance was then performed. In some cases, data for groups consisting of less than five animals are presented. These data are not included in overall analyses of variance,

but are separately examined utilizing t-tests, and in some cases are separately compared to the sham animals.

Table 1. Coordinates of coronal knife cuts (KCs). Incisor bar placement is expressed in mm below interaural line and coordinates for anterior-posterior placement [with respect to Bregma (B) or Lambda (L) line], lateral placement from midline, and dorsal-ventral placement (below skull surface) are all with respect to knife guide tip. Knife "length" and "pitch", respectively, refer to length knife extends in the ventral and lateral directions. All KCs were performed with knife extending in the medial direction into brain tissue. With respect to the pontine KCs, histology from animals in earlier experiments indicated that in some rats the knife failed to substantially sever brain tissue. This appeared to be due to the fact that the KCs attempted to sever tissue adjacent to the brachium conjunctivum and facial nerve, which are rather large, heavily-myelinated fiber tracts. Therefore, two different knife lengths were used to produce pontine KCs and are denoted by 1.8/2.5. Also, pontine KCs involved a two-stage cutting procedure and this is designated by the two dorsal-ventral parameters (see text for details).

Table 1

Label of KC	Incisor Bar Placement	Anterior/ Posterior	mm Lateral to Midline	Dorsal/ Ventral	Height of KC	Knife Length	Knife Pitch
Posterior Hypothalamic Cuts							
Midline KC	0.0	+5.2L	1.4	-9.2	4.0	2.5	1.5
Periformical KC	0.0	-3.0B	2.5	-9.6	3.0	2.0	1.3
Midbrain Cuts							
Dorsal KC	-4.0	-6.0B	2.1	-6.9	3.0	2.5	1.2
Lemniscal KC	-4.0	-6.0B	2.1	-8.0	3.0	2.5	1.2
Ventral KC	-4.0	-6.0B	2.1	-8.7	2.7	2.5	1.2
Pontine Cuts							
Rostral Pontine KC	-4.0	0.0L	2.5	-7.0	3.4	1.8/2.5	1.5
				-8.0	3.2	1.8/2.5	1.5
Caudal Pontine KC	-4.2	-2.5L	2.8	-7.4	3.1	1.8/2.5	1.5
				-8.0	3.2	1.8/2.5	1.5

Chapter 3

Clonidine-Induced Feeding: Where are the Central Sites of Action and Fiber Projections Mediating this Response?

Since the original studies of Grossman (1962a), a number of investigators have shown that hypothalamic injections of norepinephrine (NE) elicit a robust feeding response (Booth, 1968; Slangen & Miller, 1969; Leibowitz, 1980). This NE-induced effect is mediated by alpha-adrenergic receptors, since injection of alpha-adrenergic blocking agents, but not beta-adrenergic, dopaminergic, or serotonergic blockers, prior to NE injection, attenuate or abolish NE eating (Booth, 1968; Leibowitz, 1975c; Ritter & Epstein, 1975; Slangen & Miller, 1969). The largest feeding response, in terms of amount consumed, results from injection of NE to the medially located paraventricular nucleus (PVN). Using groups of rats with cannulae directed to various locations in the diencephalon, injections to the PVN produce the greatest feeding response (Davis & Keesey, 1971; Matthews, Booth, & Stolerman, 1978; Leibowitz, 1978b) and cannula placements 0.5 mm away from the PVN in any plane yield significantly smaller eating responses (Leibowitz, 1978b). In fact, feeding can be elicited from the PVN at doses as low as 4 ng (Leibowitz, 1978b), and PVN lesions essentially abolish feeding to NE infused intracerebroventricularly (Leibowitz, Hammer, & Chang, 1983).

Clonidine (CLON), an alpha-adrenergic agonist (Starke, 1977), elicits feeding when administered peripherally to mice (Beales, Callahan, & Oltmans, 1982), rats (Atkinson, Kirchertz, & Peters-Haefeli, 1978; Mauron, Wurtman, & Wurtman, 1980; Sanger, 1983), and monkeys (Schlemmer, Casper, Narasimhachari, & Davis, 1979). This response appears to be

mediated through an $alpha_2$-adrenoceptor, since peripheral injection of the $alpha_2$-adrenergic receptor blocker, yohimbine, attenuates CLON-induced hyperphagia (Sanger, 1983; Schlemmer, Elder, Casper, & Davis, 1981), while an $alpha_1$-adrenergic antagonist, prazosin, has no effect (Schlemmer, Elder, Casper, & Davis,1981). CLON also elicits feeding when injected intracerebroventricularly (Holman, Shillito, Vogt, 1971; Ritter, Wise, & Stein, 1975), or when injected into the anterolateral hypothalamus (Broekkamp and van Rossum, 1972). It is plausible that CLON's effect is mediated through the alpha-adrenergic PVN system, since PVN injections of CLON also elicit feeding (Fahrbach, Tretter, Aravich, McCabe & Leibowitz, 1980; McCabe, DeBellis & Leibowitz, 1982).

As originally hypothesized, $alpha_1$- and $alpha_2$-adrenoceptors were characterized, respectively, as acting upon pre- and post-synaptic processes (Langer, 1977; Starke, 1977). However, more recent developments suggest $alpha_2$-adrenoceptors may be situated post-synaptically, as well as pre-synaptically (Starke, 1981; Langer, 1981), and CLON binding studies suggest that this adrenergic agonist may act post-synaptically in a number of brain areas, including the hypothalamus (U'Prichard, Bechtel, Rovot, & Synder, 1979). Also, $alpha_2$ sites are found to be particularly dense in medial hypothalamic regions, in contrast to $alpha_1$ sites, which are more diffusely distributed throughout medial and lateral hypothalamic areas (Leibowitz, Jhanwar-Uniyal, Dvorkin & Makman, 1982; Young & Kuhar, 1980).

There is a variety of evidence to suggest that NE, as well as CLON, may be acting post-synaptically within the PVN to stimulate eating behavior. For example, the response to injected NE is not dependent

upon pre-synaptic stores of NE, since it continues to occur after pre-treatment with alpha-methyl-p-tyrosine (Leibowitz, Arcomano & Hammer, 1978), as well as after electrolytic and 6-hydroxydopamine damage to major noradrenergic fiber projections that innervate the PVN (Leibowitz & Brown, 1980b). In contrast to NE, feeding induced by the antidepressants, which appear to act through the release of presynaptic NE, is abolished by alpha-methyl-p-tyrosine injection or brainstem lesions (Leibowitz, Arcomano & Hammer, 1978; Leibowitz & Brown, 1980b).

With regard to CLON, evidence suggests that this drug elicits feeding via the PVN (Fahrbach, et. al., 1980; McCabe, et. al., 1982) and that this drug is also acting post-synaptically. First, the eating response induced by this alpha-agonist can occur at doses (<25 μg; McCabe, et. al., 1982) below the level at which it would be expected to stimulate alpha$_1$ sites (Anden, Grabowska & Strombom, 1976). Second, CLON can also reliably stimulate eating in animals that have received PVN 6-hydroxydopamine injections and consequently experienced a profound depletion of endogenous NE (Leibowitz, Shor-Posner, & Azar, unpublished data). Third, peripheral injections of CLON (50 μg/kg) reduce NE levels, but fail to decrease NE turnover, specifically in the PVN and locus coeruleus, suggesting a post-synaptic site of action, while decreasing NE turnover (a pre-synaptic effect) in several other hypothalamic and extrahypothalamic sites (Jhanwar-Uniyal, McCabe, Levin & Leibowitz, 1983). Finally, pharmacological analysis shows that eating elicited by PVN injections of NE and CLON are <u>both</u> antagonized by alpha$_2$-adrenergic blockers (yohimbine and rauwolscine), but not by alpha$_1$-adrenergic blockers (corynanthine and prazosin), and that PVN injections of yohimbine significantly suppress feeding normally elicited by systemically

injected CLON (Marino, DeBellis, & Leibowitz, 1983).

The present experiments had two major objectives. The first objective was to determine whether CLON stimulates eating through a mechanism similar to NE, that is, via the PVN alpha-adrenergic receptor system. For this aspect of the experiment, the impact of PVN lesions, as compared to other hypothalamic lesions, on feeding induced by peripheral CLON injection was examined. The second major objective of these experiments was to identify the precise course of caudally-directed fiber projections that mediate feeding stimulated by NE and CLON. This objective was achieved by placing KCs at several levels of the neuraxis and testing the impact of these KCs on drug-induced feeding. Results of the lesion studies indicate that PVN lesions, as opposed to dorsomedial nucleus and perifornical lesions, attenuate feeding induced by CLON. The KC findings demonstrate that efferent fibers of the PVN alpha-adrenergic system, which mediate feeding stimulation, take a dorsomedial course through the rostral brain stem and then move laterally at the intercollicular level to pass through the parabrachial region and then caudally towards the dorsal vagal complex.

Experiment 1

In this first study, several dose levels of CLON (0.0-100 μg/kg) were administered to rats in order to assess dosages where CLON stimulates feeding when administered under the present experimental procedures.

Method.

Subjects. Nineteen naive rats, sustaining no surgical procedures, were used. Animals were maintained on the mash diet and water was available ad libitum.

Procedure. Animals were pretreated for one hour with fresh mash before testing began. To reduce spontaneous meals initiated by handling, animals were handled when given the fresh mash and also 30 min later in the middle of the pretreatment period. After pretreatment, animals received an intraperitoneal injection of either normal saline (1 ml/kg) or 1.25, 3.13, 12.50, 50.0, or 100.0 μg/kg clonidine hydrochloride (Boehringer-Ingelheim) dissolved in saline (1 ml/kg). Mash dishes were weighed at time of injection and two hour after injection. Due to the consistency of the mash, spillage was negligible and no correction of food intake measures was warranted. All animals received two tests at each dosage level, and from these scores a mean feeding response for each animal was calculated.

Results and Discussion

Figure 2 presents food intake responses to several dosage levels of peripherally administered CLON. At different dosages, the nineteen rats consumed significantly different amounts of mash by two hr post-injection [$F(5,90) = 27.1$; $p<0.001$]. Dunnett's t-statistic (Winer, 1971) indicated that administration of 12.5 [$t(6,90) = 5.55$; $p<0.01$] and 50.0 μg/kg [$t(6,90) = 7.36$; $p<0.01$] of CLON significantly increased consumption, while injections of 1.25, 3.13, and 100 μg/kg did not increase amount of food intake above baseline level. These results show, as oth-

ers have reported, that peripheral administration of CLON elicits feeding (Atkinson, et. al., 1978; Mauron, et. al., 1980; Sanger, 1983; Schlemmer, et. al., 1979; 1981).

One question is whether computation of an <u>average</u> food intake response ignores the possibility that 2 or 3 administrations of CLON results in the development of tolerance to this drugs effect upon feeding. Data from this dose response study is insufficient to answer this question since animals did not receive more than 2 injections of any dose level. However, a small sample of six different rats (utilized in another experiment) received 3 trials with CLON (50 μg/kg), and failed to exhibit a significant change in feeding response to CLON over trials [$F(2,10)= 2.04$; $p>0.10$]. As in the dose response study, therefore, data in all later studies will not separately consider whether rats show differences in response after first, second, and possibly third administration of CLON, but rather average food intake response to 2-3 tests of each drug will be computed.

Experiment 2

In Experiment 2, electrolytic lesions were aimed at either the PVN, dorsomedial nucleus, or the perifornical area. Since the PVN is the primary site for stimulating eating with central NE injections (Leibowitz, 1978b), it may also mediate CLON-induced feeding and lesions of this site may disrupt feeding stimulated by CLON administration. How destruction of the PFH region or the dorsomedial nuclei affects CLON-induced feeding are also of interest. The PFH region mediates anorexia

induced by dopaminergic and adrenergic agonists (Leibowtiz, 1978a), and the dorsomedial nucleus plays a stimulatory role in feeding (Bernardis, Bellinger, & Brooks, 1979; Dalton, Carpenter, & Grossman, 1981). Results show that total destruction of the PVN abolished feeding to peripherally administered CLON (50 μg/kg), while dorsomedial nucleus and perifornical hypothalamic lesions did not attenuate drug response.

Method

Subjects. A total of 39 rats were used and sustained either sham surgery (n=19), or electrolytic lesions aimed at the PVN (n=8), the dorsomedial nuclei (n=6), or the perifornical region (n=6).

Surgery. Using epoxylite coated electrodes with 0.5 mm exposed tips, lesions were made by passing approximately 1 mA anodal current for 12-15 sec. With the electrode tilted 4° in the lateral direction, PVN lesions were made using coordinates +0.7 mm AP/ 0.8 mm LAT/ -8.7 mm DV. For dorsomedial nucleus lesions, the same 4° electrode angle was used, except that the electrode was placed slightly more posterior to 0.3 mm behind bregma suture (-0.3 mm AP). Perifornical lesions were made with the electrode lowered perpendicular to the skull surface using the coordinates: -0.6 mm AP/ 1.2 mm LAT/ 8.8-9.2 mm DV. Sham animals received similar treatment, except that the electrode was only lowered to within 3.0 mm of the targeted sites, and no current was passed through the electrode. For all surgeries the incisor bar was set 3.1 mm (Krieg) above the interaural line.

Daily Food Intake. During the first three weeks after surgery, daily food intake was measured in some of the rats that sustained PVN, PFH, and DMN lesions. The purpose of these measures were to assess that

all animals were in good health and were able to consume enough mash every day to sustain themselves. It should be noted that in this experiment, and in all subsequent instances where food intake data are reported, groups were not matched for level of food intake or body weight before surgery and therefore caution must be taken when comparing groups sustaining different lesions or KCs.

Test Procedure. The electrolytic lesion experiment used the same procedure as the dose response study, except animals were tested for level of food intake to saline (1 ml/kg) and one dose level of CLON (50 μg/kg/1 ml saline). The 50 μg/kg dose was selected based upon results from Experiment 1 where this dosage produced the highest level of food intake. After completion of all experiments, animals sustaining electrolytic lesions were anesthetized and perfused, and each rats brain was removed for histological study.

Results and Discussion

Figure 3a illustrates an on-target PVN lesion, which destroys all evidence of tissue containing magnocellular PVN neurons. These lesions also extended beyond the PVN, and destroyed at least some of the periventricular zone rostral and caudal to the PVN and produced some damage in the dorsomedial nucleus. Dorsomedial nucleus (DMN) lesions tended to destroy the periventricular area adjacent to the ventricles and portions of the nucleus reuniens in the thalamus just dorsal to the DMN. These lesions usually did not destroy the lateral-most aspects of the DMN. The perifornical lesion extended from the caudal level of the PVN and through the entire level of the ventromedial nucleus and typically destroyed the medial half of the medial forebrain bundle, as well

as the lateral aspects of the ventromedial and dorsomedial nuclei.

Table 2 shows how electrolytic lesions to the PVN, dorsomedial nucleus (DMN), and perifornical (PFH) area affect feeding stimulated by peripherally injected CLON. With the exception of the PVN lesion group, sham animals, as well as DMN and PFH lesion animals, all exhibited significantly greater eating responses following CLON than after normal saline administration. That is, analysis of variance indicated that the group [$F(3,35)= 9.02$; $p<0.001$], drug factor [$F(1,35)= 96.05$; $p<0.001$], and also the group x drug interaction [$F(3,35)= 7.73$; $p<0.01$] were significant. Tests on simple main effects on the drug factor indicated that compared to intake after saline administration, sham [$F(1,35)= 39.11$; $p<0.001$], DMN lesion [$F(1,35)= 55.05$; $p<0.001$], and PFH lesion rats [$F(1,35)= 9.33$; $p<0.01$] all exhibited significant increases in food intake after CLON. In contrast, the PVN lesion animals failed to significantly increase consumption after CLON [$F(1,35)= 1.80$; $p>0.10$]. Comparisons using difference scores indicated the increase in consumption after CLON (minus saline intake) for the sham group was significantly greater than the PVN lesion group [$F(1,35)= 6.68$; $p<0.05$], but less than the DMN lesion group [$F(1,35)= 7.42$; $p<0.05$].

The effects of off-target and incomplete lesions on CLON feeding response are also of interest. It appears that almost the entire PVN region must be destroyed in order to observe a loss of the feeding response to CLON. Animals sustaining total unilateral PVN lesions, but minimal or only partial contralateral damage generally did not exhibit a decreased feeding response (data not shown). Finally, animals that sustained lesions that were dorsal to the PVN (Figure 3b), or that were

ventral to the PVN and destroyed anterior hypothalamic tissue, exhibited feeding responses similar to sham animals.

Table 3 presents the average daily food intake of lesioned and sham rats during the first three weeks after surgery. Analysis of variance indicated that the sham, PVN lesion, and DMN lesion groups differed significantly [$F(2,21)= 3.87$; $p<0.05$] and that the groups exhibited a significant change in average daily amount of intake over the three week period [$F(2,42)= 4.91$; $p<0.05$]. Inspection of Table 3 shows that the PVN lesion group consumed significantly more mash than the sham group. The highest level of food intake for the PVN lesion group was during the first week after surgery, and this apparently accounts for the significant overall decrease in consumption for the three groups over weeks. In the present experiments, measures of water intake were not made to assess daily water consumption by PVN lesioned rats. These animals did not appear to suffer from diabetes insipidus, however, since they did not exhibit polyurea. Others have reported that this lesion increases daily water intake, but to a degree proportionate with food intake, and dibetes insipidus is not observed (Aravich, 1983; Leibowitz, Hammer, & Chang, 1981). Table 3 also shows the PFH lesion group consumed significantly less food than their sham animals. These results concur with the findings of others that PVN lesions increase daily food intake (Aravich, 1983; Eng, Gold, & Nunez, 1979; Leibowitz, Hammer, & Chang, 1981; Sclafani & Aravich, 1983), while lesions of the perifornical area (Morgane, 1961) and midlateral hypothalamus (Oltmans & Harvey, 1976) result in hypophagia. When rats are maintained on chow diets, DMN lesions produce transient (Dalton, et. al., 1981) or chronic hypophagia (Bernardis, et. al., 1979). The present food intake data does not concur with these

reports. This may be related to the fact that the DMN lesion in this study did not destroy the entire DMN. Also, perhaps the use of the palatable mash diet, that is high in carbohydrates, masked the initial hypophagia. DMN lesioned rats preferentially decrease intake of high fat and high protein diets, but not high carbohydrate diets, compared to sham rats in a self-selection feeding paradigm (Bernardis & Bellinger, 1981). As noted earlier, food intake data is reported here primarily to verify that brain manipulations were not excessively debilitating. Extreme hypophagia would suggest that an attenuated drug response is a consequence of general malaise. These data should be interpreted with caution since body weight is not available to aid in assessing the effect of brain manipulations upon daily food intake. Instances of hyperphagia, for example, may result from animals attempting to recover their original pre-surgical body weight.

The present findings provide evidence that suggests the PVN is the principal mediator of CLON-induced feeding. Of three hypothalamic regions examined, only electrolytic lesions of the PVN appear to attenuate CLON-induced feeding. The present results do not, however, definitively show that PVN lesions completely abolish feeding stimulated by CLON since only one dose level was examined. The 50 µg/kg dose was selected for this study based upon the results of Experiment 1 which indicated this dose produced the greatest feeding response in terms of amount of mash consumed by 2 hr post-injection. It is possible that higher dosages of CLON may stimulate feeding in PVN lesioned rats. However, higher dosages of CLON are known to increase sedation and one would expect that PVN lesioned rats would be as susceptable to sedation as sham animals (Clough & Hatton, 1980; Holman, Shillito, & Vogt, 1971).

Conversely, PVN lesions may have indirectly attenuated feeding at the 50 ug/kg dose by increasing sensitivity to the sedative properties of CLON. Observation of the PVN lesioned animals, however, indicated that these rats were not grossly sedated to the extent that they were ataxic.

Lesions of the DMN and PFH region did not abolish CLON response and, in fact, results suggest that the DMN lesion, perhaps by damaging periventricular catecholamine afferents to the PVN (Lindvall & Bjorklund, 1974; however, also see Clavier, Chambers, & Coscina, 1983) enhanced drug response via denervation supersensitivity (Reisine, 1981). It is possible, however, that facilitation resulted from another mechanism not directly related to supersensitivity (Schwartz, Costentin, Martes, Protais, & Baudry, 1978). It is interesting that animals sustaining perifornical lesions are responsive to CLON. The PFH region is known to be the primary site for suppression of food intake in hungry rats by means of central injections of amphetamine (Leibowitz, 1975a) and catecholamines (Leibowitz & Rossakis, 1979b). The present study suggests that alpha-adrenergic stimulation of feeding does not depend upon the integrity of this PFH feeding suppression system. Also of interest is the fact that in spite of their chronic hypophagia (Table 3) and lower body weights (data not shown), animals with PFH lesions responded to CLON. This is consistent with an earlier study (Berger, Wise, & Stein, 1971) that anorectic LH-lesioned animals were also responsive to alpha-adrenergic stimulation of feeding via NE injections to the ventricles.

Experiment 3

The previous experiment indicates that destruction of the PVN disrupts CLON-induced feeding. In the next three experiments, an attempt was made to delineate possible efferent pathways that mediate noradrenergically-stimulated feeding via the PVN. Animals received either PVN injections of NE or CLON, or intraperitoneal administration of CLON, and sustained coronal knife cuts (KCs) in the caudal hypothalamus, midbrain, or pons. If a major efferent pathway mediating noradrenergic feeding courses through a region of the brain where a KC has been made, then feeding stimulated by central NE and CLON injections will be disrupted. Results of the immediately following study show that KCs in the caudal hypothalamus do not disrupt CLON- nor NE-induced feeding.

Method

Subjects. Rats sustained either midline KC (n=11), perifornical KC (n=9), or sham surgery (n=11). An additional 30 rats received PVN cannula implants and either midline KC (n=13) or sham surgery (n=17). No PVN cannulated rats received perifornical KCs.

Surgery. Anesthetized rats received a cannula implant, with the cannula tip directed to the PVN, using the procedure outlined in the General Methods section (cf., p. 19).

The knife cut surgical procedure (Sclafani, 1971) was used to produce a midline KC, where the guide tip was placed +5.2 mm anterior to lambda line/ 1.4 mm LAT/ -9.2 mm below skull surface, and the knife was raised and lowered 4.0 mm. Other rats sustained perifornical KCs (PFH

KCs) using coordinates: 3.0 mm caudal to bregma/ 2.5 mm LAT/ -9.6 mm below skull surface, and the knife was moved dorsally 3.4 mm. Sham animals were similarly treated, where the guide was lowered into the brain using either the coordinates for the midline KC or the PFH KC, but the knife was not extended. For all surgeries the nosebar was set 0.0 mm with respect to interaural line.

Test Procedure. The testing procedure for peripherally administered CLON, with the noncannulated KC and sham animals, was identical to the method used in the dose response and lesion studies of Experiments 1 and 2. Animals were given fresh mash for one hr before testing, were injected with either CLON (50.0 µg/kg) or saline (1 cc/kg), and food intake measures were made two hr postinjection.

Using the central drug-injection procedure outlined in the General Method section, animals with PVN cannulas were tested over a period of three weeks for level of food intake response to 40 nM NE (12.8 µg l-norepinephrine bitartrate in 0.5 µl sterile saline), 7.5 nM CLON (2 µg clonidine HCl in 0.5 µl sterile saline), and 0.5 µl sterile saline (pre-KC). The three drug treatments were administered once in the first pre-KC week, and then again (but in different orders of presentation) to each animal during the second and third pre-KC weeks. Selected rats (exhibiting >3.0 g response to either NE or CLON) were paired in terms of level of response to NE and CLON and one animal in the pair was each assigned to the KC or sham group. After KC surgery, animals again underwent the three-week (post-KC) testing procedure.

Results and Discussion

In order to simplify the presentation of data involving central drug adminstration, raw data results were first examined with two questions in mind. First, it would simplify matters if saline trials in the central drug injection studies were subtracted from each rats mean NE and/or CLON score (difference scores) so that within- and between-group comparisons before and after KC (or sham) surgery would involve single data points, rather than saline plus drug scores. This appears to be a justified procedure since the majority of rats do not eat after saline injection in the testing paradigm used here, and saline scores are generally negligible. For example, the average amount of mash comsumed after PVN injections of saline for the 22 sham animals utilized in Experiments 4 and 5 (see below) were 0.2 ± 0.1 (mean $\pm$ standard error of the mean) and the average for 15 of the 22 rats was zero. Within- and between-group comparisons, therefore, will utilize difference scores where each rats average feeding response to saline is subtracted from their NE and CLON scores. (Tables also provide raw data.)

A second question is whether animals tend to exhibit a significant decrease (or increase) in feeding response to central drug injections over time, so that the pre-KC and post-KC feeding responses should be reported on a weekly basis rather than as single pre-KC and post-KC variables. Analysis of NE response of the sham groups in Experiments 4 and 5 over Weeks 1, 2, and 3 failed to suggest that this variable should be taken into consideration. That is, when NE response during the three (pre-KC) weeks was treated as a trial factor, analysis of variance failed to show a significant change [$F(2,18) = 1.54$; $p > 0.10$]. Therefore,

data will not be presented for the separate weeks as a nested factor under either the pre- or post-KC variables.

Figure 4 provides a schematic representation of the midline and PFH KCs. Both KCs severed tissue in the caudal hypothalamus, but differed with respect to the medial-lateral extent of the cuts. Midline cuts severed tissue from midline to 0.7 mm lateral to midline. The PFH KC severed tissue extending from 0.7 mm to 1.1 mm lateral to midline. In terms of dorsal-ventral extent, both cuts severed tissue almost from the base of the brain (or to the base of the brain) to the dorsal-most aspect of the hypothalamus.

Table 4 presents the average daily mash intake of the sham and KC groups during the initial three weeks after KC surgery. As can be seen, the PFH KC group exhibited a significant degree of hypophagia during the first week post-KC, while the midline KC failed to produce any significant change in food intake. These data concur with other studies that report coronal posterior hypothalamic KCs do not lead to significant changes in daily consumption in male rats (Aravich, 1983; Grossman & Hennessy, 1976; Sclafani, 1982).

The results of peripheral administration of saline and CLON to midline, PFH KC, and sham animals is presented in Table 5. Analysis of variance indicated a significant group effect [$F(2,28) = 5.99$; $p<0.01$], as well as drug effect [$F(1,28) = 102.42$; $p<0.001$], but the interaction of these factors was not significant [$F(2,28) = 2.24$; $p>0.10$]. Individual comparisons indicated that all three groups consumed significantly more mash after CLON than after saline injection [$F(1,28) = 21.23$; 57.77; 29.04; $p<0.001$; sham, midline KC, and PFHKC groups, respectively). The

increase in amount consumed by the three groups in terms of difference scores, however, was not significantly different [$F(2,29)=2.89$; $p>0.10$]. The significant group factor results from the midline KC group exhibiting higher overall consumption than the sham group [$F(1,28)= 11.76$; $p<0.01$].

Tables 6 and 7, respectively, present the results of PVN injections of NE and CLON. Analysis of variance of NE results indicated a significant pre-/post-KC factor [$F(1,28)= 4.61$; $p<0.05$], while the group and group x pre-/post-KC interaction were not significant [$F(1,28)= 2.80$; 3.53; respectively; both $0.05<p<0.10$]. Tests on simple main effects showed the sham group, but not the midline KC group, exhibited a significant decrease in NE response after surgery [$F(1,28)= 9.35$; $p<0.01$; $F(1,28)= 0.31$; respectively]. With respect to central CLON administration (Table 7), no significant changes between the sham and midline KC groups were observed over the entire experiment [group factor: $F(1,28)= 0.20$; pre-/post-KC factor: $F(1,28)= 1.20$; interaction: $F(1,28)= 0.06$].

The preceding experiments show that coronal KCs in the posterior hypothalamus do not disrupt feeding responses to NE, administered centrally, and CLON, injected either centrally or peripherally. These results appear congruent with other recent findings (Aravich, Sclafani, and Leibowitz, 1982) which found coronal KCs in the posterior hypothalamus, as well as parasagittal perifornical KCs, fail to influence feeding stimulated by NE injections to the PVN. The present findings, in fact, extend these results since eating to centrally and peripherally administered CLON were not disrupted by KCs in this region. The sham group, in the NE experiment, exhibited a significant decrease

in feeding response to NE after KC surgery. The cause of this effect is not known but has been observed by others (Aravich, et. al., 1982). Decreased NE response may not result from the surgical procedure per se, but to the gradual development of tolerance to NE stimulation after repeated central injections, or to changes in the patency of the cannula preparation. For example, damage to neural tissue around the cannula tip may induce gliosis, which may partially prevent NE from reaching neuronal tissue, or the brain may recede slightly from the cannula. It is estimated that only 1- 2.7% of centrally-infused drugs, in a 0.5 µl injection volume, actually reach tissue surrounding the cannula tip (Myers & Hoch, 1978). In the pre-/post-KC experiments reported later (Experiment 5), sham groups also exhibit some decrements in NE response over time, but it was not as large as the change seen here. It should be noted that the midline KC can produce a significant disruption of feeding elicited by a higher 10 µg central injection of CLON (data not shown). Although no empirical measures were made, observation of the midline KC animals indicated that these animals exhibited pronounced sedation after 10 µg PVN injections of CLON. This suggests that the observed response decrement in the KC group in this case was not specific to eating since sedation competed with the capability of these rats to eat (Clough & Hatton, 1981; Holman, et. al., 1971). Similar to feeding stimulation with CLON, sedation is also mediated by alpha$_2$ adrenergic receptors (Drew, Gower & Marriott, 1979; Strombom & Svensson, 1981; Timmermans, Shopp, Kwa & Van Zwieten, 1981).

Experiment 4

In spite of the fact that KCs in the posterior hypothalamus did not efectively disrupt feeding induced by NE and CLON, KCs were next placed in the midbrain and rats were tested for feeding response to peripheral administration of CLON. This study is important since a major efferent pathway of PVN fibers traverses the region just dorsal to the medial lemniscus in the midbrain (Conrad & Pfaff, 1976; Swanson, 1977). Animals received one of three midbrain KCs, that differed with respect to anatomical placement. Similar to the results from the previous study with hypothalamic KCs, midbrain cuts did not disrupt the ability of CLON to elicit feeding.

Methods

Subjects. A total of 29 rats received either Dorsal (n=9), Ventral (n=10), Far-Ventral (n=3), or sham KC surgery (n=7).

Surgery. The three midbrain manipulations, the Dorsal, Ventral, and Far-Ventral KCs, were all produced with the nosebar set 4.0 mm below interaural line. Coordinates for the Far-Ventral midbrain KC were 6 mm posterior to bregma/ 2.1 mm lateral to midline/ and the knife was extended while the tip of the guide was moved between 8.7 to 6.0 mm below skull surface. The coordinates for the Ventral midbrain and the Dorsal midbrain KCs were identical to those used for the Far-Ventral midbrain KC, except the dorsal-ventral extent of the cuts, respectively, were 7.0-4.0 mm and 6.9-3.0 mm. Sham animals underwent surgery where the guide was lowered into the brain 6 mm behind bregma and 2.1 mm lateral to midline, but the knife was not extended.

Behavioral Testing. Following three to five days recovery, animals were tested for level of feeding responses to saline and CLON utilizing the previously outlined procedure. That is, on each testing day animals were given fresh mash for one hr and handled. Following this pretest satiation period, animals were then injected with either saline or CLON (50.0 μg/kg) and two hr later food intake was measured. At the end of their experimental testing, animals were sacrificed and perfused, and brains were removed for histology.

Results and Discussion

Figure 5 depicts the positions of the three midbrain KCs. Generally, all three midbrain cuts extended from 0.7 mm to 1.1 mm lateral to midline. The Dorsal cut, positioned just caudal to the level of the red nucleus, severed tissue ventrolateral to the mesencephalic central gray, from the upper half of the red nucleus and 1.5 mm dorsal. The Ventral midbrain KC, positioned just caudal to the red nucleus, extended from 0.7 mm- 1.4 mm lateral to midline. This cut generally did not appear to significantly affect tissue below or through the medial lemniscus, but particularly severed the region immediately dorsal to the medial lemniscus. The Far-Ventral KC, in contrast, severed tissue only below the medial lemniscus, in the ventral tegmental area and the most medial portion of the substantia nigra, pars reticulata.

Table 8 shows that the sham and three midbrain KC groups all exhibited significant feeding responses to CLON. That is, the sham, Ventral, and Dorsal KC groups consumed more mash after CLON injection [drug factor: $F(1,23)= 47.16$; $p<0.001$], and failed to exhibit significant group or group x drug differences [$F(2,23)= 0.91$; $F(2,23)= 0.82$, respec-

tively]. The Far-... ... ...c group, examined separately due to small sample size, also significantly increased food intake after CLON administration [t(2)=9.21; p<0.02].

Table 9 presents the daily food intake of the midbrain KC and sham groups. Individual comparisons indicated that the dorsal KC rats were hyperphagic, compared to sham rats, during Weeks 2 and 3 (see table legend). The observed increased feeding of the dorsal KC group appears to concur with others that coronal KCs in the dorsolateral tegmentum produce increased daily consumption (Box, Bascom, & Mogenson, 1979; Grossman & Grossman, 1977; Oltmans, Lorden, & Margules, 1977; Solafani & Berner, 1977). The results with respect to the Dorsal KC are of interest since they are similar to the findings of Aravich (Aravich, et. al., 1982) where rats with KCs in the hypothalamus which induced hyperphagia (and obesity) continued to eat following alpha-adrenergic stimulation of feeding via PVN NE injections. As this other study suggested, it appears that KC-induced hyperphagia can be functionally dissociated from the system that mediates alpha-adrenergically stimulated feeding (Aravich, et. al., 1983).

Experiment 5

A final series of KCs were made in the pontine region in order to assess how these KCs affect feeding stimulated by NE and CLON. Separate groups of animals sustained either a more rostrally-placed pontine KC, positioned just in front of the level of the locus coeruleus (LC), labeled the "rostral pontine KC," or a more caudally placed cut just

behind the level of the LC, labeled the "caudal pontine KC."

Method

Subjects. Noncannulated rats received either the rostral pontine cut (RPKC; n=11), the caudal pontine KC (CPKC; n=14), or sham surgery (n=14) and were subsequently tested for feeding response to peripheral CLON. A total of 40 PVN cannulated rats were utilized in a pre-/post-KC study and received either RPKC (n=9), CPKC (n=10), or sham surgery (n=22).

Surgery. Animals receiving central injections of NE and CLON received PVN cannula implants as described in the General Methods. The RPKC was produced at the level of lambda line, 2.5 mm lateral to midline and the dorsal-ventral extent of the cut was produced in two stages. The guide was first lowered 7.0 mm below skull surface, the knife extended, and a cut made from this point dorsally to 3.6 mm below skull surface. The knife was then retracted, the guide lowered to 8.0 mm below skull surface, and the knife was extended and a cut made at this point and dorsally to 4.8 mm below skull surface. This two-stage cut was made on both sides of the brain. The CPKC, produced in other animals, was similarly made using coordinates: 2.5 mm caudal to lambda line and 2.8 mm lateral to midline. The dorsal-ventral extent of the two-stage CPKC were 8.0 mm below skull surface up to 4.8 mm, and 7.4 mm to 4.3 mm with respect to guide tip, and the knife, 1.5 mm in pitch, extended either 1.8 or 2.5 mm lateral. Two-stage cuts were required since the wire knife was unable to sever tissue around the brachium conjunctivum in the case of the RPKCs, and the facial nerve in the case of the CPKCs. Sham animals underwent similar procedures, however, the

knife was not extended and the guide was lowered to points 3.0 mm below skull surface.

Testing Procedure. Noncannulated KC rats were tested for feeding response to peripherally injected CLON as outlined earlier, and PVN cannulated animals were tested for level of feeding response to centrally administered saline, NE, and CLON for three weeks pre-KC, assigned to the KC or the sham condition, and re-tested for three weeks post-KC. Therefore, in terms of comparisons of results with peripheral and central injection experiments, both types of experiments involved tests during the three week period immediately after KC surgery.

Results and Discussion

Histological study indicated that animals sustaining KCs could be categorized into four subgroups according to the anterior-posterior position of their KC, that is, RPKC or CPKC, but also in terms of medial-lateral extent of the cut (see Figures 6 and 7). Animals with either the rostral or caudal pontine KC, therefore, were further categorized with respect to placement of their KC either medial or lateral to the LC. The "medial RPKC" extended either from midline, or 0.5 mm lateral to midline, and as far lateral as the level of the LC (1.3 mm LAT; Pellegrino, Pellegrino & Cushman, 1979). Other animals sustained RPKCs that were primarily lateral to the level of the LC (extending 1.3-1.9 mm lateral to midline) and were designated "lateral RPKCs". The lateral RPKC severed fibers just dorsal to the brachium conjunctivum at this level, but extended largely ventral to this fiber tract. The cut severed tissue within the ventral parabrachial nucleus,

and extended below this structure. Similarly, CPKC rats (see Figure 7) exhibited cuts either from midline (or beginning 0.5 mm lateral to midline) to the level of the LC (medial CPKC), or KCs lateral to the LC, extending from 1.3-1.9 mm lateral to midline (lateral CPKCs). The CPKC was positioned just caudal to the motor nucleus of the fifth cranial nerve. This KC extended from the ventral extent of the motor nucleus of the fifth nerve and also dorsal to it so that it almost reached the dorsal surface of the brain.

Table 10 presents the food intake measures of the sham, medial RPKC, and lateral RPKC groups after peripheral injection of CLON and saline. Analysis of variance of the sham and lateral RPKC groups indicated that the overall group main effect [$F(1,14)= 15.47$; $p<0.01$], as well as the drug factor [$F(1,14)= 21.47$; $p<0.001$] were significant. The group x drug interaction was also significant [$F(1,14)= 10.11$; $p<0.01$], and main effect tests showed that the sham group consumed significantly greater amounts of mash after CLON [$F(1,14)= 30.53$; $p<0.001$], while the lateral RPKC group failed to show a significant increase in feeding to CLON than after saline injection [$F(1,14)=1.06$; $p>0.20$]. The sham and lateral RPKC groups also differed when directly compared using difference scores [$F(1,14)= 10.25$; $p<0.01$]. The small number of medial RPKC animals exhibited feeding responses to CLON, however, this increase failed to reach significance [$t(2)= 1.41$; $p<0.20$]. The upper panel of Figure 8 presents actual tracings of three lateral rostral pontine KC animals that exhibited negligible eating after peripheral injection of CLON.

Table 11 presents the effects of RPKCs upon NE-induced feeding. As

in Experiment 3, difference scores between NE and saline baseline are presented and are used to compare groups. Analysis of variance of response to NE indicated the sham and lateral RPKC groups failed to differ significantly [$F(1,14)= 3.91$; $p>0.10$], but that the pre-/post-KC factor [$F(1,14)= 11.79$; $p<0.005$] and the group x pre-/post-KC interaction [$F(1,14)= 18.04$; $p<0.005$] were significant. Tests on main effects showed that before KC surgery the sham and lateral RPKC groups failed to differ from each other [$F(1,19)= 0.05$], but that after KC surgery the lateral RPKC group exhibited a significantly attenuated response to NE compared to the sham group [$F(1,19)= 11.75$; $p<0.005$]. The medial RPKC animals (analyzed separately) failed to exhibit a significant decrease in feeding response to NE post-KC [$t(2)= -0.47$]. Figure 9a shows a photomicrograph from an animal that exhibited a diminished NE response after KC surgery.

Response level to centrally-injected CLON (Table 12) involved two animals which sustained lateral RPKCs, and both animals exhibited large decreases (-75% and -95%) in CLON response post-KC, while sham animals did not exhibit a significant decrease in response level after surgery [$t(6)= -0.91$). Similarly, the two medial RPKC rats exhibited decreases in response level to CLON post-KC, but this decrease did not appear to be as severe (-52% and -32%) as the decrease seen in lateral RPKC rats.

Table 13 presents the feeding response to CLON administration of the sham and CPKC rats. Analysis of variance of the sham and lateral CPKC groups indicated that the groups failed to differ significantly [$F(1,15)= 0.69$], but that overall there was a significant drug effect [$F(1,15)= 7.93$; $p<0.01$], as well as a significant group x drug interac-

tion [$F(1,15)= 4.93$; $p<0.05$]. Tests on main effects showed that sham animals consumed a significantly greater amount of mash after CLON than after saline [$F(1,15)= 11.97$; $p<0.01$], but the lateral CPKC rats failed to do so [$F(1,15)= 0.90$]. The lateral CPKC group, in terms of difference scores, exhibited a significantly smaller feeding response to CLON than the sham rats [$F(1,15)= 8.78$; $p<0.01$]. With respect to the animals sustaining the medial CPKC (n=3), although increased intake after CLON was observed, this response is not significantly greater than after saline injection [$t(2)= 2.55$; $p>0.20$].

Table 14 presents the results of administering NE to sham and CPKC rats. Using difference scores to compare groups, the sham, medial CPKC, and lateral CPKC rats did not differ overall [$F(2,18)= 1.13$; $p>0.30$], but exhibited a significant pre-/post-KC effect [$F(1,18)=8.88$; $p<0.01$], as well as a significant group x drug interaction [$F(2,18)= 4.58$; $p<0.03$]. Main effect tests indicate that sham [$F(1,18)= 0.01$], as well as the medial CPKC rats [$F(1,18)= 0.82$] failed to exhibit a significant decrease in feeding response to NE post-KC, while the decrease observed in the lateral CPKC group was significant [$F(1,18)= 13.70$; $p<0.005$]. Also, the post-KC scores of the lateral CPKC group [$F(1,36)=5,78$; $p<0.05$], but not the medial CPKC rats [$F(1,36)= 2.22$; $p>0.10$], were significantly lower than sham rats. Figure 8 provides a tracing of three rats that failed to exhibit feeding to peripheral administration of CLON, and Figure 9b presents an CPKC rat that did not respond to NE after KC surgery.

Table 15 presents the feeding responses of sham and medial CPKC rats to PVN injections of CLON. (No lateral CPKC rats were tested.) The

sham and medial CPKC rats did not differ from each other [$F(1,11)= 0.004$], and failed to exhibit differential responses to CLON after surgery [interaction factor: $F(1,11)= 0.007$]. Combined, however, these groups showed a significant and relatively equivalent decrease in response magnitude after surgery [pre-/post-KC factor: $F(1,11)= 6.24$; $p<0.003$].

Table 16 shows that all groups significantly increased their food intake over Weeks 1-3. The data suggest also that the RPKC group gradually become hyperphagic. [Although the interaction effect failed to reach significance $F(4,52)= 1.69$; $p>0.15$].

Knife cuts in the pontine region appear to sever crucial fibers participating in noradrenergically-stimulated feeding. Rostral pontine KCs, severing tissue 1.3-1.9 mm lateral to midline in the region of the parabrachial nucleus, just rostral to the LC, and caudal pontine KCs, extending 1.3-1.9 mm lateral to midline, just behind the motor nucleus of the fifth cranial nerve, decreased responsiveness to peripherally administered CLON and feeding to PVN injections of NE.

General Discussion

The present series of experiments have utilized the ability of CLON to elicit feeding in order to localize components of a PVN feeding circuit. The PVN appears to be crucial for eliciting feeding with CLON, since electrolytic lesions of this brain site significantly attenuated drug-induced feeding. CLON was used in conjunction with central PVN injection of NE to then describe a descending PVN feeding circuit. KCs

in the pontine region decreased feeding responses to peripherally administered CLON as well as to PVN injections of NE.

Figure 2 demonstrated that peripheral injections of CLON increase feeding in a dose-dependent manner. The dose response curve is quite similar to the results of Sanger (1983), where by 2 hr postinjection, satiated rats ate when CLON dosage was either 0.01 or 0.03 mg/kg, but not after receiving 0.1 mg/kg of CLON. Rats do eat following a 0.1 mg/kg administration of CLON by the fourth hr post-injection (Sanger, 1983). Higher doses are increasingly sedative and this most likely explains the failure to initially observe eating after high doses (Debarre & Schmitt, 1971; Drew, et. al., 1979; Holman, et. al., 1971).

Autoradiography and immunohistochemistry have been applied to delineate PVN efferent fibers as they descend through the neuraxis and, generally, two routes are seen (Buijs, 1978; Conrad & Pfaff, 1976; Swanson, 1977; Sofroniew & Weindl, 1978; Saper & Loewy, 1976). Most PVN efferent fibers course laterally from the PVN to enter the medial forbrain bundle and descend through the ventral half of the neuraxis. A second, smaller projection, however, courses directly caudal in the periventricular hypothalamus and mesencephalic periventricular area, and then through the midbrain central gray and pontine central gray just medial and anterior to the LC. Both caudally-directed PVN systems then terminate in the LC, the parabrachial nucleus, and the dorsal vagal complex, as well as continue to the spinal cord.

Knife cut placements in Experiment 3 attempted to disrupt ventrally-coursing PVN efferents as they traverse the medial posterior hypothalamus. These KCs were unable to disrupt feeding elicited by PVN

injections of NE and CLON, and peripherally injected CLON. Parasaggital KCs just lateral to the PVN that sever efferents entering the medial forbrain bundle likewise do not attenuate eating stimulated by PVN NE injections (Aravich, et. al., 1982). The results of the present study, in conjunction with the findings of Aravich (Aravich et. al., 1982), are significant since they indicate that PVN efferents coursing through the medial ventral hypothalamus and the medial forebrain bundle do not play a direct role in NE feeding.

The present findings leave open the alternative that the second descending efferent system of the PVN, which courses directly caudal and medially through the periventricular area, may mediate NE feeding. Consistent with this possibility, Experiment 4 reported that extensive KCs in the midbrain, which severed ventrally-coursing fibers of the PVN, but did not affect the midbrain periventricular system, did not disrupt CLON feeding. More recently, direct evidence confirms that the smaller descending periventricular PVN system mediates noradrenergically-stimulated feeding, since KCs that sever the periventricular region in the midbrain abolish feeding to PVN injections of CLON and NE (Weiss & Leibowitz, 1983).

In contrast to the lack of effect following midbrain KCs, pontine KCs were found to effectively alter CLON- and NE-induced feeding. That is, both the more laterally placed rostral and caudal pontine KCs attenuate feeding that normally follows NE injection to the PVN, and peripherally injected CLON. These results support a caudally projecting circuit that, at midbrain levels, travels through the perventricular gray (Weiss & Leibowitz, 1983), and at the collicular level apparently

takes a sharp lateral course to pass through the parabrachial region just rostral to the level of the LC. Crucial fibers then continue in the caudal direction just lateral and ventral to the LC. In support of this hypothesized route of projection are anatomical studies that have described PVN efferents that descend through the periventricular system and terminate in the parabrachial nuclei and dorsal vagal complex (Buijs, 1978; Conrad & Pfaff, 1976; Swanson, 1976).

How noradrenergic stimulation of the PVN elicits eating via this hypothesized descending PVN-brain stem system has not been elucidated. One possibility is that NE injections to the PVN stimulates the vagus nerve to release insulin (or possibly some other substance) that subsequently elicits feeding (see discussions by Gold, Jones, & Sawchenko, 1977; Leibowitz, 1978). As noted by one study, a relationship between hypothalamic stimulation and insulin secretion has a long history (Sawchenko, Gold, & Leibowitz, 1981). For example, Vonderhae (1937) proposed stimulation of the PVN activates caudal brainstem regions that mediate insulin release. Barris and Ingram (1936) showed lesions in the vicinity of the PVN produced hypoglycemia. More recently, PVN lesions have been shown to induce hyperinsulemia (Steves & Lorden, 1982), and injection of NE in the hypothalamus releases insulin in less than one minute (DeJong, Strubbe, & Steffens, 1977). Finally, stimulation of the dorsomedial nucleus of the vagus (Larsson, 1954) and vagus nerve (Penaloza-Rojas, Barrera-Mera, & Kubli-Garfias, 1969) elicits feeding. Congruent with this model, Sawchenko, Gold, and Leibowitz (1981) showed that NE-induced feeding is at least partially mediated by an efferent vagal mechanism. Peripheral administration of atropine methyl sulfate, which blocks efferent vagal release of insulin, abolished NE feeding

normally elicited by NE injections to the PVN. Also, coeliac vagotomy which severed fibers specifically to the pancreas (and some fibers to the small intestine) inhibited NE-induced feeding, while a combined hepatic plus gastric vagotomy did not significantly attenuate NE induced feeding. The results of several related studies, however, appear inconsistent with proposals that CLON elicits feeding by stimulating pancreatic secretion. For example, CLON injected intracerebroventricularly inhibits pancreatic secretion (Roze, Chariot, Appia, Pascaud, & Vaille, 1981) and alpha$_2$ -adrenergic receptors located in pancreatic tissue also play an antisecretory function (Nakadate, Nakaki, Muraki, & Kato, 1980a,b). An efferent PVN-vagal nerve mechanism is congruent, however, with neuroanatomical descriptions since the PVN is the sole forebrain projection to the dorsal motor nucleus of the vagus nerve (Rogers, Kita, Butcher, & Novin, 1980) and this projection is monosynaptic (Saper, Loewy, & Swanson, 1976).

The ability of pontine KCs to interfere with noradrenergic stimulation of feeding may not depend solely upon disruption of a monosynaptic descending PVN circuit, but may also result from disruptions of multiple brainstem systems that coordinate eating and/or also require severing afferents to the PVN. Several authors have emphasized the role played by sensorimotor systems in appetitive benavior (Bindra, 1978; Jacquin & Zeigler, 1983; Pfaff, 1980; Schallert, DeRyck, & Teitelbaum, 1980; Teitelbaum, 1982). Both pontine KCs apparently sever fibers interconnecting the parabrachial region, the nucleus of the solitary tract, and the dorsal motor nucleus of the vagus (Norgren, 1978; Norgren & Leonard, 1973; Ricardo & Koh, 1978; Rogers, et. al., 1980; Saper & Loewy, 1980). Taste, oral somatosensory, and visceroceptive information mediated by

these structures is crucial for coordinated and sustained feeding (Jacquin, 1983; Jacquin & Zeigler, 1982, 1983; Miller, 1981; Novin & VanderWeele, 1977). Likewise, these KCs sever reciprocal afferent projections ascending from medullary structures to the PVN and other forebrain structures (Blessing, Jaeger, Ruggiero, & Reis, 1982; Berk & Finkelstein, 1981; Tribollet & Driefuss, 1981; Saper & Loewy, 1980; McKellar & Loewy, 1981; Ricardo & Koh, 1978; Swanson & Hartman, 1980; Swanson & Sawchenko, 1982; 1983). Though decerebrate rats demonstrate adequate capacities for ingesting and rejecting food (Grill & Norgren, 1978), food seeking depends upon a variety of forebrain structures (Swanson & Mogenson, 1981), but not solely the hypothalamus (Norgren & Grill, 1982). The KCs may have disrupted noradrenergically stimulated feeding, therefore, because they interrupted crucial medullary-forebrain connections that coordinate "anticipatory" mechanisms involved in food seeking (Rogers, et. al., 1980).

It is evident that severing fibers of this PVN feeding circuit are not essential to feeding behavior since the pontine KCs and periaquaductal cuts (Weiss & Leibowitz, 1983) that interfere with PVN-stimulated feeding permit these animals to survive and maintain their daily food intake. The PVN, however, due to its pivotal role of receiving and sending fibers from both autonomic and neuroendocrine systems (Sawchenko & Swanson, 1981), plays a part in monitoring and coordinating responses to metabolism and food intake. These KCs are sufficient to disrupt a neural system apparently crucial in the initiation stage of feeding stimulated by catecholamines.

Figure 2. Dose response of CLON (0.0-100.0 μg/kg) in satiated rats. Points represent the mean (±standard error of the mean: SEM) mash intake of 19 rats. Administration of 12.5 and 50.0 μg/kg dosages of CLON resulted in significantly greater amounts of food intake (2 hr post-injection) than was seen after saline administration (*: $p<0.01$, compared to intake after saline administration). Administration of 1.25, 3.13, and 100 μg/kg of CLON did not significantly increase consumption.

Figure 3. Photomicrograph of PVN electrolytic lesion and a lesion just dorsal to the PVN. Figure 3a presents an animal that sustained bilateral PVN lesions and did not respond to peripheral administration of CLON. Figures 3b is a photograph from an animal whose lesion was localized just dorsal to the PVN. This animal exhibited a normal feeding response to CLON.

Table 2. Food intake responses (grams) to normal saline and CLON, 2 hr postinjection (mean±SEM). Rats sustaining sham treatment, DMN lesions, or PFH lesions exhibited significant increases in food intake after CLON injection, compared to their food intake response after saline injection (**$p<0.001$; *$p<0.01$). PVN-lesioned animals, in contrast, failed to exhibit significant increases in food intake after CLON ($p>0.10$). The increase in consumption after CLON compared to after saline (in terms of difference scores) for the DMN lesion group is significantly greater than the sham group (#$p<0.05$), and the small increase in consumption observed with the PVN lesion animals is significantly less than the drug response observed in the sham group (#: $p<0.005$).

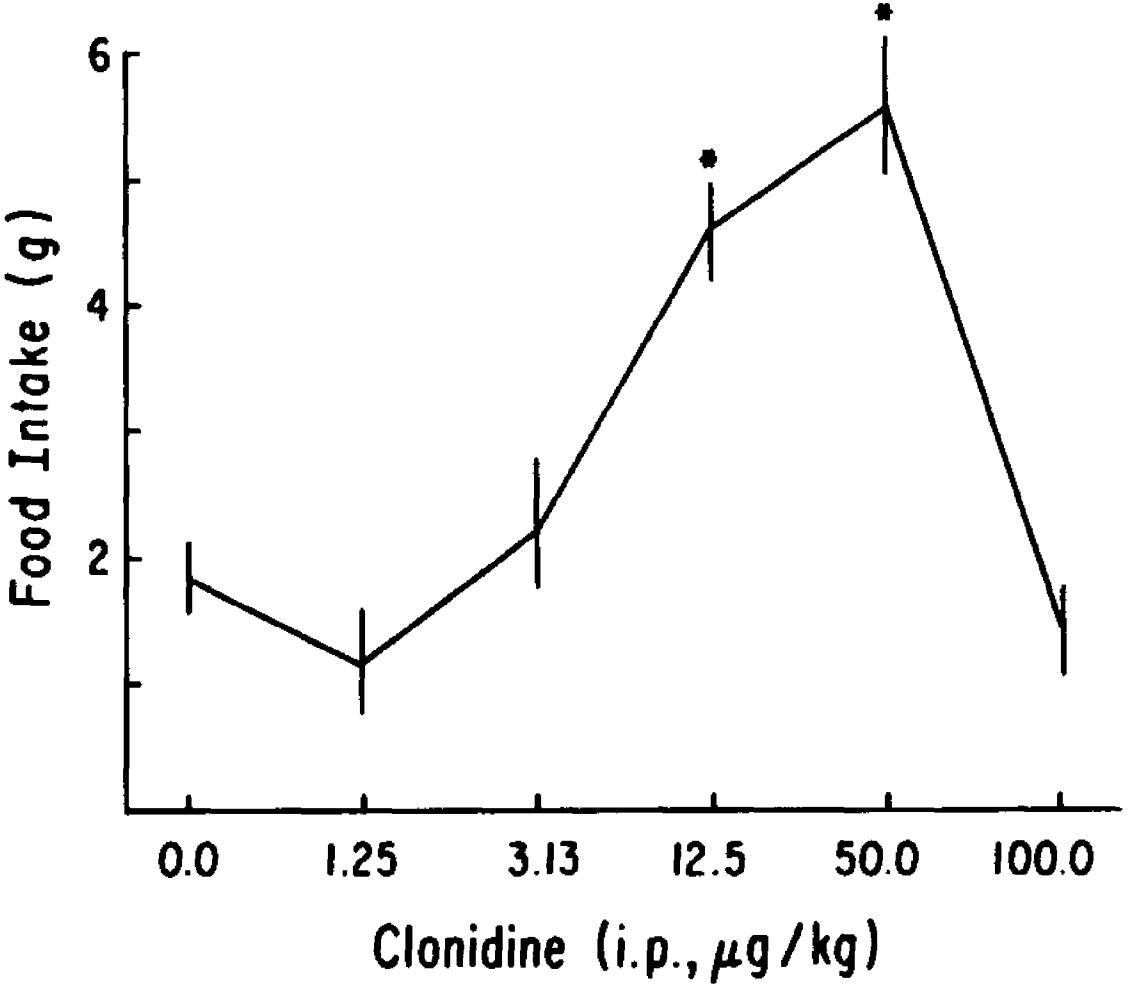


Figure 2

Figure 3

3a

3b

Table 2

Feeding Responses After Intraperitoneal Injection of Saline and Clonidine

Group	n	Saline	CLON	Difference Scores
Shams	19	0.8±0.1	3.9±0.4**	3.1±0.4
PVH Lesions	8	1.1±0.3	2.0±0.6	0.9±0.8♯
DMN Lesions	6	1.6±0.6	7.3±0.8**	5.6±0.9#
PFH Lesions	6	0.8±0.3	3.2±0.9*	2.3±0.8

* p<0.01; ** p<0.001 Within-group comparisons.
♯ p<0.05 Intake significantly less than shams.
# p<0.05 Intake significantly greater than shams.

Table 3. Average daily mash intake during the first three weeks after sustaining sham, or PVN, DMN, or PFH lesion surgery. With respect to the PVN and DMN lesion groups, the group and Week factors were significant (see text) and Neuman-Keuls comparisons indicated the average daily food intake of the PVN lesion group over the three weeks was significantly greater than both the DMN and sham animals ($p<0.05$). With respect to the significant main effect for the Week factor, Neuman-Keuls comparisons indicated consumption of mash during Week 1 was significantly greater than the level exhibited during Week 3.

Compared to their sham group, the PFH lesion rats were hypophagic [$F(1,19)=12.93$; $p<0.005$]. In addition, analysis of variance indicated a significant change in intake for the sham and PFH lesion groups over the three week period [$F(2,38)= 22.31$; $p<0.001$], and inspection of weekly food intake data shows that this increase occurs between Weeks 1 and 2. This appears to result primarily from the increase in consumption by the PFH lesion group over Weeks 1 and 2 [although the group x Weeks interaction failed to reach significance: $F(2,38)= 3.16$; $p<0.10$].

Table 3

Food Intake During First Three Weeks After Surgery

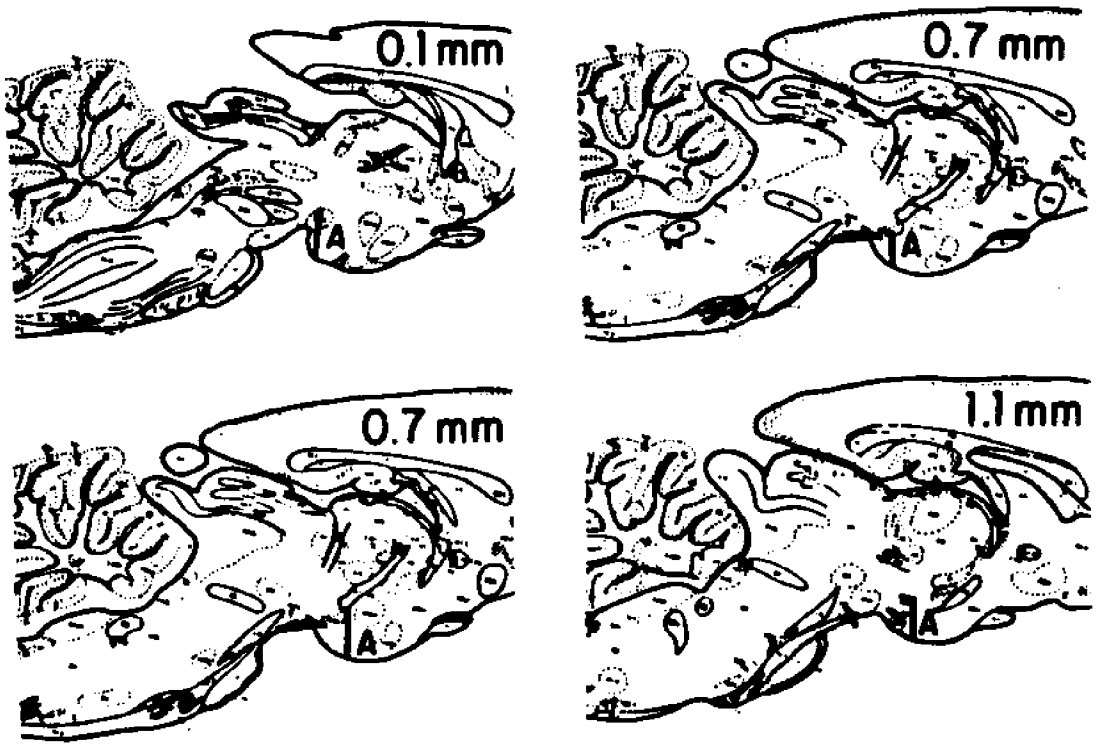
Group	n	Week 1	Week 2	Week 3
Shams	11	33.0±0.7	32.0±0.7	31.7±0.8
PVN Lesions	6	41.9±4.7**	37.7±3.0*	35.7±3.3
DMN Lesions	7	31.7±2.7	30.5±1.8	27.9±3.5
Shams	10	32.7±1.2	34.8±1.1	39.65±1.9
PFH Lesions	11	18.8±3.5‡	26.7±3.0	27.6±2.3‡

** $p<0.05$ Daily intake greater than saline group.
‡ $p<0.005$ Daily intake less than saline group.

Figure 4. The sagittal illustrations (Pelligrino, et. al., 1979) depict the site of the midline and PFH KCs at two levels lateral to midline. The KC is shown as the heavy line to the left of the letter, "A". While the midline and PFHKC overlapped at 0.7 mm lateral to midline, note the midline cut severs tissue medial to this level, while the lateral (PFH) KC severs tissue from 0.7-1.1 mm lateral to midline.

Table 4. Summary of daily food intake (grams) over Weeks 1-3 post-KC of sham, midline KC and PFH KC rats. Analysis of variance indicated that the group x Weeks interaction was significant [$F(4,66) = 6.02$; $p<0.001$]. Selected simple main effect tests indicated that during Week 1, the three groups exhibited significant differences in average daily food intake [$F(1,56) = 54.7$; $p<0.001$], and individual comparisons of the data from Week 1 showed the PFH KC rats were significantly hypophagic compared to sham rats [$F(1,66) = 15.90$; $p<0.001$], while the sham and midline KC rats failed to differ significantly [$F(1,66) = 3.07$; $p>0.10$].

Figure 4



MIDLINE KC

PFH KC

Table 4

Food Intake During First Three Weeks After Surgery

Group	n	Week 1	Week 2	Week 3
Shams	9	35.7±1.2	32.7±1.8	37.8±2.0
Hypothalamic Knife Cuts				
Midline KC	16	28.6±2.4	34.8±2.5	39.3±2.5
PFH KC	11	18.3±3.8♯	30.6±2.9	36.6±4.0

♯ $p<0.001$ Food intake less than sham group.

Table 5. Feeding responses (grams) to intraperitoneal injection of saline and CLON. Both hypothalamic KC groups, as well as sham rats, exhibited significant feeding responses to intraperitoneal administration of CLON compared to after saline administration (**$p<0.001$).

Table 6. Feeding responses (grams) to PVN injections of NE and saline before (pre-KC) and after (post-KC) sustaining midline KCs or sham surgery. The midline KC failed to disrupt feeding stimulated by PVN injection of NE. The sham group exhibited a significant attenuation of NE response after sham surgery (*$p<0.05$). NE scores are based on the difference between feeding responses to NE and saline baseline intake since each rats feeding response to saline was less than one gram.

Table 7. Feeding responses (grams) to PVN injection of CLON and saline before and after KC or sham surgery. Both sham and midline KC rats failed to exhibit significant changes in response to PVN injections of CLON after surgery (post-KC).

Table 5

Feeding Responses to Peripheral Administration of Saline and Clonidine (50 μg/kg)

Group	n	Saline	CLON	Difference Score
Snam	11	0.5±0.1	3.7±0.5**	2.8±0.4
Hypothalamic Knife Cuts				
Midline KC	11	1.4±0.3	6.1±0.8**	4.7±0.6
PFH KC	9	0.8±0.1	4.5±0.8**	3.7±0.9

** $p<0.001$ Greater than feeding response after saline.

Table 6

Feeding Responses to Central Administration of Saline and Norepinephrine (NE: 40 nM)

Group	n	Saline	NE	Difference Score
Shams				
Pre-KC	17	0.4±0.1	6.3±0.4	6.0±0.4
Post-KC	17	0.2±0.1	4.7±0.5	4.5±0.4*
Midline KCs				
Pre-KC	13	0.8±0.3	7.4±0.8	6.6±0.8
Post-KC	13	0.8±0.3	7.4±0.8	6.6±0.8

* $p<0.01$ Decreased NE response comparing pre- and post-KC.

Table 7

Feeding Responses to Central Administration of Saline and Clonidine (CLON: 7.5 nM)

Group	n	Saline	CLON	Difference Score
Shams				
Pre-KC	7	0.1±0.0	6.5±0.9	6.4±0.9
Post-KC	7	0.1±0.1	4.9±1.3	4.8±1.4
Midline Knife Cuts				
Pre-KC	4	0.5±0.3	5.9±0.6	5.5±0.8
Post-KC	4	0.0±0.0	4.4±2.1	4.4±2.1

Figure 5. Schematic drawing of the three midbrain KCs. The midbrain KCs generally severed tissue between 0.5 mm to 1.1 mm lateral to midline. The Dorsal cut (labeled "A"), severed tissue caudal to the level of the red nucleus and extended approximately 1.5 mm dorsal to the red nucleus and behind the dorsal half of this structure. The Ventral KC (B) severed tissue also caudal to the red nucleus, but the dorsal-ventral extent of this KC was from the dorsal aspect of the medial lemniscus and dorsal as far as the ventral half of the red nucleus. The Far-Ventral cut (C) was positioned below the level of the medial lemniscus.

Table 8. Feeding response (grams) of the midbrain KC and sham groups to intraperitoneal injection of saline and CLON (mean±SEM). All groups exhibited significant increases in food intake after CLON injection. That is, simple main effect tests indicated the sham, Ventral KC, and Dorsal KC groups consumed significantly greater amounts of mash (**$p<0.01$) after CLON injection than after saline injection [$F(1,23)=$ 9.78; 15.48; 22.72, respectively). Due to the small sample size of the Far-Ventral KC group ($n=3$), it was not included in the analysis of variance. These animals also responded to CLON (*$p<0.02$).

Table 9. Average (±SEM) daily mash intake of sham and midbrain KC rats during Weeks 1-3. Week, and the group x Week factors were significant [$F(2,56)=$ 16.87; $F(6,56)=$ 5.09; respectively, both $p<0.001$]. Individual comparisons showed the Dorsal midbrain group consumed significantly more mash during Weeks 2 and 3 than sham rats [$F(1,84)=$ 30.61; 13.35, both $p<0.001$].

Figure 5

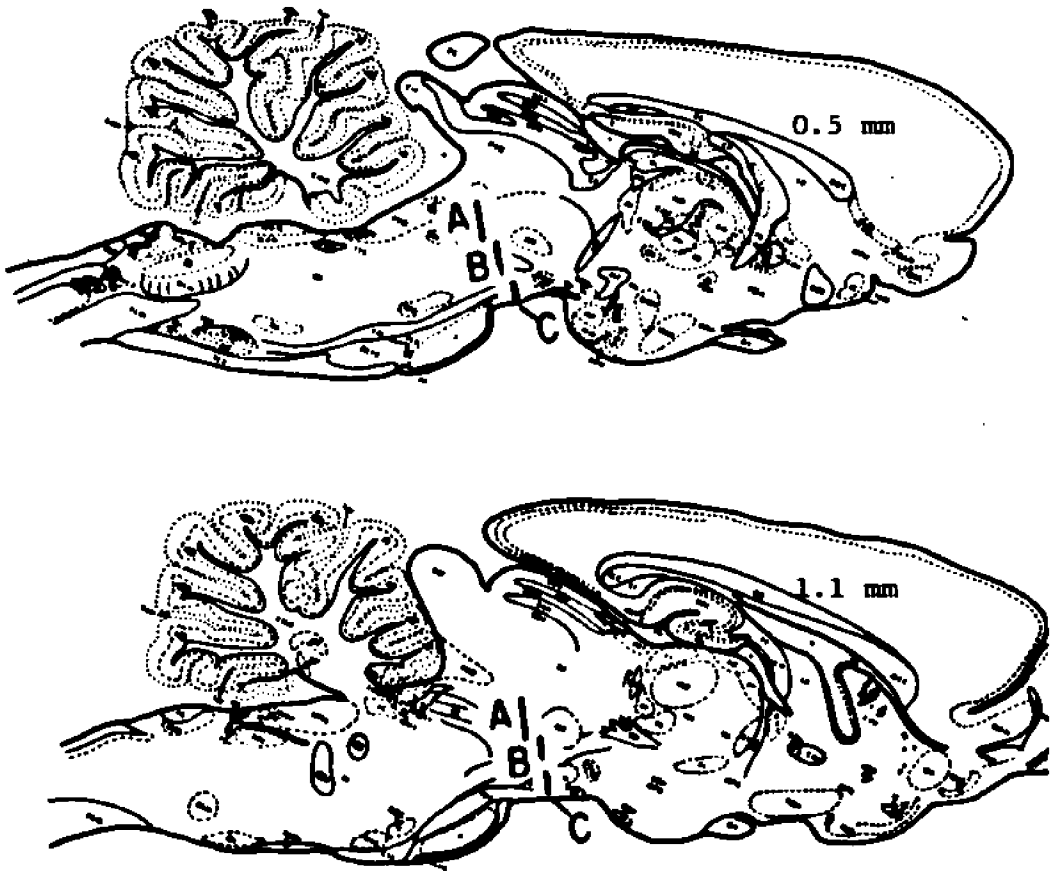


Table 8

Feeding Responses to Intraperitoneal Injection of Saline and Clonidine (CLON)

Group	n	Saline	CLON	Difference Score
Shams	7	1.6±0.5	3.7±0.6**	2.1±0.9
Midbrain Knife Cuts				
Dorsal KC	9	1.7±0.3	4.5±0.6**	2.2±0.4
Ventral KC	10	1.9±0.4	4.1±0.4**	4.2±0.1
Far-Ventral KC	3	0.8±0.3	5.0±0.4*	2.8±0.7

** $p<0.001$; * $p<0.02$ CLON intake greater than after saline.

Table 9

Food Intake During First Three Weeks After Surgery

Group	n	Week 1	Week 2	Week 3
Shams	13	31.9±1.0	31.9±1.6	35.0±1.8
Midbrain Knife Cuts				
Dorsal KC	6	30.7±2.2	46.9±0.9**	45.0±1.6**
Ventral KC	7	28.5±3.7	33.7±1.8	32.8±1.4
Far-Ventral KC	6	31.1±1.6	31.8±1.4	35.0±3.3

** $p<0.001$ Daily intake greater than sham group.

Figure 6. Schematic diagram (adapted from Pelligrino, Pelligrino, & Cushman, 1979), depicting the position of the medial RPKC and the lateral RPKC. The KCs are just to the left of the letter, "A". The medial RPKC severed tissue in the dorsal pons between 0.5 mm and 1.1 mm lateral to midline (and some animals sustained cuts that extended to midline). The lateral RPKC, located at the same level of the pons as the medial RPKC, severed tissue principally between 1.5- 1.9 mm lateral to midline (and at times extended as far medial as 1.1 mm).

Figure 7. Schematic diagram depicting the position of the medial and lateral CPKCs. The KC is located to the left of the letter, "A". Similar to the two RPKC subgroups, animals sustaining damage medial to the LC (at least between 0.5- 1.1 mm lateral to midline) were designated as medial CPKCs. The lateral CPKC severed tissue lateral to the LC, between 1.5- 1.9 mm lateral to midline.

Table 10. Feeding responses (mean+-SEM) to intraperitoneal injection of CLON and saline. The sham group exhibited a significantly greater feeding response to CLON than to saline, while the lateral RPKC group failed to significantly increase feeding after CLON. The CLON response of the lateral RPKC group is also significantly less than the sham group response in terms of difference scores (** $p<0.01$). The medial RPKC group ($n=3$) appear to increase their mash consumption after peripheral injections of CLON, but this response failed to reach significance.

Figure 6

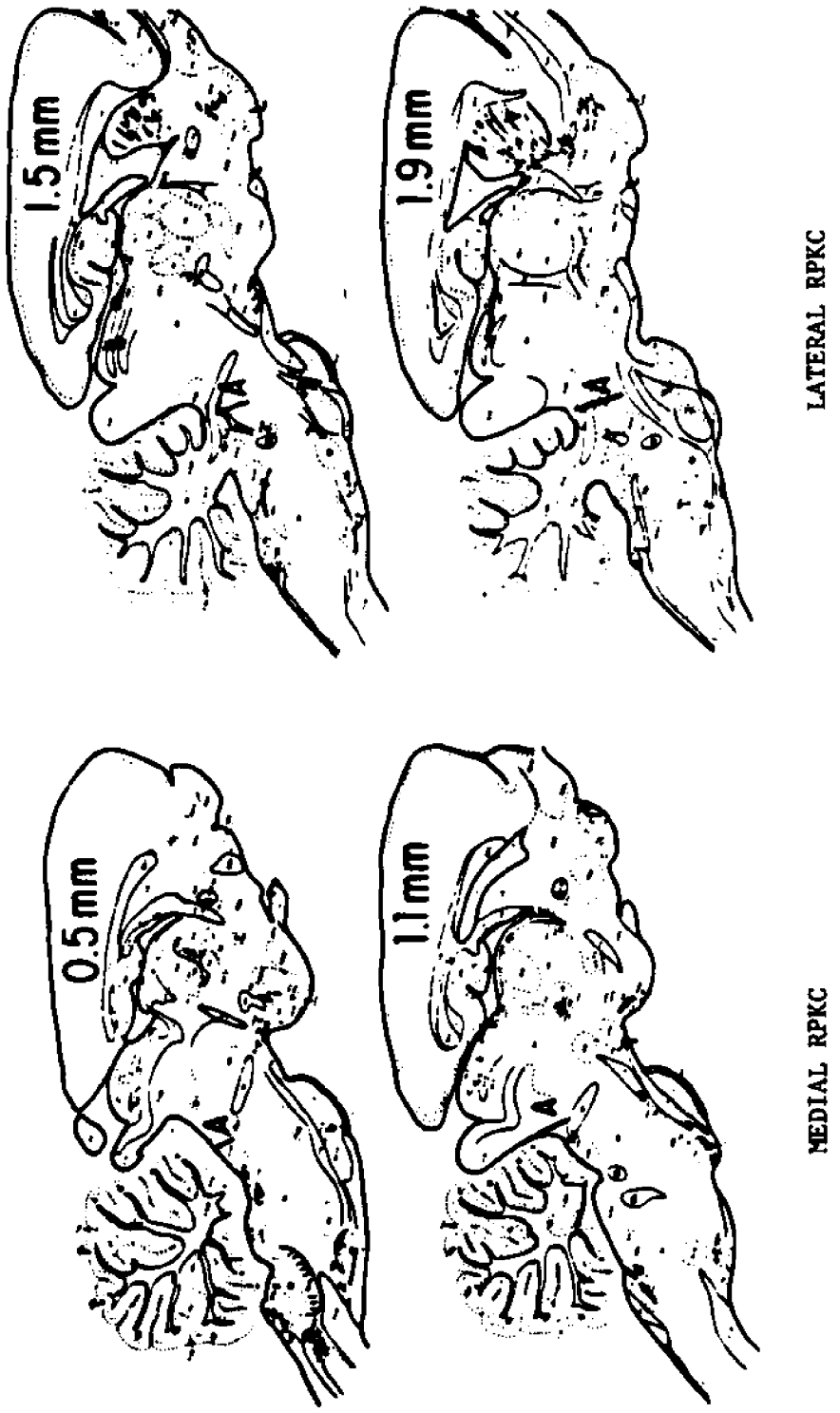


Figure 7

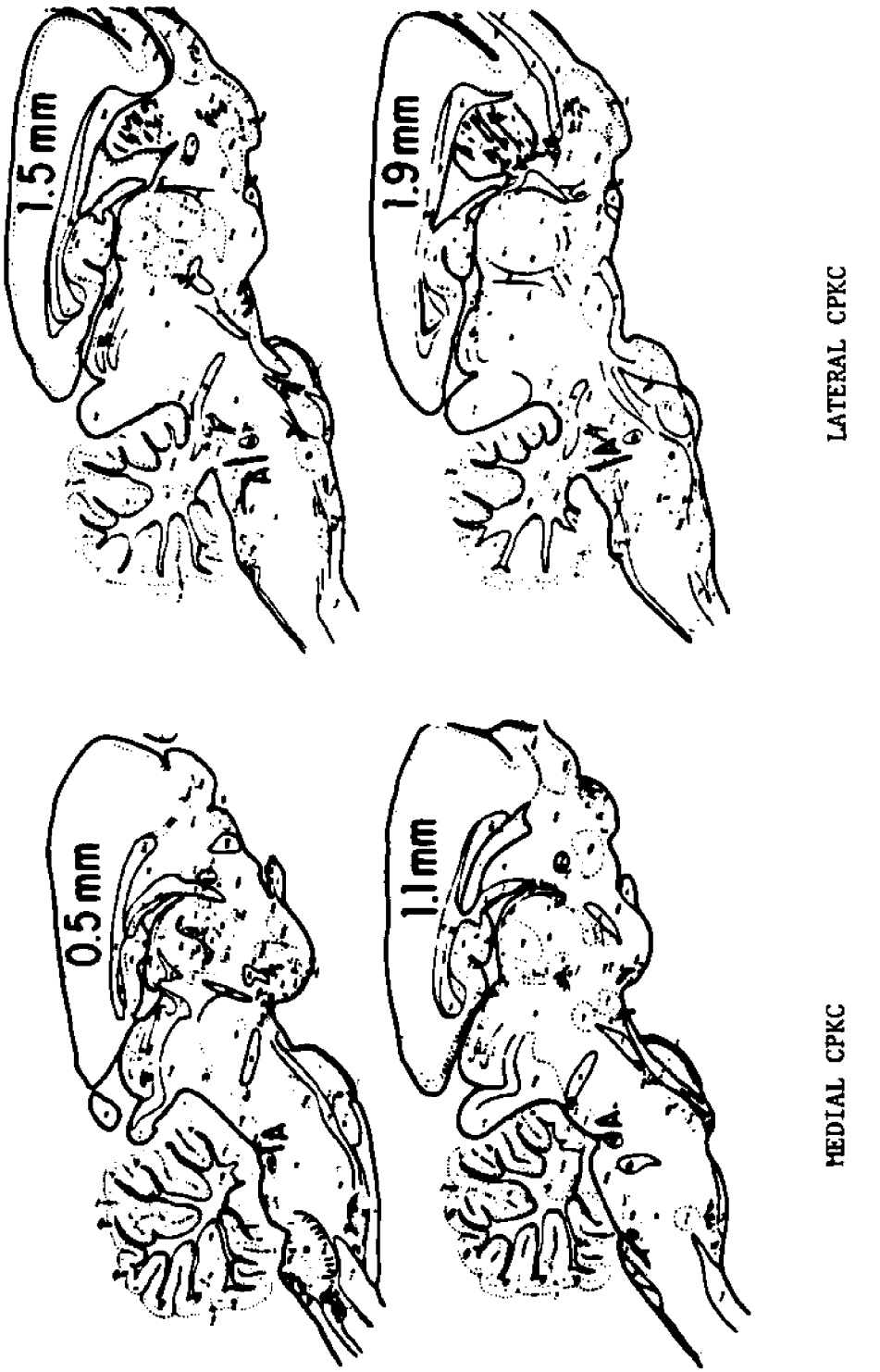


Table 10

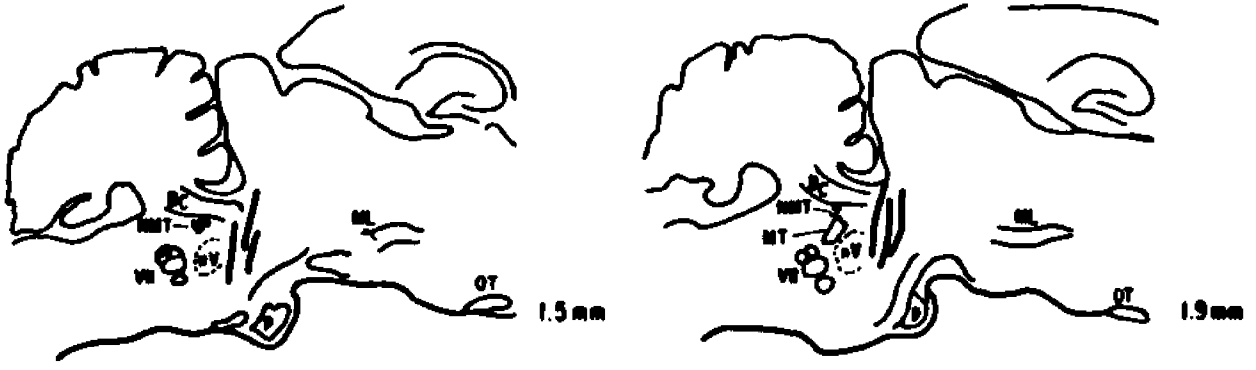
Feeding Responses to Peripheral Administration
of Saline and Clonidine (50 μg/kg)

Group	n	Saline	CLON	Difference Score
Sham	8	0.8±0.2	3.9±1.1**	3.0±0.5
Rostral Pontine KCs				
Lateral RPKC	8	0.9±0.9	1.4±1.2	0.5±0.6‡
Medial RPKC	3	1.4±1.1	5.5±1.8	4.1±1.3

** $p<0.001$ Greater than feeding response after saline.
‡ $p<0.01$ Response to CLON less than sham group.

Figure 8. These sagittal drawings of the rat brain, at approximately 1.5 and 1.9 mm lateral to midline (Pelligrino, Pelligrino, & Cushman, 1979), show actual tracings of KCs from three rats with lateral RPKCs and three rats with lateral CPKCs. These animals exhibited almost no (<1.0 g) feeding response to peripherally administered CLON and their cuts suggest that CLON-induced feeding is mediated by a longitudinal, caudally-directed fiber sustem, that courses through the dorsal pons. (Abbreviations: BC- brachium conjunctivum; ML- medial lemniscus; MT- mesencephalic tract of the motor nucleus; NMT- motor nucleus of the trigeminal nerve; nVII- nucleus of the facial nerve; p- pons; r- red nucleus; VII- facial nerve).

ROSTRAL PONTINE KC



CAUDAL PONTINE KC

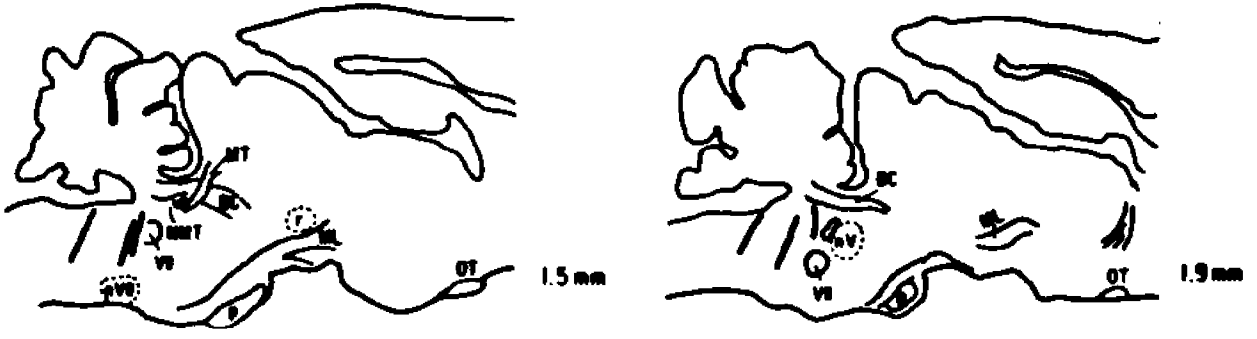


Figure 8

Table 11. Feeding responses (grams) after central administration of NE and saline. Sham rats continued to respond to NE injections post-surgery, while lateral RPKC animals exhibited a significant decrement in magnitude of response to NE post-KC (ǂ $p<0.005$).

Figure 9. Photomicrographs of the pontine KCs. The lateral RPKC (9a), rostral to the level of the LC, severed tissue both dorsal and ventral to the brachium conjunctivum and extended from 1.3 mm to 1.9 mm lateral to midline. The lateral CPKC (9b) was positioned just behind the fifth motor nucleus, and extended almost to the dorsal surface of the brain. This cut extended from 1.3 to 1.9 mm lateral to midline.

Table 12. Food intake responses after PVN injection of saline and CLON. The sham group continued to consume mash after sham surgery. Animals receiving RPKCs appear to exhibit decreased feeding responses to PVN injections of CLON post-KC.

Table 11

Food Intake After PVN Injection
of Saline and Norepinephrine (NE)

Group	n	Saline	NE	difference score
Shams				
Pre-KC	10	0.2±0.1	6.5±0.8	6.2±0.9
Post-KC	10	0.4±0.2	6.6±0.9	6.2±0.9
Lateral RPKC				
Pre-KC	6	0.2±0.2	6.1±0.8	6.0±0.9
Post-KC	6	0.0	1.6±0.7	1.6±0.7ǂ*
Medial RPKC				
Pre-KC	3	0.5±0.3	7.7±1.6	7.2±1.6
Post-KC	3	0.1±0.1	7.5±1.2	7.2±1.2

ǂ $p<0.005$ Feeding response less than shams (post-KC)
* $p<0.005$ Less than pre-KC score.

Figure 9

9a

9b

Table 12

Food Intake After PVN Injection
of Saline and Clonidine (CLON)

Group	n	Saline	CLON	Difference score
Shams				
Pre-KC	7	0.2±0.2	8.9±1.5	8.7±1.6
Post-KC	7	0.4±0.2	7.3±1.5	6.9±1.5
Lateral RPKC				
Pre-KC	2	0.0	9.9±1.2	9.9±1.2
Post-KC	2	0.0	1.4±0.8	1.4±0.8
Medial RPKC				
Pre-KC	2	0.8±0.4	6.1±0.1	5.3±0.3
Post-KC	2	0.2±0.0	3.1±0.2	2.9±0.2

Table 13. Feeding response of sham and CPKC rats to intraperitoneal injection of CLON and saline. The lateral CPKC rats exhibited a decreased response to CLON administration, compared to the sham group (♯ $p<0.01$). The medial RPKC group appear to exhibit an increase in food intake after CLON injection, but apparently due to small sample size, this increase is not statistically significant [$t(2)= 1.41$; $p<0.02$].

Table 14. Food intake (grams) of sham and CPKC rats after PVN injections of NE and saline. After KC surgery, the lateral CPKC rats exhibited a significantly attenuated response to NE compared to sham rats (♯ $p<0.005$).

Table 15. Feeding responses to PVN injection of saline and CLON. Both the sham and medial CPKC rats responded to CLON after surgery, and failed to differ significantly. No lateral CPKC rats were tested with CLON.

Table 16. Daily mean (±SEM) food intake of sham and pontine KC groups during the first three post-KC weeks. The main effect of the Weeks factor was significant [$F(2,52)= 20.10$; $p<0.001$] and is reflected primarily in the increase in food intake of the RPKC and CPKC groups across Weeks 1- 2. This was supported by tests on the Weeks main effect that indicated the three groups significantly increased their mash consumption from Week 1 to Week 2 [$F(1,52)= 28.80$; $p<0.001$], while the average food intake for all three groups did not differ significantly from Weeks 2 to 3 [$F(1,52)= 1.50$; $p>0.20$]. The group and group x Weeks factors were not significant.

Table 13

Feeding Responses to Peripheral Administration of Saline and Clonidine

Group	n	Saline	CLON	Difference Score
Sham	6	1.0±0.3	4.2±0.7**	3.2±0.7
Caudal Pontine KCs				
Lateral CPKC	11	1.8±0.5	2.4±0.6	0.6±0.5‡
Medial CPKC	3	1.2±0.6	5.4±2.0	4.2±1.7

** $p<0.01$ Greater than feeding response after saline.
‡ $p<0.01$ Response to CLON less than sham group.

Table 14

Food Intake After PVN Injection
of Saline and Norepinephrine (NE)

Group	n	Saline	NE	Difference Score
Shams				
Pre-KC	11	0.2±0.1	7.8±1.0	7.5±1.0
Post-KC	11	0.4±0.2	7.8±1.2	7.5±1.3
Lateral CPKC				
Pre-KC	5	0.6±0.6	9.0±1.1	8.4±1.3
Post-KC	5	0.4±0.4	3.4±1.2	3.1±1.3ǂ*
Medial CPKC				
Pre-KC	5	0.5±0.2	6.7±1.3	6.0±1.1
Post-KC	5	0.7±0.3	5.0±1.4	4.7±1.3

ǂ $p<0.05$ Feeding response less than shams (post-KC)
* $p<0.005$ Less than pre-KC score.

Table 15

Food Intake After PVN Injection
of Saline and Clonidine (CLON)

Group	n	Saline	CLON	Difference Score
Shams				
Pre-KC	7	0.2±0.2	7.1±0.6	6.9±0.5
Post-KC	7	0.3±0.2	5.3±0.9	5.0±1.1
Medial CPKC				
Pre-KC	6	0.5±0.2	7.4±1.1	6.9±1.0
Post-KC	6	0.2±0.2	5.1±1.5	4.9±1.4

Table 16

Food Intake During First Three Weeks After Surgery

Group	n	Week 1	Week 2	Week 3
Shams	12	25.3±1.6	31.9±1.6	33.6±1.2
Pontine Knife Cuts				
Rostral KC	9	23.6±3.1	38.2±5.6	42.8±2.4
Caudal KC	9	21.5±2.0	32.9±1.9	32.5±1.5

Chapter 4

Determination of the Course of Catecholamine Fibers Mediating Amphetamine Anorexia: Hypothalamic Lesions and Knife Cuts

Research conducted to date has provided some evidence that the well-known anorectic effect of amphetamine (AMPH) is mediated, at least in part, by hypothalamic catecholaminergic mechanisms. Booth (1968) first demonstrated that hypothalamic injections of AMPH suppress feeding. Leibowitz has utilized the central injection technique to more precisely determine the anatomical site where central injection of AMPH most effectively inhibits feeding in hungry rats (Leibowitz, 1975a; Leibowitz & Rossakis, 1978a). Direct injection to the perifornical lateral hypothalamus (PFH), specifically at the level of the ventromedial nucleus, induced the greatest anorectic response. Injections to many other hypothalamic sites, as well as extra-hypothalamic sites, were relatively ineffective. Additionally, central injections of catecholamine antagonists to the PFH area were found to reliably block anorexia induced by perifornical AMPH injections, as well as by peripherally injected AMPH.

Consistent with the hypothesis that AMPH acts through hypothalamic mechanisms are the findings of hypothalamic lesion studies. These studies have demonstrated that lesions in the lateral hypothalamus (LH), which damage the medial forebrain bundle, attenuate or abolish the anorectic effect to peripherally administered AMPH (Blundell & Leshem, 1974; Campbell & Baez, 1974; Carlisle, 1964; Fibiger, Zis, & McGeer, 1973; Leshem, 1981; Russek, Rodriquez-Zendejas, Teitelbaum, 1973). In

some cases, LH lesioned cats and rats have actually been found to increase their feeding in response to AMPH injection (Stricker & Zigmond, 1976; Wolgin, Cytawa, & Teitelbaum, 1976; Wolgin & Teitelbaum, 1978). In contrast to the effect of LH lesions, animals sustaining damage to other hypothalamic regions, including the ventromedial hypothalamic area or anterior hypothalamus, are reported to enhance the anorectic effect of AMPH (Cole, 1966; Cole & Hudspeth, 1964; Epstein, 1959; Pecile, Olgiati, & Netti, 1977; Reynolds, 1959; Stowe & Miller, 1957).

In the present study, a more detailed analysis of the impact of hypothalamic manipulations on AMPH's anorectic action was conducted. Of particular interest were how midlateral PFH lesions, as opposed to far-lateral hypothalamic lesions that damage the dopaminergic nigrostriatal bundle, would affect drug response. The PFH area, the brain site maximally sensitive to AMPH's anorexigenic effects, was therefore lesioned, and the effects of these lesions were compared with those of lesions of the PVN. In addition, coronal wire knife cuts (KCs), which produce greater damage to fibers while sparing local cell bodies, were used to examine potential projection routes taken by axons mediating AMPH anorexia. Cuts through the medial and midlateral hypothalamus, at the caudal hypothalamic level, were investigated. In these studies, feeding suppression was induced by intraperitoneal as well as perifornical hypothalamic injections of AMPH. For comparison, fenfluramine (FENF), an anorectic drug which appears to act through the release of serotonin (Garratini & Samanin, 1978), was also studied.

The results of these experiments provide strong evidence to suggest

that AMPH anorexia is mediated, at least in part, by the perifornical region of the hypothalamus. Lesions in this area, as opposed to lesions to more medial, anterior, or dorsal hypothalamic sites, abolished anorexia to peripheral AMPH administration. Furthermore, KCs which sever fibers coursing to the perifornical region likewise attenuated feeding suppression induced by peripheral and perifornical AMPH injections. Neither brain manuipulation that attenuated the AMPH response, however, affected anorexia produced by peripherally injected FENF.

Experiment 1

In the first experiment, dose response studies were undertaken where AMPH and FENF were injected intraperitoneally in order to determine a dosage that produced a partial suppression of feeding in the present experimental paradigm.

Methods

Subjects. Five and eight unoperated rats, respectively, underwent dose response tests with AMPH and FENF. Animals always had access to the mash diet, except on test days, and water was available ad libitum.

Test Procedure. Animals in the AMPH dose response study received intraperitoneal injections of saline (1 ml/kg) or 0.25, 0.50, 1.0, or 2.0 mg/kg/ml of d-amphetamine sulfate (Smith, Kline, & French) dissolved in saline. Animals in the FENF dose response study received intraperitoneal injections of saline (1 ml/kg) or 0.22, 0.66, 2.0, 6.0, or 12.0 mg/kg/ml of fenfluramine hydrochloride (A.H. Robins) dissolved in saline. Each dose level was administered to every animal 2 or 3 times

and at least 3 different dosages, in different animals, were tested on any single day. On each test day, rats were food deprived for four hr prior to drug injection. The animals were then injected with drug or saline, and fifteen min later were given a pre-weighed dish of fresh mash. Consumption was measured by weighing each rats mash dish 60 min post-injection.

Results and Discussion

Figure 10 presents the mean feeding responses (± standard error of the mean) after peripheral injection of several dosages of AMPH. Analysis of variance indicates a significant suppressive effect of AMPH on feeding behavior [$F(4,16)=33.83$; $p<0.001$]. Dunnett's t -statistic indicated that animals ate less food, compared to saline, after injection of every AMPH dose ($p<0.01$), except 0.25 mg/kg [$t(5,16)= -0.31$; -4.99; -7.50; -9.06; for 0.25, 0.50, 1.0, and 2.0 mg/kg, respectively].

Figure 11 presents food intake responses after FENF as a function of dose. Analysis of variance indicated a significant change in amount consumed at different dosages [$F(6,42)= 11.17$; $p<0.01$], and Dunnett's t -statistic indicated that food intake after the 2.0, 6.0, and 12.0 mg/kg dosages were significantly less than after saline [$t(6,35)= -3.68$, -2.70, -4.18; respectively, all $p<0.05$]. The amount of food intake after adminstration of 0.22 and 0.66 mg/kg failed to differ significantly from saline baseline intake [$t(6,35)= 0.96$; -1.50; both $p>0.10$].

The dose responses to AMPH and FENF generally show that increasing dose level significantly decreases the amount consumed by mildly food-

deprived rats. It is not clear why no greater feeding suppressive effect was observed with the 2.0 to 12.0 mg/kg doses of FENF, but it is perhaps due to the development of tolerance to this drug's anorectic action which occurs rapidly, but not completely, at higher dosages (Heffner & Seiden, 1979; Lewander, 1981; Rowland, Antelman, & Kocan, 1982).

Experiment 2

In the next experiment, animals sustained electrolytic lesions to the PFH area or to the PVN to observe their effects on anorexia induced by peripheral administration of AMPH and FENF.

Methods

Subjects. A total of 28 rats received PFH lesions and 14 received PVN lesions. During the first three weeks after surgery, daily food intake was measured in some of these animals. Ten rats sustained sham surgery.

Surgery. PVN lesions were made as described earlier. Perifornical lesions were made -0.6 mm behind bregma suture/ 1.2 mm LAT/ 8.8-9.2 mm DV, with a straight carrier. Sham animals received similar treatment, except that the electrode was only lowered to approximately 3 mm from the targeted sites, and no current was passed.

Behavioral Testing. Beginning 3 to 5 weeks after surgery, rats were tested for level of feeding suppression to AMPH and FENF administration. On each test day, animals were initially food deprived for four hours. The animals were then administered saline (1 cc/kg), AMPH

(0.5 mg/kg), or FENF (2.0 mg/kg). These specific dosages were selected from the dose response studies since they decrease food intake by a moderate 50%. AMPH tests were generally administered in the third to fifth weeks after surgery and then the FENF tests were undertaken. Fifteen min after drug injection, animals received a preweighed mash dish, and consumption was measured 60 min later. Every animal received two or three tests with saline, AMPH, and FENF and were then anesthetized and perfused in order to study brain lesion placements.

Results and Discussion

A total of 10 of the original 28 rats that underwent PFH lesion surgery sustained on-target PFH lesions. The lesions were located in the midlateral hypothalamus, damaging the fornix and surrounding tissue (Figure 12a). The lesions, in terms of anterior-posterior extent, were centered at the level of the ventromedial nucleus and extended as far anterior as the level of the PVN, and at times as far caudal as the caudal extent of the posterior hypothalamus. Often the lesions damaged the ventrolateral edge of the dorsomedial nucleus, as well as the dorsolateral edge of the ventromedial nucleus, and extended into the medial half of the medial forebrain bundle and zona incerta. Some animals sustained unilateral PFH lesions (n=9), with little or no damage apparent on the contralateral side or in two cases, a small contralateral lesion in the area of the ventromedial nucleus. Six animals sustained bilateral lesions that missed the PFH region entirely. Three of these animals sustained dorsal lesions, which were centered in the zona incerta and which damaged the dorsal half of the DMN and nucleus reuniens

in the thalamus. The other three animals sustained anterior lesions, which were centered around the fornix at the level of the PVN and extended to the rostral extent of the anterior hypothalamus (Figure 12b). These lesions also extended laterally to destroy the medial half of the medial forebrain bundle and extended caudally to the caudal extent of the PVN. The distinguishing characteristic of the anterior PFH lesion, compared to the PFH lesion, was that it did not produce significant damage to the PFH area at the level of the ventromedial nucleus.

Four animals sustained PVN lesions. These lesions destroyed the entire PVN region and extended lateral to the PVN to the medial extents of the fornix. In one of these animals, the lesion also produced extensive damage to the anterior hypothalamic region, and one other animal exhibited lesions located on the left side of the brain that extended dorsally and damaged the medial aspect of the zona incerta. The remaining 10 rats that sustained PVN lesion surgery sustained unilateral PVN lesions, partial (bilateral) PVN lesions, or lesions ventral to the PVN. (The data from these animals is not reported.)

The results of intraperitoneal administration of saline and AMPH to rats sustaining hypothalamic lesions are presented in Table 17. As noted in Chapter 2, tests of homogeneity of variance indicated that in many cases an analysis of variance using raw scores involved pooling heterogeneous error variances. Lesion and KC groups are therefore compared using percent inhibition of food intake (AMPH score minus saline score, divided by saline score), although average responses to drug and saline are also reported. With respect to the present study, the per-

cent suppression measure is also heterogenous (as are raw scores and difference scores) and therefore the PFH lesion group is compared with the unilateral PFH group. Analysis of variance showed that the degree of anorexia exhibited by the PFH lesion group is significantly less than the response observed in the unilateral PFH lesion group [$F(1,17)= 9.84$; $p<0.01$]. With respect to the anterior lesion and dorsal lesion groups, t -tests showed these groups decreased their mash intake after AMPH compared to after saline [$t(2)= -9.53$; -12.57, respectively, both $p<0.02$]. The PVN lesion rats also consumed less food after AMPH [$t(3)= -6.00$; $p<0.01$], and in fact comparing the percent suppression scores of these animals to sham rats indicated a significant enhancement of AMPH-induced feeding suppression [$F(1,14)= 15.90$; $p<0.005$].

Table 18 presents the effects of FENF administration on food intake in lesioned rats. Analysis of variance showed that the degree of anorexia, in terms of percent suppression scores, after FENF for the sham group did not differ significantly from the degree of anorexia observed in PFH lesion rats [$F(1,14)= 1.57$; $p>0.10$]. Since unilateral PFH and anterior/dorsal lesion groups consisted of small sample sizes they were not compared to sham animals. However, t-tests indicated that all groups ($p<0.001$) except the anterior/dorsal group ($n=2$) exhibited significant decreases in food intake after FENF compared to after saline administration.

Results from the present study indicate that bilateral destruction of the PFH region disrupts the ability of AMPH, but not FENF, to suppress food intake. Lesions that did not destroy the PFH in its entirety on both sides of the brain, however, did not disrupt AMPH's

effectiveness. Although only a small number of animals (n=4) sustained bilateral PVN lesions, these animals exhibited enhanced responsivity to AMPH. This finding concurs with other studies that have shown medial hypothalamic damage enhances AMPH's effect (Cole, 1966; Cole & Hudspeth, 1964; Epstein, 1959; Pecile, et. al., 1977; Reynolds, 1959; Stowe & Miller, 1957). The mechanism mediating this effect is not known. However, it may be that destruction of the PVN results in the loss of a stimulatory action of AMPH on feeding, via this drug releasing NE from the PVN, that ordinarily partially counteracts AMPH's anorectic action.

One noticable additional effect of unilateral and bilateral PFH lesions is that this brain manipulation greatly increases saline baseline feeding. Generally, this effect has not been reported previously (Fibiger, Zis, & McGeer, 1973; Russek, Rodriquez-Zendejas & Teitelbaum, 1977), which is perhaps due to the present experiment utilizing a 4-hr rather than a 24-hr food deprivation schedule. One study, however, shows recovered rats that sustained far-LH lesions consumed great amounts of food after saline or AMPH administration (Zigmond & Stricker, 1980). This effect does not appear to be correlated with denervation of the PFH region, since KCs just caudal to this brain region did not also produce increased feeding during the one-hour testing sessions (see following experiment).

PFH lesions had little impact on FENF-induced anorexia. This supports the findings of others that no hypothalamic lesion effectively blocks this drugs effect (Blundell & Leshem, 1974; Fibiger, Zis & McGeer, 1973). In the case of midlateral hypothalamic lesions, Blundell and Leshem (1974) demonstrated an enhanced anorectic response after

FENF, while far-LH lesions (Fibiger, et. al., 1973) did not produce a significantly larger anorectic response, although results appear to be in this direction. In the present study, the level of food intake after FENF injection was equal for the sham and PFH lesion groups, and anorexia expressed in terms of percent suppression of food intake to FENF versus saline, failed to indicate a potentiation of anorexia after PFH lesions.

Experiment 3

Numerous studies have shown that AMPH anorexia requires the release of endogenous catecholamines since depletion of brain catecholamines with alpha-methyl-p-tyrosine or 6-hydroxydopamine decreases the anorectic response to AMPH (Baez, 1974; Clineschmidt & Bunting, 1980; Fibiger, et. al., 1973; Heffner & Seiden, 1979; Heffner, Zigmond, & Stricker, 1977; Samanin, Bernasconi, & Garrattini, 1975). If the PFH region is the primary site mediating AMPH-induced anorexia, as suggested by the last experiment, then coronal KCs that sever ascending catecholamine fibers just caudal to the PFH area should also disrupt AMPH's ability to suppress feeding. In the next experiment, KCs were placed in the caudal hypothalamus just behind the PFH region. These KCs abolished AMPH's ability to suppress feeding while more medially placed cuts did not change drug response.

Methods

Subjects. A total of 51 rats received either KC surgery (n=37) or sham surgery (n=14).

Surgery. Two hypothalamic KCs were made, as described earlier, in different groups of rats. Animals were first anesthetized and then received either bilateral perifornical KCs (PFH KCs), midline KCs, or sham surgery. For the midline KC, the knife guide tip was directed to a point +5.2 mm anterior to lambda line/ 1.4 mm LAT/ -9.2 mm DV, with the nosebar set horizontal with interaural line. The knife (2.5 mm length; 1.5 mm pitch) was then extended, and raised and lowered 4.0 mm. The PFH KC used a knife measuring 2.0 mm (length) by 1.3 mm (pitch). For the PFH KC, the nosebar was set horizontal to interaural line and the guide was lowered into the brain using coordinates: -3.0 mm AP with respect to bregma/ 2.5 mm LAT/ -9.6 mm DV. The knife was then extended and raised 3.4 mm. Sham animals were similarly treated where the guide was lowered into the brain to within 3.0 mm dorsal to where cuts were made; but the knife was not extended.

Some PFH KC and midline rats also received cannula implants. In this case, the incisor bar was set 3.1 mm (Krieg) above interaural line and the cannula tip was aimed at the perifornical region using coordinates: -1.5 mm AP/1.5 mm LAT/ -8.7 mm DV. The cannula was then fixed to the skull using stainless steel hooks and acrylic cement.

Behavioral Testing. Noncannulated rats received 2-4 weeks of testing with AMPH and FENF using the four-hour food deprivation schedule. Fifteen min before animals received fresh mash, they received intraperitoneal injections of either 0.5 mg/kg AMPH, 2.0 mg/kg FENF, or saline (1 ml/kg). Food intake was measured 60 min postinjection.

Animals that had received PFH cannula implants were tested for response to central and peripheral administration of AMPH. On testing

days, PFH cannulated rats were food deprived for four hr, and fifteen min before food delivery they received either a central 150 nM injection of AMPH (55.32 µg in 0.5 µl sterile saline) or a peripheral injection of 0.5 mg/kg AMPH. Animals were also tested where only saline was injected either to the PFH (0.5 µl) or peripherally (1 cc/kg). Food intake was measured 60 min postinjection. After all drug tests, animals were sacrificed, perfused, and brains were removed for histological study.

Results and Discussion

As depicted in Figure 13, animals that sustained PFH KCs (n=12) exhibited bilateral cuts positioned in the caudal hypothalamus, just posterior to the level of the VMN. These cuts often extended as far lateral as the medial half of the medial forebrain bundle (1160 µ, with respect to Koenig and Klippel, 1974) and as far medial as the level of the mammilothalamic tract (580 µ, Koenig and Klippel, 1974). Generally, these cuts extended below the level of the fornix, although they did not always course to the base of the brain, and they extended dorsally as far as the dorsal extent of the dorsomedial nucleus (DMN). Some animals (n=7) sustained the midlateral KC on only one side of the brain (unilateral PFH KCs). The contralateral side severed tissue only medial to the fornix. Three animals, each, sustained bilateral KCs that were either anterior or dorsal to the PFH region. The anterior KC animals sustained quite sizeable KCs which severed through the VMN and DMN and extended almost as far dorsal as the anterior aspect of the habenula. The dorsal KC rats sustained KCs that did not fall as ventral as the level of the VMN. These cuts severed tissue at the very caudal aspect of the zona incerta and in the fields of Forel, and extended as dorsal as the ventromedial nucleus of the thalamus.

The midline KC (Figure 13) severed tissue, on both sides of the brain, from midline to approximately 740 μ lateral. These cuts, therefore, overlapped the region cut by the most medial extents of the midlateral KC, but did not sever tissue to the lateral aspect of the fornix or farther lateral. Figures 14a and 14b, respectively, are photomicrographs of the PFH KC and the midline KC. Note that the midline KC procedure frequently produced a hole at the base of the brain (Figure 14b).

Table 19 shows that bilateral PFH KCs in the posterior level of the hypothalamus disrupt feeding suppression by AMPH. That is, analysis of variance of percent suppression scores of the sham (n=14), PFH KC (n=12), and midline KC (n=12) groups indicated that the hypothalamic KC groups differed [$F(4,46)= 17.01$; $p<0.001$], and simple comparisons showed the PFH KC group exhibited a significantly attenuated anorectic response to AMPH compared to sham animals [$F(1,46)= 51.84$; $p<0.001$].

Table 20 presents results from injections of AMPH directly to the PFH region. In this experiment, animals sustaining sham (n=10), PFH KC (n=9), anterior/dorsal KC (n=6), or midline KC (n=2) surgery were tested. Since there were only two midline KC rats, these animals were not included in statistical analyses. However, it should be noted that both rats exhibited anorectic responses to AMPH with respect to their saline baseline responses (-35% and -30%). The three remaining groups, the sham, PFH KC, and anterior/dorsal KC groups, exhibited significantly different responses to AMPH in terms of percent suppression scores [$F(2,22)= 7.86$; $p<0.01$], and individual comparisons showed the PFH KC group exhibited a significantly attenuated response to PFH injections of AMPH, while the sham and anterior/dorsal KC rats failed to differ

[F(1,22)= 0.93].

Table 18 shows that the PFH KC group decrease their food intake after FENF administration. In fact, analysis of variance of percent suppression scores indicated the PFH KC group exhibited a significantly enhanced anorectic response compared to sham animals [F(1,14)=6.04; p<0.05].

The present results indicate that KCs severing fibers in the perifornical region of the caudal hypothalamus were able to disrupt AMPH's ability to decrease feeding in mildly food-deprived rats. KCs in this region also decrease AMPH [illegible] when this drug is injected directly into the PFH region. In contrast, KCs that severed tissue anterior, dorsal, or medial to the area severed by the midlateral PFH KC did not affect response to AMPH, whether this drug was injected peripherally or directly to the PFH region.

In terms of peripheral administration of AMPH, the results of the present study do not concur with the single other study where animals with coronal KCs in the caudal hypothalamus, as well as parasagittal cuts placed just medial to the fornix, were tested for response to AMPH (Sclafani & Berner, 1979). However, this may be the result of three methodological differences. Sclafani and Berner (1979) used a 4 hr/day feeding schedule and a high-fat diet whereas the present study utilized a 4 hr food deprivation regimen and a sweet milk-mash diet. Also, the present study utilized a lower 0.5 mg/kg dose in contrast to 1.0 mg/kg. The results of the Experiment 1 indicate that with a 4 hr food deprivation schedule, 1.0 mg/kg decreases feeding significantly below the level of food intake after 0.5 mg/kg. Perhaps use of a higher dose in the

present experimental paradigm, that is, in conjunction with a mild degree of food deprivation, would have concealed differences in drug responses between groups. Finally, the PFH KCs in the present experiment sever more tissue just lateral to the fornix, while KCs in the study by Sclafani and Berner (1979) appear to remain at just the lateral edge of the fornix, and were slightly more anterior so that they invaded the ventromedial nucleus. It is interesting to note that the posterior KC group in the Sclafani and Berner (1979) experiment appeared to be almost significantly less sensitive to AMPH at the 1.0 and 2.0 mg/kg dosages when food intake was measured 1 hr postinjection (A. Sclafani, personal communication).

With respect to FENF administration, the present findings indicate midlateral KC animals exhibit greater anorexia than sham animals. The mechanism mediating these changes in drug sensitivity are unknown, but are perhaps related to the fact that serotonin fibers coursing through the medial forebrain bundle have been damaged (Steinbusch, 1981). Alternatively, other investigators have suggested that dopaminergic systems may ordinarily antagonize serotonergic systems, and, when dopamine fibers are damaged, this may disinhibit serotonin's anorexigenic action (Fibiger, et. al, 1973). Likewise, since this drug is believed to have significant peripheral effects that may be related to its anorectic action (Turner, 1979), perhaps the PFH KC produces significant changes in the gastrointestinal system which subsequently enhances this drugs effect in the gastric mucosa.

Discussion

Results of the present experiment support the hypothesis that the perifornical region of the hypothalamus mediates AMPH-induced anorexia. Electrolytic lesions to the PFH region, at the level of the ventromedial nucleus, decreased feeding suppression induced by AMPH administered peripherally. This effect appeared to be specific to this site, since lesions that focused damage within the medial hypothalamus, specifically the PVN, did not attenuate AMPH's effect. Likewise, lesions just anterior or dorsal to the PFH region did not affect the drug response. Coronal KCs that severed fibers traversing the PFH area, at the level of the caudal hypothalamus, were also able to attenuate AMPH's effect, whether this drug was administered peripherally or directly to the PFH region. A variety of other cuts had no effect; namely, lateral cuts anterior to the level of the VMN, or cuts in the zona incerta region. These results suggest that AMPH's anorexigenic action is mediated by the perifornical region, and that fibers crucial for drug response course through the midlateral caudal hypothalamus as they pass between the midbrain and the PFH region. In contrast, the PFH lesions and PFH KCs that disrupted AMPH anorexia did not attenuate feeding suppression from FENF administration. In fact, the PFH KC significantly enhanced FENF-induced anorexia. The degree of anorexia exhibited by the PFH lesion rats, however, was not significantly enhanced as reported in other studies which have examined lateral, as well as midlateral hypothalamic lesions (Blundell & Leshem, 1974; Fibiger, Zis & McGeer, 1973; Pecile, Olgiati, & Netti, 1977). However, this is most likely due to the fact that the present study uses a percent suppression score to compare groups. If one compares the PFH lesioned rats to sham animals using <u>difference</u>

scores, a significantly enhanced response is seen in PFH-lesioned animals (analysis not reported). Note that this difference results solely from the fact that the PFH lesion animals consumed larger quantities of mash in the saline condition, and that their FENF response, as opposed to that observed in the PFH KC group, is not significantly less than the sham group (cf., Table 18).

It should be noted that the present experiment has utilized testing conditions quite different from those of most previous studies in this area of research. For example, all studies that have examined the effects of LH lesions on AMPH-induced anorexia have used 16-24 hr food deprivation schedules (in contrast to 4 hrs in the present study). This severe schedule, which has been questioned in terms of its physiological relevance to normal food intake regulation (Blundell & Latham, 1982), has been employed in conjunction with injections of AMPH at a relatively high (1-2 mg/kg) dose range (Carlisle, 1964; Blundell & Leshem, 1974; Fibiger, Zis, & McGeer, 1973; Leshem, 1981; Russek, et. al., 1973). In the present experiment, a lower 0.5 mg/kg dose of AMPH was used. This lower dose was chosen to minimize AMPH's stimulatory action on locomotion, which at higher dose levels clearly plays an increasingly predominant role in this drug's feeding suppressive effect (Lyons & Robbins, 1975). Similarly, use of a milder 4-hr food deprivation paradigm would be expected to attenuate AMPH's stimulatory effect, which is known to be greatly potentiated by extended food deprivation periods (Campbell & Fibiger, 1971).

The possibility exists that, in addition to the PFH, extrahypothalamic areas may be involved in AMPH-induced anorexia.

Hypothalamic manipulations used in the present study may have damaged fibers which pass between the forebrain and hindbrain. Dopaminergic systems in two forebrain structures, specifically, the striatum and nucleus accumbens, are believed to mediate two of AMPH's behavioral effects, namely, stereotypy and locomotor activity (Asher & Aghajanian, 1974; Carey, 1983; Costall, Marsden, Naylor, & Pycock, 1977; Creese & Iversen, 1974; Groves & Rebec, 1976; Kelly, Seviour, & Iversen, 1975; Koob, Riley, Smith, & Robbins, 1978; Van Rossum, Broekkamp, & Pijnenburg, 1977). Some investigators suggest that AMPH anorexia may be an indirect consequence of AMPH's locomotor effect, rather than result from direct action on catecholaminergic feeding mechanisms (Blundell & Latham, 1980; Cole, 1972,1979: Lyon & Robbins, 1975; Stricker & Zigmond, 1976; Winn, et. al., 1982). However, a variety of evidence counters the proposal that these extra-hypothalamic, forebrain structures are primary mediators of drug-induced feeding suppression. With regard to the striatum, AMPH injections directly to the striatum have no suppressive effect on feeding (Leibowitz, 1975a) and can actually elicit feeding (Winn, Williams, & Herberg, 1982). Second, destruction of dopaminergic innervation to the striatum, with local administration of the neurotoxin 6-hydroxydopamine, has little effect on anorexia induced by peripheral AMPH administration (Samanin, Bendotti, Bernasconi, Borroni, Garattini, 1977). Also, ventral noradrenergic bundle lesions, which leave intact primary dopaminergic projections to the forebrain (Fallon & Moore, 1978), reliably attenuate AMPH-induced anorexia (Ahlskog,1974; Carey, 1976; Leibowitz & Brown, 1980; Samanin, et. al., 1977), without affecting AMPH's stimulation of locomotor activity (Quattrone, Bendotti, Recchia, & Samanin, 1977; Samanin, et. al., 1977). As will be demonstrated

in the following chapter, ventrally-placed pontine KCs, which are caudal to all dopamine cell groups, also attenuate AMPH-induced anorexia.

Consistent with this dissociation of AMPH's locomotor and feeding effects is the evidence that bilateral destruction of the nucleus accumbens itself effectively attenuates AMPH's motor-stimulatory action, but does not alter the feeding suppression (Koob, et. al., 1978). LH lesions, as well as alpha-methyl-p-tyrosine treatment, are reported to decrease AMPH-induced anorexia, but not change this drug's arousal effects (Campbell & Baez, 1974; Cox & Maickel, 1975). Likewise, injections of AMPH to the nucleus accumbens can stimulate locomotor behavior (Jackson, Anden, & Dahlstrom, 1975; Pijnenburg, Honig, Van Der Heyden, Van Rossum, 1976; Van Rossum, Broekkamp, & Pijnenburg, 1977), but there is no evidence that AMPH administration directly to this structure suppresses feeding (Leibowitz, 1975a). Finally, the anterior PFH lesion in the present study (Figure 12b), which would be expected to damage dopaminergic fibers ascending to the nucleus accumbens (Fallon & Moore, 1978), had little effect on anorexia from peripheral AMPH administration.

In contrast, there is ample evidence that the PFH mediates AMPH feeding suppression. First, direct injections of AMPH to the PFH produces the strongest degree of feeding suppression of any brain site tested and has no apparent impact on locomotor behavior (Leibowitz, 1975a; Leibowitz & Rossakis, 1978a). In fact, injections of catecholaminergic blockers to this brain site disrupt anorexia induced by peripheral injection of AMPH (Leibowitz, 1975b). Finally, it is reported that the binding characteristics of ^{3}H-AMPH in the hypothalamus are

highly correlated with the feeding suppressive properties of various phenylethylamine anorectics; however, these binding sites are not related to the potencies of these anorectics in stimulating locomotor behavior (Paul, Hulihan-Giblin, & Skolnic, 1982).

The results from the present study also demonstrate a hypothalamic site of action for AMPH, specifically the PFH. First, lesions to the PFH and KCs just caudal to this region abolish anorexia induced by peripheral injection of AMPH at a relatively low dose. These brain manipulations show anatomical specificity for the PFH region, since lesions just rostral to the PFH or in the medial hypothalamus did not affect drug response. Also, Experiment 3 in the present study demonstrated that PFH KCs not only abolished anorexia resulting from peripheral administration of AMPH, but similarly affected the anorectic potency of AMPH injected directly into the PFH. These effects of PFH lesions and KCs were drug specific, since they did not decrease the anorectic potency of FENF. These findings, therefore, along with other evidence described above, strongly support the existence of a hypothalamic mechanism for feeding suppression; that is, a catecholaminergic system within the PFH region, which plays a major and direct role in mediating the anorectic action of AMPH, particularly at low dose levels. Since AMPH at higher dose levels may induce anorexia, at least in part, as a consequence of its locomotor stimulating action (Lyon & Robbins, 1975), the possibility still exists that extra-hypothalamic systems may also contribute to AMPH's anorectic action at these higher dose levels. Consistent with the proposal of a direct AMPH effect on a feeding behavior mechanism, human subjects injected with AMPH have reported decreases in hunger sensation at low doses that do not induce arousal sensations

(Blundell & Rogers, 1980; Silverstone, Wells, & Trenchard, 1983).

Mapping studies with catecholamine agonists have provided evidence that the PFH catecholaminergic receptor system involved in feeding suppression and AMPH anorexia exists specifically at the level of the ventromedial nucleus, immediately dorsal, lateral, or ventral to the fornix (Leibowitz & Rossakis, 1979b). Sites in the lateral aspects of the medial forebrain bundle, in the medial hypothalamus, or in the PFH area rostral or caudal to the ventromedial nucleus, do not appear to be involved. Sensitivity to locally administered AMPH follows a similar anatomical pattern (Leibowitz, 1975a; Leibowitz & Rossakis, 1978a), and results of the present lesion experiments confirm the conclusion that brain tissue in the immediate vicinity of the fornix, specifically at the level of the ventromedial nucleus, is crucial to the phenomenon.

In addition to defining the terminal site of catecholamine receptor sensitivity, the present study has also attempted to outline the course, within the hypothalamus, taken by fibers mediating catecholamine and AMPH feeding suppression. The KC results indicate that these fibers follow a relatively straight course through the midlateral caudal hypothalamus, that is, along the perimeter of the fornix within the most medial aspect of the medial forebrain bundle. Although the present study does not differentiate whether these fibers are ascending or descending, preliminary data obtained with direct PFH injection of dopamine indicate that caudal PFH KCs, which abolish AMPH anorexia, leave intact the feeding suppressive effect of dopamine, a post-synaptic receptor phenonemenon (Leibowitz & Brown, 1980a). Thus, a viable hypothesis (see next chapter) is that the crucial fibers severed by the cuts are ascend-

ing catecholaminergic fibers that course rostrally from the ventral midbrain and pons into the midlateral hypothalamic region and terminate in the perifornical region at the level of the ventromedial nucleus. This PFH area is dense with catecholamine fibers (Leibowitz & Brown, 1980a) and is the area most sensitive to local injection of the catecholamines (Leibowitz & Rossakis, 1979b), as well as to AMPH, which presumably acts via the release of endogenous catecholamines which subsequently inhibit feeding (Leibowitz, 1975a,c). Evidence obtained in the next series of experiments, and supported by earlier studies at the midbrain level (Leibowitz & Brown, 1980a), suggest that the catecholamine fibers ascending to the PFH region originate from the ventral medullary noradrenergic and ventral mesencephalic dopaminergic cell groups and assume a relatively ventral and midlateral position through the entire brainstem.

Figure 10. Dose response study of feeding responses to intrapertoneal (i.p.) injection of d-amphetamine sulfate, following 4 hr food deprivation. Dunnett's t -statistic ($^{*}p<0.01$) indicated the 0.5 - 2.0 mg/kg dosages produced significant decreases in mash intake compared to after saline injection. Points represent the average mash intake of 5 rats and horizontal bars represent the standard error of the mean (SEM). Consumption was measured 60 min after drug injection.

Figure 11. Dose response study of feeding response to fenfluramine hydrochloride (0.0- 12.0 mg/kg). Dunnett's t -statistic ($^{*}p<0.05$) showed intraperitoneal injection of 2.0- 12.0 mg/kg of FENF significantly decreased food intake (60 min post-injection) below levels seen following injection of saline. Administration of 0.22 mg/kg or 0.66 mg/kg of FENF failed to significantly change food intake response. Points represent the average feeding responses of eight rats, and bars depict the SEMs.

Figure 10

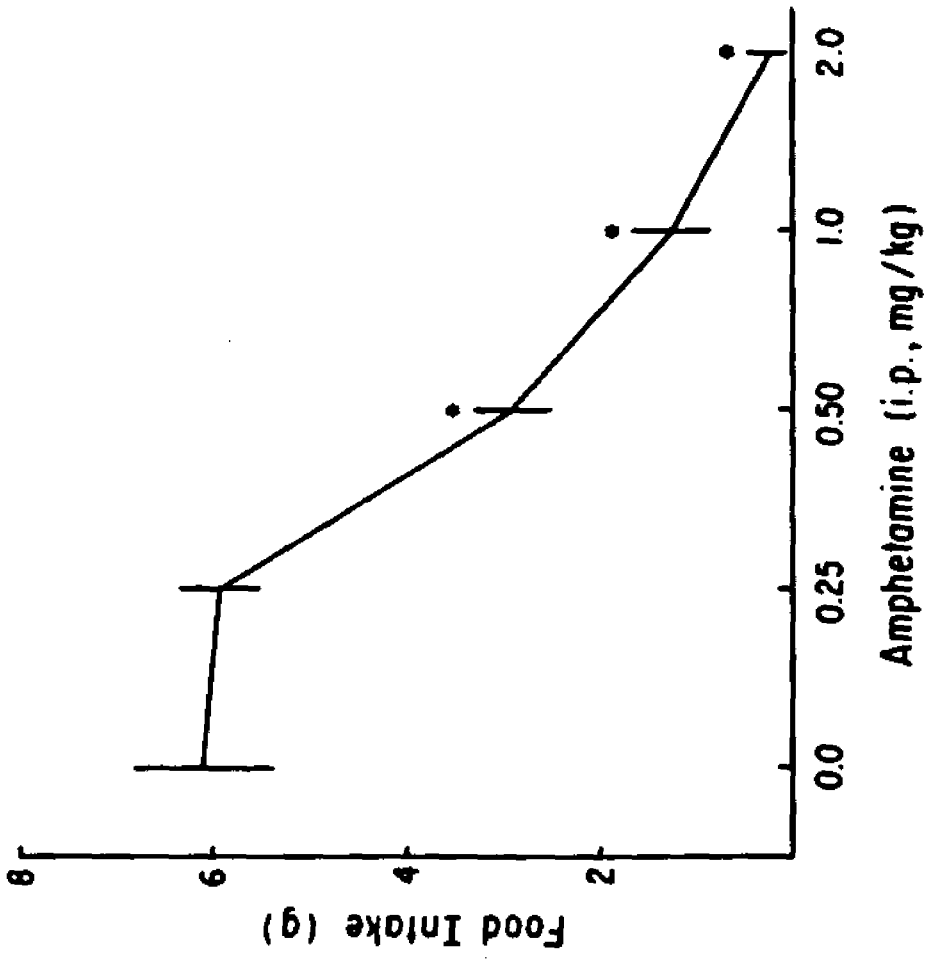


Figure 11

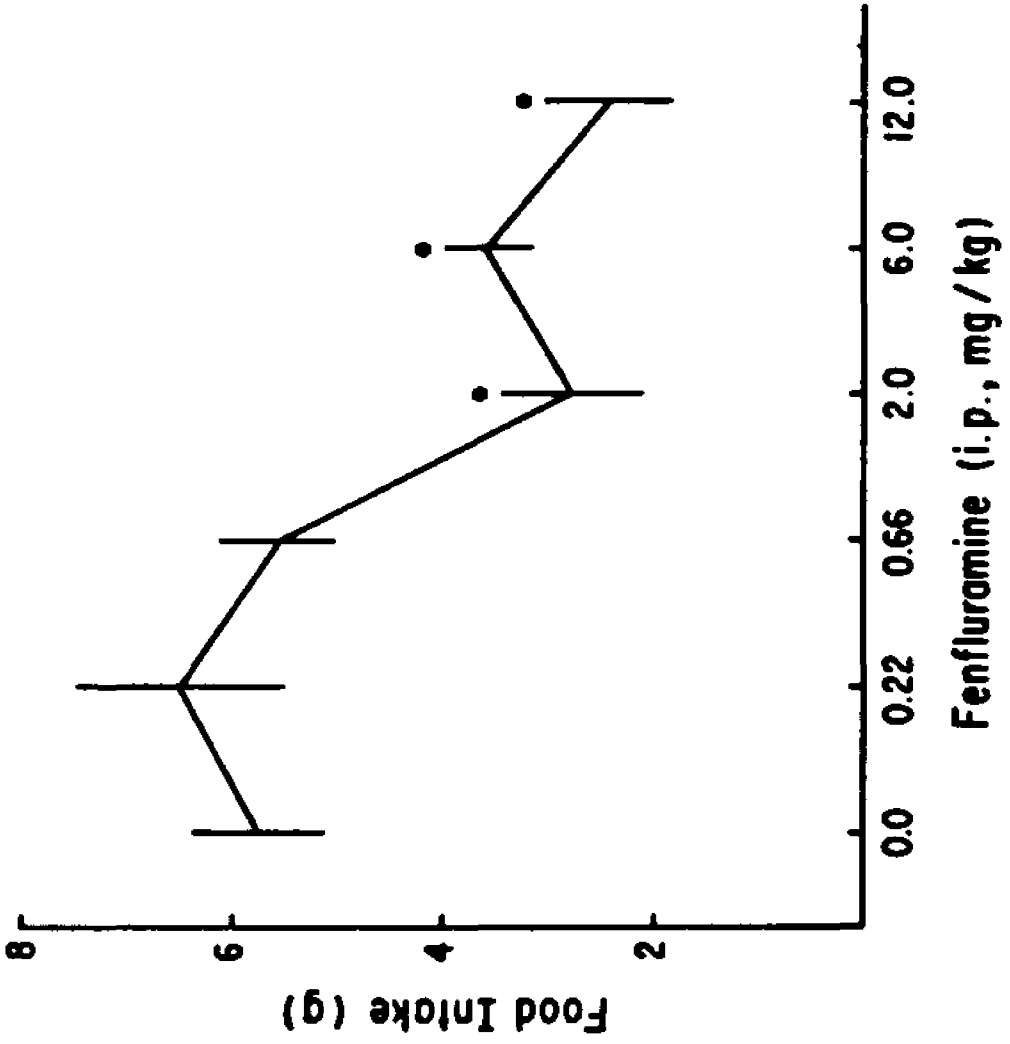
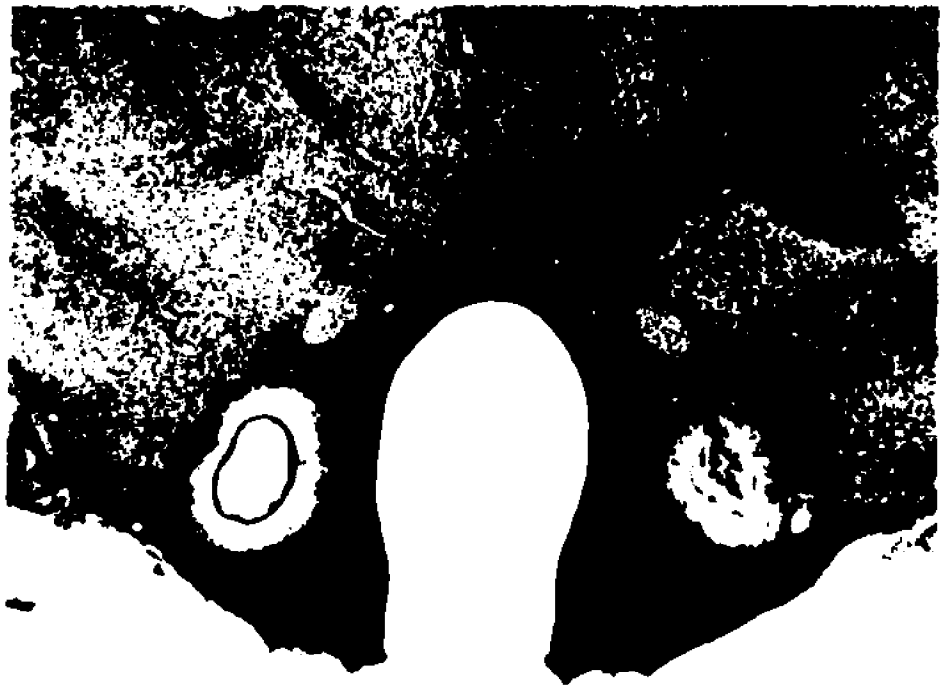
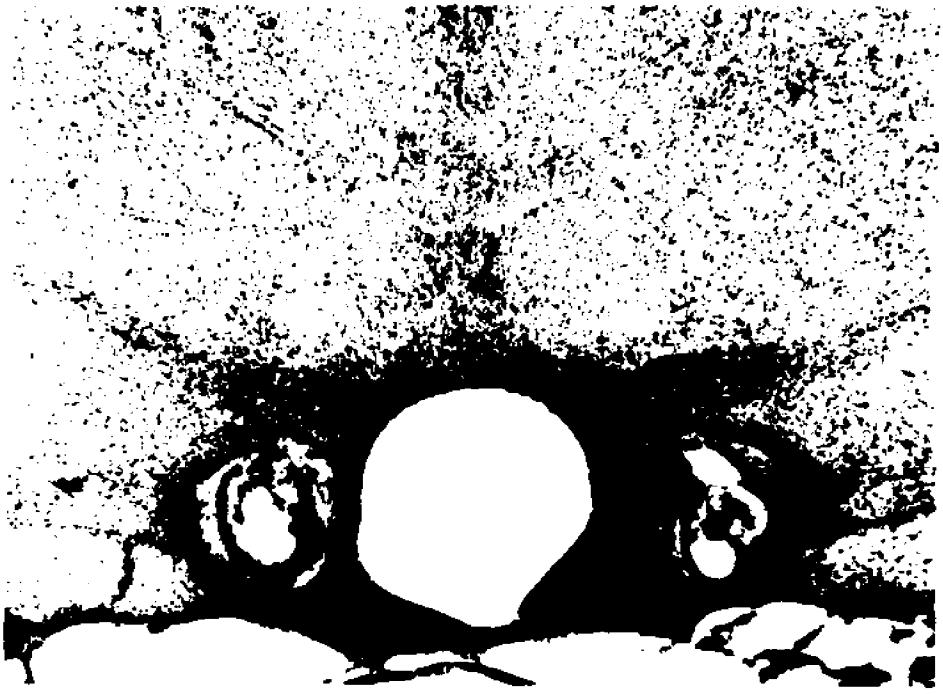


Figure 12. Figure 12a is a photomicrograph of an on-target PFH lesion. These lesions damaged the entire PFH region from the level of the PVN through the level of the ventromedial nucleus, and at times as caudal as the anterior portion of the posterior hypothalamic area. Tissue damage also extended into the medial half of the medial forebrain bundle and medial portions of the dorsomedial and ventromedial nuclei. Figure 12b shows an anterior PFH lesion which damaged the PFH region at the level of the PVN, but did not produce significant tissue damage at the level of the ventromedial nucleus.

Figure 12



12a



12b

Table 17. The effect of various electrolytic lesions upon feeding response to 0.5 mg/kg AMPH administered intraperitoneally (i.p.). Rats were food deprived for 4 hr prior to drug adminstration. Values listed below Saline and AMPH are the group mean feeding response (±standard error of the mean), and % Suppression represents the groups average decreased level of food intake after AMPH injection (% suppression equals AMPH score minus saline score, divided by saline score). Note that the groups' percent suppression score does not equal the value derived from using the averages of the raw score saline and AMPH values since the value given in the table is based upon the percent suppression scores calculated for individual rats. Since percent supression scores with respect to PFH lesion and sham groups were hetergenous, the PFH lesion rats were compared to the unilateral PFH lesion group alone. On the average, PFH lesion rats exhibited a significantly attenuated anorectic response to AMPH compared to unilateral PFH lesion rats (‡ $p<0.01$). Note that the four PVN lesion animals, compared to sham rats, exhibited a significantly greater anorectic response to AMPH (* $p<0.005$).

Table 17

Feeding Responses After Intraperitoneal Injection of Saline and Amphetamine

Group	n	Saline	AMPH	% Suppression
Shams	12	5.2±0.6	2.5±1.0	-50.9±4.4
PFH Lesions	10	12.2±1.4	12.9±2.2	+3.4±12.0‡
Unilateral PFH Lesions	9	10.8±1.1	5.7±0.9	-46.3±10.1
Anterior Lesions	3	9.5±2.6	3.9±2.5	-68.0±17.6
Dorsal Lesions	3	3.2±0.7	1.5±0.7	-60.0±17.6
PVN Lesions	4	6.4±1.4	0.9±0.6	-85.0±6.0*

‡ $p<0.001$ Percent suppression score less than unilateral PFH group.
* $p<0.005$ Percent suppression score greater than sham group.

Table 18. Food intake (grams) of sham rats and animals sustaining hypothalamic lesions or KCs after intraperitoneal injection of FENF (2.0 $\mu g/kg$) and saline. In terms of percent suppression scores, PFH lesion rats exhibited an anorectic response to FENF that failed to differ from sham rats ($p>0.10$). PFH KC rats, however, exhibited a significantly enhanced anorectic response to FENF compared to sham rats (* $p<0.05$).

Table 18

Responses to Saline and Fenfluramine

Group	n	Saline	Fenfluramine	%-Suppression
Shams	10	6.0±0.6	3.2±0.7	-52.0±7.4
Lesions				
PFH Lesions	6	11.0±1.7	3.5±0.8	-61.0±11.8
Unilateral PFH Lesions	4	8.8±1.2	5.3±0.8	-39.8±1.4
Anterior/ Dorsal Lesion	2	7.3±2.4	4.9±2.3	-32.9±0.2
Perifornical Knife Cut	6	5.8±0.7	1.4±0.4	-78.0±5.5#

# $p<0.05$ Percent suppression score greater than sham group.

Figure 13. The upper figure is a coronal drawing of the rat brain at the level of the caudal hypothalamus [approximately 3180 μ with respect to the Koenig and Klippel (1974) atlas]. The left side of this figure depicts the region severed by the PFH KC. As can be seen, the PFH KC severed tissue in the medial forebrain bundle (MFB) as well as tissue medial to the fornix (F). The midline KC is represented on the right side of the upper drawing and shows that the midline cut extended to the midline of the brain and to the medial aspect of the fornix. The lower drawing depicts a sagittal view of the rat brain (approximately 950 u lateral to midline). Three actual on-target PFH KCs are traced on the drawing and can be seen to sever tissue in the caudal hypothalamus. (Abbreviations: AC- anterior commissure; BC- brachium conjunctivum; CC- crus cerebri; F-fornix; FR- fasciculus retroflexus; LM- medial lemniscus; MFB- medial forebrain bundle; MTT- mamillothalamic tract; PC- posterior commissure; PFH KC- perifornical KC; r- red nucleus; vIII- third ventricle; VII- seventh cranial nerve).

Figure 14. Photomicrograph 14a depicts the PFH KC which abolishes the feeding suppressive effect of AMPH (see text). The PFH KC severed tissue in the caudal hypothalamus between 0.7 mm and 1.1 mm lateral to midline. In contrast, the midline cut (14b) severed caudal hypothalamic tissue from midline to 0.7 mm lateral to midline.

Figure 13

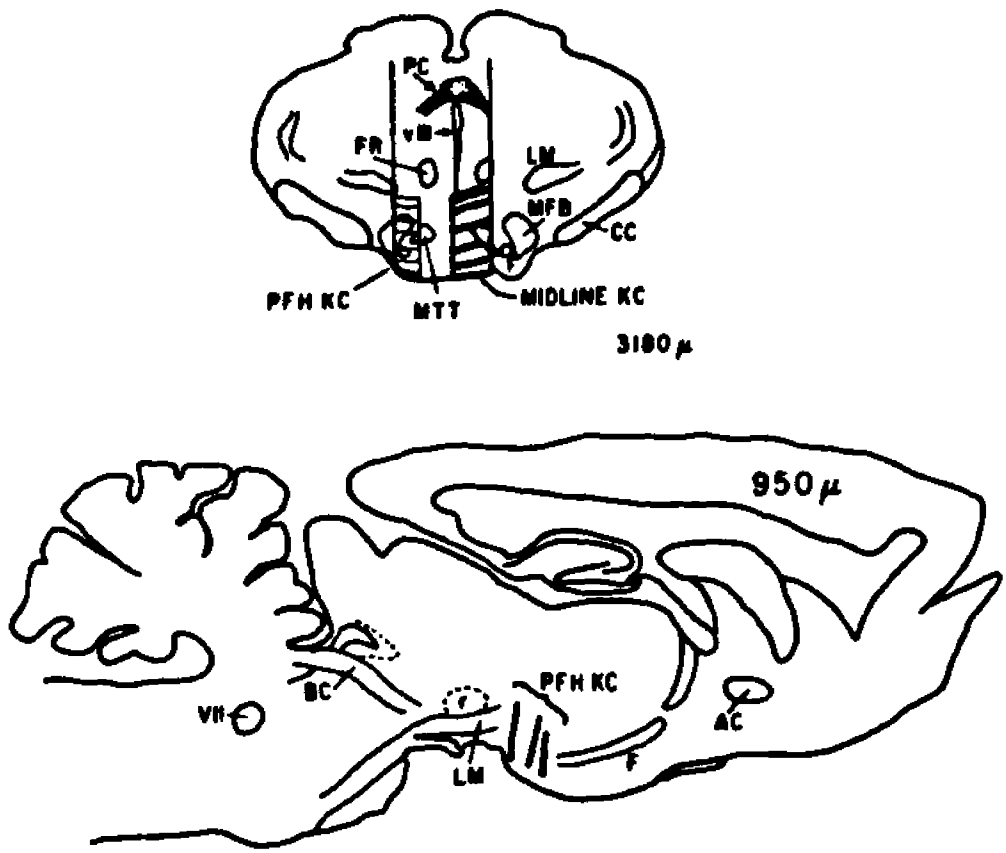

PC
VH
FR
LM
MFB
CC
PFH KC
MTT
MIDLINE KC
3180 μ
950 μ
BC
VH
PFH KC
AC
LM
F

Figure 14

14a

14b

Table 19. Feeding responses mean (±SEM) of sham and several KC groups after i.p. injection of saline and AMPH. In terms of percent suppression scores, sham animals failed to differ from unilateral PFH KC rats, and rats sustaining KCs anterior or dorsal to the region severed by the PFH KC, and midline KC rats. In contrast, the PFH KC group exhibited a significantly attenuated anorectic response to AMPH (♯ $p<0.001$).

Table 20. Mean (±SEM) feeding responses (grams) to saline and AMPH administered intracranially (i.c.) to the PFH region. Sham animals and rats with KCs either anterior or dorsal to the PFH region failed to differ in terms of anorectic response to PFH injections of AMPH. The PFH KC rats, however, exhibited a significantly attenuated anorectic response to AMPH compared to sham rats (♯ $p<0.001$).

Table 19

Feeding Responses After Peripheral
Injections of Saline and Amphetamine

Group	n	Saline	Amphetamine	%-Suppression
Shams	14	6.0±0.6	3.0±0.3	-54.8±7.8
Hypothalamic KCs				
PFH KCs	12	5.2±0.4	5.7±1.1	-7.2±6.7‡
Midline KCs	12	6.2±0.8	3.2±0.3	-45.1±3.5
Unilateral KC	7	5.3±0.9	2.2±0.3	-55.6±3.9
Anterior/ Dorsal KC	6	7.4±1.2	3.5±1.0	-54.8±8.2

‡ $p<0.001$ Percent suppression scores less than sham group.

Table 20

Feeding Responses After Central
Injections of Saline and Amphetamine

Group	n	Saline	Amphetamine	%-Suppression
Shams	10	6.8±0.7	5.5±0.8	-19.5±8.4
PFH KCs	9	5.3±0.7	5.7±0.6	+11.8±5.7‡
Anterior/ Dorsal KCs	6	7.0±1.5	5.0±1.3	-30.5±8.2

‡ $p<0.001$ Percent suppression scores less than sham group.

Chapter 5

Determination of the Course of Catecholamine Fibers Mediating Amphetamine Anorexia: Midbrain and Pontine Knife Cuts

Previous studies have demonstrated that the lateral perifornical region of the hypothalamus (PFH), at the level of the ventromedial nucleus, plays a role in mediating AMPH-induced anorexia. The PFH is the primary site where injections of AMPH and catecholamine agonists maximally suppress feeding, in contrast to less potent effects obtained when injections are made to over 20 other hypothalamic and extrahypothalamic sites (Leibowitz, 1975a; Leibowitz & Rossakis, 1978a). In addition, the previous study has shown that electrolytic lesions of the perifornical region, in contrast to medial hypothalamic lesions, abolish AMPH-induced feeding suppression and that coronal PFH knife cuts placed in the caudal hypothalamus disrupt anorexia produced by peripheral, as well as after central PFH, injections of AMPH. These findings suggest that fibers crucial for AMPH-induced anorexia course through the midlateral caudal hypothalamus and terminate in the perifornical region at the level of the ventromedial nucleus.

Pharmacological experiments have demonstrated that endogenous catecholamines mediate AMPH anorexia (see Garratini & Samanin, 1978). First, AMPH is known to release and block re-uptake of the catecholamines norepinephrine (NE), epinephrine (EPI), and dopamine (DA: Burgess & Tessel, 1980; Copeland, Aulakh, Bhattacharyya, & Pradhan, 1980; Roffman, Cassens, & Schildkraut, 1978). Second, depletion of brain catecholamines, by intracerebroventricular administration of 6-hydroxydopamine,

that destroys presynaptic catecholamine terminals (Jonsson, 1980), impairs AMPH's ability to suppress feeding (Fibiger, Zis, & McGeer, 1973; Heffner & Seiden, 1979; Hollister, Erwin, Cooper, & Breese, 1975; Samanin, Bernasconi, & Garratini, 1975; Stricker & Zigmond, 1976). Additionally, administration of alpha-methyl-p-tyrosine, which temporarily depletes catecholamine stores (Spector, Sjoerdsma, & Udenfriend, 1965), decreases AMPH's anorectic action (Baez, 1974; Frey & Schulz, 1973; Holtzman & Jewett, 1971; Leibowitz, 1975b; Weissman, Koe, & Tenen, 1966).

Previous studies indicate that both DA and NE (or EPI) play a role in AMPH-induced anorexia. First, administration of both dopaminergic and adrenergic agonists, as well as the catecholamine precursor, l-dopa, either peripherally or directly to the PFH, induces anorexia (Baez, 1974; Bazaghi, Gropetti, Hantegazza, & Muller, 1973; Dobrzanski & Doggett, 1976; Goldman, Lehr, & Friedman, 1971; Hansen & Whishaw, 1973; Leibowitz & Rossakis, 1979a,b; Lehr & Goldman, 1973; Sanghvi, Singer, Friedman, & Gershon, 1975). Both dopaminergic and beta-adrenergic antagonists can antagonize catecholamine- and AMPH-induced feeding suppression, including when the blocker is administered to the PFH and AMPH injected intraperitoneally (Blundell & Latham, 1980; Clineschmidt & Bunting, 1980; Kruk, 1973; Leibowitz, 1975b, 1978; Leibowitz & Rossakis, 1978a; Zis & Fibiger, 1975). Similarly, central injection of alpha-methyl-p-tyrosine and the compound, FLA-63 (a dopamine-beta-hydroxylase inhibitor that reduces NE synthesis) antagonize anorexia induced by AMPH injection to the PFH (Leibowitz, 1975b). Peripheral administration of FLA-63, however, potentiates AMPH-induced anorexia (Franklin & Herberg, 1977). Studies with <u>central</u> adminstration of various beta-adrenergic

antagonists to the PFH region clearly block anorexia induced by either central or peripheral AMPH (Leibowitz, 1975b; Leibowitz & Rossakis, 1978b). Although there is one positive report showing blockade of AMPH anorexia after peripheral administration of the beta-adrenergic agonist, propranolol (Sanghvi, et. al., 1975), other studies have failed to obtain this effect (Dobrzanski & Doggett, 1979; Kruk, et. al., 1976; Lehr & Goldman, 1973; Schmitt, 1973). These inconsistencies may be due to the fact that peripherally administered propranolol, by itself, inhibits feeding and disrupts the catabolism of AMPH (Shoeman, Sirtori, & Azarnoff, 1974; Willner & Towell, 1982).

In support of the suggestion that both dopaminergic and adrenergic systems are involved in AMPH anorexia, there are a number of investigations which have damaged hindbrain catecholamine cell groups and found AMPH's feeding suppressive effect to be attenuated or abolished. Some studies have suggested that the DA neurons originating in the substantia nigra mediate AMPH-induced anorexia, since electrolytic (Carey & Goodall, 1975) and 6-hydroxydopamine lesions of this structure (Fibiger, Phillips, & Clouston, 1973; Fibiger, Zis, & McGeer, 1973) attenuate the action of peripherally administered AMPH. Other studies have reported that the ventral noradrenergic bundle mediates amphetamine feeding suppression, since electrolytic as well as 6-OHDA lesions to this fiber bundle, caudal to the midbrain dopamine cell bodies, also disrupt drug induced anorexia (Ahlskog, 1974; Ahlskog & Hoebel, 1973; Borsini, Bendotti, Carli, Poggesi, & Samanin, 1979; Samanin, Bendotti, Bernasconi & Garratini, 1977).

Using the central injection technique to administer the catecholam-

ine agonists EPI and DA, as well as AMPH, directly to the perifornical region, Leibowitz has obtained evidence (Leibowitz & Brown, 1980a; Leibowitz, Brown, & Hammer, 1980) that the ventral adrenergic fiber system and DA fibers arising from the DA-containing A8 and A9 cell groups, mediate AMPH's anorectic effect. Electrolytic and 6-hydroxydopamine lesions to these catecholamine fiber projections disrupted anorexia induced by PFH, as well as peripheral, injections of AMPH, while actually potentiating anorexia by central administration of DA and EPI. Lesions that damaged other ascending catecholamine fiber systems did not affect responsiveness to AMPH.

From these recent studies, it is apparent that damage to ascending catecholamine fiber systems disrupts AMPH feeding suppression. Additionally, data from the previous study suggests that, at the level of the hypothalamus, the adrenergic and dopaminergic fibers which mediate AMPH-induced anorexia course through the midlateral caudal hypothalamus and terminate in the PFH area. The present study has attempted to further map the brain catecholamine systems mediating AMPH response by determining the specific trajectory of crucial fibers at pontine as well as midbrain levels of the brainstem. The rats in this study sustained bilateral coronal knife cuts in the midbrain and pons, and were tested with central PFH injections of AMPH and DA, as well as with peripheral injections of AMPH and FENF.

The results of these studies indicate that the catecholaminergic fibers mediating AMPH anorexia follow a relatively straight, ventral and midlateral course through the brainstem on their way to the PFH area. It is proposed that the noradrenergic or adrenergic fibers involved in

the response originate from the ventrolateral, and perhaps dorsolateral, medullary cell groups, whereas the dopaminergic fibers originate from ventrolateral midbrain DA cells scattered caudal to the substantia nigra.

Experiment 1

The previous paper has delineated regions in the hypothalamus that are crucial for AMPH-induced anorexia. Fibers appear to ascend within the medial forebrain bundle and the midlateral perifornical region of the caudal hypothalamus and terminate in the perifornical region of the hypothalamus at the level of the ventromedial nucleus. In the next experiment, KCs were placed in the midbrain region to sever catecholamine fibers as they course through the brain just caudal to the level of the red nucleus. Results indicate that ascending fibers crucial for the mediation of AMPH response pass just dorsal, and perhaps through, the medial lemniscus just ventral to the red nucleus.

Methods

Subjects. A total of 55 rats were used in this experiment. During the entire study, animals were maintained on the mash diet and water was available ad libitum.

Surgery. As outlined in the Methods section of the CLON paper, three midbrain KCs were made in different groups of animals. A total of 9 rats received the Far-Ventral KC severing tissue at the base of the brain, while 16 rats each received either the Ventral or the Dorsal KC. Some of the Far-Ventral and Ventral KC rats also received PFH cannula

implants.

Test Procedure. Noncannulated rats were tested for response to saline (1 cc/kg), AMPH (0.5 mg/kg), and FENF (2.0 mg/kg) after 4 hrs of food deprivation. Animals that were implanted with a PFH cannula were tested for response to saline (0.5 µl), 150 nM amphetamine sulfate (55.32 µg in 0.5 µl sterile saline), or 150 nM dopamine (DA: 28.45 µg in 0.5 µl sterile saline). On days when DA was administered, animals also received a 15 mg/kg intraperitoneal injection of pargyline. These trials were conducted in conjunction with baseline tests on other days where pargyline was given with PFH injections of saline. As in previous studies, food intake was measured 60 min after drug administration.

Results and Discussion

Figure 15 presents a schematic illustration of the three midbrain KCs. Animals that sustained the Far-Ventral KC procedure had small cuts that severed tissue in the very medial aspects of the substantia nigra and in the ventral tegmental area. These cuts generally damaged the base of the brain and extended as far dorsal as the level of the medial lemniscus. The Ventral KC severed tissue around the medial lemniscus, but particularly seemed to damage tissue just dorsal to the medial lemniscus. These cuts generally were just caudal to the level of the red nucleus and extended from approximately 0.5 mm to 1.1 mm lateral to midline (with respect to the atlas of Koenig and Klippel, 1974). The Dorsal KC was positioned dorsal to the Ventral cut and severed tissue beginning just behind the most caudal tip of the red nucleus and

extended approximately 1.8 mm dorsal to sever tissue just ventrolateral to the central grey. Similar to the Ventral cut, this KC extended between approximately 0.5 to 1.1 mm lateral to midline. Figures 16a-16c present sagittal photomicrographs of (respectively) the Dorsal, Ventral, and Far-Ventral midbrain KCs.

Table 21 presents the results of peripheral injections of AMPH and, as can be seen by examining the percent suppression scores, the Ventral KC group as opposed to the sham and other KC groups, exhibited a smaller anorectic response to AMPH. Analysis of variance indicated that the average percent suppression of the sham and KC groups were significantly different [$F(3,51)= 5.76$; $p<0.01$], and individual comparisons showed that the Ventral midbrain KC group's response was significantly less than sham rats [$F(1,51)= 13.9$; $p<0.01$]. The degree of feeding suppression of the Far-Ventral KC rats and the Dorsal KC group failed to differ from sham animals [$F(1,51)= 0.46$; 0.0001, respectively]..

Table 22 presents the feeding responses of sham and midbrain KC groups after administration of FENF and saline. No Far-Ventral KC animals were tested with this drug. Analysis of variance of percent suppression scores indicated the sham, Dorsal KC, and Ventral KC groups failed to differ significantly [$F(2,18)= 1.32$; $p>0.25$],

Table 23 presents the results of PFH injections of AMPH. Analysis of variance indicated the three groups failed to differ significantly in terms of percent suppresion scores [$F(2,27)= 1.40$; $p>0.05$]. It should be noted that the sham and Far-Ventral KC rats, comparing their respective feeding responses to saline and AMPH, both exhibited significant decreases in food intake after AMPH [$t(11)= -3.56$; $p<0.001$; $t(7)= -2.67$;

$p<0.05$, respectively), while the decrease observed for the Ventral KC group failed to be significant [$t(9)= -0.90$]. These data suggest that in spite of the larger and significant anorectic responses to PFH injections of AMPH by the sham and Far-Ventral KC group the attenuated and non-significant anorectic response observed after the Ventral KC was not sufficient to significantly block AMPH induced anorexia when this drug is injected centrally. Table 24 summarizes the response pattern of the sham, Ventral KC, and Far-Ventral KC groups after PFH injection of saline and DA. Analysis of variance of percent suppression scores indicated that the sham, Ventral KC, and Far-Ventral KC groups failed to differ significantly [$F(2,16)= 1.60$; $p>0.10$].

At the midbrain level, the crucial region through which catecholamine fibers course that mediate AMPH anorexia pass between the medial lemniscus and the red nucleus. Knife cuts that severed tissue in this region attenuated AMPH response to the extent that anorexia induced by peripheral administration of AMPH no longer decreased food intake. This result was in contrast to cuts severing tissue in the ventral tegmental area and very medial substantia nigra (the Far-Ventral cut), as well as Dorsal midbrain cuts placed just ventrolateral to the central grey, which did not disrupt feeding suppression to AMPH. The present results, with central injections of AMPH, however, are not clear cut. As can be seen in Table 23, the Ventral midbrain KC group, on the average, do decrease their feeding after AMPH by -14% compared to a decrease of -34% by the sham rats. Closer inspection of the data from the Ventral midbrain KC and sham groups does suggest that the majority of rats in the Ventral KC group exhibit an attenuated response to AMPH. Specifically, assessing each animals degree of anorexia to AMPH as a percentage of

their food intake after saline, 7 of the 10 Ventral KC rats (70%) decreased their response to AMPH by less than 25%, while 4 of the 12 (33%) of the sham rats exhibited percent suppression scores less than 25%.

Experiment 2

In this study, animals sustained one of three coronal KCs at the pontine level, namely, a Rostral Pontine KC (RPKC), Dorsal Caudal Pontine KC (Dorsal CPKC), or a Ventral Caudal Pontine KC (Ventral CPKC). The dorsally placed RPKC and CPKC did not affect response to AMPH. The larger, and more ventrally-placed, Ventral CPKC significantly attenuated AMPH-induced anorexia.

Methods

Subjects. A total of 29 rats were used in this study. Five sham animals and Dorsal CPKC animals also received cannula implants to the PFH region. Cannulated animals were then tested for level of response to centrally injected DA and AMPH.

Surgery. The pontine KCs were made using a knife that extended 1.8 or 2.5 mm lateral to the guide and 1.5 mm below the guide (pitch). The nosebar was set 4.0 mm or 4.2 mm below interaural line for, respectively, the Rostral and Caudal pontine KCs. As noted earlier, these cuts were made in two stages.

Behavioral Testing. Noncannulated rats were food deprived for four hr before receiving injections of either saline (1 ml/kg) or AMPH (0.5 mg/kg). Only RPKC and sham animals received tests involving FENF, and a 3.0 mg/kg dose was used. Animals that received PFH cannula implants

were tested for response to saline (0.5 µl), 150 nM AMPH (55.32 µg in 0.5 µl sterile saline), and 150 nM dopamine (28.45 µg in 0.5 µl sterile saline). Fifteen min prior to central DA injection, or saline trials for comparison to DA response, rats received a 15 mg/kg injection of pargyline. As in the previous experiments, food intake was measured 60 min after drug administration.

Results and Discussion

The three pontine KCs are illustrated in Figure 17. A total of 10 animals sustained RPKCs. This cut was positioned anterior to the level of the locus coeruleus and severed tissue, in different animals, from as medial as 0.5 mm to 1.9 mm lateral to midline. The dorsal-ventral extent of the cut severed tissue from the ventral half of the periaqua-ouctal grey to just below the ventral aspect of the ventral parabracnial nucleus. Six rats received the Dorsal CPKC. This cut was positioned just caudal to tne level of the motor nucleus of the fifth cranial nerve. Cuts in different animals severed tissue from 0.5 mm to 1.9 mm lateral to midline and extended from the dorsal surface of the pons and as far ventral as the ventral level of the motor nucleus of the fifth nerve. Animals (n=5) that sustained the Ventral CPKC possessed tissue damage generally at the same anterior-posterior level as the Dorsal CPKC, and extended from 1.3 mm-1.9 mm lateral to midline. In contrast to the Dorsal CPKC, the Ventral CPKC extended farther ventral, and reached to the dorsal surface of the nucleus of the seventh cranial nerve. Photomicrographs of the three pontine cuts are presented in Figure 18.

Table 25 presents the results of peripheral administration of AMPH

to sham and pontine KC rats. Analysis of variance of percent suppression scores indicated the groups exhibited significantly different degrees of anorexia [$F(3,25)= 3.82$; $p<0.05$]. Tests comparing the sham and Ventral CPKC group indicated the latter group exhibited a significantly attenuated response to AMPH [$F(1,25)= 4.93$; $p<0.05$], while the RPKC group and the Dorsal CPKC group failed to differ from shams [$F(1,25)= 1.22$; 0.94, respectively].

Table 26 summarizes the results of 3.0 mg/kg administration of FENF to animals that sustained sham or RPKC surgeries. As can be seen, both sham and RPKC rats were responsive to FENF and analysis of variance of percent suppression scores indicated that these groups failed to differ significantly in terms of the degree of anorexia induced by FENF [$F(1,12)= 0.007$].

Table 27 presents the results of PFH injections of AMPH and saline to sham and Dorsal CPKC rats. Both groups decreased their feeding after AMPH and analysis of variance indicated the groups failed to differ [$F(1,10)= 3.40$; $P<0.1$]. Table 28 presents the results of PFH injections of saline and DA to sham and Dorsal CPKC rats. Both groups consumed less mash after combined injections of pargyline and DA than after pargyline and saline and, in fact, the degree of anorexia exhibited by the Dorsal CPKC rats was greater than the sham rats [$F(1,10)= 6.82$; $p<0.05$].

The present study suggests that coronal KCs placed in the caudal pons that sever tissue extending as far ventral as the dorsal surface of the nucleus of the seventh nerve disrupts anorexia elicited by peripheral administration of AMPH. In contrast, KCs severing tissue only in the dorsal half of the pons, either rostral or caudal to the level of

the LC, did not affect response to AMPH, and with respect to the caudal cut, whether this drug was administered peripherally or directly to the PFH. The caudal cut, likewise, did not disrupt anorexia that follows PFH injection of DA. The RPKC, as well as the Dorsal CPKC, would be expected to sever dorsal ascending catecholamine fibers that arise from pontine and medullary catecholamine cell bodies, but these KCs did not affect response to AMPH. In contrast, the Ventral CPKC severed ventrally-coursing lateral tegmental catecholamine fibers, and in comparison to the dorsal cuts, this manipulation decreased anorexia to AMPH below the degree seen in the sham animals. These results suggest that catecholamine fibers, ascending through the pons, at least partially mediate AMPH feeding suppression. Crucial fibers mediating AMPH response, therefore, ascend in the ventral portion of the pons and apparently ascend from catecholamine cell bodies positioned caudal to the effective KC.

General Discussion

Tne results of the present study indicate that knife cuts placed at specific sites in the midbrain and pons disrupt anorexia stimulated by peripheral administration of AMPH. The effective cuts, at both midbrain and pontine levels, were located in the ventral aspects of the brainstem, while KCs placed in more dorsal portions of the brain did not antagonize AMPH's effect upon feeding. In addition, PFH cannulated animals with Ventral midbrain KCs were unresponsive to PFH injections of amphetamine but exhibited anorexia after PFH injections of dopamine. This conclusion with respect to Ventral midbrain KCs attenuating anorexia induced by PFH injections of AMPH must be interpreted with caution since the degree of anorexia exhibited by these animals was not

significantly less than what was observed in the sham group. With respect to other findings at the midbrain level, midbrain KCs severing tissue ventrolateral to the central gray or in the ventral tegmental area and very medial substantia nigra did not affect drug induced anorexia whether AMPH was administered peripherally or to the PFH. Similarly, dorsally-placed pontine KCs, either anterior or posterior to the level of the LC, did not affect anorexia induced by peripheral administration of amphetamine, or PFH injections of AMPH or dopamine. Based upon these findings, it is proposed that fibers mediating AMPH anorexia traverse the ventral brainstem. In particular, crucial fibers course through the pons just dorsal to the nucleus of the seventh cranial nerve, and then traverse the midbrain, at the level of the red nucleus, just dorsal to (and perhaps through) the medial lemniscus.

In contrast to these findings with AMPH, where some KCs disrupted drug-stimulated anorexia, no brain manipulation affected response to FENF. Anorexia from this drug depends upon its action on serotonergic fibers, where it releases and blocks re-uptake of serotonin (Garratini and Samanin, 1978). These findings suggest that the KC placements in this study did not significantly damage fibers that mediate this response.

In terms of this study with AMPH, one question is whether the proposed ventrally-coursing pathway mediating AMPH's feeding suppressive effect is an ascending or descending system. The results obtained with central PFH injections of AMPH and DA would seem to suggest an ascending, presumably catecholaminergic fiber system. That is, the midbrain KC appears to disrupt anorexia induced by PFH injection of AMPH, but had

no effect upon DA-induced anorexia. Since DA, in contrast to AMPH, is believed to act postsynaptically at receptor sites located in the PFH region (Leibowitz & Brown, 1980a), the present results suggest that efferent fibers from the PFH region were not severed by the effective midbrain KC. Instead, since AMPH is believed to act presynaptically and rely upon the release of endogenous catecholamines (CAs) to induce anorexia (Garratini & Samanin, 1978), it appears that the midbrain KC severed ascending fibers that mediate anorexia from PFH administration of amphetamine. This conclusion is consistent with the finding that ventral midbrain injection of 6-hydroxydopamine, which selectively damages CA fibers, are also effective in abolishing AMPH anorexia (Leibowitz & Brown, 1980).

This study concurs with previous research suggesting hindbrain fiber systems mediate AMPH-induced anorexia, and has further defined the specific route taken by these crucial fibers. Since PFH injections of DA-antagonists and beta-adrenergic antagonists both attenuate anorexia induced by PFH or peripheral injection of AMPH, it has been proposed that dopamine-containing, as well as noradrenergic/adrenergic-containing fiber systems, play a role in AMPH anorexia (Leibowitz, 1980; Leibowitz & Brown, 1980a). In the present study, KCs that disrupted AMPH response severed brainstem tissue where both dopamine and noradrenergic/adrenergic fibers are distributed (Lindvall & Bjorklund, 1978; Ungerstedt, 1971). With respect to which particular DA cell group mediates AMPH feeding suppression, the present findings suggest that fibers which arise from the "A8" dopamine cell group (Dahlstrom & Fuxe, 1964; Leibowitz & Brown, 1980a), mediate AMPH-induced anorexia. That is, the Ventral midbrain KC, positioned just dorsal to the medial

lemniscus, severs fibers apparently arising from "A8" DA neurons. Counter to several other reports, the present findings suggest that fibers from dopamine cells within the traditionally defined substantia nigra, pars compacta, or "A9" cell group do not directly mediate AMPH anorexia. This conclusion is based upon the fact that the Ventral midbrain cut is caudal to the majority of the substantia nigra, but effectively abolished AMPH feeding suppression. The present study therefore confirms the findings of Leibowitz and Brown (1980a) that crucial DA fibers that mediate AMPH feeding suppression arise from DA cells caudal and ventrolateral to the substantia nigra. In terms of studies which have suggested that the substantia nigra, pars compacta, at least partially mediates AMPH anorexia, an alternative explanation seems plausible. First, the descriptions of the magnitude of the substantia nigra lesions were not complete. For example, electrolytic lesions (Carey & Goddall, 1975) as well as 6-hydroxydopamine lesions to the substantia nigra (Fibiger, Zis & McGeer, 1973; Fibiger, Phillips & Clouston, 1973) that attenuated anorexia to AMPH dramatically reduced tyrosine hydroxylase activity in the striatum, indicating significant damage to the nigrostriatal system. However, in the Carey and Goddall (1975) study, it was not indicated whether the lesion extended to the caudal substantia nigra and affected "A8" neurons in addition to destroying the substantia nigra proper. Likewise, Fibiger's work (Fibiger, Zis & McGeer, 1973; Fibiger, Phillips & Clouston, 1973) verified that 6-OHDA infusions to the substantia nigra depleted striatal tyrosine hydroxylase activity, but no histology was provided (since the midbrains were used for tyrosine hydroxylase assays) and therefore the full extent of neuronal damage was not determined. Electrolytic lesions of the substantia nigra,

in fact, (which did not reduce tyrosine hydroxylase activity in the striatum as well as substantia nigra 6-hydroxydopamine infusions) did not significantly attenuate AMPH response (Fibiger, Phillips & Clouston, 1973). It is notable that in the latter study electrolytic lesions of the substantia nigra also did not damage tissue just dorsal to the medial lemniscus; the site which appears crucial for disrupting AMPH response. Other factors that counter proposals that the nigrostriatal system mediates AMPH feeding suppression were discussed at length in the previous chapter. Namely, lesions to the striatum do not affect AMPH response (Sawanin, et. al., 1977) and AMPH injections to this region do not induce anorexia (Leibowitz, 1975; Leibowitz & Rossakis, 1978b). It should be noted, however, that there is no well-defined distinction between the A8, and A9 or A10 DA cell groups and that they form a relatively continuous and extended midbrain dopamine cell system (Beckstead, Domesick, & Nauta, 1979; Fallon & Moore, 1978; Leibowitz & Brown, 1980; Lindvall & Bjorklund, 1978). However, a distinction can apparently be made behaviorally, since the present findings suggest that DA neurons which mediate AMPH-induced anorexia are positioned in the ventrolateral tegmentum, caudal to the level of the substantia nigra.

In addition to the Ventral midbrain KC, the Ventral pontine cut also affected drug response. Specifically, the Ventral pontine KC, which severed tissue just dorsal to the level of the nucleus of the seventh cranial nerve, significantly attenuated anorexia to peripheral administration of AMPH. In contrast, the dorsally-placed rostral pontine cut, as well as the Dorsal caudal pontine cut, did not affect AMPH response. These findings suggest that fibers which mediate AMPH feeding suppression course through the ventral pons. Since the Ventral pontine

cut is caudal to DA cell groups (Dahlstrom & Fuxe, 1964; Lindvall & Bjorklund, 1978), CA fibers severed by this cut are noradrenergic/adrenergic, and, as others have suggested, the present findings indicate that these fibers also mediate AMPH anorexia (Ahlskog, 1974; Ahlskog & Hoebel, 1973; Carey, 1976; Leibowitz & Brown, 1980a; Leibowitz, Hammer & Brown, 1980; Samanin, et. al., 1977). The present findings, in addition, suggest which catecholamine cell groups mediate drug response. First, the results indicate that the A6 and A7 CA cell groups, located in the locus coeruleus and rostral lateral tegmental area (Lindvall & Bjorklund, 1978), respectively, do not mediate AMPH-induced feeding suppression. If fibers arising from these cell groups were crucial, the rostral pontine KC would have attenuated AMPH feeding response. In addition, it appears the fibers arising from the rostral ventrolateral pons, the A5 catecholamine cell group, can be eliminated as a possible mediator of AMPH response. This is due to the fact that these cell bodies, that extend as far caudal in the pons as the rostral aspect of the nucleus of the seventh nerve, are <u>rostral</u> to the effective Ventral pontine cut. The remaining candidates in terms of which catecholamine group(s) mediate AMPH response are the neurons situated in the dorsal medulla, the A2/C2 catecholamine cell group, and the catecholamine neurons positioned in the ventrolateral medulla, the A1/C1 catecholamine cell group (Lindvall & Bjorklund, 1978; Hokfelt, Fuxe, Goldstein, & Johansson, 1974). As originally proposed by Hokfelt (Hokfelt, et. al., 1974), the C1 and C2 cell groups refer to adrenergic (epinephrine-containing) neurons which are partially contiguous with NE cell bodies in the A1 and A2 CA regions. Based upon anatomical descriptions of the ascending course of fibers from these CA cell groups, it is

difficult from the present study to conclude if AMPH feeding suppression is mediated by either, or both, of these cell groups. According to Sawchenko and Swanson (1982), the majority of A2/C2 ascending catecholamine fibers, arising from the dorsal medulla, initially take a ventrolateral course to a position just dorsal to the A1/C1 cell group. Here, some of the dorsal medullary fibers terminate in the A1/C1 cell group in the ventrolateral medulla, while others turn rostrally to ascend, intertwined with ascending A1/C1 fibers, through the lateral tegmental field. The present findings, however, do suggest that the secondary ascending output from the dorsal medulla (A2/C2 cell group), which ascends through the dorsal pons, does not mediate AMPH anorexia, since the Dorsal caudal pontine KC did not affect drug response.

Combining several assumptions with less than direct evidence, however, suggests that the ventral C1 cell group mediates drug response. First, evidence indicates that AMPH anorexia is at least partially mediated by a beta-adrenergic mechanism localized in the PFH. This suggests, but does not prove, that PFH anorexia is mediated by an adrenergic mechanism, rather than noradrenergic inputs. [The PFH area, in fact, is described as receiving a major innnervation from adrenergic neurons (Hokfelt, Fuxe, Goldstein & Johnsson, 1974).] If one assumes that adrenergic mechanisms mediate AMPH anorexia, then this suggests that either the C1 or C2 adrenergic cell groups are involved, rather than A1 or A2 NE systems. Between C1 and C2, lesions of the ventrolateral medulla, in comparison to lesions of the dorsolateral medullary region, result in a significant loss of adrenergic content in the hypothalamus (Palkovits, Mezey, Zaborsky, Feminger, Versteeg, Wijnen, DeJong, Fekete, Herman, & Kanicska, 1980). This suggests that the bulk

of adrenergic innervation originates from adrenergic neurons in the ventrolateral medulla (Palkovits, Mezey, Zaborsky, Feminger, Versteeg, Wijnen, DeJong, Fekete, Herman, & Kanicska, 1980). Additionally, the ventrolateral medullary CA group apparently contains the larger collection of adrenergic neurons (Hokfelt, et. al., 1974), and this cell group also provides a greater degree of noradrenergic innervation to the hypothalamus than the dorsolateral cell group (Palkovits, Zaborsky, Feminger, Mezey, Fekete, Herman, Kanyicska, & Szabo, 1980). Finally, the sole study that has examined the effect of lesions in the medulla upon AMPH induced anorexia indicates that lesions to the area postrema (albeit a minor portion of the total dorsal medullary CA system) enhanced response to AMPH (Carlisle & Reynolds, 1964). Additional experimental work is needed to examine the question of whether AMPH response is mediated singly by the ventral or dorsal medullary CA cell group, or whether both systems are involved. Anatomical studies appear to suggest the PFH receives innervation from both the dorsal and ventrolateral catecholamine cell groups (Takagi, Shiosaka, Tohyama, Senba, & Sakanaka, 1980), but this question has not been directly addressed. In any case, the present findings suggest that crucial fibers from these medullary CA neurons ascend in the ventral half of the pons and midbrain.

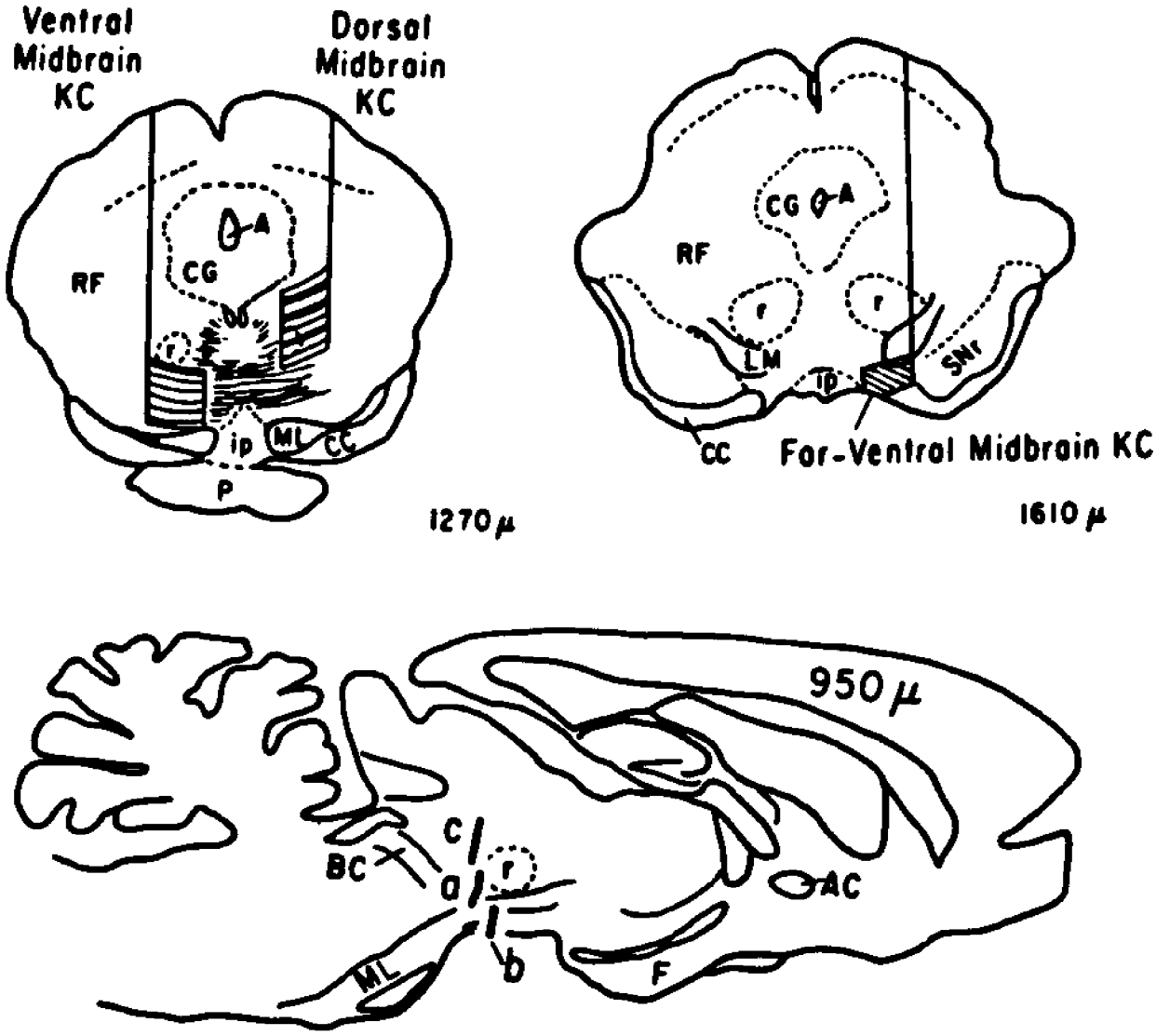
Fine unmyelinated central neurons, including catecholamine systems, have remarkable adaptive and regenerative capacities (Bjorklund & Stenevi, 1979; Reisine, 1981). For example, neurochemical studies show that following a post-lesion period of 2-3 weeks, enzyme activities in some damaged brain regions may return to almost normal levels (Acheson & Zigmond, 1981; Emson, Bjorklund, & Stenevi, 1977; Stricker & Zigmond,

1976). Anatomical observations suggest central neurons also regenerate, and that this capacity in conjunction with neurochemical adaptation may account at least partially for functional recovery af... ... ...issue damage (Bjorklund, Dunnett, Stenevi, Lewis, & Iversen, 1980; Bjorklund & Stenevi, 1979; Foerster, 1982; Katzman, Bjorklund, Owman, Stenevi, & West, 1971; Stricker & Zigmond, 1976). With respect to the present knife cut experiments, it is important to keep in mind that drug tests were conducted for as long as 2-3 months postsurgery, and that during this time some damaged neuronal systems may have regenerated or have been significantly modified. The present experiments, however, still provide information about the anatomical trajectories of fibers mediating drug-induced feeding and anorexia. In cases where a lesion or KC has significantly affected drug-induced feeding responses, the crucial fiber system that modulated a particular drug response must have been nearly completely destroyed and inoperative at time of testing. Alternatively, a crucial feeding circuit may have been only partially severed. In this case, in spite of possible adaptive changes in any remaining neurons, the system was unable to sufficiently execute the drugs usual neuronal effect. Results where KCs disrupted drug-induced eating or anorexia suggests that these brain manipulations therefore are at least sufficient under these testing conditions to alter drug response, but it has not been determined whether KC rats would have been at least partially responsive if such factors as drug dose, diet, or feeding schedule were modified. It should be noted that severed fibers are believed to "detour" around scars left by wire knife-cut procedures, but that when the area around a cut is more than approximately 400 um larger than the boundary through which the fiber tract normally

traverses, re$_{e}$eneration is rarely observed (Foerster, 1982).

Figure 15. Rat brain drawings depicting the location of the three coronal midbrain KCs. The upper two brain drawings depict the three midbrain cuts in the coronal plane. The ventral midbrain cut extended from the dorsal surface of the medial lemniscus (ML) and as far dorsal as the ventral half of the red nucleus (r). The dorsal midbrain KC was positioned above the ventral cut, and severed tissue just ventrolateral to the central grey (CG). The Far-Ventral KC (right upper drawing) was positioned below the medial lemniscus (LII) and severed tissue in the ventral tegmental area and medial substantia nigra. These cuts are depicted in the lower right sagittal drawing as heavy vertical lines (a- Ventral KC; b- Far-Ventral KC; c- Dorsal KC). The approximate coordinates of these cuts in terms of anterior-posterior position (1270 and 1610 μ) refer to the Koenig and Klippel (1974) atlas. The sagittal drawing is approximately 950 μ lateral to midline. (Abbreviations: a- Ventral midbrain KC; b- Far-Ventral KC; c- Dorsal KC; A- cerebral aquaduct; AC- anterior commisure; BC- brachium conjunctivum; CC- crus cerebri; CG- central grey; F- fornix; ip- interpeduncular nucleus; LII and ML- medial lemniscus; p- pons; r- red nucleus; RF- reticular formation; SNr- substantia nigra, <u>pars reticulata</u>).

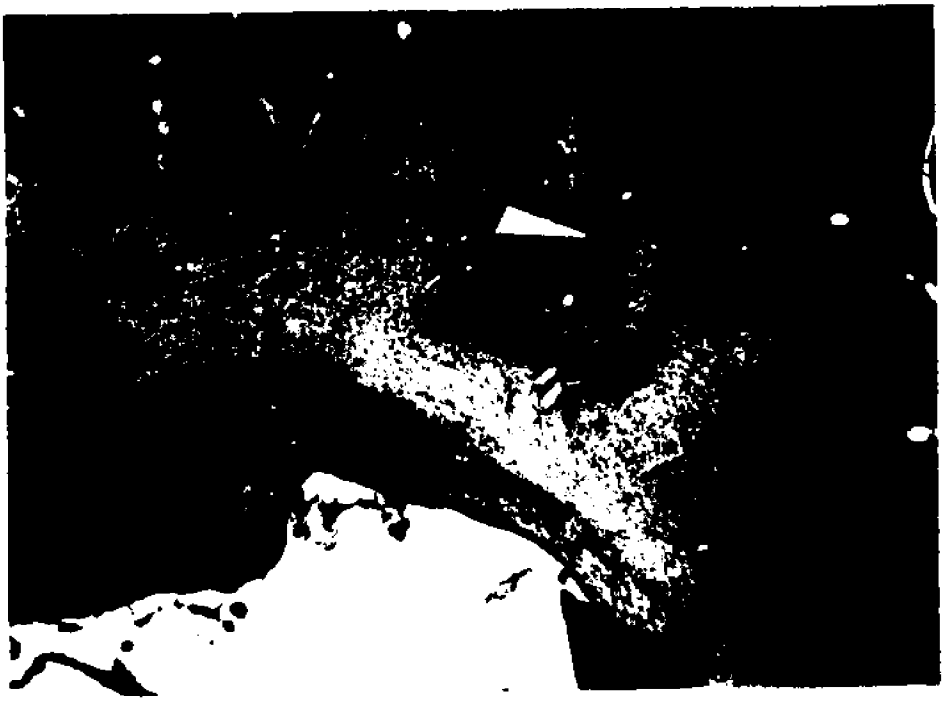
Figure 15



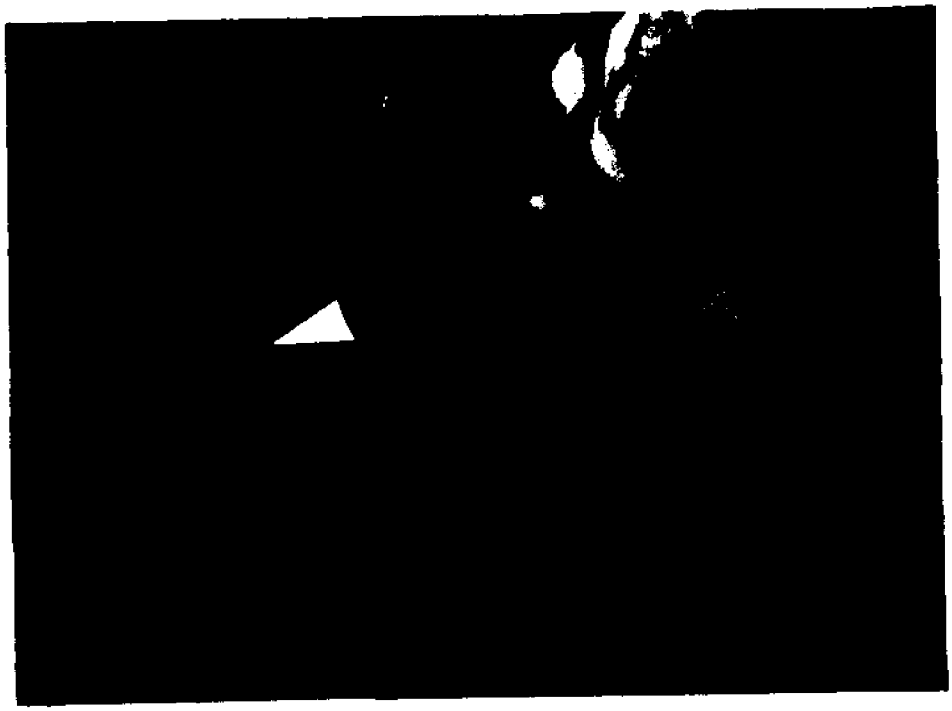
a – Ventral Midbrain KC
b – For-Ventral Midbrain KC
c – Dorsal Midbrain KC

Figure 16. Photomicrographs of midbrain KCs. The Far-Ventral KC (16c) produced a small cut located in the ventral tegmental area and medial substantia nigra. This cut did not extend dorsal to the medial lemniscus but usually extended to the ventral surface of the brain. The Ventral KC (16b), severed tissue caudal to the red nucleus and just dorsal to the medial lemniscus. The Dorsal KC (16a) severed tissue caudal to the red nucleus and extended from the mid-dorsoventral level of this nucleus and approximately 1.2 mm dorsal. All three midbrain cuts extended from approximately 0.5 to 1.1 mm lateral to midline.

Figure 16



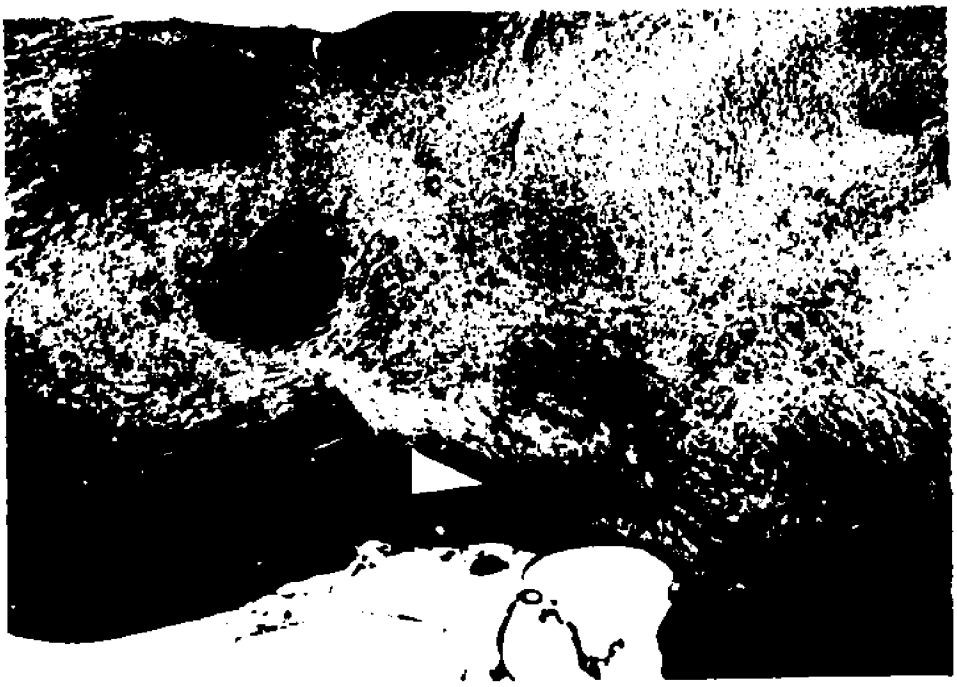
16a



16b

Figure 16

(continued)



16c

Table 21. Feeding responses of midbrain KC rats after intraperitoneal (i.p.) administration of saline and AMPH (0.5 mg/kg). In terms of percent suppression scores, the Ventral midbrain KC significantly attenuated anorectic response to AMPH (♯ $p<0.01$), while the Dorsal and Far-Ventral cuts failed to significantly affect drug response. Note that the mean percent suppression scores presented in the right collumn are calculated from the suppression scores of individual animals and do not equal the values calculated by using the mean saline and AMPH scores presented in the table.

Table 22. Food intake (grams) of sham rats and animals with midbrain KCs after administration of FENF tests. All groups exhibited decreases in food intake after FENF and in terms of percent suppression scores, the degree of anorexia exhibited by the Dorsal and Ventral KC groups failed to differ from the sham rats.

Table 21

Feeding Responses to Intraperitoneal Injection of Saline and Amphetamine (AMPH)

Group	n	Saline	AMPH	% Suppression
Shams	18	6.3±0.6	3.7±0.6	-45.1±5.2
Midbrain Knife Cuts				
Ventral KC	16	9.4±1.1	8.0±1.1	-14.3±7.0♯
Dorsal KC	12	11.1±0.9	6.1±0.7	-45.0±5.6
Far-Ventral KC	9	5.6±0.7	3.4±0.6	-38.4±8.5

♯ $p<0.01$ Percent suppression scores less than sham group.

Table 22

Feeding Responses After Peripheral Administration of Saline and Fenfluramine (2 mg/kg)

Group	n	Saline	Fenfluramine	%-Suppression
Shams	10	6.0±0.6	3.2±0.7	-52.0±7.4
Dorsal KC	6	11.0±1.3	2.9±1.0	-70.0±8.2
Ventral KC	5	11.7±4.5	3.7±0.5	-63.6±9.7

Table 23. Feeding responses of sham and midbrain KC rats after PFH injections of and AMPH (150 nM). The degree of anorexia in terms of percent suppression exhibited by sham and midbrain KC rats, fails to demonstrate a significant attenuation of AMPH-induced anorexia by the Ventral KC rats. However, note that the Ventral midbrain KC rats were the only group that did not exhibit a significantly decreased level of intake after AMPH compared to their response to saline.

Table 24. Feeding responses of sham and midbrain KC rats after PFH injection of saline and DA (150 nM). The sham, Ventral KC, and Far-Ventral KC groups failed to exhibit significantly different responses to PFH injections of DA.

Table 23

Food Intake After PFH Injections
of Saline and Amphetamine

Group	n	Saline	AMPH	% Suppression
Shams	12	6.3±0.8	4.5±1.1	-33.7±8.1
Ventral KC	10	7.4±1.2	6.7±1.5	-13.7±12.5
Far-Ventral KC	8	4.7±0.5	3.0±0.3	-36.6±10.7

Table 24

Food Intake After PFH Injections
of Saline and Dopamine (DA)

Group	n	Vehicle	DA	% Suppression
Shams	7	4.6±0.4	2.5±0.6	-47.3±9.8
Midbrain Knife Cuts				
Ventral KC	6	5.7±0.3	2.1±0.5	-65.5±8.4
Far-Ventral KC	6	5.4±0.9	3.3±1.0	-28.0±19.0

Figure 17. Coronal and sagittal drawings of the positions of the rostral, Dorsal caudal, and Ventral caudal pontine KCs. The upper right coronal brain drawing shows the approximate location of the rostral pontine KC. This cut severed tissue around and through the region occupied by the brachium conjunctivum (BC). The middle drawing on the right depicts the Dorsal CPKC that was positioned caudal to the motor nucleus (see lower figure). The Ventral CPKC was larger than the Dorsal CPKC, and extended farther ventral to the dorsal aspect of the nucleus of the seventh nerve (n VII). The lower parasagittal drawing shows the positions of the RPKC (a), Dorsal CPKC (b), and Ventral CPKC (c). The upper coronal sections are labeled P1.0-1.5 mm and P3.9 mm and refer to the approximate position of the KCs with respect to the atlas of Palkovits and Jacobowitz (1974). The sagittal drawing is positioned approximately 1.7 mm lateral to midline according to the atlas of Pelligrino, Pelligrino, and Cushman (1979). (Abbreviations: a- Rostral pontine KC; b- Dorsal caudal pontine KC; c- Ventral caudal pontine KC; A- cerebral aquaduct; AC- anterior commissure; BC- brachium conjunctivum; IC- inferior colliculus; LL- medial lemnicus; MFB- medial forebrain bundle; MLF- medial longitudinal fasciculus; OT- optic tract; PBv- ventral parabrachial nucleus; RF- reticular formation; SN- substantia nigra; TSV- spinal tract of the trigeminal nerve; nV- motor nucleus of the trigeminal nerve; nVII- nucleus of the facial nerve; ntVd- nucleus of the spinal tract of the trigeminal nerve; p- pons; VII- facial nerve).

Figure 17

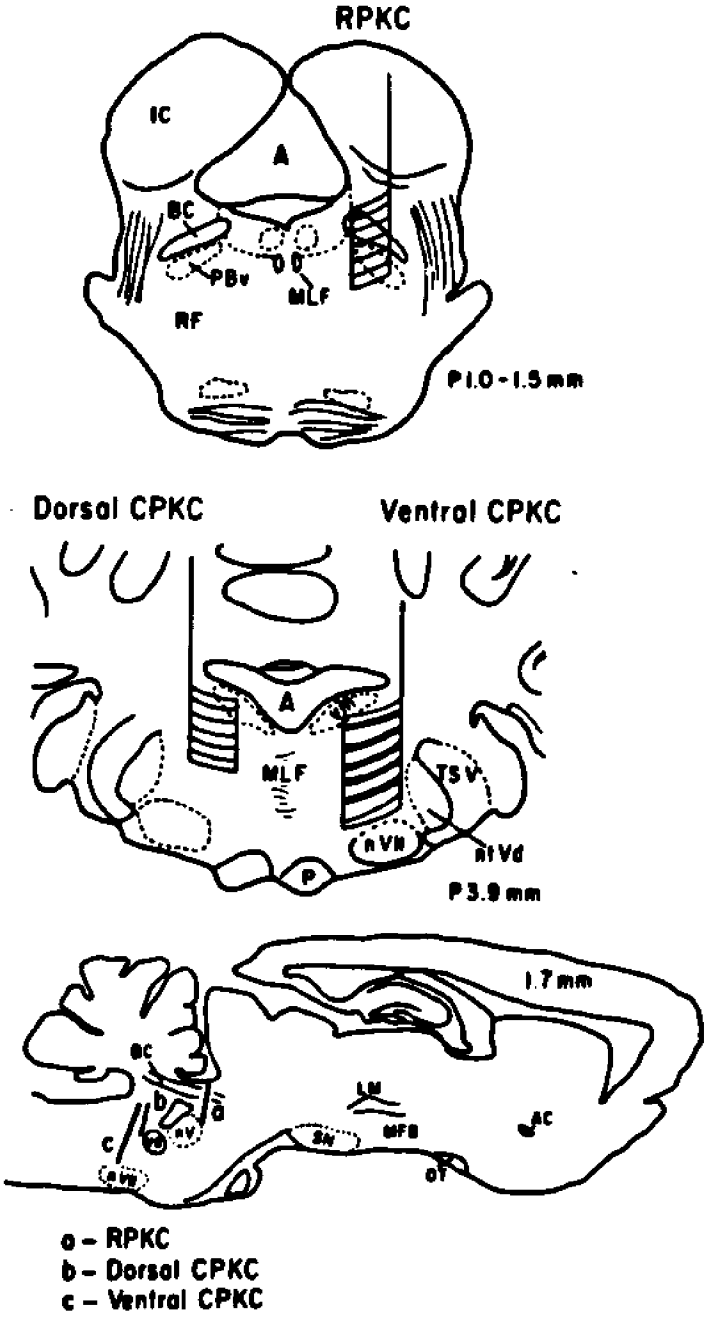
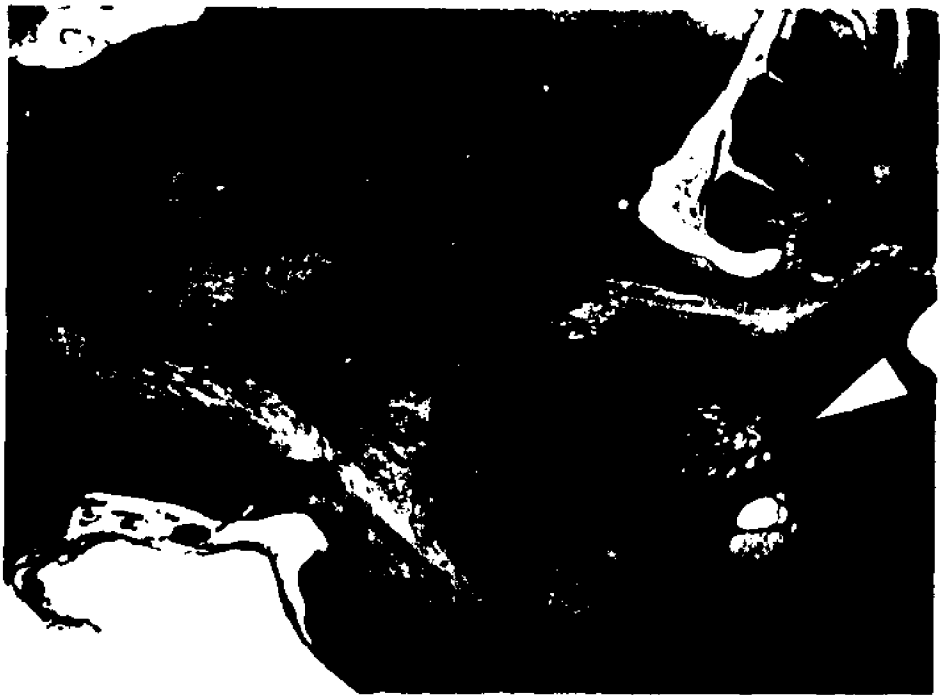

RPKC
IC
A
BC
PBv
MLF
RF
P1.0-1.5 mm
Dorsal CPKC
Ventral CPKC
A
MLF
TSV
nVM
nVd
P
P3.9 mm
1.7 mm
BC
b
a
c
nV
SN
MFB
LM
AC
OT
a - RPKC
b - Dorsal CPKC
c - Ventral CPKC

Figure 18. Parasagittal photomicrographs of the three pontine KC groups. Figure 18a is an example of a rostral pontine KC, positioned anterior to the LC, which severs tissue in the midlateral tegmentum. The Dorsal caudal pontine KC (18b) severed tissue only behind and dorsal to the level of the motor nucleus of the fifth nerve. In contrast, the ventral CPKC (18c) extended to the ventral half of the pons and severed tissue to the dorsal aspect of the nucleus of the seventh cranial nerve.

Figure 18

18a



18b

Figure 18

(continued)

18c

Table 25. Feeding responses of sham and pontine KC rats to peripherally administered AMPH (0.5 mg/kg) and saline. The sham, RPKC, and Dorsal CPKC rats exhibited similar anorectic responses to AMPH, while the Ventral CPKC rats were significantly less responsive to AMPH compared to sham rats (♯ $p<0.05$).

Table 26. Food intake (grams) of RPKC and sham rats after peripheral administration of FENF (3.0 mg/kg) and saline. Both groups exhibited decreases in food intake after FENF and failed to differ from each other in terms of degree of feeding inhibition.

Table 25

Feeding Responses to Peripheral Administration of Saline and Amphetamine

Group	n	Saline	AMPH	% Suppression
Sham	8	6.3±1.1	2.9±0.6	-51.9±7.2
RPKC	10	5.3±0.6	2.0±0.5	-62.5±9.7
Dorsal CPKC	6	5.5±1.1	1.9±0.6	-64.8±7.6
Ventral CPKC	5	5.8±1.0	4.9±1.2	-20.6±10.1♯

♯ $p<0.05$ % suppression scores less than sham group.

Table 26

Feeding Responses After Peripheral
Injection of Saline and Fenfluramine (3 mg/kg)

Group	n	Saline	Fenfluramine	%-Suppression
Shams	9	6.4±0.8	2.4±0.4	-62%
Rostral KC	5	4.3±0.3	1.9±1.1	-69%

Table 27. Feeding responses of sham and dorsal CPKC rats after PFH injection of AMPH. Both groups consumed less food after central injection of AMPH than after receiving saline injections. It appears that the Dorsal CPKC may potentiate sensitivity to central AMPH injections, but this difference failed to reach significance (see text).

Table 28. Feeding responses of sham and dorsal CPKC rats after PFH injection of DA. The anorectic responses of the Dorsal CPKC rats were significantly greater than the sham rats with respect to the percent suppression measure (* $p<0.05$).

Table 27

Feeding Responses After PFH Injections of Saline and Amphetamine

Group	n	Saline	AMPH	%-Suppression
Shams	7	4.9±0.4	3.0±0.5	-40.1±7.7
Dorsal CPKC	5	4.2±0.4	1.5±0.8	-67.4±13.9

Table 28

Feeding Responses After PFH
Injections of Saline and Dopamine

Group	n	Saline	DA	%-Suppression
Shams	7	4.8±0.6	2.7±0.4	-37.6±12.8
Dorsal CPKC	5	4.1±0.5	0.8±0.5	-84.2±12.1*

* $p<0.05$ Percent suppression scores greater than sham group.

Chapter 6

General Discussion

The present series of experiments were conducted in order to delineate an hypothesized feeding circuit, arising from the PVN, that can be activated by central and peripheral administration of alpha-adrenergic agonists. In addition, an attempt was made to determine which of several brainstem catecholamine cell groups mediate feeding inhibition produced by AMPH administration.

PVN neurons appear to send efferents to the caudal brainstem that mediate noradrenergically-stimulated feeding. A schematic diagram (Figure 19) depicts the proposed trajectory of these fibers which, based upon the present studies, mediate alpha-adrenergically stimulated feeding induced by either PVN injections of NE or peripheral administration of CLON. The present study has not determined the precise initial course of these neurons, but it would appear that crucial fibers do not course through the midlateral hypothalamus and midbrain (see also Aravich, et. al., 1982). Rather, crucial fibers from the PVN that mediate noradrenergically-stimulated feeding apparently course dorsal and directly caudal from the PVN and then traverse the periventricular region of the midbrain (Weiss & Leibowitz, 1983). At the level of the colliculi, these fibers then take a sharp lateral turn, and pass caudally through the parabrachial region and dorsal half of the pons. One prominent terminus for the fibers would appear to be the dorsal vagal complex since this structure plays a role in feeding behavior and receives direct innervation from the PVN (Buijs, 1978; Conrad & Pfaff, 1976; Hyde, Eng, & Miselis, 1982; Swanson, 1976).

With respect to feeding inhibition with AMPH (Figure 20), fibers containing EPI or NE originate from the catecholamine groups A1/C1 and/or possibly from A2/C2, and apparently not from the catecholamine cell groups A5, A6, or A7. DA Neurons also mediate AMPH-induced anorexia, and these fibers appear to arise from the ventrolateral tegmentum, especially from the A8 cell group. The crucial fibers from the medulla then ascend through the ventral pons and midbrain where they are joined by DA fibers in the midbrain, and crucial fibers traverse the midlateral aspect of the caudal hypothalamus before terminating in the PFH region.

Suprisingly, the present studies have not detected evidence of an interdependence between alpha-adrenergically stimulated feeding and AMPH-induced anorexia. For example, PVN lesion rats were unresponsive to CLON but this brain manipulation did not affect AMPH response. Likewise, PFH lesions disrupted AMPH feeding suppression, but not feeding stimulated by CLON. Several other studies have suggested that medial and lateral hypothalamic systems mediating feeding do not interact directly. For example, lateral hypothalamic self-stimulation is not altered by parasagittal KCs that sever connections between the medial and lateral hypothalamus (Sclafani, Gale, & Maul, 1974), and stimulation of the ventromedial hypothalamus, which inhibits feeding, is not changed by lateral hypothalamic lesions or by KCs between ventromedial and lateral hypothalamic areas (Sclafani & Maul, 1974). Similarly, PVN injections of phentolamine, which attenuate NE-induced feeding, do not alter stimulus-bound feeding (Halperin, Gatchalian, Adachi, Carter, & Leibowitz, 1983). These studies do not show, however, that the PVN and PFH independently mediate feeding, but suggest these systems are not

interactive by means of a simple serial mechanism. In the past few years, neuroanatomical studies have demonstrated the incredible complexity of the PVN region. At least 10 subregions of the PVN have been described, and this brain region interacts with hormonal systems, by means of connections with the pituitary and median eminence, and with brainstem autonomic centers, including the dorsal vagal complex and the parabrachial area, as well as preganglionic neurons in the spinal cord (Armstrong, Warach, Hatton, & McNeill, 1980; Conrad & Pfaff, 1976; Koh & Ricardo, 1980; Swanson & Kuypers, 1980; Swanson & Sawchenko, 1983). Less is known concerning the PFH region (Palkovits, 1975). Due to its position between the medial and lateral segments of the hypothalamus, and its contiguity to longitudinal fibers of the fornix and the medial forebrain bundle, suggests this brain region will prove to also possess extensive intra- and extra-hypothalamic connections. The complexity of the PFH region is suggested by observations that relatively slight (0.5 mm) changes in the positions of perifornical parasagittal KCs significantly affects daily food intake and body weight. That is, parasagittal KCs just medial to the fornix (1.0 mm lateral to midline) induced greater hyperphagia and obesity than cuts just lateral (1.5 mm lateral to midline) to the fornix (Sclafani, Berner, & Maul, 1975).

Determining the anatomical course, as well as crucial cell bodies, that mediate drug-induced stimulation and inhibition of feeding is an important task. This information provides clues to how specific hypothalamic regions, determined as mediating drug-induced changes in feeding behavior, are integrated with other brain areas that coordinate feeding and allows one to consider what forms of neuronal messages between brain structures are mediating drug-induced feeding behavior.

In the last several years, the theoretical orientation of what regulates feeding has broadened from an initial hypothalamo-centric view of feeding in terms of "hunger" and "satiety", to include not only other brain areas, but also other behavioral concepts, such as arousal, learning, reward, and expectancy. In addition, organismic processes, such as sensory and motor responses, and neurochemical, autonomic, metabolic, and "peripheral" or visceral mechanisms, are recognized as fully participating in the regulation of food intake, and cannot be ignored when trying to envision what makes an animal eat and not eat; and do so with such apparent "regulation." The present experiments have examined only one aspect of food intake regulation; but (one wishes) these data will be contributory.

Figure 19. Schematic drawing of the hypothesized efferent PVN feeding circuit that mediates noradrenergically-stimulated feeding. Fibers arise from the PVN and course directly caudal through the medial regions of the thalamus or dorsal hypothalamus and enter the periventricular region of the midbrain. As they approach the level of the inferior colliculus, crucial fibers then apparently move in a lateral direction to pass through the region of the parabrachial nuclei. Fibers then continue in a caudal direction in the midlateral-dorsal aspect of the pons and perhaps terminate in the dorsal vagal complex. (Abbreviations: BC- brachium conjunctivum; ML- medial lemniscus; NST- nucleus of the solitary tract; r- red nucleus; PVG- periventricular grey; nVII- nucleus of the seventh nerve; VII- seventh nerve; VIId- descending fibers of the seventh nerve).

Figure 19

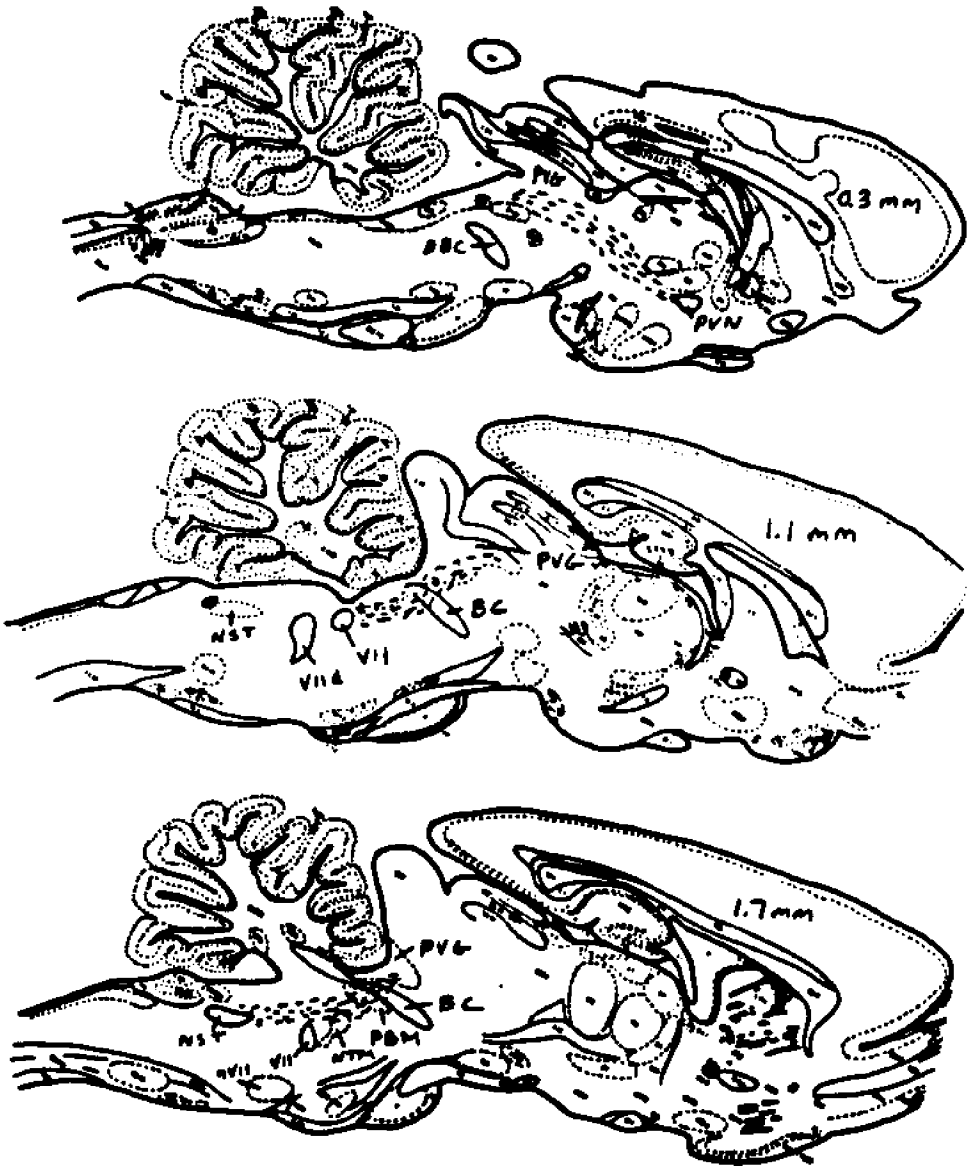
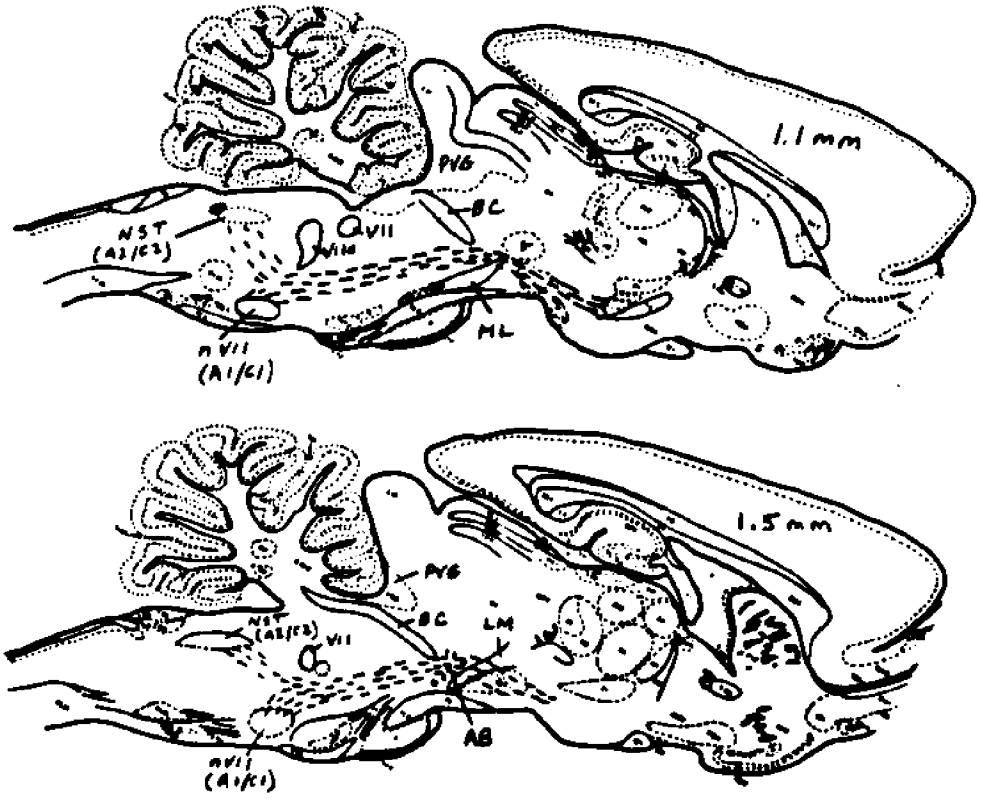


Figure 20. This drawing depicts the hypothesized route of the ascending catecholamine fibers that mediate AMPH-induced feeding suppression. Adrenergic/noradrenergic fibers arise from the A1/C1 and/or the A2/C2 medullary catecholamine cell groups, positioned, respectively, within the nucleus of the seventh nerve and the nucleus of the solitary tract. Fibers ascend in the midlateral ventral pons, possibly on a direct course, to the ventral midbrain. At this level, DA-containing fibers (labelled "A8")crucial to AMPH response possibly join the medullary fibers and pass just dorsal to the medial lemniscus. Fibers then enter the medial forebrain bundle and ascend to the PFH region. (Abbreviations: A1/C1- noradrenergic and adrenergic cell bodies in the ventrolateral medulla; A2/C2- noradrenergic and adrenergic cell bodies in the dorsomedial medulla; A8- midbrain dopamine cell bodies; BC- brachium conjunctivum; ML- medial lemniscus; NST- nucleus of the solitary tract; PVG- periventricular grey; nVII- nucleus of the facial nerve; r- red nucleus; VII- facial nerve VIId- descending root of the facial nerve).

Figure 20


1.1 mm
PYR
BC
NST
(A1/C2)
VII
nVII
(A1/C1)
ML
1.5 mm
PYR
BC
LM
NST
(A1/C2)
VII
AB
nVII
(A1/C1)

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