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The Lateral Hypothalamus and Energy Balance: Facilitation and Inhibition of Perifornical Self-Stimulation by Long- and Short-Term Metabolic Signals

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ABSTRACT

The Lateral Hypothalamus and Energy Balance: Facilitation and Inhibition of Perifornical Self-Stimulation by Long- and Short-Term Metabolic Signals

Stephanie E. Fulton

Electrical stimulation of certain brain areas produces a robust effect that directs the animal towards obtaining more stimulation. This phenomenon is believed to result from the activation of neural circuits that direct the animal towards naturally-occurring reinforcers such as food. The lateral hypothalamus (LH) is considered a particularly good site for obtaining vigorous self-stimulation in rats. There is evidence that chronic food restriction enhances the reward value of LH electrical stimulation when the electrode is positioned in the perifornical region. The objective of the present work was to characterize the reward circuitry activated by perifornical stimulation by examining the effects of long- and short-term energy balance manipulations within the same subjects. In this regards, Experiment 1 was carried out to replicate the findings that chronic food restriction facilitates perifornical self-stimulation. In accordance with previous results, chronic food restriction decreased self-stimulation thresholds in a subset of subjects with electrodes located in the dorsal or dorsolateral quadrant of the perifornical LH. It was questioned whether the reward neurons that were responsive to chronic food restriction were similarly sensitive to an acute manipulation of food deprivation. Thus, Experiment 2 was carried out to investigate the effect of a 48 hour period of food deprivation on self-stimulation. This short-term deprivation period failed to facilitate self-stimulation for all subjects. This finding suggests that the reward substrate that was responsive to chronic food restriction is preferentially

sensitive to long-term regulatory signals arising from chronic food restriction and body weight loss. Leptin is an adiposity signal implicated in the long-term regulation of energy balance. To examine whether leptin signaling is involved in the process whereby food restriction enhances the rewarding effect of the perifornical stimulation the effect of an intraventricular infusion of leptin on self-stimulation was tested in Experiment 3. Interestingly, in a manner that is consistent with its effect on feeding, leptin decreased the rewarding effect of the stimulation in the same subset of subjects that were responsive to the effects of food restriction. Conversely, leptin increased the reward effectiveness of the stimulation for subjects that were unresponsive to food restriction. These results suggest that leptin may act as a long-term regulatory signal linking changes in energy balance to adjustments in the sensitivity of reward-related circuitry activated by perifornical stimulation. Furthermore, the findings suggest the presence of different reward substrates that can be activated by stimulating the LH, at least one of which is sensitive to long-term energy balance signals arising from by chronic food restriction and leptin.

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DEDICATION

This thesis is dedicated to my grandmother Amy Fulton. She gave me overwhelming encouragement for my endeavors and took endless pride in my accomplishments. The courage, strength and integrity she embodied has set a standard in my own life.

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INTRODUCTION

A hungry rat rummages through its cage with hopes of encountering a morsel of food. Although the animal makes no discovery it remains in active anticipation of something to relieve its voracious appetite, for it has been a day since its last meal. The cage door opens. At last, food has arrived. The rat approaches the first morsel, sniffs it, grasps it between its paws, and then begins to eat vigorously. The food is very rewarding to the rat, each bite seems to whet the rat's appetite for more. Consider the same rat's behavior while performing for rewarding electrical brain stimulation. After learning that some arbitrary act produces the rewarding stimulation the rat will repeatedly perform this act. The rewarding stimulation seems to produce appetitive behavior just as the morsel of food. Thus, the question is raised: What is the relationship between the rewarding effects of electrical brain stimulation and natural goals objects such as food?

Electrical stimulation of the lateral hypothalamus (LH) is considered particularly rewarding. Rats will stimulate the LH to the point of self-starvation (Routtenberg & Lindy, 1965; Spies, 1965) and will choose LH stimulation over food and water in conditions of severe deprivation (Rossi & Stutz, 1978). Furthermore, there is evidence showing that responding for lateral hypothalamic self-stimulation (LHSS) can be modified by energy balance manipulations that alter food intake. Accordingly, it has been proposed that LHSS mimics the rewarding properties of food (Hoebel, 1968; Hoebel & Thompson, 1969; Routtenberg & Lindy, 1965)

The energy balance signals necessary to produce reward changes in LHSS are not well understood. Manipulations of energy balance have often been categorized as either chronic

or acute. Chronic food restriction is generally considered a manipulation of long-term energy balance due to the resulting fat depletion, whereas acute food deprivation is considered one that affects short-term energy signals such as a glucose. Chronic food restriction has been shown to decrease LHSS thresholds at particular stimulation sites in the LH (Blundell & Herberg, 1968; Carr & Wolinsky, 1993). Acute food deprivation has been shown to produce a small effect at certain placements (Carr & Simon, 1984), whereas others have found no effect or inhibition (Atrens & Sinden, 1975; Giovino & Wise, 1986; Olds, 1958) or only an effect after 48 hours of deprivation (Atrens, Williams, Brady & Hunt, 1982). Until some of these inconsistencies are understood no clear statement about the effects of acute deprivation on LHSS can be made.

It has recently been emphasized that the effects of chronic food restriction on LHSS are seen only when the electrode is positioned in a specific area of the LH (Carr and Papadouka, 1994; Carr & Wolinsky, 1993). It is not known, however, whether acute food deprivation can alter self-stimulation at these same sites. Furthermore, whether other metabolic signals regulating energy balance can alter self-stimulation at these sites is unknown. The present investigation set out to test the sensitivity of LHSS to metabolic changes arising from chronic food restriction, acute food deprivation and leptin, a circulating hormone implicated in the control of food intake and body weight regulation. The design of the present study was such that it was possible to test the effects of all three manipulations in the same subjects, thus allowing a comparison of their effects without the confounding influence of stimulation site.

Although the notion of the LH as the "feeding center" has fallen out of favor, researchers have continued to investigate the function of the LH in the neuronal control of

food intake. Along with the research performed with brain stimulation reward (BSR), there are several lines of evidence for LH involvement in feeding derived from neuroanatomical studies, lesion studies, electrophysiology and chemical stimulation studies. In part, this research provided a rationale underlying the investigation of a relationship between the LH and feeding for BSR studies. Thus, before discussing the BSR research implicating a relationship between the LH and feeding, other work concerning this relationship will be reviewed.

Neuroanatomy

The LH is a heterogeneous region of cells and fibers dispersed among the fibers of the medial forebrain bundle and surrounded by various neuronal structures. The LH has been categorized into three rostro-caudal zones: anterior, tuberal and posterior (Saper, Swanson & Cowan, 1979). Throughout the rostro-caudal length, the LH it is bound medially by the fornix and dorsally by the zona incerta. At the anterior level it is bound medially by the dorsomedial nucleus as well, and laterally by the substantia inominata. At the tuberal level the ventromedial nucleus lies medially to the LH, and the optic tract and the internal capsule laterally. At rostral levels, the LH reaches the base of the brain, whereas at caudal levels it lies dorsal to the supraoptic nucleus and the optic chiasm (Saper et als., 1979).

Support for the idea that the LH is involved in feeding emerges from neuroanatomical studies demonstrating connections between the LH and other structures known to play a role in feeding. The LH and the amygdala are sites of convergence for numerous neural pathways. Gustatory afferent pathways, from the nucleus of the tractus solitaris (NTS) and

the pontine relay, terminate in the LH, amygdala and in the substantia innominata (Norgren, 1976). Vagal and parasympathetic pathways, from the gastrointestinal tract and the liver, overlap the gustatory pathways and the NTS by way of the dorsal motor nucleus of the vagus; these trajectories terminate in the periventricular nuclei of the LH (Novin, Rogers & Hermann, 1981) and the basolateral nuclei of the amygdala (Ricardo & Koh, 1978). Pathways descending to the parasympathetic system have been identified from the LH and the basolateral amygdala (Saper, Loewy, Swanson & Cowan, 1976) and are implicated in gastric secretion and actions of the pancreas (Rogers, Kita, Butcher & Novin, 1980). The olfactory bulb sends projections to the anterior amygdala and to the piriform cortex of the LH (McLeod, 1971). Finally, recent evidence shows connections between the LH and the pariventricular nucleus (PVN) of the hypothalamus, a crucial structure for the action of certain feeding-related peptides (Larsen, Hay-Schmidt & Mikkelsen, 1994).

Lesion Studies

Although neuroanatomical studies suggest that the LH senses and relays information involved in feeding, lesion studies were the first to implicate this area in feeding. Anand and Brobeck demonstrated in 1951 that a bilateral electrolytic lesion of the LH in the rat resulted in a post-operative period of aphagia and adipsia. Thereafter, similar findings were reported documenting the consistent symptomology of LH electrolytic lesions, which collectively became known as the LH syndrome (Teitelbaum and Stellar, 1954). Rats recover from this aphagia-adipsia syndrome in a predictable sequence of stages consisting of a hypophagic period wherein the rat slowly starts feeding and its body weight

eventually reaches 75-80% of control levels (Teitelbaum and Epstein, 1962). The syndrome is further characterized by a failure of recovered rats to overeat in response to insulin (Epstein and Teitelbaum, 1967) and 2-deoxy-D-glucose (2-DG) (Epstein, 1971), two manipulations that produce hypoglycemia-induced feeding in the normal rat. When placed in a cold environment, however, these rats eat in a normal manner (Epstein and Teitelbaum, 1967)

Research performed in the early 1970's drew attention to the idea that some, or even all, of the behavioral effects produced by the lesions were caused by damage to the substantia nigra dopaminergic (SNDA) system, not to intrinsic LH neurons (Ungerstedt, 1970; Zigmond & Stricker, 1972). It was later shown that injection of 6-hydroxydopamine (6-OHDA), an excitotoxin that selectively targets DA neurons, into the SNDA, produces a selective deficit in feeding similar to that produced by electrolytic lesions (Marshall & Teitelbaum, 1973). As in the electrolytically lesioned rats, there were impairments in response to 2-DG and insulin, and a normal overeating response when animals were placed in a cold environment. In addition to this finding, however, there was another that demonstrated that injections of 6-OHDA into the LH produced a less severe eating disability than electrolytic lesions (Myers and Martin, 1973). This evidence suggests that destruction of dopaminergic fibers was not the sole factor governing the behavioral deficits produced by the electrolytic lesion

Later it became possible to selectively lesion LH neurons without destroying any fibers of passage. Lesions in the LH produced by kainic acid (KA) and ibotenic acid (IBO), excitotoxins that cause destruction of cell bodies without interfering with fibers of passage, resulted in abnormalities resembling the ones produced by electrolytic lesions;

although the effect of KA and IBO was less severe (Winn, Tarbuck & Dunnett, 1984; Dunnett, Lane & Winn, 1985) and only lasted about 10 days (Brit & Wise, 1981; Nadler, 1979). It was hypothesized that the cause of the long-term behavioral deficits seen with electrolytic lesions arises from the destruction of both fibers of passage and intrinsic LH neurons (Brit & Wise, 1981).

In summary, electrolytic LH lesions produce an aphagia-adipsia syndrome typified by a sequence of recovery stages culminating in the maintenance of a new, lower body weight. This outcome was later thought to be due to destruction of DA fibers coursing through the LH because of the similar feeding deficits produced by lesions of the SNDA. However, subsequent work using excitotoxins selective to LH cells showed that feeding disturbances still appear, albeit in a less severe manner. Collectively, this evidence suggests that LH neurons are, at least, in part, responsible for the resulting behavioral abnormalities produced by electrolytic lesions.

Electrophysiology

One of the distinguishing behavioral outcomes of LH lesions, produced either electrolytically or chemically, is the failure of rats to respond to a glucoprivic challenge, a finding that suggests the involvement of the LH in glucose sensing. There is a powerful relationship between blood glucose levels and the onset of feeding. Under free-feeding conditions, every meal is preceded by a 6-8% drop in the blood glucose level approximately 5-6 minutes prior to meal initiation (Louis-Sylvestre & Le Magnen, 1980). When the preprandial hypoglycemia is prevented by glucose infusion, the subsequent meal is delayed until glycemia drops again (Campbell, Brandon & Smith, 1985).

The earliest evidence that LH neurons were sensitive to glucose levels came from the work of Anand, Dua and Singh (1961). When rats were rendered hyperglycemic by glucose infusion there was a decrease in activity in the LH and an increase in activity in the VMH. In another study, Himmi, Boyer and Orsini (1988) recorded single unit activity in the LH and surrounding areas along while measuring transient changes in blood glucose level. They found that 1/3 of the LH neurons recorded changed activity as a result of transient blood glucose changes and ventricular glucose injection, whereas only one neuron in neighboring structures (in VMH) showed a change in firing rate. These findings suggest that the LH is more sensitive to glucose changes than neighboring sites.

Single unit recordings in the LH have also been made in response to food stimuli. Rolls, Burton & Mora, (1976) showed that in the monkey some LH and substantia inominata (SI) neurons respond to either the sight of food, some to taste and some to both sight and taste of food. Evidence that these neurons may be involved in the initiation of feeding when food is seen and tasted comes from the observation that they only responded when the monkey was hungry (Burton, Rolls & Mora, 1976), and that it was possible to predict the response of the hungry monkey to food from the response of these neurons (Rolls, Sanghera & Roper-Hall, 1979). In addition, the responses became weaker as the monkey became satiated. These findings suggest that the LH neurons that respond during feeding may be important in mediating the rewarding effects of food. This idea is upheld by the finding that food-reactive neurons in the LH and SI as well are activated by electrical self-stimulation at sites in the LH and nucleus accumbens (Rolls, 1976; Rolls, Burton and Mora, 1980). These data were later confirmed in the rat with the finding that most LH neurons that were inhibited by feeding were also suppressed by rewarding MFB

stimulation (Kazuosasaki, Ono, Muramoto, Nishino and Fukada, 1984). Rolls et als. (1980) interpreted this evidence to suggest that self-stimulation at these sites mimics the rewarding effect of food in hungry animals.

To summarize, electrophysiology studies have established that certain factors that mediate food intake can change the firing patterns of a subset of LH neurons. Transient changes in blood glucose level, known to correlate with meal initiation, alter the activity of a population of LH neurons, without having effects on neurons in adjacent hypothalamic regions. A subset of LH neurons respond to the taste and sight of food when the animal is hungry and the responses become weaker as the animal becomes satiated. This evidence, along with evidence that many of LH neurons that are inhibited by food are inhibited by MFB rewarding stimulation, has lead some to imply that these neurons are responsible for the rewarding effects of food.

Chemically-induced feeding

Another approach to the examination of LH involvement in feeding comes from the investigation of different compounds that produce changes in food intake when directly infused into the LH. A variety of chemicals have been shown to produce changes in feeding when administered into different brain sites. Indeed, many recent studies have focused on the investigation of novel peptides that alter food intake when centrally administered. While these compounds do not produce their effects solely within the LH, recent evidence suggests the LH is particularly sensitive to neurochemicals that induce changes in feeding. In the next section some of this research will be reviewed. It can be

noted that many studies have been concerned with the perifornical hypothalamus (PFH), a region that seems critically important in chemically induced changes in feeding.

Evidence that cathecholamines (CA) in the LH suppress hunger-stimulating mechanisms comes from the work of Leibowitz and coworkers. They injected amphetamine (AMPH), an anorectic drug known to enhance synaptic availability of dopamine (DA), into numerous hypothalamic sites via locally implanted cannulae. The results indicate that the PFH is the area most sensitive to the inhibitory effect of AMPH on feeding (Leibowitz, 1975). Interestingly, fluorescence microscopy studies have distinguished a cluster of CA varicosities surrounding the fornix (Hokfelt, Fuxe, Goldstein & Johansson, 1974; Jacobowitz & Palkovits, 1974). These varicosities contain DA, norepinephrine (NE) and epinephrine (EPI) (Hokfelt et al., 1974).

To further investigate the inhibitory effect of CA on feeding, Leibowitz and Rossakis (1979) examined the sensitivity of 24 different brain areas to different CA. The highest suppression of feeding (50-70%) in response to both DA and EPI was found in the perifornical region of the LH, extending from the caudal portion of the PVN to the caudal portion of the VMN. Although anatomical specification can be difficult in studies using intracranial injections because of diffusion around the cannula tip, in this case cannulae were placed in many locations surrounding the fornix. It was found that dorsal, lateral, or ventrolateral movement away from the fornix into the zona incerta or the MFB portion of the LH caused a dramatic reduction in effectiveness. It is worthwhile to mention that an investigation into the nature of the receptors mediating the DA feeding effects in the PFH has indicated that the D2 receptor is involved (Carruba, Nisoli, Garosi, Pizzi, Memo & Spano, 1991).

The PFH has also become the focus of the orexogenic effects of neuropeptide Y (NPY), a neurochemical found in high concentrations in the brain. When injected intraventricularly it has been shown to be one of the most potent stimulants of feeding (Clark, Kalra, Crowley & Kalra, 1984). Subsequent work has revealed that the PVN contains high concentrations of NPY (Chronwall, DiMaggio, Massari, Pickel, Ruggiero & O'Donohue, 1985). Moreover, it has been demonstrated that PVN injections of NPY produces a stronger eating response than ventricular injections (Stanley & Leibowitz, 1984). However, by implanting cannulae in many areas of the hypothalamus to map out the effectiveness of NPY, the greatest stimulation of feeding was found in the PFH (Stanley, Magdalin, Seirafi, Thomas & Leibowitz, 1993). The medial PFH, at the level of the caudal PVN, was the most sensitive site for NPY-induced feeding. Furthermore, NPY effects in the PFH are restricted to the eating response, whereas in the PVN NPY also induces changes in the respiratory quotient (Currie and Coscina, 1995). Taken together, this evidence suggests the importance of the PFH for NPYs orexigenic effects.

Glutamate is one of the primary excitatory neurotransmitters in the brain. When injected into the LH it elicits substantial eating in satiated rats (Stanley, Ha, Spears & Dee II, 1993). Multiple cannula sites were tested to localize the effectiveness of the excitatory amino acids glutamate, kainic acid (KA), amino-3-hydroxyy-5-methyl-isoxazole propionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) to stimulate feeding within the hypothalamus (Stanley, Willett, Donias, Ha & Spears, 1993). The results showed, that across doses and agonists, the most effective sites were within the LH between the PFH and the lateral LH.

Stanley and coworkers further demonstrated that the LH, and more so the PFH, were highly sensitive to another chemical that elicits feeding, the second messenger cAMP (Gillard, Khan, Haq, Grewal, Mouradi & Stanley, 1997; Gillard, Khan, Grewal Mouradi, Wolfsohn & Stanley, 1998). They showed that when either 8-bromo-cAMP, an analog of cAMP, or compounds that increase endogenous cellular cAMP were microinjected into the LH, PFH and bracketing areas, the greatest amount of feeding was elicited from the PFH. In fact, following microinjection of compounds that increase endogenous cAMP the feeding effect was exclusive to the PFH (Gillard et al, 1998).

By and large, this recent evidence shows the PFH and LH to be "hot spots" for an array of neurochemical effects on feeding. DA, AMPH, EPI, NPY and cAMP all produce their greatest effects when injected into the PFH compared to their effect in bracketing areas. Taken together, these data further portray the LH as being an important substrate for the control of food intake. Particularly, they show the PFH to be crucial to chemical stimulation of feeding. This area has received some attention in self-stimulation studies. Although, little work has been done to examine the effects of different homeostatic challenges on self-stimulation in this area.

Electrical stimulation

Although rewarding self-stimulation can occur with electrodes placed throughout the LH, this by no means implies a homogeneous substrate. Unfortunately, it is not known which of the dozens of fiber systems coursing through this area contribute to the rewarding effect of the stimulation. Electrical stimulation via an electrode implanted into the brain may activate local cell bodies in addition to fibers of passage, thus, it can invade

distant cell bodies and terminals. The search for the fibers responsible for BSR has been narrowed by the use of paired-pulse stimulation techniques that can characterize these fibers (Yeomans & Davis, 1975; Gallistel, Shizgal & Yeomans, 1981). It is now reasonably well established from paired-pulse studies that BSR depends upon the direct activation of small-diameter myelinated fibers, at least some of which, course the length of the MFB and conduct in a rostral-caudal direction (Bielajew & Shizgal, 1982; Bielajew & Shizgal, 1986; Gallistel et al., 1981; Shizgal, Bielajew, Corbett, Skelton & Yeomans, 1980)

It is a wonder that electrical stimulation of certain brain sites can produce a meaningful signal, but the fact that animals will learn to self-administer the rewarding stimulation is evidence that the brain imposes some sort of order on the abnormal situation created by the stimulation. With different stimulation parameters, stimulation of the LH can have two apparently distinct motivational effects. If given in the presence of natural goal objects it can produce a drive-like state by inducing feeding, drinking, copulation and other familiar behaviors (Delgado & Anand, 1953; Roberts, Steinberg & Means, 1967). If given after the occurrence of some act such as lever pressing, it can produce a reinforcing effect increasing the frequency of responding for it (Olds & Milner, 1954). Moreover, stimulation-induced behaviors and LHSS have be demonstrated using the same stimulating electrode (Coons & Cruce, 1968; Hoebel & Teitelbaum, 1962). It is believed that the stimulation activates neural systems underlying natural appetitive behavior. Thus, electrical brain stimulation has been used to study the processing of goal-directed behavior.

Delgado and Anand (1953) demonstrated that electrical stimulation of the LH resulted in vigorous feeding behavior in cats. It was later established that the phenomenon of

stimulation-induced feeding (SIF) only occurred with electrodes at certain sites in the LH (Miller, 1957). Mapping studies have shown that electrodes supporting SIF to be concentrated in the perifornical region of the LH (Hernandez and Hoebel, 1989; Murzi, Hernandez & Hoebel, 1986; Roberts, 1980). When the electrode is correctly placed, SIF has been shown to induce a 2.5-fold increase over previous food intake (Steinbaum & Miller (1965). Similarly, it was reported that long-term electrical stimulation of the LH in rats resulted in extreme obesity (Steffens, 1975).

Subsequent research demonstrated that electrical stimulation of the LH may elicit drinking, gnawing or even sexual activity instead of feeding (Valenstein, Cox, Kakolewski, 1968; Valenstein & Cox, 1970). Valenstein and coworkers found that if feeding, rather than drinking, was the initial response of the stimulation, repeated exposure to water could induce stimulus-bound drinking. The initial response is maintained as long the sensory stimuli relevant to the particular behavior is present (Huang & Mogenson, 1972). These findings led to the hypothesis that the signal elicited by the stimulation is non-specific and that it produces an active state in which animals learn to respond to objects in their environment (Fray, Koob & Iversen, 1982). Furthermore, because feeding can be obtained from many electrode sites, Valenstein (1980) proposed that stimulation-bound behaviors are not governed by the same tissue needs that govern regular behavior, thus, they are not motivated by a natural "drive state".

An important characteristic of SIF is that it only occurs in some animals and that its emergence is not due to differing electrode sites but to individual differences (Wise, 1971). With the use of moveable electrodes in the dorsal-ventral plane of the LH, Wise tested 243 electrode sites. Out of these, 46 supported SIF and stimulation-induced drinking and 8

supported stimulation induced drinking alone. Moreover, all of these positive sites were obtained from a subset of subjects. Unresponsive animals were unresponsive at all sites tested, whereas responsive animals remained responsive at all tested sites within the LH. Given the observation that stimulation at particular sites did not reliably induce feeding in all animals, but that when it was observed stimulation at other sites within the same animal produced similar results, Wise hypothesized that the response of an animal to the stimulation may be involved in learning. Responses to the stimulation such as eating are reinforced by subsequent rewarding stimulation. This learned eating response could then be transferred to other stimulation sites within the same rat. This idea is consistent with the hypothesis that the signal produces an active state in which animals learn to respond to objects in their environment (Fray et al., 1982).

The study of rewarding self-stimulation, on the other hand, does not analyze the behaviors elicited by the stimulation, instead it examines the reward effectiveness of the stimulation by measuring its ability to reinforce future responding for it. Since the discovery by Olds and Milner (1954) that electrical self-stimulation of certain brain areas is rewarding, BSR has been used as a tool to examine the facets of goal-directed behavior. Studies from many laboratories have confirmed the phenomenon of BSR in a wide variety of species ranging from goldfish (Boyd & Gardiner, 1962) to humans (Bishop, Elder & Heath, 1963). It was soon discovered that animals will self-stimulate when electrodes are placed in many areas of the brain, although, the LH is considered a particularly good site for obtaining vigorous self-stimulation in rats. It has been reported that some rats will press a bar for LH stimulation until the point of "self-starvation" (Routtenberg & Lindy,

1965; Spies, 1965) and will choose it over food and water in conditions of severe food deprivation (Rossi & Stutz, 1978).

What is the relationship between LHSS and food reward? Does the signal produced by the stimulation mimic that triggered by food (Hoebel, 1968; Routtenberg & Lindy, 1965) or does it simply share some of the same appetitive properties as food (Conover & Shizgal, 1994a; 1994b; Conover, Woodside & Shizgal, 1994; Green & Rachlin, 1991)? Most of the work addressing this question can be categorized by two major measures that were used: 1) the ability of LHSS to divert goal-directed behavior from natural rewards and 2) changes in the reward effectiveness of LHSS following homeostatic challenges.

The earliest account of the self-deprivation phenomenon was described by Olds (1958) who demonstrated that rats would self-stimulate until the point of exhaustion while neglecting other bodily needs. According to Olds (1962), the rewarding properties of BSR represent the activation of the neural systems that serve natural rewards. Similarly, subsequent studies demonstrated that rats with electrodes in the so-called "feeding-center" of the LH preferred LHSS to food even during periods of severe food deprivation (Spies, 1965; Routtenberg & Lindy, 1965). Spies (1965) suggested that in the case of self-depriving animals, LHSS acts on the neural circuitry that signals food intake, thus "short circuiting" a hunger reduction mechanism.

Further studies found self-deprivation when LHSS was placed in competition with food reward in food-deprived animals (Eckert & Lewis, 1967), water reward in water-deprived animals (Falk, 1961) and salt reward in adrenalectomized, salt-deprived animals (Eckert & Lewis, 1967). This evidence suggests that the self-deprivation phenomenon may be dependent on the particular drive state induced, one that need not be specific to

hunger. However, later work argued that self-deprivation arises from a non-specific action. Examining two discrete forms of deprivation in reference to one stimulation loci, Rossi and Stutz (1978) showed that rats that preferred LHSS to food also preferred LHSS to water. These finding suggests that self-deprivation may be independent of specific drive states.

In summary, studies examining the self-deprivation phenomenon have shown that LHSS can override choice for different bodily needs under conditions in which the particular resource is deprived. The observation that animals will neglect both food while food deprived and water while water deprived for LHSS at one stimulation locus does not support the idea that the self-deprivation phenomenon is goal specific. Specifically, the evidence that LHSS can produce neglect for goals other than food does not support the hypothesis that LHSS mimics the rewarding effects of food.

The studies mentioned above examined the ability of BSR to generate neglect of physiologically needs, a second body of research describes the effects of different homeostatic challenges on responding for LHSS. Two types of challenges have been studied: the effects of postingestional factors, and the effects of deprivation.

Hoebel (1968) investigated the effects of gastric loading on LHSS. By means of an intragastric tube, food was delivered to the gut and allowed to accumulate. Rats were then given access to a lever providing stimulation on a continuous reinforcement schedule. He found that the gastric loading decreased response rates for LHSS but not for septum stimulation in the same subjects. Because only LH self-stimulation rates were suppressed, Hoebel concluded that the effects of gastric loading were due to changes in the reward value of LH stimulation, not to changes in activity level. Due to the observation that septal

self-stimulation was unaffected by the gastric loading Hoebel suggested that the LH is specific to feeding. However, with this paradigm it is difficult to account for any activity level changes that may specifically affect responding for LH stimulation; nor can a strong anatomical argument be made about the specificity of reward when comparisons are made with one other brain structure.

In a similar series of experiments Hoebel and Thompson (1969) examined self-stimulation and escape rates that were recorded on two active levers following gastric loading of food. They found that when rats accumulated a 100g or more of food the mean escape rate tripled making self-stimulation rates vary inversely with escape rates. On the basis of these results, Hoebel and Thompson suggested that the rewarding effect produced by LH stimulation is identical to the rewarding effect of feeding.

Conover and Shizgal (1994b) also investigated the influence of postingestive feedback on LHSS. They adopted two preparations that provided advantages over those used in previous studies: 1) the use of an intraoral catheter, so as to render the gustatory reward analogous to the BSR and 2) a gastric cannula which could be open or closed, so as to maintain control of the postingestional effects of the sucrose. Rats were given a choice between LH stimulation, which varied in intensity from trial to trial, and a sucrose reward alone or a compound reward consisting of a sucrose plus a fixed train of stimulation. The resulting rate-frequency functions related the number of reinforcers obtained to the intensity of the reward, allowing one to distinguish changes in reward value from changes in motoric capacity produced by the gastric loading. They found that LHSS remained stable in the face of gastric loading of sucrose compared to responding for sucrose which was substantially suppressed by gastric loading. These results imply that a sucrose reward

and LHSS are quite different from one another in one respect. Based on the results that the two rewards were modulated differently by gastric loading of sucrose, Conover and Shizgal (1994a; 1994b) suggest that metabolic signals that modulate food intake exert their effect upstream from the point where the rewarding effects of LH stimulation and gustatory stimuli are combined.

How does one account for the differences between the postingestive feedback results of Conover and Shizgal (1994b) and those of Hoebel (1968) and Hoebel and Thompson (1969)? One possible explanation arises from the dissimilar measurement techniques implemented in these studies. The rate measures used by Hoebel and colleagues have been criticized for their inability to factor out changes in motoric capacity (Edmonds & Gallistel, 1974; Hodos & Valenstein, 1962; Miliaressis, Rompre, Laviolette, Philippe & Coulombe, 1986). Another possible explanation, as pointed out by Hoebel (1968), is that the gastric loading could have enhanced an aversive effect produced by the LH stimulation, and not inhibited the rewarding effect of the LH stimulation. According to Conover and Shizgal (1994b), aversive effects produced by the gastric loading in Hoebel's study may have been caused or intensified by the fact that Hoebel's subjects received experimenter administered loads which were larger and given over a short time period. On the other hand, in Conover and Shizgal's study subjects self-administered the sucrose reward over a relatively longer time period. Another possible explanation for the different results could be the location of the stimulating electrodes in each study. Recent evidence suggests that the electrode must be placed in a particular area of the LH, the perifornical region, to obtain enhancement of LHSS following food restriction, thus perhaps the same applies to postingestive feedback manipulations.

While Conover and Shizgal's postingestive feedback experiment demonstrated important differences between food and LH stimulation, other evidence derived from their work illustrates that gustatory stimuli and LH stimulation have something in common. Conover and Shizgal (1994a) demonstrated that when LH stimulation and sucrose are pitted against one another the two rewards can compete and summate. Using a forced-choice preference paradigm, they observed that rats could choose between the two rewards in a manner that implied that the gustatory stimuli and the electrical stimulation were being evaluated along a common dimension. The subjects in their experiment chose among the two rewards as if they were being evaluated by a currency function that signifies the value of different inputs on a common scale. This common scale of evaluation is an essential antecedent of choice among goals with multidimensional properties. It represents a point where the different properties of goals are reduced to a single, common dimension. While the "general currency" exists to allow choice among many alternatives, there must also be local currencies which compute the value of specific goods such as food and water (Shizgal, 1998). Such a configuration, consisting of multiple stages of local currency evaluations specific to food, would be an orderly way for the brain to process and evaluate the multiple properties of food stimuli so as to meet energy requirements.

Another approach to the study of the relationship between the LH and feeding is that taken in the work relating LHSS to deprivation states. A deprivation state is produced when a vital physiological resource is withheld for a period of time during which the resource is depleted. In the case of acute food deprivation, food is withheld for a brief period of time (e.g., 24 to 48 hours). Acute deprivation can produce weight loss, but very

little fat depletion, thus it is believed that short-term energy stores are modulated. On the other hand, chronic states of food deprivation are implemented when daily food consumption is limited to smaller quantities for long periods of time. The outcome is a significant reduction in body weight mostly due to fat loss, implying that long-term energy stores are modified.

The effect of negative energy balance on LHSS is still a matter of considerable disagreement. Margules and Olds (1962) were the first to examine the effects of acute food deprivation on LHSS. Measuring response rate on a continuous reinforcement schedule, they reported increased responding for LHSS following 24 hours of food deprivation. Similar findings were reported by other investigators using a related paradigm (Deutsch & DiCiara, 1967). However, in addition to the reports that acute deprivation enhances LHSS, there are numerous reports of no change or even inhibitory effects (Atrens & Sinden, 1975; Giovino & Wise, 1986; Olds, 1958).

Among the reports that food-deprivation enhances LHSS, there is still considerable disagreement as to what minimum period of deprivation is required to witness the effect. In two cases the effect was only marginal after 24 hours (Carr & Simon, 1984; Rossi & Panksepp, 1992), whereas another study suggests that 48 hours of deprivation is needed to produce a facilitation of LHSS (Atrens, et al., 1982). In addition, it is still not clear where the electrode must be placed to obtain such effects. Hernandez and Hoebel (1978) have insisted that the PFH is the only region where deprivation effects, both acute and chronic can be seen, but others have argued otherwise. A case in point is the work by Atrens et al (1982) who investigated the hypothesis that there may be dissimilar effects produced by 24 and 48 hour food deprivation periods on LHSS. Utilizing a fixed interval

schedule to examine both initiation and escape from LH stimulation in a shuttle box, they discovered that 24 hour food deprivation did not affect stimulation initiation or stimulation escape rates, whereas 48 hours of deprivation enhanced them. Furthermore, they observed these effects "over virtually the entire anterior-posterior extent of the LH" in subjects who displayed stimulation-bound feeding as well as in those who did not (Atren et als., 1982). This finding contradicts the notion that the effect is restricted to the PFH. Thus, it seems as though the effects of acute food deprivation on LHSS remain to be elucidated.

Blundell and Herberg (1968) discovered that chronic food restriction is more efficient at facilitating LHSS than acute deprivation. Moreover, they recognized that the effect was localized to the PFH. Carr and his collaborators have shown that at certain PFH sites thresholds for LHSS can decrease in a manner that correlates with body weight (Carr & Wolinsky, 1993). Carr and coworkers have proposed that manipulations that generate "acute tissue need" fail to affect LH stimulation at the food restriction responsive sites (Carr, 1996). Administering 2-DG caused a two-fold increase in blood glucose levels yet failed to alter LHSS thresholds (Cabeza de Vaca, Holiman & Carr, 1998). In addition, they showed that blocking the mobilization of free-fatty acids with nicotinic acid decreased metabolic rate and increased food intake yet failed to alter LHSS thresholds (Cabeza de Vaca et al., 1998). On the basis of their studies, Carr and his collaborators propose that the perifornical area is preferentially sensitive to long-term metabolic signals arising from chronic tissue need.

What is the difference between the short- and long-term regulation of energy balance? The fact that most normal weight and obese animals maintain relatively stable body weights over long periods of time suggests that some form of weight regulation is

occurring (Keesey, Mitchell & Kemnitz, 1979). Accordingly, food intake is seen as a regulatory mechanism that contributes to body energy homeostasis. Energy balance regulation has commonly been divided into short- and long-term regulation. Short-term regulation of energy balance seems to be involved in the monitoring of transient states of energy balance. Thus, short-term metabolic signals are involved in the bodies response to immediate metabolic needs. While the factors modulating this form of regulation have not been fully elucidated, glucose has been implicated as a likely candidate. The brain has an immediate need for glucose, it is one of the brains primary sources of energy (Sokoloff et als., 1977). There is evidence that there are neurons in the hypothalamus that directly sense and alter their firing rates as glucose availability changes (Oomura, 1983). Furthermore, meal initiation has been linked to slight changes in plasma glucose levels (Louis-Sylvestre & Le Magnen, 1980).

Whereas short-term regulation requires adjusting current intake to meet immediate metabolic need, long-term regulatory mechanisms are in action to achieve a constancy of body fat mass and body weight. Fat reserves are used to buffer short-term positive and negative energy availability. The size of the fat store is under the control of lipostatic mechanisms that affect food intake and energy metabolism (Kennedy, 1953). These lipostatic mechanisms are implemented on a short-term basis through adjusting metabolic rate and food intake, yet they serve as long-term regulators of body fat mass. Insulin and leptin have been implicated as two lipostatic mechanisms.

The present study explored the hypothesis that perifornical self-stimulation is preferentially sensitive to long-term metabolic signals. In light of the fact that chronic food restriction produces a significant amount of body fat loss, it is believed to influence

metabolic signals regulating long-term energy balance. On the other hand, acute food deprivation produces very little fat loss, yet it creates a state of negative energy balance by modifying metabolic signals involved in the short-term regulation of energy balance. A strong test of the hypothesis that perifornical self-stimulation is preferentially sensitive to long-term regulatory signals would be to measure the ability of acute and chronic deprivation to modulate self-stimulation within the same subjects. Thus, the aim of Experiment 1 was to replicate the findings of Abrahamsen et als. (1995) showing that chronic food restriction decreases thresholds for LHSS at certain electrode placements in the PFH. Experiment 2 was carried out to test whether an acute period of 48 hour food deprivation would also decrease thresholds for LHSS in the same subjects. If, as Carr (1996) proposed, LHSS is only sensitive to manipulations that affect chronic tissue need then one would expect that acute food deprivation would not decrease thresholds at sites that are responsive to chronic food restriction.

If the stimulation sites responsive to food restriction are preferentially sensitive to long-term regulatory signals, what are the specific signals mediating the effect? Presumably, they are related to changes in body weight or body fat mass produced by chronic food restriction. To test this hypothesis, Experiment 3 was carried out to determine if leptin, a satiety signal implicated in the long-term control of energy balance, affects LHSS in a manner that corresponds to its inhibitory effects on feeding. If it is found that leptin reduces the reward effectiveness of LH stimulation only in subjects that show facilitation of self-stimulation by chronic food restriction it will suggest that leptin contributes to the process whereby food restriction enhances LHSS.

The three experiments were carried out to gain a better understanding of: (1) the nature of the relationship between LHSS and food reward, (2) the ability of long-and short-term metabolic signals to affect LHSS, and (3) the long-term metabolic signals that are involved in the process whereby food restriction enhances LHSS. An important aspect of this work is that all three manipulations were performed within the same subjects, permitting one to characterize the neurons responsible for the rewarding effect without the confounding influence of stimulation site.

Experiment 1

Does electrical stimulation of the LH produce its rewarding effect by mimicking the rewarding properties of a piece of food? One approach researchers have taken to answer this question concerns the examination of the effects of food deprivation on LHSS. Early reports indicated that chronic food restriction was better than acute food deprivation at enhancing LHSS (Blundell & Herberg, 1968). Similarly, recent studies that used more reliable response measures have demonstrated that the threshold for LHSS is decreased by chronic food restriction when the electrode is placed in a particular area of the LH (Abrahamsen et al, 1995).

If the signal induced by the electrode is identical to the one produced by a rewarding piece of food then we would expect LHSS to be modulated by manipulations that alter the reward effectiveness of food. The present study set out to test whether chronic food restriction, resulting in 20-25% body weight loss, would decrease the threshold for LHSS.

Method

Subjects

Ten male Long Evans rats of the Charles River Breeding Farms (St. Constant, Quebec) with initial weights ranging between 300 to 400 grams served as subjects. They were housed individually in plastic solid floor cages with *ad libitum* access to food and water. All subjects were placed on a 12 hour dark/light reverse cycle in a temperature controlled room. Behavioral testing was conducted in the dark phase of the cycle.

Surgery

Surgery was performed under Pentobarbitol anesthetic (Somnotol 75 mg/kg i.p.) and Atropine Sulfate (0.5 mg/kg s.c.) to reduce bronchial secretions. Prior to surgery, deep anesthesia was assessed by tail pinch reflex, and subjects were secured into a stereotaxic device. With skull landmarks bregma and lamda positioned on the same horizontal plane, rats were implanted with bilateral monopolar electrodes aimed at the perifornical region of the LH (3 mm posterior to bregma, 1.6 mm lateral to the midsagittal sinus, 7.8 mm below the dura mater) and a stainless steel 24 gauge guide cannula (11 mm in length.) aimed at the right lateral ventricle (0.4 mm posterior to bregma; 1.6 mm lateral to the midsagittal sinus; 4 mm below the dura mater). Electrodes were constructed by insulating Size 00 stainless steel insect pins to within 0.5 mm of the tip. Four jewelers screws embedded into the skull served as anchors for the assembly, and a wire wrapped around one of the screws served as the return. Following electrode and cannula insertion dental cement was applied to fix them. Male amphenol pins attached to copper wire soldered to the electrode and the return wire were inserted into a nine-pin connector. The entire assembly was then fixed

onto the animals head with dental cement. Obturators were fastened to the intracerbroventricular (ICV) guide cannulae to prevent infection. Following surgery subjects received a post-operative analgesic (Bupremorphine 5.0 mg/kg s.c.) and topical antibiotic to prevent infection around the incision. Subjects recovered for at least three days before behavioral testing commenced.

Testing Apparatus

Prior to testing subjects were screened for self-stimulation in wooden boxes (25cm x 25 cm 70 cm) with Plexiglas front panels and wire mesh floors. A lever, positioned 3 cm above the floor, protruded from the middle of one wall. A key light was positioned 5 cm above the lever to signal the availability of the reward. An electrical lead connected a 7-channel, slip-ring commutator located at the top of the box, to the assembly on the rat's head. Electrical stimulation was generated by dual constant current amplifiers and controlled by hand-operated circuit pulse generators

Actual testing took place in an isolated computer-controlled testing room. Test chambers were similar to those used for training except they were made entirely of Plexiglas and were equipped with a white house light positioned at the top of the box. Each test chamber was enclosed in a 50 cm x 50 cm x 90 cm sound attenuating foam box. Temporal stimulation parameters for each test cage were managed by a microprocessor and monitored on an oscilloscope in an adjoining room. Subjects were supervised during testing by means of a remote-controlled video camera.

Procedure

Behavioral Training

Before behavioral testing began rats were shaped to press a lever for a 1 s train of cathodal, rectangular, constant-current pulses, 0.1 ms in duration, on a continuous reinforcement schedule (CRF). In the beginning, stimulation parameters were set to low, currents and frequencies. If the rat displayed signs of aversion (e.g., vocalizations, freezing, jumping) training was discontinued; otherwise, rats were shaped to press the lever. Stimulation frequencies were adjusted to produce optimal levels of responding at 200-250 or 400-450 μA. Self-stimulation was tested for both stimulating electrodes, but only that which produced vigorous lever-pressing was chosen for further testing.

Subjects were then moved to the testing apparatus for further training. At this stage rats were accustomed to responding in sessions where the current was held constant and the stimulation frequency decreased from one trial to the next. Data were collected relating response rate to the stimulation frequency (rate-frequency curves). Accordingly, stimulation frequencies were adjusted so as to produce maximal (asymptotic) responding for the first few trials. The training phase lasted approximately one week.

Behavioral Testing

In the testing phase, with the current held constant at either 200-250 or 400-450 μA, seven rate-frequency curves were collected, the first one considered as a warm-up. Rate-frequency curves were determined by recording the number of lever-presses per 60 s trial on a CRF schedule over a range of stimulation frequencies. The frequency decreased from trial to trial by 0.033 $\log_{10}$ unit steps from values that produced maximal responding to

one that yielded less than 5 responses. Each trial was preceded by a 10 s inter-trial interval wherein 5 priming trains of stimulation were delivered with identical parameters as those available during the trial.

Behavioral testing was conducted during two phases of the experiment. In the first phase rats were placed on a restricted-access feeding paradigm wherein they received 10g of standard lab chow daily until their body weights stabilized at 75-80% of the original values (approx. 2-3 weeks). Rate-frequency data were collected through out this phase. During the second phase of the experiment *ad libitum* feeding was allowed and data were collected when body weights stabilized at age-matched, control values.

Histology

Following the completion of the this and the next two experiments, marking lesions were made at the stimulation sites by delivering 100 μA of direct current for 15 s, with the depth electrode serving as the anode. The deposited iron produced by the lesion was stained according to the Prussian Blue method. Accordingly, rats were injected with a lethal dose of sodium pentobarbitol (100mg/kg, IP) and perfused with phosphate-buffered saline followed by a mixture of 10% formalin (100ml), trychloriacetic acid (0.5g), potassium ferrocyanide (3g) and potassium ferricyanide (3g). The brain was removed and stored in 10% formalin. After a 24 hr immersion period in 20% sucrose formalin, 30μm coronal sections were cut using a cryostat and mounted on gelatin coated slides (Fisher Scientific). Sections were stained for Nissl substance using formal thionin. The location of the stimulation sites was identified with the aid of a stereotaxic atlas (Paxinos and Watson, 1997).

Data analysis

The raw data collected consisted of the number of lever-presses and the number of reinforcers obtained for a given trial. Since the rats did not procure the stimulation at every lever press, the number of reinforcers obtained per minute was used the dependent variable because it provides a measure of how many times the rat's behavior was reinforced. Therefore, the data were plotted as the number of reinforcers per minute as a function of the stimulation frequency in $\log_{10}$ units. For all intents and purposes this curve is still considered a rate-frequency curve, the only difference being that rate of reinforcement is used instead of response rate as the dependent variable. Of the seven rate-frequency curves obtained during one test day the first one was considered a warm-up and was eliminated to yield six curves for the analysis. A three-segment broken-line function with a horizontal lower asymptote, linear sloping segment, and horizontal upper asymptote was fit to each curve. From the broken-line function the stimulation frequency required to maintain half-maximal reinforcers was calculated. This value was used as the threshold point. In addition to the threshold, the slope and maximal rate of reward were calculated derived from the broken-line function. For the purpose of curve fitting and statistical analysis, all calculations used the common logarithm of the stimulation frequency.

Results

Food restriction produced a 20-25% decrease in body weight. Free-feeding weights ranged between 512-600g and food restricted weights ranged between 372-466g. Half of the subjects showed significant decreases in self-stimulation thresholds following food restriction as shown in Figure 1. On the other hand, the remaining subjects showed relatively stable thresholds following food restriction. Figure 2 illustrates the rate-frequency curves for subjects that were responsive to the effects of food restriction. The leftward, horizontal displacement of the food restriction curves relative to the baseline curves is indicative of reduced thresholds produced by food restriction. Figure 3 illustrates the rate-frequency curves for the remaining subjects who did not show any significant changes in threshold following food restriction. The magnitude of mean threshold changes, the standard error of the mean (SEM) and significance values are represented in Table 1. Tables 2 lists any changes in the maximum number of reinforcers obtained (the upper asymptote of the broken-line function) and slope (the rising portion of the broken-line function) for each food restriction session versus baseline.

Stable threshold values on two consecutive days were chosen from both the food restriction and baseline conditions. The threshold values were used to examine any horizontal displacement of the curves produced by food restriction. The six values taken from each of the two baseline days were grouped to yield twelve threshold values. These twelve values were compared with the twelve obtained from grouping the threshold values of the two food restriction days using a one-way ANOVA. The slope of the

Figure 1 Bar graph illustrating the magnitude of the mean threshold change (stimulation frequency in $\log_{10}$ units) produced by chronic food restriction relative to baseline threshold values.

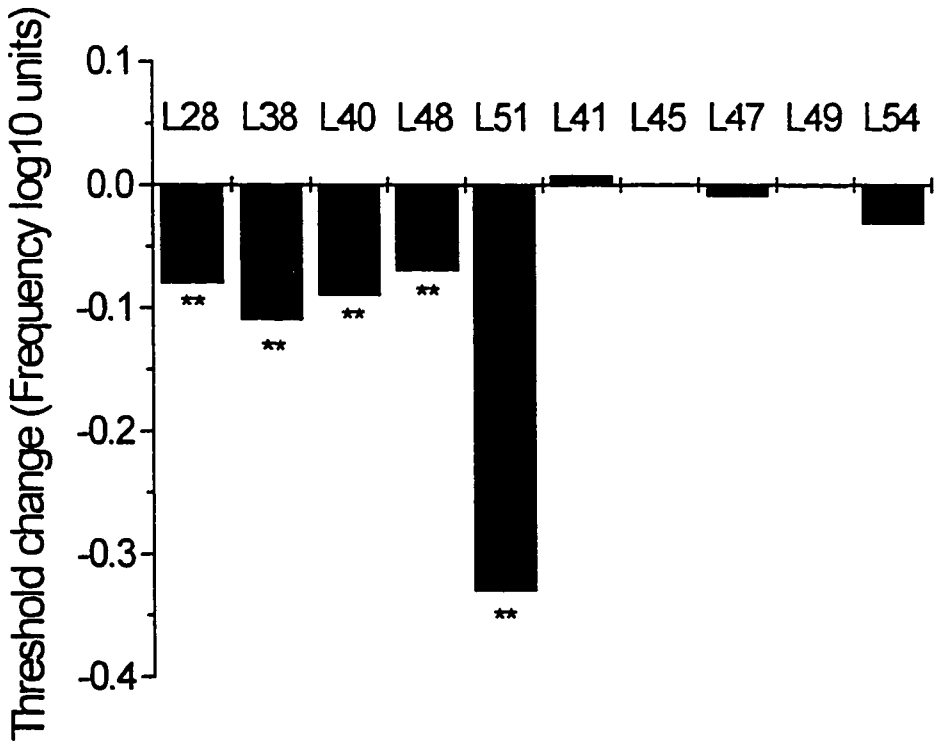
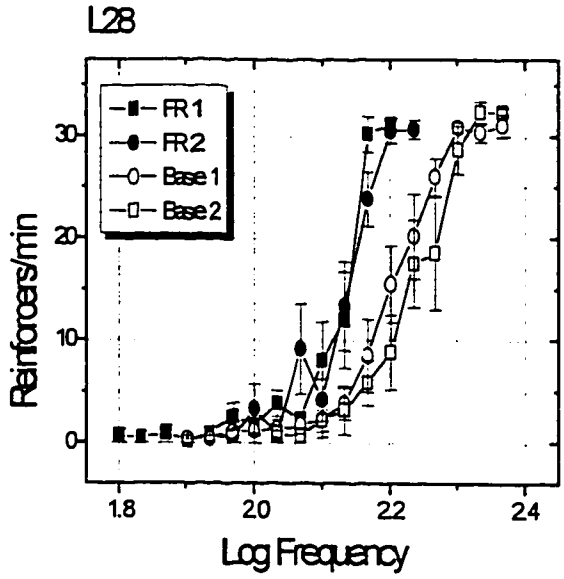
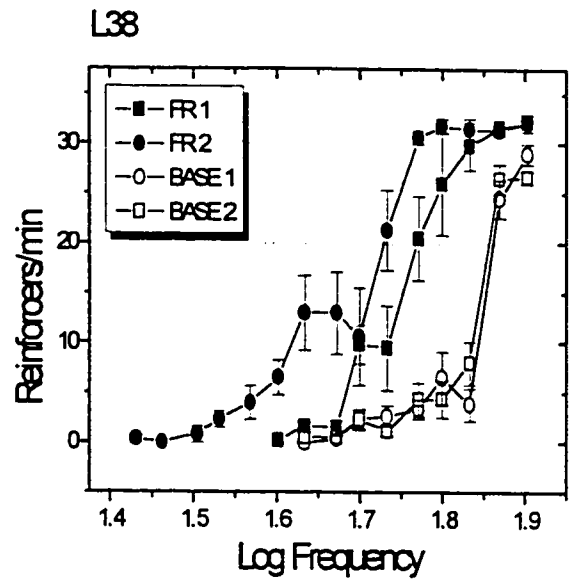
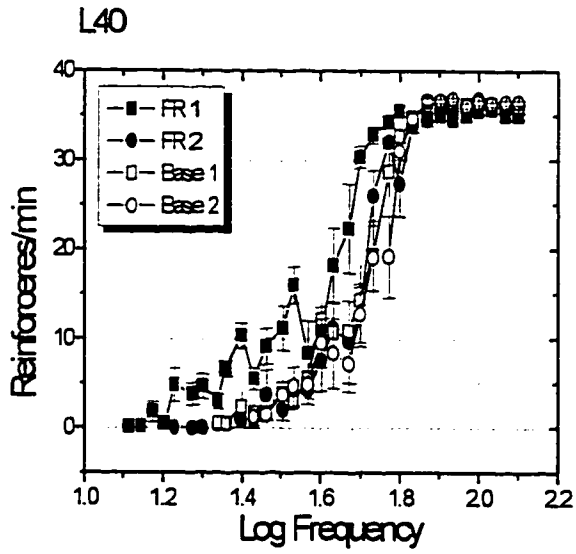
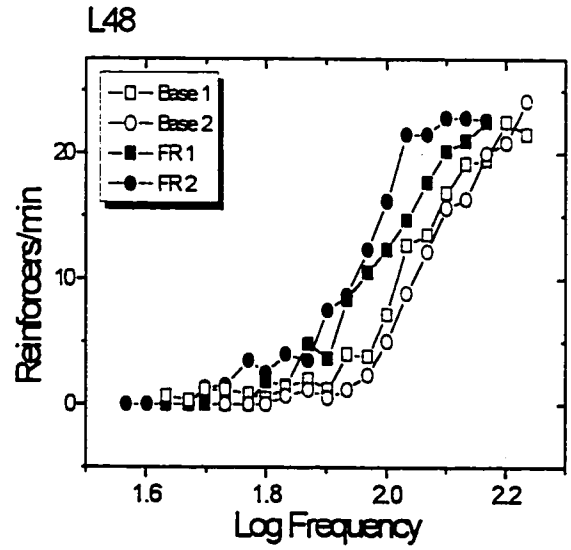

Threshold change (Frequency log10 units)
0.1
0.0
-0.1
-0.2
-0.3
-0.4
L28
L38
L40
L48
L51
L41
L45
L47
L49
L54
**
**
**
**
**

Figure 2. Rate frequency curves for subjects that showed decreased thresholds following food restriction. Two rate-frequency curves represent self-stimulation responding during baseline (base) and the other two represent responding during food restriction (FR). Each curve represents the average of six curves from a daily session and relate the number of reinforcers obtained per minute as a function of the stimulation frequency in $\log_{10}$ units. Vertical bars surrounding each point represent the standard error of the mean number of reinforcers obtained. Numbers in the legend refer to the session day.


L28
FR1
FR2
Base1
Base2
Reinforcers/min
Log Frequency


L38
FR1
FR2
BASE1
BASE2
Reinforcers/min
Log Frequency


L40
FR1
FR2
Base1
Base2
Reinforcers/min
Log Frequency


L48
Base1
Base2
FR1
FR2
Reinforcers/min
Log Frequency

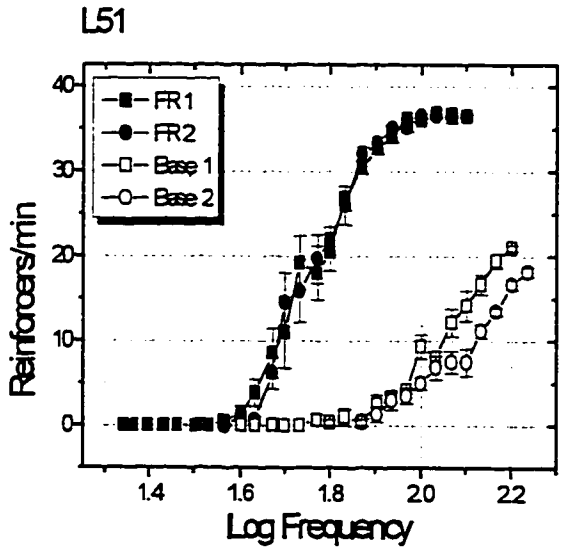

L51
FR1
FR2
Base1
Base2
Reinforcers/min
Log Frequency

Figure 3 Rate frequency curves for subjects that showed stable thresholds following food restriction. Two curves represent self-stimulation responding during baseline (base) and the other two represent responding during food restriction (FR). Each curve represents the average of six curves from a daily session and relate the number of reinforcers obtained per minute as a function of stimulation frequency in log10 units. Vertical bars surrounding each point represent the standard error of the mean number of reinforcers obtained. Numbers in the legend refer to the session day.

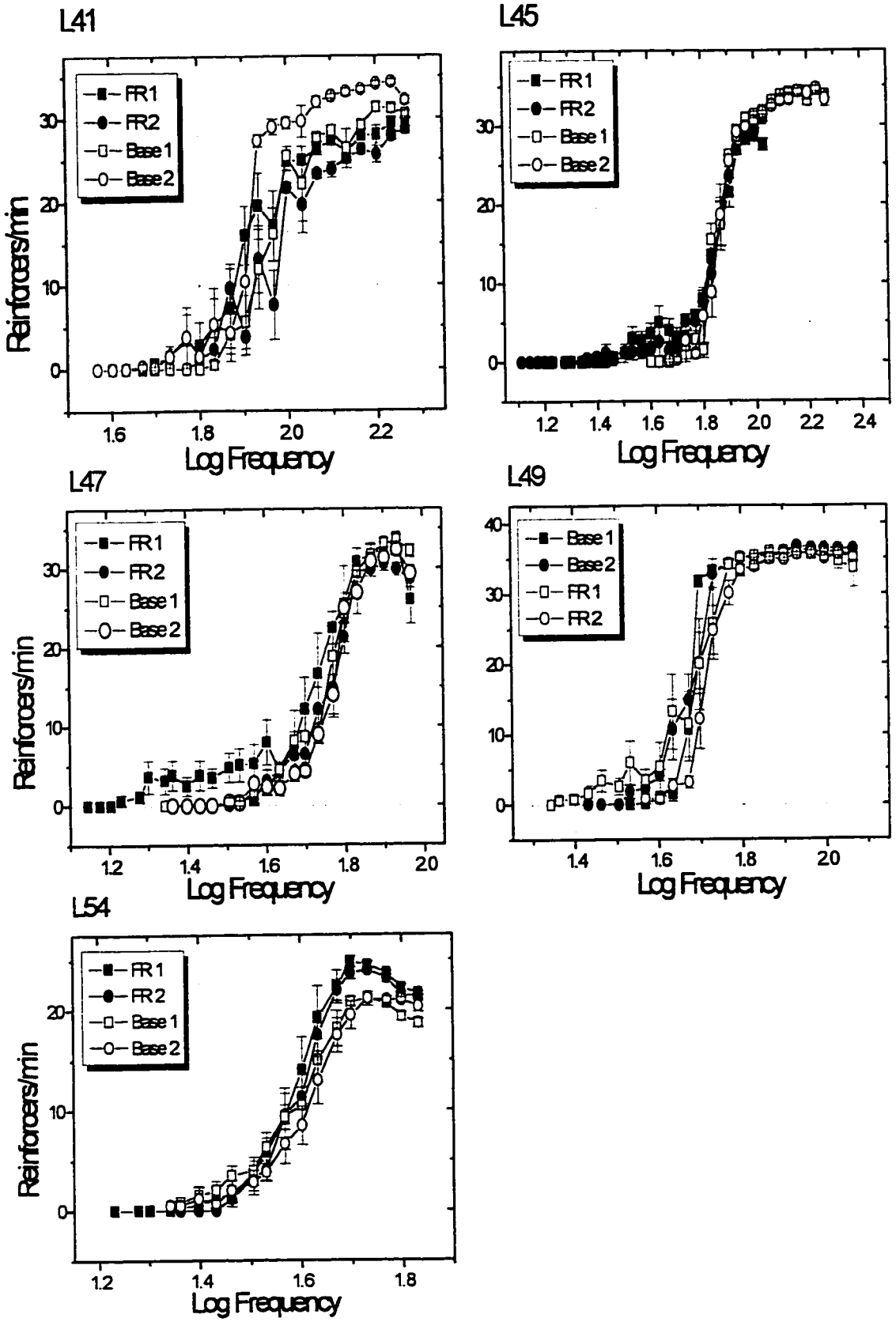

L41
FR1
FR2
Base1
Base2
Reinforcers/min
Log Frequency
L45
FR1
FR2
Base 1
Base 2
Log Frequency
L47
FR1
FR2
Base 1
Base 2
Reinforcers/min
Log Frequency
L49
Base 1
Base 2
FR1
FR2
Log Frequency
L54
FR1
FR2
Base 1
Base 2
Reinforcers/min
Log Frequency

Table 1

Magnitude and significance of curve-shifts produced by food restriction

Rat	Mean Shift (Log10 pulses)	SEM	*df*s	*F* value	p
L28	-0.08	0.007	1, 21	33.86	0.000**
L38	-0.11	0.017	1, 23	103.29	0.000**
L40	-0.09	0.006	1, 23	44.99	0.000**
L48	-0.07	0.01	1, 23	13.38	0.001**
L51	-0.33	0.009	1, 23	356.97	0.000**
L41	0.008	0.01	1, 23	0.13	0.718
L45	0.001	0.005	1, 17	0.01	0.94
L47	-0.01	0.006	1, 23	0.57	0.456
L49	-0.003	0.009	1, 23	0.03	0.866
L54	-0.032	0.008	1, 22	3.85	0.063

*p < .05. **p < .01

Table 2

Food restriction changes in the slope and maximum reward rate derived from broken-line functions for all subjects.

Rat	Food restriction Session	Slope: Mean change from baseline values	Max.rate: Mean change from baseline values
L28	1	48.12	-0.93
	2	101.97	-0.30
L38	1	6.14	4.86*
	2	21.73	4.71*
L40	1	58.06	-1.13
	2	124.02	-0.98
L41	1	133.1	-3.93*
	2	226.18	-4.79*
L45	1	19.63	-3.58*
	2	45.11	-3.04*
L47	1	75.93	-1.73
	2	127.25	-0.68
L48	1	1.22	0.95
	2	100.20*	0.04
L49	1	137.35	-0.16
	2	67.8	-1.87
L51	1	14.02	18.10*
	2	3.16	17.95*
L54	1	21.65	2.61*
	2	15.58	1.5*

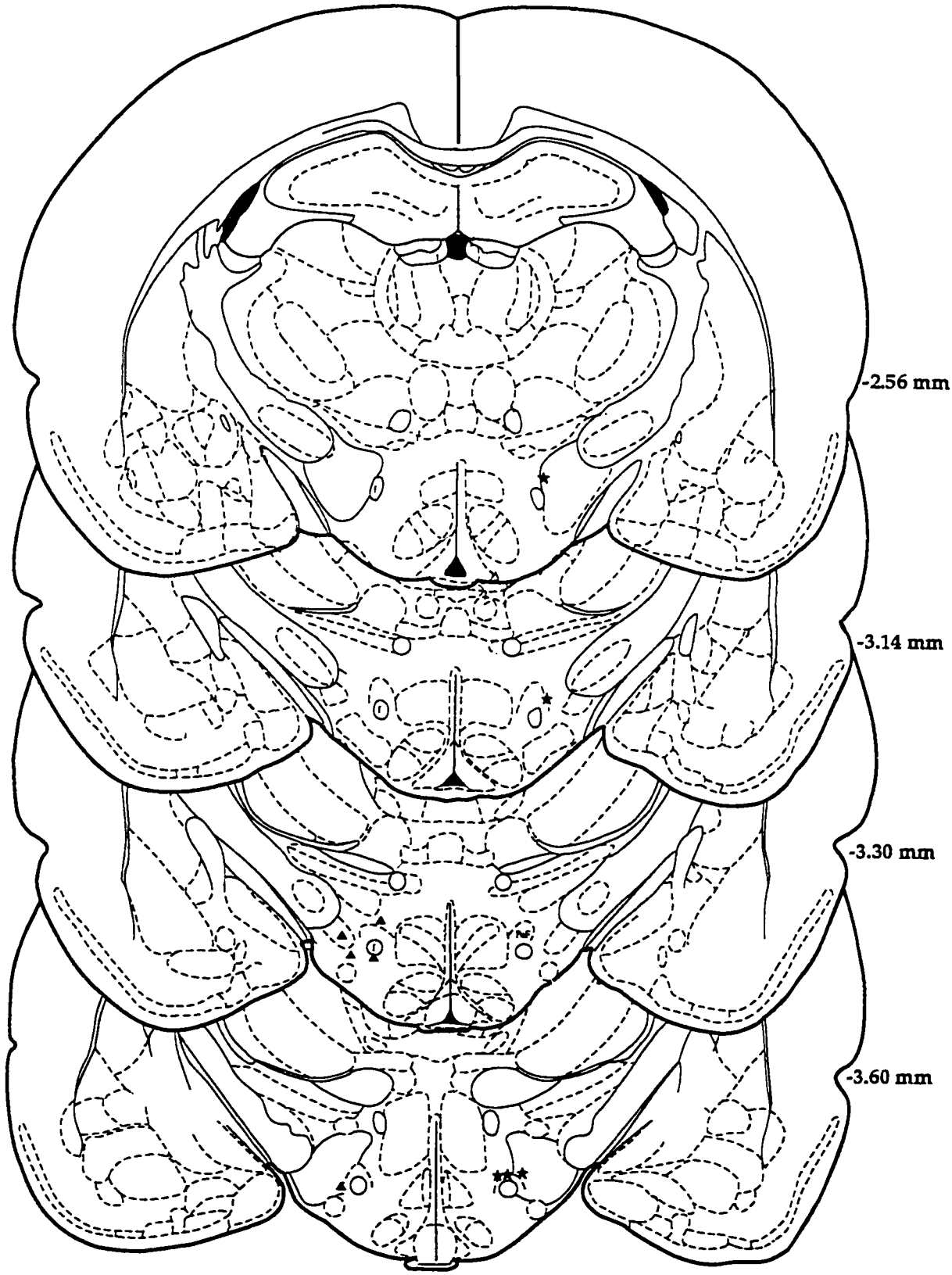
*$p < .05$.

broken-line function was calculated by dividing the change in stimulation frequency (log_{10}) by the change in the number of reinforcers. The maximum number of reinforcers was the value from the upper asymptote of the fitted broken line functions. The six slope values and the six maximum number of reinforcers values obtained on each food restriction day were compared to baseline values using the Dunnett test for multiple comparisons.

Histological Results

The five subjects that were responsive to the effects of food restriction had electrode tips located immediately dorsal or dorso-lateral to the fornix in the perifornical region of the LH (Figure 4). In the remaining subjects who showed relatively stable thresholds following food restriction, electrode tips were located in extra-perifornical sites: 1 was located just outside the dorsal border of the PFH , 3 were in the midlateral LH and 1 was ventral to the fornix in the LH.

Figure 4. Coronal sections illustrating electrode placements for subjects who were responsive the effects of food restriction (right side: star), and subjects who were unresponsive to the effects of food restriction (left side: triangle).


-2.56 mm
-3.14 mm
-3.30 mm
-3.60 mm

Discussion

The illustration of the leftward horizontal displacement of food restriction rate-frequency curves relative to baseline curves in a subset of subjects indicates that chronic food restriction enhanced the rewarding effect of LHSS. These results are consistent with those obtained elsewhere (Abrahamsen et al., 1995). The fact that only a portion of the subjects were responsive to this manipulation emphasizes the importance of the placement of the stimulating electrode in producing this effect. Histological observation revealed that electrode tips for responsive subjects were located immediately dorsal or dorsolateral to the fornix, whereas electrode tips for unresponsive subjects were located ventral to the fornix or lateral to the PFH. This finding is consistent with previous evidence showing that electrodes located dorsally and dorsolaterally to the fornix are responsive to the effects of chronic food restriction (Carr & Wolinsky, 1993).

What does this evidence imply for the relationship between food reward and LHSS? On the basis of these types of results it has been proposed that the stimulation at these sites mimics the rewarding effect of food (Hoebel, 1968; Hoebel and Teitelbaum, 1969). Proponents of this idea have implied that the signal elicited by the stimulation produces one that is identical to that produced by a piece of food. The present findings cannot provide support this hypothesis, rather they suggest that food restriction produces reward value changes for food and LHSS of the same direction. One could support Hoebel and colleagues hypothesis by examining choice among gustatory stimuli and LHSS in a manner that would indicate the relative change in reward value for these two stimuli. If food restriction would reduce responding for food and LHSS to the same degree, one could

more appropriately propose that the signal produced by the stimulation is like that produced by a piece of food. Moreover, to support this hypothesis it is necessary to illustrate the modulation of LHSS by a variety of other manipulations that alter food intake, such as acute food deprivation.

Conover and Shizgal (1994a, 1994b) and Conover et al. (1994) proposed that the reward signal generated by the stimulation is a general one that is not specific to any one type of goal object. In their studies they demonstrated that LHSS remained stable in the face of two acute manipulations: postingestive feedback of sucrose and sodium deprivation. They speculated that any chronic need state, such as that implemented by food restriction, could activate emergency mechanisms that influence this reward substrate to increase the animal's likelihood of procuring the essential goal object. During chronic food restriction a fair amount of body fat is lost. The fat store is the most valuable to the hungry animal because it can be mobilized to provide energy in cases where short-term stores are depleted. Thus, when fat stores are low the animal must take action to procure food in order to survive. Perhaps under these circumstances emergency mechanisms are employed that enhance the reward value of food, thus, increasing the animals chances of survival.

In order to characterize the neural substrate responsible for the rewarding effects of the stimulation it is necessary to investigate the influence of various energy balance manipulations on LHSS. Carr (1996) has suggested that signals that affect "chronic tissue need" have a more potent effect on LHSS. Carr and colleagues demonstrated that inducing cellular glucopenia and blocking the synthesis of free-fatty acids both fail to alter LHSS (Cabeza de Vaca et als., 1998). Although there is no consistent evidence of such, it

could be that acute metabolic signals modulate reward in other areas of the LH. Perhaps there are different subsystems in the LH that modulate the rewarding effects of different energy states.

Because of the apparent heterogeneity of LH reward fibers, it is difficult to interpret the results of experiments using different subjects and thus, different stimulation sites. A strong test of the hypothesis that certain electrode sites in the LH are preferentially sensitive to long-term metabolic signals would be to measure the ability of acute and chronic energy balance manipulations to modulate LHSS within the same subjects. Therefore, one of the goals of the present work is to test the influence of acute food deprivation on self-stimulation thresholds in subjects that are responsive to food restriction.

Experiment 2

The previous experiment demonstrated that chronic food restriction enhances the reward effectiveness of LHSS for a subset of subjects. The observation that only a portion of the subjects show this effect suggests the presence of dissimilar reward circuitry that can be activated by stimulating different LH sites. That the activity of a subset of reward neurons was modified by chronic food restriction implies that some reward circuitry is sensitive to metabolic signals involved in the long-term regulation of body weight. To characterize these neurons as preferentially sensitive to long-term metabolic signals requires examining the influence of acute energy balance manipulations on LHSS.

Chronic food restriction generates long-term regulatory signals of adiposity and body weight regulation. However, in certain restriction paradigms animals are fed a limited amount of food once a day, thus, there is likely a modulation of short-term metabolic signals as well. The subjects in this study and in the studies of Carr and colleagues are fed a small quantity of food once per day (Carr & Wolinsky, 1993; Carr & Papadouka, 1994). Furthermore, it was observed that rats consumed the food immediately in the present study. Therefore, one could just as well argue that the enhancement of LHSS following chronic food restriction is due to short-term signals arising from acute food deprivation. In order to characterize this region as exclusively responsive to long-term signals or a combination of both short- and long-term signals, it is necessary to observe the stability of LHSS in the face of acute food deprivation alone.

Acute manipulations are those that affect short term energy stores. Early reports indicated that LHSS could be modulated by acute signals arising from food deprivation (Margules & Olds, 1958) and postingestive consequences of food (Hoebel, 1968; Hoebel & Teitelbaum, 1969). Conversely, recent reports using more reliable response measures have shown that responding for LHSS remains stable in the face of gastric loading of sucrose (Conover & Shizgal, 1994b), sodium deprivation (Conover, Woodside & Shizgal, 1994), cellular glucopenia induced by 2-DG (Cabeza de Vaca et als., 1998) and decreases in plasma free-fatty acids induced by nicotinic acid (Cabeza de Vaca et als., 1998). The effect of acute food deprivation on LHSS is still a matter of disagreement. There are numerous reports of no change or even inhibitory effects following food deprivation (Atrens & Sinden, 1975; Giovino & Wise, 1986; Olds, 1958). Among the cases where the effect is reported there is disagreement concerning the minimum necessary length of the deprivation period. In two cases food deprivation effects were found after 24 hours (Carr & Simon, 1984; Rossi & Panksepp, 1992). Another study, however, found that 48 hours of food deprivation is required to observe an enhancement of LHSS (Atrens, Williams, Brady & Hunt, 1982).

Experiment 2 was carried out to test the effect of 48 hours of food deprivation on LHSS in subjects who were either responsive or unresponsive to the effects of long-term food restriction. A 48 hour period was chosen over a shorter period to be certain that the subjects were sufficiently food deprived. An advantage of the present investigation is that the same subjects were used to test the effects of acute deprivation as those employed to test the effect of chronic food restriction in Experiment 1. Accordingly, the stimulation site

remains the same, permitting one to characterize the reward substrate that is activated by the stimulation at that particular site.

Method

Subjects and Surgery

Nine of the ten rats that participated in the previous experiment were used as subjects. See Subjects and Surgery pp. 26.

Procedure

The equipment used for this experiment was identical to that described in Experiment 1.

Employing stimulation parameters and a testing procedure identical to that used in Experiment 1 (see Procedure pp. 27), rate-frequency data were collected during two phases of the experiment. During the first phase rats were fed *ad libitum* and rate-frequency data were collected daily until stable baseline measures were obtained. During the second phase, all food was removed from the home cage and rats were food-deprived for a period of 48 hours. During this time no testing was performed. Following the deprivation period rats were re-tested for self-stimulation.

Results

With the exception of one subject, a 48 hour period of food deprivation did not enhance self-stimulation thresholds as shown in Figure 5. An examination of the rate-frequency curves for subject L28 that showed a statistically significant decrease in threshold illustrates that the points of the food deprivation curve are not positioned to the left of the points of the baseline curves (Figure 6). Broken-line functions were fit to the six individual curves obtained per day to calculate threshold values. Figure 7 illustrates that, in one case, a broken-line function did not provide an accurate representation of the data. Thus, it is argued that the apparent change in threshold for L28 is not due to and actual change in the stimulation frequency required to maintain half-maximal responding, rather, the change in threshold is due to the poor fit of the broken-line functions to the data. One other subject (L38) displayed a lowered threshold that was statistically significant, but the magnitude of change considered a very small and meaningless change. Furthermore, by examining the rate-frequency curves for this subject one can see that there is no leftward lateral displacement of the deprivation curve relative to baseline curves. Finally, subject L45 showed a statistically significant increase in thresholds following food deprivation. The remaining subjects displayed relatively stable rate-frequency curves following food deprivation. The rate-frequency curves for the subjects that were responsive to the effects of food restriction in Experiment 1 are represented in Figure 6. The rate-frequency curves for the subjects that were unresponsive to the effects of food restriction in Experiment 1 are represented in Figure 8. Table 3 shows the magnitude of mean threshold changes and their significance values for all subjects. Table 4 lists any changes in the maximum number

Figure 5 Bar graph representing the mean changes in threshold (stimulation frequency) and their significance levels produced by acute food deprivation for all subjects.

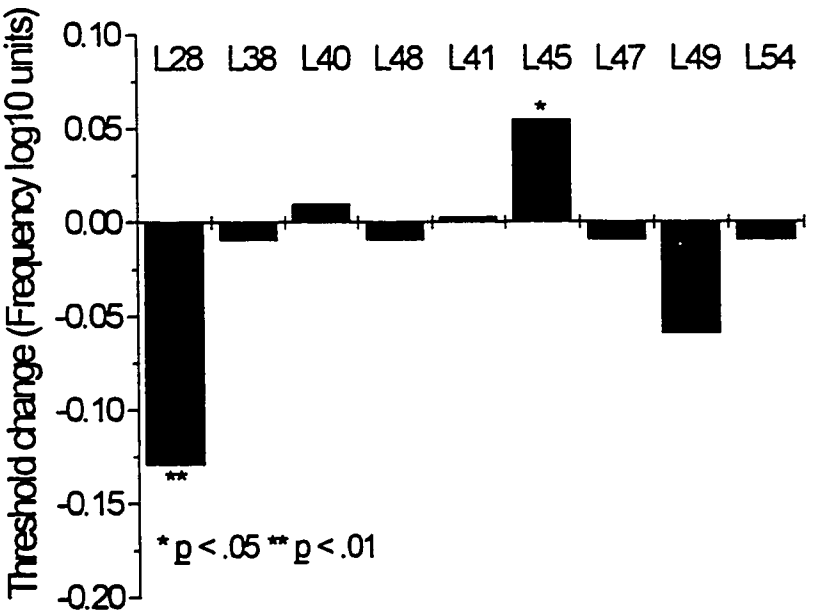

Threshold change (Frequency log10 units)
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
L28
L38
L40
L48
L41
L45
L47
L49
L54
*
**
* p < .05 ** p < .01

Figure 6 Rate frequency curves for subjects that were responsive to the effects of food restriction. Two curves represent the self-stimulation responding during baseline (base) and the other represents responding after 48 hours food deprivation. Each curve represents the average of six curves from a daily session and relate the number of reinforcers obtained per minute as a function of the stimulation frequency in log10 units. Vertical bars surrounding each point represent the standard error of the mean. Numbers in the legend refer to the session day.

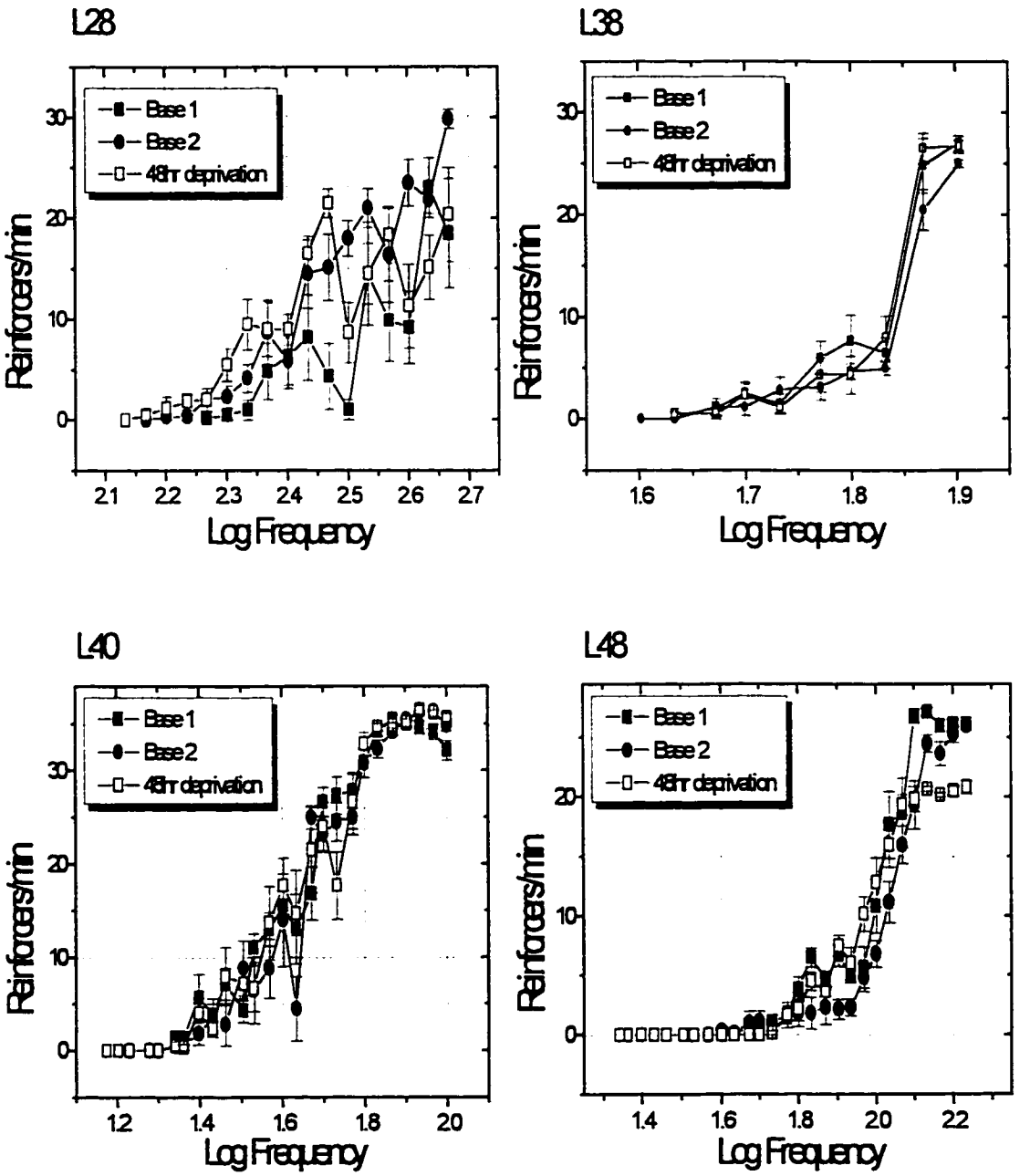
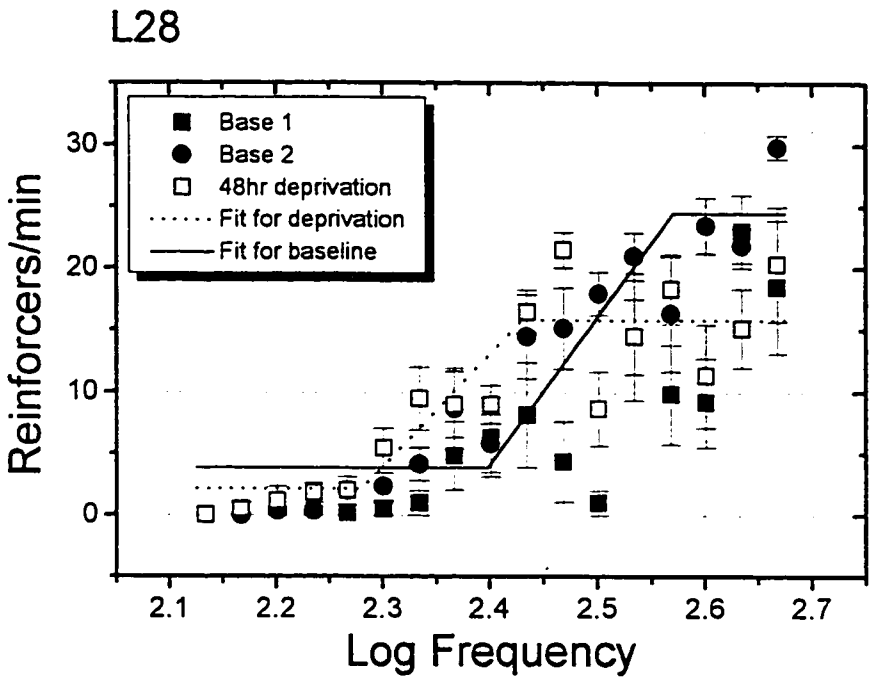

L28
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency
L38
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency
L40
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency
L48
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency

Figure 7 Top: Illustration that the broken-line function provides a poor representation of the rate-frequency data of L28. Bottom: An example of the broken-line function providing a good representation of rate-frequency data of L54.


L28
Base 1
Base 2
48hr deprivation
Fit for deprivation
Fit for baseline
Reinforcers/min
30
20
10
0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
Log Frequency

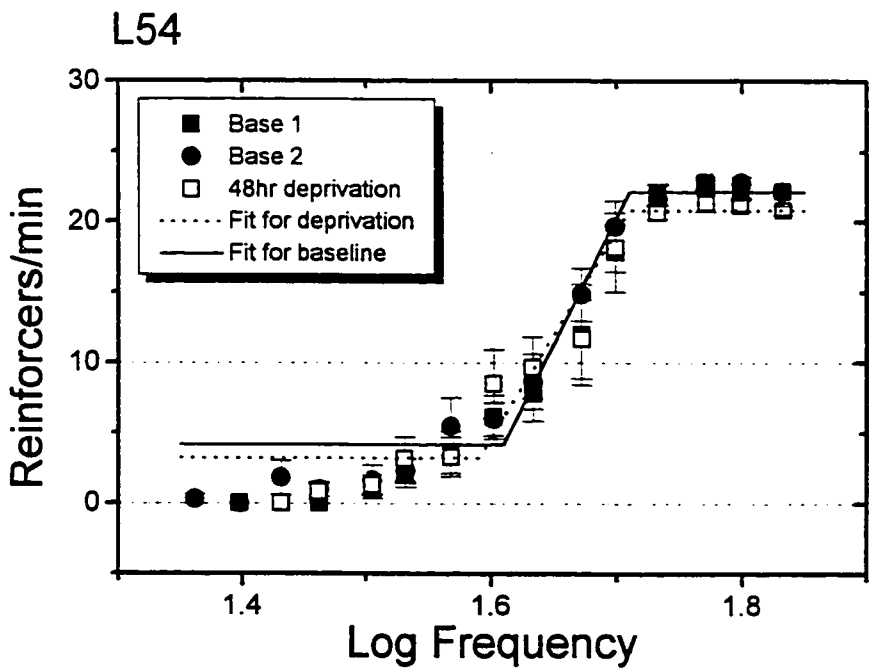
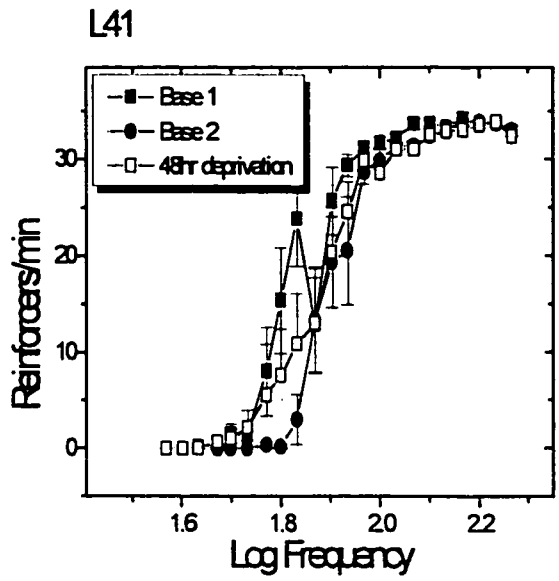
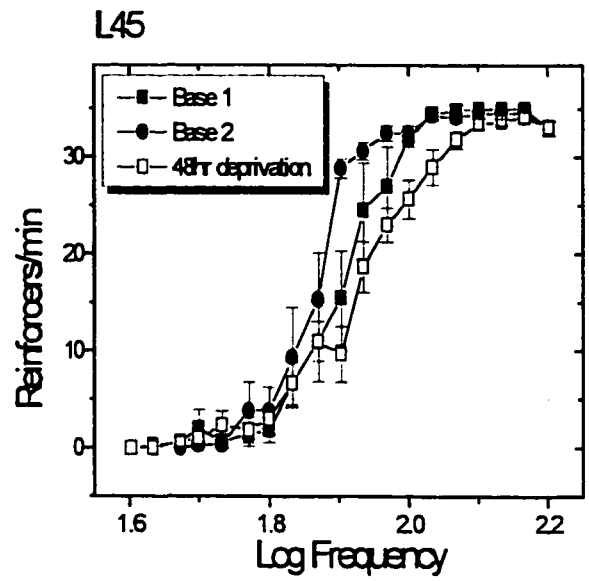
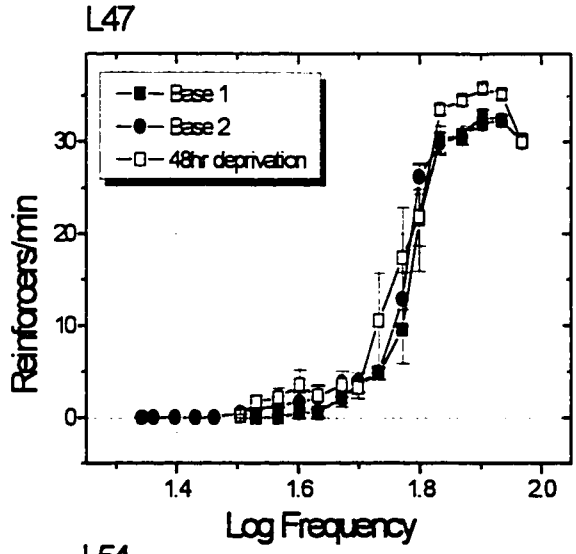
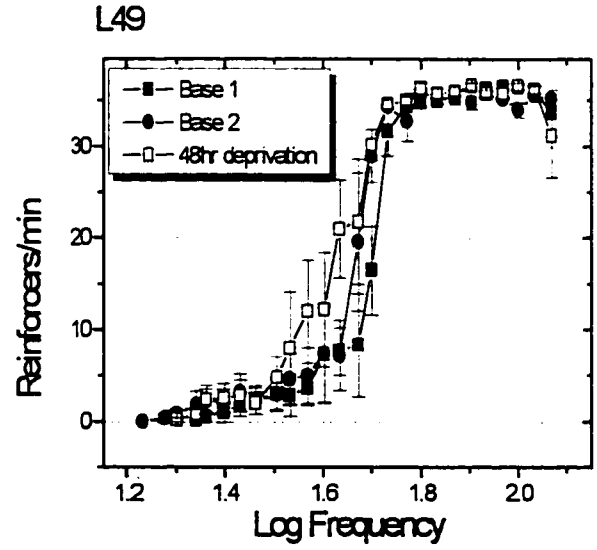

L54
Base 1
Base 2
48hr deprivation
Fit for deprivation
Fit for baseline
Reinforcers/min
30
20
10
0
1.4
1.6
1.8
Log Frequency

Figure 8 Rate frequency curves for subjects that were unresponsive to the effects of food restriction. Two curves represent the self-stimulation responding during baseline (base) and the other represents responding after 48 hours food deprivation. Each curve represents the average of six curves from a daily session and relate the number of reinforcers obtained per minute as a function of the stimulation frequency in log10 units. Vertical bars surrounding each point represent the standard error of the mean. Numbers in the legend refer to the session day.


L41
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency


L45
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency


L47
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency


L49
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency

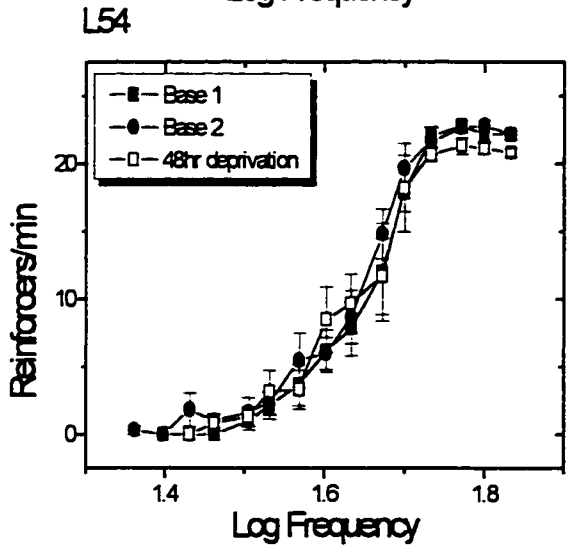

L54
Base 1
Base 2
48hr deprivation
Reinforcers/min
Log Frequency

Table 3

Magnitude and significance of curve-shifts caused by 48hr food deprivation

Rat	Mean Shift (Log10 pulses)	SEM	*df*s	*F* value	p
L28	-0.13	0.02	1, 16	9.49	0.008**
L38	-0.01	0.002	1. 16	7.29	0.016*
L40	0.01	0.009	1, 17	0.16	0.692
L48	-0.01	0.002	1, 16	3.56	0.056
L41	0.003	0.014	1, 17	0.01	0.922
L45	0.055	0.01	1. 17	7.15	0.017*
L47	-0.01	0.006	1, 17	0.61	0.445
L49	-0.06	0.017	1, 17	2.62	0.125
L54	-0.01	0.009	1, 17	0.32	0.58

*p < .05. **p < .01

Table 4

Food deprivation changes in the slope and maximum reward rate derived from broken-line functions for all subjects.

Rat	Baseline Session	Slope: Mean Change from Baseline	Max.rate: Mean Shift from Baseline
L28	1	188.89	-6.64
	2	183.98	-10.84*
L38	1	44.15	2.6
	2	55.99	1.66
L40	1	81.79	1.86*
	2	8.13	0.36
L41	1	34.89	-0.48
	2	138.58	-0.12
L45	1	19.63	-0.08
	2	45.11	-0.74
L47	1	374.07*	2.9*
	2	239.13	2.19*
L48	1	214.22*	-5.82*
	2	25.14	-5.04*
L49	1	18.97	0.49
	2	32.79	0.3
L54	1	161.85	-1.04
	2	22.96	-1.55*

* $p < .05$

of reinforcers obtained and slope for between curves obtained during baseline and after food deprivation.

The treatment of the raw data is explained in Experiment 1 (pp. 30). Half-maximal threshold values were used to examine any horizontal displacement of the curves produced by food deprivation. Twelve threshold values were obtained from grouping the values obtained on two consecutive baseline days. These were compared to the six threshold values obtained following food deprivation using a one-way ANOVA. The slope of the broken-line function was calculated by dividing the change in stimulation frequency (log) by the change in the number of reinforcers. The maximum number of reinforcers was the value from the upper asymptote of the fitted broken line functions. The six slope values and the six maximum number of reinforcers values obtained on each food restriction day were compared to baseline values using the Dunnett test for multiple comparisons, calculated with a statistical expert system (RS/1).

Discussion

If short-term food deprivation enhanced the rewarding effect of the stimulation one would expect a leftward lateral shift in the rate-frequency curves relative to baseline rate-frequency curves. However, this was not what was found. There was only subject (L28) that had a statistically meaningful decrease in threshold, but when one examines the rate-frequency curve for this subject there is no apparent leftward shift following deprivation. As illustrated in the results, the extreme variability of the rate-frequency curves for L28 confounded the broken-line fitting used to calculate threshold values. Thus, the statistical outcome is believed to be a result of this variability rather than an actual curve shift. In the case of L45, a subject that showed a slight elevation in threshold, the rate-frequency curves illustrates a rightward lateral shift. In this case it can only be hypothesized that signals arising from food deprivation inhibited the neurons responsible for the rewarding effect of LHSS at this stimulation site.

How do the present results compare to those reported in other studies? For the most part, investigations into the effects of food deprivation have been pursued without the use of response measures that can differentiate reward from performance capacity. This can be problematic in cases where activity levels may change as a result of the manipulation. One exception is the work of Giovino and Wise (1986). They also employed rate-frequency curves similar to the ones used here to examine the effects of deprivation on LH stimulation. They found that deprivation did not decrease self-stimulation thresholds, nor did it alter the response capacity of the rat. Likewise, the findings of the present experiment indicate that LHSS was not facilitated by food deprivation. However, unlike

the study of Giovino and Wise, two subjects (L48 and L54) in the present investigation demonstrated a decrease in asymptotic reward rates, indicative of reduced response capacity, while one other (L47) showed increased reward rates indicative of enhanced response capacity (Table 4). Its not understood why these discrepancies exist between the two studies, however, the occurrence of the response changes in the present study can help to explain how rate-dependent response measures can confound the results of a study.

Numerous investigators have inferred increases in reward value from increases in response rate when examining the effects of acute food deprivation on LHSS (Atrens et al., 1982; Deutsch & DiCiara, 1967; Margules & Olds, 1962). The data in the present study illustrate that response parameters can change independent of changes in reward value. An inspection of the rate-frequency curves for subjects L47 and L48 reveals that the upper asymptote of curves can shift irrespective of any lateral shift in the rising portion of the curve. On the other hand, other studies inferred changes in reward effectiveness produced by food deprivation by employing a method of limits (Carr & Simon, 1984; Rossi & Panksepp, 1992). Accordingly, thresholds are determined by increasing and decreasing stimulation frequencies until they meet a certain response criterion. Unlike the rate measures, this measure does not rely on changes in response rate to infer changes in reward value. However, this method is still unable to distinguish changes in responding from changes in threshold. Thus, the results of the studies using a method of limits may have the confounding influence of change in motor capacity that has been observed following acute food deprivation. Otherwise, changes in threshold seen after acute food deprivation in these studies could be the result of changes in reward effectiveness. As is

discussed later, it could be that acute manipulations of energy balance affect self-stimulation at sites in the LH other than the ones tested in the present study.

The current findings shed light on the nature of the reward substrate that is activated at sites that are responsive to the effects of food restriction. In the preceding experiment it was confirmed that a subset of subjects with stimulation sites in the PFH are responsive to chronic food restriction. It is likely that the long-term food restriction produced significant decrease in adiposity, which is believed to generate long-term metabolic signals. However, in Experiment 1, short-term metabolic signals were also likely mobilized because the rats consumed their daily ration in a brief period of time, remaining without food until the following day. It could be argued that it is the stimulation of short-term signals that mediates the enhancement of LHSS by food restriction. The present findings indicate that this is not the case. A 48 hour period of food deprivation failed to enhance LHSS in subjects that were responsive to food restriction. In light of these results, it can be more reasonably argued that neural signals arising from stimulation of the PFH are sensitive to either long-term metabolic signals arising from food restriction or a combination of short- and long-term signals produced by acute deprivation and chronic food restriction.

The present findings are consistent with demonstrations that short-term signals fail to alter LHSS. Acute manipulations of sodium deprivation (Conover et al, 1994), gastric loading of sucrose (Conover & Shizgal, 1994b), cellular glucopenia (Carr, 1996) and lipoprivation (Carr, 1996) all fail to alter LHSS. On the basis of their work, Conover & Shizgal (1994b) proposed that the circuitry responsible for the rewarding effects of the stimulation is a general reward substrate. One of the proposals that Conover and Shizgal provide in response to the finding that chronic food restriction enhances LHSS is that

chronic manipulations such as these may activate emergency mechanisms. These mechanism would serve to bias the reward circuitry so as to strengthen goal-directed behavior for the vital resource that is being withheld. Given that acute food deprivation is not as threatening to the animal as chronic food restriction and that it did not enhance LHSS, this proposal may still apply. A strong test of this hypothesis would be to implement a physiological emergency unrelated to feeding, such as water deprivation induced by a diuretic. If water deprivation would enhance LHSS in a similar manner one could provide support for the idea that these reward neurons are not specific to feeding. This study is being considered for future research.

What do these results suggest about the nature of the rewarding signal produced by the stimulation? Since chronic food restriction, but not acute food deprivation, alters self-stimulation in certain sites it is not plausible to think of a signal identical to that created by a rewarding piece of food. Such a signal would imply that all manipulations that alter choice for food would alter self-stimulation. Alternatively, there could exist numerous reward subsystems related to feeding. Although, histological examination of the sites tested in this study reveal placements in various areas of the tuberal LH, perhaps there are sites apart from those tested that are responsive to signals arising from acute manipulations. In this regard, it is interesting to note that acute manipulations of cellular glucopenia produced by 2-DG infusion decrease rate-frequency determined thresholds for LHSS at sites in the anterior LH (Frutiger and Drinkwine, 1992). To determine if there are sites responsive only to short-term signals one could examine the effects of other acute manipulations on self-stimulation at 2-DG responsive areas in the anterior LH. To account for a reward system that has discrete regions that are sensitive to different metabolic

signals one can speculate that there are local currencies that evaluate food stimuli in accordance with the state of energy balance. This type of currency evaluation would not carry the multi-sensory information about the food, instead, it might evaluate limited information about the food such as its capacity to renew either short- or long-term energy stores. Evaluations of the multidimensional properties of the food would be made at points upstream from the local currency evaluation.

What other signals implicated in the long-term regulation of energy balance alter LHSS at sites responsive to the effects of food restriction? It is not yet understood which signals are mediating the process whereby food restriction enhances perifornical self-stimulation. Experiment 3 was performed to investigate whether leptin, a hormone implicated in the control of food intake and long-term body weight regulation, is involved.

Experiment 3

The preceding two experiments provided support for the hypothesis that the rewarding effect of PFH stimulation is preferentially sensitive to long-term regulatory signals. Chronic food restriction was shown to enhance the rewarding effect produced by PFH stimulation, whereas acute food deprivation failed to affect the reward effectiveness of the stimulation regardless of stimulation site. Which long-term regulatory signals are involved in the process whereby food restriction facilitates perifornical self-stimulation?

Previous investigations have suggested that an opioid mechanism is mediating the effects of food restriction on LHSS. Carr and Wolinsky (1993) have shown that ICV administration of naltrexone, an opiate antagonist, reversed the enhancement of LHSS by food restriction. These results were only present in subjects that showed decreased thresholds in response to food restriction, suggesting that opioids are involved in the specific process whereby LHSS is facilitated. Opioid agonists have also been demonstrated to play a role in ingestive behavior. When opioids are directly injected into the LH they stimulate food intake (Gosnell & Levine, 1996; Gosnell, Morley & Levine, 1986). Furthermore, there is evidence that ICV infusion of opioid antagonists reduces NPY-induced feeding (Kotz, Grace, Billington & Levine, 1993). Perhaps an opioid mechanism is interacting with the NPY feeding system to mediate the effect of food restriction on LHSS. This raises the possibility that other signals that mediate NPY-induced feeding are also involved.

One such signal that is closely tied to the NPY feeding system is the recently identified protein product of the mouse obesity gene, leptin (Zhang, Proenca, Maffei, Barone, Leopold & Friedman, 1994). Secreted by adipocytes, leptin informs the brain about the size of the fat depot, providing information that appears to be crucial to match metabolism and food intake to energy demands. Direct administration of leptin into the hypothalamus potently reduces food intake and stimulates body weight loss in rats (Satoh et al., 1997). Leptin receptors are expressed in hypothalamic areas important for the control of food intake, including the arcuate nucleus (ARC), PFH and LH (Hakansson, Brown, Ghilardi, Skoda & Meister, 1998). This evidence suggests that the brain is the primary target of the anorexic effects of leptin. The long form of its receptor (Ob-R) is located in high concentrations in the ARC where its inhibitory actions on food intake take place, in part, by inhibiting NPY gene expression (Schwartz, Seeley, Campfield, Burn & Baskin, 1996). In normal rats leptin administration blunts the effects of fasting to increase hypothalamic NPY messenger RNA (mRNA) levels (Schwartz et al., 1996). More remains to be understood about the mechanisms responsible for the reduction in food intake induced by leptin. Particularly, it not known if leptin influences the brain reward mechanisms governing goal-directed behavior for food. If, as can be assumed, leptin does affect brain reward circuitry, it is of interest to determine which components of this circuitry are involved.

The idea that leptin acts as a long-term regulatory signal is derived from several lines of evidence. First, leptin is secreted in direct proportion to adipose mass (Maffei et al., 1995). Weight loss after repeated leptin administration appears to be due entirely to loss of fat (Chen et al.,1996). Finally, leptin secretion is not stimulated acutely in response to

meals as are glucose and insulin (Korbonits et al., 1997). In light of these long-term signaling characteristics, Experiment 3 was carried out to investigate whether ICV leptin administration would inhibit perifornical self-stimulation It is of interest to find out whether leptin could be involved in the enhancement of self-stimulation produced by food restriction. Presumably, leptin levels are low during periods of low body weight and fat depletion, thus the absence of the inhibitory actions of leptin could be contributing to the process whereby food restriction enhances LHSS.

Method

Subjects and Surgery

Nine out of the 10 rats that participated in Experiment 1 were used as subjects in the present experiment. An additional group of 6 male Long Evans rats, with weights ranging between 450-550 grams, were used to assess the effects of an ICV leptin dose on food intake. These subjects were housed individually in plastic hardfloor cages with access to *ad libitum* food and water, and placed on a 12 hour dark/light reverse cycle in a temperature controlled room. All behavioral testing was conducted in the dark phase of the cycle. Electrodes and cannulae were implanted in these subjects in the same manner as those described previously (See Surgery pp. 26).

Apparatus

Food intake was measured with the use of a digital scale (Mettler PJ360 Deltarange) that provided readings within 0.0001 grams. Powdered food was placed in cups attached to aluminum sheets, that were molded to hang on the wall of the cage. A flat-bottomed bowl was fixed underneath each food cup to catch any falling food.

The equipment used for the BSR component of this experiment is identical to that described in Experiment 1.

Procedure

Leptin pilot testing

Adequate placement of ICV cannulae was tested by infusing Angiotensin II (2μg) intraventricularly and observing a substantial drinking response. Following a week of

habituation to a powdered food diet, rats received an intraventricular injection of either 2 μg of murine leptin (Peprotech Inc.) or vehicle (0.1 mol/l Tris, pH 7.35) given within 2 min in a volume of 1.6 μl. All injections were given between 1:00 and 2:00 p.m. in accordance with self-stimulation testing times. Food intake was measured for 4 following hours.

Behavioral testing

The effect of leptin LHSS was measured, first, after a period of chronic food restriction wherein body weights declined to 75-80% of baseline values and, then, after a period of *ad libitum* feeding wherein body weights were allowed to return to normal. The procedure that follows applies to both the food restriction and *ad libitum* feeding conditions. One hour prior to testing, vehicle (0.1 mol/l Tris, pH 7.35) or leptin (2 μg) was injected over a 2 min period in a volume of 1.6 μl. Employing similar stimulation parameters and testing procedure to those used in Experiment 1, rate-frequency data were collected following vehicle injection for two days preceding leptin administration. Data obtained from this baseline were compared to those collected on the day of leptin injection and days that followed. In cases where an inhibitory effect of leptin was observed, testing lasted 4 days. In all other cases testing was performed for two days following leptin administration.

Results

A 2 μg ICV injection of leptin produced a significant overall reduction in food intake relative to vehicle injected controls, $F(1, 7) = 6.56$, $p = .04$. As shown in Figure 9, leptin treated rats consumed less food than vehicle treated rats over four hours.

Food restriction decreased body weights by 20-25% from baseline values with initial values ranging between 534-615 g and food restricted values ranging between 396-489. Figure 10 represents the mean change in the thresholds produced by leptin during food restriction for all subjects. The graph on the left represent the subject that were responsive to the effects of food restriction that showed increased thresholds on all 4 testing days following leptin administration. Four out of the five subjects in this group had significant increases in thresholds for two or more days following leptin injection. Figure 11 illustrates the rate-frequency curves for the subjects that were responsive to food restriction. Table 5 displays the mean change in threshold values along with significance values for these same subjects. The graph on the right of Figure 10 represents subjects that were unresponsive to food restriction and that showed decreased thresholds following leptin administration. Figure 12 illustrates the rate-frequency curves for these subjects. Table 6 displays the mean change in threshold values along with significance values for these same subjects. None of the subjects that were unresponsive to food restriction showed a significant increase in threshold following leptin during food restriction. On the contrary, 3 out of the 4 subjects that were unresponsive to chronic food restriction exhibited significant decreases in threshold on at least one of the two days tested. Table 7

Figure 9. Mean food intake as a function of hour post-injection for leptin (2 μg) treated rats (n = 3) and vehicle treated rats (n = 3).

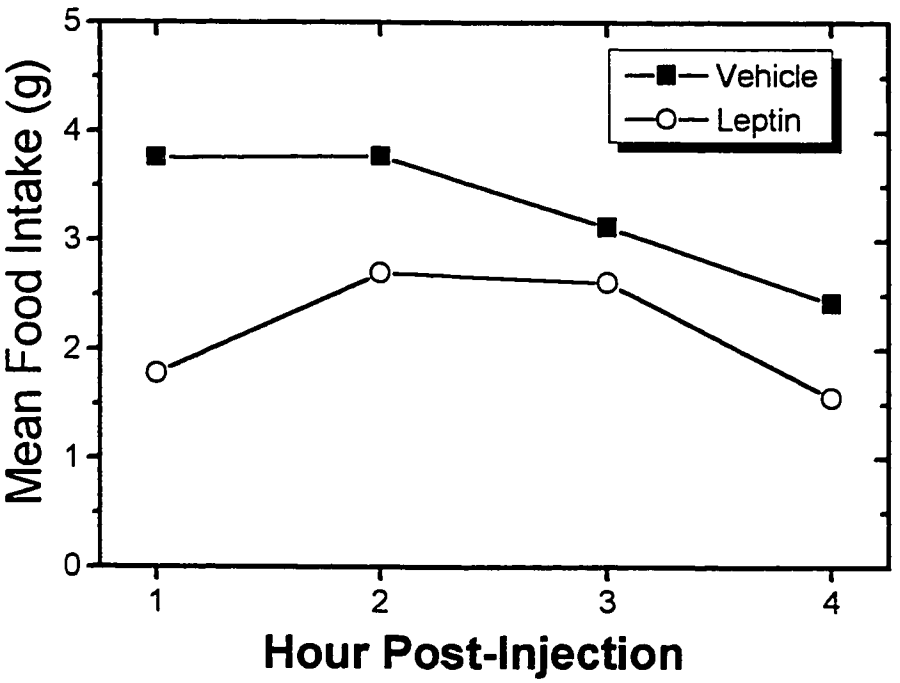
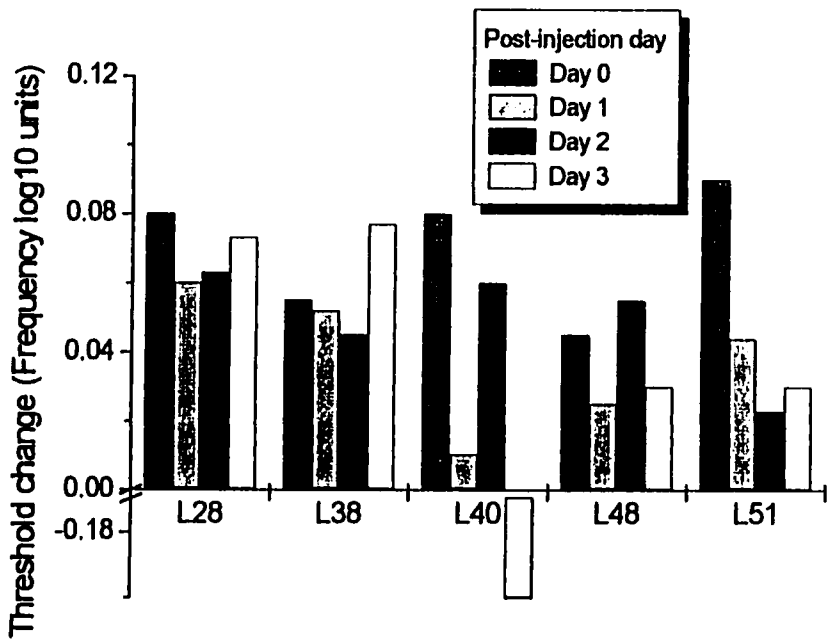

Mean Food Intake (g)
0
1
2
3
4
5
1
2
3
4
Hour Post-Injection
Vehicle
Leptin

Figure 10 Bar graph representing the magnitude of threshold changes produced by leptin during food restriction as compared to vehicle threshold values. Subjects that were responsive to the effects of food restriction are represented in the graph on the left whereas subjects that were unresponsive to the effects of food restriction are represented in the graph on the right.


Post-injection day
Day 0
Day 1
Day 2
Day 3
Threshold change (Frequency log10 units)
0.12
0.08
0.04
0.00
-0.18
L28
L38
L40
L48
L51

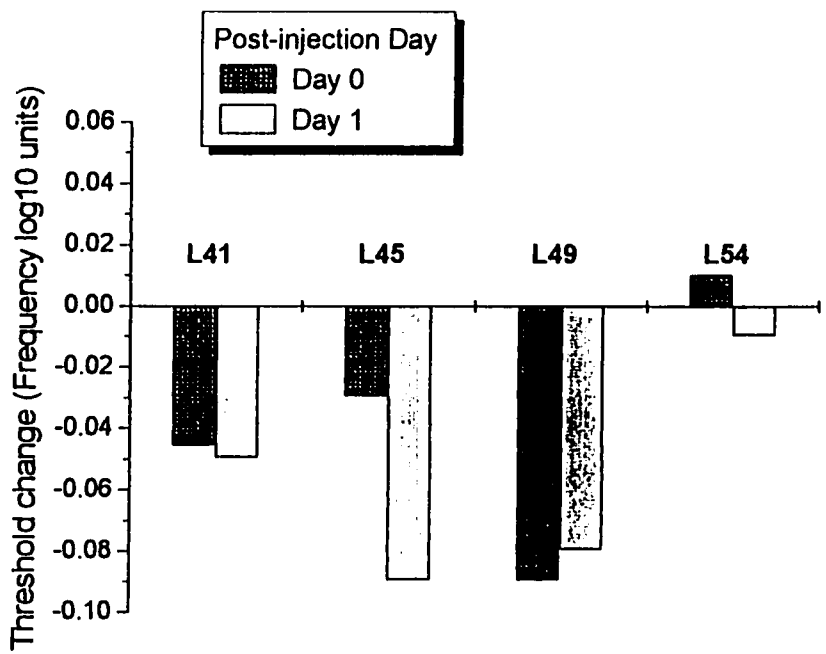
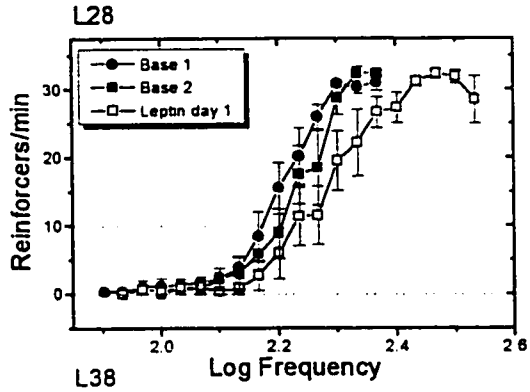
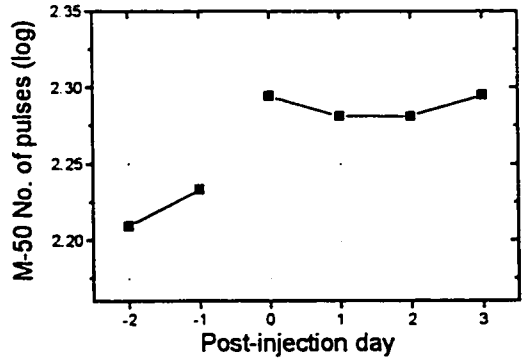
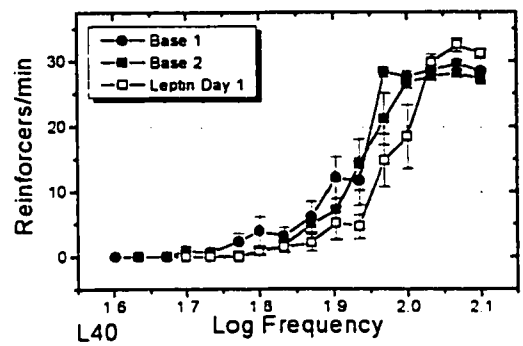
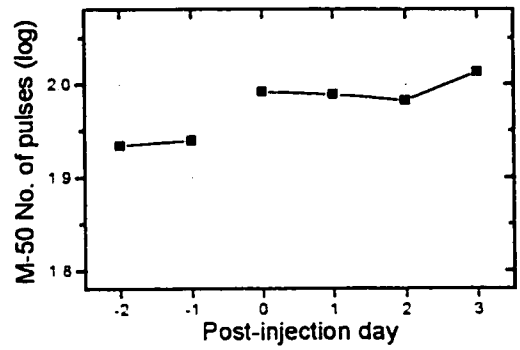

Post-injection Day
Day 0
Day 1
Threshold change (Frequency log10 units)
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
L41
L45
L49
L54

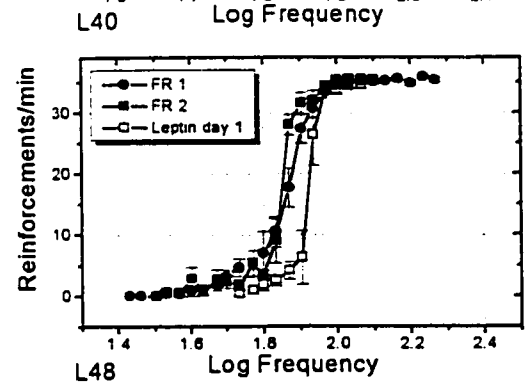
Figure 11. **Left**: Rate frequency curves for subjects that were responsive to the effects of food restriction in Experiment 1. These curves represent the effect of leptin during food restriction. Curves were created by averaging over the six curves of a daily session. Each curve relates the number of reinforcers obtained per minute as a function of the stimulation frequency in log10 units. Vertical bars surrounding each point represent the standard error of the mean. Numbers in the legend refer to the session day. **Right**: Mean threshold values for two days of vehicle treatment and for the four consecutive days following leptin administration. Each point represents the mean stimulation frequency (log10 units) required to maintain half-maximal reward rate.

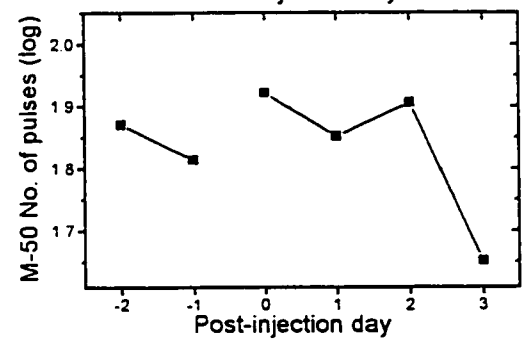

L28
Base 1
Base 2
Leptin day 1
Reinforcers/min
Log Frequency

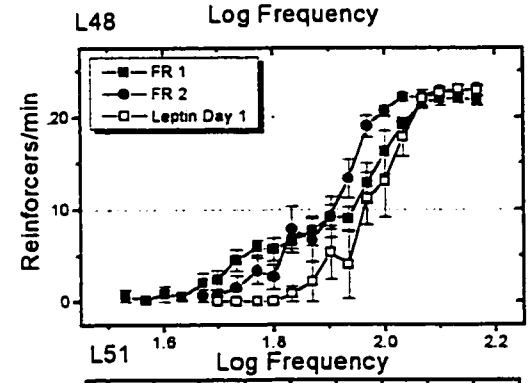

M-50 No. of pulses (log)
Post-injection day

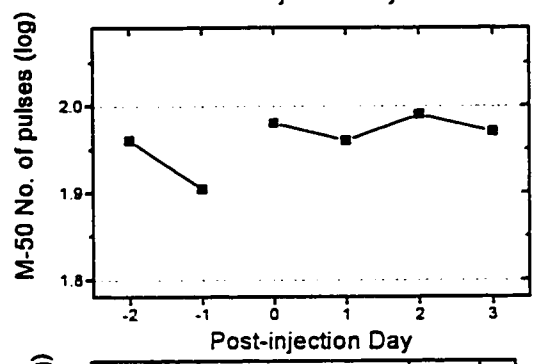

L38
Base 1
Base 2
Leptin Day 1
Reinforcers/min
Log Frequency

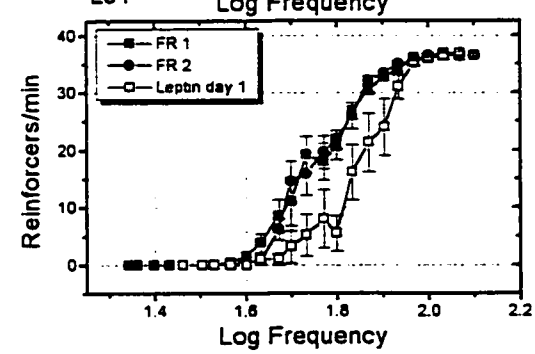

M-50 No. of pulses (log)
Post-injection day


L40
FR 1
FR 2
Leptin day 1
Reinforcements/min
Log Frequency


M-50 No. of pulses (log)
Post-injection day


L48
FR 1
FR 2
Leptin Day 1
Reinforcers/min
Log Frequency


M-50 No. of pulses (log)
Post-injection Day


L51
FR 1
FR 2
Leptin day 1
Reinforcers/min
Log Frequency

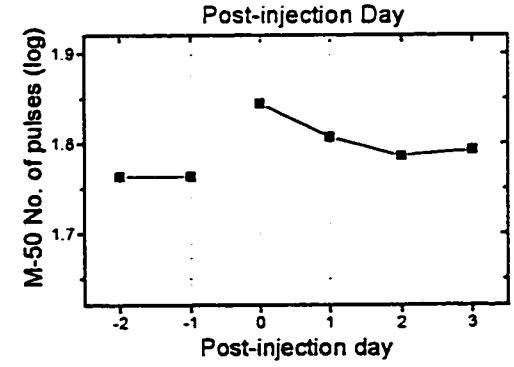

M-50 No. of pulses (log)
Post-injection day

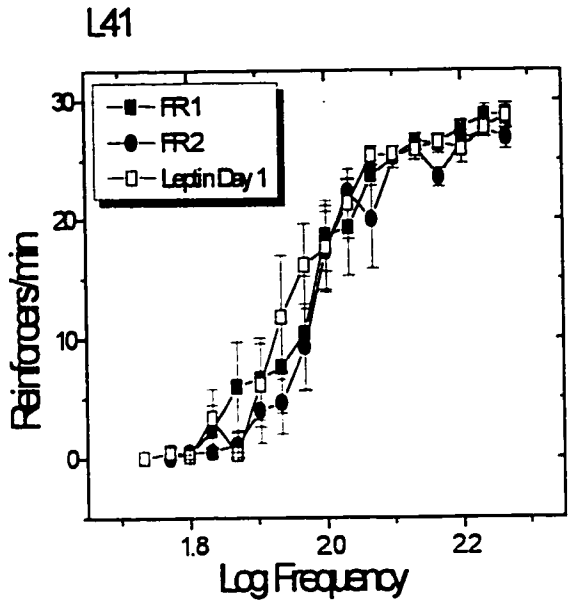
Table 5

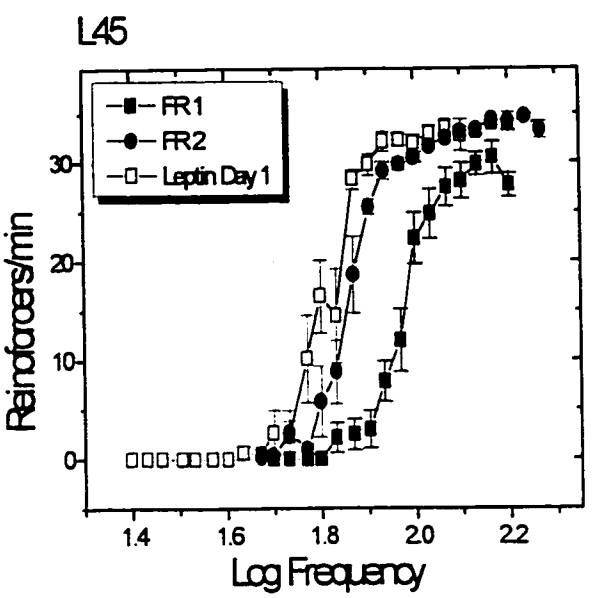
Magnitude and significance of curve shifts caused by leptin during food restriction for subjects that were responsive to the effects of food restriction.

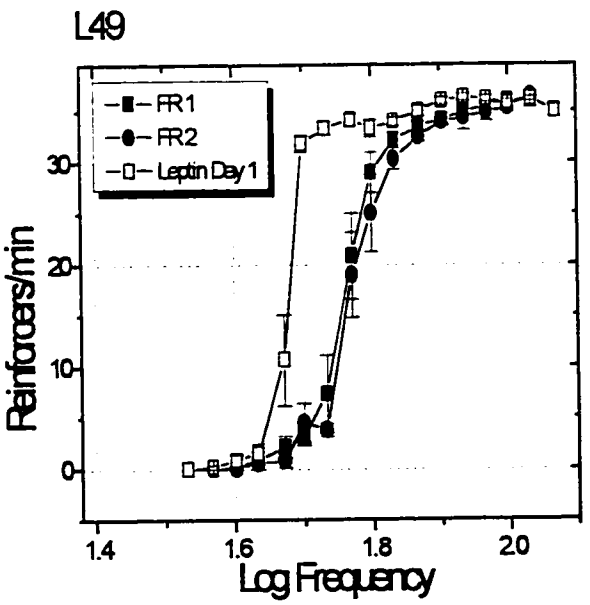
	Post-injection Day															
	1				2				3				4			
Rat	Shift (Log10 pulses)	SEM	*df*s	d	Shift (Log10 pulses)	SEM	*df*s	d	Shift (Log10 pulses)	SEM	*df*s	d	Shift (Log10 pulses)	SEM	*df*s	d
L28	0.08	0.021	1, 17	3.42**	0.06	0.021	1, 17	2.83**	0.063	0.021	1, 17	2.95*	0.073	0.021	1, 17	3.45**
L38	0.055	0.015	1, 17	3.59**	0.052	0.015	1, 17	3.42**	0.045	0.015	1, 17	2.96**	0.077	0.015	1, 17	5.03**
L40	0.08	0.017	1, 17	4.9**	0.01	0.016	1, 16	0.59	0.06	0.016	1, 17	3.76**	-0.19	0.017	1, 16	11.98**
L48	0.045	0.026	1, 17	1.69	0.025	0.026	1, 17	0.93	0.055	0.025	1, 17	2.1*	0.03	0.025	1, 17	0.97
L51	0.09	0.023	1, 17	3.87**	0.044	0.023	1, 17	1.89*	0.023	0.023	1, 17	0.98	0.03	0.023	1, 17	1.28

*$p < .05$. **$p < .01$.

Figure 12. Rate frequency curves for subjects that were unresponsive to food restriction in Experiment 1. These curves represent the effect of leptin during food restriction. Curves were created by averaging over the six curves of a daily session. Each curve relates the number of reinforcers obtained per minute as a function of the stimulation frequency in log10 units. Vertical bars surrounding each point represent the standard error of the mean. Numbers in the legend refer to the session day.


L41
FR1
FR2
Leptin Day 1
Reinforcers/min
Log Frequency


L45
FR1
FR2
Leptin Day 1
Reinforcers/min
Log Frequency


L49
FR1
FR2
Leptin Day 1
Reinforcers/min
Log Frequency

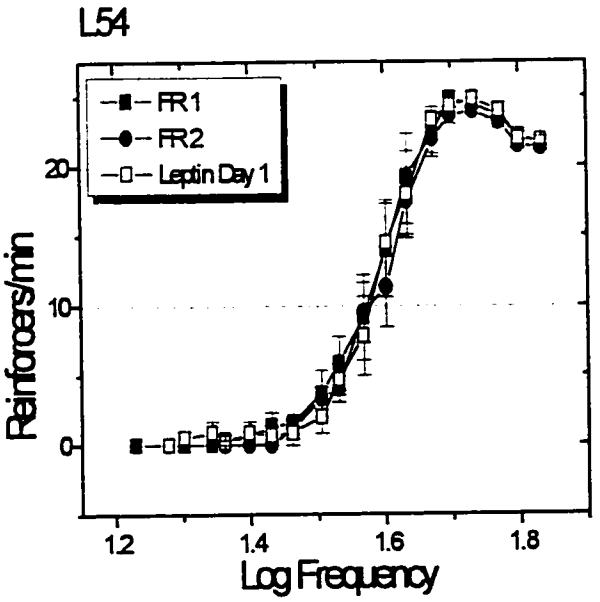

L54
FR1
FR2
Leptin Day 1
Reinforcers/min
Log Frequency

Table 6

Magnitude and significance of curve shifts caused by leptin during food restriction for subjects that were unresponsive to the effects of food restriction.

	Post-injection Day							
	Day 1				Day2			
Rat	Shift (log10 units)	SEM	*df*s	d	Shift (log10 units)	SEM	*df*s	d
L41	-0.046	0.019	1, 17	2.4*	-0.05	0.019	1, 17	2.58**
L45	-0.03	0.025	1, 17	1.33	-0.09	0.025	1, 10	3.71**
L49	-0.09	0.011	1, 17	8.52**	-0.08	0.011	1, 17	7.53**
L54	0.01	0.022	1, 16	0.47	-0.01	0.021	1, 17	0.77

*$p < .05$. **$p < .01$.

Table 7

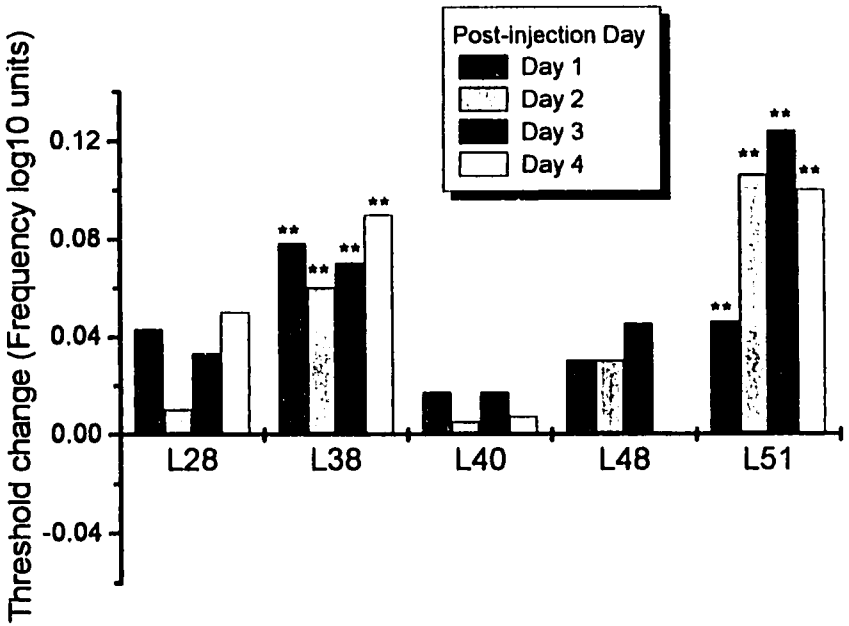
Leptin during food-restriction changes in the slope and maximum reward rate derived from broken-line functions for all subjects.

Rat	Post-injection Day	Slope: Mean Change from Baseline	Max.rate: Mean Shift from Baseline
L28	0	271.72	4.06*
	1	34.93	0.78
	2	51.16	3.32
	3	180.59	1.08
L38	0	21.58	3.12*
	1	1.21	0.83
	2	0.24	1.23
	3	139.31	-1.07
L40	0	501.23*	-0.73
	1	212.84	-0.84
	2	82.87	-1.28
	3	223.63	-0.76
L41	1	103.21	0.76
	2	34.02	0.84
L45	1	19.05	-1.73
	2	158.64	3.35*
L48	1	149.81*	0.67*
	2	17.15	1.66*
L49	1	301.61	0.8
	2	29.98	1.69*
L51	0	272.89*	-0.78
	1	55.76	0.39
	2	109.75	-0.79
	3	153.23	-0.83
L54	1	0.99	-0.89
	2	39.64	0.43

represents any changes in the maximum number of reinforcers obtained and the slope for leptin during the food restriction condition. Leptin was also tested after *ad libitum* feeding was reintroduced and body weights of the rats returned to normal values. Figure 13 represents the mean change in thresholds produced by leptin during *ad libitum* feeding for all subjects. The graph on the left illustrates the threshold changes for subjects that were responsive to the effects of food restriction. All of these subjects displayed elevated thresholds, however, only two of them displayed significant increases in threshold. Figure 14 illustrates the rate-frequency curves for these subjects. Table 8 provides the values of the mean threshold changes and their significance values. The right side of Figure 13 illustrates the threshold changes produced by leptin during *ad libitum* feeding for subjects that were unresponsive to food restriction. Figure 15 illustrates the rate-frequency curves for these subjects. Table 9 displays the values for the mean threshold changes and the significance values for these same subjects. Two of the 4 subjects displayed significant decreases in threshold following leptin administration. One other showed an increase threshold on the second day of testing. Table 10 displays any corresponding changes in maximum reward rate and slope for the free-feeding condition.

The data were analyzed in the same manner for both the *ad libitum* feeding and food restriction condition. The treatment of the raw data is explained in Experiment 1 (pp. 30) The threshold values were used to examine any lateral displacement of the curves. The six threshold values collected for each baseline day were grouped and compared to the six values obtained on each post-injection day. The threshold values along with the slopes and maximal reward rates were compared by a statistical expert system (RS/1) using the Dunnett statistic for multiple comparisons.

Figure 13 Bar graph representing the magnitude of threshold changes produced by leptin during *ad libitum* feeding as compared to vehicle threshold values. Subjects that were responsive to the effects of food restriction are represented in the graph on the left whereas subjects that were unresponsive to the effects of food restriction are represented in the graph on the right.


Post-injection Day
Day 1
Day 2
Day 3
Day 4
Threshold change (Frequency log10 units)
0.12
0.08
0.04
0.00
-0.04
L28
L38
L40
L48
L51

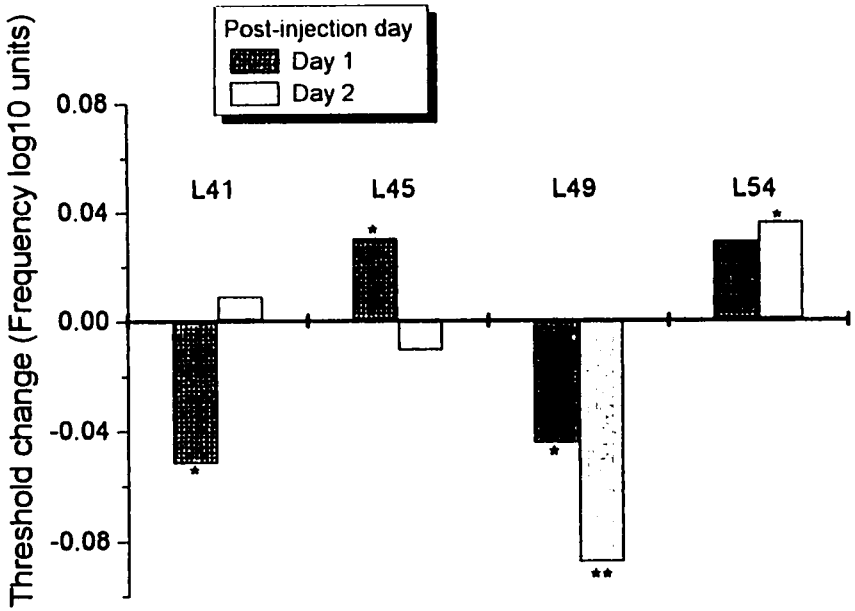
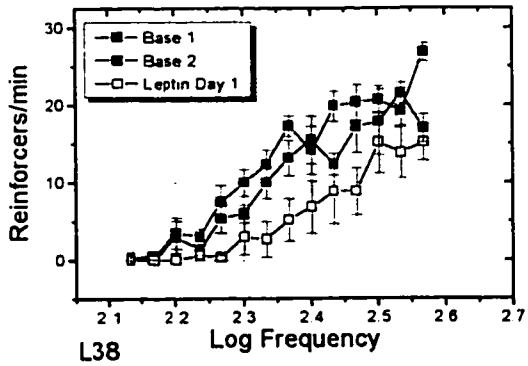
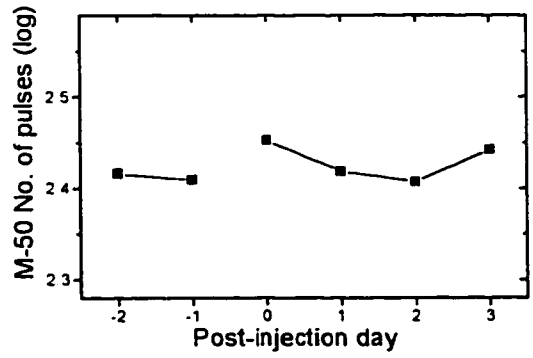

Post-injection day
Day 1
Day 2
Threshold change (Frequency log10 units)
0.08
0.04
0.00
-0.04
-0.08
L41
L45
L49
L54

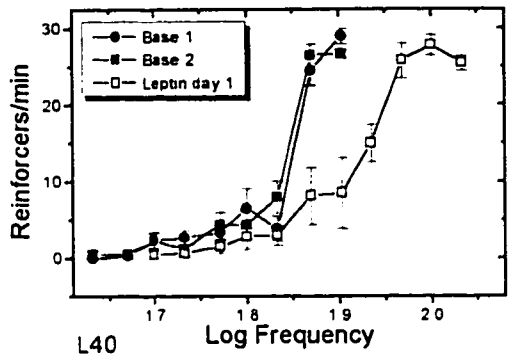
Figure 14 **Left**: Rate frequency curves for subjects that were responsive to the effects of food restriction in Experiment 1. These curves represent the effect of leptin during *ad libitum* feeding. Curves were created by averaging over the six curves of a daily session. Each curve relates the number of reinforcers obtained per minute as a function of the stimulation frequency in log10 units. Vertical bars surrounding each point represent the standard error of the mean. Numbers in the legend refer to the session day. **Right:** The corresponding mean threshold values for two days of vehicle treatment and for the four consecutive days following leptin administration. Each point represents the mean stimulation frequency (log10 units) required to maintain half-maximal reward rate.

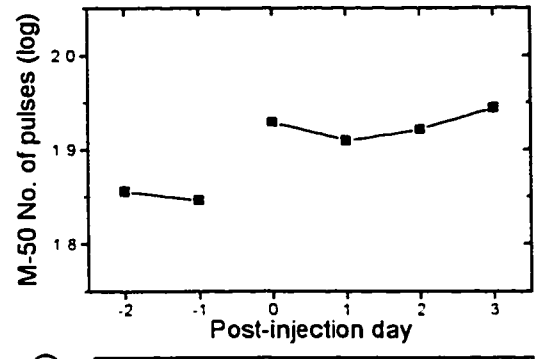
L28



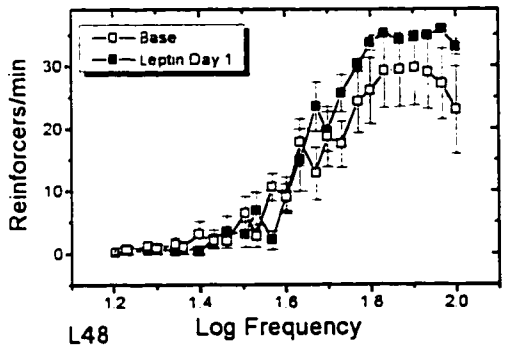


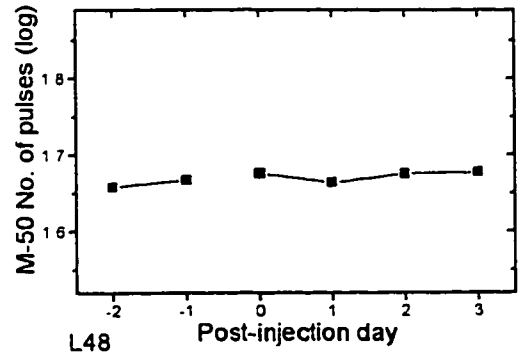
L38



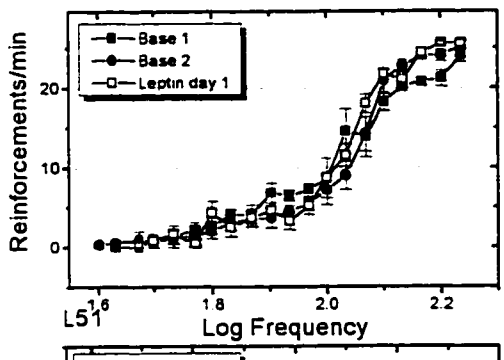


L40

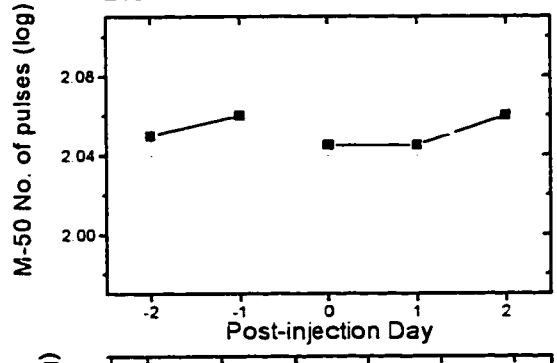




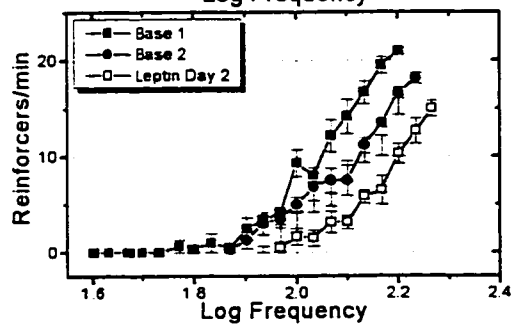
L48



L48



L51



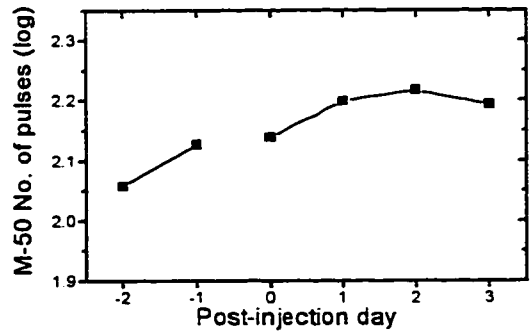


Table 8

Magnitude and significance of curve shifts caused by leptin during *ad libitum* feeding for subjects that were sensitive to the effects of food restriction.

	Post-injection Day															
	1				2				3				4			
Rat	Shift (Log10 pulses)	SEM	*df*s	d	Shift (Log10 pulses)	SEM	*df*s	d	Shift (Log10 pulses)	SEM	*df*s	d	Shift (Log10 pulses)	SEM	*df*s	d
L28	0.043	0.032	1, 17	1.31	0.01	0.032	1, 17	0.27	0.003	0.032	1, 17	0.10	0.05	0.032	1, 17	1.6
L38	0.078	0.013	1, 17	4.25**	0.06	0.013	1, 17	4.25**	0.07	0.013	1, 17	5.1**	0.09	0.013	1, 17	6.79**
L40	0.017	0.024	1, 17	0.70	0.005	0.024	1, 17	0.19	0.017	0.024	1, 17	0.67	0.019	0.024	1, 17	0.77
L48	0.01	0.016	1, 17	0.74	0.02	0.016	1, 17	1.29	0.00	0.016	1, 17	0.18	NA	NA	NA	NA
L51	0.046	0.018	1, 17	2.5**	0.106	0.018	1, 17	5.81**	0.124	0.019	1, 16	6.39**	0.1	0.019	1, 16	5.14**

*p < .05. **p < .01.

Figure 15. Rate frequency curves for subjects that were unresponsive to food restriction in Experiment 1. These curves represent the effect of leptin during *ad libitum* feeding. Curves were created by averaging over the six curves of a daily session. Each curve relates the number of reinforcers obtained per minute as a function of the stimulation frequency in log10 units. Vertical bars surrounding each point represent the standard error of the mean. Numbers in the legend refer to the session day.

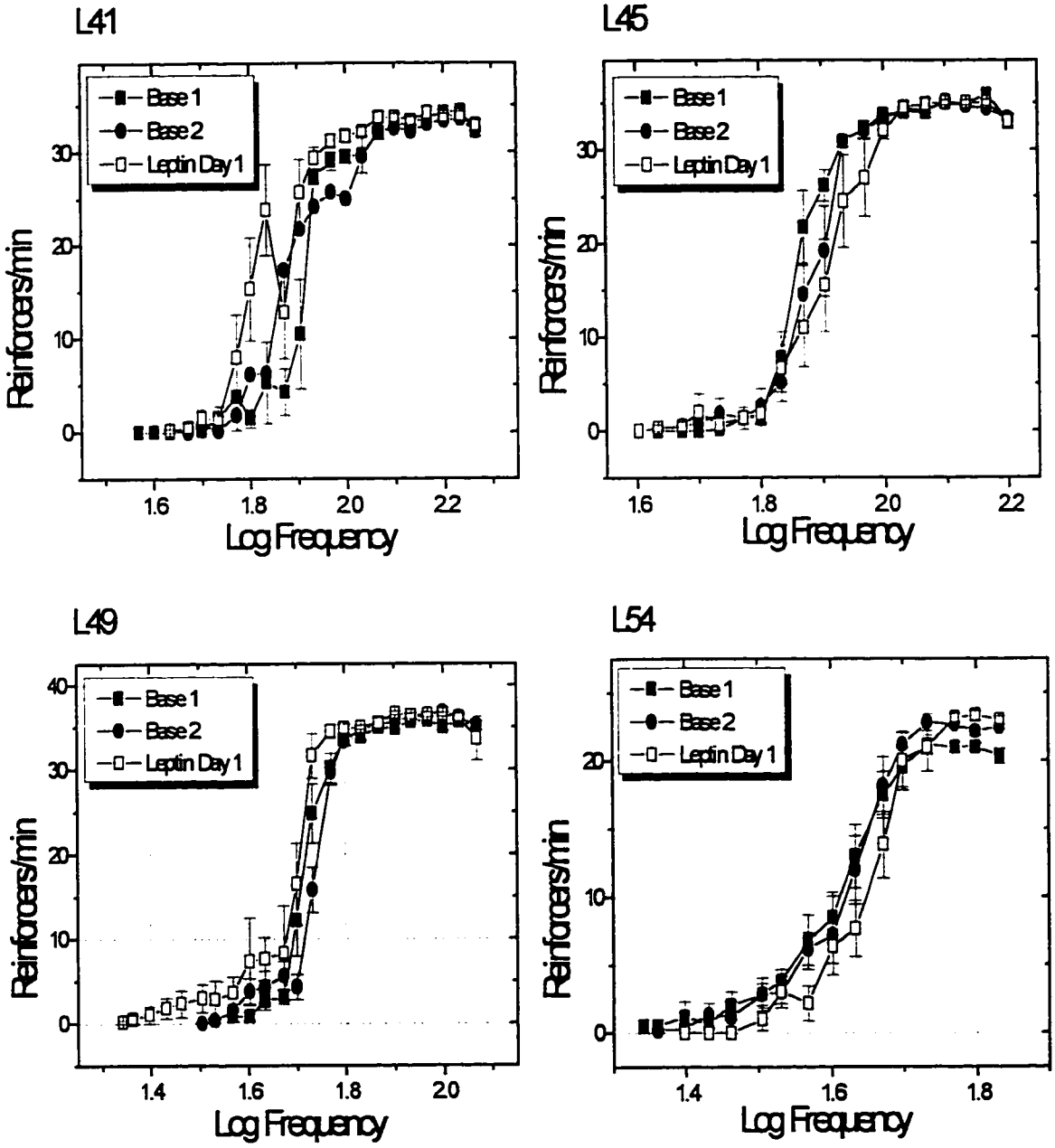

L41
Base 1
Base 2
Leptin Day 1
Reinforcers/min
Log Frequency
L45
Base 1
Base 2
Leptin Day 1
Reinforcers/min
Log Frequency
L49
Base 1
Base 2
Leptin Day 1
Reinforcers/min
Log Frequency
L54
Base 1
Base 2
Leptin Day 1
Reinforcers/min
Log Frequency

Table 9

Magnitude and significance of curve shifts caused by leptin during *ad libitum* feeding for subjects that were unresponsive to the effects of food restriction.

	Post-injection Day							
	Day 1				Day 2			
Rat	Shift (log10 units)	SEM	*df*s	d	Shift (log10 units)	SEM	*df*s	d
L41	-0.052	0.021	1, 17	2.45*	0.009	0.021	1, 17	0.43
L45	0.03	0.017	1, 17	1.78*	-0.011	0.017	1, 17	0.67
L49	-0.045	0.026	1, 17	1.78*	-0.088	0.026	1, 17	3.28**
L54	0.029	0.018	1, 17	1.63	0.036	0.018	1, 17	1.98*

*$p < .05$. **$p < .01$.

Table 10

Free-feeding leptin changes in the slope and maximum reward rate derived from broken-line functions for all subjects.

Rat	Post-injection Day	Slope: Mean Change from Baseline	Max.rate: Mean Shift from Baseline
L28	0	122.52	-2.18
	1	6.71	-4.35
	2	2.09	-0.27
	3	230.07	-2.85
L38	0	7.13	-1.69
	1	28.43	-3.82*
	2	33.42	0.15
	3	92.91	1.59
L40	0	324.06*	1.75*
	1	74.65	3.25*
	2	0.99	3.62*
	3	7.14	3.54*
L41	1	163.65	0.98
	2	171.09	-0.37
L45	1	19.69	-0.36
	2	137.97	-0.47
L48	1	1.65	0.17
	2	141.22	2.77*
L49	1	139	0.95
	2	50.34	0.16
L51	0	61.01	-2.57
	1	7.48	-2.35
	2	15.32	-2.82
	3	18.48	-3.95*
L54	1	20.84	1.04
	2	105.18	1.56*

* $p < .05$

Discussion

A 2 μg ICV injection of leptin decreased feeding for four hours relative to vehicle treatment in *ad libitum* fed rats. This result is consistent with previous reports that centrally administered leptin produces hypophagia in lean rats (Cusin, Rohner-Jeanrenaud, Stricker-Krongrad & Jeanrenaud, 1996; Halaas, et al., 1995).

The observation of a rightward lateral shift of rate-frequency curves for subjects that were responsive to the effects of food restriction is indicative of the decreased reward effectiveness produced by leptin. Significant increases in thresholds were observed anywhere from one to four days of testing. In contrast, leptin did not produce an increase in LHSS thresholds during food restriction in subjects that were unresponsive to the effects of food restriction. Interestingly enough, 3 out the 4 subjects showed a significant decrease in threshold, suggesting that leptin enhanced the reward effectiveness of LHSS during food restriction for these subjects.

The effect of leptin on the reward effectiveness of LH stimulation was also tested after rats were re-fed and allowed to return to normal body weights. The results for both groups of subjects were in the same direction as those obtained during food restriction, however, the effects were less robust. As during food restriction, leptin decreased the rewarding effect of the stimulation for the food restriction responsive animals during *ad libitum* feeding, although, these effects were only significant for two animals. In these two animals threshold changes were significant on all four days of testing. As during food resriction, leptin administration during *ad libitum* feeding produced an increase in reward effectiveness for two subjects that were unresponsive to food restriction. On the other

hand, another subject that showed a decrease in threshold by leptin during food restriction did not show a similar effect during *ad libitum* feeding.

The observation that leptin decreased the reward effectiveness of perifornical stimulation corresponds with the fact that leptin supresses food intake. On the contrary, the observation that leptin facilitated self-stimulation for subjects that were unresponsive to chronic food restriction is not consistent with leptin's effects on feeding. There is no other evidence of leptin stimulating neural activity anywhere in the literature. In light of the finding that leptin excited reward neurons that were unresponsive to both chronic and acute food deprivation, it is speculated that leptin stimulates reward circuitry that is not involved in feeding. Given the lack of information concerning this phenomenon, no further speculation can be provided.

The demonstration that leptin did not produce as robust an inhibitory effect on LHSS during *ad libitum* feeding as it did during food restriction may be explained by the number of functional leptin receptors in the brain. There is evidence that the long form of the leptin receptor (Ob-Rb), that which is implicated in feeding, is up-regulated in the hypothalamus of mice lacking functional leptin (*ob/ob*) (Baskin et al., 1998; Huang, Lin & Zhang, 1997). Furthermore, there is evidence that a 48 hour fast reduces circulating leptin and increases Ob-Rb in the hypothalamus of mice (Baskin et al., 1998). Leptin levels are also likely to be low during a prolonged period of food restriction and body fat loss. This idea is supported by the finding that amount of leptin in plasma is proportionate to the amount of body fat (Maffei et als., 1995). Because leptin levels in the subjects of the present study were likely lowered by prolonged food restriction, it is speculated that Ob-Rb was up-regulated. Accordingly, leptin would be more potent in rats that are food

restricted then ones that are not, which provides a possible explanation for the weaker effect of leptin during *ad libitum* feeding than during food restriction in the present study.

For the most part, leptin produced a decrease in thresholds that lasted more than one day. This finding suggests that centrally administered leptin can produce long-lasting changes in the activity of reward neurons. This is consistent with the finding that an ICV bolus of leptin can produce a reduction in body weight lasting up to six days (Cusin et al., 1996). It is not understood how leptin produces these long-lasting changes. Nevertheless, the current finding may be related to the evidence showing that leptin produces "long-lasting" changes in membrane potential and excitability (Spanswick, Smith, Groppi, Logan & Ashford, 1997). The study demonstrated that leptin applied to glucose-responsive hypothalamic neurons in vitro produced hyperpolarization of ATP-sensitive K+ channels that continued long after leptin was washed out of the bath. Subsequent research investigating the signaling properties of leptin will hopefully elucidate the observation of attenuated threshold changes in the present study.

A particularly interesting finding of the present work is the demonstration that leptin reduced the reward effectiveness of the stimulation for subjects that were responsive to the effects of food restriction. It suggests that leptin may be involved in the process whereby food restriction enhances LHSS. It is not understood what sequence of mechanisms contribute to this outcome, for we know that leptin could be affecting the expression of numerous peptides involved in feeding. However, previous work by Carr and coworkers has indicated that opioids may also play a role in the physiological adaptations to chronic food restriction. The lowering of self-stimulation thresholds by food restriction is reduced by the nonspecific opioid antagonist naltrexone (Carr &

Wolinsky, 1993), the antagonist TCTAP and the antagonist nor-binaltorphimine (Carr & Papadouka, 1994). How can leptin be exerting its effects on LHSS in a manner that is consistent with this evidence?

The process whereby leptin inhibits feeding is commonly identified with its influence on NPY. Central NPY administration promotes a state of positive energy balance and increased fat storage with the most sensitive sites located in the PVN and the PFH. Fasting results in increased NPY gene expression in the ARC and increased NPY release into the PVN (Sahu, Kalra & Kalra, 1988). Several lines of evidence suggest that NPY is normally inhibited by negative feedback provided by leptin. First, leptin receptors are concentrated in the arcuate nucleus where NPY exists in high concentrations (Schwartz, Seeley, Campfield, Burn & Baskin, 1996). NPY is overexpressed in the ARC of leptin-deficient ob/ob mice and leptin resistant *db/db* mice (Schwartz et al., 1996; Stephens et al., 1995). Finally, in normal rats, leptin administration blunts the effects of fasting to increase hypothalamic NPY messenger RNA (mRNA) levels (Schwartz et al., 1996; Seeley et al., 1996).

Perhaps leptin acts via NPY to modulate LHSS. Several lines of evidence supports the idea of an NPY-opioid feeding circuit that could be, in part, modulated by leptin. NPY-induced feeding is reduced by ventricular injection of μ and κ opioid antagonists (Kotz, Grace, Billington & Levine, 1993; Lambert, Wilding, Al-Dokhayel, Gilbey, Ghatei & Bloom, 1994). Furthermore, naloxone blocks the inhibitory effect of NPY on brown fat thermogenesis (Kotz, Grace, Briggs, Levine & Billington, 1995). Targets of NPY-containing neurons of the ARC include the perifornical LH and the DMH (Gray, O'Donohue & Magnuson, 1986) which are sensitive sites for both NPY- (Stanley et al.,

1993) and opioid-induced feeding (Gosnell et al., 1986; Stanley, Lanthier & Leibowitz, 1989). Fos-like immunoreactivity (FLI) has been used to mark areas of neuronal activation in response to naltrexone during food restriction (Carr, Park, Zhang & Stone, 1998). In concordance with NPY cellular and target areas, FLI was found to be most heavily concentrated in the ARC, LH and DMH. Furthermore, the suggestion that NPY release is a stimulus for opioid release in the DMH and the LH is supported by evidence showing that food restriction increases NPY mRNA levels in the ARC (Brady, Smith, Gold & Herkenham, 1990) and increases prodynorphin-derived peptide levels in the DMH and LH (Berman, Devi & Carr, 1994). Taken together, this evidence favors the idea of an opioid-NPY feeding circuit. In consideration of the evidence that opioids are involved in the facilitatory actions of food restriction on LHSS and the findings mentioned above, the possibility arises that the increases in threshold observed after leptin administration may, in part, be occurring through its inhibitory actions on NPY neurons. This hypothesis provides a possible explanation for the involvement of both leptin and opioids in the enhancing effects of food restriction on LHSS. In order to test this hypothesis it will be necessary to determine in future studies whether NPY facilitates LHSS.

Ongoing research is highlighting the LH and PFH as important hypothalamic regions for the neural control of food intake. The emphasis of recent studies has concerned the identification and analysis of new peptides that are either produced or act in these areas. Recent discoveries include hypothalamic cocaine-amphetamine-related peptide (CART), an anorectic peptide that is regulated by leptin (Kristensen et al., 1998). Obese ob/ob mice, which lack functional leptin, show a down-regulation of CART mRNA expression in the ARC, LH and DMH as compared with heterozygous mice. After leptin injection these

same animals showed increased mRNA CART expression in these areas accompanied with a decrease in food intake. Furthermore, the stimulatory effect of NPY is suppressed by CART in a dose-dependent manner. This evidence suggests that leptin's ability to suppress food intake is controlled not only by a reduction of NPY, but also in a reduction of CART. Taking into account that leptin stimulates CART expression and that CART suppresses NPY expression raises the prospect of CART involvement in the mediation of leptin effects on perifornical self-stimulation and introduces a potential avenue for investigation in future studies.

Other targets of leptin signaling include galanin (GAL), melanin concentrating hormone (MCH), proopriomelanocortin (POMC) and neurotensin. Specifically, central injection of leptin decreases hypothalamic GAL, MCH and POMC gene expression and increases NT gene expression (Sahu, 1998). These compounds have all been implicated in the neuronal control of food intake. Various neuropeptides including β-endorphin are derived from the protein product of the POMC gene (Eipper & Mains, 1980) and stimulate feeding in rats (Grandison & Guidotti, 1977). Similarly, central injection of GAL stimulates feeding (Leibowitz, 1991) and central NT administration inhibits feeding in rats (Morley, 1987). The finding that leptin increases MCH gene expression (Sahu, 1998) is of particular interest because MCH-containing neurons are located exclusively in the zona incerta, PFH and LH (Skofitsch, Jacobowitz & Zamir, 1985), and leptin receptors are located on many of these MCH-containing neurons (Hakansson et al., 1998). Furthermore, central MCH administration stimulates feeding in rats (Qu, 1996). Given this evidence, MCH should be viewed as another possible candidate mediating the effects of leptin on perifornical self-stimulation.

In addition to MCH, the only other peptide known to be exclusively produced in the LH and PFH are the orexins, a family of neuropeptides whose mRNA are abundantly and specifically expressed in this area (Sakurai et al., 1998). An orexin bolus administration into the ventricles stimulates feeding 6- to 10-fold in a dose-dependent manner. After a 48 hour fast, orexin mRNA is up-regulated 2.4-fold as compared with fed controls. What is intriguing about this peptide is that many orexin-containing cells of the LH and PFH have leptin receptors (Peyron, et als., 1998). There is immunocytochemical evidence that the MCH- and the orexin-containing cells of the LH and PFH are discrete systems (Peyron et als., 1998). Thus, while both orexin and MCH cells have leptin receptors, there is no evidence of connections between orexin and MCH cells. Leptin may be acting directly on the cells of the LH and PFH to influence brain reward circuitry, if this is the case, it would be of interest to determine whether MCH or orexin cells are involved in the influence of leptin on reward circuitry.

The identification of these various peptides and their receptor systems have not only aided the study of feeding, but have increasingly been viewed as potential targets for anti-obesity drugs. The discovery of gene mutations in animal models of obesity that alter these biochemical pathways has intensified efforts to develop drugs that would suppress or eliminate obesity. The most significant new target is the product of the obesity gene, leptin. Genetically obese ob/ob mice are deficient in leptin production and show a marked reduction in food intake and body weight when provided with exogenous leptin (Campfield, Smith, Guisez, Devos & Burn, 1995). Thus, the search for an anti-obesity drug in humans has been directed towards one that would influence leptin signaling pathways. However, except for a few rare cases, obese humans have increased serum

levels of leptin suggesting that they have a decreased sensitivity to leptin rather than a deficiency in its production. This form of obesity is similar to that seen in *db/db* mice or *fa/fa* rats, two animal models of obesity arising from a deficiency in leptin receptor pathways. The *fa/fa* (Zucker) rat provides a useful model in which to examine impaired leptin sensitivity in the brain. Specifically, it would be of interest to determine what aspects of the neural mechanisms governing feeding are impaired in these animals. This question could be indirectly addressed by investigating the modulation of perifornical self-stimulation by food restriction or leptin administration in *fa/fa* rats as compared to non-obese rats, an idea that is being considered for future research.

General Discussion

The primary objective of this work was to examine the influence of long-term energy balance signals on LHSS. In accordance with previous results, Experiment 1 demonstrated a site-specific enhancement of LHSS in response to chronic food restriction. A food restriction-induced loss of body weight lowered stimulation thresholds when the electrode was positioned immediately dorsal or dorso-lateral to the fornix indicating that metabolic signals arising from chronic food restriction enhance the reward value of the stimulation at these sites. Chronic food restriction is commonly distinguished from acute food deprivation by the severity of the need state that is induced. It was of interest to examine whether a similar distinction is made on the part of the reward neurons activated by PFH stimulation. Thus, the secondary objective of this work was to gain a better understanding of the functional role of these reward neurons by examining the potential effects of short-term deprivation on self-stimulation. A 48 hour period of food deprivation failed to decrease thresholds in all subjects tested in Experiment 2. Most importantly, these results show that food deprivation, which would likely substantially increase food intake, failed to enhance LHSS in subjects that showed increased responding following chronic restriction. This finding suggests that self-stimulation in this area is preferentially sensitive to signals mediating long-term energy balance. Finally, the third aim of this work was to pursue this hypothesis and examine specifically what signals are involved in the facilitating effects of food restriction. Because food restriction, resulting in a 20-25% reduction in body weight,

most likely produces a significant loss of fat, the search was directed towards an adiposity signal as a likely candidate. The protein product of the obesity gene, leptin, meets this criterion and its effect on LHSS was analyzed. In a manner consistent with its effect on feeding, leptin suppressed the reward effectiveness of the stimulation for subjects that were responsive to the effects of food restriction, thus, leptin produced changes in threshold of the opposite direction than those produced by food restriction. Another intriguing finding of this experiment is that leptin increased the reward effectiveness of the stimulation for subjects that were unresponsive to food restriction. Due to the lack of consistency of this effect of leptin with it's effect on feeding, and the fact that the facilitatory effect was observed in subjects that were unresponsive to both the energy balance manipulations that were studied in the present work, it is speculated that leptin may stimulate reward circuitry that is not involved in feeding .

These data further imply a functional differentiation between the different reward neurons activated by stimulating different sites. Stimulation sites in the immediate perifornical area were responsive to the long-term signaling induced by leptin and food restriction in a manner that is consistent with their effects on feeding. In contrast, stimulation sites outside of this area were unaffected by chronic food restriction and affected by leptin in a manner that is inconsist0ent with its effect on feeding behavior. Taken together, the findings of the three experiments imply the existence of different reward substrates, at least one of which is susceptible to energy balance signals arising from food restriction and leptin. It is understood that there are many different factors that are changing during body weight loss that could play a role in the process whereby food

restriction enhances perifornical self-stimulation. It can now be suggested that leptin may be involved in this process.

This evidence favors the conception of the LH as a functionally heterogeneous area. On the basis of these results we can propose that there exists different types of reward systems that can be activated through stimulation of the LH. If we are to assume that the responsive substrate is specific to food then one can speculate that there is a reward system that carries information about the nutritive value of food stimuli and how it meets current energy balance needs. Alternatively, this same system may not be specific to food, but a general one that responds to emergency signals arising from chronic need states (Conover & Shizgal, 1994b). In order to determine if this reward substrate is unique to food it will be necessary to investigate the potential effects of other chronic need states unrelated to feeding. Whichever way one looks at it, the evidence supports the conception of different reward substrates that can be accessed in the LH. Thus, a particularly interesting experiment would be to examine the differential Fos activation produced by stimulating perifornical vs. extra-perifornical sites. This study will most likely give us information about whether the responsive reward substrate is specifically related to feeding since we have a priori knowledge of which feeding-related structures to examine.

The anatomical distinction between responsive and unresponsive sites is consistent with the functional one implied by other studies. The neuroanatomical connections of the perifornical region may explain its associations with feeding. Structures that are involved in feeding, including the pontine parabrachial nucleus and the bed nucleus of the stria terminalis, innervate the dorsal hypothalamus lateral to the fornix (Veening et al., 1982). Extra-perifornical sites such as portions of the LH ventral to the fornix and the zona

incerta are not similarly innervated. The perifornical LH also has dense reciprocal connections with the VTA (Berk & Finkelstein, 1982; Veening et al., 1982), where the meso-accumbens dopamine pathway originates and plays a role in the positive reinforcing effects of food (Hernandez & Hoebel, 1988). In addition, mapping studies have shown electrodes sites supporting stimulation-induced feeding to be positioned lateral and dorso-lateral to the fornix (Hernandez & Hoebel, 1989; Murzi, Hernandez & Babtista, 1986).

Similarly, recent data have distinguished the PFH, relative to adjacent regions, as particularly sensitive to chemically-induced changes in feeding. By microinjecting compounds into numerous hypothalamic sites researchers can determine their effectiveness relative to nearby areas. Accordingly, one of the most potent stimulants of feeding, NPY, was found to produce the most robust eating response in the PFH relative to bracketing areas (Stanley et al., 1993).When cAMP analogs or compounds that increase endogenous cAMP are injected into different hypothalamic sites, they most effectively stimulate feeding in the PFH (Gillard et al., 1997). What's more, catecholamines have been demonstrated to produce their strongest inhibitory effects on feeding when injected into the PFH (Leibowitz and Rossakis, 1979). Taken together, this evidence portrays the PFH as an important substrate for the neural control of food intake. As the neuroanatomical foundation for the uniqueness of this area is further established and more is revealed about its neurochemistry we will likely gain a better understanding of the functional significance of this region in the control of food intake.

With the advent of new peptides that are involved in an evidently, intricate network of molecules and receptor systems comes an increasing number of possible interactions that remain to be uncovered to further our understanding of the neural mechanisms of feeding.

With the production of receptor- and peptide-specific immunoreactive agents the task of teasing apart this network is achievable. Furthermore, by employing BSR it is possible to investigate a crucial part of the feeding system, the brain reward mechanisms that forge the final path leading to goal-directed behavior. The present findings have brought us a little closer to explaining the neurobiological components of feeding reward by distinguishing a reward substrate that is sensitive to long-term metabolic signals arising from chronic food restriction and leptin. Incorporating chemical stimulation and immunocytochemistry techniques with BSR, as is proposed in prospective studies, will hopefully further promote our understanding of the neural reward mechanisms governing feeding.

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