# Effects Of Naltrexone on Milk Intake In Genetically Obese and Lean Mouse Pups

by

Kathleen Marie Feldkircher

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A thesis 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

Genetically obese rodents have elevated pituitary and plasma B-endorphin levels (Morley, Levine, Yim, & Lowy, 1983) and opiate antagonists (e.g., naloxone) selectively abolish overeating in these animals (Margules, Moisset, Lewis, Shibuya, & Pert, 1978). Other recent work has demonstrated that the endorphin system is established before birth in rodents (Bayon, Shoemaker, Bloom, Mauss, & Guillemi, 1979). Because opiate antagonists suppress food intake in adult obese (ob/ob) mice and because endorphinergic mechanisms are functional early in rodent development (Kehoe & Blass, 1986), it was hypothesized that suppression of milk consumption would occur in preobese pups if these pups were differentially sensitve to naltrexone compared to lean (+/+ and +/?) pups. Pups from whole litters (derived from matings of homozygous lean (+/+) and heterozygous lean (+/ ob) breeders in our colony, yielding 76 whole litters) were randomly assigned to be tested on Postnatal Day 6 or 12. Pups were removed from their dams 4 h prior to testing and placed with a nonlactating virgin female mouse. After pups' weights and rectal temperatures (Tre's) were recorded, subcutaneous injections of an appropriate dose of naltrexone (0.5, 1.0, 4.0 mg/kg body weight) or .15 M saline were administered. Pups were weighed immediately before being cross-fostered successively to two milk-replete dams for 1 h

each. After testing, pups Tre's and weights were retaken. Pups were weaned at 23 days and weighed weekly until the ob phenotype was clearly visible in at least one mouse per ob/+ litter. Factorial analysis revealed that overall preobese pups ingested less than lean pups ($p < 0.0006$), although saline-injected ob/obs ingested more than similarly treated leans (+/+); and 12-day-olds consumed less than 6-day-olds ($p < 0.0011$). A phenotype x drug dose x test age interaction ($p < 0.0002$) was found. At both 0.5 and 4.0 mg/kg doses of naltrexone preobese pups consumed less than lean pups. The results that naltrexone significantly altered the amount of milk ingested by preobese pups suggest that opiate receptors are functional early in their development in this strain and prior to the onset of their obesity.

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# Introduction

In the United States, 200,000 female adults suffer from severe obesity (i.e., more than 100% overweight). In addition, these individuals suffer from hypertension, diabetes, and hyperlipidemias, with a 12-fold increase in mortality for obese persons aged 25-34 years (United States Vital and Health Statistics, 1983). Obesity is also a serious health problem among children in industrialized societies. Approximately 25% of American children are overweight (Forbes, 1975). Their obesity is associated with a decrease in growth hormone release, hyperinsulinemia, hyperlipidemia, hypertension, and carbohydrate intolerance. Furthemore, 80% of obese children become obese adults. The problem of obesity requires early identification and immediate intervention (Brownell, 1984). A 1976 report entitled "Research on Obesity" compiled by the United States Department of Health and Social Security pointed out that the cause of obesity in the majority of cases is influenced by the interaction of genetic, metabolic, endocrine, and psychological factors. Future research, the report contended, should be directed towards disentangling the relative importance of each component. Stellar, Henning, Rodin, Rozin, and Wilson (1980) believed that future research using animal models that address genetic factors determining food choices and the regulation of caloric

intake, with emphasis on the underlying mechanisms, is needed. The aim of the present research is the investigation of the early ingestive behavior of an animal model of genetic obesity and its pharmacological modification.

Genetically Transmitted Obesity: The Obese (ob/ob) Mouse

The Bar Harbor obese-hyperglycemic mouse (C57Bl/6J, ob/ob) has been widely studied as an animal model for some forms of human obesity. The ob/ob was first described by Ingalls, Dickie, and Snell (1950) and inherits its obesity as an autosomal recessive mutation on Chromosome 6. The obese phenotype is usually not visually recognizable until the postweaning period or the fourth postpartum week (Bray & York, 1971). Numerous biobehavioral characteristics are associaed with the development of the obese syndrome in this strain, such as hyperphagia, gross adiposity, hypoactivity, hyperglycemia, hyperinsulinemia and insulin resistance, hypothermia, impaired oxygen consumption, impaired fertility, and endocrine abnormalities (Bray & York, 1979). The primary defect responsible for this syndrome is still under investigation.

Although the obese phenotype is not detectable by visual inspection until Days 25-28, characteristics of the obese genotype are present early in development. Two of the earliest biological abnormalities found in preobese mice are

hypothermia and decreased oxygen consumption. Decreased core temperature is evident as early as 10-14 days for preobese mice subjected to either cold exposure (12-14°C) or normal laboratory temperatures (21-25°C), and a 2.0-2.5°C difference is maintained between adult obese and lean mice housed under standard laboratory conditions (Boissonneault, Hornshuh, Romsos, & Leveille, 1976). The body temperature of obese mice housed at 34 C, however, is normal. Thus, at thermoneutrality, resting metabolic rates are similar in obese and lean animals (Trayhurn & James, 1980). The lowered core temperature suggests that the metabolic rates in obese mice may be lower than in lean animals. To substantiate this suggestion, Van der Kroon, van Vroonhoven and Douglas (1977) found oxygen consumption reduced in preobese mice by 5 days postpartum. Together lowered core temperature and oxygen consumption have been suggested as early tests to differentiate preobese mice from age-matched lean pups (Kaplan & Leveille, 1974; Thurlby, Trayhurn, & James, 1978).

Another correlate of the gross adiposity of the obese mouse is its hypoactivity, although evidence of its primary or secondary role in the development of obesity is equivocal. Yen and Acton (1972) found locomotor activity of obese mice decreased significantly only after the obese mice became corpulent, thereby signifying it as a secondary

consequence of obesity. On the other hand, Joosten and Van der Kroon (1974) found a reduction in locomotor activity in preobese pups from the beginning of the second week onwards, before excessive deposition of white fat has occurred. Their results suggest that the inactivity of obese mice results from the genetic condition leading to obesity, rather than obesity itself. They further contend that lower activity and decreased metabolic rate keep caloric expenditure low for ob/ob's and, therefore, leave more calories available for an increase in body weight.

Endocrine and Neurotransmitter Defects

In addition to these thermogenic and behavioral abnormalities, a wide range of endocrine and neurotransmitter abnormalities have been detected in the ob/ob, which may either contribute to, or result from, its obesity. A small increase in serum insulin followed by hypoglycemia is observed in the ob/ob during Days 17-21 (Dubuc, 1977). While the serum insulin levels increase, there is a transition from hypoglycemia to hyperglycemia. The increase in insulin results from both hypertrophy and hyperplasia of the beta cells of the pancreas (Bray & York, 1979). Although the underlying mechanism of hyperinsulinemia remains unclear, Beloff-Chain (1979) has suggested that there may be an excessive production of pituitary factors that stimulate insulin secretion in the

obese mouse. Best, Atkins, Bailey, Flatt, Newton, and Matty (1977) have suggested that the insulin hypersecretion results from excessive stimulation by gastric or intestinal peptide hormones.

Both male and female obese mice have lower pituitary prolactin (PRL) levels (Larson, Sinha, & Vanderlaan, 1976; Sinha, Salocks, & Vanderlaan, 1975). In comparison to lean littermates, pituitary levels of luteinizing hormone (LH) are also lower in obese mice (Swerdloff, Batt, & Bray, 1976). Growth hormone (GH) and follicle stimulating hormone (FSH) levels are elevated in the ob/ob (Naeser, 1974). On the other hand, serum levels of PRL, LH, GH, and FSH have been found to be lower in obese mice compared to lean littermates (Sinha et al., 1975; Swerdloff et al., 1976). The discrepancy in pituitary and serum levels of these hormones suggests that the ob/ob might have deficits in the synthesis and release of pituitary hormones (Lorden & Oltmans, 1977). Serum corticosterone is elevated around Day 17 (Dubuc, 1977; Naeser, 1974) and continues to increase before decreasing in older obese mice. Elevated ACTH levels have also been found in the ob/ob.

Hypothalamic norepinephrine (NE) was significantly elevated (52%) in 6-month-old obese mice compared to age-matched lean controls with no statistically significant differences in hypothalamic dopamine (DA) levels (Lorden &

Oltmans, 1977), although others could find no differences in hypothalamic NE in older animals (Nemeroff, Bisette, & Kizer, 1978). The pituitaries of the ob/ob mice were smaller than lean controls, and pituitary DA content was 50% higher in the obese mice, although pituitary NE was not significantly different (Lorden & Oltmans, 1977). In addition, Garthwaite, Kalkhoff, Guansing, Hagen, and Menahan (1980) found significantly elevated levels of serotonin in the brains of obese mice compared to lean controls. Norepinephrine, dopamine, and serotonin have all been implicated in the regulation of food intake (Morley, 1982), and their alteration in the brains and pituitaries of ob/ob mice have lead to speculation that they may be primary contributors to the development of obesity in this strain.

Food Intake

Food intake and growth patterns have been investigated in obese and lean mice. Hyperphagia increases dramatically during the dynamic phase of obesity (1st-6th month) in the ob/ob (Bray & York, 1979), with adult obese mice having a 44% higher food intake than lean mice (Joosten & Van der Kroon, 1974). In addition, some researchers contend that differences in body weight can be detected prior to the postweaning period. Joosten and Van der Kroon (1974) calculated both a mean body weight and growth rate in a group of 100 mice from birth until Day 16 postpartum.

Differences in body weight between preobese and lean pups increased from the day of birth onward and reached statistical significance on Day 10. The relative growth rate of obese mice was higher than that of lean mice on Days 6-7. Others have shown that obese mouse pups are already depositing more energy as fat as early as 8-10 days postpartum even if body weights do not differ significantly (Boissoneault et al., 1976). Joosten and Van der Kroon (1974) have speculated from these data that ob/ob are congenitally hyperphagic, although evidence on the appearance of hyperphagia in the ob/ob before weaning has been equivocal. Lin, Romsos, and Leveille (1977) found no difference in milk intake between 6 h-deprived obese and lean mice from 7-21 days of age. In the Lin et al. study (1977), however, each pup was in competition with their littermates for the available source of food and only a single dam was used as a milk supply. Friedman (1975) has shown that deprived rat pups ingest more milk than nondeprived pups only if given access to enough milk. If milk supplies are limited, the effect of increasing deprivation on increasing milk intake is masked by an artificial ceiling placed on the amount of milk consumed. Thus, the possibility exists that if the preobese mouse is given an unlimited milk supply, hyperphagia could be detected at an earlier age, although it may not be a primary

contributor to its early adiposity. Similar to the results of the Lin et al. study, Rath and Thenen (1979) found no differences in 24-h milk intake of preobese and lean mice at Days 10 and 15. However, the litter size in their study varied from 2-11 pups. Caution should be applied to interpreting the results of this study, because the litter size was not standardized. To control for these factors, litter size should be standardized and pups should be provided with a series of milk-replete dams to ensure that there is an unlimited source of milk (Drewett, Statham, & Wakerley, 1974).

Endogenous Opiates

The discovery of stereospecific opiate receptors that mediate opiate activity was followed by the identification of the endogenous opioid peptides (Hughes, 1975). The first report of the presence of two pentapeptides in the brain (i.e., leucine (leu)- and methionine (met)-enkephalin) with opiate action on smooth muscle launched numerous investigations of the physiological role of endogenous opioids (Hughes, Smith, Kosterlitz, Morgan, & Morris, 1975). Margules (1979) has speculated that an endogenous opioid-mediated regulatory system (i.e., endorphinergic system) and a system antagonistic to its action (i.e., endoloxonergic system) be incorporated as additional subdivisions of the autonomic nervous system. He contended

that an endorphinergic division employs endogenous opioids to increase the influx of energy and to decrease its efflux, whereas the endoloxonergic division employs endogenous naloxone-like substances to decrease the influx of energy and to increase its expenditure. Because beta-endorphin stimulates feeding behavior when administered peripherally or centrally and because genetically obese mice and rats display both hyperphagia and elevated pituitary and blood levels of beta-endorphin, leu-enkephalin, and dynorphin, Margules proposed that the obesity of these rodents is a message of impending famine that causes their overeating and hyperphagia. When famine is expected, the organism will be stimulated to build up stores by increasing its food intake. Pre-famine feeding is associated with hyperinsulinemia. To accomplish this end, the endorphinergic system releases beta-endorphin and ACTH from the pituitary gland. Beta-endorphin also reduces overall energy expenditure by reduction of thyrotrophin release (which lowers the body temperature set-point and increases the set-point for CO2 tension in the blood). This hypothesis is appealing in its ability to encompass many aspects of the ob/ob syndrome under a single explanatory system.

In support of Margules' theory, strong evidence has linked endorphins, feeding behavior, and obesity. (a) For example, opioid peptides stimulate feeding in sated rats.

Intracerebral microinjections of B-endorphin directly into the paraventricular nucleus (PVN) induce feeding (Leibowitz & Hor, 1980). Similarly, Grandison and Guidotti (1977) found that microinjection of B-endorphin into the ventromedial nucleus of the hypothalamus (co-implicated with the PVN in satiety control) elicited food consumption in satiated rats. Enhancement of food intake by intracerebral B-endorphin may be a direct effect of the endorphin acting at opiate receptors in this region of the brain. In this regard, Morley, Levine, Gosnell, and Billington (1984) have provided evidence for a B-endorphin-epsilon receptor system in the PVN, which modulates food intake. However, opiate enhancement of feeding may occur indirectly through the opiate modulation of one, or more, of the monoamines involved in the regulation of feeding. For example, dopamine, the primary transmitter substance of fibers in the nigrostriatal bundle, has been recognized as playing a major part in the initiation of feeding (Morley, 1982). Pharmacological or physiological damage along any portion of this path causes hypophagia and subsequent weight loss (Morley, 1980). Recently, opiate binding sites have been localized on the terminals of dopaminergic neurons of the nigrostriatal bundle (Pollard, Lorens, Schwartz, Gros, & Dray, 1978), and activation of these receptors increased striatal dopamine synthesis and turnover (Urwyler &

Tabakoff, 1981), thereby laying the foundation for an opioid-dopamine interaction in feeding.

(b) B-endorphin is one of the most extensively studied endogenous opioids, which has been implicated in the control of food intake. As mentioned previously, centrally administered B-endorphin stimulates feeding. It has been found that genetically obese rodents have elevated pituitary and plasma B-endorphin levels (Morley, Levine, Yim, & Lowy, 1983). Davis, Lowy, Yim, Lamb, and Malven (1983) found plasma B-endorphin levels significantly elevated in rats during conditions that can induce opiate-related hyperphagias (i.e., 2-deoxy-D-glucose, food deprivation, and darkness), thereby demonstrating that a peripheral component may be physiologically relevant to opiate-induced feeding (Yim & Lowy, 1984). In addition, Ferguson-Segall, Flynn, Walker, and Margules (1982) have found that when compared to those of lean littermates the posterior pituitaries of ob/ob mice contain approximately twice the level of dynorphin (an endogenous ligand for kappa-type receptors and a potent appetite stimulant). Dynorphin has high concentrations in the ventromedial hypothalamus (VMH) and PVN, areas of the hypothalamus in which a microinjection of B-endorphin stimulates feeding (Yim & Lowy, 1984). Leu-enkephalin, another kappa agonist, has also been shown to be in excess in the posterior pituitaries of ob/ob mice compared to lean

littermates (Ferguson-Segall et al., 1982). The demonstration of elevated levels of B-endorphin, dynorphin, and leu-enkephalin in obese rodents provides additional evidence for opioid involvement in feeding regulation.

(c) Opiate antagonists (such as naloxone and naltrexone) suppress spontaneous food intake and weight gain in rats (Brands, Thornhill, Hirst, & Gowdy, 1979) and food intake in food-deprived rats and mice (Brown & Holtzman, 1979). Naloxone, a highly specific antagonist at mu and kappa opiate receptors, in 1.0-10.0 mg/kg BW doses, reduced food consumption in food-deprived rats (Holtzman, 1974). In additional work, Holtzman (1979) showed that 0.3-10.0 mg/kg doses of naloxone supressed eating and drinking in rats that had been food deprived for 48 h or water deprived for 24 h. Intracerebral naloxone or naltrexone injections into the VMH and naloxone injections into the LH decreased 90-min food intake in food-deprived (20 h) rats, as did subcutaneous naloxone injections (Thornhill & Saunders, 1984). Because naloxone suppression of feeding occurs after either central or peripheral administration, it is likely that both central and peripheral opiate receptors are involved in feeding regulation.

(d) Opiate antagonists also suppress food intake in obese rodents (Ferguson-Segall et al., 1982). Margules, Moisset, Lewis, Shibuya, and Pert (1978) found that small doses of

the opiate antagonist naloxone selectively abolished overeating in genetically obese mice (ob/ob) and rats (fa/fa). A dose of naloxone as small as 0.25 mg/kg selectively depressed food intake of these obese animals by 30%, with no effect on lean mice. Similarly, Atkinson (1982) found that a bolus dose of 15 mg of naloxone suppressed food intake of massively obese human subjects by 29% but had no effect in lean human subjects.

Recent research has shown that the endorphin system is established before birth. The regional distribution of endorphins is similar to the adult conformation by Embryonic Day 16 (Bayon, Shoemaker, Bloom, Mauss, & Guillemin, 1979), and opiate receptors have been found to be present in the rat brain by Embryonic Day 14 (Kent, Pert, & Herkenham, 1982). Because the endorphin system develops pre- and postnatally, the endogenous opiates may influence the regulation of food intake from early in ontogeny. Margules (1979) has speculated that endorphins play an important role coordinating early growth and development. Some evidence supporting these speculations comes from the work of Sewell (1980). He administered SC injections of naloxone (either 0.3, 1.0, or 2.0 mg/kg) to 5-day-old Wistar rats and measured changes in their body weights up to 36 h after drug administration, when pups were left with their mothers. Changes in body weight, especially in infant rodents,

provide reliable, indirect measures of their milk intake (Houpt & Epstein, 1973; Lytle, Moorcroft, & Campbell, 1971). Naloxone antagonized milk intake in a graded fashion, with weights of all pups returning to control values within 36 h. Similarly, Aroyewun and Barr (1982) injected doses of naloxone (5, 10, 30 mg/kg) and naltrexone (10, 30, 50 mg/kg) intraperitoneally into 10-, 12-, 14- and 19-day-old Long-Evans rat pups. The animals were deprived for 4 h and following the injection of the drug were returned to their mother for 1 h. After the test period, pups were reweighed. Both naloxone and naltrexone reduced food intake in 14- and 19-day-old pups, but had no effect on younger animals. These results are similar to the naloxone suppression of feeding in adult mice and rats, although the findings suggest effects later in the development of rat pups compared to the Sewell (1980) study.

The influence of naloxone on the appetitive component of suckling in infant rodents (viz., nipple attachment) has also been studied. Spear and Ristine (1982) found no influence of naloxone on nipple attachment in 4-day-old rat pups. Because suckling can be dissociated into nutritive and non-nutritive components (Brake, 1979), which appear to respond to different stimuli (Lorenz, Ellis, & Epstein, 1982), the contrary effects of naloxone on attachment in the Spear et al. (1982) study and on intake in the Sewell (1980)

study are not surprising. Sinha and Wilson (1983), however, demonstrated that naloxone (at 0.3 and 1.0 mg/kg BW doses) depressed suckling by increasing nipple attachment latencies and decreasing length of attachment in 6- and 15-day-old lean (+/?) and preobese (ob/ob) mouse pups. The incongruence of the Spear et al. (1982) and the Sinha et al. (1983) study, however, can be attributed to methodological factors. Spear et al. (1982) used an idiosyncratic procedure for monitoring nipple attachment (i.e., number of attachments were tallied every 5 min, as opposed to a continuous recording). Also the experimental animal used in both studies differed. Spear et al. (1982) used rat pups, whereas Sinha et al. (1983) used mouse pups. Preliminary results from Hall and Browde (1986) suggested that the early ingestive behaviors of mice and rats differ. Therefore, differences in results may be attributable to the animal model under investigation. In summary, endorphin systems are present early in rodent development and appear to have an impact on early ingestion.

Because (a) the endorphin system has been established prior to birth, (b) naloxone influences food intake in adult animals and may affect milk intake in preweanling rodents, and (c) naloxone differentially suppresses food intake in lean and obese rodents, the present study examined whether naltrexone suppresses milk intake in mouse pups and whether

preobese pups are more sensitive than lean pups to naltrexone's effects, as has already been demonstrated in adult ob/ob mice. The purpose of the present study was to determine naltrexone's effect on milk intake (as measured by percentage body weight gain) in preobese (ob/ob) and lean (+/?, +/+) mice. Varying doses of naltrexone (0, 0.5, 1.0, and 4.0 mg/kg BW) were administered SC to 6- and 12-day-old pups prior to successively cross-fostering them to two milk-replete dams. It was expected that preobese ( ob/ob ) pups would not consume as much milk as their lean counterparts (+/+) when treated with naltrexone and therefore, that they would show a smaller percentage increase in body weights across testing. The +/? pups would probably consume less milk than +/+ pups, but more milk than ob/ob pups when treated with the same dose of naltrexone, although the presence of a gene-dosage phenomenon might make comparisons with +/? mice subject to greater error variance.

## Method

### Subjects

Forty-three litters born from matings of adult male and female mice ( Mus musculus, strain C57Bl/6J, ob/ +, Jackson Laboratories, Bar Harbor, ME, USA) and 33 litters born from matings of adult male and female mice (strain C57Bl/6J, +/+, Jackson Laboratories, Bar Harbor, ME, USA) were used in the present study. Following the dams' removals from the breeding cages of male mice, their nesting cages were checked daily between 1600-1700 h; litters were designated as born on that day if all pups had been delivered by that time (day of birth = Day 0). Only litters containing between 8-11 pups were used in the experiment. The litters were housed from birth until weaning with their natural dams in clean polypropylene nesting cages with sufficient wood chip bedding, in a mouse colony room cycled on a 12 h light/dark cycle (lights on at 0800 h). Room temperature was maintained between 22-24 $^{\circ}$ C, and relative humidity varied between 30-40%. Food (F6 Rodent Blox, Wayne Pet Food Division, Chicago, IL, USA) and water were available continuously to all mice.

### Apparatus and Procedure

Litters were randomly assigned to be tested on either Day 6 or Day 12 postpartum. A litter was tested during daylight hours and was not tested on more than one day. Of the 43

ob/ + litters used, 22 litters were tested on Day 6, and the remaining 21 litters were tested on Day 12. Of the 33 +/+ litters used, 18 litters were tested on Day 6, and the remaining 15 litters were tested on Day 12.

Ob/ + litters and +/+ litters were randomly assigned to one of the following test drug conditions by someone other than the experimenter: (a) 0.15 M saline (SAL) solution; (b) 0.5 mg/kg body weight (BW) dose of naltrexone (0.5 NLTX); (c) 1.0 mg/kg BW dose of naltrexone (1.0 NLTX); and (d) 4.0 mg/kg BW dose of naltrexone (4.0 NLTX). All pups were removed from their dams 4 h prior to testing and placed with a nonlactating virgin female mouse (i.e., aunt). Maternal care (i.e., stimulation of reflexive micturation and maintenance of thermal homeostasis) was provided by the aunt, while pups were deprived of nutrition (Richards, 1967; Wilson, Chang, Henning, & Margules, 1981). These procedures yielded a 4 x 2 (drug dose x test age) between groups experimental design.

At testing, pups were removed from their aunt and stimulated to urinate and defecate, immediately before the initial weighing, by gently stroking the anogenital area with a cotton-tipped swab. This procedure was followed to reduce any variability in weight loss due to excretion during the testing (Hall, Cramer, & Blass, 1975). All pups were then tail-marked with a water-based nontoxic marking

pen for future identification. Pups were weighed (pre-injection weight) to the nearest 0.01 g on a Mettler digital balance (Mettler Model PB300). Rectal temperature (pre-injection temperature) was recorded to the nearest 0.1 $^{o}$C using a digital readout thermometer (Bailey Inst. Inc., Model BAT-12). A lubricated thermocouple (diameter 0.0064 cm) was inserted into the anus and gently pushed approximately 5 mm into the rectum. After body weight and rectal temperature were recorded, subcutaneous (SC) injections of the appropriate drug dose (Margules, June, 1985) were administered in volumes of 1 cc/100 g BW through a 1/2-in(1.2 cm) 26-ga. needle attached to a microliter syringe into the nuchal fold. An individual other than the experimenter, who was unaware of the hypothesis of the experiment, prepared all solutions for the experimenter on the day of testing. The appropriate dose of naltrexone hydrochloride (Dupont Pharmaceuticals Wilmington, DE, USA) was weighed on a Sartorius analytical balance (Model 125646) and dissolved in 0.15 M sterile saline solution. The solution was placed in coded injection vials, with the code made available to the experimenter only after completion of the experiment. Pups were reweighed immediately following injections (post-injection weight) before whole litters were cross-fostered successively to two-milk replete dams for 1 h each. Post-injection weights were taken because weight loss

due to excretion may have occurred during the injection or an increase in weight may have occurred due to the injected solution.

The milk-replete surrogate dams had given birth to their own litters within 48 h of the test litters' birth dates. On the day of testing, the surrogate dam had her litter placed with a non-lactating virgin female, leaving the surrogate without pups for 6-8 h. The size of the dam's milk supply can be increased by separating the dam from her pups for 8 h, thereby optimizing the milk supply for test pups (Friedman, 1975; Jara-Almonte & White, 1972). In no instance were litters tested on their own dams.

Before the pups were crossed-fostered to the second surrogate, their body weights were recorded. After 1 h with the second surrogate, pups were reweighed. Weight gain served as an indirect measure of milk intake (Houpt & Epstein, 1973; Lytle, Moorcroft, & Campbell, 1971), and the total weight gain was used as an index of total milk intake. Temperatures were recorded at the end of testing, and all litters were returned to their natural dams. Tail markings were rechecked and renewed up to Day 22. At weaning (Day 22) pups were ear-punched for permanent identification and sexed. The animals were rehoused by sex and litter assignment with no more than five mice per cage and were

maintained ad lib on food and water until the obese phenotype was clearly visible in at least one mouse per ob /+ litter. The mice were reweighed at 28 and 35 days to verify the results of the visual inspection and were sacrificed by ether inhalation. If no obese mice were identified in a litter derived from ob/ + breeding, data concerning that particular litter were discarded from the data analysis. Upon identification of the obese mice in each ob/ + derived litter, data were regrouped in one of two ways to yield an additional genotype ( ob/ob, +/+) or an additional phenotype ( ob/ob, +/?, +/+) factor to the original 4 x 2 (Drug Dose x Test Age) design.

Statisical Analysis

Two types of data were analyzed in this study: body weights and rectal temperatures. Body weight (g) as an index of milk intake (dependent variable) was converted into a percentage change from pretest body weight from the following formula:

$$\% \text{ body weight gain} = \frac{(\text{posttest weight} - \text{postinjection weight})}{\text{postinjection weight}} \times 100$$

Because pups may differ in their pretest body weights, expressing intake as a percentage of pretest body weight

assesses intake more accurately in relation to differing baseline body weights. In a similar fashion, the change in temperature (°C) over testing was also converted into a percentage change from baseline (or pretest) temperature:

$$\% \text{ change temp} = ((\text{posttest temperature} - \text{pretest temperature}) / \text{pretest temperature}) \times 100$$

Because preobese mice have lower core temperatures, expressing temperature as a percentage of pretest rectal temperature assesses temperature more accurately in relation to differing baseline temperatures.

The data were analyzed in two different ways: (a) raw intake and temperature data, in which individual pups' intake and temperature scores contributed to the analysis and (b) mean intake and temperature data (after Abbey & Howard, 1973). Within each data set, phenotype ( ob/ob vs. +/? vs. +/+) and genotype comparisons ( ob/ob vs. +/+) were evaluated in separate analyses.

The raw intake data were analyzed using a 3 x 4 x 2 (Phenotype x Drug Dose x Test Age) and a 2 x 4 x 2 (Genotype x Drug Dose x Test Age) factorial analysis of variance (ANOVA) on the variable, total percentage body weight gain.

Because the design is unbalanced by incorporating different cell sizes, a general linear model solution to the ANOVA was used. The Statistical Analysis System (SAS) package contains a general linear model (GLM) solution if an unbalanced design is used. Mean data in the present study were analyzed using a GLM Type III test procedure (SAS, 1982).

Abbey & Howard (1973) developed a technique to equate maternal contributions to interlitter variability in animal developmental research. According to their method, the mean score on each dependent measure within a litter serves as a single data point. For example, the average percentage body weight gain for all ob/ob mice within a particular litter (and thereby within a single drug dose x age combination) constituted one data point. Similarly, the mean percentage body weight gain for all lean (+/?) mice in that same litter formed a lean data point for that drug dose x age combination. Mean intake data were analyzed using a 3 x 4 x 2 (Phenotype x Drug Dose x Test Age) and a 2 x 4 x 2 (Genotype x Drug Dose x Test Age) factorial ANOVA on the variable, total percentage body weight gain.

Both raw and mean temperature data were analyzed using a 3 x 4 x 2 (Phenotype x Drug Dose x Test Age) and a 2 x 4 x 2 (Genotype x Drug Dose x Test Age) factorial ANOVA on the variable, percentage change in body temperature.

Post hoc comparisons of significance between group means used both the Scheffe and Tukey-Kramer tests. A series of linear contrasts aided comparisons of group means on significant interaction effects (Mount, July, 1986). The alpha value for all comparisons was set at $p < .05$. It should be noted that only main effects were analyzed using the Scheffe and Tukey-Kramer tests. Interaction effects were analyzed by (using a SAS procedure) linear contrasts in which each particular contrast was specified. A new ANOVA table was generated and significant interaction effects were listed.

# Results

## Intake Data: Raw Data

Phenotype comparisons: ob/ob versus +/? versus +/+. Total percentage body weight gain was significantly affected by phenotype, $F$ (2,681)= 12.77, $p < 0.0001$, drug dose, $F$ (3, 681)= 3.26, $p < 0.0209$, and test age, $F$ (1,681)= 18.62, $p < 0.0001$. Post hoc comparisons showed that obese pups gained a smaller percentage body weight in testing (and thereby consumed less milk) than either +/+ or +/? pups. Drug comparisons showed an overall impact of NLTX on intake, although no clear cut dose-dependent effect appeared. That is, although pups given naltrexone tended to consume less milk, only pups in the 1.0 NLTX drug condition consumed less than pups in the 0.5 NLTX drug condition. In addition, the overall impact of test age on percentage body weight gain showed that 12-day-olds consumed less than 6-day-olds. Table 1 provides a summary of mean percentage change in body weight for each phenotype under each drug condition at both test days.

Insert Table 1 about here

Phenotype x test age, $F$ (2,681)= 8.50, $p < 0.0002$, and phenotype x drug dose interactions, $F$ (6,681)= 3.24, $p < 0.0038$, were also found (see Appendix A-1 for ANOVA summary table on phenotype raw intake data). Linear contrasts showed that obese pups consumed less than +/+ and +/? pups at both the 0.5 and 4.0 NLTX drug conditions and that +/? pups consumed less than +/+ pups at the 4.0 NLTX drug dose. A dose-dependent trend was noted for obese pups (i.e., a systematic decrease in intake was observed as the dose of NLTX increased). This dose-dependent effect was not found for either +/+ or +/? pups.

A phenotype x drug dose x test age interaction, $F$ (6,681) = 11.54, $p < 0.0001$, was also found. Linear contrasts showed that obese and +/? pups ingested more than +/+ pups at 6-days in the SAL condition. At 12 days, however, +/+ pups ate more than +/? pups in the SAL condition. Obese pups consumed less than +/? pups at 6 days in the 0.5 NLTX drug condition, and +/? consumed more than +/+ pups under the same test conditions. Twelve-day-old obese pups ingested less than +/+ pups in the 0.5 NLTX drug condition. The +/? pups in the 1.0 NLTX drug condition consumed more than 6-day-old +/+ pups. Six-day-old obese pups consumed less than both +/+ and +/? pups in the 4.0 NLTX drug condition, and +/? pups consumed less than +/+ pups under the same test conditions. At 12 days obese pups ingested

less than +/? pups in the 4.0 NLTX drug condition, and 12-day-old +/? pups ingested more than +/+ pups. Linear contrasts of different drug dose levels within each phenotype x age combination revealed that at 6 days both +/? and obese pups consumed less in the 4.0 NLTX drug condition than in the SAL condition, while +/+ pups ate more under 4.0 NLTX than in the SAL condition. In fact, +/+ pups consumed more at the 4.0 NLTX than at either 0.5 or 1.0 NLTX conditions. They also ate more after 0.5 NLTX than after SAL injection. In addition, +/? pups consumed less under both 1.0 and 4.0 NLTX drug conditions compared to the 0.5 NLTX group. By 12 days, however, +/? pups ate more at 0.5 and 1.0 mg/kg doses than saline-injected pups. But, in contrast to their pattern as 6-day-olds, 12-day-old +/+ pups ingested less under both 1.0 and 4.0 mg/kg doses than at either SAL or 0.5 mg/kg NLTX.

Genotype comparisons: ob/ob versus +/+. The total percentage body weight gain was significantly affected by test age, $F$ (1,367)= 4.55, $p < 0.0337$. The overall impact of test age on percentage body weight gain showed that 12-day-olds ($M$ = 6.55) ate less than 6-day-olds ($M$ = 7.09). Although a significant genotype main effect was not found, overall obese pups ($M$ = 6.25) tended to consume less than +/+ pups ($M$ = 6.96). Genotype x drug dose, $F$ (3,366)= 4.59, $p < 0.0038$, and drug dose x test age, $F$ (3,367)=3.35, $p <$

0.0191, interactions were also found. Obese pups ate less than +/+ pups in the 4.0 NLTX drug condition. A drug-dependent trend was noted for obese pups (i.e., as the dose of naltrexone increased, the amount of milk ingested decreased). A genotype x drug dose x test age, $F$ (3,366)= 7.29, $p < 0.0001$, interaction was also found (see Appendix A-2 for ANOVA summary table on genotype raw intake data). In the SAL condition, 6-day-old obese pups ingested more than their lean counterparts. In the 4.0 NLTX condition, 6-day-old obese pups consumed less than +/+ pups. Similarly, 12-day-old obese pups ingested less than +/+ pups in the 0.5 NLTX drug condition.

**Intake Data: Mean Data**

Phenotype comparisons: ob/ob versus +/? versus +/+. The total percentage body weight gain was significantly affected by phenotype, $F$ (2,95)= 8.01, $p < 0.0006$, and test age, $F$ (1,95)= 11.39, $p < 0.0011$.

Insert Figure 1 about here

Unlike the analysis of raw data, naltrexone did not produce a main effect on milk intake. Figure 1 illustrates the mean percentage body weight gain for ob/ob, +/+, and +/? mouse

pups as a function of the amount of naltrexone administered. Overall, obese pups (M = 6.40) ingested less than +/? pups (M = 7.52), and 12-day-olds (M = 6.50) consumed less than 6-day-olds (M = 7.35) during testing (see Appendix C-1 and C-2 for summary of the mean percentage change in body weight for each phenotype under each drug at both test ages).

Insert Figure 2 about here

Unlike the ANOVA on the raw data, no two-way interactions achieved significance, when mean data were analyzed. Figures 2 and 3, however, illustrate the significant phenotype x drug dose x test age interaction, F (6,95)= 4.88, p < 0.0002,

Insert Figure 3 about here

similar to that found with the raw data analysis (see Appendix B-1 for ANOVA summary table on phenotype mean intake data). At 6 days, obese pups ingested more than +/+ pups in the SAL condition. Similarly, +/? pups ingested more than 6-day-old +/+ pups in the SAL condition (+/+ consumed less than ob/ob, who consumed less than +/?).

Obese 6-day-olds ate less than +/? pups in the 0.5 NLTX drug condition, and +/? pups consumed more than +/+ pups. Six-day-old obese pups ingested less than +/? and +/+ pups in the 4.0 NLTX drug condition, and +/? consumed less than +/+ pups ( ob/ob consumed less than +/?, who consumed less than +/+). At 12 days obese pups consumed less than +/? pups in the 4.0 NLTX drug condition. Linear contrasts of different drug dose levels within each phenotype x age combination showed that at 6 days, both +/? and obese pups consumed less in the 4.0 NLTX condition than in the 0.5 NLTX condition. In addition, obese pups ingested less in the 4.0 NLTX drug condition than in SAL or 1.0 NLTX drug conditions. On the other hand, +/+ pups ate more under the 4.0 NLTX condition than in the SAL, 0.5 NLTX or 1.0 NLTX conditions. The +/? pups consumed less in the 1.0 NLTX condition compared to the 0.5 NLTX condition. By 12 days, however, +/+ pups ingested less in the 4.0 NLTX drug condition than in the SAL condition and 0.5 NLTX drug condition. No significant differences were found for obese and +/? pups among drug conditions at 12 days.

Genotype comparisons: ob/ob versus +/+. Mean percentage body weight gain was significantly affected by genotype x drug dose, $F$ (3,60)= 3.42, $p < 0.0226$, drug dose x test age, $F$ (3,60)= 2.81, $p < 0.0461$, and genotype x drug dose x test age, $F$ (3,60)= 8.03, $p < 0.0002$, interactions (see Appendix

B-2 for ANOVA summary table on genotype mean intake data). Obese pups consumed less in the 4.0 NLTX drug condition than their lean counterparts. A dose-dependent trend was noted for obese pups (as the dose of NLTX increased the amount ingested by obese pups decreased). Figure 2 graphically depicts that 6-day-old obese pups ingested more milk than +/+ pups in the SAL condition, although at 12-days this difference was no longer significant (see Figure 3). Six-day-old obese pups consumed less milk than their lean counterparts in the 4.0 NLTX drug condition. Similarly, obese 12-day-olds ate less than +/+ pups in the 0.5 NLTX drug condition.

**Temperature Data: Raw Data**

Phenotype comparisons: ob/ob versus +/? versus +/+. Percentage change in rectal temperature was significantly affected by drug dose, $F$ (3,654)= 6.67, $p < 0.0002$, and test age, $F$ (1,654)= 78.58 $p < 0.0001$. Overall, changes in rectal temperatures over testing were less for pups in the 0.5 NLTX group than for pups in either the SAL, 1.0, or 4.0 NLTX groups. The latter pups did not differ among themselves on this measure. The rectal temperatures of 12-day-olds showed a greater increase ($M$ = 3.11) over testing than those of younger pups ($M$ = .44). Table 2 provides a summary of mean percentage change in rectal

temperature for each phenotype under each drug condition and at both test days. Drug dose x test age,

Insert Table 2 about here

$F$ (3,654)= 8.30, $p < 0.0001$, and phenotype x drug dose x test age, $F$ (6,654)= 12.45, $p < 0.0001$, interactions were also found (see Appendix A-3 for ANOVA summary table on phenotype raw temperature data). Table 3 and 4 summarize preinjection and postinjection rectal temperatures, respectively, for each phenotype under each drug condition and at both test ages.

Insert Tables 3 and 4 about here

Genotype comparisons: ob/ob versus +/+. Percentage change in temperature was significantly affected by drug dose, $F$ (3,340)=3.20, $p < 0.0232$, test age, $F$ (1,340)=33.53, $p < 0.0001$, drug dose x test age, $F$ (3,340)=3.05, $p < 0.0283$, and genotype x drug dose x testage interactions, $F$ (3,340)=11.27, $p < 0.0001$ (see Appendix A-4 for ANOVA summary table on genotype raw temperature data). A greater

change in rectal temperature was found with pups in the SAL condition compared to the 0.5 NLTX drug condition. Twelve-day-olds showed a greater change in temperature over testing than did 6-day-olds. These data reflect similar significant trends as those revealed by the phenotype comparisons.

**Temperature Data: Mean Data**

Phenotype comparisons: ob/ob versus +/? versus +/+. Mean percent change in temperature was significantly affected by test age, $F$ (1, 93)=29.48, $p < 0.0001$, although the drug effect revealed in the raw data ANOVAs did not achieve significance here. A drug dose x test age, $F$ (3,93)=3.54, $p < 0.0177$, and a phenotype x drug dose x test age interaction, $F$ (6,93)=2.65, $p < 0.0202$, also occurred (see Appendix B-3 for ANOVA summary table on phenotype mean temperature data). As previous comparisons emphasized, older pups were able to raise their rectal temperatures over testing more than younger pups (Ms = 2.97 and .30, respectively). Figures 4 and 5 graphically show mean temperature interactive effects.

Insert Figures 4 and 5 about here.

Genotype comparisons: ob/ob versus +/+. Mean percentage change in temperature was significantly affected by test age, $F$ (1,58)=13.38, $p < 0.0006$, and by a genotype x drug dose x test age interaction, $F$ (3,58)=4.07, $p < 0.0108$ (see Appendix B-4 for ANOVA summary table on genotype mean temperature data). Both obese and lean 12-day-olds demonstrated a greater change in temperature compared to 6-day-olds.

## Discussion

B-endorphin has been extensively studied and implicated in the control of food intake. Genetically obese rodents have elevated pituitary and plasma B-endorphin levels (Morley et al., 1983) and opiate antagonists (e.g., naloxone) selectively abolish overeating in these animals (Margules et al., 1978). Because opiate antagonists suppress food intake in adult obese rodents, it was hypothesized that suppression of milk consumption would occur in preobese pups if these pups were differentially sensitive to naltrexone compared to lean (+/+ and +/?) pups. The results of this study suggest that administration of the opiate antagonist naltrexone is effective in decreasing the amount of milk ingested by milk-deprived preobese pups.

Overall, preobese pups consumed less than +/+ and +/? lean pups. On the surface this finding is consistent with data of several researchers, who have contended that hyperphagia characterizing the adult ob/ob does not develop until the pup becomes free-feeding (Contaldo, 1981; Lin, Romsos, & Leveille, 1977; Rath & Thenen, 1979). A closer examination, however, revealed that preobese pups in the saline (no drug) condition ingested more milk than lean (+/+) pups and a similar quantity of milk compared to a lean sample representing an unknown proportion of heterozygous (

ob /+) to homozygous (+/+) pups. Because several studies have emphasized the likelihood of a gene-dosage effect of the ob gene in influencing the metabolic phenotype of lean mice to approach that of obese mice (Connally & Carnie, 1984), it is not surprising that significant differences in intake (no drug condition) occurred for genotype, and not phenotype, comparisons. The finding of greater intake in preobese than +/+ pups supports recent work in which preobese pups at 6, 12, and 18 days postpartum ingested more milk than leans when cross-fostered to milk-replete dams (Wilson, 1983) and substantiates Joosten & Van der Kroon's (1974) contention that obese mice are congenitally hyperphagic.

At the smallest dose of naltrexone (0.5 mg/kg), however, 6-day-old preobese pups ate less than +/? lean pups. Similarly, at the highest dose of naltrexone (4.0 mg/kg), 6-day-old preobese pups ingested less than both +/+ and +/? lean pups. The response to naltrexone was similar for 12-day-old preobese pups. At the smallest dose of naltrexone, preobese pups ate less than homozygous (+/+) lean pups, and at the highest dose of naltrexone preobese pups consumed less than both +/+ and +/? lean pups. The result that naltrexone significantly altered the amount of milk ingested by preobese pups compared to their lean littermates suggests that opiate receptors are functional

early in development in this strain and prior to the onset of obesity.

Differences in milk intake were found between +/? and +/+ lean pups. Although there is no known technique to identify the genotype of +/? mice (other than by breeding), statistically two-thirds of the +/? lean mice should be carriers of the ob gene. The results suggest that a single obese gene can influence milk consumption in this strain if appropriate conditions are met for its expression. At both the 0.5 mg/kg and 1.0 mg/kg doses of naltrexone 6-day-old lean (+/?) pups ingested more than +/+ pups. However, at the highest drug dose of naltrexone (4.0 mg/kg) 6-day-old +/? pups consumed less than +/+ pups.

Drug comparisons showed an overall impact of naltrexone on intake. Pups in the 1.0 mg/kg naltrexone condition consumed less than pups in the lowest drug condition (0.5 mg/kg naltrexone). These results are comparable to those of Sewell (1980), who found that naloxone antagonized milk intake in 5-day-old rats in a graded fashion (depending on the dose of naloxone administered).

The dose of naltrexone administered influenced the amount of milk ingested by mouse pups. Preobese 6-day-olds consumed less when given the highest dose of naltrexone (4.0 mg/kg) compared to any other drug condition (saline, 0.5

mg/kg, or 1.0 mg/kg). In general, as the dose of naltrexone increased, the amount ingested by preobese pups decreased. Differing levels of naltrexone also influenced milk consumption in lean (+/?) pups. Similar to preobese pups, 6-day-old +/? pups ate less when given the highest dose of naltrexone than +/? pups in the saline condition. Also, 6-day-old +/? pups in the 1.0 mg/kg and 4.0 mg/kg naltrexone conditions consumed less than pups in the 0.5 mg/kg naltrexone condition. Again, these results with +/? pups lend support to the idea that a single obese gene has an influence on the early development of eating behavior in this strain of mice.

Unlike drug comparisons for either ob/ob or +/? pups, the 6-day-old lean (+/+) pups ate more at the highest dose of naltrexone (4.0 mg/kg) compared to any other drug condition (saline, 0.5 mg/kg, and 1.0 mg/kg). This finding provides some evidence that the suppression of milk consumption observed with preobese and lean (+/?) pups is due to their genetic make-up and to the influence of the drug on a mechanism involved in the suppression of ingestion and not due to some side effect (i.e., nausea) of the drug, which depresses intake by debilitating the pup.

Overall, 12-day-olds consumed less than 6-day-old pups. This age main effect is misleading, unless it is noted that

approximately 3/4 of all pups tested at each age were treated with naltrexone. Numerous studies (Bayon et al., 1979; Kent et al., 1982; Khachaturian, Alessi, Munfakh, & Watson, 1983) have identified the presence of opiate receptors before birth with the number of receptors increasing until the time of weaning. Therefore, it is not surprising to find 12-day-olds ingesting less than 6-day-olds (as a reflection of the greater effectiveness of naltrexone on a more mature endorphin system at 12 days). These results mirror the work of Aroyewun & Barr (1982), who found that naloxone and naltrexone suppressed milk intake in 14- and 19-day-old rat pups, but had no effect on younger animals.

Low body temperature is one of the earliest indicators of the ob/ob genotype and is detectable 7-10 days after birth (Carlisle & Dubuc, 1982). This hypothermia contributes to obesity because a greater percentage of ingested diet is diverted to fat rather than wasted on thermogenesis (Hoyenga & Hoyenga, 1982). Lower than normal pretest rectal temperatures were found with all pups. During the deprivation period litters were housed with a nonlactating virgin female mouse. Although this artificial situation closely simulates the natural environmental condition, while eliminating milk as a food source, Henning & Gisel (1980) have shown that "aunts" do not maintain rat pups'

temperatures during deprivation as close to nest values as do nipple-ligated dams. A similar effect may have contributed to the lower-than-normal pretest rectal temperatures observed here.

Naltrexone significantly affected posttest rectal temperatures. Overall, a greater change in temperatures was found for animals in the saline condition, 1.0 mg.kg, and 4.0 mg/kg naltrexone conditions compared to the lowest naltrexone (0.5 mg/kg) condition. Overall, 12-day-olds showed a greater increase in rectal temperature than did 6-day-olds. At the two highest naltrexone doses (1.0 mg/kg and 4.0 mg/kg) 6-day-olds exhibited an increase in rectal temperatures (with the exception of lean (+/+) pups tested in the 4.0 mg/kg naltrexone condition). Under all possible drug conditions, 12-day-old preobese and lean (+/+ and +/?) pups demonstrated an increase in rectal temperature (with the exception of lean (+/+) pups tested in the 0.5 mg/kg naltrexone condition). Although it was expected that preobese pups would demonstrate lower body temperatures in comparison to lean (+/+ and +/?) littermates (Trayhurn, Thurlby, & James, 1977), neither a phenotype nor a genotype main effect was found, demonstrating no differences among obese and lean pups in ability to alter their rectal temperatures during testing.

Opiates and opiate antagonists influence thermoregulation in adult mice. Rosow, Miller, Poulsen-Burke, & Cochin (1982) found that naloxone reversed the hypothermic effect that followed a B-endorphin injection in mice. If the preobese pups in this study had higher levels of circulating B-endorphin compared to their lean counterparts, or earlier developing endorphin systems, the administration of naltrexone could have antagonized the thermic depression that otherwise would have been observed with the preobese pups.

The intake results of this study lend support to the idea of early endorphin involvement in ingestive behavior in this strain of mice. Antagonism of milk consumption was detected as early as 6 days of age. These results contribute to the developing body of literature suggesting a behaviorally functional opioid system early in ontogeny (Hamm & Knisely, 1984; Panksepp, 1984; Zagon, 1984). Kehoe & Blass (1986) found neonatal rats, as early as 5 days of age, were sensitive to the affective consequences of an opioid, morphine. A neutral stimulus (a novel taste or odor) that predicted a positive morphine state became highly preferred by preweanling rats. The positive associations formed with the novel taste (saccharin) received during suckling were prevented with an opioid antagonist (naloxone) when administered prior to conditioning. Therefore, the opioid

system appears to be tapping into the motivational system, which may serve as a reward mechanism leading to learning and conditioning. Kehoe et al. (1986) contended that this may be an important component in mother-infant bonding.

The results of the present study support the hypothesis that preobese pups are differentially sensitive to naltrexone compared to lean (+/+ and +/?) pups. This was evidenced by the preobese pups consuming less milk than their lean counterparts (as shown by a smaller percentage increase in body weight across testing). This provides evidence that opiate receptors are functional early in the development in this strain. Future research can continue looking at how the endorphinergic system modulates food intake, as well as how the endorphinergic system taps into the motivational system in which food intake is implicated.

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# Appendix A

## ANOVA Summary Tables: Raw Data

Appendix A-1

Raw Data in a 3 x 4 x 2 (Phenotype x Drug Dose x Age) Design

Percentage Change in Body Weight

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Phenotype | SS= 114.88<br>MS= 57.44 | 12.77 | 2, 681 | 0.0001 |
| Drug Dose | SS= 44.02<br>MS= 14.67 | 3.26 | 3, 681 | 0.0209 |
| Testage | SS= 83.74<br>MS= 83.74 | 18.62 | 1, 681 | 0.0001 |
| Phenotype x Drug Dose | SS= 87.46<br>MS= 14.58 | 3.24 | 6, 681 | 0.0038 |
| Phenotype x Testage | SS= 76.46<br>MS= 38.23 | 8.50 | 2, 681 | 0.0002 |
| Drug Dose x Testage | SS= 15.09<br>MS= 5.03 | 1.12 | 3, 681 | 0.3411 |
| Phenotype x Drug Dose x Testage | SS= 311.41<br>MS= 51.90 | 11.54 | 6, 681 | 0.0001 |
| Error | SS= 3063.22<br>MS= 4.50 | | | |

Appendix A-2

Raw Data in a 2 x 4 x 2 (Genotype x Drug Dose x Age) Design

Percentage Change in Body Weight

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Genotype | SS= 11.13<br>MS= 11.13 | 2.84 | 1, 367 | 0.0927 |
| Drug Dose | SS= 19.89<br>MS= 6.63 | 1.69 | 3, 367 | 0.1664 |
| Testage | SS= 17.81<br>MS= 17.81 | 4.55 | 1, 367 | 0.0337 |
| Genotype x Drug Dose | SS= 54.00<br>MS= 18.00 | 4.59 | 3, 367 | 0.0038 |
| Genotype x Testage | SS= 9.86<br>MS= 9.86 | 2.52 | 1, 367 | 0.1135 |
| Drug Dose x Testage | SS= 39.33<br>MS= 13.11 | 3.35 | 3, 367 | 0.0191 |
| Genotype x Drug Dose x Testage | SS= 85.66<br>MS= 28.55 | 7.29 | 3, 367 | 0.0001 |
| Error | SS= 1437.78<br>MS= 3.92 | | | |

Appendix A-3

Raw Data in a 3 x 4 x 2 (Phenotype x Drug Dose x Age) Design

Percentage Change in Rectal Temperature

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Phenotype | SS= 28.93<br>MS= 14.46 | 1.37 | 2, 654 | 0.2560 |
| Drug Dose | SS= 212.11<br>MS= 70.70 | 6.67 | 3, 654 | 0.0002 |
| Testage | SS= 832.38<br>MS= 832.38 | 78.58 | 1, 654 | 0.0001 |
| Phenotype x Drug Dose | SS= 14.48<br>MS= 2.41 | 0.23 | 6, 654 | 0.9677 |
| Phenotype x Testage | SS= 16.04<br>MS= 8.02 | 0.76 | 2, 654 | 0.4695 |
| Drug Dose x Testage | SS= 263.65<br>MS= 87.88 | 8.30 | 3, 654 | 0.0001 |
| Phenotype x Drug Dose x Testage | SS= 791.61<br>MS= 131.94 | 12.45 | 6, 654 | 0.0001 |
| Error | SS= 6927.95<br>MS= 10.59 | | | |

Appendix A-4

Raw Data in a 2 x 4 x 2 (Genotype x Drug Dose x Age) Design

Percentage Change in Rectal Temperature

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Genotype | SS= 13.38<br>MS= 13.38 | 1.10 | 1, 340 | 0.2944 |
| Drug Dose | SS= 116.61<br>MS= 38.87 | 3.20 | 3, 340 | 0.0232 |
| Testage | SS= 406.77<br>MS= 406.77 | 33.53 | 1, 340 | 0.0001 |
| Genotype x Drug Dose | SS= 9.22<br>MS= 3.07 | 0.25 | 3, 340 | 0.8591 |
| Genotype x Testage | SS= 7.19<br>MS= 7.19 | 0.59 | 1, 340 | 0.4420 |
| Drug Dose x Testage | SS= 111.07<br>MS= 37.02 | 3.05 | 3, 340 | 0.0283 |
| Genotype x Drug Dose x Testage | SS= 410.00<br>MS= 136.67 | 11.27 | 3, 340 | 0.0001 |
| Error | SS= 4124.48<br>MS= 12.13 | | | |

# Appendix B

## ANOVA Summary Tables: Mean Data

Appendix B-1

Mean Data in a 3 x 4 x 2 (Phenotype x Drug Dose x Age) Design

Percentage Change in Body Weight

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Phenotype | SS= 27.40<br>MS= 13.78 | 8.01 | 2, 95 | 0.0006 |
| Drug Dose | SS= 9.57<br>MS= 3.19 | 1.87 | 3, 95 | 0.1390 |
| Testage | SS= 19.47<br>MS= 19.47 | 11.39 | 1, 95 | 0.0011 |
| Phenotype x Drug Dose | SS= 17.15<br>MS= 2.86 | 1.67 | 6, 95 | 0.1364 |
| Phenotype x Testage | SS= 8.80<br>MS= 4.40 | 2.57 | 2, 95 | 0.0815 |
| Drug Dose x Testage | SS= 4.04<br>MS= 1.35 | 0.79 | 3, 95 | 0.5071 |
| Phenotype x Drug Dose x Testage | SS= 50.04<br>MS= 8.34 | 4.88 | 6, 95 | 0.0002 |
| Error | SS= 162.48<br>MS= 1.71 | | | |

Appendix B-2

Mean Data in a 2 x 4 x 2 (Genotype x Drug Dose x Age) Design

Percentage Change in Body Weight

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Genotype | SS= 3.87<br>MS= 3.87 | 2.68 | 1, 60 | 0.1068 |
| Drug Dose | SS= 5.13<br>MS= 1.71 | 1.18 | 3, 60 | 0.3225 |
| Testage | SS= 4.76<br>MS= 4.76 | 3.29 | 1, 60 | 0.0745 |
| Genotype x Drug Dose | SS= 14.83<br>MS= 4.94 | 3.42 | 3, 60 | 0.0226 |
| Genotype x Testage | SS= 3.64<br>MS= 3.64 | 2.52 | 1, 60 | 0.1178 |
| Drug Dose x Testage | SS= 12.20<br>MS= 4.07 | 2.81 | 3, 60 | 0.0461 |
| Genotype x Drug Dose x Testage | SS= 34.82<br>MS= 11.61 | 8.03 | 3, 60 | 0.0002 |
| Error | SS= 86.71<br>MS= 1.44 | | | |

Appendix B-3

Mean Data in a 3 x 4 x 2 (Phenotype x Drug Dose x Age) Design

Percentage Change in Rectal Temperature

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Phenotype | SS= 7.33<br>MS= 3.66 | 0.53 | 2, 93 | 0.5925 |
| Drug Dose | SS= 46.74<br>MS= 15.58 | 2.24 | 3, 93 | 0.0877 |
| Testage | SS= 205.30<br>MS= 205.30 | 29.48 | 1, 93 | 0.0001 |
| Phenotype x Drug Dose | SS= 4.50<br>MS= 0.75 | 0.11 | 6, 93 | 0.9953 |
| Phenotype x Testage | SS= 2.88<br>MS= 1.44 | 0.21 | 2, 93 | 0.8138 |
| Drug Dose x Testage | SS= 73.88<br>MS= 24.63 | 3.54 | 3, 93 | 0.0177 |
| Phenotype x Drug Dose x Testage | SS= 110.94<br>MS= 18.49 | 2.65 | 6, 93 | 0.0202 |
| Error | SS= 647.70<br>MS= 6.96 | | | |

Appendix B-4

Mean Data in a 2 x 4 x 2 (Genotype x Drug Dose x Age) Design

Percentage Change in Rectal Temperature

Main Effects

| Source | Statistic | F | df | p |
|---|---|---|---|---|
| Genotype | SS= 6.86<br>MS= 6.86 | 0.84 | 1, 58 | 0.3643 |
| Drug Dose | SS= 30.11<br>MS= 10.04 | 1.22 | 3, 58 | 0.3095 |
| Testage | SS= 109.84<br>MS= 109.84 | 13.38 | 1, 58 | 0.0006 |
| Genotype x Drug Dose | SS= 1.89<br>MS= 0.63 | 0.08 | 3, 58 | 0.9670 |
| Genotype x Testage | SS= 0.84<br>MS= 0.84 | 0.10 | 1, 58 | 0.7499 |
| Drug Dose x Testage | SS= 46.19<br>MS= 15.40 | 1.88 | 3, 58 | 0.1422 |
| Genotype x Drug Dose x Testage | SS= 100.34<br>MS= 33.45 | 4.07 | 3, 58 | 0.0108 |
| Error | SS= 476.10<br>MS= 8.21 | | | |

# Appendix C

Appendix C-1

Mean Data: Mean (± SEM) Percentage Change in Body Weight

| Drug Dose | |
|---|---|
| **Homozygous Lean (+/+)** | |
| 0.0 saline (vehicle) | 6.61 (.46) |
| 0.5 Naltrexone | 7.18 (.41) |
| 1.0 Naltrexone | 6.35 (.56) |
| 4.0 Naltrexone | 7.63 (.66) |
| **Lean (+/?)** | |
| 0.0 saline (vehicle) | 7.41 (.53) |
| 0.5 Naltrexone | 8.09 (.39) |
| 1.0 Naltrexone | 7.46 (.57) |
| 4.0 Naltrexone | 7.13 (.50) |
| **Obese (ob/ob)** | |
| 0.0 saline (vehicle) | 7.16 (.23) |
| 0.5 Naltrexone | 6.59 (.42) |
| 1.0 Naltrexone | 6.43 (.50) |
| 4.0 Naltrexone | 5.44 (.37) |

Appendix C-2

Mean Data: Mean ($\pm$ SEM) Percentage Change in Body Weight

| Drug Dose | Age (in days) 6 | 12 |
|---|---|---|
| **Homozygous Lean (+/+)** | | |
| 0.0 saline (vehicle) | 5.54 (.45) | 7.68 ( .17) |
| 0.5 Naltrexone | 6.97 (.86) | 7.39 ( .19) |
| 1.0 Naltrexone | 6.05 (.71) | 6.75 (1.04) |
| 4.0 Naltrexone | 9.04 (.45) | 5.52 ( .58) |
| **Lean (+/?)** | | |
| 0.0 saline (vehicle) | 8.67 (.39) | 6.16 ( .57) |
| 0.5 Naltrexone | 9.26 (.42) | 7.11 ( .18) |
| 1.0 Naltrexone | 7.68 (.97) | 7.18 ( .59) |
| 4.0 Naltrexone | 7.42 (.76) | 6.79 ( .68) |
| **Obese (ob/ob)** | | |
| 0.0 saline (vehicle) | 7.69 (.16) | 6.64 ( .28) |
| 0.5 Naltrexone | 7.29 (.82) | 6.00 ( .22) |
| 1.0 Naltrexone | 7.16 (.78) | 5.56 ( .31) |
| 4.0 Naltrexone | 5.39 (.17) | 5.51 ( .83) |

Appendix C-3

Mean Data: Mean (± SEM) Percentage Change in Rectal Temperature

| Drug Dose | Age (in days) 6 | 12 |
|---|---|---|
| **Homozygous Lean (+/+)** | | |
| 0.0 saline (vehicle) | 1.33 (1.57) | 3.53 (1.29) |
| 0.5 Naltrexone | 2.25 ( .88) | - .12 ( .75) |
| 1.0 Naltrexone | - .10 ( .97) | 3.91 (1.56) |
| 4.0 Naltrexone | .12 (1.71) | 5.55 (2.26) |
| **Lean (+/?)** | | |
| 0.0 saline (vehicle) | -1.77 ( .93) | 5.17 (1.74) |
| 0.5 Naltrexone | - .78 (1.50) | 1.61 ( .77) |
| 1.0 Naltrexone | 1.41 ( .63) | 2.68 ( .43) |
| 4.0 Naltrexone | 1.05 ( .59) | 3.00 ( .49) |
| **Obese (ob/ob)** | | |
| 0.0 saline (vehicle) | -1.62 ( .74) | 5.82 (1.39) |
| 0.5 Naltrexone | - .93 (1.77) | 1.57 (1.21) |
| 1.0 Naltrexone | .87 (1.09) | 2.26 ( .88) |
| 4.0 Naltrexone | 1.87 (1.16) | 1.58 ( .77) |

Appendix C-4

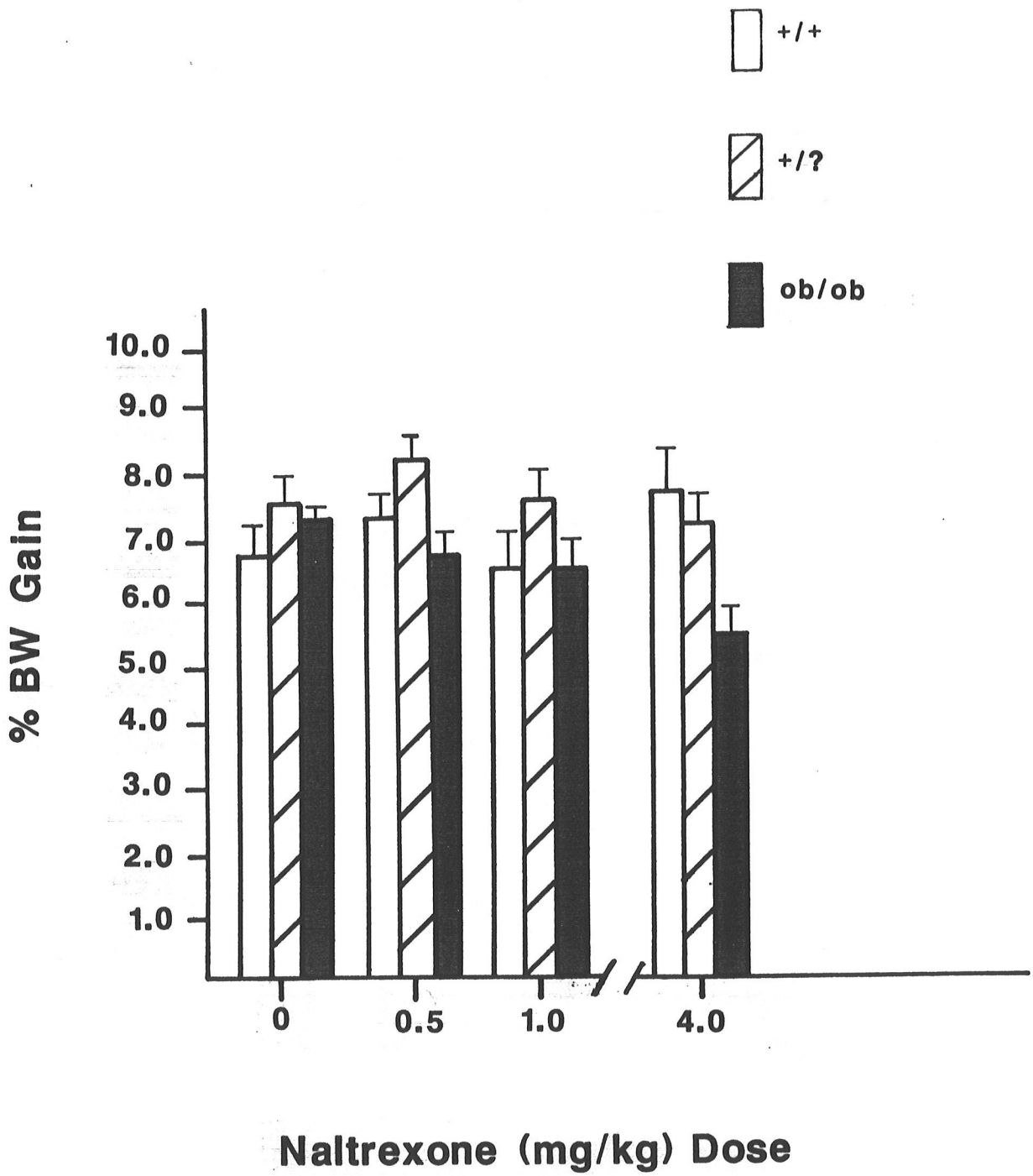
Mean Data: Mean (± SEM) Preinjection Rectal Temperature

| Drug Dose | Age (in days) 6 | 12 |
|---|---|---|
| **Homozygous Lean (+/+)** | | |
| 0.0 saline (vehicle) | 33.99 (.39) | 34.42 (.21) |
| 0.5 Naltrexone | 33.40 (.19) | 34.91 (.29) |
| 1.0 Naltrexone | 34.42 (.32) | 34.41 (.34) |
| 4.0 Naltrexone | 33.58 (.31) | 33.79 (.68) |
| **Lean (+/?)** | | |
| 0.0 saline (vehicle) | 34.16 (.10) | 33.56 (.55) |
| 0.5 Naltrexone | 34.20 (.17) | 34.91 (.24) |
| 1.0 Naltrexone | 33.94 (.19) | 34.38 (.23) |
| 4.0 Naltrexone | 33.77 (.28) | 34.41 (.23) |
| **Obese (ob/ob)** | | |
| 0.0 saline (vehicle) | 33.87 (.13) | 33.15 (.38) |
| 0.5 Naltrexone | 33.99 (.11) | 34.63 (.40) |
| 1.0 Naltrexone | 34.16 (.30) | 34.10 (.29) |
| 4.0 Naltrexone | 33.29 (.37) | 34.38 (.43) |

Appendix C-5

Mean Data: Mean (± SEM) Posttest Rectal Temperature

| | Age (in days) | |
|---|---|---|
| Drug Dose | 6 | 12 |
| **Homozygous Lean (+/+)** | | |
| 0.0 saline (vehicle) | 34.50 (.13) | 35.62 (.27) |
| 0.5 Naltrexone | 34.14 (.20) | 34.85 (.21) |
| 1.0 Naltrexone | 34.38 (.28) | 35.72 (.21) |
| 4.0 Naltrexone | 35.60 (.33) | 35.61 (.08) |
| **Lean (+/?)** | | |
| 0.0 saline (vehicle) | 33.56 (.40) | 35.25 (.15) |
| 0.5 Naltrexone | 33.92 (.44) | 35.47 (.37) |
| 1.0 Naltrexone | 34.41 (.29) | 35.29 (.17) |
| 4.0 Naltrexone | 34.11 (.17) | 35.43 (.23) |
| **Obese (ob/ob)** | | |
| 0.0 saline (vehicle) | 33.20 (.23) | 35.06 (.24) |
| 0.5 Naltrexone | 33.68 (.63) | 35.16 (.38) |
| 1.0 Naltrexone | 34.44 (.24) | 34.87 (.43) |
| 4.0 Naltrexone | 33.88 (.31) | 34.90 (.30) |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+/?
ob/ob
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9.0
8.0
7.0
6.0
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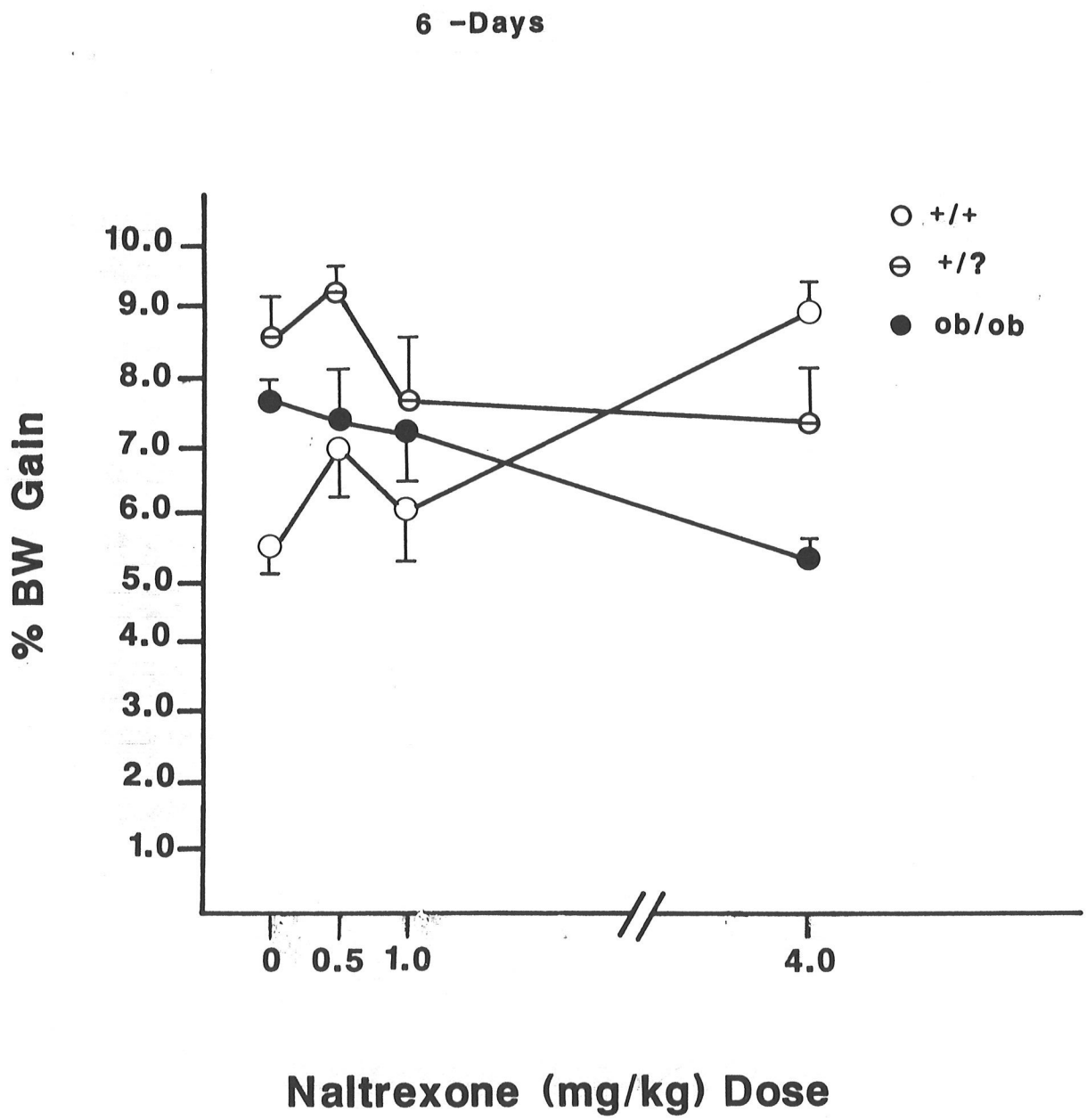
3.0
2.0
1.0
0
0.5
1.0
4.0
Naltrexone (mg/kg) Dose

Fig. 1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1.0
4.0
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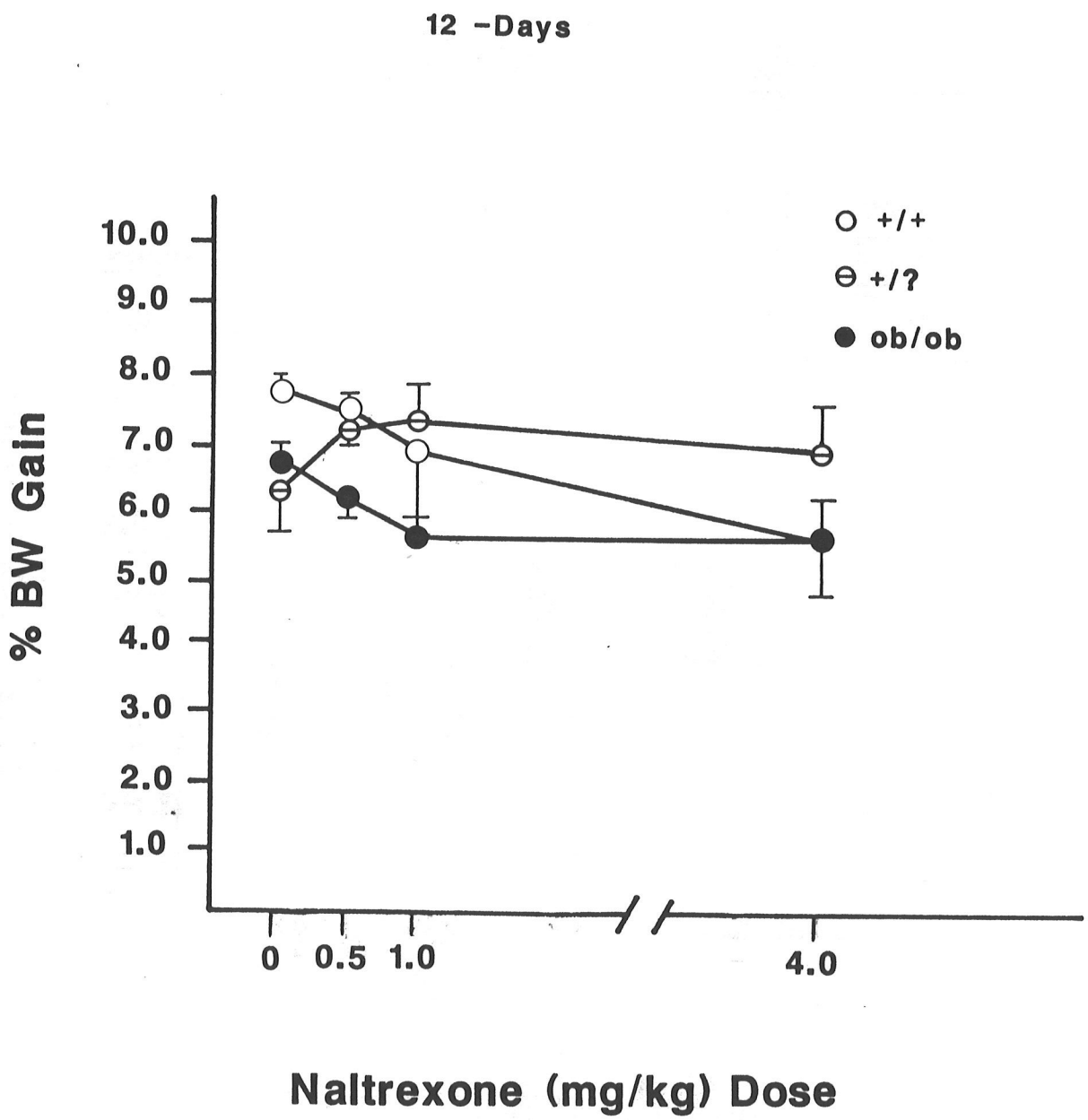
ob/ob
Naltrexone (mg/kg) Dose

Fig. 2


12 -Days
% BW Gain
10.0
9.0
8.0
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3.0
2.0
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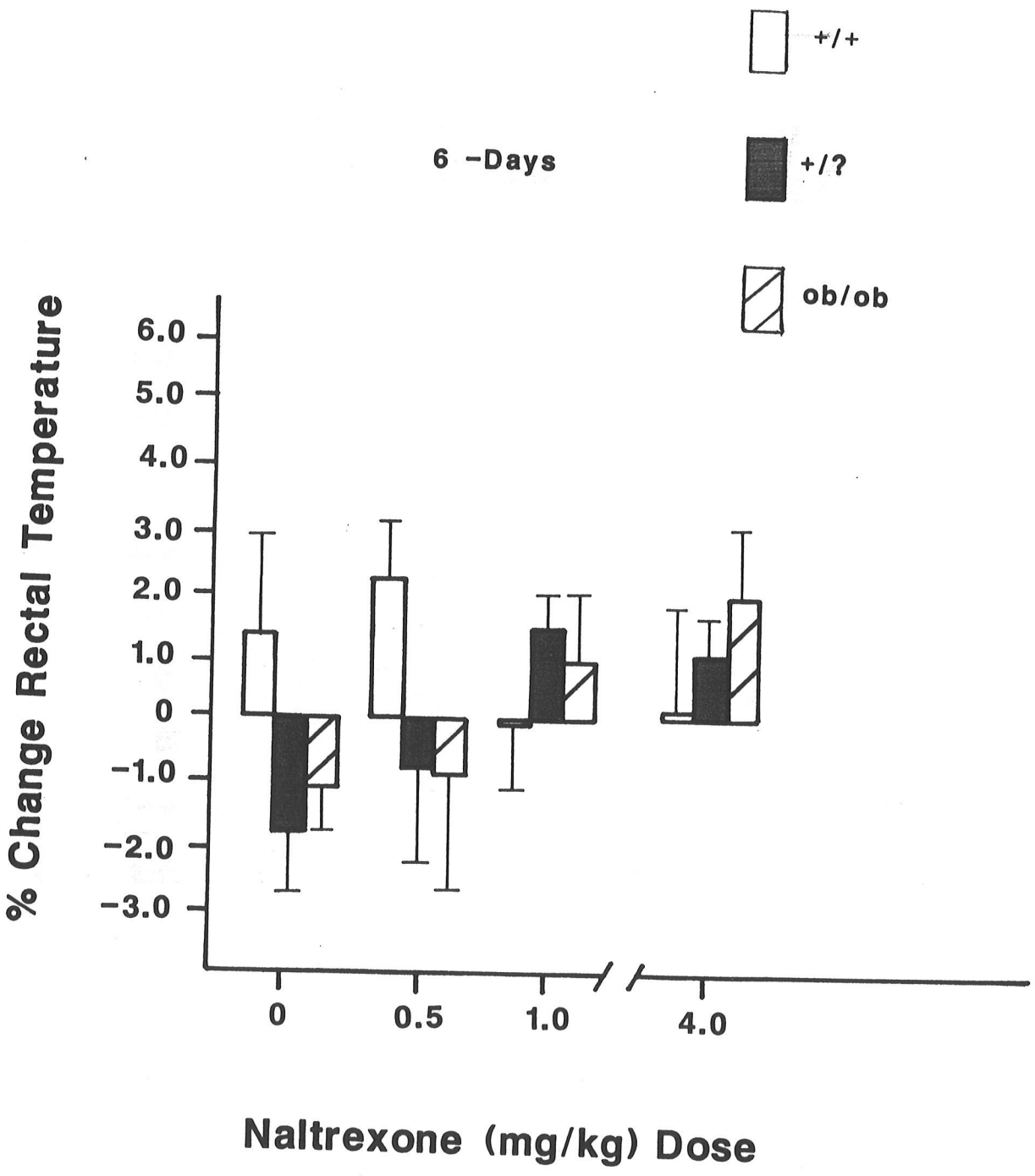
0
0.5
1.0
4.0
Naltrexone (mg/kg) Dose

Fig. 3


+/+
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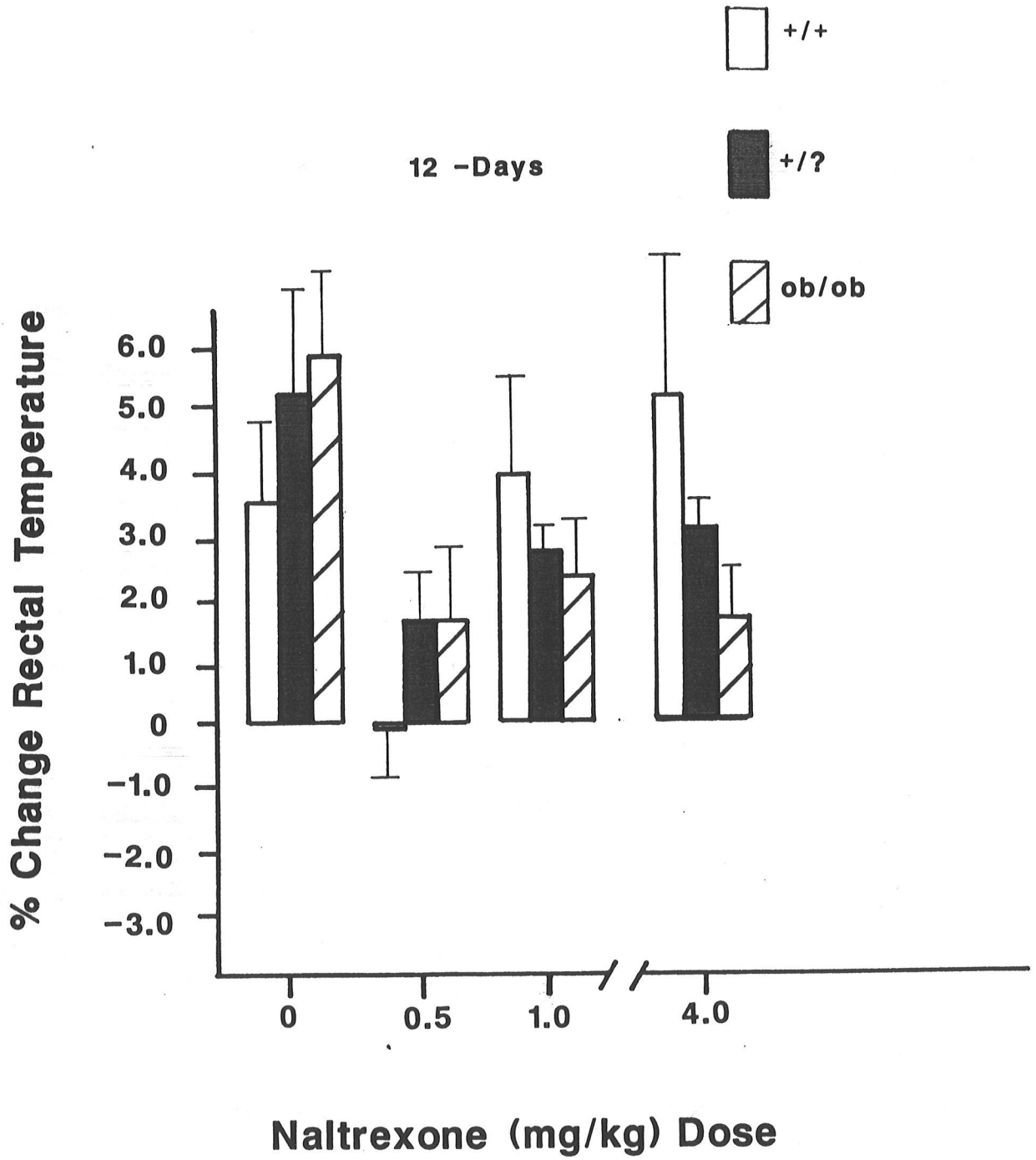
ob/ob
6 -Days
% Change Rectal Temperature
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
-3.0
0
0.5
1.0
4.0
Naltrexone (mg/kg) Dose

Fig. 4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% Change Rectal Temperature
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
-2.0
-3.0
0
0.5
1.0
4.0
Naltrexone (mg/kg) Dose

Fig. 5

Table 1

Raw Data: Mean (± SEM) Percentage Change in Body Weight

| Drug Dose | Age (in days) 6 | 12 |
|---|---|---|
| **Homozygous Lean (+/+)** | | |
| 0.0 (saline vehicle) | 5.58 (.34) | 7.65 (.22) |
| 0.5 Naltrexone | 6.94 (.33) | 7.40 (.28) |
| 1.0 Naltrexone | 6.05 (.45) | 6.30 (.40) |
| 4.0 Naltrexone | 8.85 (.28) | 5.48 (.31) |
| **Lean (+/?)** | | |
| 0.0 (saline vehicle) | 8.67 (.35) | 6.11 (.37) |
| 0.5 Naltrexone | 9.25 (.37) | 7.10 (.23) |
| 1.0 Naltrexone | 7.78 (.44) | 7.07 (.29) |
| 4.0 Naltrexone | 7.33 (.42) | 6.56 (.28) |
| **Obese (ob/ob)** | | |
| 0.0 (saline vehicle) | 7.78 (.14) | 6.55 (.25) |
| 0.5 Naltrexone | 7.00 (.64) | 5.91 (.25) |
| 1.0 Naltrexone | 7.12 (.67) | 5.73 (.32) |
| 4.0 Naltrexone | 5.42 (.21) | 5.12 (.53) |

Table 2

Raw Data: Mean (± SEM) Percentage Change in Rectal Temperatures (° C)

| | Age (in days) | |
|---|---|---|
| Drug Dose | 6 | 12 |
| **Homozygous Lean (+/+)** | | |
| 0.0 (saline vehicle) | 1.33 (.70) | 3.50 (.59) |
| 0.5 Naltrexone | 2.30 (.42) | - .08 (.41) |
| 1.0 Naltrexone | .03 (.44) | 4.12 (.74) |
| 4.0 Naltrexone | - .37 (.54) | 5.41 (.78) |
| **Lean (+/?)** | | |
| 0.0 (saline vehicle) | -1.75 (.50) | 5.28 (.68) |
| 0.5 Naltrexone | - .62 (.68) | 1.56 (.38) |
| 1.0 Naltrexone | 1.39 (.34) | 2.63 (.34) |
| 4.0 Naltrexone | 1.37 (.42) | 3.07 (.40) |
| **Obese (ob/ob)** | | |
| 0.0 (saline vehicle) | -2.02 ( .67) | 5.92 (1.22) |
| 0.5 Naltrexone | -2.05 (1.48) | 1.90 (1.12) |
| 1.0 Naltrexone | 1.49 ( .99) | 2.59 ( .81) |
| 4.0 Naltrexone | 2.36 (1.46) | 2.02 ( .62) |

Table 3

Raw Data: Mean (± SEM) Preinjection Rectal Temperatures (° C)

| Drug Dose | Age (in days) 6 | 12 |
|---|---|---|
| **Homozygous Lean (+/+)** | | |
| 0.0 (saline vehicle) | 33.94 (.15) | 34.42 (.12) |
| 0.5 Naltrexone | 33.40 (.12) | 34.89 (.12) |
| 1.0 Naltrexone | 34.42 (.14) | 34.36 (.18) |
| 4.0 Naltrexone | 33.65 (.11) | 33.82 (.24) |
| **Lean (+/?)** | | |
| 0.0 (saline vehicle) | 34.16 (.11) | 33.53 (.21) |
| 0.5 Naltrexone | 34.20 (.12) | 34.90 (.12) |
| 1.0 Naltrexone | 33.94 (.12) | 34.39 (.13) |
| 4.0 Naltrexone | 33.60 (.16) | 34.25 (.13) |
| **Obese (ob/ob)** | | |
| 0.0 (saline vehicle) | 33.91 (.18) | 33.12 (.32) |
| 0.5 Naltrexone | 33.94 (.42) | 34.37 (.34) |
| 1.0 Naltrexone | 33.87 (.25) | 34.17 (.31) |
| 4.0 Naltrexone | 33.30 (.42) | 34.22 (.25) |

Table 4

Raw Data: Mean (± SEM) Posttest Rectal Temperatures (° C)

| Drug Dose | Age (in days) 6 | 12 |
|---|---|---|
| **Homozygous Lean (+/+)** | | |
| 0.0 (saline vehicle) | 34.51 (.20) | 35.61 (.13) |
| 0.5 Naltrexone | 34.15 (.12) | 34.85 (.12) |
| 1.0 Naltrexone | 34.42 (.14) | 35.75 (.13) |
| 4.0 Naltrexone | 33.50 (.12) | 35.59 (.01) |
| **Lean (+/?)** | | |
| 0.0 (saline vehicle) | 33.57 (.20) | 35.25 (.11) |
| 0.5 Naltrexone | 33.98 (.20) | 35.44 (.16) |
| 1.0 Naltrexone | 34.40 (.14) | 35.29 (.11) |
| 4.0 Naltrexone | 34.04 (.12) | 35.28 (.12) |
| **Obese (ob/ob)** | | |
| 0.0 (saline vehicle) | 33.23 (.27) | 35.06 (.23) |
| 0.5 Naltrexone | 33.26 (.72) | 35.00 (.33) |
| 1.0 Naltrexone | 34.36 (.27) | 35.05 (.41) |
| 4.0 Naltrexone | 34.04 (.24) | 34.90 (.19) |