EFFECTS OF NALOXONE ON TAIL-FLICK ANALGESIA IN GENETICALLY OBESE (B6.V-lep^{ob}/lep^{ob}) 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

Studies of strain-dependent pain sensitivity in mice have established greater sensitivity of B6.V mice compared to most other strains using several pain assessment techniques, including tail-flick: the spontaneous withdrawal of the tail from a radiant heat source. Surprisingly, the pain response of the genetically obese B6.V-lep^{ob} mouse, a mutation occurring on the B6.V background strain, remains unknown. Elevated central levels of opioids and heightened opioid receptor affinity found in the ob mouse may contribute to an altered pain response. Furthermore, ob mice are more sensitive to the effects of pharmacological manipulation of their opioid systems, particularly to antagonists, such as naloxone. Therefore, even in the absence of different pain phenotypes, ob mice might be more sensitive to the hyperalgesic effects of naloxone. In the current study, 12 lean (B6.V, +/?) male mice (*Mus musculus*) and 12 obese mice, 10 male and 2 female (B6.V-lep^{ob}, lep^{ob}/lep^{ob}), aged 10-15 weeks, were tested in a tail-flick analgesia test using a 50°C infrared heat source. Following 4-5 days of adaptation to the testing protocol, each mouse received either an intraperitoneal

injection of saline or 2.0 mg/kg naloxone hydrochloride 10-30 min before testing.

Although greater pain sensitivity was expected in ob mice due to possible tolerance to their elevated β-endorphin levels, the phenotypes did not differ in their tail-flick latencies, $F(1,20) < 1$, nor did naloxone have a significant effect, either alone or differently between strains, $F(1,20) < 1$. Greater control for stress-induced analgesia and larger sample size will be examined for future research.

Effects of Naloxone on Tail-Flick Analgesia in Genetically Obese (B6.V-lep^{ob}/lep^{ob}) Mice

The genetically obese mouse of the B6.V-lep^{ob} strain, hereinafter called ob, has a recessive mutation on Chromosome 6. The non-mutated ob gene codes for the hormone leptin, as identified by Zhang, Proenca, Maffei, Barone, Leopold, and Friedman (1994). Relatively recently, the mechanism causing obesity in this strain of mice was discovered, although the full manifestation of the syndrome is not completely understood. As a result of the mutation, the ob mouse fails to produce leptin and suffers behavioural, physiological, and hormonal abnormalities. An absence of circulating leptin in the mutant may contribute to an altered endogenous opioid system because leptin may be a regulator of POMC (pro-opio-melanocortin) expression, a precursor molecule to the opioid β-endorphin, in the hypothalamus of the ob mouse (Inui, 1999). Endogenous opioids are neuropeptides produced by the body that mimic the action of exogenous opiates such as morphine. Among their many roles, opioids such as β-endorphin, dynorphin, and enkephalins are part of the body's natural analgesic response. Each kind of opioid binds to its own receptor sub-type. Whether a specific receptor sub-type is part of a mechanism of analgesia is determined by the nociceptive

(pain) stimulus being experienced, such as a thermal, chemical, or mechanical stimulus. If the lean counterparts (+/?) are compared to the ob mice in a specific nociceptive test, the level of pain sensitivity may differ because of the ob mouse's altered opioid profile. Thus, the study of analgesia in the ob mouse may demonstrate another characteristic of this phenotype.

The recessive mutation in the ob mouse is expressed in many ways, including hyperphagia, increased adiposity, hyperinsulinemia, impaired thermogenesis, and infertility (Bray & York, 1971). Aspects of the syndrome appear as hypothalamic and pituitary-adrenal axis abnormalities. The hypothalamus has decreased neuronal size in the dorsomedial and ventrolateral subdivision of the ventromedial hypothalamic nucleus, altered dendritic orientation in the lateral and ventromedial areas, increased α-1 noradrenergic receptor density, and increased levels of norepinephrine, largely in the paraventricular and ventromedial nuclei (Bereiter & Jeanrenaud, 1980; Currie & Wilson, 1992; Feldman, Blalock, & Zern, 1979). Hypertrophic adrenals, increased adrenal and pituitary corticosteroid levels, elevated secretion of adrenocorticotropic hormone (ACTH) (Bray & York, 1979) and hypothalamic corticotrophin (Bina & Cincotta, 2000) suggest a high susceptibility to

stressors.

The ob syndrome is also characterized by an altered endogenous opioid profile. The effects of opioids depend upon the class of opioid and the opioid receptor subtype with which it interacts, causing a range of effects including decreased pain sensitivity, body temperature and blood pressure, and increased feeding and drinking. Margules, Moisset, Lewis, Shibuya, and Pert (1978) found that ob mice have an excess of β-endorphin in their pituitary and this was linked to hyperphagia (overeating), in that small doses of the opiate antagonist naloxone selectively abolished the hyperphagia. Other studies in ob mice followed, finding elevated levels of both β-endorphin and dynorphin and increased affinity to their binding sites in the pituitary (Khawaja, Bailey, & Green, 1989) and in areas traditionally implicated in regulating energy balance (Gosnell, 1987). Moreover, Khawaja, Chattopadhyay, and Green (1991) found levels of β-endorphin were increased two-fold in the ventromedial nuclei and five-fold in the dorsomedial nuclei of the hypothalamus as compared to the lean mice. A five-fold increase in dynorphin concentration was found in the dorsomedial hypothalamic nuclei of the mutant. Despite evidence of central and systemic opioid

elevations in this mutant, researchers have not yet measured the impact of this altered opioid profile on analgesia in the ob mouse.

The exaggerated opioid profile of the ob mouse does seem consistent with an increased stress response because pituitary ACTH (which releases stress hormones, glucocorticoids) is co-released with β-endorphin. Beta-endorphin and ACTH not only are in greater quantities in storage together in the pituitary gland, but are also cleaved from a common precursor molecule, POMC (Mains, Eipper, & Ling, 1977), and are released concomitantly (Guillemin et al., 1977). Accordingly, a stressor may trigger simultaneously the release of large amounts of ACTH, resulting in elevated glucocorticoid levels, in concert with the exaggerated β-endorphin profile known in the ob syndrome. Surwit, Feinglos, Livingston, Kuhn, and McCubbin (1984) found high levels of the glucocorticoid corticosterone in the ob mouse, although secretion of the corticosterone was not differentially affected by stress in the ob mice compared to lean mice. In addition to the activation of the hypothalamic-pituitary-adrenal axis, the stress response also includes hyperglycemia when epinephrine mobilizes glycogen stores (Nelson, 2000). Based on the hyperglycemic profile of the ob mouse, Surwit et al.

suggested that an exaggerated increase in blood glucose levels is another measure of stress in ob mice.

In two separate experiments, Surwit et al. (1984) measured blood glucose and corticosterone levels simultaneously after stressor or non-stressor conditions. The first experiment compared lean and obese mice after 60 min of immobilization or 5 min of shaking in a mechanical shaker to animals that remained undisturbed in their home cages. The second experiment compared lean and obese mice after a single dose injection of epinephrine that represented an environmental stressor, or after a saline injection. For both experiments, the ob mice had higher elevated corticosterone levels compared to the lean mice in the non-stress conditions. Both the ob and lean mice had increased corticosterone in response to the stressor conditions of the two experiments, but the response to the stressor in the two groups was not different. This suggests that stressors do not evoke an exaggerated glucocorticoid response in the ob mouse. The glucose measurements yielded low blood glucose levels in both lean and ob mice for the non-stress conditions. As expected, both phenotypes had elevated glucose levels in response to the stress conditions; however, the ob mice showed higher blood glucose levels compared with lean mice to the stressors.

Surwit et al. suggested that increases in ob mouse blood glucose levels measured after stressors were an example of stress-induced hyperglycemia. Their findings are significant because they demonstrate that the ob mouse is more susceptible to stressors and, therefore, may also be more likely to display another feature of the stress response, stress-induced analgesia. Analgesia induced by a stressor has been attributed, in part, to the concomitant release of the endogenous opioid analgesic, β-endorphin with ACTH, although non-opioid mechanisms have also been implicated, depending upon the stressor involved and the gender of the animal (Olson, Olson, & Kastin, 1995). Accordingly, taken together with the elevated opioid profile of the ob mouse, stress-induced analgesia from the stress of the tail-flick test may result in less pain sensitivity and longer latency in the tail-flick test of analgesia.

By the hyperglycemic response of the ob mouse in their experiment, Surwit et al. (1984) enhanced our profile of the ob mouse as an animal model of Type II diabetes. In humans, Type I and Type II diabetes are associated with greater pain sensitivity (Lithner, Bergenheim, Borssen, Dahlen, & Nilsson, 1995; Morley, Mooradian, Levine, & Morley, 1984). Overall, because the opposing influence of

opioids and diabetes, the ob mouse may not have altered pain sensitivity. Kamei, Kawashima, Hitosugi, Misawa, Nagase, and Kasuya (1993) tested the effect of diabetes on β-endorphin as an analgesic. IRC mice made diabetic (Type I) via streptozotocin (200 mg/kg, i.p.) were compared to IRC mice injected with a vehicle. Pain sensitivity was tested using the tail-flick assay. A beam of light was focused on the tail and the latency until the tail moved to escape the heat source was measured up to a maximum cut-off point (to prevent tissue damage) of 10 s. A longer latency indicated less pain sensitivity. In four trials, β-endorphin was administered intracerebroventricularly (i.c.v.) using one of four doses ranging from 0.125 to 1.0 μg for each trial. The same procedure was repeated with β-endorphin by intrathecal (i.t.) administration. The i.c.v. and i.t. administration of β-endorphin both produced a dose-dependent decrease in pain sensitivity in the diabetic and non-diabetic mice and the i.c.v. administration also resulted in a comparatively greater analgesic response in the diabetic mice. In contrast, i.t. administration produced less analgesia in the diabetic mice than the non-diabetic mice. Since the tail-flick assay is a measure of spinal pain mechanisms, the results suggest that diabetes may

modulate β-endorphin-induced antinociception differently at the supraspinal (i.c.v.) level than the spinal level (i.t.). The ob mouse, which is diabetic, may also display greater pain sensitivity in the tail-flick assay; however, Kamei et al. were testing a model of Type I diabetes and not Type II, which may yield different results. Moreover, sustained β-endorphin levels in the ob mouse compared to trial administration of the opioid in Kamei et al.'s experiment would also affect the outcome because the ob mouse may have already developed tolerance to its own β-endorphin, predicting increased pain sensitivity. Nonetheless, the ob hyperglycemic, diabetic profile may contribute to greater pain sensitivity.

Since the ob mouse has a physiological profile suggesting a greater susceptibility to painful stimuli, the pain sensitivity found in other strains of mice, including the B6.V strain on which the ob mutation occurred, would be an important relative measure to predict the degree of pain sensitivity in the ob mouse. There is evidence of differences in pain sensitivity among strains of mice. Mogil, Lichensteiger, and Wilson (1998) reproduced these results using many strains of mice across an array of tests, with special interest in the formalin test because

there were no previous studies using inflammatory stimuli to test strain-dependent pain sensitivity. Mogil et al. included 11 strains of mice in the first part of their experiment, the formalin test. The second part of the experiment used multiple tests with only two strains. The A/J mouse was chosen from the first part of the experiment due to its extremely low pain sensitivity compared to the other strains; whereas, the B6.V mouse was chosen because it is highly sensitive to pain and because it represents a commonly used strain of mouse in the scientific community. The goal of this line of research was to identify and ultimately understand the genetic basis of pain sensitivity with two well-known strains of mice used as models.

The first part of Mogil et al.'s (1998) study tested 11 strains of mice, three outbred (i.e., randomly bred) and eight inbred (JAX substrains). The outbred strains were CD-1, ICR, SW (of Harlan Sprague-Dawley) and the JAX-sublines were A/J, AKR/J, C57BL/6J (B6.V), BALB/cJ, C3H/HeJ/ C58/J, CBA/J, DBA/2J, RIIIS/J, and SM/J. The first test habituated the mice in a Plexiglas tube for an hour before each mouse was presented with a cardboard restrainer, which most mice entered voluntarily. The test consisted of a subcutaneous (s.c.) injection of 5% formalin (25 µl volume) into the hindpaw of the mouse, which causes a biphasic response

described as an early or "acute" nociceptive response followed by a late or "tonic" nociceptive phase. The acute phase was 0-10 min after the formalin injection, and the tonic phase was 10-60 min post-injection. Both nociceptive phases were characterized by licking of the hindpaw, but not the forepaws because that is also a grooming behaviour. There were main effects of genotype in the acute phase, and the tonic phase, of the first experiment. The A/J strain was the least pain sensitive, especially in the tonic phase, as measured by the smallest total amount of licking of the hind paw in the set time periods. Although B6.V mice were not the most responsive to inflammatory pain stimuli, Mogil et al. chose the B6.V strain for further investigation because it established a consistently more pain-sensitive model relative to the less pain-sensitive model, the A/J mouse, representing a difference in pain response between two common experimental models.

The second part of Mogil et al.'s (1998) experiment focused on further characterizing the differences between the A/J and B6.V strains in pain sensitivity by comparing both in other nociceptive tests. They compared the A/J and the B6.V mice on the tail-flick, hot-plate, abdominal constriction, and the bee venom tests of nociception. The tail-flick test measured the latency in seconds until the

mouse, under gentle restraint, responds with the reflexive flick of the tail from water maintained at 49 $\pm$ 0.2°C. The B6.V mice were more sensitive in the tail-flick test at a latency of 3.2 s than A/J mice at 6.8 s. The hot-plate test placed the mice on a 14 X 14 cm aluminum surface maintained at 52 $\pm$ 0.2°C and tested the latency in seconds until a hindpaw lick or a shake/flutter. The hot-plate test does not use front paw licking for a cut-off time because it is also a grooming behaviour. Again, the B6.V mice showed increased pain sensitivity in the hot-plate test with a latency of 14.2 s and A/J mice had a latency of 22.6 s. In the bee venom test, 0.1 mg of venom is injected s.c. into the hindpaw (same procedure as the formalin test) and unlike the previous tests, the A/J mice were more sensitive with a latency until licking of 85.0 s and a latency of 743.6 s in the B6.V mice. The abdominal constriction test has the mice injected i.p. with 0.6 % glacial acetic acid, followed by counting the number of writhes 30 min post-injection. Abdominal constriction was the only test where no difference was found between the two strains. A forced swim test was included to ensure that the strain differences found between the A/J and B6.V mice were measuring pain sensitivity and not stress-induced analgesia (SIA). The test placed the mice in a cylinder filled with

20°C water at a depth of 10 cm to swim for 3 min. Both strains, however, responded similarily; so SIA did not affect the results. The tests in this experiment demonstrate that endogenous analgesia is not only different among strains of mice, but it is also determined by the pain test administered. The B6.V mice were more sensitive to the nociceptive stimulus of the formalin, tail-flick, and hot-plate tests; whereas, the A/J mice were more sensitive to pain than the B6.V mice in the bee venom test. Again, no difference was found in the abdominal constriction test. The results may suggest genetic mediation of pain response. Research beyond this study is necessary to understand the genetics underlying individual strain responses. Mogil and colleagues demonstrated generally higher pain sensitivity in the B6.V strain compared to other strains of mice. Although these experiments are not on the B6.V-lep^{ob} specifically, they suggest that the obs would experience pain sensitivity at least as great as the B6.V's due to the genotype of the background strain on which their single-point mutation developed.

Many studies involving pain sensitivity and analgesia use morphine, which binds to μ-receptors in the brain and spinal cord to induce analgesia, just as β-endorphin, the

natural ligand to μ-receptors, does. Administration of either β-endorphin or morphine in an animal model can alter its pain sensitivity. Therefore, the chronically elevated levels of endogenous β-endorphin in ob mice may alter their pain sensitivity, but it is not known whether analgesia or hyperalgesia will result. The ob mouse may display hyperalgesia because tolerance to opioids and exogenous opiates, such as morphine, typically develop after chronic exposure. Among the different types of tolerance, those caused by cellular mechanisms and drug disposition tolerance (increased rate of metabolism) are associated with reduced sensitivity to analgesic agents, although tolerance is primarily attributed to cellular mechanisms (Feldman, Meyer, & Quenzer, 1997). Consistent with opiate literature reviewed (Petruzzi, Ferraro, Kurschner, Golden, & Berrettini, 1997), the current study hypothesizes that elevated opioid levels may result in tolerance through opioid receptor down-regulation. Mu-receptors are reduced in number with desensitization occurring through functional "uncoupling" of the G-protein and the receptor, which modifies the second messenger system in the cell (Cox, 1994; Feldman, et al., 1997), thereby reducing β-endorphin binding. Although researchers have not yet looked at the ob

mouse and possible tolerance to its own β-endorphin, there are numerous morphine tolerance studies using mice that may offer insight into β-endorphin and tolerance in the ob mouse.

In that light, Oliverio and Castellano (1974) confirmed a differential tolerance to morphine among the inbred strains, BALB/cJ, DBA/2J, and B6.V. The comparison of the B6.V and DBA/2J is often revisited because they are the most genetically dissimilar of existing inbred strains (Taylor, 1972). First, a baseline analgesia measurement was made with the hot-plate test to establish endogenous analgesia levels for the assay in the three strains. The hot-plate test measured latency until either the forepaw or the hindpaw was licked or the 30-s interval on the hot-plate ended. The latencies between the strains were not significantly different for the baseline analgesia measurement. Morphine-induced analgesia was then tested in the three strains in the hot-plate test where they all displayed dose-dependent increased analgesia with BALB/cJ mice showing the greatest analgesia and B6.V mice showing the least. Tolerance was observed after three injections of one dosage of morphine (either 5.0, 10.0, and 20.0 mg/kg i.p. injections) and the B6.V mice showed the greatest tolerance, followed by the DBA/2J and the BALB/cJ mice.

Since both morphine and β-endorphin bind to the μ-receptor, it follows that the B6.V mouse may develop tolerance to β-endorphin as well as it does to morphine. Heightened sensitivity to nociceptive stimuli in the B6.V mouse, as found by Mogil, Lichensteiger, and Wilson (1998), and Oliverio and Castellano's finding of B6.V tolerance to morphine-induced analgesia in the hot-plate assay, suggest that the B6.V may have exaggerated pain sensitivity due to tolerance to their own β-endorphin and this could be true for the B6.V-lep^{ob} as well. Although Oliverio and Castellano used the hot-plate test, a test of supraspinal contributions to analgesia, extrapolations must be made carefully because the B6.V mouse may not exhibit the same tolerance to morphine-induced analgesia in other nociceptive assays, such as the tail-flick test, which primarily assesses spinal control of analgesia.

Belknap, Noordewier, and Lamé (1989) also explored the response of the B6.V mouse to morphine-induced analgesia compared to other strains of mice (DBA/2J and C3H/HeJ) in the hot-plate assay. In addition, they used different mice than tested in the hot plate assay to compare measures of opioid receptor-binding and brain morphine concentrations in the three strains. In the hot-plate assay, two doses

i.p. of morphine sulphate were administered, 6.25 or 12.5 mg/kg, and saline for the control group. Each of the groups receiving the different injections were divided between two hot-plate tests: the first test was measured 30 min post-injection and the second, in 15-min intervals starting 15 min before and ending 90 min after injection. The cut-off time was measured by a hindpaw lick. A significant strain effect was found in both the single and repeated measure hot-plate test. The DBA/2J showed the greatest analgesic effect, followed by the C3H/HeJ. The B6.V was least sensitive to morphine-induced analgesia in the hot-plate assay, confirming the results of Oliverio and Castellano (1974).

Belknap et al. (1989) measured opiate receptor-binding in the whole brains of the mice by means of a naloxone-binding assay: the fewest opiate receptors were found in the brains of B6.V mice compared to the two other strains of mice. Another part of the study measured brain morphine concentrations in mice of each strain, 30 min after 12.5 mg/kg i.p. injections of morphine. As fewer opiate receptor numbers would indicate, the B6.V mice had the highest brain morphine concentration. Belnap et al.'s study suggests profile of B6.V mice with smaller numbers of opioid-receptors to explain their relative insensitivity to

morphine analgesia.

Varied pain sensitivity among mouse strains can be accounted for, in part, by genetic influence. The use of different protocols between experimental designs makes it difficult to compare genetic differences in strains and consequently can make an inaccurate profile of a mouse strain. Mogil, Kest, Sadowski, and Belknap (1996) sought to address this issue by identifying differences in genetic mediation of sensitivity to morphine in known opiate-sensitive models by comparing seven strains of mice in four nociceptive assays using the same dosage of morphine and time interval for each. The nociceptive tests included hot-plate, tail-flick, abdominal constriction, and formalin; and the subjects were B6.V, DBA/2J, CXBK, HA, LA, HAR, and LAR mice. The HA and HAR mice were specifically bred for a high analgesic response, as the LA and LAR were for a low analgesic response. The results for the strains bred for specific analgesic responses will not be compared to the B6.V, DBA/2J, and CXBK strains because the goal of comparing different strains of mice is to demonstrate naturally occurring pain sensitivity and establish a pattern for the B6.V mouse relative to the DBA/2J and CXBK strains within the Mogil et al. experiment to relate to findings of previous studies. In each test, morphine

sulphate was administered i.p. in an injection volume of 10 ml/kg 20 min before the application of the nociceptive assay, except for s.c. administration in the abdominal constriction test. The experimenters found the same relationship between the DBA/2J mice and the B6.V mice as Oliverio and Castellano (1974) and Belknap et al. (1989) had, where the B6.V mice did not respond to morphine-induced analgesia as much as the DBA/2J mice in the hot-plate test. The current experiment found CXBK mice responded the least of the three strains to morphine-induced analgesia. Mogil et al. found the magnitudes of analgesia for all four assays can be represented as DBA > B6.V > CXBK. The finding that the CXBK mouse has more tolerance for morphine analgesia than the B6.V mouse can probably be attributed to the fact that CXBK mice have a small number of μ-receptors relative to the B6.V mouse (Baran, Shuster, Eleftheriou, & Bailey, 1975).

Based on these experimental results, a profile of pain sensitivity emerges with the B6.V mouse identified as a strain with greater pain sensitivity that is less sensitive to morphine analgesia in a number of nociceptive assays compared to other mouse strains. Since the B6.V mouse has a relatively high pain sensitivity and tolerance to morphine, specifically in thermal pain assays, the ob (B6.V-lep^{ob})

mouse may respond in a similar manner. The response to pain within a specific nociceptive assay is species-dependent; whereas the pain response between assays is, in part, regulated by the receptor(s) type tapped into by the test. In the ob mouse, β-endorphin is elevated and has increased affinity for its receptors (μ) in the hypothalamus (Khawaja, Bailey, & Green, 1989); so, studying endogenous opioids through analgesia in this model would require a nociceptive assay in which pain is mediated through the μ-receptors. If thermal nociceptive assays are mediated through μ-receptors, either the hot-plate or tail-flick assay would be a logical method if the ob model response is similar to the B6.V mice in thermal testing.

Researchers have established that the type of receptor(s) that mediate a nociceptive test is dependent on whether the test is thermal or non-thermal (Tyers, 1980). Ward and Takemori (1983) used the tail-flick and acetic acid-induced writhing tests to represent a thermal and a chemical stimulus with Swiss-Webster mice. They employed mu, kappa, and delta opioid agonists: morphine (μ), ethylketazocine (κ), nalorphine (κ), [D-Ala^2, D-Leu^5]enkephalin (δ) or DADLE, and [D-Ala^2, Met^5]enkephalinamide (μ, δ) or DAME. The antinociceptive

effects of all of the agonists were measured in both assays, then naloxone was administered s.c. (in every trial) to deduce which agonists were causing antinociception and if there was possible receptor interaction. They concluded that a μ and κ agonist interaction will result in an antinociceptive effect in the acetic acid-induced writhing test, but only μ agonists will be effective for a tail-flick test, with a possible δ-receptor interaction.

Ward and Takemori (1983) found that between μ- and κ-receptors, only using ligands to μ-receptors, and possibly δ, resulted in analgesia in a thermal assay, although they only tested the tail-flick assay. Mogil, Richards, O'Toole, Helms, Mitchell, and Belknap (1997) went further to demonstrate which specific opioid receptors may mediate the hot-plate assay and identified a related strain and a gender interaction. The study used B6.V and DBA/2J hybrids, B6 and D2. Analgesia was measured with the hot-plate test and receptor antagonists were administered to determine their effect on hot-plate latency. Of four populations (B6 and D2 of both sexes), each of the four populations were divided into six opiate receptor-specific drug groups and a saline group (s.c. administration). A high dose of

$naloxone_{high}$ (10 mg/kg) was to block all opioid receptors, as the higher the NAL dose, the less it has receptor specificity, since the NAL_{low} ((0.1 mg/kg) selectively blocks μ-receptors. Nor-binaltorphimine (nor-BNI; 5 mg/kg) blocks κ-receptors. Naltrindole (NTI; 5 mg/kg) blocks all δ-receptors, whereas, 7-benzylidenenaltrexone (BNTX; 0.7 mg/kg) blocks δ_1-receptors and naltriben (NTB; 1 mg/kg) blocks δ_2-receptors. Mice were tested in the hot-plate assay 30 min after drug administration. As compared with previous studies, the B6.V hybrid, B6 displayed greater pain sensitivity than the DBA/2J hybrid, D2 (in the saline treatment). $Naloxone_{high}$ D2 mice showed increased pain sensitivity, but the B6 mice were, again, unaffected. The μ and κ antagonists did not affect the latencies of either strain in the test. Naltrindole and NTB (general δ-antagonist and δ_2-antagonist), not the δ_1-receptor blocker, BNTX, reduced the latencies of both strains in a strain- and gender-dependent manner. The D2 males were affected most by the δ-blockers, then B6 males, followed by the D2 females and the B6 females, suggesting that hot-plate analgesia may be mediated by δ_2-receptors, but not μ, κ, or δ_1-receptors.

In summary, the combined results of Ward and Takemori

(1983) and Mogil et al. (1997) indicate that the tail-flick assay should be used to test how the ob pain sensitivity is affected by their opioid profile. Ward and Takemori demonstrated that the tail-flick test is mediated by μ-receptors. Although it may also induce an interaction between δ-receptors, according to the Mogil et al. experiment, there was no mediation at all by μ-receptors in the hot-plate assay. If elevated levels of β-endorphin in the ob mouse will alter their pain sensitivity, employing the tail-flick assay will demonstrate the effect because it is mediated by the natural receptor for β-endorphin, the μ-receptor. Furthermore, a number of experiments show B6.V mice, the background strain for ob mice, having greater pain sensitivity and tolerance for morphine in the tail-flick test, predicting the same results in ob mice if they are tolerant to morphine. Therefore, the tail-flick assay is ideal to test ob pain sensitivity.

Although exaggerated levels of β-endorphin in the ob mouse clearly suggest less pain sensitivity than their lean counterparts (+/+), other evidence also indicates an alternate hypothesis where the ob mouse has increased pain sensitivity. Roane and Porter (1986) demonstrated increased pain sensitivity or opioid tolerance in another genetically

obese species. They focused on the rat model of genetic obesity, the obese (fa/fa) Zucker rat. Despite the physiological anomalies in their genetic mutations not being identical, the ob mice and the Zucker rats both have elevated levels of pituitary β-endorphin. As researchers in the ob mouse have also done, Roane and Porter have investigated the nature of endogenous opioid system in the obese Zucker rat by testing the effects of elevated β-endorphin on their food intake, but have gone further by testing their response to nociception. Their experiment employed both the tail-flick and tail-pinch methods. In both assays, all rats were given morphine sulphate s.c. at dosages of 1.0, 2.0, 4.0, 8.0, and 16 mg/kg based on the weight of the lean animal of each lean/obese pair so that each animal in a pair received the same absolute quantity of the drug. After 30 min, the animals were tested for latency of response in both assays. In both tail-flick and pinch, the obese rats had a shorter latency response than the lean animals. A greater difference was seen in the tail-pinch test where the mean time for the obese animals was 0.7 s (SE 0.16) compared to 10.8 s (SE 2.6) for the lean; whereas, in the tail-flick test, the obese rats had a latency of 6.33 s (SE 0.40) compared to 8.24 s (SE 0.73). Evidently, elevated β-endorphin levels or exogenous ligands

to μ-receptors like morphine, as used in Roane and Porter's study, do not result in reduced pain sensitivity in a genetically obese rat model. Whether this is true in the genetically obese mouse model has yet to be tested.

Further evidence suggesting possible elevated pain sensitivity in the ob mouse comes from a study of morphine tolerance due to receptor down-regulation in the ob background strain, B6.V. Petruzzi, Ferraro, Kurschner, Golden, and Berrettini (1997) studied the effect of chronic morphine administration on μ-receptors in the B6.V and DBA/2J. Research to date has shown high tolerance to morphine in the B6.V strain compared to low tolerance in the DBA/2J strain (Belknap, Noordewier, & Lamé, 1989; Mogil, Kest, Sadowski, and Belknap, 1996; Oliverio & Castellano, 1974). Petruzzi et al. gave mice of each strain a single daily s.c. injection of morphine sulphate or saline for a period of 7 days. The mice were euthanized 6 h after the last injection and the brain was removed for dissection of the frontal cortex and striatum because they are areas with relatively high μ-receptor density. The second phase of the study used DAMGO (D-Ala^2, N-Me-Phe^4, Gly^5-ol), a μ-receptor agonist, to bind to the receptors to assess μ-receptor density with chronic morphine. For the

saline treatment groups, μ-receptor density in the striatum for the B6.V and DBA/2J did not differ, but there was a difference between the two strains in the frontal cortex, with more binding in the DBA/2J group. Another study (Belknap et al., 1995) had also shown μ-receptor binding in whole brain homogenates revealed DBA/2J mice having higher overall density of μ-receptors than the B6.V strain. For the repeated morphine injection group, a strain effect was found in that the B6.V mice exhibited a decrease in striatal binding indicating a μ-receptor density down-regulation by approximately 45%; whereas no change in binding was observed in the DBA/2J mice. DAMGO binding in the frontal cortex showed no differences in μ-receptor number between the strains, meaning there was no tolerance through down-regulation occurring in either strain in this area. Although B6.V mice only demonstrated morphine tolerance in the striatum, Petruzzi et al. explained that these results are the focus in this experiment because the frontal cortex is much larger proportionally, so measuring μ-opioid receptor differences at this level may have masked more discrete effects that can be found in smaller areas such as the striatum. Therefore, in attributing morphine tolerance through μ-receptor down-regulation, Petruzzi et

al. explained a mechanism for B6.V morphine tolerance and DBA/2J morphine sensitivity.

The current experiment focused on the altered opioid profile of the B6.V-lep^{ob} genetically obese mouse. Since opioids, such as β-endorphin, are known as endogenous analgesics, studying pain sensitivity is an effective means of observing the properties of an opioid system through behavioural methods. In the B6.V-lep^{ob}, their chronically elevated β-endorphin levels may mediate analgesia and reduce pain sensitivity in a pain assay, or their β-endorphin levels may induce tolerance resulting in greater pain sensitivity in a pain assay. Tolerance to morphine (which binds to μ-receptors as β-endorphin does) was demonstrated in the background strain to the ob mouse (B6.V mice) through down-regulation of the μ-receptors. Chronic exposure to β-endorphin has also been shown to develop tolerance and down-regulate μ-receptors (Wu, Wilcox, & McLoon, 1994). Therefore, it follows that a possible consequence of exaggerated β-endorphin levels in the ob mice could be a blunted response to endogenous analgesia due to tolerance to its own endorphins. The ob mouse could also experience greater pain sensitivity due to elevated glucose levels from Type II diabetes, as is found in humans.

The present study examined the endogenous analgesic response and naloxone's (μ-receptor blocker) effect on altering pain sensitivity in ob and lean mice using the tail-flick method. The latency between laying the mouse tail on the heat source and the tail-flick measured the amount of analgesia experienced by the subject. The ob mice would have had longer tail-flick latencies compared to the lean mice if they were experiencing reduced pain sensitivity due to their elevated β-endorphin levels. Shorter tail-flick latencies were anticipated from the ob mice due to possible tolerance to their own opioids, as well as increased pain sensitivity from their Type II diabetic hyperglycemic profile. Furthermore, the opioid profile of the ob mouse suggests a hyperalgesic response to the naloxone condition due to hypersensitivity of down-regulated receptors, resulting in shorter tail-flick latencies, as compared to the tail-flick latencies measured in the saline condition.

Method

Subjects

Obese male (B6.V-lep^{ob}, lep^{ob}/lep^{ob}; n=10), obese female (B6.V-lep^{ob}, lep^{ob}/lep^{ob}; n=2) and lean male (B6.V, +/?, n=12) adult mice (Mus Musculus), aged 10-15 weeks at the start of the experiment, were obtained from the Jackson Laboratory, Bar Harbour, ME, USA and from the Mouse Colony, Department of Psychology, University of Manitoba.

The mice were housed individually in 18 x 18 x 25 cm polypropylene mouse nesting cages with ample wood chip bedding. Nestlets were provided for nest-building. Cardboard paper tubes were both for play and adaptation to the apparatus. The ob mouse tubes were 4 cm in diameter and the lean mouse tubes were 3 cm in diameter to closely accommodate body sizes. The food (Lab Diet Prolab 5P00 Rat/ Mouse / Hamster 3000) and water were provided ad libitum. The mice were housed in a room with a 12 hr light-dark cycle with lights on at 0800 hr, the temperature at 22-25°C, and relative humidity kept between 40-60%.

Apparatus

The tail-flick test, as modified from the method by D'Amour and Smith (1941), was used to evaluate analgesic response. An Ugo Basile (Model 7360) tail-flick analgesiometer measured 42.0 X 20.3 cm. A stainless steel

casing enclosed a variable radiant heat source, which was recessed 2.0 mm below a 1.0 cm diameter aperture in the top surface of the casing.

A "cave" for the mice to enter unrestrained and to keep them stationary during the tail-flick test was constructed of upholstery fabric (personal communication, Dr. Jeff Mogil, February 18, 2003). A cardboard tube was placed in each mouse home cage for the mice to acclimatize before the later placement of the tube in the fabric pocket for tail-flick testing. All tubes measured 7.5 cm long; whereas the diameters of the tubes were 3.0 cm for the lean mice and 4.0 for the ob mice. The cardboard tube from their cage was placed inside an upholstery-fabric pocket measuring 9.0 cm long and 10.5 cm wide. The tail was placed over the aperture through which the infrared heat source was focused at a distance of approximately 2 cm from the base of the tail. A pedal-operated digital timer was used to measure the latency for removal of the tail from the heat source. The mice were weighed with a Mettler PM480 Delta Range digital balance, weighing to the nearest 0.01 g.

Drugs and Drug Preparation

Naloxone hydrochloride (Sigma), a μ-receptor blocker, was administered intraperitoneally (i.p.) at a dose of 2.0

mg/kg. The drug was dissolved in a sterile isotonic saline solution (0.15 M NaCl) at a standard injection volume of 0.5 ml/100 g body weight. Drug dosage was selected based upon studies measuring analgesia in B6.V mice. Drugs were prepared fresh daily and coded blind to the experimenter.

Procedure

Mice were acclimated to the colony room for 3-5 weeks before the study began and were handled daily for 2 min to accustom them to the experimenter. They were first habituated to a cardboard tube placed in their home cages for four days before the experiment. Over the two days before tail-flick testing began, mice were adapted to the fabric pocket with the cardboard tube placed in it that would hold them during testing for a minimum of 2 min each day. All testing was between 1100 and 1400 hr. Mice were weighed and rank ordered by body weight within phenotype. The mice were assigned to either saline or naloxone groups by a balanced, randomized block design to ensure the mean weight within a phenotype for each drug condition was not significantly different. For example, the heaviest mouse within a phenotype was randomly assigned to either the saline or naloxone condition, and then the next heaviest mouse was assigned to the other drug condition. This procedure continued until all mice were assigned within a

phenotype. Adaptation followed the same randomized order used for the experiment. Mice were brought individually to the test room and presented with the cardboard tube in the fabric pocket for 2 min each. The mice entered the cave-like structure readily and head-first, so that the base of their tails were at the entrance of the pocket and their tails lay outside of it. The mouse in its pocket was placed on the tail-flick apparatus, so that its tail lay over the aperture of the tail-flick apparatus. Most mice remained in the pocket for 2 min, although lean mice backed out of the tube often, after which they were removed gently from the pocket into their home cages and returned to the colony. In addition, tail lengths and circumferences were measured in centimeters during the first day of habituation to the pocket, to determine that approximately the same tail locale was stimulated across testing.

On the 5th day, mice were randomly assigned to one of two drug treatments. Mice were weighed and received intraperitoneal injections of either the saline or naloxone treatment. Testing began 10-30 min after injection. Naloxone is distributed systemically 10 min post-injection (Margules, Moisset, Lewis, Shibuya, & Pert, 1978). Mice were re-introduced to the cardboard tube in the fabric pocket and their tails were positioned with the aperture

overlying the infrared heat source approximately 2 cm from the base of the tail. A digital timer started when the mouse's tail was released, and the dependent variable, the tail-flick latency, was measured from the tail's release until the mouse moved it laterally from the heat source, or 10 s, whichever occurred first. Ten seconds was chosen as the cutoff point to prevent tissue damage to the tail from overheating (Paul, Bodnar, Gistrak, & Pasternak, 1989). All procedures involving mice conformed to the guidelines of the Canadian Council for Animal Care, Ottawa.

The independent variables were phenotype ($\underline{lep}^{ob}/\underline{lep}^{ob}$, +/?) and treatment drugs (saline, naloxone), with dependent variables of tail-flick latency, tail length, and tail diameter. Data were analyzed using a 2 X 2 (Phenotype X Treatment Drug) between groups ANOVA (SPSS 9.0 for Windows). A posteriori analysis was also run that excluded subjects that did not display a clearly identifiable tail-flick (i.e., tail movement from body movement and not a quick reflex response to the heat stimulus). Comparisons of tail length and tail diameter between phenotype were made with independent-samples $\underline{t}$ tests. The alpha level was set at $\underline{p} < .05$.

Results

A 2 X 2 (Phenotype X Drug Treatment) between groups ANOVA showed no main effect by Phenotype, $F(1,20) = .631$, $p = .436$, by Drug Treatment, $F(1,20) = .846$, $p = .369$, or a Phenotype X Drug interaction, $F(1,20) = .074$, $p = .789$. As shown in Figure 1, neither phenotype nor drug treatment affected tail-flick latencies. During testing, the tail-flick measure was repeated until there was a clearly defined tail-flick latency or a measurable tail movement in lieu of a clear tail-flick. The tail movement was measured in more active animals, usually lean, that continually backed out of the tube, making a true tail-flick difficult to identify in several instances. The latency closest to appearing like a tail-flick was used, with the most trials on a single animal reaching four measurements because more may have caused tissue damage. A chi-square revealed a phenotype-dependent difference in number of clear tail-flicks, $\chi^2(1, N = 24) = 4.44$, $p < .05$. Therefore, re-analyses omitted latencies that were not clear tail-flicks, but yielded the same results. The latencies of 9 animals, 7 lean and 2 ob, were omitted in the re-analyses. There was no Phenotype effect, $F(1,11) = .000$, $p = .985$, Drug Treatment effect, $F(1,11) = .029$, $p = .867$, nor interaction, $F(1,11) = .207$, $p = .658$. Table 1 presents the

means and standard deviations of tail-flick latencies with all cases included, as well as the clear tail-flick cases calculated alone.

In addition, the same 2 X 2 ANOVA was run including all subjects except the two female ob mice to decrease any detectable variability based on gender-specific effect on analgesia. Again, there was no significant Phenotype effect, $F(1,18) = .690$, $p = .417$, Drug Treatment effect, $F(1,18) = .843$, $p = .317$, nor interaction found, $F(1,18) = .027$, $p = .870$. Two female ob mice were used to increase the sample size. Because the ob mutation results in infertility, the females do not have estrous cycles, which could add variability to the results. Although both increased and decreased tail-flick latencies have been reported in different stages of the estrous cycle in rats and mice (Fillingim & Ness, 2000), the absence of a cycle in female ob mice should not have affected the overall results.

A univariate analysis of variance of body weights within a given phenotype did not differ across drug treatment conditions. The ob body weights were consistent across saline and naloxone treatments, ($M = 57.86$, $SD = 4.69$; $M = 59.87$, $SD = 4.82$, respectively) as were the lean body weights ($M = 29.94$, $SD = .95$; $M = 30.82$, $SD = 2.09$).

The lack of difference across drug treatment within each strain attested to the effectiveness of the randomized balanced assignment of mice to the treatment groups.

There was no significant difference in tail diameter between the lean (M = .372, SD = .026) and the ob mice (M = .378, SD = .021) at the location of the aperture placement (approximately 2 cm from the base of the tail). However, the lean mice (M = 8.16, SD = .108) had longer tails than the ob mice (M = 7.57, SD = .410), F (1,22) = 12.73, P = .002.

Discussion

The ob mouse has chronically elevated opioid levels (Khawaja, Bailey, & Green, 1989; Khawaja, Chattopadhyay, & Green, 1991) that may result in tolerance to their own opioids. In addition to possible opioid tolerance, their Type II diabetic hyperglycemic profile (Surwit, Feinglos, Livingston, Kuhn, & McCubbin, 1984) suggests that the ob mouse might display shorter tail-flick latencies, or greater pain sensitivity, than their lean counterparts in the tail-flick assay. Alternatively, ob mouse opioid levels may reduce pain sensitivity, or display longer tail-flick latencies than lean mice if opioid tolerance does not develop. The results of the current study did not demonstrate different pain sensitivity between the B6.V-lep^{ob}/lep^{ob} and its background strain, the B6.V, in the tail-flick analgesia test. Although the reported mean tail-flick latency of the lean mice is consistent with tail-flick measures taken in other studies with B6.V mice, there are no equivalent studies in B6.V-lep^{ob}/lep^{ob} mice for comparison (Baran, Shuster, Eleftheriou, & Bailey, 1975; Jacob, Nicola, Michaud, Vidal, & Prudhomme, 1986; Lariviere et al., 2002). If ob mice are tolerant to their own opioids, a hyperalgesic response, or shorter tail-flick latency was anticipated in the naloxone condition as compared to the

saline condition, or baseline tail-flick latency. Unexpectedly, the lean and ob tail-flick latencies did not differ after naloxone injections by drug or phenotype effect. Although the predicted results were not revealed, possible methodological considerations may strengthen the relationship in this paradigm.

One limitation of the current study is the small sample size. A larger sample size might lessen the impact of omitting measures not considered "true" tail-flick measures. In the current study, nine cases that could not be identified as clear tail-flicks were omitted from part of the data analysis. These cases were phenotype-dependent because only 2 were ob mice while 7 were lean. Habituating the mice to the cardboard tube in the fabric pocket longer might increase the likelihood of them remaining stationary long enough to measure a tail-flick attributed to a tail flexion and not a movement of the whole mouse. Methods of limiting activity are necessary, in particular, for lean mice due to their frequent attempts to turn around or back out of the apparatus; whereas, all ob mice readily entered the apparatus with most remaining still throughout testing. Nonetheless, extending habituation may also be an effective control in ob mice if stress-induced analgesia contributed to longer tail-flick latencies. However, it is unlikely

that stress-induced analgesia confounded the results because all mice were handled for 3-4 weeks before apparatus habituation. Only residual phenotypic responses to handling should influence data at this point, such as the exaggerated hyperglycemic response to environmental stressors exhibited by ob mice (Surwit, Feinglos, Livingston, Kuhn, & McCubbin, 1984).

The data may accurately represent the comparative pain sensitivity between the lean and ob mouse. As previously mentioned, the reported mean baseline tail-flick latency (saline condition) of the lean mice is consistent with baseline tail-flick measures taken in other studies with B6.V mice. In the current study, the mean tail-flick latency for all lean mice in the saline condition was 2.57 s. Other studies employing the radiant heat tail-flick method yielded comparable baseline tail-flick latencies in B6.V mice. Lariviere et al. (2002) measured 3.7 s (SE = 0.01); Jacob, Nicola, Michaud, Vidal, and Prudhomme (1986) measured 3.03 s (SE = 0.12); and Baran, Shuster, Eleftheriou, and Bailey (1975) measured 2.0 s. These studies yielded an average latency of 2.91 s compared to the 2.57 s mean tail-flick latency of the current study. Although the three studies using the radiant heat tail-flick assay are all based on modifications of the D'Amour

and Smith (1941) technique, not all reported the temperature of the radiant heat source. Tail-withdrawal studies reporting similar temperature of water as the radiant heat used in the current study also had comparable tail-flick latency results. Mogil, Lichensteiger, and Wilson (1998) found tail-flick withdrawal latencies of B6.V mice averaged 3.2 s (SE = 0.2) in an assay where mouse tails are dipped into water maintained at 49 ± 0.2°C. Two later studies by Mogil and associates used the same assay and resulted in tail-flick latencies of 2.2 s (SE = 0.1) for the B6.V mice in both studies (Kest, Hopkins, Palmese, Adler, & Mogil, 2002; Mogil et al., 1999), thus yielding an across-study average of 2.53 s. This latency corresponds closely to the mean tail-flick latency in the present study. Although the heat stimulus used in Mogil's studies was water at 49°C and the current study used an infrared heat source at 50°C, the methods employed between the researchers yielded tail-flick latencies that did not differ. Crabbe, Wahlsten, and Dudek (1999) have demonstrated that even with meticulous effort to equate laboratory environments, three different laboratories will get significantly different results from the same strain of mouse in behavioural tests. For example, Crabbe et al. found significantly different scores between laboratories

in B6.V mice in tests of anxiety and locomotor activity, although analgesia was not tested. Despite differing apparatus between the current study and studies by Mogil et al. and Kest et al., tail-flick/withdrawal latencies recorded between laboratories were similar. This similarity, however, does not rule out the possibility of laboratory environment impacting the results of the current experiment.

Since naloxone was expected to increase pain sensitivity by blocking μ opioid receptors to endogenous β-endorphin, the absence of a naloxone effect in both phenotypes may indicate that a systemically administered dosage greater than 2 mg/kg of naloxone may be required to block analgesia in B6.V and B6.V-lep^{ob}/lep^{ob} mice. Vaccarino, Tasker, and Melzack (1988) found a dosage as high as 10.0 mg/kg naloxone could not induce significant hyperalgesia in B6.V mice in a tail-withdrawal assay. Vacarrino et al. measured tail-withdrawal latencies with water maintained at 49°C with a cut-off time of 15 s to avoid tissue damage. A total of 18 female B6.V mice were divided into three drug conditions: s.c. administration of saline, 0.3, or 10.0 mg/kg naloxone hydrochloride. The mean tail-withdrawal latency was 3.80 s for mice in the saline condition, 3.85 s for 0.3 mg/kg naloxone condition, and 3.1 s in the 10.0

mg/kg naloxone condition. Although Vacarrino et al. found 10.0 mg/kg naloxone was not a sufficient dosage for hyperalgesia, studies have demonstrated effective phenotype-dependent, lower doses of naloxone in other paradigms. Naloxone doses as low as 0.25 mg/kg can depress food intake in ob mice (Margules, Moisset, Lewis, Shibuya, & Pert, 1978). Gilson and Wilson (1989) also demonstrated that systemic naloxone at both .5 and 2.0 mg/kg selectively abolishes caloric intake from fat in obese mice without affecting intake of lean mice at either dose. Since intake and pain are both systems involving opioid mediation (which overlap in areas of brain associated with affective evaluation of reward), it was a reasonable first approach to adopt a naloxone dosage that reliably attenuates food intake in ob mice and that reliably differentiates the phenotypes. Margules et al. demonstrated a dose-dependent decrease in food intake in both lean and ob mice, ranging from 0.25 to 5.0 mg/kg naloxone, with ob mice responding at low doses which had no effect on lean mice. If, as predicted in one of the hypotheses of the present thesis, ob mice do experience tolerance to their own elevated opioid levels, and this tolerance generalizes to mechanisms supporting analgesia as well as energy balance, they should respond to lower doses of naloxone compared to lean mice.

The results of the current study suggest that an experiment with a larger sample size may yield a significant hyperalgesic response from the ob mice to 2 mg/kg of naloxone. Additionally, despite similarities between components of pain and energy balance, different systems controlling different behaviours may have different antagonism thresholds.

Another methodological consideration in the current study is a drawback of the i.p. method of drug administration. This method is unable to identify the precise site (either spinal or cerebral) of naloxone's action. Both tail-withdrawal and the tail-flick test are spinally-mediated, so naloxone would have to act on μ-receptors in the spinal cord to induce analgesia (Tjolsen & Hole, 1997). It is well documented that the descending endogenous analgesia system, including the periaqueductal gray, plays an important role in modulation of nociceptive transmission and morphine-produced analgesia (Besson & Chaouch, 1987; Pasternak, 1993). The periaqueductal gray has been shown to receive input from the hypothalamus in the rat, so it follows that elevated β-endorphin in the ventromedial nuclei and dorsomedial nuclei of the hypothalamus in the ob mice may affect the descending analgesic pathway to the spinal cord (Beitz, 1982; Willis &

Westlund, 1997). While systemic methods of drug administration limit the ability of identifying the location of drug action, nociceptive studies have used s.c. administration of naloxone to block tail-flick analgesia. Paul, Bodnar, Gistrak, and Pasternak (1989) found systemic administration of naloxone (s.c.) effectively blocked intrathecal (spinal) administration of μ-agonist, DAMGO, in tail-flick in CD1 mice. Although the mice were not B6.V mice, their study demonstrates that spinally-mediated nociceptive assays can be blocked by systemic administration of naloxone. Research has established that naloxone readily crosses the blood-brain barrier, thus having both peripheral and central actions (Vaccarino, Tasker, & Melzack, 1988). Therefore, i.p. administration of naloxone should be an effective substitute for intrathecal administration even though the tail-flick assay is spinally mediated.

In the current experiment, the baseline analgesic response of the B6.V and the B6.V-lep^{ob}/lep^{ob} mouse in the tail-flick assay was measured. As previously discussed, strain-dependent pain sensitivity across a wide variety of nociceptive assays has been extensively studied. Notably, Belknap et al. (1995) mapped the genes influencing strain variability in morphine-induced antinociception in DBA and

B6.V mice. Using the method of quantitative trait locus mapping, they localized the genes responsible for morphine analgesia to Chromosome 10. The recessive mutation in the B6.V-lep^{ob} strain is located on Chromosome 6. Given that Belknap et al. identified the region on Chromosome 10 involved in pain sensitivity in the background strain of the ob mouse, several predictions about differential analgesic sensitivity between ob and lean mice emerge. First, because the ob mutation is on Chromosome 6, obs may not differ from lean mice in analgesic sensitivity to the tail-flick assay. Likewise, naloxone may not act differently in modifying analgesia on this assay between the two strains. Second, even though the ob mutation appears on Chromosome 6, it may still influence the expression of the morphine-analgesia site on Chromosome 10 by establishing an altered neurohormonal environment for action of the products of the morphine analgesia site. This possibility predicts altered pain response between the two strains, either in baseline testing or in testing following a morphine or naloxone challenge, or both.

A serendipitous finding from the present research was shorter tail lengths in the ob mice as compared to the lean mice. Previous work (Laharrague et al., 1998) has implicated leptin in bone growth and development, and has

added to the anatomical phenotype of the ob mouse as stunted in musculo-skeletal development (Steppan, Crawford, Chidsey-Frink, Ke, & Swick, 2000). Typically, naso-rectal length is shorter in ob than in lean mice, as is femoral length. Steppan et al. (2000) found no change in femur length (15 mm) in lean mice after 3 weeks of leptin administration, while ob mice femoral length increased by 5.4% from 13.75 mm to 14.5 mm. The present study is the first to report reliably shorter (on average, 0.5 cm) tail base-to-tail tip lengths in ob mice than in their lean controls. To the extent that tail growth may be an early developmental indicator of the ob phenotype, this measure may provide an innocuous and easy-to-use technique for phenotyping newborn mice from heterozygous ob litters. This application is preferable to other techniques, such as more time-consuming assays like PCR testing, wherein the time to conduct the phenotyping overshoots important early developmental windows.

The present study sought to further characterize the altered opioid profile in the B6.V-lep^{ob} mouse. Given that chronically elevated levels of opioids in the ob mouse could result in tolerance, comparing pain sensitivity between the ob mice and their lean counterparts might have demonstrated another characteristic of this phenotype.

Employing the tail-flick assay allowed an indirect measure of β-endorphin because it is mediated by μ-receptors and subsequently might have suggested tolerance developing in ob mice if their pain sensitivity was greater than lean mice. Moreover, naloxone blocks μ-receptors, so opioid tolerance was expected to cause hyperalgesia in the ob mice at a dose lower than one that would cause hyperalgesia in lean mice. Tail-flick latencies were compared between ob and lean mice after saline or naloxone administration. Latencies were not different between phenotypes for the saline or the naloxone condition. Interestingly, ob tails were shorter than lean mice, demonstrating the decreased stature (skeletal development) of the ob mouse due to a lack of circulating leptin.

Future studies could increase the sample size to determine the impact of omitting measures not considered "true" tail-flicks. Furthermore, longer habituation to the apparatus may reduce activity in lean mice thereby increasing the observation of clear tail-flicks. Alternatively, more trials within subjects might yield less variability in tail-flick latency threshold. Either increasing the naloxone dosage, generating a dose-effect curve for naloxone, assessing pain sensitivity in more than one type of assay, or a combination of these could clarify

the threshold for the hyperalgesic effect of naloxone in B6.V and ob mice. Lastly, exploration of non-opioid mechanisms of analgesia may reveal new directions in understanding the ob mutant.

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Table 1

Mean (SE+) Tail-Flick Latency All Cases Included and Clear Tail-Flick Cases Only.

All Cases Included

(n=24)

	Saline	Naloxone	
Ob	3.42 (± 2.10)	3.93 (± 2.24)	3.68 (± 2.09)
Lean	2.57 (± 1.60)	3.52 (± 1.81)	3.04 (± 1.70)
	2.99 (± 1.83)	3.73 (± 1.95)	

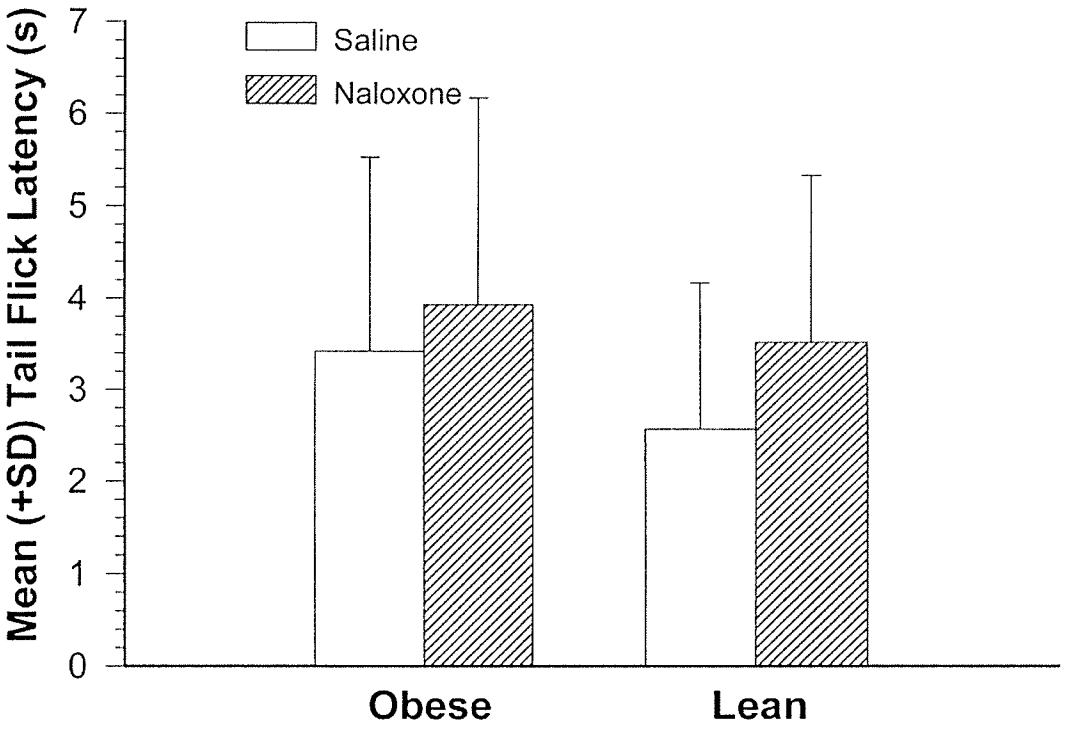
Clear Tail-Flick Cases Only

(n=15)

	Saline	Naloxone	
Ob	3.48 (± 2.34)	3.20 (± 1.50)	3.34 (± 1.86)
Lean	3.05 (± .071)	3.67 (± 1.36)	3.42 (± 1.02)
	3.36 (± 1.92)	3.38 (± 1.37)	

Figure Caption

Figure 1. Mean Phenotype Difference of Tail-Flick Latency Across Drug Treatment (n=24).


Mean (+SD) Tail Flick Latency (s)
0
1
2
3
4
5
6
7
Saline
Naloxone
Obese
Lean