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Capuano, Christopher A.

THE PHARMACO-ONTOGENY OF HYPOTHALAMIC RECEPTOR SYSTEMS MEDIATING INDEPENDENT FEEDING IN THE RAT

City University of New York PH.D. 1987

THE PHARMACO-ONTOGENY OF HYPOTHALAMIC RECEPTOR SYSTEMS MEDIATING INDEPENDENT FEEDING IN THE RAT

by

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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.

1987

This manuscript has been read and accepted by the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

12, FEBRUARY, 1987 — Date

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Abstract

THE PHARMACO-ONTOGENY OF HYPOTHALAMIC RECEPTOR SYSTEMS MEDIATING INDEPENDENT FEEDING IN THE RAT

by

Christopher A. Capuano

Advisor: Gordon A. Barr, Ph.D.

Studies in adult rats have provided evidence suggesting that two separate catecholamine systems are involved in regulating feeding behavior. The first is a noradrenergic system that stimulates feeding through interaction with alpha-noradrenergic receptors located in the medial (paraventricular) hypothalamic region. The second is an adrenergic and dopaminergic system that suppresses feeding through interaction with beta-adrenergic and dopaminergic receptors located in the lateral (perifornical) hypothalamic region. Further evidence exists that neuropeptides also play a role in hypothalamic regulation of feeding in the rat. Other studies have identified an independent feeding system in the rat pup that mediates ingestion of milk off the nipple and appears to be developmentally continuous with feeding in adult rats. The present research was undertaken to assess the pharmaco-ontogeny of hypothalamic catecholamine and neuropeptide receptor systems mediating independent feeding in the rat. Sated and deprived rat pups ranging in age from 2 to 15 days were stereotaxically implanted with a unilateral cannula directed at the

hypothalamic paraventricular nucleus, ventral third ventricle, perifornical hypothalamus or more rostral anterolateral hypothalamus. The next day, pups were implanted with an intra-oral cannula for infusion of milk or water. Following injection of a single dose of either the alpha-noradrenergic receptor agonists norepinephrine or clonidine, the beta-adrenergic receptor agonists epinephrine or salbutamol, the dopaminergic receptor agonist apomorphine, the neuroactive peptides cholecystokinin or neuropeptide Y, or the vehicle, milk or water intake was assessed in a 1 hr test. The results indicated that receptors mediating independent feeding in the rat are behaviorally fuctional prior to the onset of weaning. Except for cholecystokinin, receptors mediating the feeding response to norepinephrine, epinephrine, dopamine and neuropeptide Y neurochemical agents do so as early as 2 days postpartum. Moreover, consistent with studies in adult rats, this work has identified the paraventricular nucleus and perifornical hypothalamus as primary hypothalamic areas in the infant rat that are sensitive to the effects of the neurochemical agents tested. Collectively, the present findings strongly suggest that the independent feeding system in the infant, while not used during normal development, is developmentally continuous with feeding in the adult and represents an appropriate starting point for developmental analysis of neurochemical systems regulating adult feeding behavior in the rat.

Acknowledgements

At the outset of this work and on several occasions since then, I wondered how I would feel when sitting down to write this final section of my doctoral thesis. Having completed just the first sentence, I can assure you that it feels great. That is, great to finally have the opportunity to thank all who contributed to this work and/or made it possible in their own way.

First, I would like to thank Drs. Gordon Barr and Sarah Leibowitz, my co-mentors and very good friends. Thank you for your help in generating first rate research ideas, for encouraging me to see them through and for supporting this work both emotionally and financially. This research and my recent success in seeking employment is a reflection of both of you.

Next, I thank the remaining members of my supervisory committee, Drs. Turkewitz, Hofer and Gamzu, for making my thesis defense a very enjoyable and intellectually stimulating experience.

I also thank Dr. Jim Giordano and soon to be Drs. Harry (Eddie) Hughes and Nina Goodless, my former colleagues and very special friends. Thank you for making the atmosphere in the lab a most humorous and always enjoyable one. I'll never forget the good times we had both out on the town and out of town.

A very special thanks goes to my parents, Italo and Nina Capuano, who are a major part of this work, my success

and my life. Thank you for everything you have done for me and my family. I love you both very much.

Finally, I thank my lovely wife Susan. Thank you for your love and support and for tolerating my many mood swings as well as the long and lonely hours incurred during the last 1 to 2 years of this work. Most of all, thank you for giving me the most precious thing in my life, our daughter Marissa Christina. It is to her that I dedicate this thesis.

Table of Contents

List of Tables

List of Figures

General Introduction

The behaviors involved in the acquisition of food are of vital importance to the survival of all organisms. They are, however, merely the end points of complex biological control systems geared towards regulating body levels of nutrients and energy. Numerous scientific investigations have significantly advanced our understanding of these control systems and have provided a clearer appreciation of the multitude of variables that come to bear on the expression of feeding behavior. Through the years, the hypothalamus, lying at an interface between the brain and periphery, has attracted interest from many points of view and has been demonstrated to have an essential function in the regulation of feeding. Clearly, the hypothalamus does not operate autonomously in this function, but as part of a whole brain circuitry that is organized at various integrative levels and involves complex interactions between these levels through multiple neurochemical processes.

Neurochemical Regulation of Feeding Behavior

Feeding has long been recognized as an extremely complex process, the regulation of which involves a variety of peripheral inputs integrated within the central nervous system. The integration process is one that makes use of multiple neurotransmitters. The four major families of neurotransmitters - amines, amino acids, neuropeptides and prostaglandins - have all been demonstrated to play a role

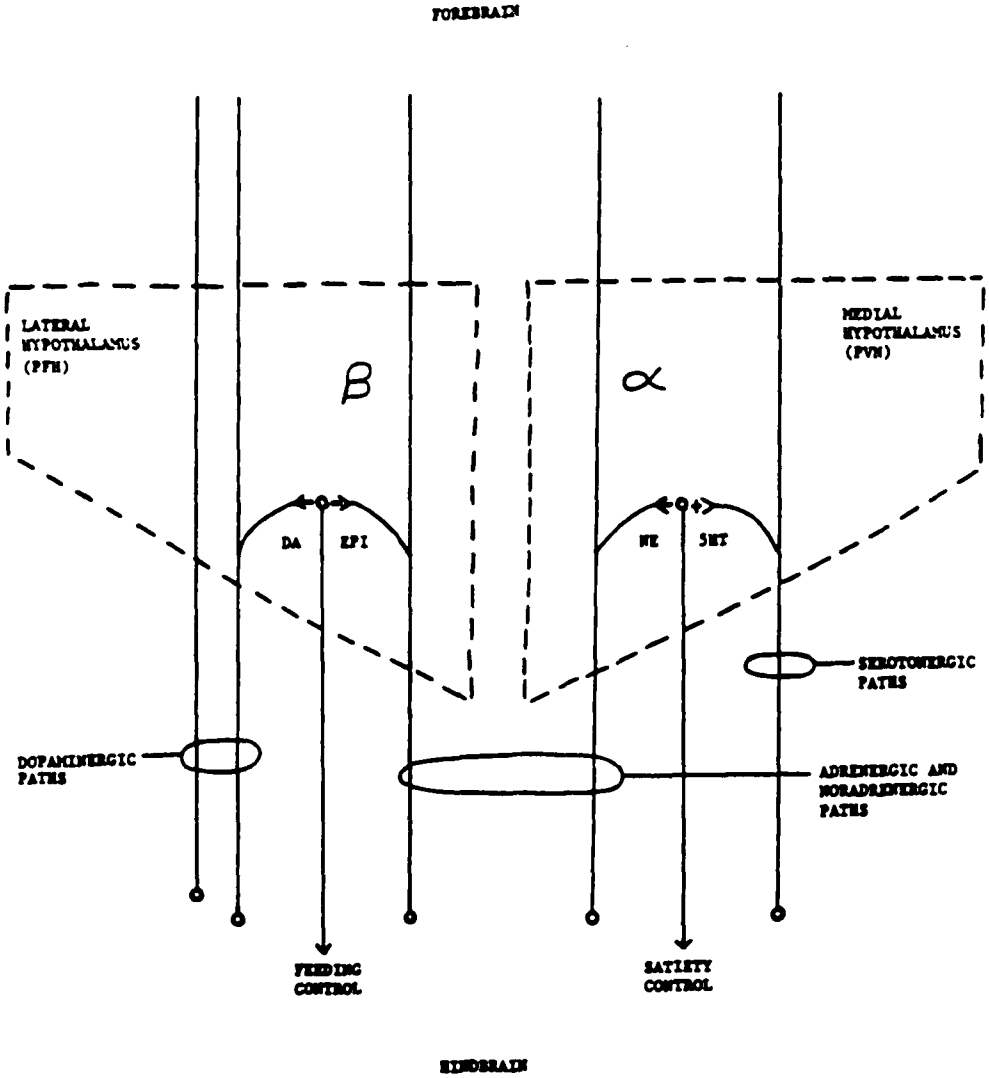
in the integration of the whole system.

Catecholamine Systems.

In support of the involvement of amines in the regulation of feeding behavior, extensive evidence has accumulated over the past decade or so implicating a role for hypothalamic catecholamine (CA) systems in the regulation of natural feeding behavior in the adult rat (Leibowitz, 1980, 1986). Peripheral and hypothalamic administration of CA agonists, antagonists, releasers and synthesis inhibitors have been found to significantly affect food intake and appetite, and 6-hydroxydopamine (6-OH-DA) lesion studies have revealed an essential role for hypothalamic CAs in regulating feeding, as well as drug effects on feeding (Leibowitz, 1982). The evidence has suggested that two separate CA systems are involved in regulating feeding in adult rats. As depicted in Figure 1, the first is a noradrenergic system that stimulates feeding through interaction with alpha-noradrenergic receptors located in the medial (paraventricular) hypothalamic region and the second is an adrenergic and dopaminergic system that suppresses feeding through interaction with beta-adrenergic and dopaminergic receptors located in the lateral (perifornical) hypothalamic region (Hoebel & Leibowitz, 1981; Leibowitz, 1986).

Medial Hypothalamus. By intracerebral injection of drugs via chronically implanted cannulas in adult rats,

Figure 1. Monoaminergic regulation of feeding behavior in the adult rat; reproduced from Hoebel and Leibowitz (1981). The medial hypothalamus, specifically the PVN, contains a satiety control system. Ascending noradrenergic/adrenergic and serotonergic pathways innervate this area. NE reduces satiety (minus sign) acting through alpha-noradrenergic receptors, whereas serotonin (5-HT) facilitates satiety (plus sign). The lateral hypothalamus, specifically the PFH, contains a feeding control system. Ascending adrenergic/noradrenergic and dopaminergic pathways innervate this area. EPI reduces feeding (minus sign) acting through beta-adrenergic receptors. Similarly, DA also inhibits feeding (minus sign).


FOREBRAIN
LATERAL HYPOTHALAMUS (PFH)
β
α
MEDIAL HYPOTHALAMUS (PVH)
DA
EPI
NE
5HT
SEROTONERGIC PATHS
DOPAMINERGIC PATHS
ADRENERGIC AND NORADRENERGIC PATHS
FEEDING CONTROL
SATIETY CONTROL
HINDBRAIN

Grossman (1960, 1962a, 1962b) originally demonstrated that hypothalamic injections of the CA neurotransmitters norepinephrine (NE) or epinephrine (EPI) could induce a feeding response in fully sated rats or potentiate a feeding response in deprived rats. This noradrenergic/adrenergic feeding response has been selectively blocked by the general alpha-noradrenergic receptor blockers phentolamine and phenoxybenzamine (Booth, 1967; Grossman, 1962b; Leibowitz, 1980; Slangen & Miller, 1969). Furthermore, the noradrenergic feeding response has been more effectively obtained with injection into the medial hypothalamus, where beta-adrenergic effects appear to be absent (Hoebel & Leibowitz, 1981; Leibowitz, 1986). Leibowitz (1978a, 1980, 1986) found that the most sensitive area to noradrenergic stimulation within the medial hypothalamus is the paraventricular nucleus (PVN), the butterfly-shaped nucleus that surrounds the ventral third ventricle. Recently, injection of the α_2-noradrenergic receptor agonist clonidine into the PVN was shown to produce a potent feeding response in sated rats that was very similar to that of NE's (Goldman, Marino & Leibowitz, 1985). This response by either agonist was blocked, in a dose-dependent fashion, by local injection of the α_2-noradrenergic antagonists rauwolscine and yohimbine and the general alpha antagonist phentolamine. In contrast, this response was unaffected by hypothalamic injection of the α_1-noradrenergic antagonists prazosin and corynanthine. These results indicated that

feeding elicited by noradrenergic stimulation in the region of the PVN is mediated through α_2-type noradrenergic receptors that appear to be located postsynaptically, since the effectiveness of clonidine in eliciting eating in this same study was undisturbed by prior injection of the CA synthesis inhibitor α-methyl-p-tyrosine.

Near physiological doses of NE injected into the PVN have also been demonstrated to elicit a feeding response in rats (Leibowitz, 1980; Ritter & Epstein, 1975). Martin and Myers (1975) reported an increase in the release of endogenous NE in the medial hypothalamus during spontaneous feeding in rats. A feeding response was also reported with medial hypothalamic injection of the CA-releasing drug amphetamine (AMPH), a finding that was in direct contrast with the anorexia produced by lateral hypothalamic injection of AMPH (Leibowitz, 1975b). These findings suggest a physiological role for NE in the regulation of natural feeding behavior in the adult rat.

Tricyclic antidepressants such as desipramine, protriptyline and amitriptyline have also been found to elicit feeding in rats when injected into the PVN (Leibowitz, Arcamano & Hammer, 1978). These drugs block the presynaptic uptake of NE, thereby potentiating its action within the synapse. As with NE, the antidepressant response was abolished by the alpha-noradrenergic blockers phentolamine and phenoxybenzamine, but not by other receptor-type blockers. The antidepressant effect has also

been shown to be reversed by PVN injection of drugs that block NE synthesis, such as previously mentioned α-methyl-p-tyrosine (Hoebel & Leibowitz, 1981; Leibowitz, 1986). These findings demonstrate that the feeding response produced by the antidepressants may be mediated by the endogenous CA neurotransmitter NE. They also provide a possible explanation for the overeating sometimes observed in people receiving chronic treatment with antidepressants.

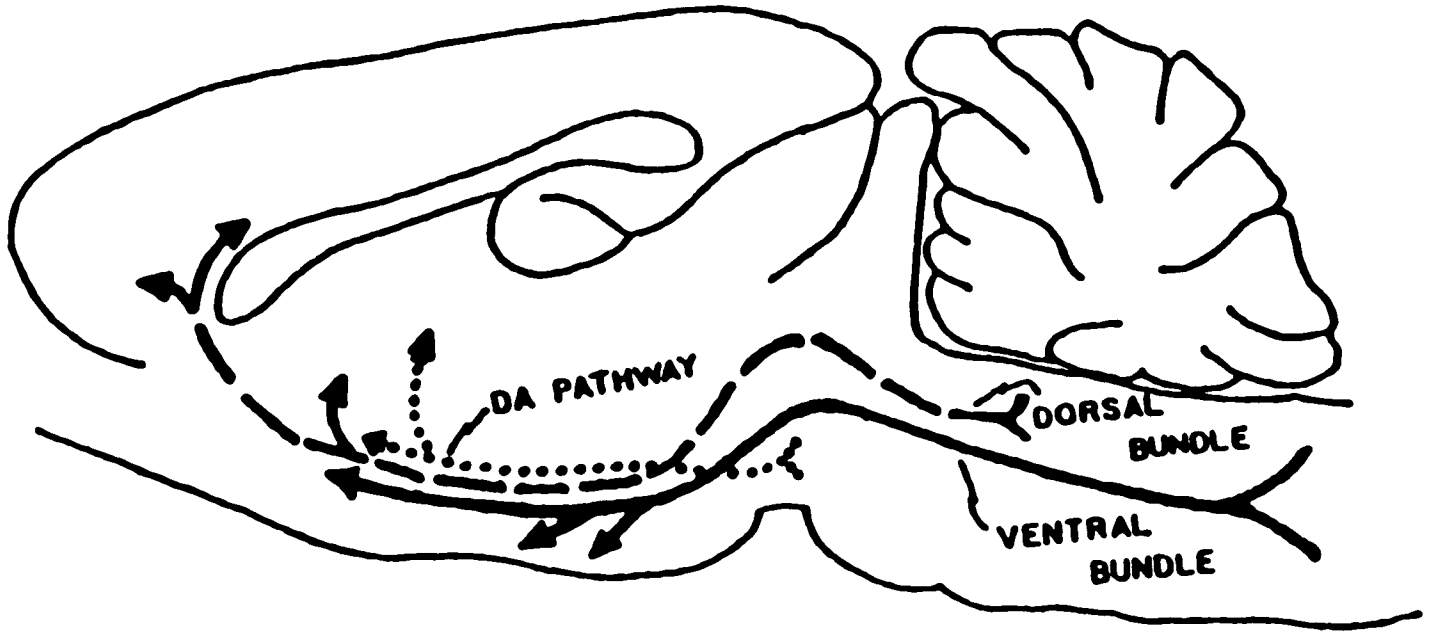
Theoretically, the alpha-noradrenergic feeding system appears to have an inhibitory influence on a medial satiety system (Hoebel & Leibowitz, 1981; Leibowitz, 1986). This is consistent with the evidence that iontophoretic infusion of NE into the medial hypothalamus of rats inhibits neuronal firing (Bloom, Oliver & Salmoiraghi, 1963). This theory is also supported by studies that assessed the effects of alpha-noradrenergic receptor agonists and antagonists injected directly into the PVN of the cat (Gaida & Kuhn, 1980) and some other species (Hoebel, 1977b; Leibowitz, 1980).

The classical effect of electrolytic lesions in the ventromedial hypothalamus is a voracious appetite, bulimia, leading to obesity. Adult rats with these lesions have been shown to regulate food intake at abnormally high body weights, and when starved down to normal weights, they subsequently overate until reattaining their abnormally high body weights (Hoebel, 1979; Hoebel & Teitelbaum, 1962; Teitelbaum, 1967). It has more recently been discovered

that medial lesions of the nearby PVN also produce hyperphagia and obesity in rats (Leibowitz, 1979, 1980; Leibowitz, Hammer & Chang, 1981). The neurochemical basis for this syndrome may, in part, be due to the destruction of satiety neurons that are normally inhibited by NE during the process of switching from satiation to eating (Hoebel & Leibowitz, 1981; Leibowitz, 1986). Consistent with this evidence is the finding that chronic infusion of NE into the PVN of rats over the course of a week caused a dramatic increase in daily food intake, meal size and body weight (Leibowitz, Brown, Tretter & Kirchgessner, 1985; Leibowitz, Weiss, Yee & Tretter, 1985; Lichtenstein, Marinescu & Leibowitz, 1984; Roosin, Rosenin & Leibowitz, 1980).

The evidence obtained thus far with respect to the neural innervation of the medial hypothalamus that mediates the noradrenergic effect on feeding suggests that the noradrenergic fibers that stimulate feeding originate from cells in the dorsal pons, either the locus coeruleus or subcoeruleus, or in the medulla (Leibowitz, 1980; Leibowitz & Brown, 1980a; Weiss & Leibowitz, 1985). As illustrated in Figure 2, at the level of the pons, these fibers maintain a relatively dorsal position within the central tegmental tract and at the level of the midbrain they turn sharply ventrally, coursing rostrally towards the hypothalamus. In the studies cited above, electrolytic or 6-OH-DA lesions in the dorsal midbrain tegmentum that damaged these fibers caused a loss of CA fluorescence in the PVN.

Figure 2. Major ascending CA pathways innervating hypothalamic areas en route to the forebrain; reproduced from Hoebel and Leibowitz (1981). Innervation is from collaterals of the dorsal and ventral bundles of the central tegmental tract and midbrain DA pathways.


DA PATHWAY
DORSAL
BUNDLE
VENTRAL
BUNDLE

Antidepressants injected into the PVN also failed to elicit feeding as they did prior to lesioning, suggesting that the neural substrate for endogenous release of the neurotransmitter had been destroyed. In addition, postsynaptic agonists including NE itself were considerably more effective in eliciting feeding after than before lesioning, thus implicating that receptor denervation supersensitivity had developed. Finally, rats with these lesions were also spontaneously hypophagic and lower in body weight, a finding that is predictable after destruction of a neurochemical system that inhibits satiety (Leibowitz & Brown, 1980a).

Lateral Hypothalamus. In support of the second of the two CA systems involved in the regulation of feeding behavior in adult rats, drug injections via chronic cannulas into the lateral perifornical hypothalamus (PFH) have revealed postsynaptic components that have been characterized as beta-adrenergic and dopaminergic receptors (Bendotti, Villa & Samanin, 1986; Borsini, Bendotti, Thurlby & Samanin, 1982; Leibowitz & Rossakis, 1978a, 1979a, 1979b; Pizzi, Coen, Memo, Missale, Carruba & Spano, 1986). The general beta-adrenergic receptor agonists EPI and isoproterenol have been shown to suppress feeding in deprived rats when injected directly into the PFH (Leibowitz, 1980; Leibowitz & Rossakis, 1978a). The β_2-adrenergic receptor agonist salbutamol was also shown to produce a potent suppression of eating. Conversely, the

general beta-adrenergic receptor antagonists propranolol and alprenolol injected into the PFH induced feeding while blocking the satiety effect of EPI and isoproterenol. The satiety effect of salbutamol was effectively antagonized by the β_2-receptor blocker butoxamine. These results indicated that the PFH is an EPI-sensitive site for inhibition of feeding with characteristics of classical beta-adrenergic receptors, specifically the β_2 subtype. Similarly, PFH injections of the dopaminergic receptor agonists apomorphine and dopamine (DA) itself have also been shown to suppress food intake in adult rats (Leibowitz, 1980; Leibowitz & Rossakis, 1979b, 1980) and dopaminergic receptor antagonists have been found to block DA satiety while stimulating food intake (Leibowitz, 1980). The dopaminergic antagonists tested in this study, haloperidol, fluphenazine, chlorpromazine and others, are neuroleptic drugs used in the treatment of psychosis that have been associated with overeating and obesity in humans, possibly due to their antagonistic action at the dopaminergic receptor site in the PFH.

Evidence of active endogenous stores for these putative neurotransmitters' satiety function also exists. AMPH and L-DOPA, a CA precursor, have both been demonstrated to suppress feeding when injected into the PFH of rats (Leibowitz, 1980; Leibowitz & Rossakis, 1979b, 1980). This satiety effect was diminished by administration of beta-adrenergic or dopaminergic antagonists, or CA synthesis

inhibitors (Leibowitz, 1975b, 1980; Leibowitz & Rossakis, 1979b, 1980). The well-known anorectic effect of AMPH injected into the PFH has also been produced with other CA excitants, such as methamphetamine, phenmetrazine and mazindol (Leibowitz & Rossakis, 1979b) and phenylpropanolamine (Hoebel, Hernandez & Thompson, 1975). This effect in the lateral hypothalamus has also been implicated in underlying the well-known anorectic properties obtained with these drugs after systemic administration (Garattini & Samanin, 1978; Hoebel, 1977a; Leibowitz, 1980).

Electrolytic lesions in the lateral hypothalamus produce the classical starvation syndrome (Grossman, 1975; Hoebel, 1971; Keesey & Powley, 1975; Mogenson, 1973; Novin, Wyrwicka & Bray, 1975; Stricker & Zigmond, 1975; Teitlebaum, 1967; Teitlebaum & Epstein, 1962). Animals with these lesions tend not to eat or react to external stimuli and may even starve to death if not maintained through intragastric tube feeding. Neurochemical evidence suggests that this deficit is the result of damage to the DA-containing nigrostriatal fibers passing through the far-lateral hypothalamus and also damage to the cell bodies or dendrites of neurons in this area that respond to local administration of CA drugs. These drugs are most effective in the lateral hypothalamus, specifically in the PFH which borders with the fornix (Hoebel & Leibowitz, 1981; Leibowitz, 1986). Kainic acid lesions of the lateral hypothalamus that specifically destroy cell bodies, but spare fibers of passage, have been

shown to produce several days of starvation (Grossman, Dacey, Halaris, Collier & Routtenberg, 1978; Stricker, Swerdloff & Zigmond, 1978), whereas loss of nigrostriatal fibers of passage blocked many other behaviors in addition to feeding. Therefore, the kainic acid lesions in these studies indicated that cells within the lateral hypothalamus itself are involved with specific aspects of feeding.

In theory, the beta-adrenergic and dopaminergic satiety system appears to have an inhibitory influence on a lateral feeding system in the adult rat (Hoebel & Leibowitz, 1981; Leibowitz, 1986). When the CA innervation of the lateral hypothalamus was destroyed by injecting 6-OH-DA into the midbrain, where ascending CA pathways course from cell bodies in the hindbrain to synapses in and beyond the PFH, hyperphagia leading to moderate obesity resulted (Ahlskog & Hoebel, 1973). As illustrated in Figure 2, the most ventral CA fibers within the midbrain tegmental tract have been shown to contain EPI, as well as NE, and can be seen passing through the area of the midbrain dopaminergic cell bodies that contribute fibers to this ascending projection system (Moore & Bloom, 1979). Electrolytic and 6-OH-DA lesions at the same midbrain site have also been shown to reduce the anorectic potency of systemic AMPH, suggesting that the lesions destroyed the neural substrate from which AMPH would normally induce the release of an endogenous neurotransmitter that suppresses feeding (Ahlskog, 1974; Leibowitz & Brown, 1980c). In a similar midbrain lesion

experiment with direct lateral hypothalamic injections of AMPH, not only did AMPH lose anorectic potency after lesioning, but direct administration of exogenous EPI or DA gained potency (Leibowitz & Brown, 1980b). These results suggested both loss of presynaptic input through which AMPH exerts its effect and compensatory development of supersensitivity of postsynaptic receptors. A loss of CA fluorescence in the limbic forebrain, including the PFH region, was also reported in this latter study. In addition, a buildup of CA fluorescence just caudal to the lesions indicated specific fiber degeneration. Thus, it was concluded that AMPH normally releases an endogenous CA neurotransmitter(s) in the lateral hypothalamus, thereby suppressing feeding. Following destruction of the ascending CA afferents, denervation supersensitivity develops in compensation for the loss of neural input, but apparently not sufficiently enough to prevent overeating (Hoebel & Leibowitz, 1981; Leibowitz, 1986).

The question of whether or not the overeating caused by 6-OH-DA lesioning results specifically from the destruction of CA neurons has also been raised. The purpose of using 6-OH-DA is that it is relatively selective in destroying CA neurons. However, this neurotoxin may also have some non-specific effects, particularly at high doses (Hoebel, 1977b; Lorden, Oltmans & Margules, 1976). In addressing this possibility, desmethylimipramine was injected systemically in combination with 6-OH-DA (Ahlskog, 1974; Ahlskog &

Hoebel, 1973; Ahlskog, Randall & Hoebel, 1975). Desmethylimipramine, an adrenergic/noradrenergic uptake blocker, is known to prevent uptake of 6-OH-DA and thereby prevents the destruction of adrenergic and noradrenergic neurons. Non-specific damage to other neurons and extraneuronal effects still result. As expected, systemic desmethylimipramine prevented adrenergic and noradrenergic depletion and also prevented hyperphagia. This finding suggested that hyperphagia is caused by specific depletion of the CAs, EPI, NE or both.

Hyperphagia due to adrenergic/noradrenergic depletion by 6-OH-DA, and not DA depletion, has also been confirmed (Hernandez & Hoebel, 1980). Desmethylimipramine or AMPH injected locally into the ventral tegmentum, just before 6-OH-DA, prevented adrenergic/noradrenergic depletion and hyperphagia. Evidently, the pretreatment blocked 6-OH-DA uptake and thereby protected the adrenergic and noradrenergic neurons needed for normal satiety. Pretreatment with local administration of the dopaminergic uptake blocker benztropine, to protect dopaminergic neurons, failed to prevent hyperphagia. This evidence confirmed that the overeating and obesity resulting from 6-OH-DA lesions in the ventral midbrain can be attributed to the specific destruction of adrenergic and noradrenergic fibers. In addition, fine-wire knife-cut lesions, that cut fibers without causing a great deal of damage to cell bodies, also induce hyperphagia when the fiber cut is located just caudal

or lateral to this PFH site (Gold, 1973; Grossman, 1975).

Despite these findings, evidence reviewed earlier suggests that the PFH contains dopaminergic receptors that suppress feeding in adult rats, apparently by a mechanism similar to that of the beta-adrenergic receptors in this same area (Leibowitz, 1980; Pizzi et al., 1986). This dopaminergic receptor system may originate from cell bodies in the ventral midbrain (Leibowitz & Brown, 1980b, 1980c). DA in the PFH does appear to play a role in AMPH anorexia, since depleting dopaminergic fibers with midbrain lesions attenuates both PFH AMPH anorexia as well as systemic AMPH anorexia (Leibowitz, 1980).

Neuropeptide Systems.

As mentioned previously, neuropeptides have also been demonstrated to play a role in the regulation of feeding behavior. In fact, a number of biologically active peptides have been found to function as neurotransmitters within the central nervous system, regulating food intake in various species. Included among them are cholecystokinin (CCK) and neuropeptide Y (NPY). Besides acting as neurotransmitters, these peptides can be released into the bloodstream, thus acting as classical hormones. Furthermore, they can also be released from non-neuronal cells in the periphery and produce an effect on neighboring cells - the so-called paracrine system. It appears that all three of these modes of action of peptides may be involved in the regulation of

feeding behavior (reviewed in Morley, Bartness, Gosnell & Levine, 1985).

Cholecystokinin. Since it was first isolated as a 33-amino acid polypeptide from the porcine gastrointestinal tract by Mutt and Jorpes (1971), CCK has been demonstrated to partially mediate taste or palatability (see Morley et al., 1985) and to have a variety of effects on the gastrointestinal tract producing satiety (Walsh, 1978). In addition to its role as a gastrointestinal hormone, CCK has been shown to be widely distributed throughout the central and peripheral nervous systems of the rat and other species (i.e., Dockray, Gregory, Hutchinson, Harris & Runswick, 1978; Lamers, Morley, Poitras, Sharph, Carlson, Hershman & Walsh, 1980). Evidence supporting a variety of effects of CCK in the central nervous system, including appetite regulation, has strongly demonstrated CCK's role as a behaviorally active neuropeptide (Morley, 1982).

Recent reviews have summarized considerable evidence supporting CCK's role as a peripheral satiety factor (i.e., Morley et al., 1985), but controversy as to whether the satiety effect of CCK is specific (Collins, Walker, Forsyth & Belbeck, 1983) or secondary to other physiological responses (Moran & McHugh, 1982; Morley et al., 1985) still exists. Despite this controversy, peripheral administration of CCK octapeptide (CCK-8) and CCK-33, the predominant forms of CCK, has been demonstrated to suppress food intake in adult rats and other species, including humans (Stacher,

Bauer & Steinringer, 1979; Stacher, Steinringer, Schnierer, Schneider & Winklehner, 1982), on numerous accounts (i.e., Anika, Houpt & Houpt, 1981; Antin, Gibbs, Holt, Young & Smith, 1975; Corp, Fitts & Woods, 1983; Falasco, Smith & Gibbs, 1979; Gibbs, Falasco & McHugh, 1976; Gibbs, Young & Smith, 1973a, 1973b; Grovum, 1981; Holt, Antin, Gibbs, Young & Smith, 1974; Houpt, Anika & Wolff, 1978; Houpt, Baldwin & Houpt, 1983; Levine, Sievert, Morley, Gosnell & Silvis, 1984; Metzger & Hansen, 1983; Savory & Gentle, 1980). Further evidence has shown that l-phenylalanine, a potent releaser of CCK (Meyer & Grossman, 1972), suppresses food intake in monkeys, whereas d-phenylalanine does not (Smith & Gibbs, 1977). Since CCK is apparently released within minutes after food enters the duodenum (Berry & Flower, 1971), Smith, Gibbs and Young (1974) have suggested that CCK is functioning as a hormone for intestinal satiety that occurs after spontaneous ingestion.

More recently, some light has been shed on yet another controversy concerning whether or not CCK produces its satiety effect at a peripheral or central site. Although it has appeared that in adult rats the major effect of peripherally administered CCK occurs in the abdomen through vagal fibers, and not directly in the brain (Lorenz & Goldman, 1982; Morley, Levine, Kneip & Grace, 1982; Smith & Cushin, 1978; Smith, Jerome, Cushin, Eterno & Simansky, 1981), Faris and Olney (1985) have recently demonstrated that low doses of CCK injected directly into the PVN

decrease feeding in a dose-dependent fashion. Similarly, Willis, Hansky and Smith (1984) reported that bilateral injections of CCK into the lateral hypothalamus also reduce food intake, and McCaleb and Myers (1980) have previously demonstrated that injections of CCK into the medial hypothalamus significantly attenuate NE-induced feeding. Despite earlier reports of little to no effect of central administration of CCK on normal feeding in adult rats (Della-Fera & Baile, 1979; Grinker, Schneider, Ball, Cohen, Strohmayer & Hirsh, 1980; Smith & Gibbs, 1979), these recent findings have provided preliminary evidence that central CCK does appear to play an important role in regulating satiety in the rat. In combination with the unequivocal data showing that the major effect of CCK is due to a direct effect on the brain in sheep (Della-Fera & Baile, 1979, 1980), pigs (Parrott & Baldwin, 1981) and chickens (Denbow & Myers, 1982), the recent evidence of central CCK effects on feeding in adult rats has renewed interest among investigators examining the effects and modes of action of CCK and other neuropeptides on feeding behavior.

Neuropeptide Y. Unlike CCK, NPY is one of few neuropeptides which have been demonstrated to enhance feeding in adult rats. NPY, a 36-amino acid member of the pancreatic polypeptide family, was first isolated from porcine brain by Tatemoto and co-workers (Tatemoto, 1982; Tatemoto, Carlquist & Mutt, 1982). NPY has a high degree of sequence homology with peptide YY (70%) and pancreatic

polypeptide (50%), the other two members of the newly recognized peptide family (Tatemoto, 1982). Although NPY has not been found in the gastrointestinal tract, it has been found to be widely distributed throughout the central nervous system with the highest concentrations in the hypothalamus, olfactory tubercle, nucleus accumbens and amygdala (Emson & DeQuidt, 1984). NPY has also been localized in coexistence with noradrenergic neurons of both the central and peripheral nervous systems and the adrenal medulla (Allen, Adrian, Polak & Bloom, 1983; Hokfelt, Lundberg, Lagercrantz, Tatemoto, Mutt, Lindberg, Terenius, Everitt, Fuxe, Agnati & Goldstein, 1983). NPY is also one of the most potent vasoconstrictors known (Emson & DeQuidt, 1984). In addition, it has also been shown to increase the number of alpha-noradrenergic receptors (Agnati, Fuxe, Benefenati, Battistini, Harfstrand, Hokfelt, Cavicchioli, Tatemoto & Mutt, 1983), inhibit NE release from the vas deferens (Lundberg, Terenius, Hokfelt, Martling, Tatemoto, Mutt, Polak, Bloom & Goldstein, 1982) and inhibit electrical contraction of the vas deferens much in the same way as dynorphan (Allen, Adrian, Tatemoto, Polak, Hughes & Bloom, 1982).

More recent studies in adult rats have shown that the concentration of NPY in the brain is higher than the concentration of any other neuropeptide (Allen, Adrian, Allen, Tatemoto, Crow, Bloom & Polak, 1983) and that the hypothalamus, specifically the PVN, has one of the densest

supplies of NPY-containing presynaptic terminals in the brain (Magnuson, O'Donohue & Gray, 1984; Olschowka, 1984). Subsequent findings have shown that NPY coexists with NE and EPI in the brainstem CA cell groups that send afferent fibers to the PVN (Hokfelt, Lundberg, Tatemoto, Mutt, Terenius, Polak, Bloom, Sasek, Elde & Goldstein, 1983; Sawchenko & Swanson, 1982). These findings, as well as the convergence of overwhelming evidence that feeding behavior in adult rats is elicited by PVN injections of exogenous NE or EPI and by release of NE into the PVN (Leibowitz, 1978a; Leibowitz et al., 1978), suggest that NPY in the PVN may act similarly to NE in regulating feeding behavior.

Not suprisingly, in addition to NPY's peripheral effects, NPY has been shown to elicit a dramatic feeding response and a small drinking response in sated adult rats when injected into the cerebral ventricles (Clark, Kalra, Crowley & Kalra, 1984; Levine & Morley, 1984) or several hypothalamic nuclei (Stanley, Chin & Leibowitz, 1985; Stanley & Leibowitz, 1984, 1985), of which PVN injections seem to produce the most dramatic effect (Stanley et al., 1985). The behavioral specificity of NPY's effect in all of these studies has implicated a physiological role for NPY in the regulation of feeding behavior.

Ontogeny of Feeding Behavior

In recent years, the developmental approach of observing and manipulating a behavioral system at different

stages during maturation in order to trace how the system is assembled and organized has been applied to the study of feeding behavior in rats (Hall, 1985; Hall & Williams, 1983). The fact that feeding is one of few behaviors of mammals that is present at birth and continues throughout life makes it an ideal system for developmental analysis. A critical assumption though is one of continuity, that the system being examined at one stage in development is the same system being examined at a later stage. Frequently, in the application of this approach, the system examined early in development is viewed as the primitive behavioral form that gradually matures into the adult pattern.

Suckling versus Independent Feeding.

Over the years, the study of the development of ingestive behaviors and their physiological controls has focused on suckling behavior (Drewett & Cordall, 1976; Friedman, 1975; Hall, Cramer & Blass, 1977; Hall & Rosenblatt, 1977; Houpt & Epstein, 1973; Houpt & Houpt, 1975; Lytle, Moorcroft & Campbell, 1971) and the emergence of independent feeding at the onset of weaning (Anderson & Patrick, 1934; Bolles & Woods, 1964; Rosenblatt, 1965; Small, 1899; Tilney, 1933). A major assumption of many of these earlier developmental studies was that suckling behavior represents a 'primitive analogue' of adult feeding behavior and gradually gives way to the adult feeding pattern during weaning (Hall et al., 1977). Also implicit

in much of this earlier work was the assumption that the emergence of physiological controls for suckling indicates the emergence of potential physiological controls for adult feeding and drinking (Hall, 1979).

Although suckling is the primary form of ingestion in newborn mammals and thus would seem to be a natural starting point for investigating the ontogeny of feeding behavior in the rat, evidence has accumulated suggesting that suckling behavior in preweanling rats differs in many ways from adult feeding behavior, despite their functional similarity in subserving the acquisition of food (Blass & Cramer, 1982; Drewett, 1978; Epstein, in press; Hall, 1979, 1985; Hall & Williams, 1983). In short, the suckling system of the infant has been demonstrated to differ from the feeding system of the adult with respect to: the motor pattern involved in appetitive responding; external controls; internal controls; experiential determinants; availability of food; and neural substrates (reviewed in Hall & Williams, 1983). Despite these differences, variables modulating suckling intake do exist and there may be some overlap between suckling and later ingestion in the rat, especially with respect to suckling after two weeks postpartum (Brake, Sager, Sullivan & Hofer, 1982; Brake, Wolfson & Hofer, 1979). In summary, interpretations have implicated that suckling is a unique behavior and probably does not represent an appropriate beginning for developmental analysis of adult feeding behavior in the rat, but rather a

highly specialized form of feeding, or in Oppenheim's (1981) terminology, 'a transient ontogenetic adaptation'.

In contrast to suckling behavior, another form of ingestion, ingestion 'independent' of the mother, has recently been identified in neonatal rats as an early precursor to adult feeding (Hall, 1985; Hall & Williams, 1983). This independent feeding system in the rat pup is one that is distinctly different from suckling and shares many characteristics with adult feeding (Bruno, 1981; Ellis, Axt & Epstein, 1984; Hall, 1979; Hall & Bryan, 1980; Hall & Williams, 1983; Johanson & Hall, 1979, 1980, 1982; Johanson, Polefrone & Hall, 1981; Johanson & Teicher, 1980; Wirth & Epstein, 1976). Furthermore, in mediating ingestion of milk off the nipple, this independent feeding system in infant rats appears to be developmentally continuous with feeding in adult rats, and while it is not used by the infant during normal development, it does seem to represent an appropriate starting point for developmental analysis of adult feeding behavior in the rat (Hall, 1985).

Catecholamine Neural Substrates. Although the evidence is not totally conclusive (Almli, 1978; Kornblith & Hall, 1979), pharmacological results suggest that differences between the suckling and independent feeding systems in the infant rat, with respect to CA involvement, do exist (Hall & Williams, 1983). For suckling, previous findings have shown that systemic administration of the CA-releasing drug AMPH suppresses milk intake in rat pups 15 days or older, but not

prior to this age (Lytle et al., 1971; Raskin & Campbell, 1981). In contrast to these findings, AMPH has also been shown to stimulate milk intake through suckling in 10-day-old (Williams & Brake, unpublished observations, cited in Hall & Williams, 1983) and 5-day-old pups (Leshem, 1981). Together, these findings suggest that a CA system(s) which enhances intake through suckling is present early in development and that a CA system(s) which suppresses intake through suckling is not functional until the onset of weaning.

With respect to intake through independent ingestion, evidence exists that both stimulatory and inhibitory CA systems mediating independent feeding in the infant rat are functional very early in development. Capuano, Barr and Leibowitz (in press) have shown an increase in milk intake via intra-oral cannulas with systemic administration of AMPH and chlorpromazine, a dopaminergic antagonist, in pups as young as 3 days of age. In contrast to these findings, suppression of milk intake via intra-oral cannulas by AMPH has also been reported in 5-day-old pups (Raskin & Campbell, 1981). Furthermore, state-dependent variables such as deprivation level, dose, ambient temperature and method of milk presentation have recently been shown to attenuate (Capuano et al., in press) and even reverse (Terry, Johanson & Wolgin, 1984) the effects of AMPH on intake through independent ingestion. These latter findings suggest that several state-dependent variables appear to be involved in

the determination of the effects of systemically administered AMPH on independent feeding in infant rats. More importantly, they also support the idea that AMPH exerts its stimulatory effect on independent feeding in the infant rat by releasing endogenous CAs in the medial hypothalamus and its inhibitory effect by releasing endogenous CAs in the lateral hypothalamus, as was demonstrated in the adult rat (Leibowitz, 1975b). If so, the alpha-noradrenergic and beta-adrenergic receptor systems of the medial and lateral hypothalamic areas respectively would need to be functionally mature very early in development in order to mediate the effects of AMPH.

In addressing this possibility, Ellis et al. (1984) recently reported that injections of NE into the ventral third ventricle increased independent milk intake via intra-oral cannulas in preweanling rats beginning abruptly at 9 to 10 days of age. Coupled with the finding that NE did not increase milk intake through suckling at any age in the same study, these results suggest that the noradrenergic system appears to modulate independent feeding in the young rat as it does in the adult (Davis & Keesey, 1971; Hoebel & Leibowitz, 1981; Leibowitz, 1986). Furthermore, this noradrenergic system that is fuctionally mature in the rat brain during the suckling period appears to be a 'nascent control' of subsequent adult feeding, rather than a functioning control of ongoing suckling (Ellis et al., 1984).

Thus, in review of the literature, an independent feeding system appears to exist in rat pups that may be present at birth and is more like the adult system for ingestion than is suckling. Unlike suckling, this independent feeding system in the pup has been demonstrated to be similar to adult feeding in its motor patterns for consumption, in its ability to support appetitive learning, in its physiological controls, and as indicated by response to the pharmacological manipulations described above, in at least some of its CA neural substrates (Hall & Williams, 1983). Furthermore, the precursors or origins of adult feeding appear much better represented by this independent ingestive system in the pup than by suckling. It is a system that seems to be developmentally continuous with adult feeding in reflecting a common CA neural substrate, but yet, more specific evidence with respect to the pharmaco-ontogeny of hypothalamic CA receptor systems mediating independent ingestion of milk in the infant rat is needed.

Despite the finding that intracerebroventricular injections of NE elicited independent feeding in infant rats (Ellis et al., 1984), a large discrepancy exists between the age of 9 days, at which this response was first demonstrated by Ellis et al. (1984), and the much earlier age of 3 days, at which systemic administration of AMPH was demonstrated to similarly enhance independent ingestion of milk in the rat pup, possibly via the alpha-noradrenergic system (Capuano et

al., in press). As mentioned earlier, these alpha-noradrenergic receptors that mediate NE's effect on food intake in the adult rat appear to reside within the PVN (Leibowitz, 1978a; Matthews, Booth & Stolerman, 1978) and have recently been identified as α_2-noradrenergic receptors (Goldman et al., 1985). Furthermore, the development of the alpha-noradrenergic receptors that mediate the feeding response to NE are already differentiated at birth into α_1- and α_2-receptor subtypes and their binding sites begin to increase from postpartum on (Morris, Dausse, Devynck & Meyer, 1980). Still further evidence suggests that the beta-adrenergic receptors that mediate EPI's inhibitory effect on feeding in the lateral hypothalamus of the adult rat are also present at birth and show a rapid postnatal rise begining at 7 days of age (Harden, Wolfe, Sporn, Perkins & Molinoff, 1977). Together, these latter findings strengthen the argument that the alpha- and beta-receptor systems that have been identified in mediating feeding behavior in the adult rat (Hoebel & Leibowitz, 1981; Leibowitz, 1986) are functionally mature very early in life, mediating independent ingestion of milk in the infant. The pharmacological evidence gathered to date seems to suggest this and certainly warrants further investigation of the pharmaco-ontogeny of these hypothalamic CA receptor systems mediating independent feeding in the rat.

Peptide Neural Substrates. While intracerebral administration of CCK and NPY have been demonstrated to

suppress and enhance feeding respectively in the adult rat, review of the developmental literature indicates a lack of evidence for central administration of these two behaviorally active neuropeptides in the infant. Despite this lack of central administration evidence, some evidence for peripheral administration of these neuropeptides in the infant does exist. Unlike pharmacological results for CA involvement, those for CCK and NPY do not clearly suggest that differences between the suckling and independent feeding systems in the pup exist with respect to regulation by these neuropeptides.

For suckling, an earlier study demonstrated that intraperitoneal injections of CCK in rat pups did not reduce deprivation-induced suckling intake via posterior tongue cannulas until 15 days of age (Blass, Beardsley & Hall, 1979). While Houpt and Houpt (1979) and Anika (1983) have shown that peripheral administration of exogenous CCK suppressed deprivation-induced suckling intake without tongue cannulas in 1- to 7-day-old pups, Anika (1983) failed to report specific suppression of suckling intake with administration of the potent releaser of endogenous CCK, l-phenylalanine, until 21 days of age. The latter findings were interpreted to suggest that while CCK receptors may be functional very early in development, endogenous release of CCK, inducing satiety, does not occur until immediately after weaning.

In addition to examining the development of CCK's

effect on suckling intake, further studies have attempted to examine the development of CCK's effect on independent ingestion of milk. Infusing milk through anteriorly placed intra-oral cannulas, Phifer and Hall (1984) have reported that specific suppression of intake by peripherally administered CCK in deprived rat pups does not appear until 15 days postpartum. In contrast, a similar study indicated that peripheral CCK was effective in specifically inhibiting intake as early as day 1 of life, when deprived pups were given free access to milk that was spread on the floor beneath them (Goldrich, Robinson, McHugh & Moran, 1984). The difference in results reported by these two studies may have been attributed to the difference in method of milk presentation, as has been demonstrated for the effects of AMPH on independent ingestion of milk in preweanling rats (Terry et al., 1984). Alternatively, differences in interpretation of CCK's specificity at early ages may also have accounted for the difference in results (Phifer & Hall, 1984). Nevertheless, these latter studies have shed some light on the development of the effects of peripherally administered CCK on independent feeding in rats.

Yet, as indicated previously, none have attempted to investigate the ontogeny of the effects of centrally administered CCK on independent feeding in rats. Among evidence reviewed earlier, the PVN was implicated as a neuroanatomical locus for the regulation of central CCK satiety in adult rats (Faris & Olney, 1985; McCaleb & Myers,

1980). Coupled with the finding that brainstem projections to the PVN and nearby hypothalamic ventromedial nucleus in adult rats contain endogenous CCK (Zaborszky, Beinfeld, Palkovits & Heimer, 1984), these results suggest that central CCK is involved in regulating satiety within the medial hypothalamus, specifically the PVN. Combined with the inconsistent results obtained for peripherally administered CCK and lack of evidence for centrally administered CCK in infant rats, the evidence gathered thus far calls for further investigation of the pharmaco-ontogeny of PVN receptors mediating the effect of CCK on independent feeding in the rat.

The behavioral effects of the second neuropeptide of interest, NPY, have not yet been examined with respect to regulation of feeding behavior in the infant rat. Among evidence reviewed earlier, NPY was reported to produce the most dramatic feeding response obtained to date in adult rats when injected into the PVN (Stanley et al., 1985). Further evidence in adult rats indicated that NPY and NE are co-localized in brainstem projections to the PVN (Hokfelt et al., 1983; Sawchenko & Swanson, 1982). Radioimmunoassay (Allen, McGregor, Woodhams, Polak & Bloom, 1984) and immunohistochemical (Woodhams, Allen, McGovern, Allen, Bloom, Balazs & Polak, 1985) analyses of the early ontogeny of the NPY system in the rat brain have more recently indicated that NPY is present in the brainstem and diencephalon, including the PVN, in embryos of 14 days

postconception. The concentrations of NPY showed a rapid postnatal rise in these regions. Furthermore, the finding of NPY early in the development of the embryonic rat brain, particularly in caudal regions, was said to have some similarities to the pattern of development of the CA system (Allen et al., 1984). In fact, areas rich in NPY immunostaining included the monoaminergic regions of the brain stem from embryonic day 13, especially the lateral reticular nucleus and the medullary reticular formation (Woodhams et al., 1985). Together, these findings suggest that NPY in the PVN may act similarly to NE in regulating feeding in the adult rat and independent milk ingestion in the infant.

Based on this speculation, upon investigating the pharmaco-ontogeny of the alpha-noradrenergic receptor system of the medial hypothalamus, the need for a similar investigation of PVN receptors mediating the effect of NPY on independent feeding in rat pups is also apparent.

Synopsis of Dissertation

In order to gain a better understanding of hypothalamic receptor systems mediating independent feeding in infant rats, the pharmaco-ontogeny of the alpha-noradrenergic and beta-adrenergic receptor systems of the medial and lateral hypothalamic areas respectivley, were investigated. In addition, the pharmaco-ontogeny of the hypothalamic receptor systems mediating the effects of the behaviorally active

neuropeptides CCK and NPY were similarly investigated.

In light of pharmacological evidence supporting the PVN and PFH as extremely sensitive sites within the medial and lateral hypothalamic areas respectively for manipulations affecting feeding behavior in adult rats, these neuroanatomical loci were selected for pharmaco-ontogenetic assessment in the present investigations. Furthermore, in light of evidence supporting developmental continuity between independent feeding in infant rats and later adult feeding, the independent feeding system in the infant rat was chosen for similar assessment in the present investigations.

General Methods and Materials

Subjects.

Male and female offspring of Long-Evans hooded rats, bred and reared in the laboratory colony, were used as subjects. In the week prior to giving birth, pregnant females were moved to individual breeding tubs containing pine shavings as bedding material. Water and Purina food pellets were freely available. The breeding colony was temperature-controlled (22°C) and kept on a 12/12 hr light-dark cycle with lights on at 7:00 AM. Mothers were checked for birth twice daily, at 0800 and 1800 hr, and rat pups found at either of these times were designated 0 days of age. All litters were culled to a minimum of 5 or maximum of 10 pups three days after birth and were otherwise left

undisturbed until the time of surgery with the exception of changing the bedding material twice each week.

Surgical and Post-surgical Procedures.

On the day prior to testing, rat pups from the same litter, ranging in age from 1 to 14 days of age, were removed from their mother, weighed and steroetaxically implanted, under Metofane anesthesia, with a chronic indwelling 26-gauge stainless-steel guide cannula (Plastic Products Co.) that terminated 1.0 mm dorsal to the targeted injection site in one of four hypothalamic areas tested. These areas included the PVN and PFH of the medial and lateral hypothalamic areas respectively, the ventral third ventricle and the anterolateral hypothalamus. The guide cannula and removable stylet were anchored to the skull with two applications. The first was a thin layer of grip cement (Dentsply International) and the second a non-iritating self-curing acrylic (Silverman's) that was used to construct a supporting crown. The stereotaxic instrument (David Kopf Instruments, Model 1430) was equipped with a custom-fitted infant stereotaxic accessory that was designed according to specifications cited in Heller, Hutchens, Kirby, Karapus and Fernandez (1979). This accessory permitted reliably accurate adjustments in all three planes. The stereotaxic coordinates for each of these hypothalamic areas were taken from a stereotaxic atlas of the infant rat hypothalamus (Valenstein, Case & Valenstein, 1969) and in pilot studies,

accurately readjusted for each age by histological confirmation. The stereotaxic coordinates used for each of these hypothalamic areas as a function of age of the pup implanted are shown in Table 1. The PVN and PFH sites represented 'on-target' injections in Experiments 1 and 2 respectively. The ventral third ventricle and anterolateral hypothalamic sites represented 'off-target' injections that were used for comparison to PVN and PFH injections in Experiments 1 and 2 respectively. The targeted site for the ventral third ventricle was medial to that for the PVN, while the targeted site for the anterolateral hypothalamus was rostral to that for the PFH. In Experiment 3, the PVN site was used for all injections.

Following surgery, pups were individually placed in small, clear plastic containers (10 x 12.2 x 12.7 cm) that were in turn placed in a post-operative incubator that was kept at nest temperature (32-34 °C) until the time of testing. Pups that were to be sated prior to testing (see Testing Procedure) were given a small, plastic spoonful of commercially available plain, low-fat yogurt that was placed on the floor in a corner of each individual container. This was carried out in an attempt to minimize any effects due to deprivation, prior to satiation. Pups that were to be deprived prior to testing received similar post-operative treatment with the exception of yogurt administration.

Post-operative weight gain and weight loss data for 2-day-old pups that were given yogurt in the initial

Table 1

Stereotaxic Coordinates for Each Injection Site as a Function of Age

Injection Site	Age (days)	Stereotaxic Coordinates (mm)		
		A-P	M-L	D-V
PVN	2	-1.2	-0.4	-4.9
	7	-1.7	-0.5	-6.3
	12	-1.6	-0.5	-6.7
	15	-1.5	-0.5	-6.9
V	2	-1.2	-0.0	-4.9
PFH	2	-2.1	-1.0	-5.4
	7	-2.9	-1.1	-7.2
	15	-2.8	-1.1	-7.8
AL	2	-1.3	-0.9	-5.1

PVN - paraventricular nucleus, V - ventral third ventricle, PFH - perifornical hypothalamus, AL - anterolateral hypothalamus. All coordinates are relative to bregma; A-P, anterior-posterior; M-L, medial-lateral; D-V, dorsal-ventral (to the surface of the skull).

investigation of Experiment 1 and pups that were not given yogurt in the initial investigation of Experiment 2 are presented in Table 2. Pups given yogurt were occasionally observed licking and mouthing the yogurt and on several occasions a pup given yogurt actually gained weight. Despite these occasional weight gains, comparison of the group means indicates that pups given yogurt and those not given yogurt both lost weight. Analysis of the data by Student's T-test indicated no significant difference in weight loss for the two groups ($t(40)=0.76, p>.05$), despite an apparent lesser weight loss for pups given yogurt. Together, these results suggest that as a group, pups that were offered yogurt post-operatively did not consume it.

Testing Procedure.

On the day following surgery, pups were removed from the post-operative incubator and individually transferred to a similar set of small, clear plastic containers that contained the same type of bedding material (pine shavings) found within their litter cages. The individual containers were then placed within a water bath that maintained the ambient temperature within the containers at nest temperature (32-34 °C) throughout each individual test period. At this time, an intra-oral cannula was implanted in the anterior portion of the mouth of each pup in order to assess independent milk or water ingestion (Hall, 1979). In doing so, a piece of polyethylene tubing (Clay Adams, PE 10)

Table 2

Post-operative Weight Gain and Weight Loss Data

for Sated and Deprived 2-Day-Olds

Sated	Deprived
-0.25	-0.26
+0.16	-0.24
-0.29	-0.18
-0.54	-0.24
-0.48	-0.26
-0.28	-0.14
-0.18	-0.22
+0.05	-0.20
-0.22	-0.18
-0.13	-0.14
-0.25	-0.27
-0.47	-0.15
-0.30	-0.16
-0.23	-0.11
+0.10	-0.26
+0.06	-0.29
-0.05	-0.29
+0.03	-0.12
-0.24	-0.23
-0.01	-0.25
-0.18 ± 0.04	-0.21 ± 0.01

These data represent weight gain (+) and weight loss (-) values in grams for pups administered (sated) and not administered (deprived) yogurt post-operatively in the First Investigations of Experiments 1 and 2 respectively. Column totals represent the mean ± SEM for both groups. Student's T-test revealed no significant difference between the two ($t(40)=0.76, p>.05$).

was flanged at one end by a flame and its other end was passed through the mouth of the unanesthetized pup, just behind the root of the lower incisors, with the aid of a piece of 30-gauge needle that was cut and angled to approximately 45°. The free end of the cannula was then attached through a larger piece of polyethylene tubing (Clay Adams, PE 50) to a 5 or 10 cc syringe that was placed in a dual infusion/withdrawal pump (Harvard Apparatus Co., Model 600-910). The pump infused milk or water continuously at rates of 0.66, 1.38, 3.30 and 3.30 cc per hr for 2-, 7-, 12- and 15-day-olds respectively. In each case, the rate of infusion was approximately equal to 10% of the pup's body weight per hr, which exceeded intake by a moderate amount. A slow, continuous infusion was chosen as opposed to a pulsating infusion in order that independent ingestion of milk in the preweanling rats tested approximate adult feeding with respect to food availability. The milk diet used for all experiments was commercially available Half-and-Half (milk and cream), which has certain similarities to rat's milk (Hall, 1979).

After voiding the pup's bladders by gently stroking their anogenital area with a moist Q-tip swab, their urogenital and anal openings were sealed with a drop of non-iritating self-curing acrylic (Silverman's) to minimize weight loss through excretion. The pups were then weighed and sated for a 1.5 hr period by infusing milk or water at the appropriate rate or continued under food deprivation for

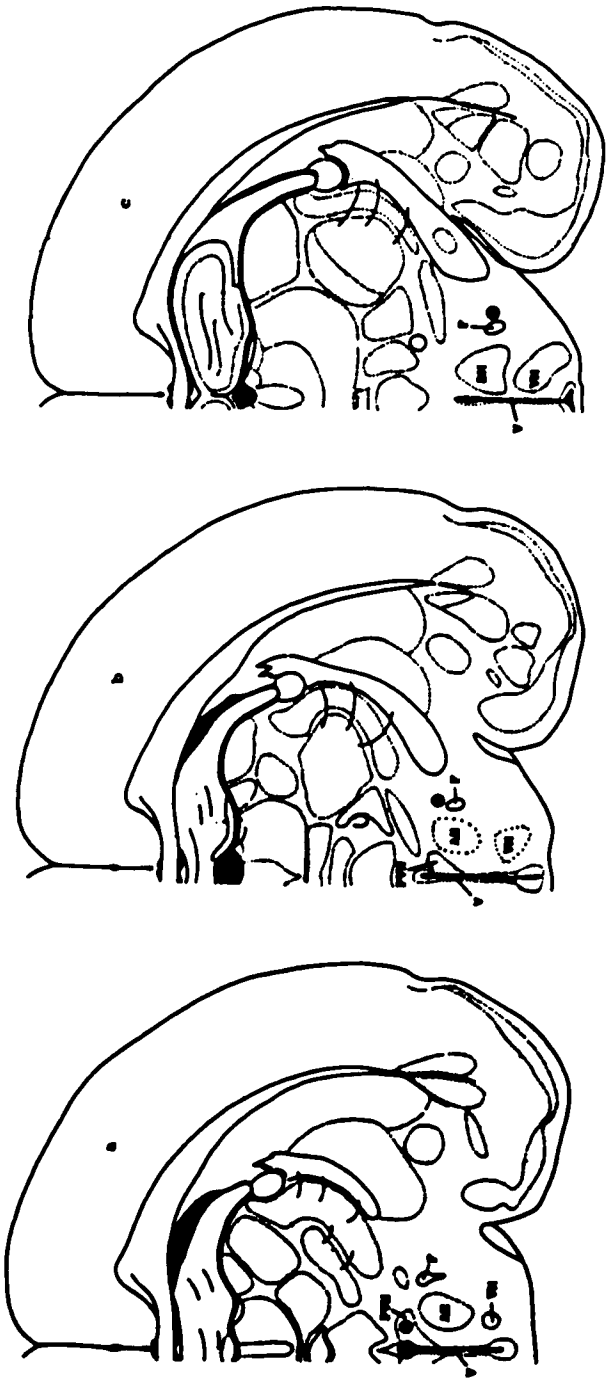
a total 22 hr period that began at the time the pups were removed from their mother on the day prior to testing. Following the period of satiation or deprivation, the pups were reweighed and intracerebrally injected, without anesthesia, with a single dose of either l-norepinephrine-d-bitartrate (NE, Sigma), clonidine HCl (Boehringer-Ingelheim), l-epinephrine-d-bitartrate (EPI, Sigma), salbutamol hemisulfate (Sigma), apomorphine HCl (Sigma), cholecystokinin octapeptide (CCK-8, Squibb 19,844), neuropeptide Y (NPY, Peninsula Laboratories, Belmont, CA) or an equivalent volume of the vehicle. The vehicle for all drugs tested was bacteriostatic saline (0.85%), with the exception of apomorphine, for which 0.02% ascorbic acid was used as a vehicle. The injection volume was 0.1 ul and injections were made directly into the brain site through a 33-gauge injector cannula (Plastic Products Co.) that extended 1.0 mm beyond the tip of the guide cannula. Immediately following the injections, the infusion pump was started and a continuous milk or water infusion was delivered for a 1 hr test period. Body weight gain was used as a measure of milk or water ingested, since it has been shown that weight gain is a valid measure of intake in suckling rats (Houpt & Epstein, 1973). Intake was assessed by measuring weight gain at the end of the test period. Increases in body weight gain of the drugged pups were compared to that of the vehicle-injected pup in each test and each pup was tested only once.

Post-testing Procedure.

Following completion of the test period, all pups received an intracerebral injection of 0.1 ul of India ink to aid histological verification of the injection sites. All pups were then perfused intracardially, under Metofane anesthesia, with isotonic saline followed by 10% buffered formalin. Brains were then excised, cut in 50 um coronal sections and stained with cresylviolet. The injection sites were determined with the aid of a stereotaxic atlas (Valenstein et al., 1969). The targeted sites are depicted in Figure 3 on plates reproduced from the Sherwood and Timiras (1970) atlas of the developing rat brain.

All cannulas terminated within or very close to the targeted brain sites in all animals reported, as was verified by the spread of diffusion of India ink. Injection sites that were verified as being very close to the targeted sites produced behavioral responses that were very similar to those for injections that terminated within the targeted areas. These findings were interpreted to suggest that the spread of diffusion of the pharmacological agents tested may have exceeded that of the India ink and/or that areas immediately boardering the targeted sites are similarly sensitive to these pharmacological manipulations. On a few occasions two or more animals from the same litter were implanted with cannulas that were verified as being far from the targeted injection site and thus results for these

Figure 3. Histological verification of injection sites as determined by the spread of diffusion of India ink. Injections into the PVN (●) and ventral third ventricle (◉) are shown in 3-a, those in the anterolateral hypothalamus (●) in 3-b and PFH (●) in 3-c. A-P (anterior-posterior) stereotaxic coordinates relative to bregma are reported for each as a function of age of the pup implanted. AH = anterior nucleus of the hypothalamus, DM = dorsomedial nucleus of the hypothalamus, F = fornix, PVN = paraventricular nucleus, V = ventral third ventricle, VM = ventromedial nucleus of the hypothalamus.



litters are not reported.

The majority of the cannulas targeted for the PVN (182/199) terminated within the dorsal (wing) portion of the nucleus (Figure 3-a) and there was no indication of spread of India ink into the third ventricle for any PVN injections. Other PVN cannuals (17/199) terminated lateral to the targeted PVN site reported, immediately lateral to the boundary of the nucleus. Cannulas targeted for the ventral third ventricle (32) terminated at approximately the same dorsal-ventral level as PVN injections ($\pm$0.1 mm) and spread of India ink was evident throughout the ventricular system. The majority of the cannulas targeted for the PFH (78/84) terminated immediately lateral to the fornix at the anterior-posterior level of the ventromedial nucleus of the hypothalamus (Figure 3-c). Other PFH cannulas (6/84) terminated slightly (0.1 to 0.2 mm) dorsal or ventral to the PFH site reported. Cannulas targeted for the anterolateral hypothalamus (16) terminated dorsolateral to the anterior nucleus of the hypothalamus, immediately posterior (0.1 to 0.2 mm) to the targeted PVN site reported (Figure 3-b).

Statistical Evaluation.

Litters were regarded as experimental units throughout all investigations and analyses. A litter was tested at one age only with pups from one litter representing a given experimental unit. A different pup from a single litter was used in each drug and vehicle (control) condition. Pups

were randomly assigned to vehicle and drug conditions at the time they were removed from their mother on the day prior to testing.

Statistical analyses were performed on weight gain in grams and on its conversion to a percentage of post-satiation or post-deprivation weight for the data of each investigation in Experiments 1-3. Appropriate one-way analyses of variance, or two- or three-way factorial analyses of variance were used to evaluate the data of each experiment. Subsequently, post hoc comparisons of drug to control conditions were made using Dunnett's Test. The lowest doses tested at the earlier ages in Experiments 2 (First Investigation) and 3 (Second Investigation) were compared to control levels using Student's T-test. These two exceptions in post hoc comparisons were permitted to account for the fact that an additional dose was tested at the earlier ages in the two investigations cited.

Experiment 1: Pharmaco-ontogeny of Alpha-noradrenergic Receptors

This initial experiment was conducted to determine the age in development at which the rat first responds, by changes in independent ingestion of milk, to injections of NE into the PVN. As reviewed in the General Introduction, NE in the medial hypothalamus or PVN has been demonstrated to enhance feeding in adult rats through interaction with alpha-noradrenergic receptors. In light of evidence in

adult rats that NE also stimulates ingestion of water (Leibowitz, 1978b; Roland, Oppenheimer, Chang & Leibowitz, 1985), NE's effect on water intake was similarly examined. In an attempt to identify the PVN as a primary site for eliciting NE's effect on milk intake, a subsequent investigation of NE's effect in the adjacently medial ventral third ventricle (Figure 3-a) was undertaken for comparison. Finally, the effect of the α_2-noradrenergic receptor agonist clonidine was assessed in an attempt to identify the receptor subtype within the PVN responsible for mediating NE's effect on independent ingestion of milk in rat pups.

Subjects and Procedure.

A total of 161 rat pups from 36 litters were tested in this first experiment. As reported by Ellis et al. (1984), pups taken directly from their mothers can ingest large volumes of milk when it is offered via an intra-oral cannula. Therefore, all pups in the present experiment were sated prior to injection, to prevent large spontaneous intake from obscuring the effects of the neurochemical agents tested. Furthermore, all pups were sated with the diet that they were to be tested for (i.e., milk or water).

In the first of four investigations, the effect of NE (0.01, 0.1, 1.0, 10.0, 20.0, 40.0 or 60.0 nmol) in the PVN on milk intake was assessed in 2-, 7- and 15-day-old milk-sated pups (n = 75). In a second investigation, the effect

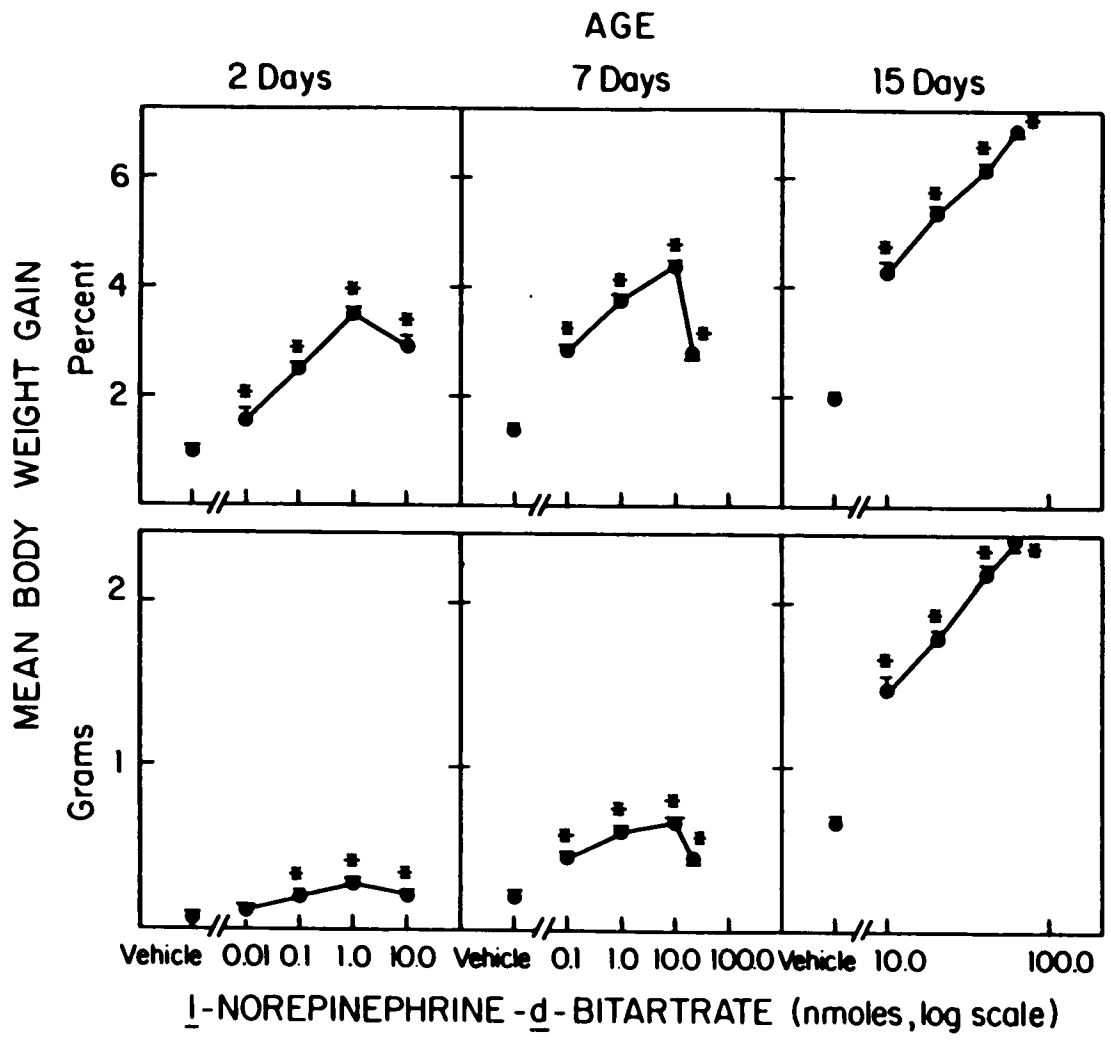
of NE (1.0 or 60.0 nmol) in the PVN on water intake was examined in 2- and 15-day-old water-sated pups (n = 16). In a third investigation, the effect of NE (0.1, 1.0, 10.0, 30.0 or 100.0 nmol) in the ventral third ventricle on milk intake was assessed in 2-day-old milk-sated pups (n = 32). In a fourth and final investigation, the effect of clonidine (0.01, 0.1 or 1.0 nmol) in the PVN on milk intake was examined in 2-day-old milk-sated pups (n = 20). As described in the Testing Procedure section of the General Methods and Materials, for each litter tested, each pup received either a single injection of one dose of the neurochemical agent or an equal volume (0.1 ul) of the vehicle.

Results and Discussion.

The results of Experiment 1 are presented in Table 3 and Figures 4-7.

First Investigation. Figure 4 shows weight gain from milk ingested in the initial investigation as a function of age and of dose of NE in the PVN. NE stimulated milk intake (as measured by weight gain in grams or its conversion to a percentage of post-satiation weight) at 2, 7 and 15 days of age. Two-way factorial analyses of weight gain (Age x Dose) indicated a significant age-effect, $F(2,12)=1190.24, p<.001$ in grams & $F(2,12)=252.73, p<.001$ as percent; dose-effect, $F(4,48)=420.01, p<.001$ in grams & $F(4,48)=709.36, p<.001$ as percent; and age-by-dose interaction, $F(8,48)=186.30, p<.001$

Figure 4. NE's effect on milk intake of sated pups as a function of age and of dose in the PVN. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-satiation weight. The error bars represent one standard error of the mean. * = $p<.01$ relative to vehicle by Dunnett's Test.


AGE
2 Days
7 Days
15 Days
MEAN BODY WEIGHT GAIN
Percent
Grams
6
4
2
2
1
Vehicle 0.01 0.1 1.0 10.0 Vehicle 0.1 1.0 10.0 100.0 Vehicle 10.0 100.0
l-NOREPINEPHRINE-d-BITARTRATE (nmoles, log scale)

in grams & $F(8,48)=76.92, p<.001$ as percent. Dunnett's post hoc comparisons revealed significant differences ($p<.01$) between the drug and control conditions for weight gain in grams and as percent post-satiation weight across all ages tested, with the exception of the lowest dose (0.01 nmol) in grams at 2 days of age ($p>.05$).

NE in the PVN enhanced milk intake in a dose-dependent mannner at all ages tested with an apparent decrease in effect at 2 and 7 days of age for the highest doses tested (10.0 & 20.0 nmol respectively). Behavioral observations indicated that at these younger ages, these higher doses produced non-appetitive behavioral activation, including squealing. Similar findings have also been observed with high doses of NE in the adult rat (Leibowitz, 1975a). Locomotor hyperactivity has also been previously reported by stimulating central α_2-noradrenergic receptors with clonidine in 7-day-old rat pups (Nomura & Segawa, 1979). Based on these findings, it seems likely that the activation produced by high doses of NE in the present investigation may have disrupted the formation of milk pools in the mouths of these young pups, thereby decreasing NE's effect on milk intake at these higher dose levels. Nevertheless, these data indicate that NE-receptive sites in the PVN that mediate enhancement of milk intake by NE in sated rat pups are functionally mature as early as 2 days postpartum.

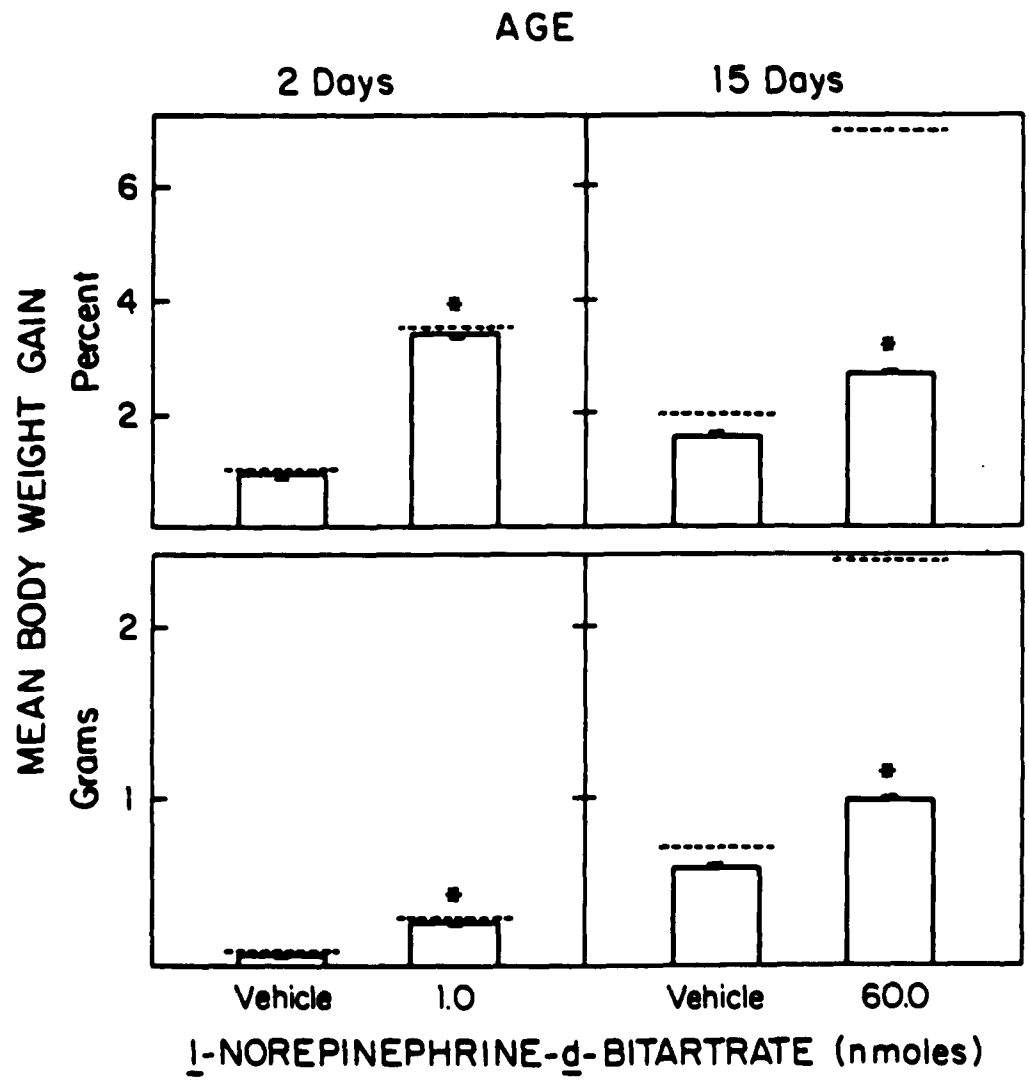
Second Investigation. Weight gain from water ingested in the second investigation as a function of age and of dose

of NE in the PVN is depicted in Figure 5. Superimposed for comparison (---) are the corresponding levels for milk intake from the initial investigation (Figure 4). NE stimulated water intake (as measured by weight gain in grams or its conversion to a percentage of post-satiation weight) at 2 and 15 days of age. Two-way factorial analyses of weight gain (Age x Drug) indicated a significant age-effect in grams only, $F(1,6)=1744.71, p<.001$ in grams & $F(1,6)=0.07, p>.05$ as percent; a significant drug-effect, $F(1,6)=320.81, p<.001$ in grams & $F(1,6)=405.73, p<.001$ as percent; and age-by-drug interaction, $F(1,6)=37.52, p<.001$ in grams & $F(1,6)=57.33, p<.001$ as percent. Dunnett's post hoc comparisons revealed significant differences ($p<.01$) between the drug and control conditions for weight gain in grams and as percent post-satiation weight at both ages tested.

Close examinatiion of percent weight gain at 2 and 15 days for water intake indicates a slight increase over age for control animals and a slight decrease for drugged animals, as compared to increases in milk intake for both groups over age. The discrepancy in direction across groups for water intake more than likely accounted for the lack of a significant age-effect obtained for weight gain analyzed as percent post-satiation weight.

In comparing the data for water intake to that for milk, it is evident that, at 2 days of age, NE in the PVN enhanced water equally as well as milk intake. This finding is consistent with previously reported data indicating that

Figure 5. NE's effect on water intake of sated pups as a function of age and of dose in the PVN. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-satiation weight. The error bars represent one standard error of the mean. Superimposed for comparison (---) are the corresponding levels for milk intake from Figure 4. * = $p<.01$ relative to vehicle by Dunnett's Test.


AGE
2 Days
15 Days
MEAN BODY WEIGHT GAIN
Percent
6
4
2
Grams
2
1
Vehicle
1.0
Vehicle
60.0
l-NOREPINEPHRINE-d-BITARTRATE (nmoles)

baby rats do not discriminate or differentiate between milk and water until about 8 days of age (Ellis et al., 1984), although evidence exists in prenatal rats suggesting that they are capable of this task prior to birth, given a different paradigm (Smotherman & Robinson, in press). More specifically, it was demonstrated by Ellis et al. (1984) that when non-drugged, deprived rat pups prior to 8 days are offered milk or water, they consume approximately equal amounts of both. Furthermore, the same study reported that when drugs (i.e., angiotensin II) that have specific dipsogenic effects in adults are administered intracerebroventricularly to pups, they produce approximately equal effects on water and milk intake prior to 8 days. Thus, the present finding of equal milk and water ingestion induced by NE in the PVN at 2 days of age supports a previously documented phenomenon, although Ellis et al. (1984) failed to demonstrate an increase in water intake with NE at any age. Collectively, these findings suggest that the ingestive response of both non-drugged and drugged rat pups prior to 8 days of age appears to be more consummatory in nature rather than appetitive. Appetitive meaning more specific to one diet (i.e., milk or water) than the other.

In further support of the early consummatory-type response of the neonatal rat, pilot data from 2-day-old pups that were tested for water intake, but cross-sated with milk, indicated similar findings. Weight gain as percent

post-satiation weight for these animals and also for their littermates, who were sated with and tested for water (Figure 5), are presented in Table 3. Comparison of the data indicates that, as a group, non-drugged pups consumed approximately equal amounts of water regardless of whether they were sated with milk or water. Comparison of drugged pups indicates the same. Histology for the cross-sated pups was not reported earlier, but all cannulas targeted for the PVN (8/8) terminated within the dorsal (wing) portion of the nucleus (see Figure 3-a) and there was no indication of spread of India ink into the ventral third ventricle for any of these injections.

Comparison of the data for water and milk intake at 15 days indicates that NE in the PVN produced a greater enhancement of milk than water intake at this age. This finding is also consistent with the developmental (Ellis et al., 1984) and adult (Grossman, 1960; Leibowitz, 1978a, 1978b; Roland et al., 1985) literature in suggesting that as pups approach the onset of weaning they begin to consume more milk (food) than water in both non-drugged and drugged states. More importantly, these findings suggest that, in discriminating or differentiating between milk and water, the ingestive response of the rat pup becomes more appetitive in nature as it approaches weaning.

Third Investigation. Figure 6 shows weight gain from milk ingested in the third investigation as a function of dose of NE in the ventral third ventricle of 2-day-old pups.

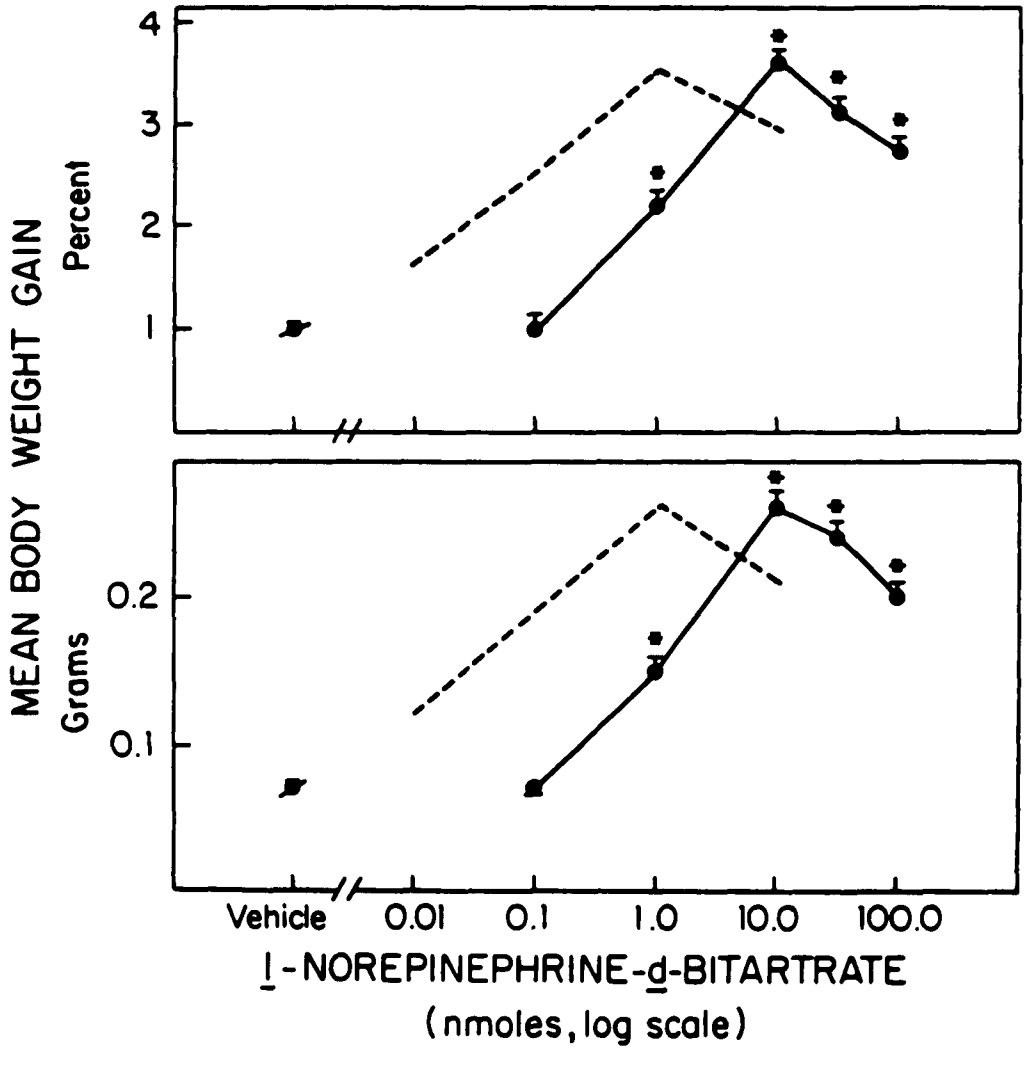
Table 3

Effect of Norepinephrine in the Hypothalamic Paraventricular Nucleus on Water Intake of Water- and Milk-Sated 2-Day-Olds

	Water-Sated	Milk-Sated
Vehicle	0.9±0.1	1.1±0.1
l-Norepinephrine-d-Bitartrate (1.0 nmol)	3.4±0.1	3.3±0.1

Values represent mean ± SEM body weight gain and are depicted as percent post-satiation weight. Values for water-sated pups are re-presented from Figure 5 for comparison to cross-sated littermates.

Figure 6. NE's effect on milk intake of sated 2-day-old pups as a fuction of dose in the ventral third ventricle. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-satiation weight. The error bars represent one standard error of the mean. Superimposed for comparison (---) are the corresponding curves for NE's effect in the PVN on milk intake from Figure 4. * = $p<.01$ relative to vehicle by Dunnett's Test.


MEAN BODY WEIGHT GAIN
Percent
Grams
4
3
2
1
0.2
0.1
Vehicle
0.01
0.1
1.0
10.0
100.0
l-NOREPINEPHRINE-d-BITARTRATE
(nmoles, log scale)

Superimposed for comparison (---) are the curves from the initial investigation (Figure 4) for NE's effect in the PVN on milk intake of 2-day-olds. The data reported in Figure 6 for NE in the ventricle were pooled from two sub-investigations. The first examined the effects of NE in the ventricle at doses of 0.0, 0.1, 1.0 and 10.0 nmol. The second similarly, at 0.0, 10.0, 30.0 and 100.0 nmol. The data of both sub-investigations indicated that ventricular injections of NE stimulated milk intake (as measured by weight gain in grams or its conversion to a percentage of post-satiation weight) at 2 days of age. One-way analyses of weight gain for both sets of data indicated significant dose-effects; first set of data, $F(3,9)=231.26, p<.001$ in grams & $F(3,9)=248.54, p<.001$ as percent; second set of data, $F(3,9)=159.51, p<.001$ in grams & $F(3,9)=248.54, p<.001$ as percent. Dunnett's post hoc comparisons revealed significant differences ($p<.01$) between the drug and control conditions for weight gain in grams and as percent post-satiation weight, with the exception of the lowest dose (0.1 nmol) for both measures ($p>.05$).

Similar to NE in the PVN, NE in the ventral third ventricle enhanced milk intake in a dose-dependent manner at 2 days of age with an apparent decrease in effect for the highest doses tested (30.0 & 100.0 nmol). Moreover, behavioral observations suggested that, for reasons indicated earlier, these higher doses in the ventricle produced non-appetitive behavioral activation that probably

disrupted feeding through intra-oral cannulas and thus decreased NE's effect on milk intake at these higher dose levels.

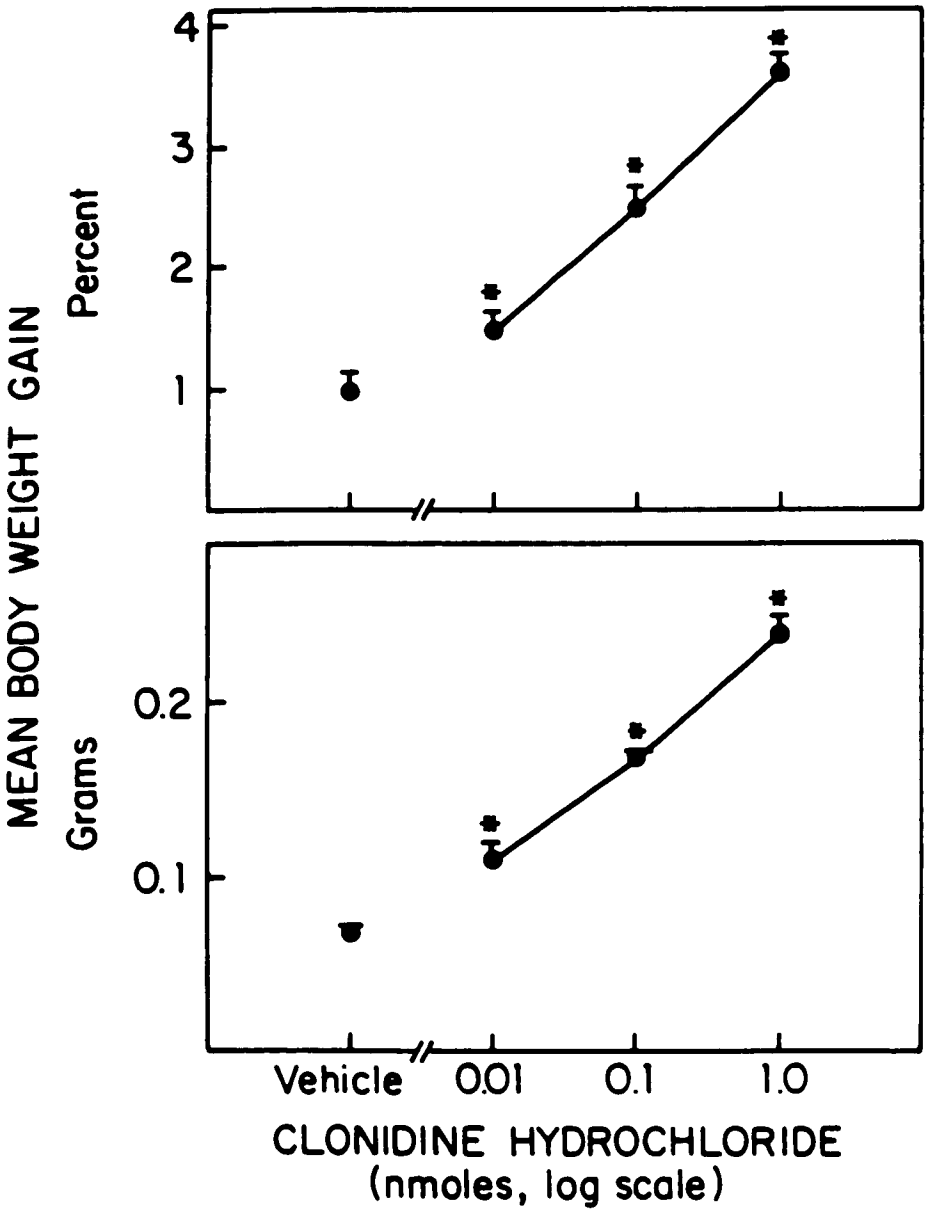
In the first investigation (Figure 4), the PVN was demonstrated to be sensitive to noradrenergic activation of feeding in 2-day-old pups, as it is in adult rats (Leibowitz, 1978a). When considering that the PVN lies on either side of the ventral third ventricle, it is not suprising that NE injected directly iuto the ventricle produces the same effect. In fact, comparison of the dose-response curves for the ventricle and PVN at 2 days of age (Figure 6) indicates that they are parallel to one another, with a rightward shift in the curve for NE in the ventricle. A dose of 0.1 nmol of NE, that produced a significant enhancement of milk intake when injected into the PVN, had no effect on milk intake when administered into the ventricle. Furthermore, 1.0 nmol of NE in the PVN produced an enhancement equal to 10.0 nmol in the ventricle and 10.0 nmol in the PVN showed diminished effectiveness similar to 100.0 nmol in the ventricle. Together, these comparisons suggest that there is approximately a 10-fold dilution factor for injections into the ventral third ventricle and, more importantly, that in rat pups as young as 2 days of age the PVN appears to be a primary site for noradrenergic stimulation of independent feeding.

Fourth Investigation. For the fourth and final investigation, weight gain from milk ingested as a function

of dose of clonidine in the PVN of 2-day-old pups is depicted in Figure 7. Clonidine stimulated milk intake (as measured by weight gain in grams or its conversion to a percentage of post-satiation weight) at 2 days of age. One-way analyses of weight gain indicated a significant dose-effect, $F(3,12)=131.86, p<.001$ in grams & $F(3,12)=159.14, p<.001$ as percent. Dunnett's post hoc comparisons revealed significant differences ($p<.01$) between the drug and control conditions for weight gain in grams and as percent post-satiation weight.

Like NE (Figure 4), clonidine, a relatively selective α_2-receptor agonist, enhanced milk intake in a dose-dependent manner in the PVN as early as 2 days of age. Coupled with the recent finding that in adult rats the stimulation of feeding by NE in the region of the PVN is also mediated through α_2-type receptors and mimicked by clonidine (Goldman et al., 1985), these results suggest that α_2-noradrenergic receptors in the PVN that mediate noradrenergic stimulation of feeding in rats are functionally mature very early in development. In addition, the present findings support the view that the neural mechanism in the PVN for noradrenergic regulation of independent feeding in neonatal rats is an underlying developmental precursor for control of subsequent adult feeding.

Figure 7. Clonidine's effect on milk intake of sated 2-day-old pups as a function of dose in the PVN. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-satiation weight. The error bars represent one standard error of the mean. * = $p<.01$ relative to vehicle by Dunnett's Test.


MEAN BODY WEIGHT GAIN
Percent
Grams
4
3
2
1
0.2
0.1
Vehicle
0.01
0.1
1.0
CLONIDINE HYDROCHLORIDE
(nmoles, log scale)

Experiment 2: Pharmaco-ontogeny of Beta-adrenergic and Dopaminergic Receptors

This experiment was undertaken to determine the age in development at which the rat first responds, by changes in independent ingestion of milk, to injecions of EPI into the PFH. As reviewed in the General Introduction, EPI in the lateral hypothalamus or PFH, in contrast to NE in the PVN, has been demonstrated to suppress feeding in adult rats through interaction with beta-adrenergic receptors. In an attempt to identify the PFH as a primary site for eliciting EPI's effect on milk intake, a subsequent investigation of EPI's effect in the anterolateral hypothalamus, a nearby but more rostral site, was conducted for comparison. Next, the effect of the β_2-adrenergic receptor agonist salbutamol was assessed in an attempt to identify the receptor subtype responsible for mediating EPI's effect in the PFH on independent ingestion of milk. A fourth and final investigation examining the effect of the dopaminergic receptor agonist apomorphine was conducted in an attempt to reveal the age of functional maturity of the dopaminergic receptor in the PFH responsible for mediating DA's suppressive effect on independent ingestion of milk.

Subjects and Procedure.

A total of 100 rat pups from 24 litters were tested in this second experiment. Upon removal from their mothers, all pups were deprived of food (milk) until the time of

testing. The 22 hr deprivation period was carried out to prevent satiety from obscuring the effects of the neurochemical agents tested. All pups were tested for milk intake in all investigations of Experiment 2.

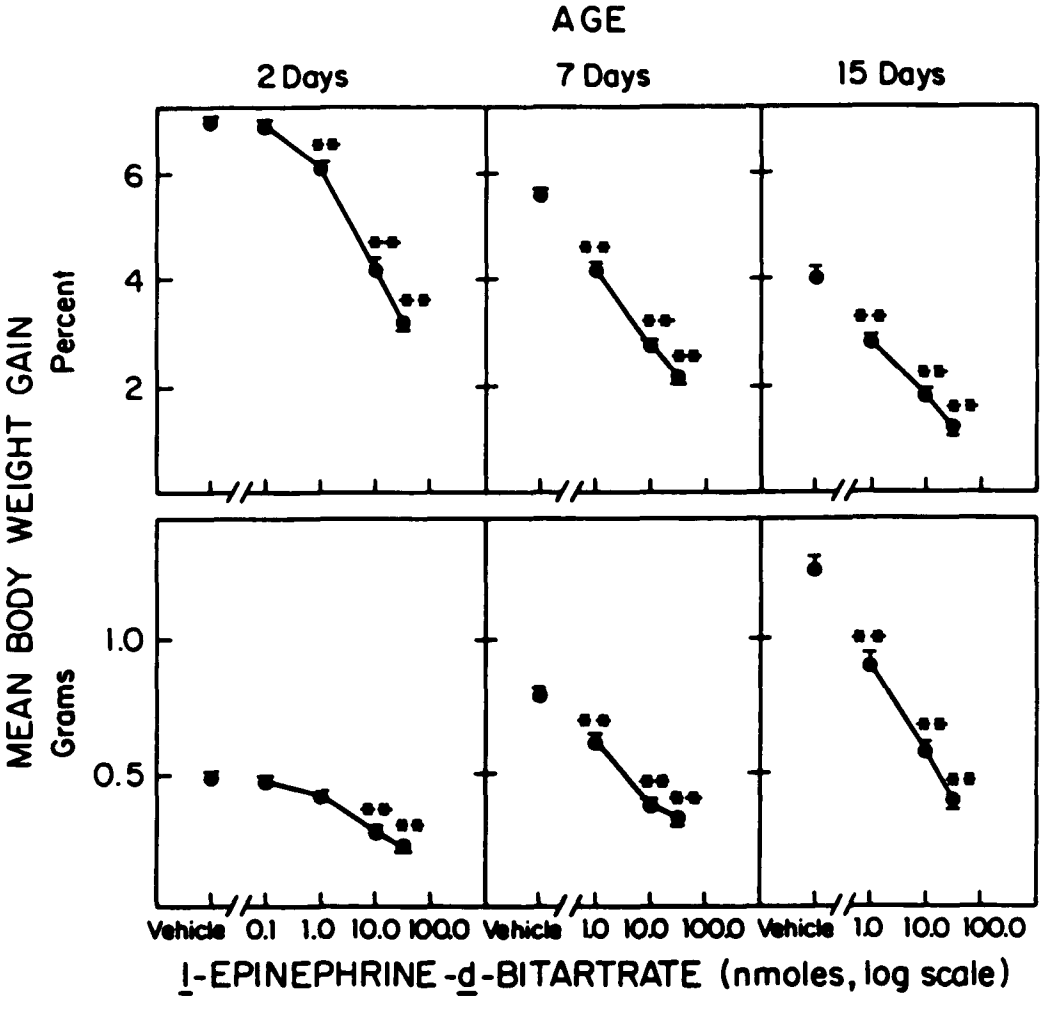
In the first of four investigations, the effect of EPI (0.1, 1.0, 10.0 or 30.0 nmol) in the PFH on milk intake was assessed in 2-, 7- and 15-day-old milk-deprived pups (n = 52). In a second investigation, the effect of EPI (0.1, 1.0 or 10.0 nmol) in the anterolateral hypothalamus on milk intake was examined in 2-day-old milk-deprived pups (n = 16). In the third and final investigations, the effects of salbutamol and apomorphine (1.0, 10.0 or 30.0 nmol) in the PFH on milk intake were assessed in 2-day-old milk-deprived pups respectively (n = 16 & 16 respectively). As for Experiment 1, for each litter tested, each pup received either a single injection of one dose of the neurochemical agent or an equal volume (0.1 ul) of the vehicle.

Results and Discussion.

The results of Experiment 2 are presented in Table 4 and Figures 8 and 9.

First Investigation. Figure 8 shows weight gain from milk ingested in the first investigation as a function of age and of dose of EPI in the PFH. EPI suppressed milk intake (as measured by weight gain in grams or its conversion to a percentage of post-deprivation weight) at 2, 7 and 15 days of age. Two-way factorial analyses of weight

Figure 8. EPI's effect on milk intake of deprived pups as a fucntion of age and of dose in the PFH. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-deprivation weight. The error bars represent one standard error of the mean. ** = $p<.01$ relative to vehicle by Dunnett's Test.


AGE
2 Days
7 Days
15 Days
MEAN BODY WEIGHT GAIN
Percent
Grams
6
4
2
1.0
0.5
Vehicle 0.1 1.0 10.0 100.0 Vehicle 1.0 10.0 100.0 Vehicle 1.0 10.0 100.0
l-EPINEPHRINE-d-BITARTRATE (nmoles, log scale)

gain (Age x Dose) indicated a significant age-effect, $F(2,9)=96.28, p<.001$ in grams & $F(2,9)=330.03, p<.001$ as percent; dose-effect, $F(3,27)=219.22, p<.001$ in grams & $F(3,27)=430.67, p<.001$ as percent; and age-by-dose interaction, $F(6,27)=22.10, p<001$ in grams & $F(6,27)=7.10, p<.001$ as percent. Dunnett's post hoc comparisons revealed significant differences ($p<.01$) between the drug and control conditions for weight gain in grams and as percent post-deprivation weight across all ages tested, with the exception of the second dose (1.0 nmol) in grams at 2 days of age ($p>.05$). Comparison of the lowest dose (0.1 nmol) to the control by Student's T-test revealed no significant difference ($p>.05$) for both measures.

EPI in the PFH suppressed milk intake in a dose-dependent manner at all ages tested. In comparing the dose-response curve obtained for the suppressive effect of EPI on feeding in the present investigation to that for the enhancing effect of NE on feeding in the first experiment (Figure 4) at 2 days of age, it is apparent that the lowest effective dose (1.0 nmol, as percent) for suppression of milk intake by EPI in the PFH is two orders of magnitude higher than the lowest effective dose (0.01 nmol, as percent) for enhancement of milk intake by NE in the PVN. This finding is consistent with the adult literature (Leibowitz, 1982) in suggesting that higher doses of EPI are required to produce a suppression of feeding in the PFH than are needed to produce an enhancement of feeding in the PVN

with NE. This rightward shift in the dose-response curve for EPI may be interpreted to suggest that EPI is not as potent in suppressing feeding in the PFH as NE is in enhancing feeding in the PVN. Alternatively, within the dose range tested in the present experiments, it may be that NE is more specific to NE-receptive sites in the PVN than EPI is to EPI-receptive sites in the PFH. Nevertheless, the results of this first investigation of Experiment 2 indicate that EPI-receptive sites in the PFH that mediate suppression of milk intake in deprived rat pups are functionally mature as early as 2 days postpartum.

Second Investigation. Weight gain from milk ingested by 2-day-old pups in the second investigation, as a function of dose of EPI in the anterolateral hypothalamus, is reported in Table 4. EPI failed to suppress milk intake (as measured by weight gain in grams or its conversion to a percentage of post-deprivation weight) in the anterolateral hypothalamus at 2 days of age. In fact, EPI enhanced milk intake at the highest dose tested (10.0 nmol) for weight gain reported as percent post-deprivation weight. One-way analyses of weight gain indicated no significant dose-effect in grams, $F(3,9)=2.18, p>.05$, but a significant dose-effect as percent, $F(3,9)=9.55, p<.01$. Dunnett's post hoc comparisons for weight gain as percent post-deprivation weight revealed no significant differences ($p>.05$) between the two lower drug (0.1 & 1.0 nmol) and control conditions, but a significant difference ($p<.01$) for comparison between

Table 4

Effect of Epinephrine in the Anterolateral Hypothalamus on Milk Intake of Deprived 2-Day-Olds

Mean Body Weight Gain	Vehicle	l-Epinephrine-d-Bitartrate (nmoles) 0.1	1.0	10.0
Grams	0.48±0.01	0.46±0.01	0.46±0.01	0.50±0.01
Percent	6.9±0.1	7.0±0.2	7.0±0.1	7.4±0.1**

Data are presented as mean ± SEM. ** = $p < .01$ relative to vehicle by Dunnett's Test.

the highest drug (10.0 nmol) and control conditions.

These results indicate that EPI in the anterolateral hypothalamus does not suppress milk intake in deprived rat pups as it does in the more caudal PFH (Figure 8). This finding of differential sensitivity is consistent with mapping studies in adult rats (Leibowitz, 1970a, 1970b, 1975b; Leibowitz & Rossakis, 1978b, 1979a), suggesting that the PFH is a brain area specifically involved in mediating suppression of feeding by EPI in both the neonatal and adult rat.

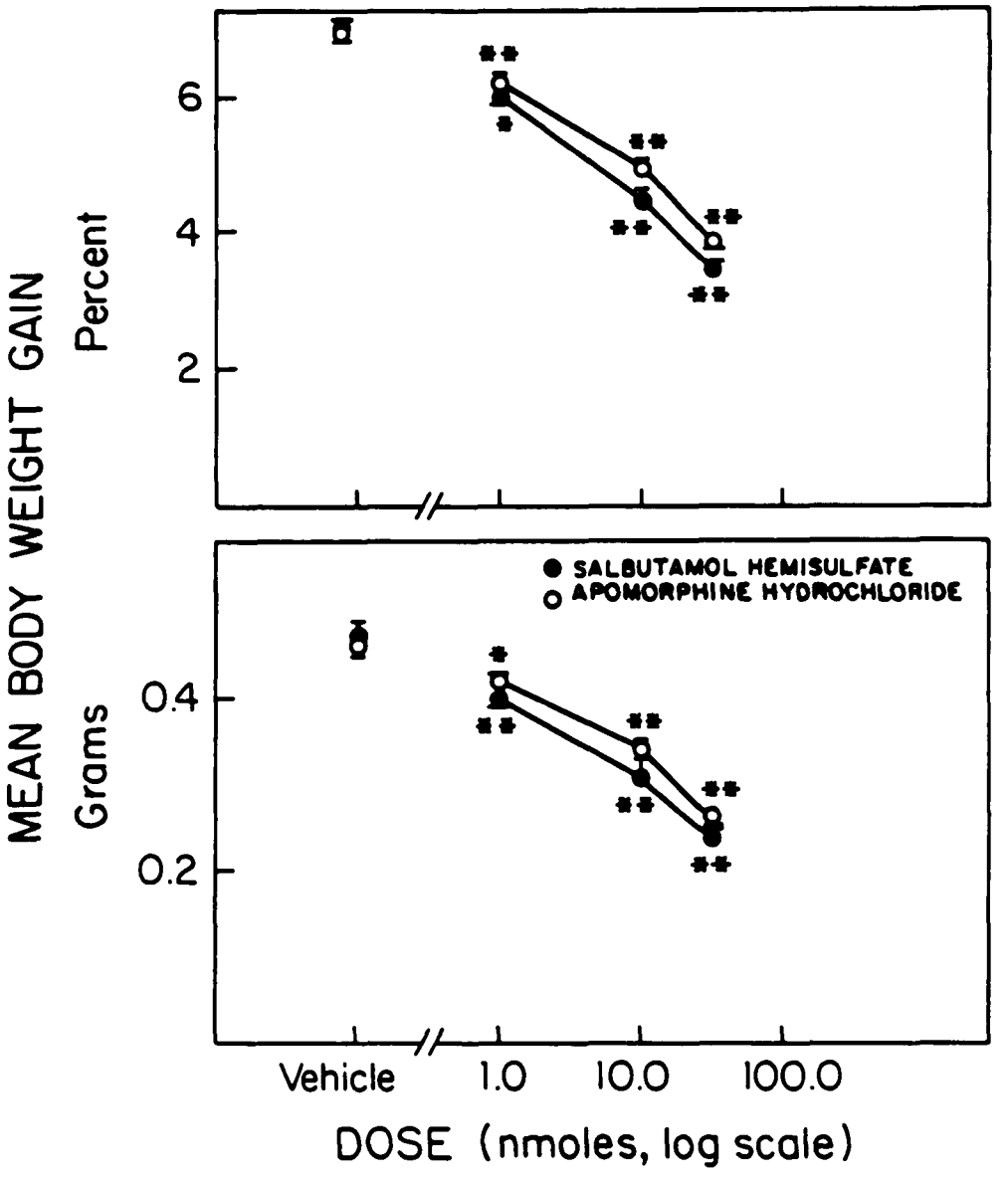
When considering that this anterolateral injection site in the 2-day-old pup is only 0.1 to 0.2 mm posterior, 0.5 mm lateral and 0.2 mm ventral to the PVN injection site tested at this age in Experiment 1 (see Table 1 & Figure 3 for comparison), it is not suprising that EPI produced an enhancement of milk intake at the highest dose tested (10.0 nmol) in the present investigation. The spread of diffusion of India ink for injections in the anterolateral hypothalamus appeared to come within 0.2 mm of the PVN, suggesting that the spread of EPI may have come even closer to, if not in contact with, the PVN. It is also likely that areas near to the PVN are sensitive to adrenergic agonists as well as noradrenergic agonists in mediating enhancement of milk intake in neonatal rats. This latter inference is consistent with findings in adult rats (Leibowitz, 1982) that have indicated that although the PVN is the primary site for noradrenergic and/or adrenergic enhancement of

feeding, other nearby hypothalamic areas, such as the anterior and ventromedial nuclei, are also sensitive to the stimulatory effects of these neurochemical agents.

Third and Fourth Investigations. Figure 9 shows weight gain in 2-day-old pups from milk ingested in the third and final investigations as a function of dose of salbutamol and apomorphine in the PFH respectively. Both salbutamol and apomorphine suppressed milk intake (as measured by weight gain in grams or its conversion to a percentage of post-deprivation weight) at 2 days of age. One-way analyses of weight gain indicated a significant dose-effect for both neurochemical agents; salbutamol, $F(3,9)=67.96, p<.001$ in grams & $F(3,9)=214.68, p<.001$ as percent; apomorphine, $F(3,9)=98.90, p<.001$ in grams & $F(3,9)=127.45, p<.001$ as percent. Dunnett's post hoc comparisons revealed significant differences ($p<.01$) between the drug and control conditions for weight gain in grams and as percent post-deprivation weight for salbutamol and apomorphine, with comparison of the low dose of apomorphine (1.0 nmol) in grams significant at the $p<.05$ level.

Similar to EPI (Figure 8), both salbutamol, a β_2-receptor agonist, and apomorphine, a dopaminergic receptor agonist, suppressed milk intake in a dose-dependent manner in the PFH as early as 2 days of age. Comparison of the dose-response curves for salbutamol and apomorphine indicates that within the dose range tested in the PFH salbutamol produced a greater suppression of milk intake at

Figure 9. The effects of salbutamol and apomorphine on milk intake of deprived 2-day-old pups as a function of dose in the PFH. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-deprivation weight. The error bars represent one standard error of the mean. * = $p<.05$ & ** = $p<.01$ relative to vehicle by Dunnett's Test.


MEAN BODY WEIGHT GAIN
Percent
6
4
2
Grams
0.4
0.2
SALBUTAMOL HEMISULFATE
APOMORPHINE HYDROCHLORIDE
Vehicle
1.0
10.0
100.0
DOSE (nmoles, log scale)

2 days of age than did apomorphine. As speculated earlier for similar comparison of EPI's and NE's effect in the PFH and PVN respectively, this result may be more a question of specificity for the receptor site than a question of potency.

Together, the present results support findings in adult rats that EPI-sensitive sites in the PFH that suppress feeding have specific characteristics of β_2-adrenergic receptors (Leibowitz & Rossakis, 1978a) and that DA-sensitive sites in the PFH that also suppress feeding are closely associated with functionally similar β_2-adrenergic receptors (Leibowitz & Rossakis, 1979a, 1979b). In addition, the present results suggest that β_2-adrenergic and dopaminergic receptors in the PFH that mediate adrenergic and dopaminergic suppression of feeding in rats are functionally mature very early in development, as early as 2 days postpartum. Furthermore, the present findings support the view that the neural mechanisms in the PFH for adrenergic and dopaminergic regulation of independent feeding in neonatal rats appear to be underlying developmental precursors for regulation of subsequent adult feeding.

Experiment 3: Pharmaco-ontogeny of Cholecystokinin and Neuropeptide Y Receptors

This third and final experiment was conducted to determine the age in development at which the rat first

responds, by changes in independent ingestion of milk, to injections of CCK and NPY into the PVN. As reviewed in the General Introduction, CCK in the PVN has been demonstrated to suppress feeding in adult rats, while NPY was shown to produce the most dramatic increase in feeding reported to date. In the initial investigation, the effect of CCK on milk intake of deprived pups was assessed. In the second investigation, the effect of NPY on milk and water intake of sated pups was examined. As for NE, NPY's effect on water intake was assessed in light of evidence in adult rats that NPY in the PVN and other hypothalamic sites stimulates water as well as food intake (Stanley et al., 1985).

Subjects and Procedure.

A total of 88 rat pups from 21 litters were tested in this final experiment. Upon removal from their mothers, pups were deprived and sated for CCK and NPY investigations respectively, for reasons previously described in Experiments 2 and 1 respectively. Pups were deprived of milk in tests for CCK's effect and sated with the diet that they were to be tested for (i.e., milk or water) in tests for NPY's effect.

In the first investigation, the effect of CCK in the PVN (0.9, 8.8 or 26.2 pmol) on milk intake was assessed in 2-, 7-, 12- and 15-day-old milk-deprived pups (n = 52). In the second investigation, the effect of NPY in the PVN (23.5, 70.5 or 235.0 pmol) on milk and water intake was

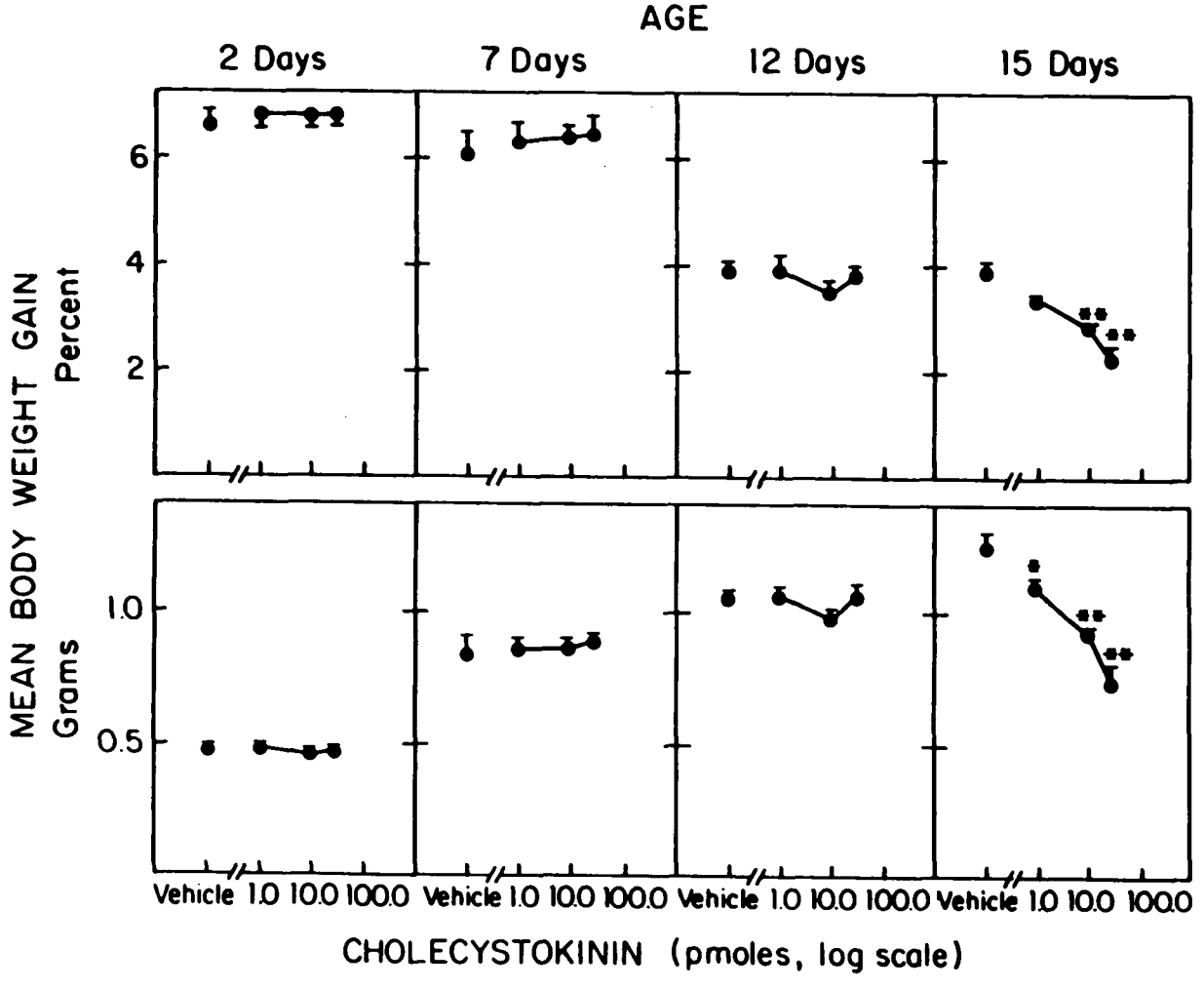
examined in 2- and 15-day-old milk- and water-sated pups (n = 36). As for Experiments 1 and 2, for each litter tested, each pup received either a single injection of one dose of the neurochemical agent or an equal volume (0.1 ul) of the vehicle.

Results and Discussion.

The results of Experiment 3 are presented in Figures 10 and 11.

First Investigation. Figure 10 shows weight gain from milk ingested in the first investigation as a function of age and of dose of CCK in the PVN. CCK, at doses known to be effective in adult rats (Faris & Olney, 1985), had no effect on milk intake (as measured by weight gain in grams or its conversion to a percentage of post-deprivation weight) at 2, 7 and 12 days of age, but suppressed milk intake at 15 days of age. Two-way factorial analyses of weight gain (Age x Dose) indicated a significant age-effect, $F(3,9)=50.78, p<.001$ in grams & $F(3,9)=107.96, p<.001$ as percent; dose-effect in grams only, $F(3,27)=8.09, p<.001$ in grams & $F(3,27)=1.90, p>.05$ as percent; and significant age-by-dose interaction, $F(9,27)=10.72, p<.001$ in grams & $F(9,27)=4.12, p<.01$ as percent. Dunnett's post hoc comparisons revealed significant differences between the drug and control conditions for weight gain in 15-day-old pups only. Results for individual weight gain comparisons at 15 days were as follows: CCK (0.9 pmol) vs. control,

Figure 10. CCK's effect on milk intake of deprived pups as a function of age and of dose in the PVN. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-deprivation weight. The error bars represent one standard error of the mean. * = $p<.05$ & ** = $p<.01$ relative to vehicle by Dunnett's Test.


AGE
2 Days
7 Days
12 Days
15 Days
MEAN BODY WEIGHT GAIN
Percent
Grams
6
4
2
1.0
0.5
Vehicle 1.0 10.0 100.0 Vehicle 1.0 10.0 100.0 Vehicle 1.0 10.0 100.0 Vehicle 1.0 10.0 100.0
CHOLECYSTOKININ (pmoles, log scale)

$p<.05$ in grams & $p>.05$ as percent post-deprivation weight; CCK (8.8 pmol) vs. control, $p<.01$ for both measures; and CCK (26.2 pmol) vs. control, $p<.01$ for both measures. All other post hoc comparisons at 2, 7 and 12 days of age revealed no significant differences ($p>.05$) for both measures.

Although a significant dose-effect resulted for weight gain analyzed in grams, the lack of such an effect for weight gain analyzed as percent post-deprivation weight may be partially attributed to the lack of and/or inconsistency in dose-response trends at ages tested prior to 15 days. Furthermore, the absence of a significant difference for the post hoc comparison of CCK (0.9 pmol, as percent) versus the control, as was not the case for this comparison in grams, most definately accounted for the lack of a significant dose-effect for weight gain analyzed as percent post-deprivation weight. Despite this small discrepancy in analyses for dose-effect, a significant age-by-dose interaction did result for weight gain analyzed in grams and as percent post-deprivation weight.

In comparing the onset of CCK's effect in the PVN to that of EPI's in the PFH (Figure 8), two conclusions are apparent. First, although the action of both neurochemical agents is a suppression of independent ingestion of milk in deprived pups, it appears that EPI-receptive sites are functional very early in development, whereas CCK-receptive sites, at least in terms of their behavioral response, remain non-functional until very late in development.

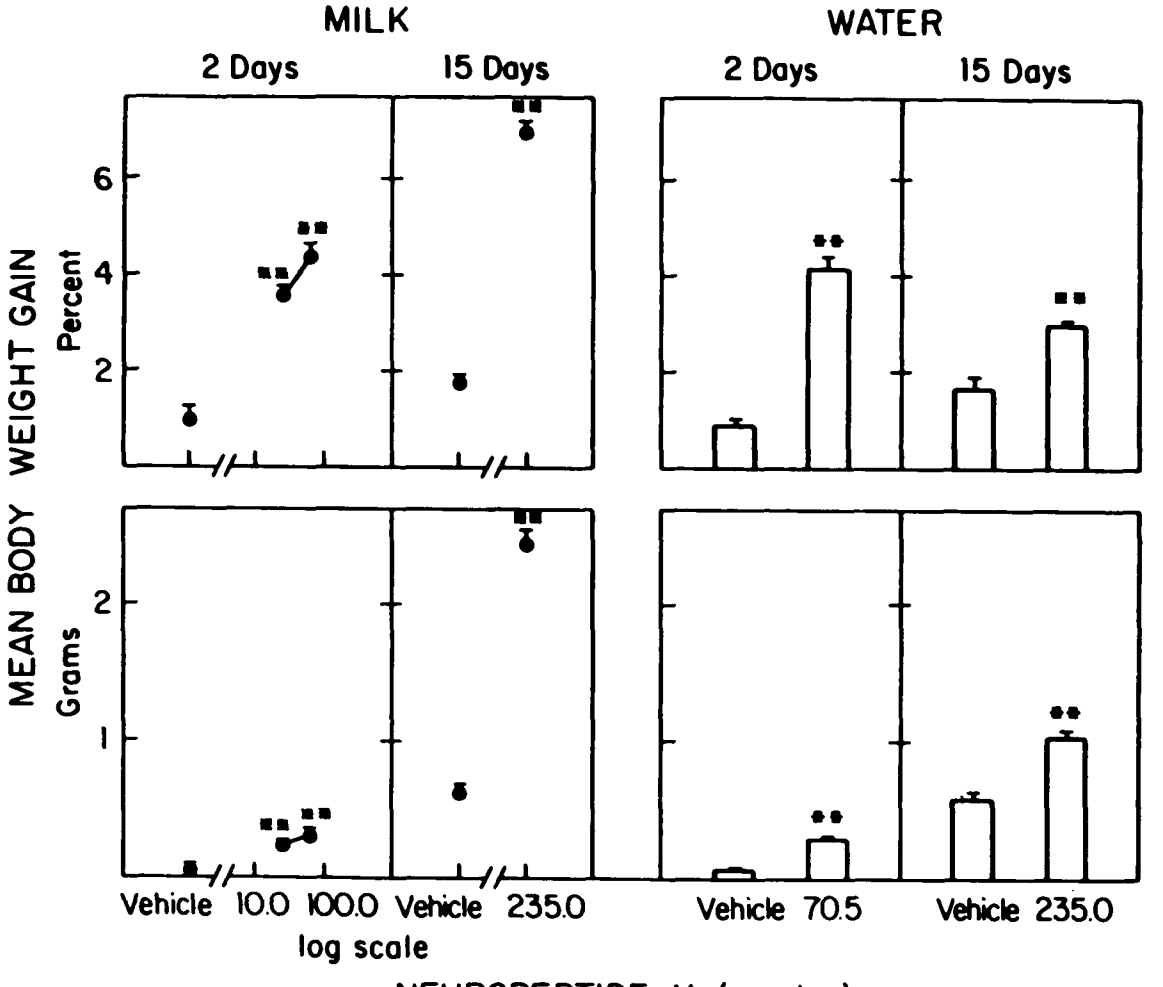
Second, their sites of action are clearly different, with EPI producing its suppressive effect in the PFH and CCK, its suppressive effect in the PVN. Furthermore, CCK is one of few neurochemical agents that have been demonstrated to suppress feeding in the PVN of deprived rats, a nucleus that has classically been associated with enhancement of feeding. In summary, the above comparisons suggest that the ontogeny of CCK and EPI receptors that mediate suppression of feeding in the rat are distinct, both in terms of onset of functional maturity and locus of action.

The failure of CCK in the PVN to suppress milk intake in 12-day-old pups, together with the dose-dependent suppression of milk intake obtained for 15-day-olds, emphasize the relatively late and rapid onset of CCK's effect on milk intake in deprived rats during development. Although the present results may seem to suggest that CCK receptors in the PVN are not functional in suppressing independent feeding in the rat until late in development, it may be that what is actually not functionally mature is the substrate (possibly release of endogenous opioids) that CCK antagonizes. In fact, the present results parallel the ontogeny of the onset of naloxone's suppression of deprivation-induced feeding in infant rats (Aroyewun & Barr, 1982). In theory, the possibility exists that CCK's action in producing satiety may occur as a result of CCK's antagonism of opioid-induced feeding. If this were the case, then CCK's action in the PVN would be dependent upon

the functional maturity of an opioid substrate that, as reported above, does not appear to be functional until the onset of weaning. Nevertheless, the present findings support developmental continuity between independent feeding in the infant and subsequent adult feeding with respect to CCK regulation in the PVN.

Second Investigation. Weight gain from milk ingested in the second investigation as a function of age and of dose of NPY in the PVN is depicted in Figure 11. NPY stimulated milk and water intake (as measured by weight gain in grams and its conversion to a percentage of post-satiation weight) at 2 and 15 days of age. Three-way factorial analyses of weight gain (Age x Diet x Drug) indicated a significant age-effect, $F(1,6)=266.17, p<.001$ in grams & $F(1,6)=8.11, p<.05$ as percent; diet-effect, $F(1,6)=178.39, p<.001$ in grams & $F(1,6)=67.39, p<.001$ as percent; age-by-diet interaction, $F(1,6)=165.60, p<.001$ in grams & $F(1,6)=43.83, p<.001$ as percent; drug-effect, $F(1,6)=431.09, p<.001$ in grams & $F(1,6)=1112.17, p<.001$ as percent; age-by-drug interaction in grams only, $F(1,6)=191.59, p<.001$ in grams & $F(1,6)=0.06, p>.05$ as percent; a significant diet-by-drug interaction, $F(1,6)=286.73, p<.001$ in grams & $F(1,6)=59.06, p<.001$ as percent; and age-by-diet-by-drug interaction, $F(1,6)=284.64, p<.001$ in grams & $F(1,6)=56.11, p<.001$ as percent. Dunnett's post hoc comparisons revealed significant differences ($p<.01$) between the drug and control conditions for weight gain in grams and

Figure 11. NPY's effect on milk and water intake of sated pups as a function of age and of dose in the PVN. The bottom panel represents weight gain in grams and the top panel its conversion to a percentage of post-satiation weight. The error bars represent one standard error of the mean. ** = $p<.01$ relative to vehicle by Dunnett's Test or Student's T-test.


MILK
WATER
2 Days
15 Days
2 Days
15 Days
MEAN BODY WEIGHT GAIN
Percent
Grams
6
4
2
2
1
Vehicle
10.0
100.0
Vehicle
235.0
Vehicle
70.5
Vehicle
235.0
log scale
NEUROPEPTIDE Y (pmoles)

as percent post-satiation weight for milk and water intake at both ages tested. Comparison of the lowest dose (23.5 pmol) to the control for milk intake by Student's T-test revealed a significant difference ($p<.01$) for both measures.

Close examination of percent weight gain at 2 and 15 days for water intake indicates a slight increase for control animals and a slight decrease for drugged animals over age. This result more than likely accounted for the lack of a significant age-by-drug interaction reported for weight gain analyzed as percent post-satiation weight. Nevertheless, a significant age-by-diet-by-drug interaction resulted for weight gain analyzed in grams and as percent post-satiation weight.

As with NE (Figure 5), NPY in the PVN enhanced milk equally as well as water intake at 2 days of age. Again, this finding is consistent with previously reported data that infant rats do not discriminate or differentiate between milk or water until about 8 days of age, whether non-drugged or drugged (Ellis et al., 1984), and further suggests that the infant's response early on in life is more consummatory than appetitive (diet specific) in nature. Comparison of the data for milk and water intake at 15 days indicates that NPY in the PVN, like NE, produced a much greater enhancement of milk than water intake at this age. Thus, similar to NE, NPY's orexigenic effect becomes apparent later in development, prior to the onset of weaning, representing more of an appetitive- than

consummatory-type response.

The similarity of NPY's action to that of NE's in the PVN on milk and water intake of infant rats and the similar onset of functional maturity of their receptors during development are consistent with findings in adult rats showing similar effects on food and water intake (Stanley & Leibowitz, 1984) and co-localization of the two neurochemical agents in brainstem projections to the PVN (Hokfelt et al., 1983; Sawchenko & Swanson, 1982). Together, these findings suggest that NPY and NE interact in the PVN to enhance independent feeding in newborn as well as adult rats. Furthermore, they support the view of developmental continuity between the independent feeding system in the infant and subsequent adult feeding.

General Discussion

This research of the pharmaco-ontogeny of hypothalamic receptor systems has demonstrated that in most cases receptors mediating independent feeding in the infant rat are behaviorally functional prior to the onset of weaning. Except for CCK, receptors mediating the behavioral response to the neurochemical agents, NE, EPI, DA and NPY, do so as early as 2 days postpartum. Furthermore, consistent with studies in adult rats, this work has identified the PVN and PFH as primary hypothalamic areas in the infant rat sensitive to the effects of the neurochemical agents tested. Collectively, the present findings strongly suggest that the

independent feeding system in the infant, while not used during normal development, is developmentally continuous with feeding in the adult and represents an appropriate starting point for developmental analysis of neurochemical systems regulating feeding behavior in the rat.

Experiment 1. In assessing the postnatal age at which the rat brain first becomes responsive to NE, Ellis et al. (1984) have previously reported that the orexigenic effect of NE appears abruptly at 9 to 10 days of age. At this age, and thereafter, a milk-sated pup will eat again after NE is deposited in its ventral third ventricle, immediately adjacent to the PVN that contains the receptors that mediate its orexigenic effect (Leibowitz, 1978a; Matthews et al., 1978). In additon, it was also reported by Ellis et al. (1984) that NE failed to increase water intake at any age, a finding inconsistent with results of other neurochemical agents tested in the same study, with the present results and with the adult literature (Grossman, 1960; Leibowitz, 1978a). Thus, according to the findings of Ellis et al. (1984), the chronology of development of the adult response to NE is that it has no effects on ingestion (milk or water) until the pup is 9 days old, at which age it becomes exclusively orexigenic.

Also inconsistent with the Ellis et al. (1984) findings are the results of the present study suggesting that alpha-noradrenergic receptors in the PVN are sensitive to the effects of NE in enhancing milk equally as well as water

intake in sated pups as early as 2 days of age. By 15 days, NE produces a much greater enhancement of milk than water, despite a small, significant water enhancement, a finding consistent with NE's effects on food and water intake in sated adult rats (Grossman, 1960; Leibowitz, 1978a).

In addressing the difference obtained in onset of NE's effect on milk intake in preweanling rats, a number of differences in methods employed are apparent when comparing the present work with that of Ellis et al. (1984). Included among the more salient differences are separation vs. absence of separation from the dam prior to testing, chronic vs. acute injection procedures and continuous vs. periodical infusions of milk. As reported in the General Introduction, Capuano et al. (in press) and Terry et al. (1984) have demonstrated that variables such as these can attenuate and even reverse drug effects on milk intake in infant rats. Among the differences listed above, separation vs. absence of separation from the dam prior to testing may be the difference in methods that accounted for the difference in results obtained. In the Ellis et al. (1984) study, pups were removed from the dam, immediately sated, acutely injected and then tested. In the present study, pups were removed from the dam, stereotaxically implanted with chronic brain cannulas, placed in a post-operative incubator for a 22 hr period, sated, injected and then tested. Despite availability of yogurt post-operatively, comparison and analysis of post-operative weight gain and

weight loss data (Table 2) indicated that as a group pups did not consume it. Thus, pups in the present study actually went without food (milk) for a 22 hr period prior to being sated. Based on these insights, it is possible that the long deprivation period prior to satiation may have activated alpha-noradrenergic receptors in the PVN of 2- and 7-day-old pups in the present study that are normally not functional until about 9 or 10 days of age (Ellis et al., 1984). This speculation may have accounted for the difference obtained in the onset of NE's effect in the region of the PVN on milk intake in preweanling rats.

The present result of enhancement of milk intake by clonidine in the PVN of 2-day-olds suggests that α_2-noradrenergic receptors mediate NE's effect on independent feeding in the infant as they do in the adult (Goldman et al., 1985). This finding of early functional maturity of α_2-noradrenergic receptors mediating independent feeding in the PVN of the rat is also consistent with the results of biochemical studies revealing the early appearance and differentiation of α_1- and α_2-noradrenergic receptors in this area (Morris et al., 1980). Further studies have demonstrated that CA terminals are present in the PVN at birth (Loizou, 1972) and that the three enzymes necessary for NE synthesis (tyrosine hydroxylase, dopa decarboxylase and dopamine β-hydroxylase) are present in the fetal rat brain at 15 days postconception (Coyle, 1974). Therefore, NE can be synthesized very early in ontogenesis. Together,

these findings provide evidence supporting a mechanism for enhancement of independent ingestion of milk, not only by exogenous NE and clonidine, as shown in the present study, but also by peripheral administration of AMPH, a CA-releasing drug, that has been previously reported to potentiate feeding in very young, deprived rat pups (Capuano et al., in press).

As theorized by Hoebel and Leibowitz (1981) and Leibowitz (1986), release of endogenous NE in the medial satiety system (PVN) of deprived adult rats produces an enhancement of feeding through interaction with α_2-noradrenergic receptors (Figure 1). Furthermore, when activated, α_2-receptors in the PVN act to block or inhibit the output of a satiety signal. This action, in combination with the absence of the inhibitory effects on feeding output produced by endogenous release of EPI and DA in the lateral feeding system (PFH) of the sated animal, results in eating in the deprived animal. As implicated from an earlier study (Leibowitz, 1975b), AMPH in the PVN of deprived animals potentiates the release of endogenous NE, producing a further enhancement of feeding. Likewise, AMPH in sated animals acts to release endogenous NE in the PVN, producing an enhancement of feeding. Thus, when considering the present finding of early functional maturity of the receptor involved in mediating this response, the subsequent issue raised is whether or not the synthesis and release of NE in the PVN is also functional at early ages in development. In

addressing this possibility, the need to assess the effect of AMPH in the PVN on independent ingestion of milk in neonatal rats is apparent.

Experiment 2. Similar to the onset of functional maturity of the alpha-noradrenergic system in the medial hypothalamus or PVN is that of the beta-adrenergic and dopaminergic system in the lateral hypothalamus or PFH. Investigation of the postnatal age at which the rat brain first becomes responsive to EPI suggests that beta-adrenergic receptors in the PFH are sensitive to the effect of EPI in suppressing milk intake in deprived pups as early as 2 days of age. The present result of suppression of milk intake by salbutamol in the PFH of 2-day-olds indicates that β_2-adrenergic receptors mediate EPI's effect on independent feeding in the infant as they do in the adult (Leibowitz & Rossakis, 1978a). Similarly, the present result of suppression of milk intake by apomorphine in the PFH of 2-day-olds suggests that dopaminergic receptors mediate DA's effect on independent feeding in the infant as they do in the adult (Leibowitz & Rossakis, 1979b).

The early fuctional maturity of β_2-adrenergic receptors mediating independent feeding in the PFH of the rat is also consistent with the results of biochemical studies supporting the early detection of beta-adrenergic receptors in the rat brain (Harden et al., 1977). In addition, a study cited previously (Loizou, 1972) also indicated that CA terminals are present in the area of the PFH, as well as the

PVN, at birth. Together with the report that the enzymes necessary for the synthesis of CAs are present in the fetal rat brain (Coyle, 1974), these findings suggest that complete neurochemical development of the adrenergic and dopaminergic systems in the PFH mediating suppression of feeding in the rat may be functionally mature very early in life as well. This possibility supports a mechanism for suppression of independent ingestion of milk by peripheral administration of AMPH, a CA releaser, also reported in very young, deprived rat pups (Raskin & Campbell, 1981).

Completing the theory of CA regulation of feeding in the rat (Hoebel & Leibowitz, 1981; Leibowitz, 1986), the release of endogenous EPI and DA in the lateral feeding system (PFH) of sated adult rats is believed to produce a suppession of feeding through interaction with β_2-adrenergic and dopaminergic receptors (Figure 1). When stimulated, β_2-adrenergic and dopaminergic receptors in the PFH act to block or inhibit the output of a feeding signal. This action, in combination with the absence of the inhibitory effect on satiety output produced by endogenous release of NE in the medial satiety system (PVN) of the deprived animal, results in the absence of eating in the sated animal. Again, as inferred from Leibowitz (1975b), AMPH in the PFH of sated animals acts to potentiate the release of endogenous EPI and DA in the PFH, producing a further suppression of feeding. Similarly, AMPH in deprived animals acts to release endogenous EPI and DA, producing suppression

of feeding. Thus, when considering the present finding of early functional maturity of the receptors involved in mediating this response, a continuation of the issue raised earlier is whether or not the synthesis and release of EPI and DA in the PFH is also functional at early ages in development. As for the noradrenergic-PVN system, the need for a subsequent study assessing the effect of AMPH in the PFH on independent ingestion of milk in neonatal rats is also evident.

In another study referred to earlier (Capuano et al., in press), it was further reported that peripheral administration of chlorpromazine, a dopaminergic antagonist, enhanced independent milk intake in sated rat pups very early in development. This finding is consistent with the effect of chlorpromazine on feeding in sated adult rats (Hoebel & Leibowitz, 1981; Leibowitz, 1986; Leibowitz & Miller, 1969; Robinson, McHugh & Bloom, 1975) and further supports the view of developmental continuity between independent feeding in the infant and subsequent adult feeding. Moreover, as inferred by the theory of CA regulation of feeding (Hoebel & Leibowitz, 1981; Leibowitz, 1986), chlorpromazine acts to enhance feeding by blocking or antagonizing the dopaminergic receptors and thus the effect of release of endogenous DA in the lateral feeding system (PFH) of sated animals. In an attempt to verify this inference during development, investigation of the effect of chlorpromazine in the PFH on independent ingestion of milk

in infant rats is also necessary.

Experiment 3. In contrast to the early onset of functional maturity of CA receptor systems, CCK receptors, at least in terms of behavioral response, do not appear to be functional in the PVN until rather late in development. This finding is consistent with results failing to report specific suppression of milk intake through intra-oral cannulas (Phifer and Hall, 1984) and suckling (Blass et al., 1979) with peripheral CCK until 15 days postpartum. As discussed previously, the possibility exists that CCK antagonizes a substrate (i.e., opioid) that is not fully mature until the onset of weaning (Aroyewun & Barr, 1982), thus making an accurate assessment of CCK receptor maturity in the PVN very difficult. Nevertheless, the present result of CCK-induced suppression of milk intake in the PVN of 15-day-old deprived rats is consistent with CCK's effect in the PVN of deprived adults (Faris & Olney, 1985). Together, these findings suggest that central CCK is involved in regulating satiety within the hypothalamus, specifically the PVN. Furthermore, CCK regulation of independent milk intake in weanling rats appears to be developmentally continuous with CCK regulation of feeding in adult rats.

Alternatively, when considering that others have shown peripheral CCK to suppress independent milk intake (Goldrich et al., 1984) and suckling intake (Anika, 1983; Houpt & Houpt, 1979) very early in life, it may be that CCK produces its effect on milk intake at various central and/or

peripheral sites and thus the PVN may not be the primary site for mediating CCK's effect on feeding, as it appears to be for other neurochemical agents.

Ulike CCK, NPY has a very early onset of receptor maturity in the PVN during development. As also discussed previously, NPY's stimulatory effect on milk and water intake in sated pups is very simflar to that of NE's, a finding consistent with the adult literature (Stanley & Leibowitz, 1984; Stanley et al., 1985). This similarity is not suprising when reminded of the prenatal detection of NPY in several brain areas, including the PVN (Allen et al., 1984; Woodhams et al., 1985) and the similarity in the pattern of rapid postnatal development of NPY systems in the brain to that of CA systems (Allen et al., 1984). Together, these findings support a functional interaction of NE and NPY in regulating food intake in the infant and adult rat. Moreover, they support the view that neurochemical regulation of independent feeding in the neonate is developmentally continuous with that of adult feeding.

Although the onset of the rat's response to CCK is considerably later in its development than that for NPY, the behavioral effectiveness of these neurochemical agents in development provide evidence that neuropeptides play an important role in hypothalamic regulation of feeding behavior in the rat. Furthermore, the functional maturity of receptors mediating their respective responses is evident in the weanling and preweanling rat, representing underlying

developmental precursors for subsequent adult feeding.

Conclusion. In summary, this work has demonstrated that, given the methods employed, α_2-noradrenergic receptors in the PVN and β_2-adrenergic and dopaminergic receptors in the PFH mediating independent feeding in the rat are behaviorally functional very early in postnatal development. NPY receptors show a similar onset of functional maturity in the PVN. In contrast, CCK receptors do not appear to be behaviorally functional in the PVN until late in development. Collectively, the present findings strengthen the argument that the physiological organization of independent feeding in the rat pup, while may be not fully mature, is developmentally continuous with adult feeding and represents the forerunner of later ingestive behavior in the rat.

Finally, in continuously inferring developmental continuity between independent feeding in infant rats and subsequent adult feeding, the present findings do not suggest that independent feeding differs in its physiological controls from suckling or that suckling is not developmentally continuous with adult feeding. As stated at the outset of this work, in light of evidence that these relationships seem to exist and that assessing intake controls using independent feeding is easier than using suckling (Hall, 1985; Hall & Williams, 1983), the independent feeding system was selected for investigation in the present experiments and inferences related to these

issues are based solely on previous ones. Furthermore, intake controls for independent feeding and suckling probably at least overlap and when better methods are discovered to assess intake controls for suckling, findings for both preweanling ingestive systems will probably concur in support of this view.

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