Running head: EFFECT OF DOPAMINE ON FOOD INTAKE IN OBESE MICE

Mesolimbic Dopamine Release in the Nucleus Accumbens: The Effect on Food Intake of Genetically Obese and Lean Mice

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Mesolimbic Dopamine Release in the Nucleus Accumbens: The Effect on Food Intake of Genetically Obese and Lean Mice

BY

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Master of Arts

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Abstract

Research suggests that norepinephrine and dopamine (DA) modulate food intake in genetically obese (B6V-*lep*^{ob}) mice. The D_1 receptor subtype alters food intake patterns, while D_2 receptors mediate motivational aspects of food intake. The effects of mesolimbic DA release in the nucleus accumbens, which is implicated in food reward, on B6V-*lep*^{ob} mice's food intake were examined in a 2 × 3 × 2 × 4 (Phenotype × Antagonist × Agonist × Time) mixed factorial design. DA agonism was expected to increase food intake, an effect which was anticipated to be attenuated by D_2 antagonism. Male, 11- to 26-week-old B6V-*lep*^{ob} and lean mice (*Mus musculus*, $N = 51$) received 1 µl intracerebroventricular infusions of a nonselective dopamine agonist (apomorphine, 1 µg) or vehicle (0.15 M NaCl) following pretreatment of a D_1 antagonist (SCH-23390, 1 µg), D_2 antagonist (raclopride, 1 µg), or vehicle infusion (0.15 M NaCl). Food intake was recorded at 1, 2, 4 and 24 hr post-infusion. Repeated measures ANOVA and post hoc *F* and *t* tests revealed apomorphine did not affect food intake in B6V-*lep*^{ob} or lean mice, nor did raclopride effect intake alone or in combination with apomorphine. This research improves over similar research in chronic central infusion and extension of temporal sampling of intake. Dose-response curves for these DA agonists and antagonists will provide a thorough examination of the D_2 receptor role in hyperphagia in B6V-*lep*^{ob} mice. Overall, DA manipulation with agonists and antagonists does not seem to affect food intake in B6V-*lep*^{ob} or lean mice under the conditions of the protocol applied to this study.

Mesolimbic Dopamine Release in the Nucleus Accumbens: The Effect on Food Intake of Genetically Obese and Lean Mice

Obesity is a debilitating disease that is widespread worldwide, and the incidence of human obesity has been increasing steadily over the past 50 years (Bray, 1985; World Health Organization, 1997). Over 3 of every 10 adults are 20% heavier than their ideal body weight (Li, Matheny, Nicolson, Tümer, & Scarpace, 1997). Increased body fat is the major contributor of body weight gain, and increased fat storage may result in an increased risk of a variety of health problems including cardiovascular disease, diabetes, and osteoarthritis (Bray; Roberts & Greenberg, 1996). Obesity may result when energy intake is greater than energy expenditure.

Energy balance consists of multiple physiological and neural mechanisms that regulate energy intake and energy expenditure in order to obtain homeostatic equilibrium between fat mobilization and energy storage (Hwa, Ghibaudi, Compton, Fawzi, & Strader, 1996). Several peptides interact in the hypothalamus in an attempt to maintain a stable body weight. These peptides are either anabolic or catabolic in function. The foremost anorectic component is leptin, a circulating satiety factor released from adipose tissue that exerts its effects on the long-form receptor located in the hypothalamus (Håkansson, Brown, Ghilardi, Skoda, & Meister, 1998; Weigle & Kuijper, 1997). When satiety signals are impaired, obesity can result over the long-term. An animal model of human obesity with non-functional leptin is the genetically obese (B6V-*lep*^{ob}) mouse (Zhang et al., 1994). This model is characterized by numerous physiological and neuroendocrine abnormalities such as hyperphagia (overeating), hyperglycemia, and impaired thermogenic response (Ingalls, Dickie, & Snell, 1950; Joosten & van der Kroon, 1974) that all contribute to the obese phenotype. One specific example of neuroendocrine

abnormality in the B6V-*lep*^{ob} mouse is an alteration in catecholamine levels (El-Refai & Chan, 1986). Noradrenergic activity, which stimulates food intake, is enhanced in the B6V-*lep*^{ob} mouse (Currie, 1992, 1993; Currie & Wilson, 1992, 1993). Alterations in serotonergic and dopaminergic systems have also been observed (Currie, 1992, 1993; Kuprys & Oltmans, 1982; Lorden & Oltmans, 1977). Dopamine (DA) is a catecholamine that modulates reward associated with food intake (Hernandez & Hoebel, 1988a, 1988b). The mesolimbic DA system is the predominant reward pathway and seems to activate mainly D_2 receptors (Seeman, 1981). Food reward involves the release of DA to the nucleus accumbens (NAc), the major terminal of the mesolimbic system (Comings & Blum, 2000). Another structure that may be involved in the mediation of reward is the lateral hypothalamus (Bray, 1985). Food intake has been shown to increase when the lateral hypothalamus is stimulated (Hernandez & Hoebel, 1988a). DA may interact with peptides in the hypothalamus, modulating mesolimbic DA release in the NAc in response to food intake. B6V-*lep*^{ob} mice may be more responsive to the effects of DA in the NAc than lean mice.

Energy Balance

Energy balance is regulated by various peptides, which control food intake and energy expenditure to maintain a stable body weight. Body weight remains stable when energy intake equals energy expenditure. If fat mass decreases to a point below equilibrium, energy conserving mechanisms are activated (Weigle & Kuijper, 1997). Conversely, when fat mass is gained, energy expenditure increases (Weigle & Kuijper). Energy is derived from carbohydrates, proteins, and fats that enter the body through ingestion of food (Bray, 1985) as well as from the breakdown of stored energy. Sensory input (such as smell and sight of food) and internal signals (nutrient levels) are the

driving forces behind food intake (Szczypka et al., 2001). In the simplest case, food intake is controlled by stomach distension and absorption of nutrients. The absorptive phase of ingestive behaviour begins with consumption and continues through digestion, absorption, and storage of nutrients. During this phase insulin, the main hormone that promotes storage, is secreted (Bray). The postabsorptive phase begins when storage is complete and ends with the onset of consumption. During this phase stored nutrients are mobilized for energy (Bray). Two-thirds of energy derived from nutrients (such as carbohydrates, proteins, and fats) is used for metabolic functions such as protein synthesis, while the remaining third provides energy for physical work (Bray). Fat storage activates the sympathetic nervous system and contributes to an increase in resting metabolism (Weigle & Kuijper), which is the amount of energy the body requires in its resting state. Resting metabolism increases as body weight increases (Bray), meaning that sympathetic nervous system activity is also increased, and this increase in sympathetic activity is related to hyperphagia (Bray). Hyperphagia is expressed through two other variables that are related to food intake: meal size and meal frequency (Baldo, Sadeghian, Basso, & Kelley, 2002; Meguid, Yang, & Laviano, 1997) both of which can be altered to maintain energy homeostasis.

Energy homeostasis is regulated by the hypothalamus (Kalra et al., 1999; Mercer, Moar, & Hoggard, 1998), which acts as part of a feedback loop that receives signals indicating the amount of energy in storage (Zhang et al., 1994) to control the release of peptides involved in food intake and energy expenditure (Sawchenko, 1998). The arcuate nucleus of the hypothalamus synthesizes peptides such as neuropeptide Y that stimulate food intake, (Kalra et al.; Weigle & Kuijper, 1997). Central injections of neuropeptide Y have been shown to cause satiated animals to ingest food and neuropeptide Y mRNA

levels are increased in animals that are food deprived (Weigle & Kuijper). Neuropeptide Y has been found in regions of the brainstem that contain catecholaminergic projections to the hypothalamus and interacts with catecholamines in this neural region (Gillard, Dang, & Stanley, 1993).

Numerous orexigenic and anorexigenic peptides interact in the maintenance of energy homeostasis. Orexigenic peptides are compounds that stimulate ingestive behaviour, fat intake, facilitate fat storage, and increase sympathetic tone while decreasing energy expenditure. Such peptides are said to have anabolic functions. Anorexigenic peptides have an opposing effect. They are catabolic and decrease energy intake, mobilize energy for expenditure, and facilitate the loss of fat stores. The primary anorexigenic signal is leptin, a protein released in the periphery that exerts its effects in the hypothalamus (Elmquist, Maratos-Flier, Saper, & Flier, 1998; Szczypka, Rainey, & Palmiter, 2000). Leptin appears to regulate food intake by modulating expression of other peptides involved in food consumption (Jeanrenaud & Rohner-Jeanrenaud, 2001; Kalra et al., 1999). Leptin decreases levels of orexigenic peptides while it increases anorexigenic expression (Jeanrenaud & Rohner-Jeanrenaud). For a summary of the different peptides involved in the regulation of ingestive behaviour, their neural sites of synthesis and action, and the effects of leptin on such peptides, see Table 1. Energy balance is maintained by the dynamic equilibrium of the opposing actions of numerous peptides. Several animal models of obesity have dysfunctional anorexigenic signaling systems, which lead to unregulated food intake and body weight gain (Kalra et al.). In the case of the B6V-*lep*^{ob} mouse the effects of orexigenic peptides remain high while anorexigenic levels are at a constant low (Jeanrenaud & Rohner-Jeanrenaud). Hyperphagia in this

model results from the disequilibria in orexigenic and anorexigenic signals and is one contributing factor leading to its perturbed state of energy homeostasis (Kalra et al.).

Leptin

The most prominent anorexigenic factor is leptin, an important hormone in the regulation of food intake, satiety, and body weight in all mammals (Saladin, Staels, Auwerx, & Briggs, 1996). The ob gene product (Li et al., 1997) is composed of 67 amino acids (Weigle & Kuijper, 1997) and appears to be a circulating satiety factor (Hwa et al., 1996; Weigle & Kuijper) that favors leanness (Jeanrenaud & Rohner-Jeanrenaud, 2001).

Leptin is an afferent signal protein (Li et al., 1997) originating primarily in white adipose tissue (Elmquist et al., 1998), although leptin mRNA is found in both white and brown adipose tissue (Saladin et al., 1996; Weigle & Kuijper, 1997). The amount of circulating leptin is correlated to the size and number of adipocytes, serving as an indicator of body fat mass, the body's major energy store (Harris et al., 1998; Hwa et al., 1996; Kalra et al., 1999; Weigle & Kuijper). Leptin is necessary to maintain energy homeostasis and neuroendocrine function in humans (Elmquist et al). When energy balance is positive, increased levels of circulating leptin inhibit food intake (Hwa et al.), indicating that energy stores are sufficient (Szczypka et al., 2000). Conversely, sensations of hunger result when circulating leptin is low (Hagan et al, 1999). Fasting depletes energy stores leading to a decrease in leptin mRNA resulting in increased food intake (Hwa et al.).

The fact that leptin receptors are found in the brain (Elmquist et al., 1998; Hwa et al., 1996) indicates that circulating leptin must gain access to the central nervous system. The large leptin molecules are unable to cross the blood brain barrier on their own, therefore a saturable transport system must exist, or access may be gained via

circumventricular organs (neural sites considered outside the blood-brain barrier) such as the median eminence. The median eminence is surrounded by hypothalamic areas containing leptin receptors including the arcuate nucleus, ventromedial hypothalamus and dorsomedial hypothalamus (Elmquist et al.). Additional leptin receptors and leptin receptor mRNA are found in the cerebellum, thalamus, parabrachial nucleus, and the nucleus of the solitary tract in rodents and humans (Elmquist et al.; Mercer et al., 1998) although primarily located in the hypothalamus (Weigle & Kuijper, 1997). The arcuate nucleus seems to be the most sensitive site for the effects of leptin, but leptin receptors have also been found to be situated in areas rich in orexigenic peptides such as neuropeptide Y, proopiomelanocortin, and melanin-concentrating hormone as well as in neurons that produce anorexigenic signals (Kalra et al., 1999).

Two forms of leptin receptors exist: the short form and the long form (Mercer et al.), which has a full-length cytoplasmic tail capable of signal transduction (Weigle & Kuijper) and seems to be involved in mediating satiety (Harris et al., 1998). Dense expression of the long form leptin receptor is found in the hypothalamus (Elmquist et al.; Weigle & Kuijper) and is associated with Golgi apparatus and glial cells, suggesting that it may also be involved in maintaining intracellular functions (Kalra et al.). Leptin may activate the long form receptor by activating ATP-sensitive potassium channels that hyperpolarize glucose-sensitive neurons in the hypothalamus (Elmquist et al.).

Leptin inhibits food intake and increases energy expenditure, thereby reducing body weight (Van Heek et al., 1996). In rodents, weight loss is predominantly due to loss of adipose tissue, as leptin increases fat oxidation (Jeanrenaud & Rohner-Jeanrenaud, 2001). Overnight fasting decreases leptin mRNA in adipose tissue in rodents (Saladin et al., 1996). Decreased circulating leptin leads to food intake, during which leptin

accumulates in the plasma until satiety is reached. Once satiated, food intake decreases, and leptin levels drop. Leptin appears to reduce food intake by affecting meal size as opposed to meal number (Kalra et al., 1999). Hypothalamic neurotransmitters that regulate intake are modulated by leptin (Brunetti, Michelotto, Orlando, & Vacca, 1999). Leptin promotes anorectic behaviour by stimulating corticotropin releasing factor and proopiomelanocortin in the hypothalamus while inhibiting peptides that stimulate intake such as neuropeptide Y and melanin-concentrating hormone (Brunetti et al.).

In addition to its effects on anorexigenic and orexigenic peptides, leptin may modulate food intake by reducing the appetitive value of food, thereby altering brain reward systems (Fulton, Woodside, & Shizgal, 2000). Leptin activity in the hypothalamus inhibits dopaminergic fibers resulting in anorectic effects (Brunetti et al.). Using brain stimulation reward to the lateral hypothalamus, Fulton et al. found that leptin decreased rewarding sensations of brain-stimulation in rats that were food deprived. The effects of leptin on brain stimulation reward lasted for four days. Because food intake can be suppressed by decreasing the reward that accompanies food intake, leptin may inhibit food intake by reducing food reward. Hyperphagia observed in the B6V-*lep*^{ob} mouse may result from an overactive reward system, since this animal model lacks endogenous leptin to inhibit the rewarding effects of food. One system that is involved in food reward is the mesolimbic dopamine system, the primary neural reward pathway (Hernandez & Hoebel, 1988a, 1988b; Terry, 1996, p.235; Zhou & Palmiter, 1995).

Defects in leptin production or signal detection create a starvation signal, although energy stores may be adequate, which may lead to obesity, diabetes, and neuroendocrine dysregulation in the long run (Elmquist et al., 1998). Pelleymounter et al. (1995) tested the hypothesis that leptin regulates adiposity by administering leptin to B6V-*lep*^{ob} and

lean mice. The B6V-*lep*^{ob} mouse is an animal model of human obesity that has a single gene mutation resulting in nonfunctional leptin (Zhang et al, 1994). Leptin was injected intraperitoneally on a daily basis for 28 days to 5-week old B6V-*lep*^{ob} and lean mice at a dose of either 0.1, 1.0, or 10.0 mg/kg. The control group received injections of phosphate-buffered saline. Body weight, food intake, and water intake were measured at 24 hr intervals throughout the treatment period. B6V-*lep*^{ob} mice in the control group experienced the most rapid weight gain. All groups who received leptin treatment demonstrated dose-dependent decreases in body weight. Weight loss that occurred in the leptin-treated group was partly due to a decrease in food intake. This effect was more obvious in B6V-*lep*^{ob} than lean mice. Leptin administration also normalized body temperature and activity level of B6V-*lep*^{ob} mice. Leptin also decreased the percentage body fat in B6V-*lep*^{ob} mice, suggesting it serves as an adiposity sensor. Taken together, these results suggest that leptin serves as a primary regulator of body weight and adiposity in mice. Leptin administration has also been shown to correct hyperglycemia, hyperinsulinemia, and sterility that accompanies obesity in B6V-*lep*^{ob} mice (Sahu, 1998) while it suppresses the overexpression of neuropeptide Y and normalizes their levels of cocaine-and amphetamine-regulating transcript and melanin-concentrating hormone (Elmquist et al.).

Genetically Obese Mouse

The B6V-*lep*^{ob} mouse is an animal model of human obesity distinguished by numerous abnormal behaviours and endocrine functions. The obese phenotype observed in the B6V-*lep*^{ob} mouse is the result of a single gene mutation (Zhang et al., 1994) resulting in an increase in the amount of adipose tissue (Halaas et al., 1995) leading to morbid obesity and Type II diabetes. The obese mutation was reported in 1950 (Ingalls et

al., 1950) and the characteristics of the obese gene resemble those exhibited in human obesity (Zhang et al.). The amino acid sequence for the obese gene is 84% the same in humans as in mice (Zhang et al.). The B6V-*lep*^{ob} mouse has a nonsense mutation in codon 105 of the obese gene resulting in an inactive form of the obese protein (Hwa et al., 1996). The B6V-*lep*^{ob} mouse is thus incapable of producing leptin and therefore has an increased limit on food consumption compared to lean mice (Weigle & Kuijper, 1997). However, the B6V-*lep*^{ob} mouse is capable of responding to exogenous sources of the satiety factor (Bray & Fisler, 1984, p.351). Leptin administration to B6V-*lep*^{ob} mice results in a significant decrease in food intake and body weight and the observed weight loss is due mainly to loss of body fat (Halaas et al.; Harris et al, 1998; Pelleymounter et al.,1995; Yu, 2000). Although the B6V-*lep*^{ob} mouse model differs from models of human obesity in that humans lack functional leptin receptors while capable of leptin synthesis (Maffei et al., 1995), it provides an animal model of aleptinemia allowing for the perturbations in energy balance resulting from a lack of leptin to be examined.

In addition to non-functional leptin, the B6V-*lep*^{ob} mouse is characterized by obesity, hyperglycemia, insulin resistance, hyperinsulinemia, hyperphagia, decreased locomotor activity, and hyperthyroidism (Joosten & van der Kroon, 1974). Hyperthyroidism may contribute to other symptoms that accompany the obese mutation such as lowered metabolic rate and impaired thermogenic response (Joosten & van der Kroon; Sclafani, 1984). In addition, B6V-*lep*^{ob} mice have decreased sympathetic output, decreased pituitary activity (Szczypka et al., 2000) and retarded growth due to decreased levels of growth hormone and prolactin (Margules, 1978). B6V-*lep*^{ob} mice also experience reproductive difficulties resulting from atrophied gonads (Margules), which

renders them sterile (Ingalls et al., 1950). The obese phenotype is apparent around 4-6 weeks of age (Ingalls et al.) and is accompanied by the early onset of hyperphagia.

During the dynamic phase of obesity, as opposed to the static phase, food intake is 44% higher in B6V-*lep*^{ob} than lean mice (Joosten & van der Kroon, 1974). Obese animals obtain a higher percentage of their total daily food intake during the light cycle than their lean counterparts. An increased amount of food consumed by B6V-*lep*^{ob} mice is transformed to body fat compared to lean mice (Joosten & van der Kroon). Taken together, the evidence shows that obese mice have higher caloric intake than energy expenditure, which is a contributing factor of the obese phenotype. Joosten and van der Kroon have also found that B6V-*lep*^{ob} mice have lower heart rates than their lean littermates as early as the first week of life, indicating differences in basal metabolic rates. Efficiency of storage mechanisms is demonstrated by decreased activity and lower metabolic rate, resulting in decreased energy expenditure, leaving more calories available for body weight gain. Other neuroendocrine alterations are characteristic of the B6V-*lep*^{ob} mouse. Noradrenergic activity is substantially increased in B6V-*lep*^{ob} mice (Currie, 1992; Currie & Wilson, 1991, 1992, 1993) whereas serotonergic activity is suppressed (Currie, 1992, 1993). Such alterations in catecholaminergic systems may be another contributor in hyperphagia of B6V-*lep*^{ob} mice.

Monoaminergic Systems in the B6V-lep^{ob} *Mouse*

Monoamines play a role in mediating behaviours necessary for survival, such as food intake (Antelman & Caggiula, 1977). Several genetically altered animal models of obesity exhibit abnormalities in hypothalamic catecholamine activity (Kuprys & Oltmans, 1982) and since monoamine systems have been implicated in the regulation of ingestive behaviour (Currie, 1992; Currie & Wilson, 1991, 1992, 1993; Kuprys &

Oltmans) it has been suggested that alterations in monoaminergic activity may contribute to altered patterns of feeding behaviour. B6V-*lep*^{ob} mice are known to have an abnormal neurochemical profile as well as altered feeding patterns. They also demonstrate altered activity and basal level of norepinephrine, serotonin, and DA (Lorden, Oltmans, & Margules, 1975, 1976; Lorden & Oltmans, 1977; Oltmans, 1983; Oltmans, Olsauskas, & Comaty, 1980), which seem to contribute to the development and/or maintenance of obesity in B6V-*lep*^{ob} mice.

Both serotonin and norepinephrine exert their modulatory effects on food intake through the paraventricular nucleus of the hypothalamus (Leibowitz, 1986; Paez & Leibowitz, 1993). When serotonergic activity in this neural region is depleted, increased food intake is observed (Currie, 1992, 1993; Paez & Leibowitz). Intracerebroventricular administration of serotonin, possibly exerting its effects in the paraventricular nucleus, suppresses food intake in both B6V-*lep*^{ob} and lean mice in a dose-dependent manner, however B6V-*lep*^{ob} mice seem to be less sensitive to the anorectic effects (Currie, 1992, 1993). Thus, abnormalities in the serotonergic system of B6V-*lep*^{ob} mice may contribute to impaired satiation.

Norepinephrine has the opposite effect on food intake. It has been suggested that the noradrenergic system in the paraventricular nucleus is activated under conditions of energy expenditure and food deprivation (Currie, 1992, 1993). Norepinehrine and clonidine (an a_2-adrenergic agonist) have been found to stimulate intake by activating a_2-noradrenergic receptors in B6V-*lep*^{ob} mice (Currie, 1992, 1993; Currie & Wilson, 1993). Norepinephrine exerts its strongest effects at the onset of the dark cycle, when feeding naturally occurs (Currie & Wilson, 1993). Alterations in the hypothalamic function of the noradrenergic system may be a contributing factor to the reduction in satiety signals

experienced by B6V-*lep*^{ob} mice. Postsynaptic stimulation of the a_2-noradrenergic receptors in the paraventricular nucleus may therefore contribute to hyperphagia. Currie and Wilson (1992) showed that clonidine increased and that yohimbine (an a_2-noradrenergic antagonist) decreased total caloric intake of B6V-*lep*^{ob} mice. In addition, they found that pretreatment with yohimbine attenuated clonidine-induced food intake. B6V-*lep*^{ob} mice exhibit an enhanced response to clonidine treatment on total energy intake compared to lean mice (Currie & Wilson, 1992, 1993). This enhanced sensitivity to the orexigenic effects of norepinephrine and clonidine suggest that noradrenergic systems in the paraventricular nucleus of B6V-*lep*^{ob} mice are altered, and may demonstrate increased activity of this catecholaminergic system, ultimately leading to hyperphagia. Studies have shown that hypothalamic norepinephrine is increased in B6V-*lep*^{ob} mice compared to lean controls (Lorden et al., 1975; Oltmans et al., 1980). Therefore, increased activity of norepinehrine may be due to increased density in a_2-noradrenergic receptors although it could also be a result of various other neurochemical mechanisms including abnormal neurotransmitter release, altered vesicular storage, or altered reuptake mechanisms. The above data provide evidence for the involvement of different catecholaminergic systems in the regulation of food intake, while indicating that B6V-*lep*^{ob} mice demonstrate altered sensitivity to pharmacologic manipulation of noradrenergic and serotonergic systems compared to lean mice.

Although serotonin and norepinephrine have opposing effects on food consumption, noradrenergic and dopaminergic systems have been hypothesized to be directly related. Kuprys and Oltmans (1982) conducted a series of studies that investigated the effects of amphetamine (a catecholamine-releasing pharmacological agent) on food intake and catecholamine levels in B6V-*lep*^{ob} and lean mice. Mice were

adapted to a 6-hr feeding schedule and experienced food-deprivation 18 hr prior to testing. Once stable baseline measures were obtained, half the obese and half the lean group received intraperitoneal injections of either vehicle or amphetamine at doses of either 3, 5, or 10 mg/kg. Food intake was measured and mice were sacrificed at the end of the study to examine brain catecholamine levels. Results indicate that amphetamine treatment resulted in a dose-dependent decrease in food intake in both B6V-*lep*^{ob} and lean mice compared to control mice. Amphetamine administration decreased hypothalamic levels of norepinephrine in both B6V-*lep*^{ob} and lean mice. They also found low doses of amphetamine (3 mg/kg) to significantly decrease hypothalamic DA levels in B6V-*lep*^{ob} mice although it had no effect on hypothalamic DA levels in lean mice. Telencephalic levels of DA were increased in B6V-*lep*^{ob} and lean mice, while telencephalic norepinephrine was reduced in B6V-*lep*^{ob} mice only. When behavioural and neurochemical data were correlated, hypothalamic DA levels were significantly increased in amphetamine-treated B6V-*lep*^{ob} mice when this group exhibited increased food intake. Hyperglycemia was normalized with amphetamine administration. These results indicate that noradrenergic and dopaminergic systems are altered in genetically obese mice.

Norepinephrine neurons may indirectly modulate DA. It has been suggested that stimulation of noradrenergic neurons may in fact facilitate DA activity (Antelman & Caggiula, 1977). Clonidine treatment has been found to potentiate the effects of DA agonists (Antelman & Caggiula); however, studies show that while differences in basal hypothalamic norepinehrine exist between B6V-*lep*^{ob} and lean mice, no differences in basal hypothalamic DA levels have been found (Kuprys & Oltmans, 1982; Lorden & Oltmans, 1977), but hypothalamic DA depletion occurs at a reduced rate in B6V-*lep*^{ob} mice (Oltmans et al., 1980). In spite of this, differences in DA levels have been found in

other neural regions and consequently provide evidence for alteration in DA systems in B6V-*lep*^{ob} mice. Levels of telencephalic DA are increased in both male and female B6V-*lep*^{ob} mice (Lorden et al., 1975) and levels of pituitary DA are 50% higher in B6V-*lep*^{ob} than lean mice (Lorden & Oltmans). In addition, DA levels in the arcuate nucleus of the hypothalamus are reduced in B6V-*lep*^{ob} mice. The arcuate nucleus is a neural region where orexigenic and anorexigenic peptides interact to modulate energy balance. DA may interact with such peptides to influence food intake. Simultaneous treatment with D_1 and D_2 agonists decreases levels of neuropeptide Y in the arcuate nucleus of B6V-*lep*^{ob} mice compared to lean mice (Bina & Hodge, 1997) while normalizing levels of corticotropin releasing factor in other hypothalamic areas (Bina & Cincotta, 2000). In addition to regulating peptides involved in food intake, treatment with a combination of D_1 and D_2 agonists ameliorates certain characteristics that accompany the obese phenotype in the B6V-*lep*^{ob} mouse such as hyperphagia, hyperglycemia, and hyperinsulinemia (Bina & Hodge) while decreasing body weight and increasing lean body mass (Cincotta, Tozzo, & Scislowski, 1997). Scislowski et al. (1999) investigated the effects of intraperitoneal administration of the D_1 agonist SKF-38393 and the D_2 agonist bromocriptine on energy balance in female B6V-*lep*^{ob} mice. Mice were treated with either the DA agonist cocktail or vehicle injection 1 hr after light onset. Food consumption was monitored during the 2-week testing period. They found that administration of both SKF-38393 and bromocriptine decreased food intake in B6V-*lep*^{ob} mice within a 24-hr period. The decrease in food intake persisted throughout testing and was associated with a decrease in body weight and improvement of hyperglycemia. Lean mice treated with the DA agonist combination did not exhibit any change in food intake, body weight, or blood glucose level. This suggests that DA may interact with neural

peptides to ameliorate characteristics of obesity in the hypothalamus, and that B6V-*lep*^{ob} mice are more sensitive to dopaminergic manipulation in this area. It follows that there may be dopaminergic differences in other neural regions that mediate food intake, such as the NAc.

Dopamine

DA is another catecholamine involved in food intake as it is thought to regulate the motivational and rewarding aspects of food (Hoebel, Hernandez, Schwartz, Mark, & Hunter, 1989; Szczypka et al., 2001). DA is synthesized from tyrosine by tyrosine hydroxylase, which brings about L-dihydroxyphenylalanine (L-DOPA), and L-aromatic amino acid decarboxylase converts L-DOPA to DA. DA is removed from the synapse either through degradation by monoamine oxidases or is recycled to synaptic vesicles (Zhou & Palmiter, 1995). DA neurons have been found to differentiate and are functional early in the life of rodents (Tirelli, 1987). DA receptors are found in the rodent brain between embryonic day 10 and 15 (Tirelli). DA binds to either D_1 receptors, which stimulate adenylate cyclase activity and are linked to cAMP production (Terry, 1996, p. 234), or D_2 receptors, which inhibit adenylate cyclase activity (Zhou & Palmiter). D_1 and D_2 receptors exhibit different sensitivities to different pharmacological compounds although they may have similar core structures (Seeman, 1981). D_1 receptor sites are found in post-synaptic neurons as opposed to DA containing neurons themselves (Seeman). D_1 agonists do not seem to act in the terminal region of the mesolimbic DA system, which is involved in ingestive and oral-motor behaviour (Terry, p. 241). In addition, D_1 receptor agonists have been suggested to effect meal number as well as satiety (Terry, p. 240). The D_2 receptor is negatively linked to cAMP production (Terry, p. 234) and D_2 receptors are situated in the mesolimbic system (Seeman). It has been

suggested that D_2 antagonists suppress feeding by altering the salience of motivational cues associated with ingestive behaviour (Terry, p. 250). D_2 antagonists have been shown to suppress food intake by increasing the latency to feed as well as decreasing meal number (Terry, p. 254). It has been suggested that D_2 receptors may be stimulated through the activation of D_1 receptors (Terry, p. 255); thus, the two receptor subtypes may interact in the regulation of feeding behaviour. Blocking of DA receptors blocks the rewarding aspects associated with food intake (Zhou & Palmiter).

The neural pathway that mediates reward and approach behavior is the mesolimbic DA system. This system arises from the ventral tegmental area in the midbrain and projects to the NAc, olfactory tubercle, and frontal cortex (see Figure 1) (Comings & Blum, 2000; Zhou & Palmiter, 1995). The ventral tegmental area receives input from the central nucleus of the amygdala, which also projects to the lateral hypothalamus and is involved in the control of emotional processes (Cardinal, Parkinson, Hall, & Everitt, 2002). The A-10 cell group of the ventral tegmental area has high D_2 activity but little D_1 activity has been found (Seeman, 1981). The NAc is the major terminal region of the mesolimbic system (Bassareo & Di Chiara, 1999) and is innervated from neurons in the ventral tegmental area (Szczypka et al., 2001). The NAc is located in the ventral striatum and is the site where information regarding affective and motivational states converge (Maldonado-Irizarry, Swanson, & Kelley, 1995). This information comes from structures of the limbic system (such as the amygdala, hippocampus, and prefrontal cortex) and once it reaches the NAc, it is converted to motor actions (Maldonado-Irizarry et al.). The NAc is made up of two distinct subregions: the core and the shell (Maldonado-Irizarry et al.; Szczypka et al., 2001). The NAc core has neural connections to the ventral pallidum, subthalamic nucleus, and substantia nigra and is involved in

modulating locomotor behavior (Maldonado-Irizarry et al.). The NAc shell seems to be involved in the mediation of rewarding behaviours (Szczypka et al., 2001) as it is associated with affective processing and viscero-endocrine responses (Maldonado-Irizarry et al.). Neurons in the shell are tonically excited, and DA suppresses this excitation, facilitating appetitive behaviour (Maldonado-Irizarry et al.). The shell appears to be involved in both appetitive and aversive motivation (Maldonado-Irizarry et al.).

DA has reinforcing effects when it is released into the NAc (Hoebel et al., 1989) and behaviours induced by reinforcers have been associated with changes in DA transmission and activity (Bassareo & Di Chiara, 1999). Natural rewards involve the release of DA to the NAc, and the reward experienced by food consumption is considered natural (Comings & Blum, 2000). The mesolimbic DA system is involved in the acquisition aspects of reward experienced as a sense of thrill, urgency or craving (Comings & Blum). Stimuli that have been conditioned to become secondary reinforcers have no effect on DA activity in the NAc in their own right (Bassareo & Di Chiara). Mesolimbic DA is also involved in mediating the rewarding and addicting properties of drugs, and may also be involved in food addiction (Hoebel et al.). Studies have shown that DA agonists administered to the NAc induce food intake (Hoebel et al.) Hernandez and Hoebel (1988a) investigated whether DA release in the NAc was sufficient and necessary to regulate reward and food-related reward. Rats were trained to lever-press for food in the presence of a light, which signaled the availability of food, as well as to self-inject with DA and amphetamine to the NAc. Extracellular DA was measured in vivo in the NAc during motivated food intake and local injections of DA and amphetamine. They found that rats will self-inject DA and amphetamine to the NAc. Both food intake and self-injection increased the concentration of dihydroxyphenylacetic acid (DOPAC; a DA

metabolite) in the NAc. When the food availability signal was lit, rats bar-pressed for and consumed the food, which resulted in increased levels of extracellular DA in the NAc. No increase in extracellular DA was observed in the absence of the signal or consumption itself; therefore, food intake is necessary for DA release. This study shows that DA is necessary in mediating reinforcement of food intake. These results add support that DA release in the NAc is involved in the motivational aspects of food reward.

In a related study, Zhou and Palmiter (1995) used DA-deficient mice, which are unable to synthesize DA, as an animal model to study the relationship between food and reward. DA-deficient mice do not consume food or liquid when placed in close proximity to these substances and will usually die of starvation if no intervening action is taken. They found that DA-deficient mice do not eat (aphagia) or drink (adipsia) at 2 weeks of age when control mice had begun to explore sources of food other than the dam. One explanation for the observed behaviour is that the aphagia seen in DA-deficient mice is due to a malfunction in the mesolimbic DA reward system. Food intake increases DA turnover in the NAc and DA release is stimulated with ingestion of palatable foods (Bassareo & Di Chiara, 1999). In DA-deficient mice, it is possible that ingestion is not perceived as rewarding, and thus food intake does not occur. These results show that DA is required for ingestive behaviour. The DA lesions that result in hypophagia (undereating) have also been attributed to lack of reward associated with food intake (Szczypka et al., 2001). The evidence indicates that the mesolimbic DA system is strongly implicated in food intake and in the reward associated with it.

The Role of the Lateral Hypothalamus in Food Intake

Another structure that may be involved in the mediation of dopaminergic reward is the lateral hypothalamus. The hypothalamus is the neural structure that integrates

information related to food regulation (Bray, 1985; Leibowitz, 1986). Destruction of the lateral hypothalamus leads to hypophagia, the extent of which is determined by the size of the lesion. Lateral hypothalamic lesions that inhibit food intake typically involve destruction of DA fibers (Bray). Rowland, Marshall, Antelman, and Edwards (1979) hypothesized that if DA is involved in obesity, then rats who have damaged DA fibers will not experience the hyperphagia nor demonstrate the body weight gain that is typical in response to lesions of the ventromedial hypothalamus. Twenty-four rats received lesions of mesencephalic dopaminergic fibers with 6-hydroxydopamine (a neurotoxin selective to catecholaminergic fibers). Because 6-hydroxydopamine affects both noradrenergic and dopaminergic fibres, rats were pretreated with desmethylimipramine to prevent noradrenergic destruction. Then half the rats underwent electrolytic lesioning of the ventromedial hypothalamus. They found that rats with only DA lesions became aphagic. Aphagia was directly related to the amount of damage. DA lesioning also suppressed hyperphagia in rats who also had ventromedial hypothalamic lesions.

Food intake increases when the lateral hypothalamus is stimulated (Hernandez & Hoebel, 1988a) and DA release is increased in the NAc (Noble et al., 1994). In addition, increased DA activity in the lateral hypothalamus can stimulate food intake (Shiraishi, 1991). It seems likely that a descending projection from the lateral hypothalamus exists and either directly or indirectly activates cells of the mesolimbic DA system, which, in turn, project to the NAc (Hernandez & Hoebel, 1988a). The lateral hypothalamus and ventral tegmental area share common axons that descend in the medial forebrain bundle, and such axons appear to be involved in reward (Hall & Stellar, 1996). DA neurons do not seem to be directly activated in response to lateral hypothalamic stimulation, but may

be excited transynaptically (Hall & Stellar) or via interneurons or other cell groups, such as the locus coeruleus (Hernandez & Hoebel, 1988b).

Hernandez and Hoebel (1988b) investigated the activation of the mesolimbic DA system during food intake and lateral hypothalamic stimulation. They hypothesized that DA turnover in the NAc of rats would be increased in each instance. Rats were trained to lever-press for food in the presence of a signal in addition to training for lateral hypothalamic stimulation. DA and its two main metabolites, DOPAC and homovanillic acid, were measured in vivo by microdialysis before, during, and after food intake and lateral hypothalamic stimulation. They found that DA turnover increased in the NAc when food was ingested, but not in the presence of the signal alone. This result shows that DA turnover resulting from food intake is increased in the mesolimbic DA system. The increase observed in DA turnover and DOPAC and homovanillic acid in response to electrical stimulation of the lateral hypothalamus was similar to that found in natural feeding situations. The results indicate that DA turnover in the NAc increases with food intake and electrical stimulation of the lateral hypothalamus. The finding that DA turnover lasted longer than the act of consuming food suggests that DA is related to an internal state of reward as opposed to simple motor behaviour. It is possible that a lateral hypothalamic-ventral tegmental pathway may exist that may be responsible for increased activity of the mesolimbic DA system in response to food intake. Output neurons of the lateral hypothalamus may project to DA terminals in the NAc or the ventral tegmental area (Parada, Puig De Parada, & Hoebel, 1995).

Statement of the Problem

Energy homeostasis is maintained through the interaction of numerous peptides, most of which exert their effects centrally (Jeanrenaud & Rohner-Jeanrenaud, 2001;

Kalra et al., 1999). Through the dynamic equilibrium of orexigenic and anorexigenic signals, food intake is regulated so that energy input equals energy expenditure and energy balance is achieved. When this equilibrium is disturbed, orexigenic signals may prevail over satiety signals, leading to hyperphagia and obesity. One transmitter that appears to interact with anorexigenic signals is DA. Szczypka et al. (2000) crossbred DA-deficient mice with B6V-*lep*^{ob} mice in order to obtain a double mutant (DD × Lep$^{ob/ob}$). DA-deficient mice were injected daily with L-DOPA for 100 days. Food consumption and body weight were monitored for three days. Food intake of DA-deficient mice and the DD × Lep$^{ob/ob}$ nearly ceased once DA had depleted 24 hr later, followed by a decrease in body weight. Their results indicate that DA is necessary for food intake when leptin is absent. They suggest that DA may modulate leptin activity involved in food intake. Therefore, if DA signaling is unavailable, the organism may not respond to orexigenic signals when leptin levels are low. As the B6V-*lep*^{ob} mouse is unable to produce leptin, and thus does not receive the satiety signal, it is possible that there may be increased stimulation of the mesolimbic DA system in this model, which would contribute to its hyperphagia. The suggestion of an existing descending projection from the hypothalamus to the mesolimbic system along with evidence of shared axons in the lateral hypothalamus and ventral tegmental area (Hall & Stellar, 1996) indicates that the mesolimbic DA system may interact either directly or indirectly with orexigenic and anorexigenic peptides in the control of food intake. The mesolimbic DA system mediates motivational and rewarding properties of food through release of DA to the NAc (Hoebel et al., 1989; Szczypka et al., 2001). Studies have shown that rats can be trained to lever-press for local injections of DA agonists to this region and that extracellular DA is increased in the NAc when food is ingested (Hernandez & Hoebel, 1988a).

Catecholaminergic systems are altered in the B6V-*lep*^{ob} mouse. Levels of pituitary DA are increased in B6V-*lep*^{ob} mice (Lorden & Oltmans, 1977) and DA depletion in the hypothalamus of B6V-*lep*^{ob} mice is reduced (Oltmans et al., 1980). In addition, hypothalamic levels of DA are increased when food intake is stimulated (Kuprys & Oltmans, 1982). Thus, alterations in DA activity have been detected in neural areas of the B6V-*lep*^{ob} mouse implicated in food intake. It is possible that alterations exist in other dopaminergic systems that regulate food intake as well. Conceivably, an increase in the release of DA in the NAc of B6V-*lep*^{ob} mice may serve to increase the appetitive value of food, and may thus result in an increase in food intake.

The purpose of the present study was to assess the contribution of mesolimbic DA release in the NAc in food intake in B6V-*lep*^{ob} mice compared to lean mice via intracerebroventricular administration of DA agonists and antagonists. It was hypothesized that administration of a nonselective DA agonist would increase food intake in both obese and lean mice compared to obese and lean mice who received a vehicle infusion. It was expected that the B6V-*lep*^{ob} mice would exhibit an increased sensitivity to the DA agonist and would ingest significantly more food than lean mice that received treatment with the DA agonist. Further, pretreatment with a selective D_2 antagonist was hypothesized to attenuate food intake potentiated by DA agonism, and this attenuation was expected to be more pronounced in B6V-*lep*^{ob} than lean mice.

Method

Animals

Genetically obese (B6V-*lep*^{ob}) ($N = 24$) and heterozygous lean (+/?) ($N = 27$) mice (*Mus musculus*) were obtained from the Jackson Laboratory, Bar Harbor, ME, USA at 5 weeks of age. Testing occurred between 11-26 weeks of age. Only male mice were

used in the study to help control for extraneous variables that would have been introduced by including females. Lean females would experience hormonal effects that the obese females would not as the estrus cycle is absent in female B6V-*lep*^{ob} mice.

The mouse room was maintained between 22-23°C with a relative humidity of 29-60%. Mice were kept on a 12 hr reverse-light cycle with lights off at 1400 hr. Mice are nocturnal and have a natural tendency to feed soon after dark onset with 80 – 90% of ingestion occurring during lights-out (Kalra et al., 1999). Testing after lights-out allowed data collection to be completed in a setting that naturally promotes food intake behaviour. Mice were individually housed in polypropylene nest boxes (27.5 cm × 16 cm × 12 cm) with woodchip bedding, Nestlets™ (pressed cotton fiber pads to facilitate nest construction), and cardboard tubes while they adapted to handling procedures and to the reverse-light cycle for a minimum of 3 weeks. Mice were transferred to hanging wire cages (25 cm × 20 cm × 21 cm) 2 weeks prior to surgery to become familiar with the testing environment. Round, tin beds were placed on the left side and food cups were placed on the right side of the hanging wire cage and held in place with a standard wooden dowel. Glass marbles were placed in the cage to provide opportunity for play. Water was available ad lib in 100-ml Wahman calibrated drinking bottles attached to the top (for nest boxes) or front (for wire cages) of the cages. Standard lab chow (Prolab® RMH 3000, PMI® Nutrition International Inc., Brentwood, MO, USA, 22.0% protein, 5.0% fat, 5.0% fiber, 6.0% ash, 2.5% minerals, metabolizable energy yield 3.2 kcal/g) was available ad lib in both housing contexts.

Surgical Procedures

Aseptic surgical procedures for intracerebroventricular infusions followed those outlined in Currie (1992) and Yu (2000). Mice were anesthetized using a Ketamine

(Bimeda-MTC, Animal Health Inc., Cambridge, ON; 100 mg/kg body weight) and Xylazine (Bayer Inc., Agricultural Division, Animal Health, Toronto, ON; 10 mg/kg body weight) combination. Anesthetic was administered with a sterile 26-ga., ½-in.stainless steel needle (Becton, Dickinson and Company, Oakville, ON) attached to a sterile 1-cc. tuberculin syringe (Becton, Dickinson and Company) via intraperitoneal injection. Mice were tested for the surgical level of anesthesia using the toe pinch technique. After the mouse had succumbed to the anesthesia, ophthalmic ointment (Tears Naturale® P.M., Alcon®, Mississauga, ON) was applied to the eyes to prevent the corneas from drying. The interaural region of the head was shaved with rodent clippers (Hair Clipper, Oster, No.80) and swabbed with Hibitane® followed by sterile physiological saline. A scalpel was used to make an incision off-midline (as the sagittal suture is not closed in mice) extending from the inter-ocular space caudally to the occiput to expose the calvarium. The underlying fascia was then incised and retracted in order to expose the bregmoidal and lambdoidal cranial sutures and to clear the calvarium for topographic placement of the guide cannula (Plastics One Inc., Roanoke, VA, USA). Bone wax was applied to the calvarium to seal seepage points and dry the skull to promote adhesion of the cranial cement and to hinder cannula loss. An aperture was made on either side of the sagittal suture, posterior to bregma and anterior to lambda, using a hand-held drill. Two screws were inserted (diameter 1.2 mm, Hilco, Winnipeg, MB) to provide an anchor for the cranial cement. Then mice were placed in the stereotax. A David Kopf stereotaxic instrument with nontraumatic mouse adaptor was used to secure head placement. Mice were placed in a Plexiglass cradle surrounded by latex gloves filled with hot water in order to minimize heat loss during surgery. The heat from the surgical lamp placed above the mouse also helped to maintain body temperature. Following

mouse placement in the stereotax, the skull was levelled by measuring its elevation where bregma and lambda intersect at the sagittal suture and by adjusting the skull until the elevations did not differ by more than 0.5 mm (Messier, Émond, & Ethier, 1999). Coordinates for cannula placement were as follows: anterior-posterior coordinate using bregma as a reference was +0.86, the medial-lateral coordinate using the sagittal suture as reference was –0.625, and the dorsal-ventral projection was –2.75 (Paxinos & Franklin, 2001). A dental drill (Dremel Mototool, Dremel Mfg. Co., Racine, WI, USA) was used to bore the aperture through the skull to expose the dura, which was then pierced with a sterile 23-ga. hypodermic needle prior to cannula placement. The guide cannula was inserted into the right lateral ventricle and adhered to the skull with Loctite™ cranial cement (Plastics One Inc.) and Jet Acrylic (Lang Dental Mfg. Co., Inc., Wheeling, IL, USA). A compatible stainless steel stylet was inserted to cover the guide cannula and keep it patent until infusion. Sutures were made anterior and posterior to the guide cannula to close the scalp incision as needed. Throughout surgery, the incised scalp was moistened with sterile saline to promote post-operative healing.

Following surgery, a topical analgesic (Emla Cream, AstraZeneca Canada Inc., Mississauga, ON) and an antibacterial cream (Furacin, Austin, Division of Vetoquinol Canada Inc., Joliette, Quebec) were applied to the suture line. The mice were removed from the stereotax and injected subcutaneously with physiological saline (two 1.5 cc volumes) to aid in postoperative rehydration. Mice were placed in clean polypropylene nest boxes lined with fresh paper towelling, half of each cage resting on a heating pad and covered with fresh surgical towel, during the postoperative period. Cage and mice temperatures were monitored by two separate thermisters (Yellow Springs Instruments, OH, USA) each fed into a Yellow Springs Instruments 12-channel telethermometer. One

thermister was inserted 2 cm into each mouse's rectum to monitor body temperature to the nearest 0.1°C every 15 min until mice regained their righting reflexes. The other thermister was attached to the side of the recovery cage to monitor ambient temperature. Mice were handled daily for 2 min following surgery. Food and water intake, overt behaviour and general physical appearance were monitored to ensure proper recovery. Five mice (1 obese, 4 lean) were excluded from the study as the guide cannula dislodged either during baseline or during infusions on the first day of testing. Four lean mice received only infusions for one agonist condition as the guide cannula was found under the cage on the interim day between test days.

Drugs

All drugs were obtained from Sigma-Aldrich Ltd. (Oakville, ON). Apomorphine hydrochloride hemihydrate was used as the nonselective DA agonist. The D_1 antagonist used was SCH-23390 [R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] and the selective D_2 antagonist was raclopride tartrate salt [3,5-Dichloro-N-(1-ethylpyrrolidin-2-ylmethyl)-2-hydroxy-6-methoxybenzamide]. All drugs were administered at a dose of 1 μg in 0.5 μl volume per infusion (Baldo et al., 2002) and reconstituted in sterile physiological saline, and prepared fresh daily by someone other than the experimenter. SCH-23390 and raclopride are photosensitive and were therefore protected from light by a protective wrapping while in storage and during reconstitution and injection. The vehicle infusions consisted of sterile physiological saline (0.15 M NaCl). Drugs and vehicle solutions were warmed to mouse temperature prior to infusion and were colour coded so that the experimenter was blind to each treatment condition during testing. Drugs were selected on the basis of previous literature (Terry, 1996,

chap.11) and recommendations from other researchers in the field (D. van der Kooy, personal communication, July 29, 2002).

Procedure

Mice were assigned to one of the three antagonist conditions using matched random assignment on the basis of pre-surgical body weight. This procedure ensured that body weight would be evenly distributed across each antagonist condition. Each mouse was handled 2 min per day using a standardized stroking procedure for a minimum of 5 weeks before undergoing stereotaxic surgery. After 4-5 days of post-operative recovery, baseline intake measures were established four days prior to testing, immediately followed by two test days separated by a drug-free day to allow any residual drug effects to dissipate before the next test session. The days on which baseline data were collected, each mouse was weighed using a Mettler digital balance (PM480) to the nearest 0.001 g, prior to dark onset and restrained in a surgical towel to imitate the infusion procedure. Remaining food was weighed at 1, 2, 4, and 24 hr post-restraint to establish stable cumulative intake measures at each of the designated times for each mouse in the absence of any pharmacological manipulation. Papers were placed under each cage to collect food spillage, which was accounted for during weighing. Each mouse served as its own control, as it received each agonist treatment condition. Different groups of mice were assigned to the antagonist pre-treatment condition, yielding a 2 (Phenotype) × 3 (Antagonist Pretreatment) × 2 (Agonist Treatment) × 4 (Time) mixed factorial design, with phenotype and antagonist pretreatment as between-groups variables and the agonist treatment and sampling time as repeated measures. Within-subjects designs offer the advantage of obtaining stable baseline measures and reliable treatment effects (Currie, 1992). This approach balances the benefits of within-subjects designs against the total

number of infusions in any one mouse. Agonist treatments were administered in a counterbalanced order for each phenotype and antagonist condition. Each mouse was weighed prior to receiving the infusion. At testing, mice were removed from their cages, and their stylets were replaced with the injection cannula. Antagonists (volume: 0.5 µl) were infused prior to administration of the agonist (volume: 0.5 µl) immediately before lights-off, yielding a total infusion volume of 1.0 µl. Mice were then returned to their cages where pre-weighed fresh lab chow and water were available. Remaining chow and spillage were weighed at 1, 2, 4 and 24 hr post-infusion.

Verification of Cannula Placement

Once behavioural testing was complete, mice were euthanized with an overdose of 1 cc. somnatol (MTC Pharmaceuticals, Cambridge, ON) and infused intracerebroventricularly with cresyl violet. When the mouse had succumbed to the overdose, it was perfused intracardially with physiological saline and neutral buffered formalin. The brain was extracted and dissected coronally on a standard polycarbonate mouse brain blocking stage at the point of entry of the guide cannula on the dorsal cortical surface. Correct placement of the cannula was determined by visual examination of the ventral extent of the cannula tracks and the presence of dye in the lateral ventricles.

Data Analysis

Cumulative intake was calculated for each of the baseline days and converted to kcal. A repeated measures analysis of variance (ANOVA) was conducted to establish which of the four days would be selected as baseline and would be used to compute the percentage of baseline intake on test days. Cumulative intake on test days was also calculated and transformed to kcal. Percentage of food intake from baseline was then computed, and an a priori analysis was conducted which consisted of omnibus

multivariate ANOVA F tests. Post hoc F and t tests were done to probe any non-significant interactions and main effects as they pertained to the specific hypotheses. Univariate statistics were used as Box's test, which tests the assumption of covariance, was violated. The alpha level for all statistical tests was $p = 0.05$.

Results

Group Assignment

A 2 × 3 × 4 (Phenotype × Antagonist × Day) repeated measures ANOVA was conducted to ensure no difference in body weight existed between the 3 antagonist conditions with day as the within-subjects factor and phenotype and antagonist as the between-subjects factors. Obese mice ($M = 54.988$, $SD = 6.711$) had significantly greater body weights than lean mice ($M = 27.673$, $SD = 1.438$), $F(1, 40) = 348.975$, $MSE = 98.566$, $p < 0.01$. No difference among body weight was found between antagonist conditions for B6V-*lep*^{ob} (vehicle: $n = 8$, $M = 53.349$, $SD = 1.275$; SCH-23390: $n = 7$, $M = 56.093$, $SD = 4.560$; raclopride: $n = 8$, $M = 55.660$, $SD = 5.686$) and lean mice (vehicle: $n = 5$, $M = 27.464$, $SD = 1.275$; SCH-23390: $n = 6$, $M = 27.449$, $SD = 1.599$; raclopride: $n = 6$, $M = 28.170$, $SD = 1.512$), $F(2, 40) = .273$, $MSE = 98.566$, $p > 0.05$.

Baseline Analysis

A 2 × 3 × 4 (Phenotype × Antagonist × Day) repeated measures ANOVA was performed to determine which of the four baseline days, if any, best represented baseline intake and against which the percentage of baseline intake would be calculated on test days. A significant Day × Time interaction was found, $F(9, 351) = 9.260$, $MSE = 1.455$, $p < 0.01$. Paired samples t tests showed that 1 hr food intake decreased from Baseline Day 1 ($M = 0.98115$, $SD = 0.46753$), to Baseline Day 2 ($M = 0.76842$, $SD = 0.60226$), $t(45) = 2.388$, $p < 0.05$ (two-tailed), to Baseline Day 3 ($M = 0.69857$, $SD = 0.26512$),

$t(45) = 3.970$, $p < 0.05$ (two-tailed), and to Baseline Day 4 ($M = 0.70344$, $SD = 0.26009$), $t(45) = 3.710$, $p < 0.05$ (two-tailed). Food intake at 24 hr increased from Baseline Day 1 ($M = 13.98151$, $SD = 3.33873$) to Baseline Day 3 ($M = 15.51303$, $SD = 3.14880$), $t(44) = -2.375$, $p < 0.05$ (two-tailed), and from Baseline Day 1 ($M = 13.92675$, $SD = 3.32225$) to Baseline Day 4 ($M = 16.48550$, $SD = 3.45369$), $t(45) = -3.888$, $p < 0.05$ (two-tailed). Food intake at 24 hr also increased from Baseline Day 2 ($M = 14.83729$, $SD = 3.60595$), to Baseline Day 4 ($M = 16.48550$, $SD = 3.45369$), $t(45) = -3.342$, $p < 0.05$ (two-tailed), and from Baseline Day 3 ($M = 15.51303$, $SD = 3.14880$) to Baseline Day 4 ($M = 16.46023$, $SD = 3.48841$), $t(44) = -2.777$, $p < 0.05$ (two-tailed). A Time × Phenotype interaction was also significant, $F(3, 117) = 30.573$, $MSE = 3.555$, $p < 0.01$. A paired samples t test revealed that B6V-*lep*^{ob} mice exhibited greater food intake at 1 hr on Baseline Day 1 ($M = 1.08675$, $SD = 0.530982$) than Baseline Day 2 ($M = 0.78122$, $SD = 0.418976$), $t(22) = 2.489$, $p < 0.05$, Baseline Day 3 ($M = 0.75047$, $SD = 0.315964$), $t(22) = 2.756$, $p < 0.05$, and Baseline Day 4 ($M = 0.74908$, $SD = 0.305931$), $t(22) = 2.714$, $p < 0.05$. In addition, food intake for B6V-*lep*^{ob} mice was greater at 24 hr on Baseline Day 4 ($M = 18.28049$, $SD = 4.009902$) than on Baseline Day 1 ($M = 15.17774$, $SD = 3.493217$), $t(22) = -2.597$, $p < 0.05$. Lean mice exhibited increased food intake at 1 hr on Baseline Day 1 ($M = 0.88152$, $SD = 0.388409$), compared to Baseline Day 3 ($M = 0.64899$, $SD = 0.202640$), $t(20) = 2.852$, $p < 0.05$, and Baseline Day 4 ($M = 0.62522$, $SD = 0.177856$), $t(20) = 2.961$, $p < 0.01$. Further, 2 hr intake for lean mice was greater on Baseline Day 1 ($M = 2.18910$, $SD = 0.966406$), than Baseline Day 3 ($M = 1.69524$, $SD = 0.415331$), $t(20) = 2.282$, $p < 0.05$, and Baseline Day 4 ($M = 1.60320$, $SD = 0.304037$), $t(20) = 2.690$, $p < 0.05$. Finally, lean mice had greater 24 hr intake on Baseline Day 4 ($M = 14.57448$, $SD = 1.255517$), than on Baseline Day 1 ($M = 12.65829$, $SD = 2.791934$), $t(20) = -3.124$, $p <$

0.01, and Baseline Day 2 (M = 12.81173, SD = 2.433534), $t(20) = -3.072$, $p < 0.01$. A time main effect revealed that food intake was greater at 2 hr than at 1 hr, at 4 hr than at 1 and 2 hr, and at 24 hr than at 1, 2, and 4 hr, $F(3, 117) = 2192.178$, $MSE = 3.555$, $p < 0.01$. Overall, B6V-*lep*^{ob} mice ate more than lean mice, Phenotype main effect, $F(1, 39) = 13.844$, $MSE = 13.914$, $p < 0.01$. No difference in baseline intake was found between antagonist conditions, $F(2, 39) = 2.446$, $MSE = 13.914$, $p > 0.05$.

Day 4 was selected as the baseline against which intake on test days would be compared (see Table 2), as food intake was increased on Day 4 (M = 5.897, SE = 0.252) from Day 3 (M = 5.510, SE = 0.189). Thus, had baseline measures continued, intake would have been expected to either continue to increase or to level off. Choosing Day 4 provided mice with maximum post-surgical recovery. To confirm that Baseline Day 4 was representative of mice's food intake, and as an additional check that the effects of drugs administered during testing had subsided, baseline data were collected on an interim day between test days and a 2 × 3 × 2 × 4 (Phenotype × Antagonist × Day × Time) repeated measures ANOVA was performed. B6V-*lep*^{ob} mice consumed more food than lean mice at 1, 2, 4, and 24 hr, $F(3, 108) = 21.269$, $MSE = 1.740$, $p < 0.01$. Intake increased from 1 hr to 2 hr, from 2 hr to 4 hr, and from 4 hr to 24 hr, $F(3, 108) = 2312.910$, $MSE = 1.740$, $p < 0.01$. No significant differences appeared for a Day × Time interaction, $F(3, 108) = 3.180$, $MSE = 1.696$, $p > 0.05$, a Day × Antagonist interaction, $F(2, 36) = 0.265$, $MSE = 7.968$, $p > 0.05$, or a Day main effect, $F(1, 36) = 0.725$, $MSE = 7.968$, $p > 0.05$. Therefore, Baseline Day 4 was used to compute percentage of baseline intake for the second test day as well as the first.

Test Data

Visual inspection of brains blocked and cut at the point of cannula entry revealed that all placements extended into the right lateral ventricle, which extended into or near the NAc, with the exception of two. Data from the mice with the errant placements were subsequently deleted from statistical analysis. A 2 × 3 × 2 × 4 (Phenotype × Antagonist × Agonist × Time) repeated measures ANOVA was done to examine the hypothesized interactions and main effects. Antagonist and phenotype were the between-subjects factors and agonist and time were the within-subjects factors. Cumulative gram intake was converted to kilocalories by multiplying each value by the metabolizable energy yield of the chow, which was 3.2. The dependent variable, percentage of baseline intake, was then calculated by dividing the food intake at each sampling time on each test day by the baseline intake, and multiplying by 100. Percentage of baseline intake for each phenotype in each antagonist pretreatment condition for each agonist treatment is displayed in Tables 3 and 4. The hypothesized 3-way interaction of Phenotype × Antagonist × Agonist (see Figure 2) did not achieve significance, $F(2, 34) = 0.236$, $MSE = 2205.690$, $p > 0.05$. Neither raclopride nor SCH-23390 affected the percentage of test day intake from baseline, either alone (vehicle-agonist condition) or in combination with apomorphine for either B6V-*lep*^{ob} or lean mice. Neither apomorphine nor vehicle treatment produced changes in food intake in either B6V-*lep*^{ob} or lean mice, Phenotype × Agonist interaction, $F(1, 34) = 1.456$, $MSE = 2205.690$, $p > 0.05$. Similarly, apomorphine did not effect percentage of baseline food intake at either 1, 2, 4, or 24 hr post-infusion, Agonist × Time interaction, $F(3, 102) = 0.816$, $MSE = 490.806$, $p > 0.05$. Pretreatment with vehicle, SCH-23390, or raclopride had no effect on food intake across agonist conditions, Antagonist × Agonist interaction, $F(2, 34) = 1.303$, $MSE = 2205.690$,

$p > 0.05$, nor was there an effect over time, Antagonist × Time, $F(6, 102) = 0.873$, $MSE = 2045.878$, $p > 0.05$. However, a Time × Phenotype interaction was significant, $F(3, 102) = 4.166$, $MSE = 2045.878$, $p < 0.05$ (see Figure 3). An independent samples t test showed B6V-*lep*^{ob} mice exhibited a greater increase in percentage of baseline intake at 1 hr post-infusion ($M = 144.22960$, $SD = 82.46410$) than lean mice ($M = 99.19740$, $SD = 32.66015$), $t(29.833) = 2.401$, $p < 0.05$ (two-tailed), and that B6V-*lep*^{ob} mice ate a lower percentage of baseline intake at 4 hr ($M = 87.72584$, $SD = 28.17379$) than lean mice ($M = 109.34122$, $SD = 23.22851$), $t(40) = -2.675$, $p < 0.05$ (two-tailed). Finally, analysis yielded no main effects, with the exception of time. Neither apomorphine, $F(1, 34) = 0.118$, $MSE = 2205.690$, $p > 0.05$, nor either raclopride or SCH-23390, $F(2, 34) = 0.841$, $MSE = 5343.163$, $p > 0.05$, altered food intake. Percentage of baseline intake was greater at 1 hr post-infusion than 4 and 24 hr, and greater at 2 hr post-infusion than at 4 and 24 hr, $F(3, 102) = 5.780$, $MSE = 2045.878$, $p < 0.01$ (see Figure 4). Percentage of baseline food intake did not differ between phenotype, $F(1, 34) = 0.012$, $MSE = 5343.163$, $p > 0.05$. Due to the absence of support for any of the hypotheses, re-analyses were conducted on gram intake (raw data), caloric intake (gram intake multiplied by 3.2), and percentage of vehicle control intake on test days. When food intake was expressed as kilocalories, phenotype, $F(1, 34) = 6.202$, $MSE = 6.864$, $p < 0.05$, affected intake, with B6V-*lep*^{ob} mice ingesting ($M = 16.70518$, $SD = 3.82881$) more across test days, on average, compared to leans ($M = 14.24772$, $SD = 1.391375$).

Discussion

It was hypothesized that administration of the nonselective DA agonist apomorphine would increase food intake in both B6V-*lep*^{ob} and lean mice compared to B6V-*lep*^{ob} and lean mice who received a vehicle infusion. It was anticipated that this

effect would be enhanced in B6V-*lep*^{ob} mice. Further, pretreatment with the D_2 antagonist raclopride was predicted to attenuate apomorphine-induced food intake. As agonists and antagonists were administered intracerebroventricularly, changes in food intake were expected to be largest at 1 and 2 hr post-infusion.

The data do not confirm the hypotheses. No difference in food intake was detected between the apomorphine and vehicle conditions in either B6V-*lep*^{ob} mice or their lean counterparts. Therefore, no effect in overall food intake was observed with raclopride pre-treatment, which is consistent with results of recent studies (Baldo et al., 2002). No apparent change in food intake was observed over any of the designated sampling times. Nonetheless, B6V-*lep*^{ob} mice displayed increased food intake as a percentage of baseline at 1 hr post-infusion, while percentage of baseline intake at 4 hr post-infusion was decreased in B6V-*lep*^{ob} compared to lean mice. Thus, B6V-*lep*^{ob} mice eat more than leans at the beginning of the dark phase of the light cycle, which is the first report of this finding. Although no significant differences were found within the agonist condition, that intake in B6V-*lep*^{ob} mice was enhanced more than lean mice at 1 hr may indicate that these mice may be more sensitive to pharmacological manipulation of the DA system, which is comparable to previous findings (Kuprys & Oltmans, 1982; Scislowski et al., 1999). Food intake as a percentage of baseline intake was greatest at 1 hr post-infusion, followed by 2 hr post-infusion in both B6V-*lep*^{ob} and lean mice. By 24 hr post-infusion, mice had returned to their baseline levels of food intake. Thus, mice appear to ingest more food at the early onset of the dark cycle when the natural tendency to feed is strongest. This result may also suggest that if any effects of agonist or antagonist treatment were experienced, they had dissipated 24 hr later. Thus central administration of DA agonists and antagonists may exert their effects at earlier time

points than later. When mean kilocaloric intake was assessed between phenotype, B6V-*lep*^{ob} mice consistently ingested more food than lean mice. This result is consistent with previous research (Currie, 1993; Currie & Wilson, 1993; Joosten & van der Kroon, 1974; Yu, 2000).

The hypotheses presented in the current study were based on the premise that mesolimbic DA release in the NAc increases food reward, and that enhancing the reward value of food via DA manipulation would lead to increased food intake. As leptin has been shown to inhibit DA release resulting from neuronal depolarization in vitro (Brunetti et al., 1999), it has been suggested that DA release in the hypothalamus (an area that interacts with the ventral tegmental area) would increase food intake, and that leptin may inhibit hypothalamic DA release. Therefore, in an animal model of obesity that lacks endogenous leptin (such as the B6V-*lep*^{ob} mouse), mesolimbic DA activity may be enhanced. Leptin may interact with the mesolimbic reward system to exert its anorectic effect by decreasing the appetitive value of food, as a result pharmacological manipulations of the mesolimbic system may not have an effect in an animal model incapable of leptin synthesis. Food reward value may thus be said to be leptin-dependent. In this model, leptin may alter the neural reward system in order to reduce food intake. Leptin has been shown to decrease reward experienced by brain stimulation in food deprived rats (Fulton et al., 2000), which suggests that leptin interacts with the DA reward system as leptin receptors are found in the lateral hypothalamus, and neuropeptides involved in food intake exert their effects in this area. Lateral hypothalamic activity may then modulate the mesolimbic DA system through a descending projection or via shared axons (Hall & Stellar, 1996; Hernandez & Hoebel, 1988a). This theory suggests that the B6V-*lep*^{ob} mouse may be an inadequate model in

which to study food reward as it may be insensitive to DA manipulation. Although leptin-dependent reward may be a possible explanation why the current experiment did not yield significant results, it does not seem likely, as DA is necessary to stimulate food intake even in leptin's absence (Szczypka et al., 2000). Experiments using a DA-deficient genetically obese double mutant mouse (DD × $Lep^{ob/ob}$) found that food intake in these mice was significantly increased when treated with L-DOPA (Szczypka et al., 2000). This result suggests that DA serves as an appetitive stimulus that increases the value of food when leptin levels are decreased. Specifically, hypothalamic DA may operate as a contributing factor in hyperphagia.

A competing theory argues that DA may exert effects similar to, and independent of leptin on food intake. This view contends that B6V-*lep*^{ob} mice may be characterized by a decrease in DA activity. Research has shown that treatment with a combination of D_1 and D_2 agonists attenuates hyperphagia in B6V-*lep*^{ob} mice, but treatment with either D_1 or D_2 agonist alone has no effect (Cincotta et al., 1997). Intraperitoneal injection of a SKF38393/ bromocriptine cocktail has been found to attenuate hyperphagia and hyperglycemia in 6-week-old B6V-*lep*^{ob} mice (Scizlowski et al., 1999). In this model, DA is anticipated to interact with peptides involved in food intake in the hypothalamus. Simultaneous administration of D_1 and D_2 agonists decrease neuropeptide Y (a peptide that stimulates food intake) levels in the arcuate nucleus of the hypothalamus (Bina & Hodge, 1997) and intraperitoneal injection of such agonists normalizes neuropeptide Y levels in the pathway projecting from the arcuate nucleus to the paraventricular nucleus of the hypothalamus (Bina & Cincotta, 2000). Research using intracerebral infusions has yielded similar results. DA infusions administered to the perifornical hypothalamus in rats have been found to attenuate food intake elicited by neuropeptide Y infusions to the

same area (Gillard et al., 1993). Therefore, DA may inhibit the orexigenic effects of neuropeptide Y. Further, DA levels in the arcuate nucleus are decreased in B6V-*lep*^{ob} mice compared to lean controls (Oltmans, 1983). It could therefore be argued that hyperphagia in B6V-*lep*^{ob} mice is a result of decreased food reward; thus B6V-*lep*^{ob} mice must ingest increased amounts of food in order to perceive the same reward perceived by lean mice.

Although these studies emphasize that DA may produce effects similar to leptin on food intake, they possess several methodological limitations. First of all, the D_2 agonist administered was bromocriptine, which also affects the serotonergic system. Serotonin has been shown to decrease food intake in B6V-*lep*^{ob} mice (Currie, 1993). If serotonergic mechanisms are being altered by bromocriptine, then the results proposing that DA attenuates food intake may in fact be modulated by a separate system. Second, the route of drug administration may produce confounding results. The DA agonists that lead to decreased food intake in B6V-*lep*^{ob} mice were administered peripherally in these studies (Bina & Cincotta, 2000; Bina & Hodge, 1997; Cincotta et al., 1997) therefore, the location of drug action remains uncertain. In an attempt to reconcile the two theories, it is possible that the effect of DA is dependent on the specific nuclei activated. Kuprys and Oltmans (1982) found that hypothalamic DA levels were decreased in B6V-*lep*^{ob} mice in response to 3 mg/kg of amphetamine; while telencephalic levels were increased.

If DA has different effects at distinct brain regions, then there are important implications pertaining to the results of this study. The stereotaxic coordinates for cannula implantation selected were more anterior to bregma than those previously used in our lab (+0.86 mm in the present study compared to -0.7 mm; Yu, 2000). This site was chosen because at this location, the tips of the lateral ventricles extend into the NAc. It

was assumed that the drugs would have the most effect in the NAc as this would be the first neural structure they would contact due the direction of CSF flow. All mice included in the analysis showed evidence of track marks penetrating the corpus callosum into the right lateral ventricle upon visual examination. However, if the placement was too far anterior or posterior, the NAc may not have been the primary neural site affected. As the drugs were administered intracerebroventricularly, the specific neural site of action cannot be determined, and activation of different neural sites may account for the nonsignificant results. In addition, drugs may have diffused across multiple brain sites where they may have a variety of effects on food intake or other competing behaviours.

The effects of DA drugs may differ depending on the neural site activated. For example, Kuprys and Oltmans (1982) found that treatment with the same dose of amphetamine had different effects on DA levels in different brain regions. They found that amphetamine decreased DA levels in the hypothalamus of B6V-*lep*^{ob} mice while it increased DA levels in the telencephalon. Not only does treatment with catecholaminergic agonists have different neural effects, but the behavioural effects resulting from DA manipulation may vary as well. DA decreases food intake when injected into the perifornical hypothalamus (Gillard et al., 1993). Conversely, when administered to the lateral hypothalamus, DA increases food intake (Shiraishi, 1991). The lateral hypothalamus and ventral tegmental area share descending axons (Hall & Stellar, 1996), thus it is possible that DA may interact with orexigenic and anorexigenic peptides in the hypothalamic area. Further, a descending projection from the lateral hypothalamus activates the mesolimbic DA system (Hernadez & Hoebel, 1988a), which terminates in the NAc. DA agonists have been shown to increase food intake when administered to the NAc (Hoebel et al., 1989).

Other modulators of food intake have yielded mixed results. Intracerebroventricular infusions of leptin have opposing effects on brain stimulation reward depending in which hypothalamic region the electrodes are placed (Fulton et al., 2000). In the NAc, DA acts as a reinforcer and consequently stimulates food intake (Hoebel et al., 1989). The NAc is the terminal region of the mesolimbic reward system. Infusion of DA agonists or of DA itself directly into this region would mimic the effects of natural DA release that occurs when the ventral tegmental area is activated. Future studies may choose an intracerebral placement aimed at the NAc so that the problem of drug dilution is minimized. Intra-accumbens cannula placement would also enable more definite conclusions regarding the rewarding effects of DA on food intake by ensuring that the mesolimbic system was, in fact, manipulated. However, even within the NAc, there may be distinct nuclei that mediate reward and others that mediate locomotor behaviour (Baldo et al., 2002).

One possible explanation why the expected results were not obtained is that activation of DA systems by pharmacological means may generate a variety of stereotypic behaviours. Some of these behaviours include gnawing, licking, sniffing and locomotion (Terry, 1996, p. 234; Tirelli, 1987; Tirelli & Witkin, 1995). Apomorphine has been shown to induce both oral and locomotor stereotypy in rodents (Arnt, Bøgesø, Hyttel, & Meier, 1988), which typically peak 20-40 min after subcutaneous injection (Battisti, Uretsky, & Wallace, 2000). Apomorphine elicits oral stereotypies in a dose-dependent manner in rats when administered peripherally (Canales & Greybiel, 2000) and also induces gnawing upon central administration in mice (Costall, Naylor, & Nohria, 1981). In the present study, apomorphine was administered intracerebroventricularly, therefore creating the possibility that stereotypic gnawing may

have affected food intake. This, however, did not seem to be the case. Gnawing was monitored during baseline and testing by observing the quantity of wood splinters chewed off the wooden dowel holding the food cups in place, and pieces of rubber from the water bottle stoppers present on the papers used to collect spillage at the designated sampling times. No increase in the amount of splinters or rubber was observed during testing.

Although apomorphine-induced gnawing did not seem to influence food intake in this study, other stereotypic behaviours that are incompatible with feeding may have surfaced. Apomorphine administered directly to the NAc has been shown to reduce spontaneous climbing behaviour in a dose-dependent manner with a maximum reduction occurring at 0.5 μg. Climbing behaviour is restored with doses between 0.8-4.2 μg (Costall, Eniojukan, & Naylor, 1983, 1985). Further, cerebral administration of apomorphine to neural sites directly above or below the NAc elicited only weak effects on climbing behaviour (Costall et al., 1983). As apomorphine in the present study was infused into the right lateral ventricle, and at a dose where restoration of climbing behaviour was observed, it follows that stereotypic climbing behaviour resulting from apomorphine treatment would be minimal. In addition, it appears that activation of both D_1 and D_2 receptors are required in order for oral and locomotor stereotypies to surface (Arnt et al., 1988). Antagonism of either D_1 or D_2 receptors is sufficient to block apomorphine-induced stereotypy (Arnt et al.; Canales & Greybiel, 2000). SCH-23390 has been shown to block behavioural effects of apomorphine (Mailman et al., 1984). In the present study, mice were pretreated with vehicle, SCH-23390 (a D_1 antagonist) or raclopride (a D_2 antagonist). Therefore, if stereotypic behaviour were an interfering factor, only mice in the vehicle-apomorphine group would have been affected. As testing

occurred during the dark cycle, it was not possible to monitor mice for such behaviour. Nonetheless, one lean mouse in the vehicle-apomorphine condition did display hyperactivity and repetitive motion at the 4 hr sampling time. This was the only instance of stereotypic behaviour observed. To ensure that no other oral or locomotor stereotypies were occurring at the selected dose of apomorphine (1 μg), an ad hoc test was conducted with an obese mouse once testing was complete. The mouse was placed in a testing chamber (23 cm × 24 cm) with a grid on the bottom covered with plastic. A standard food pellet was placed in the chamber so gnawing could be assessed. The mouse was infused with apomorphine and monitored for stereotyped behaviour for a 5 min period immediately post-infusion, and at 1 and 2 hr post-infusion using a stereotypy rating scale adapted from Creese and Iversen (1973). No evidence of increased locomotion, gnawing, rearing, wall climbing, or sniffing was evident. In this situation, it was not possible to evaluate the effects of apomorphine-induced climbing as the walls of the testing chamber were smooth. However, stereotypical behaviours are minimized when mice are housed individually (Battisti et al., 2000) as in the present study.

Since no stereotypic behaviours were observed, it could be that the test doses were not sufficient to induce such behaviour. While it is preferable that doses remain sub-threshold for stereotypy in order to reduce behaviours that may interfere with feeding, it could also be that the doses were insufficient to elicit food intake. Doses were selected on the basis of previous studies administering SCH-23390 and raclopride intracerebrally in rats (Baldo et al., 2002). Baldo et al. used doses of 1 μg or 2 μg to evaluate feeding and locomotor behaviour resulting from DA manipulation in the NAc shell and core. They observed dose-dependent effects on both behavioural aspects examined. To avoid eliciting any behaviour that may have competed with feeding (such as locomotion) in the

present experiment, the lower dose of 1 μg was selected as significant differences were found between this dose and vehicle infusions (Baldo et al.). No difference in overall food intake was found between vehicle rats and rats treated with either SCH-23390 or raclopride at 1 μg, but differences in the pattern of feeding behaviour were observed. Overall, D_1 and D_2 antagonists administered to the NAc increased meal size while decreasing meal number. The authors do suggest that although effects in feeding behaviour were observed, the doses used may not have been adequate to cause a disruption in reward mediation. One factor accounted for in selecting the doses in the present study was drug dilution from the site of infusion to the site of action, thus the amount of drug acting at the neural target site would be less than that administered. Other studies have administered intracerebroventricular doses of SCH-23390 ranging from 0.01-30 μg in the rat (Mailman et al., 1984). Thus, the initial dose may have been inadequate to affect DA reward and the dilution may have further diminished any potential effects. One reason the 2 μg dose used by Baldo et al. was not chosen is that neural differences exist between rats and mice. For example, catecholaminergic levels in numerous hypothalamic nuclei are lower in mice than in rats (Oltmans, 1983). It follows that in order to manipulate mouse catecholaminergic systems by means of pharmacological methods, drug doses should be altered. Previous studies investigating the effects of intra-accumbens DA manipulation on locomotor behaviour in mice have used doses well above the chosen dose for this study. Doses of apomorphine in these studies were as high as 17 μg and doses of SCH-23390 were administered in doses of 5 μg (Messier, Mrabet, & Destrade, 1991). Because these doses were used to investigate the effects of locomotor behaviour, our chosen dose was below that to avoid stereotyped behaviour.

Other potential reasons for inconclusive results could be that the testing period and sampling times may have been insufficient. Previous studies have found that alterations in food intake appear only after chronic treatment, while no or opposite effects surface during acute manipulation. Chronic amphetamine treatment was found to increase food intake in lean mice during the last 10 days in a 14-day test period while food intake was decreased throughout the first 4 days (Kuprys & Oltmans, 1982). Likewise, leptin was not found to attenuate brain stimulation reward when rats were acutely food deprived, but did attenuate enhanced brain stimulation reward resulting from chronic food deprivation (Fulton et al., 2000). Therefore, had apomorphine treatment occurred over a greater number of days, perhaps an effect in overall food intake would have emerged. However, Scislowski et al. (1999) have observed that alterations in food intake that are present during extended treatment surface within 24 hr. Therefore, the sampling time in the present study appears to have been adequate if any differences in food intake existed.

Sampling time at which food intake was measured may have also contributed to confounding results. It is possible that DA manipulation may not affect overall food intake, but may contribute to other aspects of feeding behaviour such as meal frequency, meal duration, and latency to feed. DA manipulation using specific D_1 and D_2 antagonists revealed that D_1 and D_2 receptor antagonism in the NAc shell and core reduced meal frequency but increased meal size (Baldo et al., 2002). It was not possible to monitor such behaviour in the current study as testing occurred during the dark phase of the light cycle and was not automated. In addition, the first measurement of food intake did not occur until 1 hr post-infusion. Some previous studies have found that changes in meal patterns occur during the first 30 min following intracerebral treatment (Baldo et al.).

Although the present study did not yield significant results, many methodological strengths were employed that were not present in other studies. First, mice were tested during the dark phase of the light cycle, immediately after lights-off, which is most conducive to natural feeding. Previous studies administered treatment 3 hr after lights-off (Kuprys & Oltmans, 1982). Other studies delivered treatment shortly after lights-on (Cincotta et al., 1997; Scislowski et al., 1999). With intracerebroventricular infusion of DA agonists and antagonists just prior to lights-off, the effects of the drug are immediate and coincide with the natural spontaneous feeding.

Second, multiple sampling times were used. Sampling occurred at 1, 2, 4, and 24 hr post-infusion. Previous studies have taken only one measure of food intake at 24 hr (Bina & Hodge, 1997; Cincotta et al., 1997; Scislowski et al., 1999) or at 3 and 6 hr after treatment (Kuprys & Oltmans, 1982). Multiple sampling times provides a more complete picture of the pattern of food intake as different effects could be occurring at different times throughout the 24-hr cycle, which is consistent with previous findings (Kuprys & Oltmans). Thus, it was possible to analyze any potential changes in food intake over time as well as any potential interactions that may have occurred between time, phenotype, agonist treatment, and antagonist pre-treatment. In addition, extending the sampling time allowed changes in food intake that may have been obscured by locomotor behaviour to surface. Baldo et al. (2002) did not observe any changes in food intake with intra-accumbens infusions of SCH-23390 and raclopride at doses of 1 μg and 2 μg. However, behaviour was only sampled for a 30-min period at 10-min intervals. It is possible that a change in overall food intake may have occurred later in the cycle. Further, testing throughout a 24-hr period allowed for verification that mice had recovered from

treatment. Previous studies may not have designated specific sampling times for the reason that peripheral injections do not exert immediate effects.

Third, the current study also employed intracerebroventricular administration as opposed to peripheral administration. As the effects of DA agonists on food intake appear to act centrally (Bina & Cincotta, 2000), administration directly to the site of action minimizes interaction with additional non-specific mechanisms that can occur with peripheral administration (Costall et al., 1983). DA agonists were infused intracerebroventricularly in conjunction with DA antagonists in an attempt to elicit food intake via a non-specific DA receptor and elucidate which receptor subtype was primarily involved. This is an improvement over studies that have used only DA antagonists to manipulate food intake (Baldo et al., 2002). DA antagonists alone have no effect on locomotor behaviour (Arnt et al., 1988) and since the NAc is involved in both reward and locomotion, this principle may apply to feeding as well.

Fourth, stress-induced food intake was minimized by pre-test handling. Food intake is modulated by a variety of orexigenic and anorexigenic peptides. Corticotropin-releasing factor, the hormone involved in eliciting a stress response, is an anorexigenic peptide that modulates food intake. Mice were handled daily, adapted to the testing environment, and adapted to the restraining procedure to minimize stress during testing. These procedures were followed to reduce the anorexigenic effect of corticotropin-releasing factor during testing, which may have been an extraneous variable affecting food intake.

The data obtained in the present experiment do not provide evidence in support of the main hypotheses regarding the role of DA in food intake of B6V-*lep*^{ob} and lean mice. However, the literature does indicate that DA is involved in food consumption (Baldo et

al., 2002; Bina & Cincotta, 2000; Hernandez & Hoebel, 1988b) although the neural mechanism through which it exerts its effects remains unclear. The current study was primarily exploratory but furthers the research as little has been done with central infusions to investigate food intake in B6V-*lep*^{ob} mice. Perhaps studies using a cannula placement aimed at specific neural structures would provide positive results. In addition, a dose-response curve for apomorphine would provide invaluable information. Overall, the study is consistent with the evidence that B6V-*lep*^{ob} mice ingest more than lean mice, but the role of DA remains unanswered. The present study reveals that central infusions of selective DA antagonists followed by nonselective DA agonists, each administered at a dose of 1 μg immediately prior to dark onset, does not seem to affect overall food intake within a 24-hr period in B6V-*lep*^{ob} or lean mice

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Table 1

Orexigenic and Anorexigenic Peptides Involved in Feeding Behaviour

Peptides	Effect on food intake	Effects of leptin on peptides	Site of synthesis	Site of action	Reference
NPY	↑	↓	ARC	PVN	Kalra et al. (1999)
MCH	↑	↓	LH	LH	Kalra et al. (1999)
Orexins	↑	↓	LH, PFH	ARC	Kalra et al. (1999)
Opioids	↑	↓	ARC, POMC	NAc	Sawchenko (1998)
CART	↓	↑	ARC, PVN	Hypothalamus	Kalra et al. (1999)
CRF	↓	↑	PVN	Pituitary	Kalra et al. (1999)
POMC (a-MSH)	↓	↑	ARC	PVN, VMH	Jeanrenaud & Rohner-Jeanrenaud (2001)

Note. ↑ = increase; ↓ = decrease; NPY = neuropeptide Y; MCH = melanin-concentrating hormone; CART = cocaine-and amphetamine-regulated transcript; CRF = corticotropin-releasing factor; POMC = proopiomelanocortin; a-MSH = a-melanocyte-stimulating hormone; ARC = arcuate nucleus of the hypothalamus; PVN = paraventricular nucleus of the hypothalamus; LH = lateral hypothalamus; PFH = perifornical hypothalamus; VMH = ventromedial hypothalamus.

Table 2

Baseline Food Intake (kcal) of Obese and Lean Mice as a Function of Assignment to Antagonist Condition

		Time (hr)			
		Obese Mice			
Antagonist	*n*	1	2	4	24
Vehicle	8	0.692	1.635	3.643	16.885
		(0.368)	(0.504)	(1.013)	(3.076)
SCH-23390	6	0.823	2.209	4.209	18.511
		(0.356)	(0.933)	(0.803)	(2.202)
Raclopride	8	0.732	3.506	6.209	19.585
		(0.238)	(4.098)	(4.776)	(5.774)
		Lean Mice			
Vehicle	8	0.672	1.669	3.884	14.618
		(0.140)	(0.242)	(0.476)	(0.741)
SCH-23390	8	0.630	1.653	3.880	14.690
		(0.243)	(0.358)	(0.510)	(1.260)
Raclopride	7	0.674	1.585	4.166	14.773
		(0.234)	(0.366)	(1.294)	(1.815)

Note. Group mean followed by standard deviation in parentheses.

Table 3

Food Intake (expressed as percentage of cumulative baseline intake) of Obese Mice in Each Agonist Condition as a Function of Assignment to Antagonist Condition

		Time (hr)			
		Apomorphine			
Antagonist	*n*	1	2	4	24
Vehicle	8	132.357	131.323	115.341	96.762
		(76.433)	(25.849)	(26.359)	(15.410)
SCH-23390	7	116.456	92.865	75.407	91.532
		(73.658)	(29.268)	(25.277)	(13.553)
Raclopride	8	180.404	105.999	96.646	94.604
		(91.934)	(46.430)	(34.578)	(28.220)
		Vehicle			
Vehicle	8	142.438	109.997	97.320	84.788
		(104.050)	(48.101)	(35.080)	(34.087)
SCH-23390	7	125.525	94.582	86.134	99.350
		(91.709)	(40.912)	(21.653)	(11.044)
Raclopride	8	135.696	83.614	79.525	91.628
		(98.376)	(36.365)	(26.031)	(23.158)

Note. Group mean followed by standard deviation in parentheses.

Table 4

Food Intake (expressed as percentage of cumulative baseline intake) of Lean Mice in Each Agonist Condition as a Function of Assignment to Antagonist Condition

		Time (hr)			
		Apomorphine			
Antagonist	*n*	1	2	4	24
Vehicle	5	111.492	119.350	107.702	96.222
		(30.911)	(20.842)	(5.808)	(7.879)
SCH-23390	6	77.473	92.865	100.281	98.861
		(25.430)	(29.268)	(17.169)	(5.363)
Raclopride	6	107.604	119.748	102.856	101.281
		(39.707)	(30.353)	(15.136)	(7.656)
		Vehicle			
Vehicle	5	110.418	121.687	115.052	92.985
		(20.319)	(12.314)	(11.529)	(7.014)
SCH-23390	6	119.965	103.519	102.383	97.690
		(28.577)	(21.700)	(14.460)	(4.227)
Raclopride	6	115.600	112.200	107.778	97.606
		(69.397)	(25.312)	(37.793)	(22.335)

Note. Group mean followed by standard deviation in parentheses.

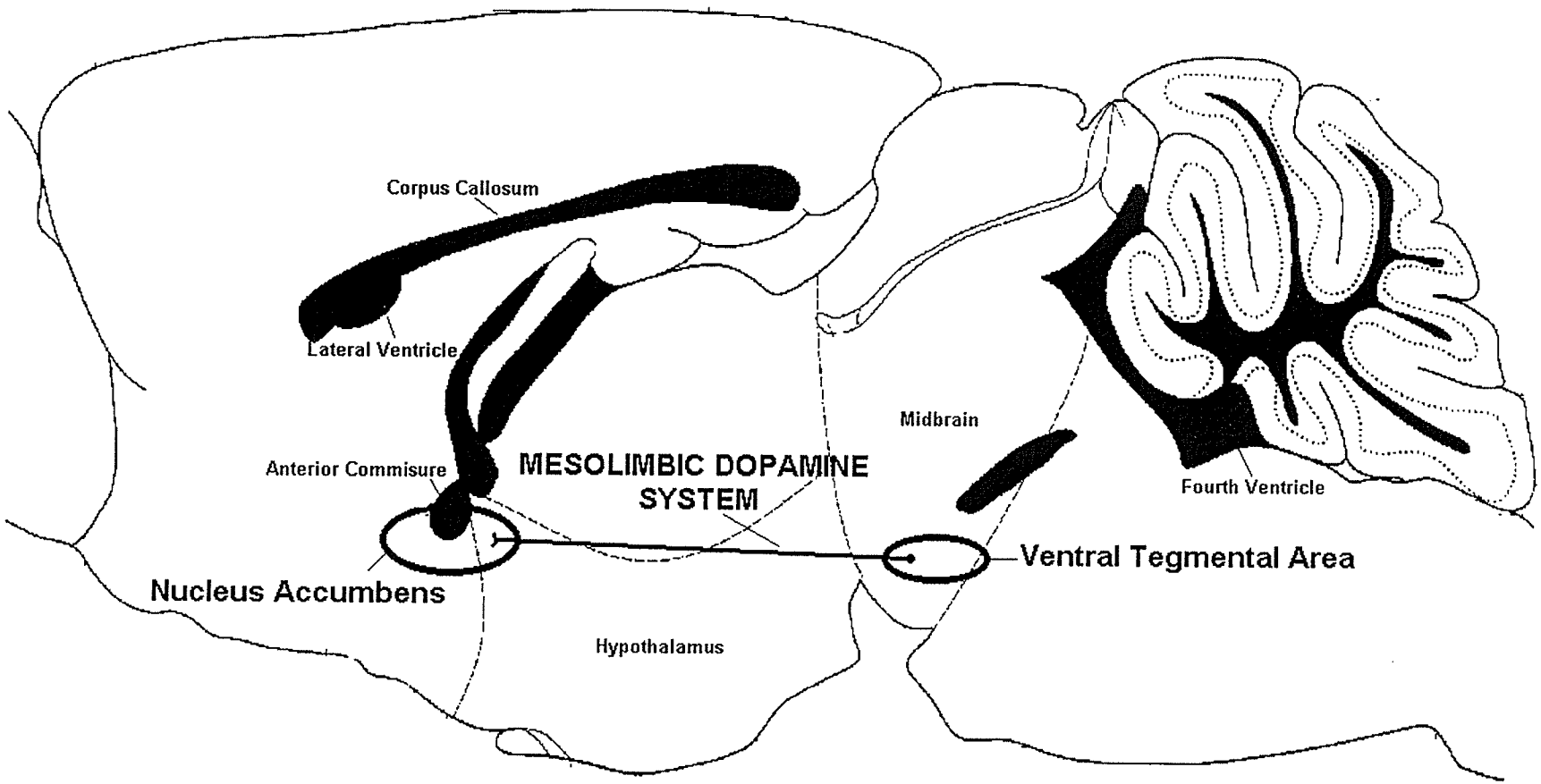
Figure Captions

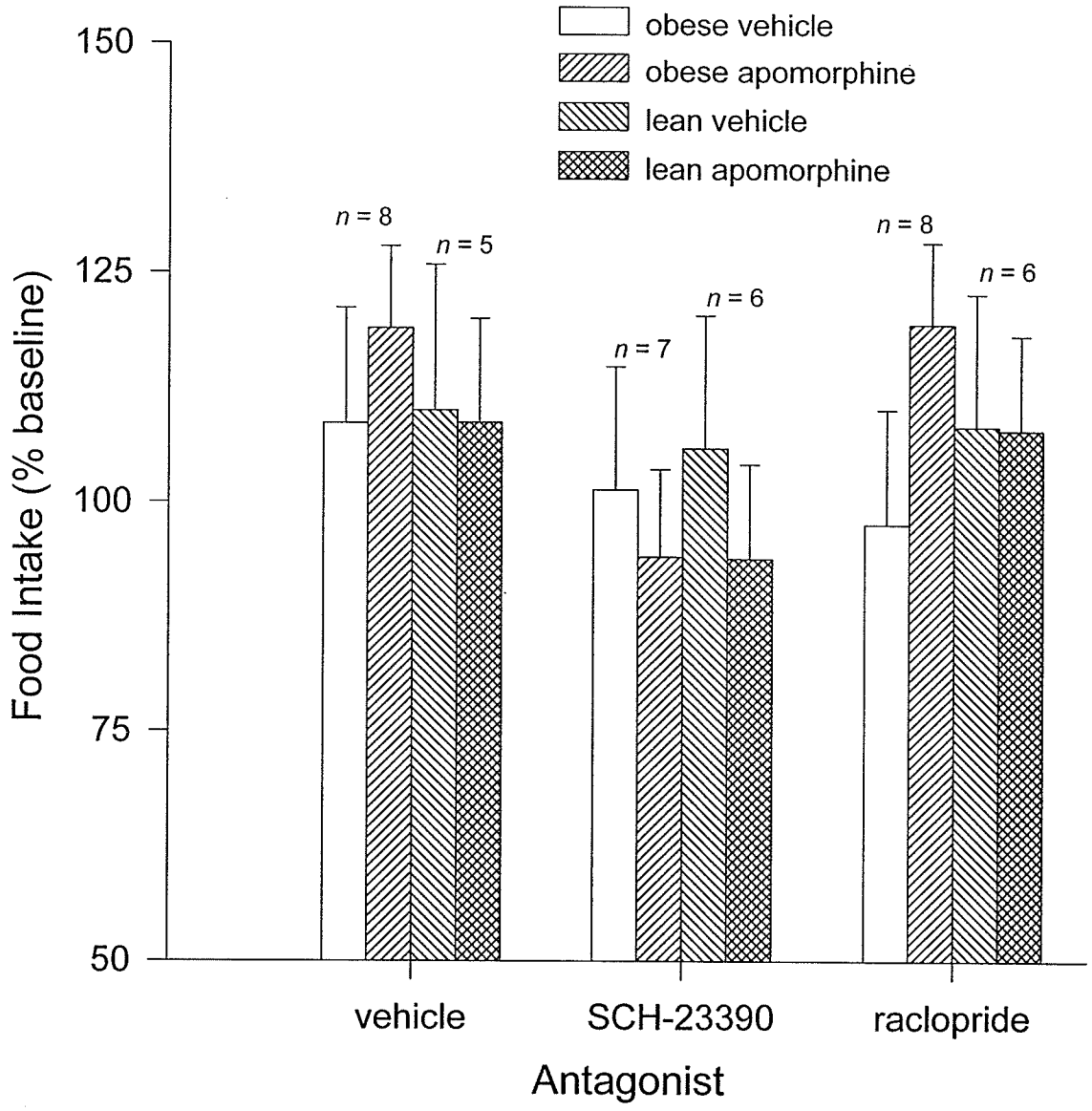
Figure 1. Projection pathway of the mesolimbic DA system as modified from *The Mouse Brain in Stereotaxic Coordinates* (p. xxv) by G. Paxinos and K. B. J. Franklin, 2001, San Diego, CA: Academic Press.

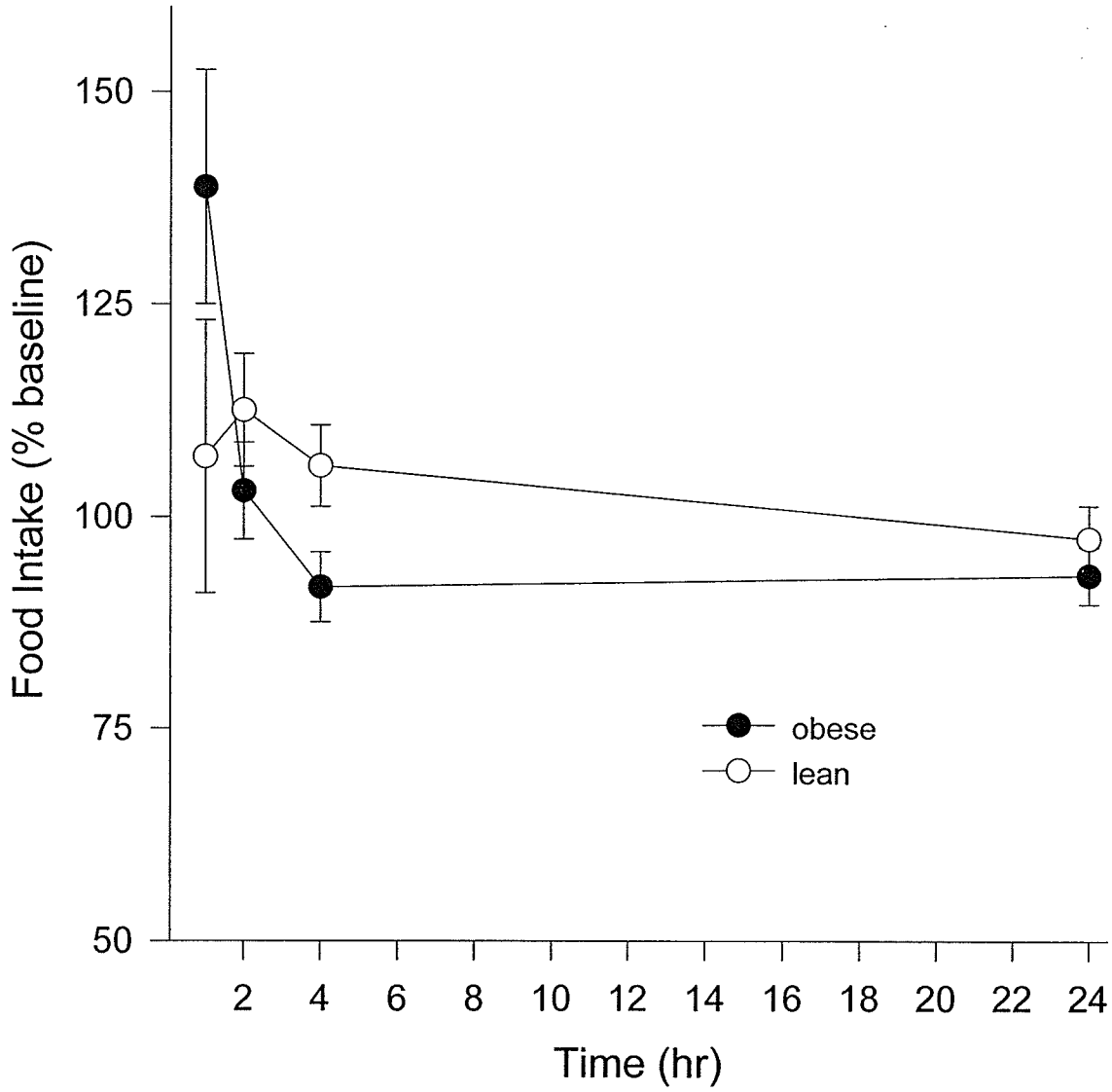
Figure 2. Food intake ($M \pm SEM$) as a function of phenotype, DA antagonist, and DA agonist. Sample sizes as indicated in superscripts.

Figure 3. Food intake ($M \pm SEM$) in obese and lean mice over time.

Figure 4. Food intake ($M \pm SEM$) over time.


Corpus Callosum
Lateral Ventricle
Midbrain
Anterior Commisure
MESOLIMBIC DOPAMINE SYSTEM
Fourth Ventricle
Ventral Tegmental Area
Nucleus Accumbens
Hypothalamus


obese vehicle
obese apomorphine
lean vehicle
lean apomorphine
150
125
100
75
50
Food Intake (% baseline)
n = 8
n = 5
n = 7
n = 6
n = 8
n = 6
vehicle
SCH-23390
raclopride
Antagonist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Food Intake (% baseline)
2
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20
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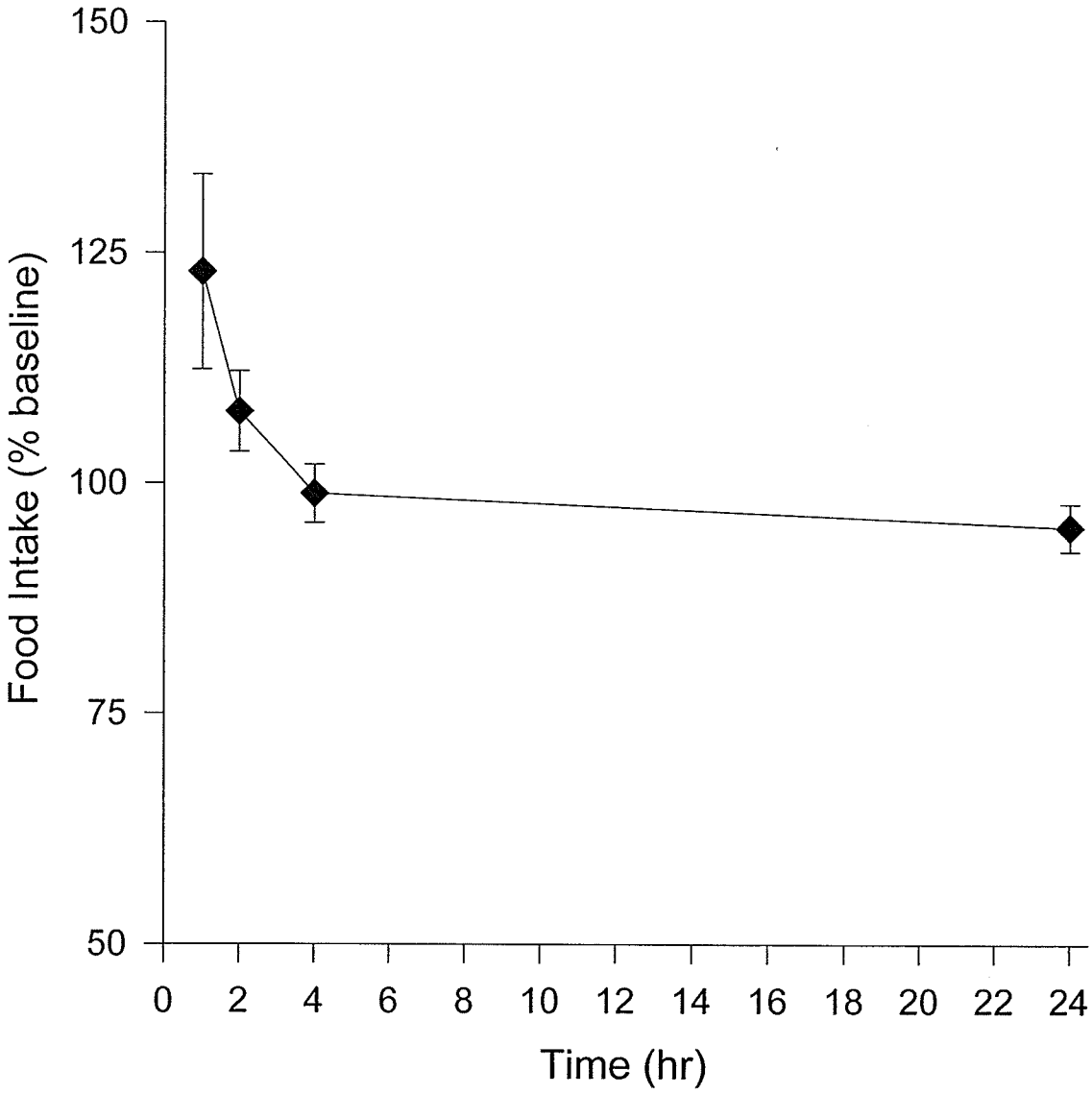
Time (hr)
obese
lean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Food Intake (% baseline)
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Time (hr)