Opiate Blocker Effects on Taste and Voluntary Morphine

Consumption in Genetically Selected Mice

by

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Abstract

The endogenous opiate system plays a role in determining voluntary morphine self-administration patterns in C57 and DBA mice. This thesis explores other factors that may contribute to morphine self-administration differences in these strains of mice. In Experiment 1, the opiate blocker naltrexone was administered to both C57 and DBA mice 15 minutes before presenting a choice between a morphine and an equally bitter quinine solution. Both strains of mice increased morphine consumption while decreasing quinine consumption, suggesting that quinine may be aversive to both strains. Experiment 2 attempted to measure differences in taste sensitivity between C57 and DBA mice using a conditioned taste aversion paradigm. However, because the conditioned taste aversion did not generalize to the more dilute solutions, aversion could not be used as a measure of absolute taste threshold. Experiment 3 examined the effects of naltrexone on voluntary saccharin consumption to elucidate strain differences in the opiate system's role in the pleasurable sensation of sweetness. Both saline treated mice and naltrexone treated C57 mice consumed approximately equal amounts of saccharin solution. However, the naltrexone treated DBAs' saccharin consumption was significantly reduced. Differences between these strains of mice are discussed.

ACKNOWLEDGMENTS

Special thanks to Dr. Barry Beyerstein and Dr. Bruce Alexander for their continued support, helpful suggestions and excellent editing skills. In addition, I would also like to thank Marilyn Courntney for her help editing this manuscript, Joathan Male for his assistance in all chemical preparations, and both Dr. Ray Koopman and Ms. Elizabeth Michno for their statistical advice.

I would like to acknowledge Debbie Nijdam for initially pointing out that quinine is not necessary a non-aversive adulterant.

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INTRODUCTION

DBA/2J and C57BL/6 inbred mice are studied by psychopharmacologists, behavioral geneticists, and neuroscientists because they exhibit theoretically-interesting behavioral, neurochemical and physiological differences (see Table 1, Appendix A). By examining differences between these strains, insights into certain brain-behavior relationships can be gained. Inbred mice also provide a useful model for studying genetically determined behavior because they typically show stable behavioral differences across subjects (Bovet, Bovet-Nitti & Oliverio, 1969; Durkin, Ayad, Ebel & Mandel, 1977).

C57 and DBA mice became popular organisms for drug self-administration research when it was discovered that they have different affinities for alcohol (see Table 1, Appendix A). C57 mice will voluntarily consume alcohol, whereas DBAs will avoid it (McClearn & Rogers, 1962; Meyers, 1969). Later, different affinities for morphine were also found, paralleling those found with alcohol (Eriksson & Kiianmaa, 1971; Horowitz & Whitney, 1975). Some researchers believe that insight into human substance abuse problems can be gained through the study of genetic differences in these mice (Rogers & McClearn, 1962; Horowitz & Whitney, 1975).

Researchers have sought to explain differences between C57 and DBA mice throught genetic explanations (Horowitz & Whitney, 1975). However, many polygenetically determined factors could contribute to these opiate self-administration differences in the two strains. This thesis explores some explanations for C57s' and DBAs' differing opiate consumption patterns.

Voluntary Opiate Consumption

As human use of illicit opiates became a more pressing social problem and the pharmacological similarities between alcohol and opiate drugs were recognized (Davis & Walsh, 1970; Eriksson & Kiianmaa, 1971; Yirmiya & Taylor, 1989), researchers began to search for animal models by examining voluntary morphine consumption in rodents known to be prone to alcohol consumption (Eriksson & Kiianmaa, 1971; Horowitz, Whitney, Smith & Stephan, 1977; Nichols & Hsiao, 1967).

Nichols and Hsiao (1967) found that individual rats, after a period of morphine pre-medication, showed varying levels of voluntary morphine consumption. Using these data, the researchers then selectively bred strains for the trait of high or low morphine consumption. Within one generation, a clear difference in the amount of morphine consumed by the offspring of the high versus low morphine consuming parents was apparent. Further selective breeding increased the differences between these strains (Nichols & Hsiao, 1967). No differences between the selectively bred rats were found when quinine, equated in initial aversiveness, was presented in a similar regimen (Nichols & Hsiao, 1967).

Prompted by the results of Nichols and Hsiao (1967), Eriksson & Kiianmaa (1971) investigated the inheritance of morphine preference (susceptibility) in CBA/Ca (CBA) and C57 mice. After chronic involuntary morphine administration, C57 mice showed higher voluntary morphine intake than CBAs. In addition, C57 female mice showed significantly greater drug seeking behavior than their male counterparts (Eriksson, 1970; Eriksson & Pikkarainen, 1968; Eriksson & Pikkarainen, 1970).

Morphine, being an alkaloid, possesses an inherently bitter taste. This usually results in low levels of voluntary oral consumption in animal models. Although forced premedication appeared to overcome this reluctance (Kumar, Steinberg & Stolerman, 1968), it was not always a successful method of inducing high consumption (Stolerman & Kumar 1970). In addition, conclusions concerning the addictive liability of a drug were difficult to reach when pre-medication paradigms were used. The definition of "voluntary" consumption varies from study to study. In the present thesis, "voluntary" will only be used when the animal is given a choice between one of two fluids.

The presentation of morphine in an aqueous solution of sodium saccharin appeared to solve the problems of low voluntary morphine self-ingestion (Horowitz, Whitney, Smith & Stephan, 1977; Kumar et al., 1968). However, the addition of saccharin created unforeseen problems. An unexpected interaction between the endogenous opiate system and non-nutritive sweeteners was soon discovered. The consumption of sweet solutions or food was found to increase endogenous opiate pools resulting in low levels of tolerance to morphine (Lieblich, Cohen, Ganchrow, Blass & Bergmann, 1983; Lieblich, Cohen,

& Ganchrow, 1985; Cooper, 1985; Zellner et al., 1985; Yirmiya, Lieblich & Liebeskind, 1988). This created a possible confound with its use as a masking agent for morphine.

The unequal morphine consumption in C57 and DBA strains likely does result in part from genetically-determined differences in the response to morphine's pre- and post-ingestional effects. But in addition, there may have been certain confounds introduced by the particular paradigms used that complicate the picture. For example, strain differences in taste sensitivity, or learning, could have been responsible for some of the observed differences in voluntary opiate consumption. These alternatives should have been ruled out before reaching the conclusion that these data indicate differences in the strains' genetic predisposition for morphine addiction (Horowitz et al., 1977).

Forgie, Beyerstein and Alexander (1988) conducted a number of experiments concerned with genetic differences in the preference for opiate solutions in C57 and DBA mice. A simultaneous control solution of quinine sulfate (QSO_4) was introduced in place of the usual water alternative because it has a similar bitter taste to that of morphine hydrochloride (MHCl). In addition, it had been used extensively as an inactive adulterant (Peters et al., 1979). To match the bitterness of morphine and $QS0_4$ solutions, blind taste tests were conducted with human tasters (Alexander, Beyerstein, Hadaway & Coambs, 1981). The MHCl at a concentration of 0.3 mg/ml was judged to be equal in bitterness to 0.06 mg/ml of QSO_4.

To study the interaction between taste and opiate consumption Forgie et al. (1988) employed a third choice condition. The synthetic opioid etonitazene, estimated to be between 1000 and 2000 times more potent than MHCl, was used (Chernov et al., 1968; Rosow, Miller, Pelikan, Cochin, 1980) along with MHCl and QSO_4. When suitably diluted, etonitazene provides an equivalent pharmacological solution to MHCl (based on withdrawal symptoms and behavioral effects) but without the bitter quality of MHCl (McMillan, et al., 1976). In this experiment (Forgie et al., 1988), C57s continued to prefer MHCl when it was paired with QSO_4, but did not prefer the nearly tasteless etonitazene. This suggests there may be differences between etonitazene and MHCl in endorphin receptor affinity that remains to be

discovered. The DBAs, appeared to be indifferent to the effects of the three solutions. They consumed from all three solutions equally.

In the second experiment by Forgie et al. (1988), she examined the effects of sweetening all of the solutions. C57 mice continued to display a clear preference for MHCl in all conditions, suggesting that the preference is based on the drug's post-ingestional effects. In an additional experiment, C57s avoided a saccharin/$QS0_4$ solution compared to a saccharin-alone solution, demonstrating that C57 mice are not insensitive to bitterness but are preferentially consuming opiates for their post-ingesitonal effects (Forgie Beyerstein & Alexander, 1988; Klein & DeFries, 1970).

The DBA mice showed no significant difference in consumption between MHCl and its controls in either of the vehicles. In Horowitz et al.'s (1977) study, DBAs consumed very little MHCl when given a choice between tap water and a MHCl solution. In the Forgie et al. (1988) study, DBAs consumed approximately equal amounts of MHCl and $QS0_4$ solutions. This result suggests that perhaps DBAs are not avoiding the pharmacological effects of MHCl, but are simply indifferent to them. In other words, their earlier aversion had been primarily based on taste rather than dislike of post-ingestional effects.

DBAs showed no preference for the $QS0_4$/saccharin solution versus the saccharin alone. This suggests that the mice avoided the sweetened MHCl solution in the Horowitz et al. (1977) study because of the post-ingestional effects and not the bitter taste. Both strains appear to be indifferent to etonitazene, suggesting that the post-ingestional effects to etonitazene are different from those of MHCl (Forgie, Beyerstein & Alexander, 1988).

In a second study (conditionally accepted for publication) Forgie and Beyerstein further investigated the apparent lack of enthusiasm for etonitazene. C57 and DBA mice were given a three-way choice test with a standard MHCl solution, one of two concentrations of etonitazene and a control solution of $QS0_4$. Again MHCl and QSO_4 solutions were equated in bitterness based on the human palate. The C57 mice consistently sought the MHCl solution over the other two choices. Post-ingestional effects appeared responsible for their preference for MHCl. The DBA mice consumed equally from all three

solutions, supporting the notion that they are indifferent to some of the central effects of opiates (Forgie et al., 1988).

The reviewers of the submitted Forgie and Beyerstein article requested evidence to support the contention that the taste of the morphine and quinine solutions was equal for the two strains. This has been a particularly trying problem. Several attempts at producing a design to test this hypothesis have been unsuccessful (Marchant, 1991). The present thesis is aimed at furthering the understanding of the underlying differences in opiate consumption between C57 and DBA mice. In other words, this thesis hopes to provide evidence that QSO_4 mixed at .06 mg/ml is an adequate control for a MHCl solution mixed at the concentration of .3 mg/ml. With this evidence the concern that there may be a residual taste confound in the results of a study submitted for publication by Forgie and Beyerstein may be nullified.

Attempts to Examine Taste Factors

The first set of experiments conducted to study possible differences in taste sensitivity between C57 and DBA mice used the ascending method of limits to measure taste psychophysics (Nijdam, 1990). DBA and C57 mice were given a choice test between low doses of either morphine or quinine solutions and water. The drug concentrations were then gradually increased over an extended time. The intent was to compare (between strains) the concentrations at which the mice stopped consuming the two tastants. Although this experiment provided some interesting information, potential problems remained. For example, although morphine and quinine were raised to very high concentrations, no point could be identified where the animals stopped consuming the tastants. In addition, acquired tolerance, habituation to the drug solutions, and the potential for an interaction between the test solution and taste made the determination of pure taste contributions to between-strain differences ambiguous.

Later, an operant conditioning paradigm was used in an attempt to test for differences in absolute taste thresholds between C57 and DBA mice (Marchant, 1991). The mice were trained to identify a drug solution (tastant) in a choice with water. After this discrimination conditioning, the concentration of the tastant was to be lowered until the mice could not reliably distinguish between water and the tastant

solution. Electric shock had been ruled out by the animal ethics committee as a technique for negatively reinforcing incorrect discrimination, so food was used as a positive reinforcer instead. However, conditioning proved unsuccessful in these rather high-strung inbred strains. Motivation problems and a potential for an interaction between the food reward and the tastant made this method not worth pursuing.

A descending voluntary choice paradigm was tried next. The drug solutions (MHCl & QSO_4) were mixed at a high concentration and given in a voluntary choice paradigm with water. The concentrations of the drug solutions were then lowered every second day until the mice were consuming approximately equal amounts of the drug and water solutions. Within the data, no clear points could be identified which could be used to indicate a threshold. Additional problems with tolerance and taste habituation also made these results difficult to interpret.

The failure of these prior attempts to clarify possible differences in taste sensitivity between C57 and DBA mice with respect to morphine and quinine, led to the present study. It was reasoned that if the central effects of morphine were blocked, the animals' subsequent consumption would most likely be the result of taste factors alone. If quinine and morphine solutions were then made equivalent in taste, this should be reflected in equal consumption of both solutions. In other words, C57 mice would no longer be consuming morphine for the post-ingestional effects and DBAs could not be said to be drinking to avoid those effects. It was felt that this approach should shed some light on possible differences between strains that may contribute to their behavioral differences, other than those resulting from differences in their endogenous opiate systems (see Table 1, Appendix A).

To pursue possible differences in taste sensitivities between DBA and C57 mice, in the present thesis the strains were conditioned to avoid either a MHCl or a $QS0_4$ solution. In a Conditioned Taste Aversion (CTA) paradigm, the avoidance of a tastant on subsequent exposure to varying concentrations constitutes the dependent measure. Comparisons of taste factors across strain, using both morphine and quinine were thus attempted as described below.

The Experiments

In Experiment One of the present thesis, the opiate antagonist, naltrexone (NAL) was used to pharmacologically block post-ingestional effects of MHCl. Naltrexone, derived from naloxone, blocks opiate receptor sites by binding to them with a greater affinity than either MHCl or endogenous opiates (Akil, Mayer & Liebeskind, 1976; Palfai & Jankiewicz, 1991). This procedure was selected because it allows one to determine the effects of taste on consumption patterns in isolation from post-ingestional effects of MHCl. Therefore preference behavior is likely to result from taste factors alone, resulting in equal consumption of morphine and quinine.

In Experiment Two, taste sensitivity and learning differences between DBA and C57 mice are studied. According to Conditioned Taste Avoidance (CTA) Theory, when a novel flavor is followed by sickness within a given interval, a strong and lasting aversion to that flavor will be produced (Garcia, Hankins & Rusiniak, 1974; Green & Garcia, 1971). The second experiment used conditioned taste avoidance (CTA) to cause an aversion to either morphine or quinine. This would be expected to limit consumption of the tastants (morphine or quinine) as long as they are detectable in the test solution. The tastant can then be given in descending concentrations, paired with a water alternative, until no distinction is made between the two fluids (Ingram, 1982). Thus a reasonable estimate of the absolute taste threshold for both morphine and quinine across strains and solutions can be made. This would provide a method to evaluate taste equivalencies between strains and solutions (MHCl and QSO_4).

Experiment Three was conducted to examine the effects of naltrexone blockage of the endorphin system on sapid solution consumption. It is conceivable that if opiates are involved in the pleasurable experience associated with sweet taste, then an opiate antagonist may also affect taste preferences. Both strains of mice were given NAL 15 minutes prior to being presented with a two-bottle choice test consisting of water versus a saccharin solution. If the reinforcing value of sweeteners is mediated by endorphinergic mechanisms, NAL might be expected to affect voluntary saccharin consumption. Differences between strain and gender may point to differences in their endorphin systems. Saline-treated

C57 mice are expected to consume a greater amount of saccharin solution than saline-treated DBA mice. There should be no difference between DBA and C57 mice when treated with naltrexone.

EXPERIMENT ONE

Methods

This experiment investigated the effects of naltrexone (NAL) administration on the voluntary consumption of morphine hydrochloride (MHCl) and quinine sulfate (QSO_4) in C57 and DBA mice. With the pharmacological (post-ingestional) effects of MHCl blocked by NAL, consumption patterns should not be affected by MHCl post-ingestional effects. Taste factors should play the major role in determining the consumption pattern.

Groups of 20 DBA and 20 C57 mice were obtained from Charles River Labs (Montreal, Quebec). At the time of arrival, all the mice were between 40-56 days of age and 15-20 grams in weight. Equal sex ratios were obtained for each group. The animals were housed with a reversed 12:12 light:dark cycle. Following a seven day adjustment and acclimatization period, the mice were eased (over eight days) onto a 2:22 hour daily drinking schedule . On days one and two of this period the mice received water only in their dark phase. On days three and four, they received water for only the last six hours of the dark phase. During days 5 and 6, the mice received water during the last three hours of the dark phase. On days 7 and 8, they were able to drink water only in the last two hours of their dark phase (2:22 hour drinking schedule).

The mice were individually housed in standard polyurethane cages (15cm x 25cm x 45cm) with wood shaving floors. Rat Chow was available ad lib. All fluids were presented through the cage top in 150 ml glass bottles with rubber stoppers and stainless steel sipping tubes. All experimental manipulations took place during the dark phase under dim red lights.

In Experiment One, the test solutions included QSO_4 (.06 mg/ml) and MHCl (.3 mg/ml) prepared with distilled water. Fresh bottles and solution were substituted every second day. This helped guard

against flavoring contamination from rubber stoppers, chemical degradation, and/or staleness. Bottles were carefully transferred to an adjoining room, and weighed daily. These procedures were followed in all experiments unless otherwise indicated.

Daily fluid consumption for each mouse was calculated by subtracting the bottle weight after the drinking period from the bottle weight prior to its last replacement on the cage. Four empty cages with two water bottles on each served as control bottles. These bottles were filled and weighted in the same manner as all other bottles. By weighing the bottles daily, the average loss resulting from handling, measuring, evaporation, and spillage could be estimated.

An 8-day water baseline was recorded after the animals had adjusted to the light and drinking schedule. The forty mice were divided into 8 groups by sex and strain and treatment (5 animals per group). Each day, for six days, half of the mice received a .2 mg/kg SC injection of NAL mixed in a isotonic saline (SAL) vehicle. The other half received a control injection of an equal volume of isotonic SAL. For injections, each cage was removed from the housing area and transported to the adjoining room where the lighting cycle was identical. After an SC injection of either NAL or SAL, the cage was returned. Fifteen minutes after the injection each mouse was presented with a bottle containing MHCl (.3 mg/ml) and a bottle containing $QS0_4$ (.06 mg/ml). The presentation time was recorded. To control for side preference, the bottles were placed in the cages on opposite sides each day (Meyers, 1969). Two hours after the presentation of the drinking solutions, the bottles were carefully removed, transported to the adjoining room and re-weighed. This procedure was then repeated for 6 days.

Fluid intake differences between the strains that might be due to weight inequities were controlled for by dividing each mouse's daily consumption score by its body weight. This produced a measure of grams of solution consumed per gram of body weight. This number was then multiplied by 100 to produce more easily readable numbers.

Average daily evaporation/spillage was calculated at .14 grams with a standard deviation of .16 grams. This amount was not deducted from the raw consumption scores because if the average of .14

grams were subtracted from all raw scores, some zeros and negative numbers would have been introduced into the statistical analysis. Within a statistical analysis this could grossly distort the true state of affairs (R. Koopman, personal communication, June, 1992).

All data recording and transformations were done using Microsoft Excel (Version 4.0) and all statistics with BMDP's 4V multivariate analysis (MANOVA) program. The factorial MANOVA procedure is designed to handle more than one dependent variable (Tabachnick & Fidell, 1989). Strain, gender and treatment (naltrexone or saline) were the 3 dependent variables (DV) examined. A MANOVA, is a generalization of analysis of variance to a situation in which there is more than one DV. An ANOVA tests whether mean differences among the groups on a single DV are likely to have occurred by chance, while a MANOVA tests whether mean differences among groups on a combination of DVs are likely to have occurred by chance. Ad hoc (or post hoc) comparisons were not necessary because there were no more than 2 levels in either of the IVs or DVs. Significant changes can be seen by simply graphing the main effects, without sacrificing any degrees of freedom.

Morphine and $QS0_4$ consumption scores were analyzed separately and together. Additional insights can often be gained when DVs are co-analyzed. Difference scores (MHCl consumption minus $QS0_4$ consumption) were also analyzed. In all cases where the independent variable was a repeated measure, the Huynh-Feldt corrected probabilities are reported. This statistical correction provides a conservative test for repeated measure factors (Huynh & Feldt, 1976).

Results (Experiment One)

Baseline Data

C57 mice are slightly heavier than DBA mice, and therefore consumed slightly more fluids. After the daily raw consumption scores were corrected for individual weight differences no significant difference in total fluid intake was found between C57 and DBA mice. The DBA and C57 mice consumed, on average, .11 grams of water per gram of mouse per day.

Morphine Data

When the MHCl scores were analyzed independently of $QS0_4$ scores, a significant main effect for the NAL treatment was seen ($F(1, 32) = 4.16$, $p<.0497$). This effect can be seen in Figure One. Naltrexone treated mice consumed more MHCl (average daily MHCl consumption = 10.48) than mice treated with SAL (average daily MHCl consumption = 8.86). In addition, there was a significant increase in MHCl consumption over days ($F(5, 160) = 6.55$, $p<.000$). This effect can be seen in Figure Two.

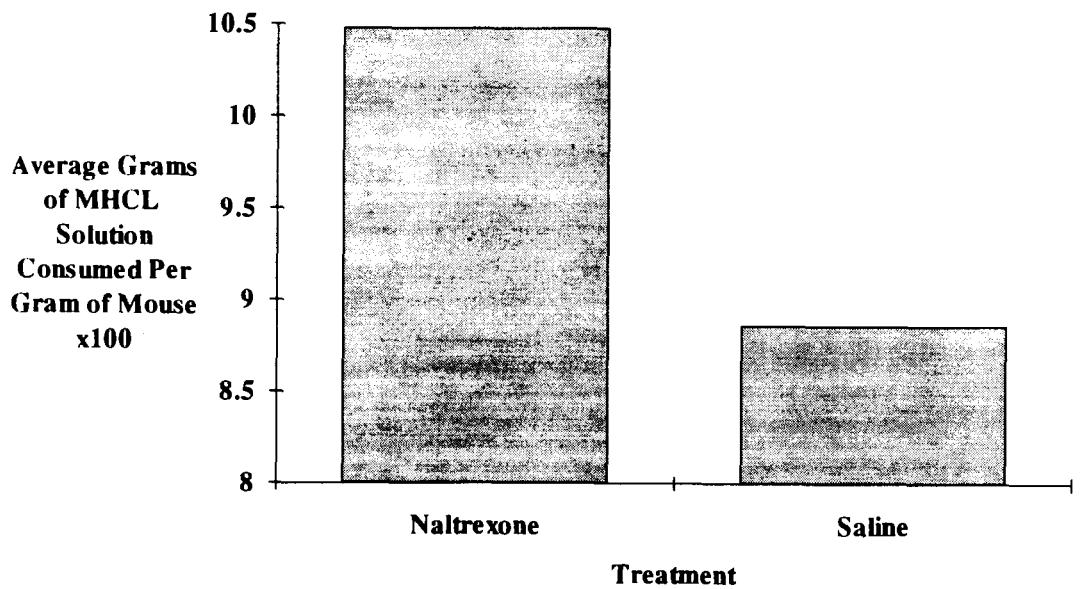

Figure 1. The effects of NAL administration on MHCl consumption collapsed across strain, over 6 days.

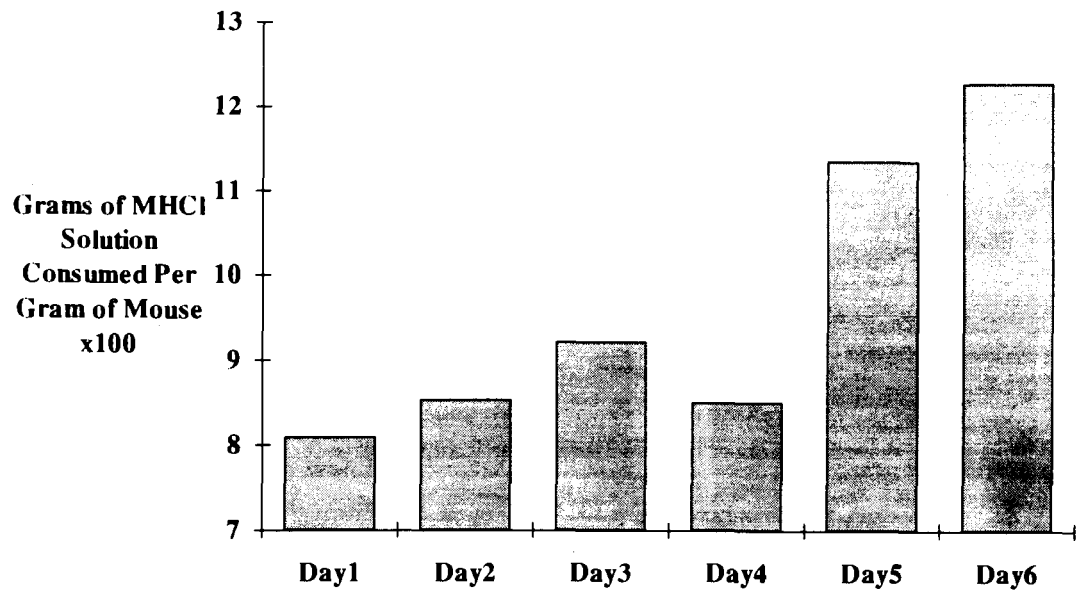

Figure 2. Morphine consumption over the 6 days of the Experiment One, collapsed across strain.

Quinine Data

When analyzed separately, the $QS0_4$ data produced a significant strain and a significant treatment main effect. In addition, a significant strain by sex interaction was also found (Figure 3). Consumption of QSO_4 by the female DBA mice was significantly higher compared to their male counterparts. DBAs in general, consumed significantly more than C57 mice ($F(1, 32) = 7.95$, $p<.0082$). There were no significant changes in $QS0_4$ consumption over days.

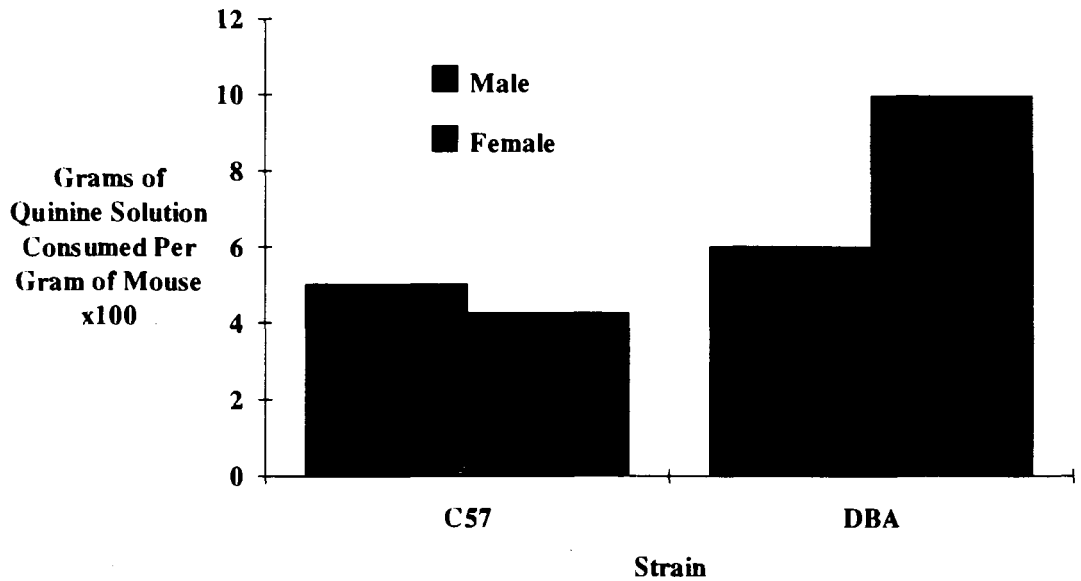

Figure 3. Quinine consumption graphed by strain and sex in Experiment One.

Difference Scores

Difference scores were obtained by subtracting the daily $QS0_4$ consumption from daily MHCl consumption. Thus a zero score indicates equal consumption of MHCl and $QS0_4$ and a positive number indicates higher MHCl consumption. The analyzed difference scores only produced a significant strain effect ($F(1, 32) = 10.09$, $p<.0033$). This simple main effect can be seen in Figure 4. The average difference between MHCl and $QS0_4$ in the DBA strains was .87 grams. In the C57 strain, the average difference was approximately 6 grams, indicating much greater MHCl consumption.

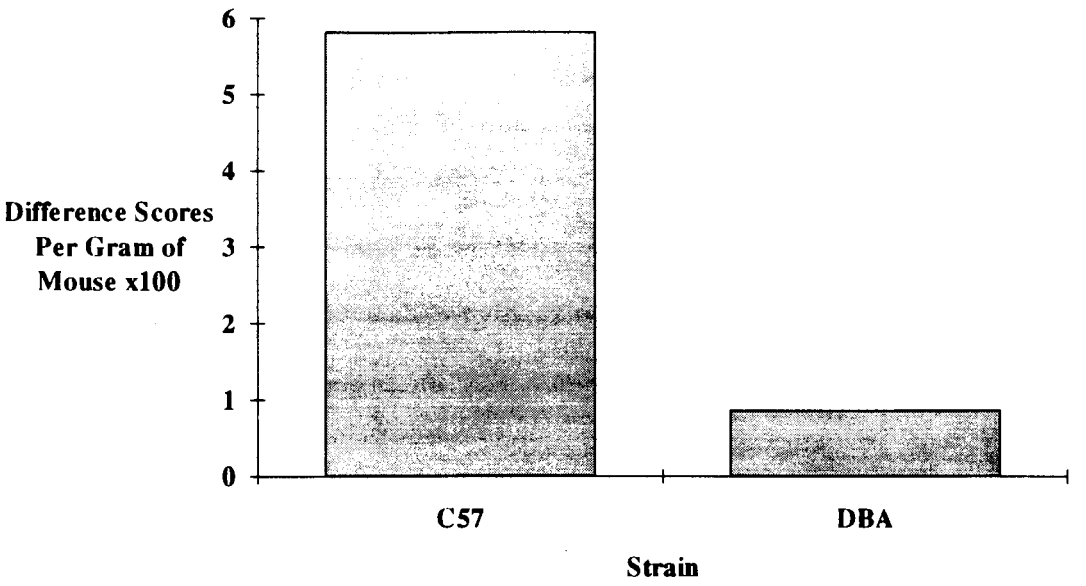

Figure 4. Difference scores obtained by subtracting $QS0_4$ consumption from MHCl consumption.

Morphine and Quinine Data Co-Analyzed

Morphine and $QS0_4$ scores were independently collapsed into averages over 6 days. These scores were then analyzed together as two separate DVs. The analysis produced significant strain by treatment ($F(1, 32) = 6.26$, $p<.0176$), strain by sex ($F(1, 32) = 18.95$, $p<.0001$) (see Figure 5b or 5a) and solution by strain interactions ($F(1, 32) = 10.04$, $p<.0002$) (see Figure 5b). A significant three-way interaction was not obtained. The simple main effects will not be discussed because of these interactions.

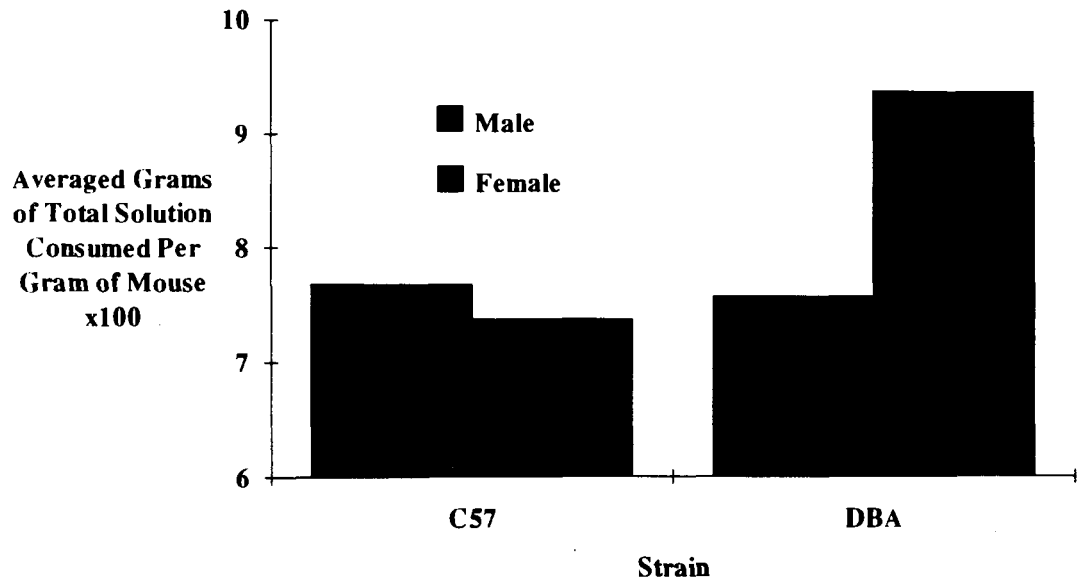

Figure 5b. Strain by sex interaction for C57 and DBA mice collapsed across solutions.

The significant strain by sex interaction occurred because the female DBA male mice consumed significantly more solution than both their male counterparts and C57 female mice (see Figure 5b). C57 MHCl consumption, DBA MHCl consumption and C57 $QS0_4$ consumption do not differ across gender. DBA $QS0_4$ consumption differs significantly across gender (see Figure 5b). DBA female mice consumed dramatically more $QS0_4$ over their male counterparts, and even greater amounts than either sex of C57 mice (see figure 5b).

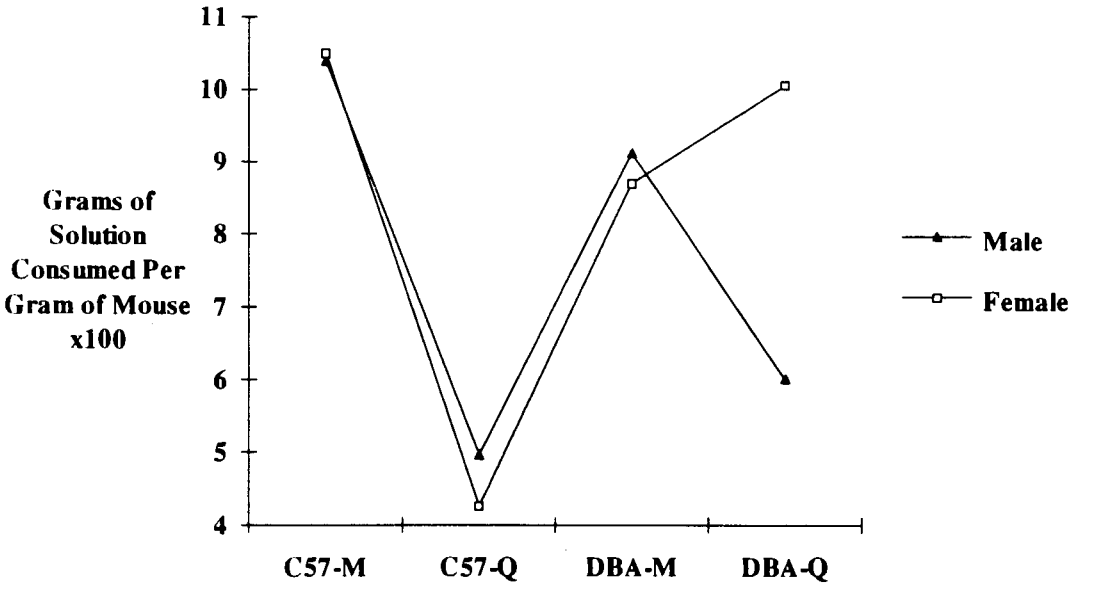

Figure 5b. Strain by solution by sex interaction. Please note that although the three-way interaction was not significant this graph best displays three two-way interactions

A strain by solution interaction resulted from differences in patterns of consumption for MHCl and QSO_4 between C57 mice and DBA mice (see Figure 6). The DBA mice consumed approximately equal amounts of MHCl and $QS0_4$ solutions, whereas C57 mice consumed significantly less $QS0_4$ solution than MHCl solution.

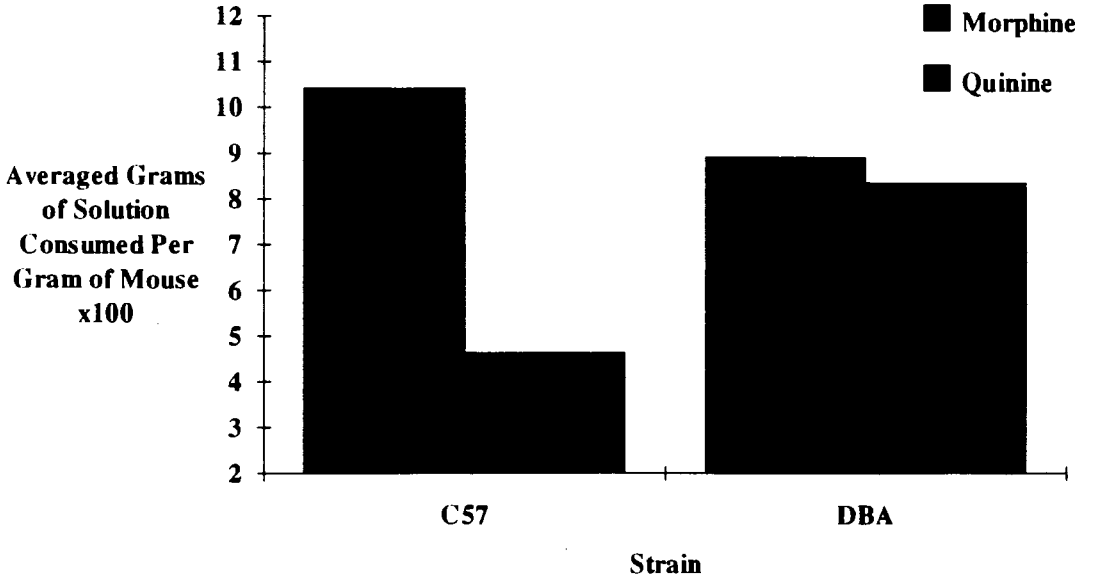

Figure 6. Morphine and $QS0_4$ consumption across strains for Experiment 1. Significant interaction between solution and strain.

The third two-way interaction is between strain and treatment (Figure 7). DBA consumption of total solutions is little affected by the NAL treatment. The C57 mice consume more solution in total when treated with NAL. Similar to the strain by sex interaction presented previously, more information is available when the solutions are not collapsed (see Figure 8).

When DBA mice were treated with SAL their MHCl and $QS0_4$ consumption were approximately equal. When NAL is administered to DBA mice their consumption of $QS0_4$ is decreased.

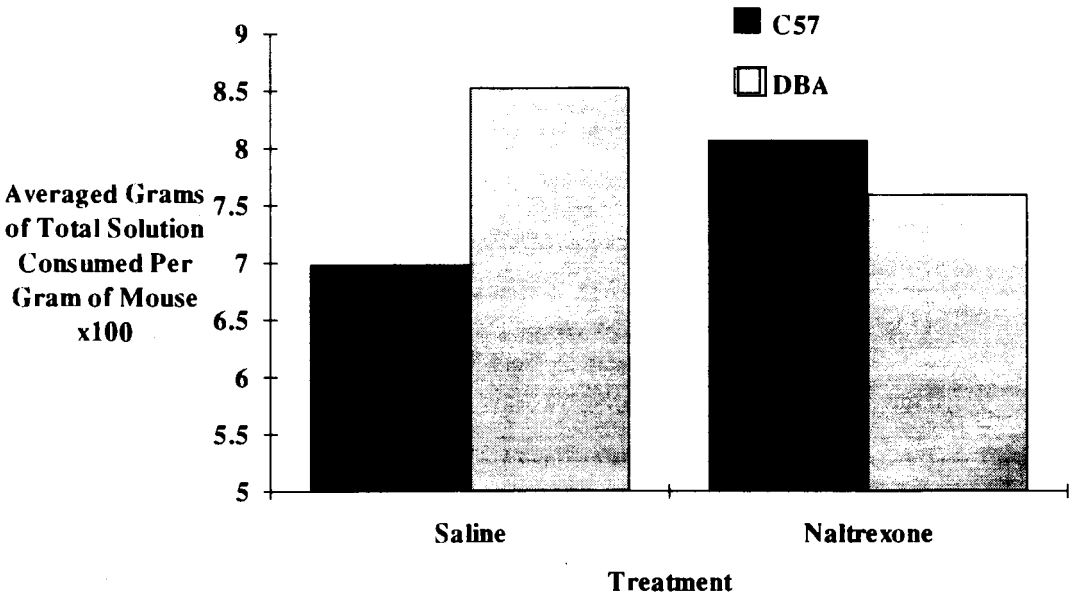

Figure 7. Strain by treatment interaction for C57 and DBA mice, collapsed across solution.

The C57 mice had a similar pattern to that of the DBA mice. C57 $QS0_4$ consumption was unaffected by the NAL treatment, however their MHCl consumption was dramatically increased (see Figure 8). When the data were re-analyzed all the days of the experiment the results were the same.

Saline Treated Mice

By examining consumption patterns of MHCl and $QS0_4$ on day one of the experiment, an indication of taste preference might be gained. All the mice would have been naive to the post-ingestional effects of both solutions. Therefore much of the initial consumption may be based on taste preference alone.

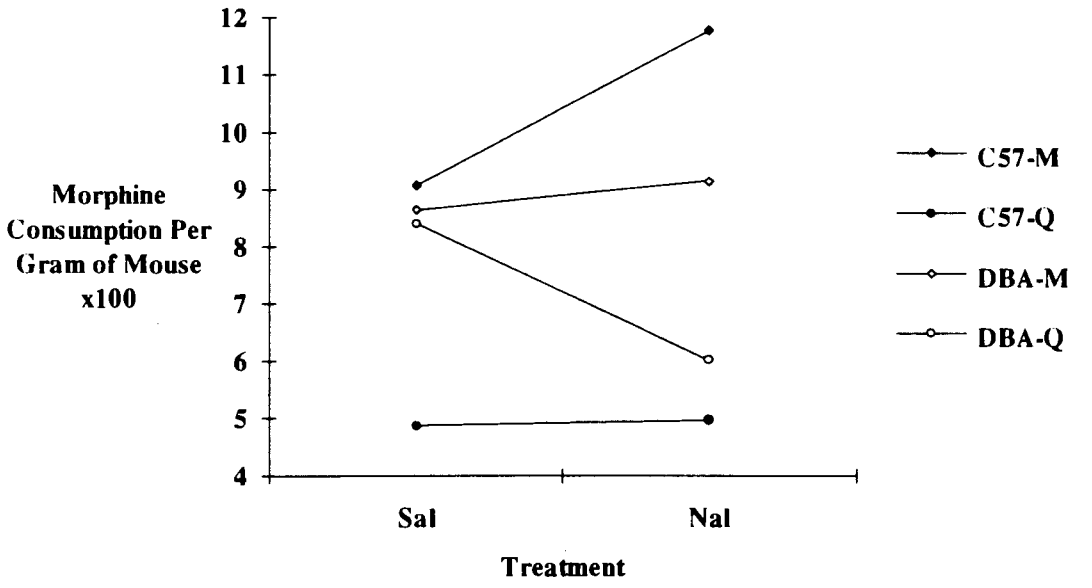

Figure 8. Strain by treatment interaction for DBA and C57 mice, averaged over the six days of Experiment One

No significant differences were found first exposure in consumption for morphine or quinine in DBA and C57 mice ($F(1, 32) = 3.80$, $p<.0601$). However, in Figure 9, it can be seen that SAL treated C57 mice consumed 48 % morphine solution compared to 52 % of QSO_4. When these scores are compared to those of the SAL treated DBA mice, we see a smaller $QS0_4$ consumption (39 %) than MHCl consumption (61 %). Based on only the first day's data, it appears that DBA mice prefer the taste of MHCl over the taste of $QS0_4$, and that C57 mice prefer $QS0_4$ slightly more than MHCl. This consumption pattern is different from that established over the six day period (see Figure 8).

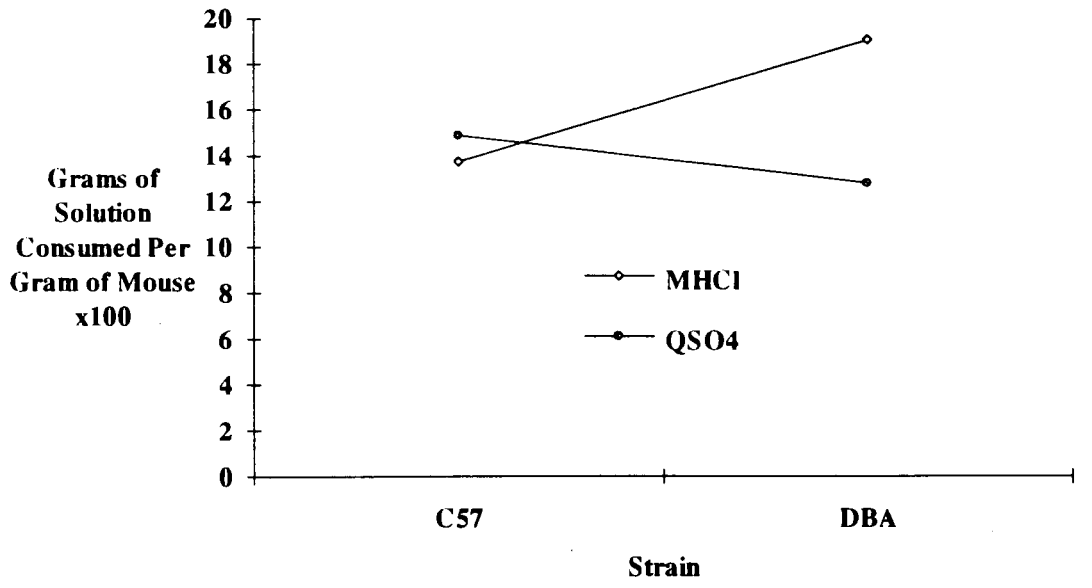

Figure 9. Day one consumption patterns in SAL treated C57 and DBA mice.

Discussion (Experiment One)

Experiment One was conducted to help validate the use of $QS0_4$ as a taste-matched control solution for MHCl. It was thought that if the post-ingestional effects of MHCl were blocked by NAL and the two solutions tasted equally bitter they would tend to be consumed more equally. As long as taste is the only salient difference, equal consumption should indicate equal bitterness. In addition, this study was designed to investigate any differential effects of NAL on MHCl and $QS0_4$ consumption in a two bottle choice test with these two strains of mice.

A number of different factors can affect free-choice drinking behavior. Taste and post-ingestional effects are the two primary factors. Taste alone likely governs initial intake when no prior information exists. However, based on these results, it appears that fluid intake is governed more by post-ingestional effects. From an evolutionary perspective, an eating or drinking regime solely governed by taste would not be adaptive. It has been repeatedly demonstrated in humans and infrahuman species that

when a pleasurable substance is followed by the experience of illness, a taste aversion develops to the erstwhile pleasurable substance. Chronic consumption patterns are likely to minimize aversive post-ingestional effects regardless of taste.

The interaction between taste and post-ingestional effects of QSO_4 has been explored by Aravich and Sclafani (1980). The researchers questioned the assumption that QSO_4 was without aversive post-ingestional effects and therefore questioned its use as a dietary adulterant. A second adulterant, sucrose-octa-acetate (SOA), was used as a control substance. SOA, like QSO_4, possesses an inherently bitter taste and is often used as a food or fluid adulterant. In this study, rats were given a free-choice between a less bitter $QS0_4$ adulterated diet and a more bitter SOA adulterated diet. The rats consistently preferred the less bitter QSO_4 diet. The same rats were then given a 4 day period of QSO_4 adulterated diet alone. When the choice situation was re-introduced, the rats stopped consuming QSO_4 altogether, and consumed only the more bitter SOA-adulterated food. This effect was not seen when the study was repeated with QSO_4 and SOA reversed. The researchers reasoned that the initial consumption was governed by taste alone, and that the toxic effects of QSO_4 were being generalized to both adulterants. However, after the forced consumption of the less bitter $QS0_4$ diet, the rats were able to properly associate its taste with its toxic post-ingestional effects. When presented with both adulterated diets again, the rats chose the more bitter tasting diet over the more toxic diet.

Aravich and Sclafani 's (1980) study demonstrates a number of important points. First, along with a number of other studies (Cooper, 1986; Heyback & Boyle, 1983; Nijdam, 1990; Peters, Wellman, Gunion, & Luttmers, 1979), it questions the belief that quinine is inert as a dietary adulterant. Second, this study demonstrates that taste and post-ingestional effects are not easily separated. Furthermore, taste is a secondary consideration to the post-ingestional effects in a choice situation. It also raises the possibility that differences in voluntary morphine and quinine consumption by DBA and C57 mice may, in part, be explained by different generalizations. For example, DBA mice may have consumed indifferently in the Forgie et al. (1988) study because of an inability to decipher what solution caused what

post-ingestional effects, or they may have simply generalized the post-ingestional effects of one of the solutions to all similar tasting solutions.

The different consumption patterns of the saline treated C57 and DBA mice in Experiment One can be explained by differences in the severity of the post-ingestional effects to MHCl. When C57 mice are given a choice between water and a morphine solution, they consume approximately 33% of their daily intake as morphine (Marchant, Beyerstein & Clement, 1993). This suggests that C57 mice do not find MHCl aversive at all, but perhaps functional in combating boredom in a barren laboratory environment (Alexander, 1990; Zellner, & Berridge, 1984). In addition, opiate receptors have been isolated on dopaminergic neurons in the mesolimbic reward pathway (Bardo, Bhatnagar & Gebhart, 1983; Cabib, Puglisi & Oliverio, 1984). Electrical and chemical stimulation of this pathway has been demonstrated to be very rewarding in rodents. Behaviorally, C57 mice become hyperactive and experience little of the analgesic qualities of morphine, again suggesting that morphine is not having a negative impact on them.

The physiological and behavioral reaction of DBA mice to MHCl indicates that MHCl is more aversive than it is to C57 mice. Morphine administration produces immobility and more analgesic effects in DBA mice (see Table 1, Appendix A). The response cost, or negative impact of opiate consumption in C57s appears to be considerably less than the response cost in DBA mice.

If C57 mice were consuming morphine for its pleasurable post-ingestional effects, NAL treatment would likely decrease the intake of MHCl, unless quinine produced aversive post-ingestional effects too (Bardo, Bhatnagar & Gebhart, 1983). In the present study, NAL increased the consumption of MHCl in both strains of mice (see Figures 2 and 8). Based on these observations, it is reasonable to assume that C57 mice are consuming more MHCl because it was less aversive than the QSO_4 solution (lower response cost). There is no evidence that I am aware of that naltrexone has any mixed agonist-antagonist properties, or is a partial antagonist. However, the possibility exists that the mice are consuming more morphine to get more of its positive effects.

If the DBA mice were opiate indifferent (i.e., are somewhat immune to the post-ingestional effects as argued by Forgie et al., 1988), NAL should have had little effect on their consumption pattern. Likewise, if DBAs were drinking due to taste factors, NAL also should not have affected their consumption patterns. These predictions assume that quinine has no aversive post-ingestional effects. This interpretation is not supported by the present data. DBA mice increased their consumption of MHCl slightly and decreased their consumption of $QS0_4$ in response to NAL administration. This result would only be expected if QSO_4 produces more aversive post-ingestional effects than morphine when morphine's its pharmacological effects are blocked by NAL. In other words, this study provides evidence that the response cost of MHCl consumption is decreased below that of QSO_4 for the DBA mice.

There is an indication that the endogenous opiate system may play an important role in drinking physiology (Sanger, 1981). In the C57 Experiment One strain's total consumption of fluids increased in response to naltrexone treatment while in the DBA mice total intake decreased. If naltrexone causes strain specific changes in fluid intake and a blockage of MHCl's aversive post-ingestional effects, then decreased QSO_4 consumption in the DBA mice is perhaps the most beneficial trade off. The C57 mice, faced with an increased fluid desire, increase their MHCl consumption over that of quinine because the former is less aversive.

Naltrexone administration is known to block conditioned taste aversion (CTA) learning when MHCl is used as a UCS (Bardo & Miller, 1984; Lieblich & Yirmiya, 1987). It is likely that the post-ingestional aversive qualities of MHCl are decreased or removed altogether as a result of the NAL treatment. This could explain the increase in MHCl consumption in both strains (see Figure 1). There is no evidence that I know of to indicate that the post-ingestional aversive qualities of $QS0_4$ would be affected by NAL.

Other explanations are also available, however. Naltrexone was developed by the manipulation of the chemical structure of naloxone (Way & Glasgow, 1978). Both NAL and naloxone are pure opiate antagonists. However, NAL has a much longer duration of action (Way & Glasgow, 1978). Zukin et al.

(1982) have shown that chronic blockage of opiate receptors by NAL leads to a two-fold increase in both mu and delta receptors in the central nervous system. The extent of the increase varies with brain area, with the largest increase occurring within the mesolimbic system and frontal cortex. Tempel, Zukin and Gardner (1982) also found increases in mu, delta and kappa opiate receptor subtypes, and little change in sigma-receptor types, after chronic NAL treatment. Since these studies, other opiate receptors have been isolated including epsilon, mu1, mu2 and sigma (Moskowitz & Goodman, 1985). Unknown differences in opiate receptor distribution and/or density sites and in the pharmacological action of NAL could be responsible for differential consumption patterns prior to any manipulations.

Opiate receptor up-regulation resulting due to opiate antagonist treatment may also explain the increased MHCl consumption. Within the C57 strain, opiate receptor up-regulation has been shown to occur within 15 minutes after antagonist administration (Bardo & Miller, 1984). If unbound naltrexone is oxidized or removed before newly formed opiate receptor sites are created, MHCl or endogenous opiates could be binding to the up-regulated receptor sites. C57 mice may consume more MHCl for its 'supposedly' desired post-ingestional effects. This explanation is unlikely because of NAL's ability to effectively block opiate motivated behavior for long periods of time (Way & Glasgow, 1978). In addition, this does not account for the observed increase in consumption seen in the DBA strain, in which opiate up-regulation occurs at a much slower pace.

Differences Over Days

When averaged over days, the differences present support the observation of Forgie et al. (1988). DBA mice appear to be non-preferers, not MHCl-avoidant (Horowitz et al., 1977). It is likely that the post-ingestional effects of MHCl and QSO_4 are approximately equivalent in the DBA strain. When the intake scores are averaged over the 6 days of the experiment, no difference between MHCl and QSO_4 are seen within the SAL group. DBAs' initial consumption pattern is likely changed after the post-ingestional effects of the two solutions are experienced.

The SAL-treated C57 mice consumed significantly more MHCl than QSO_4 solution over the 6 days of the experiment. When the effects of MHCl are blocked by NAL, MHCl consumption is increased. No change is seen in QSO_4 consumption (see Figure 8). If C57 mice consumed MHCl in pursuit of its pharmacological effects, their consumption would be expected to decrease in response to NAL. However, if the C57s' drinking strategy had been to minimize aversive post-ingestional effects, their consumption of MHCl might be expected to increase. This pattern is seen in Figure 8. The NAL treated DBA mice also increased their MHCl consumption slightly over the SAL controls and decreased their QSO_4 intake. The effects of taste over time are likely not to contribute significantly to the drinking patterns seen here. Post-ingestional effects are most likely to control consumption patterns.

Saline Treated Mice Day One

Some information concerning taste preference, without the interference of post-ingestional factors, may be gained by examining data from saline treated animals on day one of the experiment. At this point in time all mice were drug naive. In addition, the mice were drinking only during a 2 hour block. As the result of the 22 hour deprivation prior to solution presentation much of the daily intake is consumed within the first half hour (Yirmiya et al., 1988). Thus, post-ingestional experiences are not likely to play a significant role in the consumption of either solution on the first day.

On day one, C57 mice consume approximately equal amounts of MHCl and QSO_4. This suggests that MHCl and QSO_4 solutions presented had a very similar taste for C57 mice. The DBA mice actually consumed more morphine on day one, suggesting a taste preference for it. These conclusions are based on the assumption that when other information is available, such as post-ingestional effects, the mice are likely to consume the drug solutions based on taste preference alone.

Gender Effects

The gender specific effects for DBA female mice in QSO_4 consumption was unexpected (see Figure 5b). Although gender differences have been found for the consumption of palatable sweet solutions and food (Eriksson & Kiianmaa, 1971; Lieblich, et al., 1983; Valenstein et al., 1967), no gender

specific differences have be reported in the QSO_4 literature. Most research studying the effects of QSO_4 used single sex groups. Forgie et al. (1988) reported that gender accounted for little variance in oral opiate consumption within or between strains. The present study supports this finding (see Figure 5b). However, there is a significant gender effect for DBA mice. Female DBA QSO_4 consumption was significantly higher than that of the male DBAs ($p<.0001$). No gender differences were seen in the C57s' or DBAs' MHCl consumption, or in C57 QSO_4 consumption. There were no significant interactions between gender and treatment ($p<.25$).

Conclusions (Experiment One)

Naltrexone, by reducing or removing the post-ingestional effects of MHCl, caused C57 mice to increase their intake of MHCl and DBAs to decrease their consumption of QSO_4. It appears that both strains of mice are consuming the solutions based on aversive post-ingestional effects, and not because of predispositional (Horowitz et al., 1977) or taste factors.

The suitability of QSO_4 as taste-matched control for MHCl is neither supported nor refuted by this research. There are differences between the C57 and DBA pattern of consumption on both the first day (although not significant) and on the average six day consumption. Neurochemical differences in the response to morphine are likely to account for the overall drinking patterns within both strains.

The effects of MHCl are likely to be less aversive to C57 mice, considering their behavioral responses to MHCl administration (running response and weak analgesia). In addition, the lethal dose for MHCl in C57 mice is considerably higher than in DBA mice (Moskowitz et al., 1985). All told, increases in MHCl consumption following NAL treatment seem to be best explained by a decrease in morphine's aversive post-ingestional effects.

EXPERIMENT TWO

Methods

This study looked for taste sensitivity differences between DBA and C57 mice using a conditioned taste aversion (CTA) paradigm (Garcia, Hankins & Rusiniak, 1974; Green & Garcia, 1971;

Palmerio, Rusiniak & Garcia, 1980). By use of the CTA technique, it was hoped to determine the absolute taste threshold for bitterness in male and female DBA and C57 mice. The same animals that participated in Experiment One also participated in this experiment. They were tended and housed in the same manner as they were in Experiment One.

Following a period of 20 days which had elapsed between experiments, a 5-day water consumption baseline was recorded to ensure that there were no differences in mean intake. All neurological changes resulting from chronic NAL administration (for example, up-regulation) have been shown to have dissipated in mice and rats by 14 days post-administration (Bardo, Bhatnagar & Gebhart, 1983; Bardo & Miller, 1984; Chang, Lutfy, Sierra & Yoburn, 1991).

The mice were maintained on the same drinking schedule as in Experiment One. On the first day of Experiment Two, after 22 hours of water deprivation, half the mice received two hours of free access to a single bottle containing MHCl (.3 mg/ml) while the other half received a bottle containing .06 mg/ml of $QS0_4$. All solutions were prepared with distilled water. Thirty minutes after the presentation of the fluid, each mouse was removed from its cage, weighed and quickly given an injection (IP) of a .15M solution of lithium chloride (LiCl) (.19 mg/kg). After injection, the mice were returned to their home cages. The lithium chloride constituted the unconditioned stimulus (UCS) to produce the unconditioned response (UCR) of nausea. The tastant, either MHCl or $QS0_4$ was thus paired with the nausea created by the LiCl (UCS), making the tastant a conditioned stimulus (CS). The desired conditioned response (CR) was the avoidance of the tastant.

On the following day, the mice were given a two-bottle choice test between their respective CS (either MHCl or $QS0_4$) and distilled water. The concentrations were equivalent to those used during the conditioning phase. Two hours following the presentation, the bottles were removed and weighed. This procedure was repeated for a second day, alternating bottle positions. The two day period served as a control for chance occurrences or side preference. A new active solution, diluted by 50%, was mixed and paired with water every second day until the animals began to consume approximately 50% of their fluids

in the form of the solution containing the CS. The CS to water intake ratio reached 50% by the sixteenth day. At this point the mice were drinking 1.56% (dilution by 50% eight times) of the original concentration. The starting concentration was presented a second time at the end of this experiment to check for strain-specific differences in extinction.

Results (Experiment Two)

Data were entered into and transformed by Microsoft Excel and statistics performed by BMDP. The two daily scores obtained for each mouse at each solution concentration were averaged. The two-day averaged consumption scores were then expressed per unit of body weight for each mouse. Average bottle spillage was calculated to be .13 grams per day with a .14 gram standard deviation. Spillage was not deducted from their consumption scores for the same reasons explained in Experiment One. Both a consumption by weight score and a proportion of the test solution by total consumption was calculated and analyzed. A repeated measures MANOVA was used with each tested concentration being treated as a dependent variable (DV).

Overall, there were 8 DVs because the maximum concentration solution was run both at the beginning and the end of the test period. The concentrations tested were as follows: 100%, 50%, 25%, 12.5%, 6.25%, 3%, 1.5%, and 100% (of original strength). The 100% concentration were 0.3 mg/ml of MHCl or 0.06 mg/ml of $QS0_4$, respectively. There were no significant differences in baseline water consumption among the groups once intake was expressed per unit of body weight.

When LiCl was used to produce a CTA to either MHCl or $QS0_4$, it was thought the illness would result in a generalized avoidance of the CS that would persist until the bitter taste it could no longer be detected. This would allow us to compare absolute taste sensitivity between the mouse strains without the influence of post-ingestional effects.

Unfortunately, in order for this procedure to have been successful in determining absolute thresholds, there would need to have been a stronger generalization of the CTA to the more dilute

solutions. Despite the CTA failing to produce the kind of all-or-nothing avoidance needed to establish an absolute threshold, a number of results are still worth reporting.

When the overall transformed data were analyzed, the only significant difference found was an interaction between concentration and strain ($F(1,224) = p<.0392$). When each concentration was analyzed as a single dependent measure (collapsing across the two tastants) it was found that with 50% of the original concentration, consumption by C57 and DBA mice differed significantly (see Figure 10). At this concentration (.15 mg/ml of MHCl and .03 mg/ml of QSO_4), DBA mice consume significantly more test solution than C57s ($F(1, 32) = 4.04, p<.05$).

When the original taste solutions were tested for a second time at the end of the experiment, DBA mice consumed more solution than the C57s, however this difference only approached significance ($F(1, 32) = p<.08$).

Examining proportional intake data, there was a significant difference across concentration ($F(1, 32) = p<.0000$), a significant interaction of strain by concentration ($F(1, 32) = p<.0068$) and a significant interaction between concentration and flavorant ($F(1, 32) = p<.05$). The p-values reported here have been adjusted using the Huynh-Feldt correction (Huynh & Feldt, 1976).

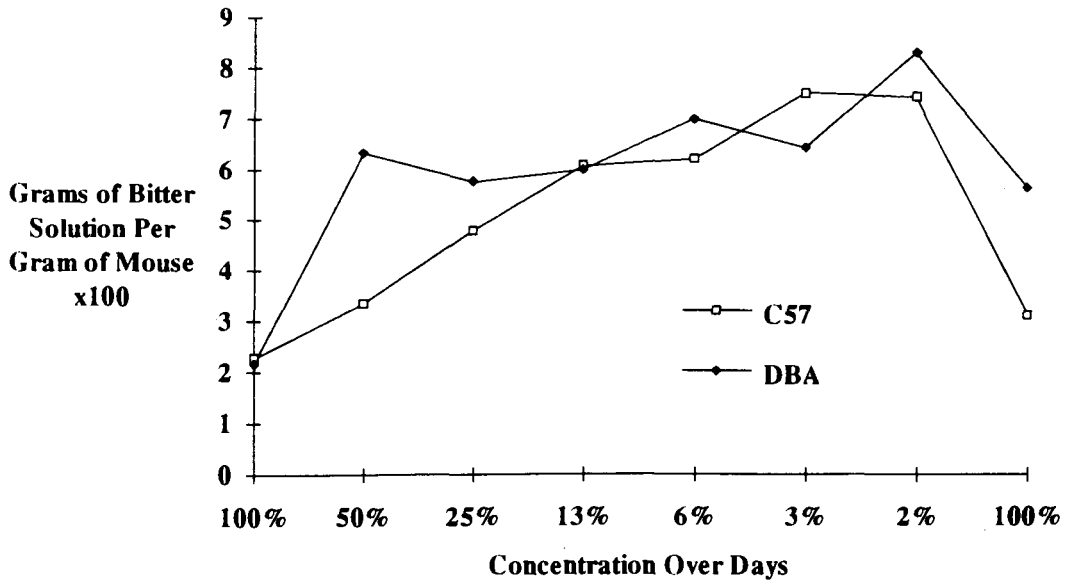

Figure 10. Consumption of the conditioned stimuli across various concentrations of solution. Each data point represents a two day average.

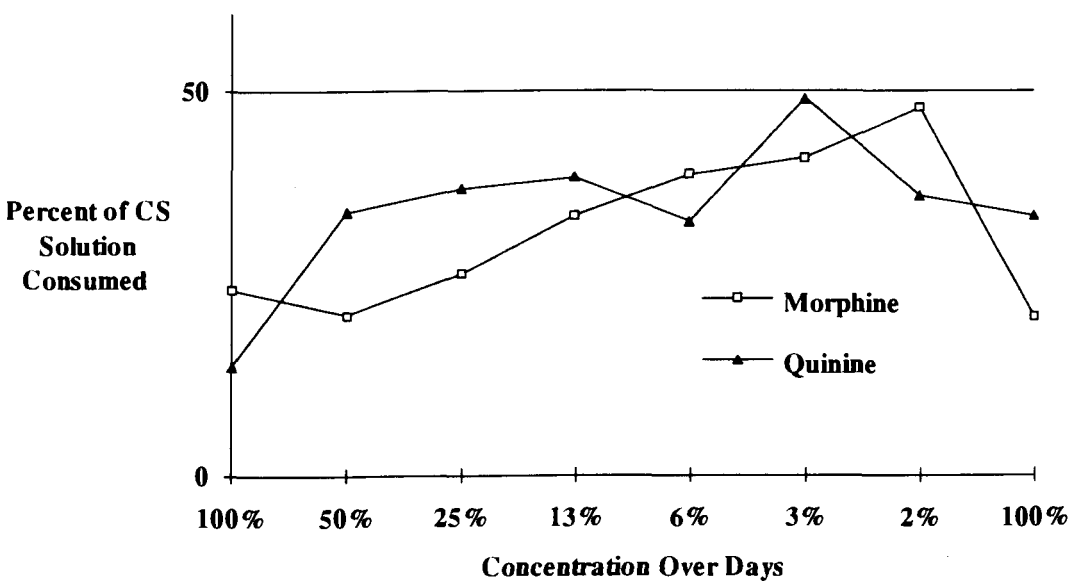

Figure 11. Proportion of total fluids comprised by conditioned stimuli across descending doses. Each data point represents a two day average at the specified solution strength..

Discussion (Experiment Two)

This experiment was conducted in hopes of determining the absolute taste threshold for MHCl and QSO_4 for C57 and DBA mice. This was attempted because doubts had been raised about the adequacy of matching of the taste of drug and control solutions in previous research. If differences exist in taste acuity, with respect to strain, gender or solution, the CTA procedure might have provided additional explanations for differing opiate self-ingestion patterns in C57 and DBA mice.

A second purpose for Experiment Two was to look for possible strain differences in CTA learning. Although the CTA learning failed to generalize to more dilute solutions, a number of the results do provide some indication of differences between C57 and DBA mice. The most informative result is that C57 mice appear to be more resistant to extinction than DBA mice (see Figure 10). The time lapse between the initial conditioning and the reinstatement of the 100% concentration was 12 days. The avoidance of the CS after 12 days was approximately equal to that of their initial avoidance in the C57 strain (collapsed across solution). The DBAs consumed nearly double the amount of solution compared to their initial 100% solution consumption (see Figure 10). Other researchers have also noted differences in learning and retention between these strains of mice (for review see Oliverio et al., 1984).

The CTA to QSO_4 was greater than to MHCl on the first days of the experiment. The MHCl CTA was retained to a greater extent than that of QSO_4. Endogenous opiates, on which these strains have been found to differ, have been implicated in memory storage and retrieval of aversive events (Castellano & Puglisi-Allegra, 1983; Oliverio et al., 1984).

Overall, this experiment provides an indication of potential differences that may exist in CTA learning in C57 and DBA mice. Ingram (1982) found no learning differences between C57 and DBA mice when using LiCl as an UCS when the CS was saccharin. However, in the present experiment, MHCl may have a strain-dependent effect on memory (Castellano & Puglisi-Allegra, 1983). For example, MHCl may impair DBAs' retention ability, and improve C57s'. In addition, higher doses of opiates administered prior to CTA learning can interfere with LiCl's (UCS) ability to produce a CR (Yirmiya, Lieblich, Liebeskind & Garcia 1988).

CTA can be used to further investigate differences in these strains of mice. For example, if MHCl and $QS0_4$ produce aversive effects in these animals, they could be used as a UCS themselves. This would provide valuable information on important relations between these substances and consumption regimes. If MHCl produced a significant conditioned avoidance in the DBA mice while not affecting the C57 mice, and the reverse held true for C57 mice, insights into their particular drinking patterns could be gained. CTA learning can provide a good indication of the aversiveness of a stimulus. In hindsight, it seems taste sensitivity could be better evaluated with repeated re-conditioning with LiCl at each concentration of interest or, better yet, with a between subjects design where each group was tested with only one stimulus concentration. This would probably overcome the lack of stimulus generalization found in the present experiment.

EXPERIMENT THREE

Experiment Three was conducted to study differences in the effects of an opiate blocker on voluntary saccharin consumption in DBA and C57 mice. Consumption of sweet solution or food has been found to interact with endogenous opiate systems (Bergmann, et al., 1985; Dum, Gramsch & Herz, 1983; Lieblich, Yirmiya & Liebeskind, 1991). It is reasoned that the hedonic pleasures produced by sapid solutions can be blocked by opiate antagonists. If C57 and DBA mice have differences in their endogenous opiate systems, it seemed reasonable to predict that voluntary consumption of sapid solutions may be differentially affected by naltrexone (Apfelbaum & Mandenoff, 1981).

Methods (Experiment Three)

The procedures in Experiment Three were similar to those used in the previous two experiments. Sixty-two subjects were run in this study. The mice were divided into 4 treatment groups and 4 control groups. Forty of the mice were from the earlier experiments, 22 were naive. The mice used in the prior

research and the naive mice were mixed so that both were represented in all groups. All groups consisted of eight subjects each, except for C57 male and female control groups which contained seven. Fifty days elapsed between the second and third experiments. Ad lib food and water was available during this time. The mice were again eased into a two hour per day drinking regimen over a 5 day period (as described in Experiment One).

Each mouse was removed from its cage and carried to an adjoining room to be weighed. After receiving a subcutaneous injection of naltrexone (NAL) (.2 mg/kg), the mice were returned to their home cages. All manipulations took place under dim red light. Fifteen minutes after the NAL injection, two weighed drinking bottles were randomly placed through the top of the cages. The bottles contained either a saccharin solution (30mM) or distilled water. After two hours, the bottles were removed and weighed to allow daily consumption to be calculated. This procedure was repeated for 4 days.

Results (Experiment 3)

This experiment was conducted to investigate the effects of opiate blockers on voluntary saccharin consumption. The data were transformed and analyzed in the same manner as in the previous two experiments. Three sets of analyses are reported, including: absolute saccharin consumption, saccharin consumed as a proportion of total fluid intake, and a log transformation of the average scores for both water and saccharin solution. All data sets were again corrected for body weight differences. The logarithmic transformation was performed because of the abnormally small F-score indicating a need for such transformation (personal communication, R. Koopman, 1992). All probabilities reported here which involve a repeated measures factor have been adjusted using the Huynh-Feldt method (1976). No significant differences were found between the naive mice and previously used mice.

When saccharin data were analyzed alone, significant strain ($F(1, 54) = 18.13$, $p<.001$), treatment ($F(1, 54) = 27.47$, $p<.0000$), strain by treatment ($p<.0000$), days ($F(1, 162) = 37.01$, $p<.0000$) and days by gender effects ($F(1, 162) = 4.76$, $p<.0154$) were found (see Figures 12 & 13). C57 saccharin

consumption was unchanged by the NAL treatment, however the DBAs' consumption was greatly reduced (see Figure 12).

Using proportional consumption scores, significant strain ($F(1, 54) = 63.32$, $p<.0000$), sex ($F(1, 54) = 12.20$, $p<.001$), treatment ($F(1, 54) = 48.87$, $p<.0000$) effects were found. In addition, significant strain by treatment ($F(1, 154) = 42.20$, $p<.0000$) and strain by gender by treatment interactions ($F(1, 54) = 4.47$, $p<.0391$) were found (see Figures 14 & 15). Significant effects for days ($F(3, 162) = 7.91$, $p<.0003$), days by strain ($F(3, 162) = 5.34$, $p<.0038$), and days by treatment ($F(3, 162) = 5.83$, $p<.0023$) were also obtained (see Figures 16 & 17).

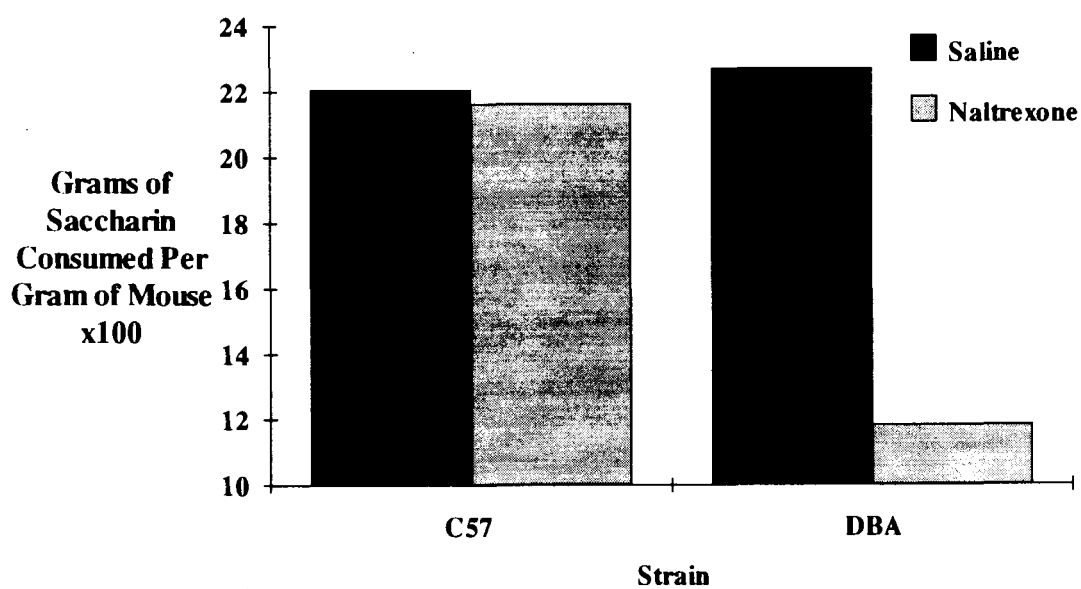

Figure 12. Saccharin consumption in C57 and DBA mice treated with either NAL or SAL for Experiment Three.

The saccharin consumption patterns within the male and female control groups were not different between the strains (see Figure 14 and 15). The C57 control group did not differ from the treatment group. The DBA control group consumed significantly more saccharin than the treatment group. The male DBA mice treated with NAL consumed, on average, 20% less saccharin and female DBA mice consumed close to 30% less saccharin than their control groups (see Figure 15).

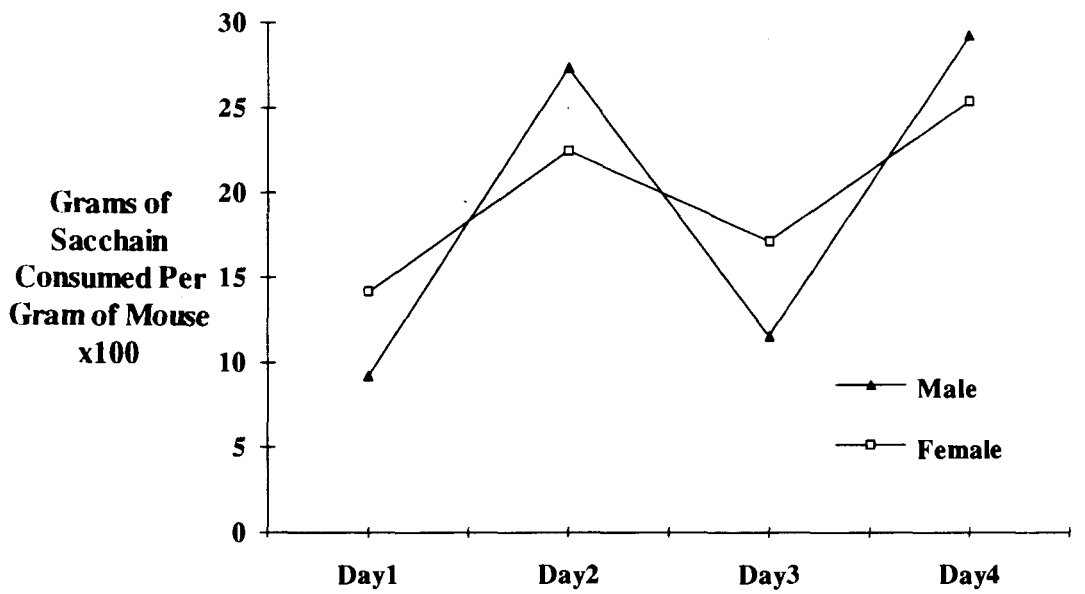

Figure 13. Saccharin consumption by gender over days, for Experiment Three.

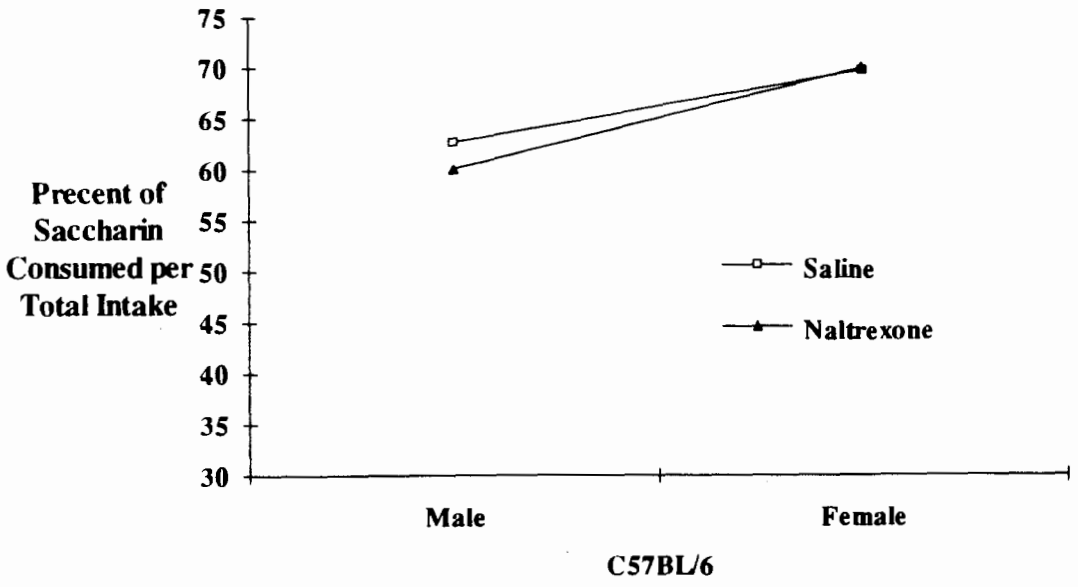

Figure 14. Consumption by gender in C57 mice in Experiment Three

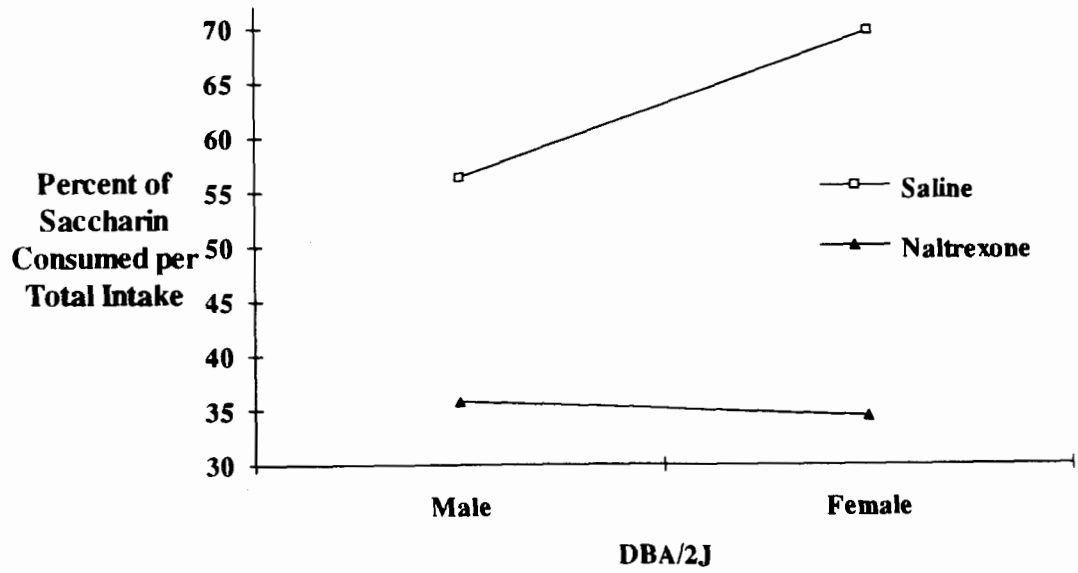

Figure 15. Consumption by gender for DBA mice in Experiment Three

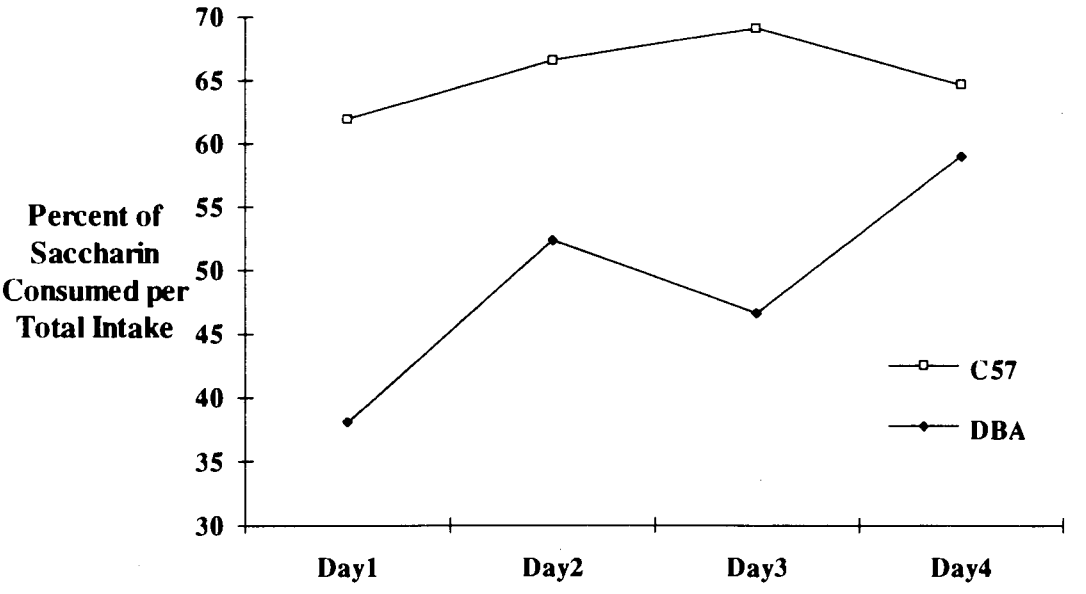

Figure 16. Strain difference in saccharin consumed across days for Experiment Three.

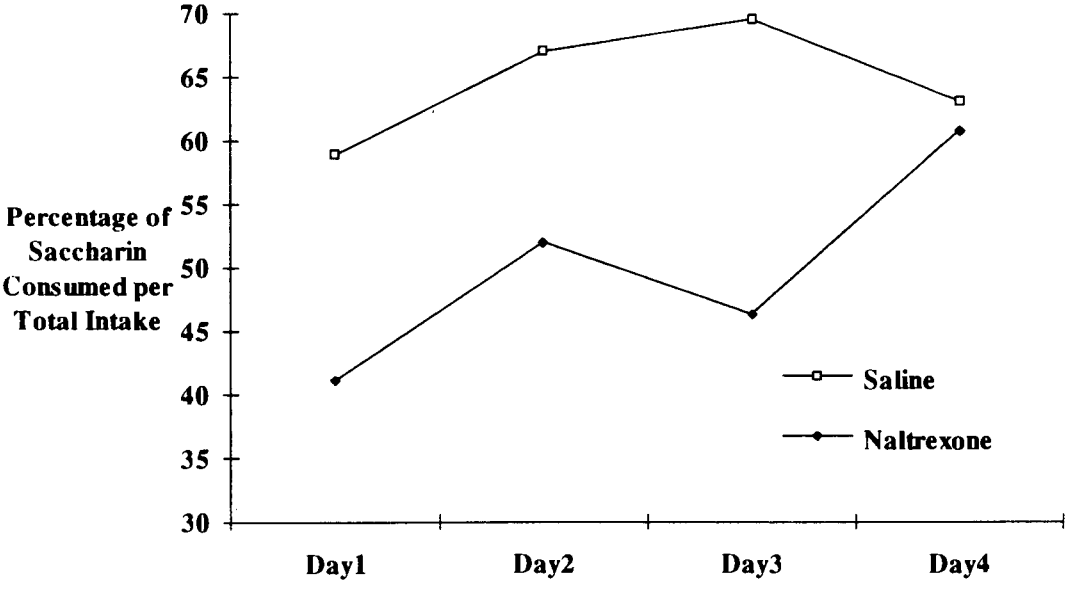

Figure 17. Treatment effects on saccharin consumed across days for experiment three.

When the averaged log water and saccharin consumption were analyzed together as two separate DVs, significant interactions between solution, strain and treatment ($F(3, 162) = 26.16$, $p<.0000$) and solution, gender and treatment ($F(3, 162) = 4.13$, $p<.0471$) emerged. For the solution by strain by treatment interaction, NAL caused a reversal in water consumption patterns across the strains. Naltrexone decreased the water consumption in the C57 strain while increasing it in the DBA strain. Saccharin consumption in the C57 mice was unaffected by the NAL treatment, whereas in the DBA mice, NAL significantly decreased saccharin consumption (see Figures 18 & 19). No significant effects on total fluid consumption were found in either strain.

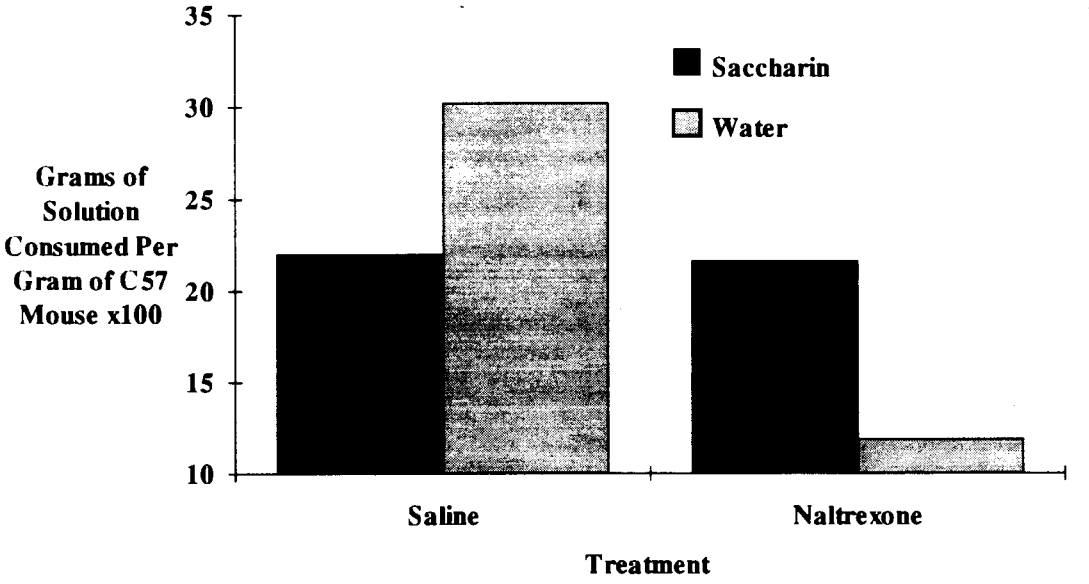

Figure 18. Saccharin vs. water consumption in NAL and SAL treated C57 mice. Three way interaction between strain, solution and treatment (see also Figure 19).

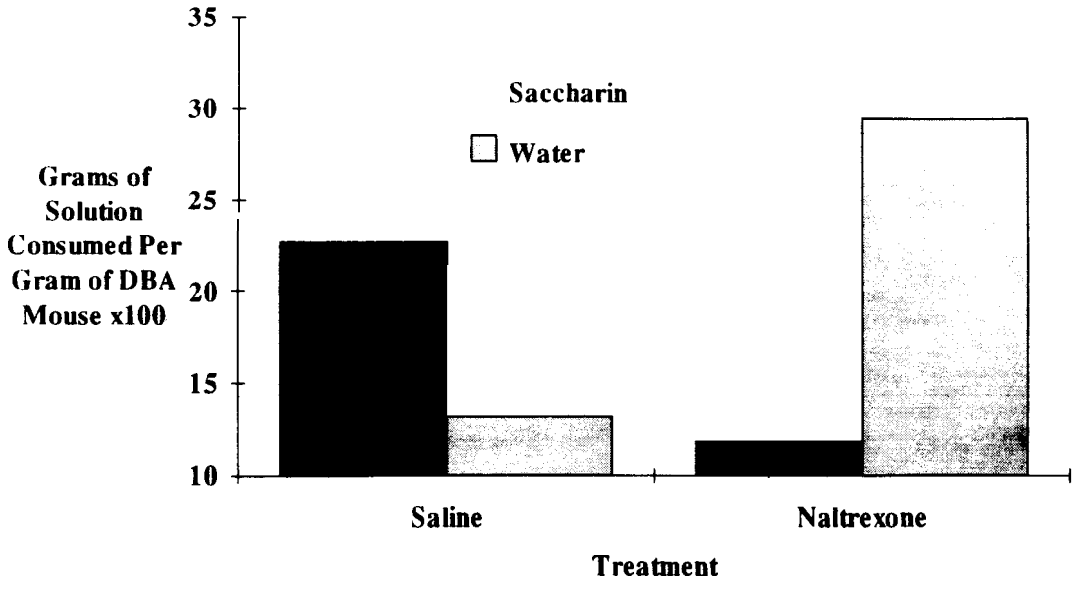

Figure 19. Saccharin vs. water consumption in NAL and SAL treated DBA mice. Three way interaction between strain, solution and treatment.

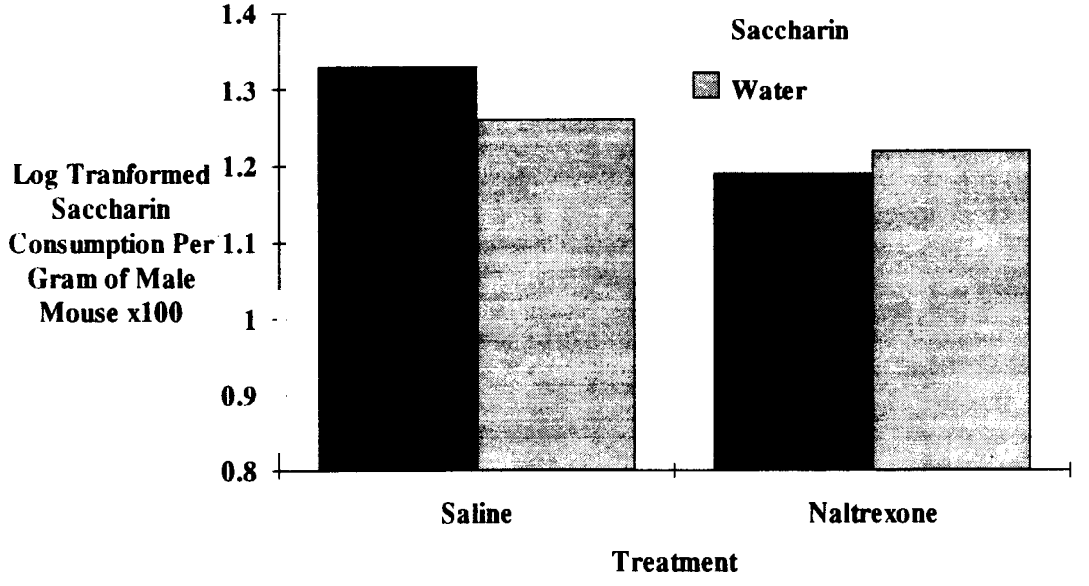

Figure 20. Saccharin and water consumption by male mice treated with NAL or SAL. Three way interaction between gender, solution and treatment (see also Figure 21).

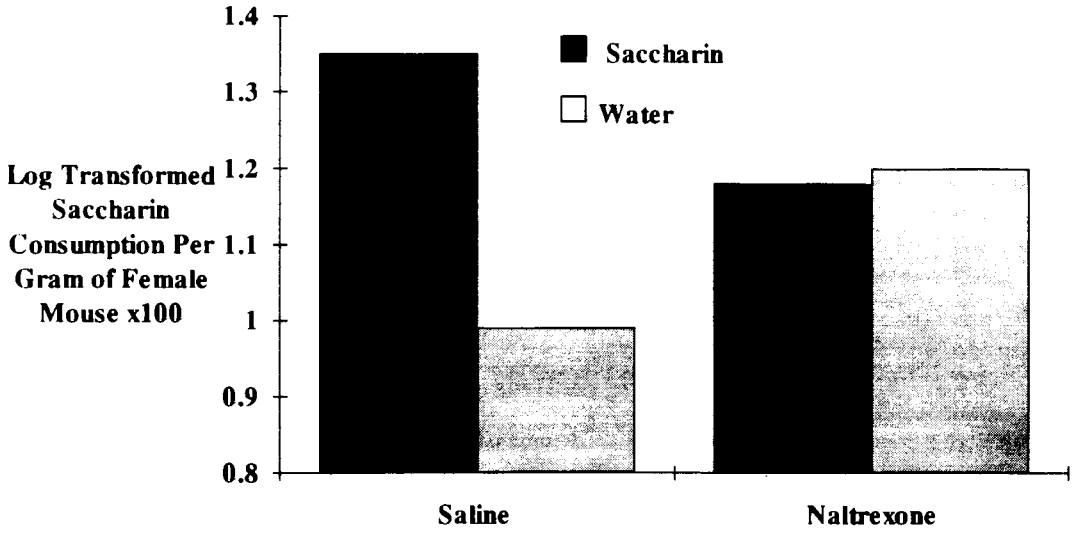

Figure 21. Saccharin and water consumption by female mice treated with NAL or SAL. Three way interaction between gender, solution and treatment (see also Figure 20)

In the second three-way interaction, between solution, gender and treatment (see Figures 20 & 21), female mice were affected by the NAL treatment more than the male mice. The female mice administered NAL significantly increased their water consumption while decreasing their saccharin intake. The male mice were similarly affected by the NAL, however, the effect was much less dramatic. In the female SAL treated mice, saccharin consumption is higher than the water consumption (see Figure 21). The male SAL-treated mice consumed only slightly more saccharin than water. This drinking pattern is the opposite of that seen in the NAL treated male mice (see Figure 20). Injected with NAL, the male mice consumed slightly more water than saccharin.

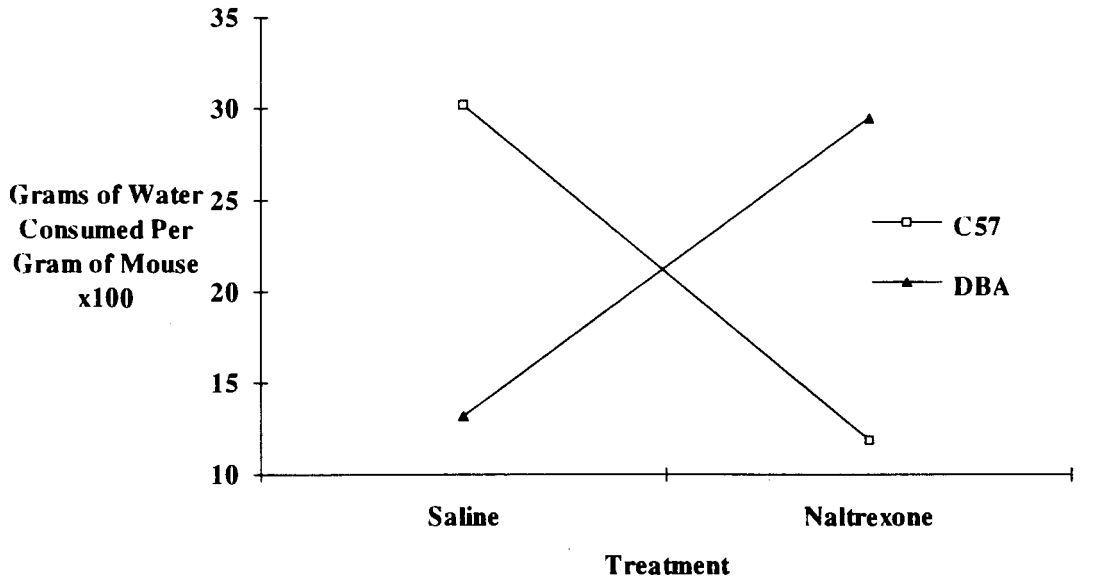

Figure 22. Water consumption in C57 and DBA mice treated with either NAL or SAL.

When average water consumption was analyzed separately, a significant strain by treatment interaction emerged ($F(3, 162) = 4.23$, $p<.04$). Naltrexone had opposite effects on the two strains of mice. In the C57 strain, water consumption was decreased by the NAL treatment, while in the DBA strain it was increased (see Figure 22).

Discussion (Experiment Three)

Overall, C57s' saccharin consumption was unaffected to by the NAL treatment, whereas the DBAs' saccharin consumption was decreased (see Figure 12). Yirmiya et al. (1988) investigated the effects of NAL on saccharin consumption in C57 and CXBK mice. Yirmiya et al. (1988) chose the CXBK mice because they are deficient in opiate receptors (Baran, Schuster, Eleftheriou & Bailey, 1975). Experiment Three adopted both the dose of NAL used and the most desirable saccharin concentration found by Yirmiya et al. (1988). The duration of their experiment was also replicated. Prior to any

manipulations, Yirmiya et al. (1988) found that the CXBK mice had a reduced saccharin preference compared to C57 mice. This initial difference in saccharin consumption was suggested to be evidence for the endogenous opiate systems involvement in the consumption of highly palatable sweet solutions (i.e., in the pleasurableness/reward value of sweet sensations). In the present study, the SAL-treated C57 and DBA mice did not differ in overall saccharin consumption as compared to the NAL-administration groups (Figure 12). Following the same logic as Yirmiya et al. (1988), this would indicate that C57 and DBA mice do not differ in opiate receptor densities. However, this is not supported by either the literature or the present experiments. For the C57 mice, saccharin consumption is unaffected by the NAL treatment, however, water consumption is decreased (see Figure 18). When the proportional data is examined, NAL decreases the proportion of saccharin to water consumed by C57 mice (see Figures 16 & 17). However, this effect appears to be the result of an increase in water consumption and not due to a change in saccharin consumption. Yirmiya et al. (1988) report only proportional data and thus may have reached a misleading conclusion. Naltrexone has been noted to affect water intake (see Sanger, 1981).

In DBA mice, both water and saccharin consumption were dramatically affected by NAL treatment (see Figure 19). DBA mice treated with NAL consumed 20% (males) to 30% (females) less saccharin than those treated with SAL. This pattern is opposite to that of the SAL-treated DBA mice. Naltrexone may produce this effect by altering mechanisms responsible for water intake or the reward associated with saccharin consumption. For example, saccharin consumption may change only as a result of changes in water intake control. This is unlikely based on the well-documented relationship between saccharin consumption and endogenous opiates (see Lieblich, Yirmiya, & Liebeskind 1991; Rockwood & Reid, 1982)

Yirmiya et al. (1988) report that NAL significantly reduced the overall preference for the saccharin solutions at the concentrations of 9, 30 and 60 mM. The present research indicates that C57 mice were not affected by the NAL treatment at the concentration of saccharin found to produce the largest effect for Yirmiya et al. (1988). If NAL affects water intake primarily, changes in the proportion

of saccharin would result. Yirmiya et al. (1988) did not report enough information to clarify this issue. In addition, they failed to report any significant differences between the C57 and the CXBK strains.

Although NAL failed to influence saccharin consumption in C57 mice, it did decrease water consumption (see Figure 18). In the DBA mice, NAL caused an inversion of the drinking patterns for both water and saccharin (see Figure 19). Whether this reversal is the result of NAL effects on water intake or saccharin intake remains unknown. The dramatic difference between C57 and DBA mice in response to NAL does provide support for endorphinergic involvement in fluid consumption (water and saccharin). Strain differences in the action of NAL on consumption of saccharin likely resulted from differences in the number and location of opiate binding sites. C57 mice, having more opiate receptor sites (Bardo, Neisewander, & Ennis, 1988), may need a higher dose of NAL in order to successfully reduce the consumption of saccharin. DBA mice, having fewer opiate receptors than C57 mice are likely to need smaller amounts of NAL to affect saccharin consumption. This explanation is questionable however, because no changes at all were seen in the C57 mice. These differences also could also have possibly resulted from differences in receptor types, opiate receptor density or opiate receptor location.

Strain-specific response to the naltrexone treatment were not expected for water consumption. For the C57 mice, the NAL treatment decreased the amount of water consumed, whereas it increased the amount of water consumed by DBA mice. Yirmiya et al. (1988) reported no significant differences in water consumption resulting from the NAL treatment. The differences in water consumption may be an indirect result of the treatment effect on saccharin consumption. However, in the C57 strain water consumption changed as a result of treatment without reciprocal changes in saccharin consumption.

Gender differences were also found. The effects of NAL on water and saccharin consumption in males were considerably less than on females (see Figures 20 & 21). However, the pattern of results was similar. In female mice, NAL increased water consumption while decreasing saccharin consumption in approximately equal proportions. In male mice, NAL caused a similar decrease in saccharin consumption; however water consumption was relatively unaffected. Gender differences in the response

to opiates and opiate antagonists have been reported though they tend to be somewhat inconsistent across studies (Eriksson & Kiianmaa, 1971; Lieblich, et al, 1983).

Differences between the Yirmiya et al. (1988) study and the present one may be accounted for by different measuring methods. If NAL affected water consumption in Yirmiya et al.'s (1988) report, it may have looked like saccharin consumption was affected when in fact it remained unchanged. Further research is necessary to understand fully the endogenous opiate systems involvement in saccharin and water consumption. With the continued use of these strains, valuable information may be gained into genetic differences in endogenous opiate systems.

General Discussion

The effects on taste of dampening the endogenous opiate system with naltrexone (NAL) were studied in Experiments One and Three. Experiment Two was conducted in an attempt to determine the absolute taste threshold for bitterness (morphine and quinine) for C57 and DBA mice. These strains of mice were used in these experiments because of the extensive literature on genetic differences in their endogenous opiate systems (Bardo et al., 1988; Oliverio et al., 1984) and because of their use in drug self-administration research (Forgie et al., 1988; Horowitz et al., 1977).

Experiment One was conducted to validate the use of quinine as a taste-matched control for morphine. In past research (Forgie & Beyerstein, submitted), reviewers asked for further evidence that a MHCl solution mixed at .3 mg/ml was equivalent in taste to the control a QSO_4 solution mixed at the concentration of .06 mg/ml for these two strains of mice. Only by using the costly and time consuming methods of taste psychophysics could this question be answered. In a different approach to answer the comparablitity of taste issue, NAL was used to block the post-ingestional effects of MHCl, thereby causing taste factors to play a more central role in determining consumption.

It has been argued here and elsewhere that the consumption of QSO_4 produces aversive post-ingestional effects in many species of rodents (Aravich & Sclarani, 1980; Heyback and Boyle, 1982;

Nijdam, 1990). The exact nature of the post-ingestional costs of QSO_4 consumption are unknown in these two strains of mice. Assuming they are not negligible, treating the mice with NAL would reduce the cost of MHCl consumption, possibly explaining the increased consumption by the DBAs. Although there is some evidence that NAL may alter taste and fluid consumption (Aravich & Sclarani, 1980; Gartside & Laycock, 1987; Kratz & Levitsky, 1978; Kratz, Levitsky & Lustick, 1978a, 1978b; Mandenoff, Fumeron, Apfelbaum & Margules, 1982; Ostrowski, Foley, Lind, & Reid, 1980), it was argued above that post-ingestional effects of MHCl and QSO_4 likely govern chronic consumption in both strains.

In C57 mice, there is both behavioral and neuropharmacological evidence that morphine consumption is somewhat pleasurable. In response to opiate administration, C57 mice become hyperactive, and experience little of morphine's analgesic effects. In addition, studies have isolated opiate receptors in dopaminergic reward pathways in the mesolimbic system (Cabib, Puglisi-Allegra & Oliverio, 1991; Gysling & Wang, 1983). Given that naltrexone is a powerful opiate antagonist, the rewarding quality of opiate consumption should have resulted in decreased in morphine consumption.

The expected decrease in voluntary morphine consumption was not seen in our experiments. The increased consumption of morphine could be explained if quinine has aversive post-ingestional effects. Because of this potential for aversive post-ingestional effects of quinine, it is not possible to evaluate at this time the validity of quinine as a taste-match control solution for morphine. It is my belief that validating quinine as a comparably tasting control for morphine is unnecessary based on what we know about condition taste avoidance theory. Taste factors are consistently secondary to the more salient post-ingestional factors (Aravich & Sclarani, 1980).

In Experiment Two, LiCl was used in a CTA paradigm to condition an avoidance to either a MHCl or QSO_4 solution. The intent was to use avoidance thresholds for these two tastants as a measure of their respective absolute sensory thresholds. The avoidance of the tastant was also necessary in order to avoid possible confounding effects of MHCl and QSO_4 intake. Morphine and QSO_4 have been shown to

interfere with both taste and fluid consumption, and therefore represent a potential confound. After conditioning to a single tastant, water and the tastant were present in a two-bottle choice test.

Despite the initial success of conditioning, it failed to generalize completely to more dilute solutions. The incomplete generalization resulted in some consumption of the tastant, making threshold determination difficult. In retrospect, taste avoidance conditioning should have taken place prior to the presentation of each new concentration. Neither strain of mouse consumed a lot of the CS solution until they were exposed to very small concentrations (2-3% of maximum solution concentration). At these low concentrations, the effects of consuming either MHCl or QSO_4 on taste is likely minimal to non-existent. The possibility exists that the absence of the desired avoidance resulted from the mice failing to discriminate, as opposed to failing to generalize the CTA. However, if this were the case, a ratio of 50:50 tastant to water would be expected. This was not the case. As the concentration of the tastant was lowered, its consumption increased.

Both strains of mice were consuming approximately 50% of the tastant at a concentration of 2% of the maximum dose (i.e., dilution by 50%, six times). No differences were found between the strains for solution consumption except at the first dilution (50% original dose). At this concentration DBA mice consumed significantly more CS than C57 mice. When the original CS (100%) was reinstated, C57 mice also showed a greater resistance to extinction than the DBA mice. There appears to be little difference between C57 and DBA mice in CTA to MHCl and QSO_4. Due to the inconclusiveness of the results in this experiment, little can be said for certain concerning absolute taste threshold for bitterness in these strains. On the other hand, there seems to be little reason to think that the taste of these two substances for these strains is greatly different either.

In Experiment Three, the effects of NAL on sapid solution consumption was examined. Based on the literature, it was hypothesized that mice which have more opiate receptors would consume greater amounts of sapid solution than mice with fewer opiate receptors (Yirmiya et al., 1988). However, no

differences between C57 and DBA mice in voluntary saccharin consumption were found despite these reported differences in opiate receptor densities (Moskowitz et al., 1985).

When treated with NAL, C57s' saccharin consumption was unaffected, whereas DBAs' saccharin consumption was significantly reduced (see Figure 12). This relationship can also be seen by examining the difference by gender (see Figures 14 & 15). A difference in the effect of NAL on water consumption was also seen (see Figure 22). This strain specific effect of NAL on water consumption has not been reported previously. In the C57 strain, water consumption was significantly reduced, whereas in the DBA mice water consumption was increased. The cause of this strain specific effect of NAL is unknown. The effects of NAL on both water and saccharin consumption alone should be further explored in these two strains of mice. Gender differences were also noted with larger changes in saccharin consumption in female mice. This is consistent with previous work on gender differences in sweet preference in rats (Valenstein, Kakolewski & Cox, 1967).

The endogenous opiate system is likely involved in control of both water and saccharin consumption. As expected, differences in saccharin consumption between C57 and DBA mice were not found in the SAL treated groups. Because C57 and DBA mice differ in opiate receptor densities, this result suggests that overall differences in receptor densities cannot account for the difference in voluntary saccharin consumption. The differences in proportional saccharin consumption found by Yirmiya et al. (1985) in C57 mice treated with NAL likely the result of changing water consumption patterns and not a direct effect of naltrexone on saccharin consumption.

Experiment Three provides evidence that the endogenous opiate system is involved in the control of fluid intake. However, because NAL affected both water and saccharin consumption, it is unknown how much of the change was due to each of these factors. Strain specific effects of NAL provide a strong indication that the C57 and DBA strains of mice have different endogenous opiate systems. The precise involvement of opiates in fluid consumption control is not well understood. Further exploration of strain-

specific differences in drinking control between these two strains may provide clues to understanding of disorders where the endorphin system has been implicated (e.g., addiction, obesity, and pain).

The exact role of endogenous opiates in the control of saccharin consumption remains uncertain; however, differences between these strains of mice may provide future insights into various regulatory mechanisms (e.g., those controlling food intake). With further investigation, the role of the endorphins in control of sapid solution consumption may be elucidated. The reward value of sweet sensations is an obvious factor to be explored in this connection.

The use of QSO_4 as a control for MHCl is not strongly refuted or supported by the present research. Saline-treated C57 and DBA groups differentially consume QSO_4, indicating that the use of QSO_4 as a control is less than optimal. Based on a review of the literature, however, both QSO_4 and MHCl produce unfavorable post-ingestional effects (Heyback & Boyle, 1982). The post-ingestional aversive aftereffects for QSO_4 seems to be roughly equal between strains. If so, the variation between C57s and DBAs is likely accounted for by different physiological and neurochemical reactions to MHCl.

The effects of taste on ingestion are likely minimal when post-ingestional effects are known to an animal. Animals who consume fluid or food based solely on taste would be disadvantaged in an evolutionary sense. Conditioned taste aversion research has demonstrated that initially palatable foods or liquids, if paired with nausea, will be avoided in the future (Klein, 1987). The difference in taste between MHCl and QSO_4 is not likely to explain or even contribute to the observed difference between C57 and DBA strains with the exception of the initial consumption pattern. Most research conducted in the SFU Drug Studies Lab is chronic, therefore, the minimal effects of initial taste preference are likely to be transcended by more important differences produced by the post-ingestional effects of each substance. In retrospect, the time and energy spent on the examination of the initial taste differences between MHCl and QSO_4 could have been better spent on researching differences in endogenous opiate systems. It is therefore the conclusion of this thesis that the issue of differences in initial taste preferences be placed to rest.

Conclusions, Limitations & Future Recommendations

Further research is necessary to substantiate the dominant role of post-ingestional effects over taste preference in MHCl-QSO_4 choice situations. Possible routes of investigation are paradigms similar to that used by Aravich & Sclafani (1980). Experiment One could be repeated, including a QSO_4 pre-exposure period. In addition, sucrose octa-acetate might also be added as a third choice or replacement for quinine in much the same manner as Forgie et al. (1988) added etonitazene. These methods may provide insight into consumption motivation. A third alternative and perhaps the easiest possibility includes monitoring initial consumption patterns in naive mice before the post-ingestional effects of either MHCl or QSO_4 can be experienced. This pattern could then be compared with chronic consumption patterns.

Elucidation of the strain-specific effects of NAL on water consumption in these two strains of mice is necessary before reaching a conclusion about its effects on voluntary saccharin consumption. Naltrexone has been shown to affect fluid consumption in some species of animals, while not in others (Arrok, Czirr & Reid, 1988; Holtzman, 1975; Ostrowski et al., 1980; Sanger, 1981; Stapleton, et al., 1979)

A possible limitation of the present research is the fact that the mice, after the initial study were not naive. Despite extended lengths of time between the experiments, potential carry-over effects may have influenced the results. Great care was taken to counterbalance (mix) the groups prior to each experiment, and no differences were seen between the new and naive subjects.

If Experiment Two were to be repeated, conditioning with the use of LiCl, should occur for each concentration tested, possibly in a between subjects design. Conditioning to the CS did not transfer to the more dilute solutions as well as had been hoped. Conditioning a new group at each level would have allowed better assessment of taste thresholds in these strains of mice.

This thesis, like most research projects, produced more questions than it answered. The research presented here does however provide some initial insights into the problems produced by with taste confounds in oral self-administration studies.

Appendix A

Studies that Examine Differences Between C57BL/6 and DBA2J Mice

Locomotion Response Differences

Badiani, Castellano & Oliverio, 1991	oxotremorine induced locomotion in chronically depressed animals	chronic stress results in sensitization of the cholinergic system in DBAs as seen by the depressant effects of oxotremorine, this is not seen in C57s
Castellano, Filibeck & Oliverio, 1976	locomotor activity under the influences to heroin, amphetamine, strychnine & ethanol	heroin produced running fits in C57s, not in DBAs amphetamines increased locomotion only in C57s heroin + amphetamines (or ethanol) increased activity in DBAs heroin and strychnine increased activity in C57s only
Cabib & Puglisi-Allegra, 1985	effects of APO on activity and climbing behavior	dose dependent reduction of locomotion activity in DBAs which C57s showed a biphasic activity curve, and an increase in climbing behavior
Castellano, Llovera & Oliverio, 1975	morphine induced running following septal lesions or brain amines modification	septal lesions antagonized analgesia in both strains while pharmacological manipulation of brain catecholamines did not effect running
Castellano, Pavone & Sansone, 1985	effects of opioid benodiazepine tifluadom (k-opiate receptor agonist) on analgesia and locomotion	exerted an depressant effect on locomotion in both DBAs and C57s, no differences in analgesic qualities found
Castellano & Puglisi-Allegra, 1982	assessed naloxone, naltrexone on locomotor behavior	naltrexone decreased activity at a lower dose, but not a a higher dose in C57 mice DBAs needed less than C57s to suppress activity
Castellano, 1981	effects of FK-33824 opioid peptides	induced dose dependent decrease in locomotor activity in DBAs in C57s it induced a biphasic effects
Filibeck, Castellano & Oliverio, 1981	combined effects of D-amino acid & morphine on analgesia & running activity	cross-tolerance between D-amino and morphine seen in DBAs to analgesia while the stimulating effects of opiates in C57s were not modified

Locomotor Response Differences (cont.)

Frigeni, Bruno, Carenzi, Racagni & Santini, 1978	analgesia and motor activity in response to ICV administration of morphine and enkephalins	C57s showed an increase in activity one hour after injections, while DBA showed no increase DBA more sensitive to the analgesic effects
Helmeste & Seeman, 1982	relationship between D_2 receptors and locomotor response to amphetamine	C57s show a greater locomotor reaction to amphetamines & slightly greater D_2 receptor binding
Kempf, Greilsamer, Mack & Mandel, 1974	correlated locomotor differences with difference in brain amines	found high correlation of noradrenalin in the pons medulla correlated with activity difference
McClearn, 1968 Rogers, 1972	voluntary alcohol consumption	C57s show a marked preference for 10% ethanol solution, whereas DBAs showed consistent avoidance
Michael-Titus, Dourmap, Caline, Costentin & Schwartz, 1989	effects of acetorphan (enkephalinease inhibitor) on activity & nociception	induced excitatory behavior in C57 and no analgesia effects DBAs its increased activity too a much greater extent and possessed analgesic capabilities
Oliverio, 1975	EEG, behavior and analgesia in normal and septal lesioned mice in response to morphine	C57s showed sharp increase in activity, EEG showed high amplitude slow wave(similar to sleep), and no analgesia DBAs showed no locomotor, or EEG correlates, but a large analgesic response
Oliverio & Castellano, 1974; Brase, 1986	sensitivity and tolerance to morphine and heroin	C57 showed the highest running and lowest analgesic response
Puglisi-Allegra, Carletti & Cabib, 1990	Ly 171,555 (D2 agonist) on locomotor behavior	induced dose-dependent catalepsy with the C57's response significantly lower than DBAs
Puglisi-Allegra, Oliverio & Mandel, 1982	effects of Ly 171,555 on activity and defensive behavior (D2 agonist)	
Sansone, Ammerasari-Teule, Renzi & Oliverio, 1981	effects of APO on locomotor activity	APO exerts a biphasic (U) effects in C57s while in DBA APO simply decreased activity
Sansone & Oliverio, 1980	effects of chlordiazepoxide & morphine	enhanced locomotion in C57s and counteracts the effects of morphine in DBAs
Siegfried, Alleva & Oliverio, 1980	tested for spontaneous locomotion response to tactile stimulation and cortical EEG desynchronization	C57s were characterized by high basal activity, reactivity and lower electrophysical arousal DBAs showed lower activity, reactivity, but higher cortical arousability

Neurochemical Differences

Cabib, Kempf, Schleef, Oliverio & Puglisi-Allegra, 1988	measured DOPAC, DA, HVA/DA & 3MT levels in the caudate putamen & accumbens septi in response to immobilization stress	immobilization stress did not produce any effects on dopaminergic metabolism in the frontal cortex in C57 s, while DBAs it caused a time dependent effects on HVA/DA ratio
Cabib & Puglisi-Allegra, 1988	apomorphine induced climbing	increased climbing behavior (biphasic fashion) seen in the C57 but not the DBAs, however a dose dependent reduction in locomotor behavior seen in the DBAs
Durkin, Ayad, Ebel. Mandel, 1977	ACh turnover rates in the brain	ACh turnover rates were significantly higher in DBA mice
Ebel, Hermetet & Mandel, 1973 Mandel, Ebel, Hermetet, Bovet & Bovet 1973	measured choline acetyltransferase and acetyltransferase	demonstrated higher activity in the DBA mice
Frischknecht, Siegfried, Riggio & Waser, 1983	comparison of three long lasting opiate antagonists which preferably bind to different opiate receptor subtypes	b-CNA blocked MHCl analgesia in DBA and motor activity in C57s, indicating that different opiate receptors are mediating different behavior in these mice
Helmeste & Seeman, 1982	relationship between D_2 receptors and locomotor response to amphetamine	C57s show a greater locomotor reaction to amphetamines & slightly greater D_2 receptor binding
Kempf, Greilsamer, Mack & Mandel, 1974	correlated locomotor differences with difference in brain amines	found high correlation of noradrenalin in the pons medulla correlated with activity difference
Lace, Schneider & Hartline, 1986	ethanol sensitivity of calcium taken up by a depolarized dependent process	no difference found between the strains
Puglisi-Allegra, Kempf & Cabib, 1990	behavioral and biochemical analysis of the effects of stress on DA functioning	C57s characterized by hypersensitivity of the mesolimbic DA autoreceptors and a dramatic increase in D1/D2 receptor ratio DBAs show hyposensitivity DA autoreceptors and no change in D1/D2 receptors
Racagni, G. F., Bruno, E., Iuliano, E. & Paoletti, R. 1979	measured turnover rates of acetylcholine (TR_{ACh}) in striatum & limbic system	morphine decrease TR_{ACh} in the limbic system of DBAs (not in the striatum) where as in C57 it on decreased TR_{ACh} in the striatum

Neurochemical Differences (cont.)

Reggiani, Battaini, Kobayashi, Spano & Trabucchi, 1980	number of m and d opiate receptors	C57 mice show a higher number of striatial and other brain areas d receptors DBAs have more m receptors
Schwab, Brückner, Castellano, Oliverio & Biesold, 1990	immunochemical measurement of cholinergic neuronal densities	DBA characterized by higher densities of cholinergic neurons in the nucleus basalis meynert, stria terminalis, hippocampus & temporal cortex
Schwab, Brückner, Castellano, Oliverio & Biesold, 1990	brain cholinergic organization using immunohistochemistry	C57s characterized by lower cholinergic densities
Tunnicliff, Wimer & Wimer, 1973	relationship between neurotransmitters, metabolism and behavior	DBAs have a greater level of AChE, while C57s show greater MAO, and COMT
Vetulani, Sansone, Oliverio, 1982	behavioral effects of APO	inhibitory effects and gnawing of high doses in DBAs (reversible with primozide), not seen in C57s
Wimer, Norman & Ebeleftheriou, 1973	stress differences in norepinephine (NE) and serotonin (5-HT)	increase in NE in the C57s but not the DBAs in the amygdala 5-HT levels in the frontal cortex increased and decreased in the amygdala to a much greater extent in C57s
Belknap & Deutsch, 1982	sensitivity to ethanol, T-butanol, 1,2-propranediol & phenobarbital on a number of different measures	DBAs consistently more neurosensitive than C57s on the grid floor & ambulatory ataxia but no difference in drug induced hypothermia
Frigeni, Bruno, Carenzi, & Racagni, 1981	development of tolerance to the analgesic qualities of morphine & and enkephalins	C57 mice developed tolerance to quicker than DBA mice DBA more sensitive than C57 mice
Marley, Witkins & Goldberg, 1991	repeated cocaine administration	C57s most susceptible to acute cocaine induced seizures, however desensitization occurred most rapidly in C57s when challenged with a high dose of cocaine
Middaugh, Zemp, 1976	effects of single and repeated methadone on brain monoamines and activity	methadone attenuated activity in DBAs, however this effect disappeared after 7 daily injections in C57 methadone increased activity (more pronounced with time) norepinephine was elevated in DBAs

Neurochemical Differences (cont.)

Moore & Kakihana, 1978 Tabakoff & Ritzman, 1979 Grieve et al., 1979	tolerance to alcohol	tolerance develops at a much greater rate in C57s
Oliverio & Castellano, 1974	sensitivity and tolerance to morphine and heroin	C57 showed the highest running and lowest analgesic response
Schneider, Trizil, & D' Andrea 1974 Kakihana, et al., 1966	alcohol sensitivity	DBAs are found to be more sensitive to alcohol
Sheppard, Albersheim & McClearn, 1968	voluntary alcohol consumption and ADH activity	C57 have a much greater alcohol oxidizing capacity, and a higher level of voluntary consumption

Voluntary Drug Consumption Differences

Forgie & Beyerstein, submitted	voluntary choice consumption of quinine , morphine & etonitazene	C57 consistently preferred morphine, whereas DBAs consumed equal amounts of all three solutions
Forgie, Beyerstein & Alexander, 1988	voluntary consumption of morphine etonitazene & quinine	C57s show a significant preference for morphine , while DBAs appear to have no preference
Horowitz, Whitney, Smith & Stephan, 1977	voluntary consumption of morphine in a saccharin vehicle	C57 consumed up to lethal amount of drug solution where DBA avoided it
Ramirez & Fuller, 1976	voluntary consumption of sucrose solution	C57 consumed more sucrose solution

Physiological and Behavioral Response Differences to Stress

Cabib, Puglisi-Allegra & Oliverio, 1985; Cabib & Puglisi-Allegra, 1985	dopaminergic plasticity following chronic stress	DBAs showed an increase in APO induced climbing, while C57s showed a clear cut reduction in this behavior
Castellano & Puglisi-Allegra, 1983	the effects of post-training immobilization with and without naloxone	immobilization stress improved C57s' memory and impaired DBAs', effects were naloxone reversible
Kulling, Frischknecht, Pasi, Waser & Siegfried, 1987	single and repeated aggressive confrontation on nociception and defensive behavior	DBAs showed a consistent encounter induced analgesia & escape response, while C57 showed no encounter analgesia after the 1st and 3rd encounter and changed their defensive strategy to submissive

Physiological and Behavioral Response Differences to Stress (cont.)

Puglisi-Allegra, Kempf & Cabib, 1990 Cabib & Puglisi-Allegra, 1988; 1985; Cabib et al., 1985, 1991; Cabib et al., 1984	behavioral and biochemical analysis of the effects of stress on DA functioning	C57s characterized by hypersensitivity of the mesolimbic DA autoreceptors and a dramatic increase in D1/D2 receptor ratio DBAs show hyposensitivity DA autoreceptors and no change in D1/D2 receptors
Shanks, Zalcman, Zacharko & Anisman, 1990	alterations of central norepinephine. dopamine and serotonin following acute stress	in the absence of footshock, norepinephine and dopamine was lower in C57s footshock increased both in C57s serotonin also increased in C57s
Wimer, Norman & Ebeleftheriou, 1973	stress differences in norepinephine (NE) and serotonin (5-HT)	increase in NE in the C57s but not the DBAs in the amygdala 5-HT levels in the frontal cortex increased and decreased in the amygdala to a much greater extent in C57s

Physiological and Behavioral Response Differences to Pain

Belknap & Lame, 1990	hot plate assay of nociception using initial response and paw lick response	no differences in initial response to the hot plate, but DBAs was much slower to paw lick
Castellano, Pavone & Sansone, 1985	effects of opioid benodiazepine tifluadom (k-opiate receptor agonist) on analgesia and locomotion	exerted an depressant effect on locomotion in both DBAs and C57s, no differences in analgesic qualities found
Collins & Whitney, 1978	effects of prior experience with test situation and nociception	DBAs showed little effects prior experience C57s were significantly effected by prior experience with the test situation
Frigeni, Bruno, Carenzi, Racagni & Santini, 1978	analgesia and motor activity in response to ICV administration of morphine and enkephalins	C57s showed an increase in activity one hour after injections, while DBA showed no increase DBA more sensitive to the analgesic effects
Kulling, Frischknecht, Pasi, Waser & Siegfried, 1987	single and repeated aggressive confrontation on nociception and defensive behavior	DBAs showed a consistent encounter induced analgesia & escape response, while C57 showed no encounter analgesia after the 1st and 3rd encounter and changed their defensive strategy to submissive

Physiological and Behavioral Response Differences to Pain

Michael-Titus, Dourmap, Caline, Costentin & Schwartz, 1989	effects of acetorphan (enkephalinease inhibitor) on activity & nociception	induced excitatory behavior in C57 and no analgesia effects DBAs its increased activity too a much greater extent and possessed analgesic capabilities
Oliverio, 1975	EEG, behavior and analgesia in normal and septal lesioned mice in response to morphine	C57s showed sharp increase in activity, EEG showed high amplitude slow wave(similar to sleep), and no analgesia DBAs showed no locomotor, or EEG correlates, but a large analgesic response

Learning Differences

Bovet, 1977 Oliverio et al., 1979	learning differences	C57 mice characterized by poorer learning patterns in a number of different tasks
Castellano & Pavone, 1985	dermorphine & [D-ala2-D-Leu] enkephalin ICV administration after passive avoidance training	the peptides impaired retention at low doses (5ng) and the performance of both strains high doses (50ng) impaired retention in DBAs but improved it in C57s

Learning Difference (cont.)

Castellano & Puglisi-Allegra, 1983	the effects of post-training immobilization with and without naloxone	immobilization stress improved C57s' memory and impaired DBAs', effects were naloxone reversible
Castellano, 1975, 1980, 1981	memory performance in response to opiate agonist and antagonists	opiate agonist improve C57's memory and impair DBAs opiate antagonists impairs C57s' memory and improves DBA's
Dudek & Fuller, 1978 Horowitz & Whitney, 1975	condition response to alcohol and acetaldehyde	DBAs exhibited stronger aversion to the normally preferred CS solution of saccharin when the UCS was ethanol or acetaldehyde
Ingram, 1982	used LiCl as a UCS to test for differences in CTA learning	no differences in CTA learning, however DBAs showed a greater resistance to extinction

Miscellaneous Differences

Broida & Svare, 1982	postpartum aggression	DBA mice exhibit a high level of maternal aggression when confronted with a intruder mouse, C57s do not
Ryan, 1984	EEG recording in free moving mice	brief spinal episodes seen in DBAs but not C57s which were punctuated by conspicuous bursts of high amplitude (6-7 cps)

References

Akil, H., Mayer, D. J. & Liebeskind, J. C. (1976). Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. Science, 191, 961-962.

Alexander, B. K. (1991). Peaceful Measures: Canada's Way out of the War on Drugs. Toronto: Toronto University Press.

Alexander, B. K., Beyerstein, B. L., Hadaway, P. F., & Coambs, R. B. (1981). Effect of early and later colony housing on oral ingestion on MHCl in rats. Pharmacology, Biochemistry & Behavior, 15, 571-576.

Apfelbaum, M. & Mandenoff, A. (1981). Naltrexone suppresses hyperphagia induced in rat by a highly palatable diet. Pharmacology, Biochemistry & Behavior, 15, 89-91.

Aravich, P. F. & Sclafani, A. (1980). Dietary preference behavior in rats fed bitter tasting quinine and sucrose octa-acetate adulterated diets. Physiology & Behavior, 25, 157-160.

Arrok, F., Czirr, S. & Reid, L. D. (1988). Small doses of MHCl and intake of water. Bulletin of the Psychonomic Society, 26(6), 556-558.

Badiani, A., Castellano, C. & Oliverio, A. (1991). Effects of acute and chronic stress and of genotypes on oxotremorine-induced locomotor depression of mice. Behavioral and Neural Biology, 55, 123-130.

Baran, A., Shuster, L., Eleftheriou, B. E. & Bailey, D. W. (1975). Opiate receptors in mice: Genetic Differences. Life Sciences, 17, 633-640.

Bardo, M. T., Bhatnagar, R. K. & Gebhart, G. F. (1983). Chronic naltrexone increases opiate binding in the brain and produces supersensitivity to morphine in the locus coeruleus in the rat. Brain Research, 289, 223-234.

Bardo, M. T., Neisewander, J. L. & Ennis, R. B. (1988). Chronic treatment with naltrexone enhances MHCl-stimulated dopamine neurotransmission: Neurochemical and behavioral evidence. Neuropharmacology, 27(11), 1103-1109.

Bardo, M. T., Bhatnagar, R. K. & Gebhart, G. F. (1983). Chronic naltrexone increase opiates binding in brain and produces supersensensitivity to MHCl in the locus coeruleus of the rat. Brain Research, 289, 223-234.

Bardo, M. T. & Miller, J. S. (1984). Opiate receptor supersensitivity producedby chronic naloxone treatment: Dissociation of MHCl-induced antinociception and conditioned taste aversion. Pharmacology, Biochemistry & Behavior, 21, 591-597.

Belknap, J. K.(1990). Physical dependence induced by voluntary consumption of morphine consumption. Pharmacology, Biochemistry & Behavior, 35(2), 311-315.

Belknap, J. K. & Deutsch, C. K. (1982). Differential neurosensitivity to alcohol and phenobarbital in C57BL/6J and DBA/2J mice. Behavioral Genetics, 12(3), 309-317.

Belknap, J. K. & Lame, M. (1990). Inbred strain differences in morphine induced analgesia with the hot-plate assay: A reassessment. Behavior Genetics, 20(2), 333-338.

Bergmann, F., Lieblich, I., Cohen, E., & Ganchrow, J. R. (1985). Influence of intake of sweet solutions on the analgesic effect of a low dose of MHCl in randomly bred rats. Behavior and Neural Biology, 44, 347-353.

Bhargava, H. N. (1978). Effects of methionine-enkephalin and MHCl on spontaneous locomotor activity: Antagonism by naloxone. Pharmacology, Biochemistry and Behavior, 9, 167-171.

Bovet, D., Bovet-Nitti, F. & Oliverio, A. (1969). Genetic aspects of learning and memory in mice, Science, 163, 139-149.

Brase, D. A. (1986). Unequal opiate cross-tolerance to MHCl in the locomotor-activation model in the mouse. Neuropharmacology, 25(3), 297-304.

Broida, J. & Svare, B. (1982). Postpartum aggression in C57BL/6J and DBA/2J Mice: Experiential and environmental influences. Behavioral and Neural Biology, 35, 76-83.

Cabib, S., Kempf, E., Schleef, C., Oliverio, A. & Puglisi-Allegra, S. (1988). Effects of immobilization stress on dopamine and its metabolites in different brain areas of the mouse: role of genotype and stress duration. Brain Research, 441, 153-160.

Cabib, s. & Puglisi-Allegra, S. (1985). Differential effects if apomorphine on climbing behavior and locomotor activity in three strains of mice. Pharmacology, Biochemistry and Behavior, 23, 555-557.

Cabib, S. & Puglisi-Allegra, S. (1988). A classical genetic analysis of two apomorphine-induced behaviors in the mouse. Pharmacology, Biochemistry & Behavior, 30, 143-147.

Cabib, S. & Puglisi-Allegra, S. (1991). Genotype-dependent effects of chronic stress on apomorphine-induced alteration of striatial and mesolimbic dopamine metabolism. Brain Research, 542, 91-96.

Cabib, S. Puglisi-Allegra, S. & Oliverio, A. (1984). Chronic stress enhances apomorphine-induced stereotyped behavior in mice: Involvement of endogenous opioids. Brain Research, 298, 138-140.

Cabib, S. Puglisi-Allegra, S. & Oliverio, A. (1985). A genetic analysis of stereotypy in the mouse: Dopaminergic plasticity following chronic stress. Behavioral and Neural Biology, 44, 239-248.

Castellano, C. (1981). Strain dependent effects of the enkephalin analogue FK 33-824 on locomotor activity in mice. Pharmacology, Biochemistry & Behavior, 15, 729-734.

Castellano, C. Espinet, B. & Oliverio, A. (1975). Morphine-induced running and analgesia in two strains of mice following septal lesions or modification of brain amines. Naunyn-Schmiedeberg's Archives of Pharmacol., 288, 355-370.

Castellano, C., Llovera, B. E. & Oliverio, A. (1975). Morphine induced running and analgesia in two strains of mice following septal lesions or modification of brain amines. Naunyn-Schmiedeberg's Archives in Pharmacol., 288, 355-370.

Castellano, C. & Pavone, F. (1985). Dose- and strain dependent effects of dermorphine and {D-Ala^2-D-Leu^5}Enkephalin on passive avoidance behavior in mice. Behavioral Neuroscience, 99(6), 1120-1127.

Castellano, C. & Puglisi-Allegra, S. (1982). Effects of naloxone and naltrexone on the locomotor activity in C57BL/6 and DBA/2 mice. Pharmacology, Biochemistry & Behavior, 16, 561-563.

Castellano, C. & Puglisi-Allegra S. (1983). Strain-dependent modulation of memory by stress in mice. Behavioral and Neural Biology, 38, 133-138.

Castellano, C. & Puglisi-Allegra, S. (1983). Strain-dependent modulation of memory by stress in mice. Behavioral & Neural Biology, 38(1), 133-138.

Castellano, C, Filibeck, U. & Oliverio, A. (1976). Effects of heroin, alone or in combination with other drugs, on the locomotor activity in two inbred strains of mice. Psychopharmacology, 49, 29-31.

Chang, S. J. L., Lutfy, K., Sierra, V. & Yoburn, B. C. (1991). Dissociation of opioid receptor up-regulation and functional supersensitivity. Pharmacology, Biochemistry & Behavior, 38, 853-859.

Chernov, H. I., Ambrose, F. G. & Plummer, A. J. (1968). Patterns of etonitazene solution consumption by naive rats. Archives in Pharmacodyne, 175(2), 309-318.

Collins, R. L. & Whitney, G. (1978). Genotype and test experience determine Responsiveness to Morphine. Psychopharmacology, 56, 57-60.

Cooper, S. J. (1986). Effects of the beta-carboline FG 7142 on saccharin preference and QSO_4 aversion in water-deprived rats. Neuropharmacology, 25(2), 213-216.

Cooper, S. J., Jackson, A. & Kirkham, T. C. (1985). Endorphins and food intake: Kappa Opioid Receptor Agonists and hyperphagia. Pharmacology, Biochemistry & Behavior, 23, 889-901.

Crabbe, J. C. (1986). Genetic differences in locomotor activation in mice. Pharmacology, Biochemistry & Behavior, 25, 289-292.

Davis, V. E. & Walsh, M. J. (1970). Alcohol, amines, and alkaloids: A possible biochemical basis for alcohol addiction. Science, 167, 1005-1007.

Dudek, B. C. & Fuller, J. L. (1978). Task-dependent genetic influences on behavioral response of mice (*Mus musculus*) to acetaldehyde. Journal of Comparative and Physiological Psychology, 92, 749-758.

Dum, J., Gramsch, C. & Herz, A. (1983). Activation of hypothalamic B-endorphin pools by reward induced by highly palatable food. Pharmacology, Biochemistry & Behavior, 18, 443-447.

Durkin, T., Ayad, G., Ebel, A. & Mandel, P. (1977). Regional acetylcholine turnover rates in the brains of three inbred strains of mice: Correlation with some interstrain behavioral differences. Brain Research, 136, 475-486.

Dykstra, L. A., Wharton, W. & McMillan, D. E. (1977). Antagonism of etonitazene's effects in rats and pigeons. Pharmacology, Biochemistry & Behavior, 6, 215-219.

Ebel, A., Hermetet, J. C., Mandel, P. (1973). Comparative study of acetylcholinesterase and choline acetytransferase enzyme activity in the brain of DBA and C57 mice. Nature, 242, 251-56.

Eriksson, K. & Kiianmaa, K. (1971). Genetic analysis of susceptibility to MHCl addiction in inbred mice. Annales Medicinae experimentalis et biologiae, 49, 73-78.

Eriksson, K., Pikkarainen, P. H. (1968). Differences between the sexes in voluntary alcohol consumption and liver ADH-activity in inbred strains of mice. Metabolism, 17, 1037-1042.

Eriksson, K., Pikkarainen, P. H. (1970). Strain and sex differences in voluntary alcohol consumption, liver ADH activity and aldehyde-oxidizing capacity inbred strains of mice. Japanese Journal of the Study of Alcohol, 5,10-15.

Filibeck, U. Castellano, C. & Oliverio, A. (1981). Cross-tolerance between D-amino acids and MHCl and mice. Brain Research, 212, 227-229.

Forgie, M. L. & Beyerstein, B. L. (submitted). A taste-controlled comparison of preference for morphine and etonitazene in C57BL/6 and DBA/2 mice. Psychopharmacology.

Forgie, M. L., Beyerstein, B. L. & Alexander, B. K (1988). Contribution of taste factors and gender to opioid preference in C57BL and DBA mice. Psychopharmacology, 95, 237-244.

Frigeni, V., Bruno, F., Carenzi, A. & Racagni, G. (1981). Difference in the development of tolerance to MHCl and D-ALA2-methionine-enkephalin in C57 BL/6j and DBA/2J mice. Life Sciences, 28(7), 729-736.

Frigeni, V., Bruno, F., Carenzi, A., Racagni, G. & Santini, V. (1978). Analgesia and motor activity elicited by morphine end enkephalins in two inbred strains of mice. Journal Pharm. Pharmac., 30, 310-311.

Frischknecht, H., Siegfried, B. Riggio, G. & Waser, P. G. (1983). Inhibition of MHCl-induced analgesia and locomotor activity in strains of mice: A comparison of long-acting opiate antagonists. Pharmacology, Biochemistry & Behavior, 19, 939-944.

Garcia, J., Hankins, W. G. & Rusiniak, W. (1974). Behavioral regulation of the milieu interne in man and rat. Science, 185, 824-831.

Gartside, I. B. & Laycock, J. F. (1987). Increased aversion to bitter-tasting fluids in the battleboro rat. Physiology & Behavior, 39, 571-577.

Green, K. F. & Garcia, J. (1971). Recuperation from illness: Flavor enhancement for rats. Science, 173, 749-751.

Gysling, K. & Wang, R. Y. (1983). Morphine-induced activation of A10 dopamine in rat. Brain Research, 277, 119-127.

Helmeste, D. A. & Seeman. P. (1982). Amphetamine-induced hypolocomotion in mice with more brain D2 dopamine receptors. Psychiatric Research, 7, 351-359.

Heyback J. P. & Boyle, P. C. (1982). Dietary QSO_4 reduces body weight and food intake independent of aversive taste. Physiology & Behavior, 29, 1171-1173.

Holtzman S. G. (1975). Effects of narcotic antagonists on fluid intake in the rat. Life Sciences, 16, 1465-1470.

Horowitz, G. P. & Whitney, G. (1975). Alcohol-induced condition aversion: Genotypic specificity in mice (*Mus musculus*). Journal of Comparative and Physiological Psychology, 89, 340-346.

Horowitz, G. P., Whitney, G., Smith, J. C. & Stephan, F. K. (1977). Morphine ingestion: Genetic control in mice. Psychopharmacology, 52, 119-122.

Huynh, H & Feldt, L. S. (1970). Condition under which the mean square ratios in repeated measures design have exact F-distributions. Journal of the American Statistical Association, 65, 1582-1589.

Ingram, D. K. (1982). Lithium chloride-induced taste aversion in C57BL/6J and DBA/2J mice. The Journal of General Psychology, 106, 233-249.

Kakihana, R., Brown, D. R., McClearn, G. E. & Tabershaw, I. R. (1966). Brain sensitivity to alcohol in inbred mouse strains. Science, 154, 1574-1575.

Kempf, E., Greilsamer, J., Mack, G. & Mandel, P. (1974). Correlation of behavioral differences in three strains of mice with differences in brain amines. Nature, 15, 453-455.

Klein, S. B. (1987). Learning: Principles and Applications. New York: McGraw-Hill Book Co.

Klein, T. W. & DeFries, J. C. (1970). Similar polymorphism of taste sensitivity to PTC in mice and men. Nature, 225, 555-557.

Kratz, C. M. & Levitsky, D. A. (1978). Post-ingestive effects of quinine on intake of nutritive and non-nutritive substances. Physiology & Behavior, 21, 851-854.

Kratz, C. M., Levitsky, D. A. & Lustick, S. (1978). Long term effects of QSO_4 on food intake and body weight in the rat. Physiology & Behavior, 21, 321-324.

Kratz, C. M., Levitsky, D. A. & Lustick, S. (1978). Differential effects of QSO_4 and sucrose octa acetate on food intake in the rat. Physiology & Behavior, 20, 665-667.

Kulling, P. Frischknecht, H. R., Pasi, A. Waser, P. G. & Siegfried, B. (1987). Effects of repeated as compared to single aggressive confrontation on nociception and defense behavior in C57BL/6 and DBA/2 mice. Physiology & Behavior, 39, 599-605.

Kumar, R., Steinberg, H. & Stolerman, I. P. (1968). Inducing a preference for MHCl in rats without premedication. Nature, 218, 564-565.

Lace, J. W., Schneider, C. W. & Hartline, R. A. (1986). The ethanol sensitivity of calcium taken up by a depolarization-dependent process in the mouse strains DBA and C57BL. Pharmacology, Biochemistry & Behavior, 24, 1137-1139.

Lieblich, I., Cohen, E., Ganchrow, J. R., Blass, E. M. & Bergmann, F. (1983). Morphine tolerance in genetically selected rats induced by chronically elevated saccharine intake. Science, 221, 871-873.

Lieblich, I. & Yirmiya, R. (1987). Naltrexone reverses a long term depressive effect of a toxic lithium injection on saccharin preference. Physiology & Behavior, 39, 547-550.

Lieblich, I., Yirmiya, R. & Liebeskind, J. C. (1991). Intake of and preference for sweet solution are attenuated in MHCl-withdrawn rats. Behavioral Neuroscience, 105(61), 965-970.

Mandenoff, A., Fumeron, F., Apfelbaum, M. & Margules, D. L. (1982). Endogenous opiates and energy balance. Science, 215, 1536-1537.

Marchant, E. G. (1991). Absolute and preference taste threshold comparison for morphine and quinine in C57BL/6 and DBA/2J mice. Unpublished Honors Thesis, Department of Psychology, Simon Fraser University.

Marchant, E. G., Beyerstein, B. L. & Clement, J. (1993). Voluntary opiate consumption of C57BL/6 mice in enriched and deprived environments. Unpublished manuscript, Department of Psychology, Simon Fraser University.

Marley, R. J., Witkins, J. M. & Goldberg, S. R. (1991). Genetic differences in the development of cocaine-kindled seizures. NIDA Research Monograph Series, no.6, pp. 72-73.

Maxon, S. C. & Trattner, A. (1981). Interaction of genotype and fostering in the development of behavior of DBA and C57 mice. Behavior Genetics, 11(2), 153-165.

McMillan, D. E., Leander, J. E., Wilson, T. W., Wallace, S., Fix, T., Redding, S. & Turk, R. T. (1976). Oral ingestion of narcotic analgesics by rats. Journal of Pharmacology and Experimental Therapeutics, 196(2), 269-279.

Meyers, R. D. (1966). Voluntary alcohol consumption in animals: Peripheral and intracerebral factors. Psychosomatic Medicine, 28(4), 484-497.

Michael-Titus, A., Dourmap, N., Caline, H., Costentin, J. & Schwartz, J. C. (1989). Role of endogenous enkephalins in locomotion and nociception in two inbred strains of mice (C57BL/6J and DBA/2J). Neuropharmacology, 28(2), 117-122.

Middaugh, L. D. & Zemp, J. W. (1976). Effects of methadone on activity and on brain monoamines in two strains of mice. Pharmacology, Biochemistry & Behavior, 5, 367-370.

Moore, J. A. & Kakihana, R. (1978). Ethanol-induced hypothermia in mice: Influence of genotype on development of tolerance. Life Science, 23(23), 2331-2337.

Moskowitz, A. S. & Goodman, R. R. (1985). Autoradiographic analysis of MU_1, MU_2, and delta opioid binding in the central nervous system of C57BL/6BY and CXBK (opioid receptor-deficient) mice. Brain Research, 360, 108-116.

Moskowitz, A. S., Terman, G. W., Cater, K. R., Morgan, M. J. & Liebeskind, J. C. (1985). Analgesic, locomotor and lethal effects of MHCl in the mouse: Strain Comparisons. Brain Research, 361, 46-51.

Moskowitz, A. S., Terman, G. W. & Liebeskind, J. C. (1983) Opioid and non-opioid forms of stress-induced analgesia in the mouse: Strain comparison. Society for Neuroscience Abstracts, 9, 795.

Nichols, J. R. & Hsiao, S. (1967). Addiction liability of albino rats: Breeding for quantitative differences in MHCl drinking. Science, 157, 561-563.

Nijdam, D. (1990). The effects of pre-exposure to bitter flavorants on subsequent consumption of morphine on opiate preferring and opiate avoiding mouse strains. Unpublished Honors Thesis, Department of Psychology, Simon Fraser University.

Oliverio, A. (1975). Genotype-dependent electroencephalographic, behavioral and analgesic correlates of MHCl: An analysis in normal mice and in mice with septal lesions. Brain Research, 83, 135-141.

Oliverio, A. & Castellano, C. (1975). Morphine sensitivity and tolerance: A genetic investigation in the mouse. Psychopharmacologia, 42, 219-224.

Oliverio, A. & Castellano, C. (1974a). Experience modifies MHCl-induced behavioral excitation of mice. Nature, 252, 229-230.

Oliverio, A. & Castellano, C. (1974b). Genotype-dependent sensitivity and tolerance to MHCl and heroin: Dissociation between opiate-induced running and analgesia in the mouse. Psychopharmacologia, 39, 13-22.

Oliverio, A., Castellano, C. & Puglisi-Allegra, S. (1984). Psychobiology of opioids. International Review of Neurobiology, 25, 227-337.

Ostrowski, N. L., Foley, T. L., Lind, M. D. & Reid, L. D. (1980). Naloxone reduces fluid intake: Effects of water and food deprivation. Pharmacology, Biochemistry & Behavior, 12, 431-435.

Palmerio, C. C. Rusiniak, K. W. & Garcia, J. (1980). Flavor-illness aversion: The peculiar roles of order and taste in memory for poison. Science, 208, 753-755.

Peters, R. H., Wellman, P. J., Gunion, M. W. & Luttmers, L. L. (1979). Acids and quinine as dietary adulterants. Physiology & Behavior, 22, 1055-1059.

Palfai, T. & Jankiewicz, H. (1991). Drugs and Human Behavior, New York: C Brown Publisher.

Puglisi-Allegra, S., Kempf, E. & Cabib, S. (1990). Role of genotype in the adaptation of the brain dopamine system to stress. Neuroscience & Biobehavioral Reviews, 14, 523-528.

Puglisi-Allegra, S, Oliverio, A. & Mandel, P. (1982). Effects of opiate antagonists on two kinds of aggressive behavior in mice. Aggressive Behavior, 8, 175-177.

Puglisi-Allegra, S. Carletti, P. & Cabib, S. (1990). LY 171555-induced catalepsy and defensive behavior in four strains of mice suggest the involvement of different D2 dopamine receptor systems. Pharmacology, Biochemistry & Behavior, 36, 327-331.

Racagni, G. F., Bruno, E., Iuliano, E. & Paoletti, R. (1979). Differential sensitivity to morphine-induced analgesia and motor activity in two inbred strains of mice: behavioral and biochemical correlations. Journal of Pharmacology and Experimental Therapeutics., 209, 111-116.

Ramirez, I. & Fuller, J. L. (1976). Genetic influence on water and sweetened water consumption in mice. Physiology & Behavior, 16, 163-168.

Reggiani, A., Battaini, F., Kobayashi, H., Spano, P. & Trabucchi, M. (1980). Genotype-dependent sensitivity to MHCl: Role of different opiate receptor populations. Brain Research, 189, 289-294.

Rockwood, G. A & Reid, L. D (1982). Naloxone modifies sugar-water intake in rats drinking with open gastric fistulas. Physiology & Behavior, 29, 1175-1178.

Rogers, D. A. (1966). Factors underlying differences in alcohol preference among inbred strains of mice. Psychosomatic Medicine, 28(4), 598-513.

Rogers, D. A. & McClearn, G. E. (1962). Alcohol preference of mice. In E. L. Bliss (Ed.), Roots of Behavior (pp. 49-63). New York, Hoeber-Harper.

Rosow, C. E., Miller, J. M., Pelikan, E. W. & Cochin, J. (1980). Opiates and thermoregulation in mice. 1. Agonists. Journal of Pharmacology and Experimental Therapeutics, 213(2), 273-283.

Ryan, L. J. (1984). Characterization of cortical spindles in DBA/2 and C57BL/6 inbred mice. Brain Research Bulletin, 13, 549-558.

Sanger, D. J. (1981). Endorphinergic mechanisms on the control of food and water intake. Appetite, 2, 193-208.

Sansone, M., Ammerasari-Teule, Renzi & Oliverio, A. (1981). Different effects of apomorphine on locomotor activity in C57BL/6 and DBA/2 mice. Pharmacology, Biochemistry & Behavior, 14, 741-743.

Sansone, M & Oliverio, A. (1980). Effects of chlordiazepoxide-morphine combinations on spontaneous locomotor activity in three inbred strains of mice. Archives in International Pharmacodyn. Therapeutics, 247, 71-75.

Sheppard, J. R., Albersheim, P. & McClearn, G. E. (1968). Enzyme activities and ethanol preference in mice. Biochemical Genetics, 2, 205-212.

Siegfried, B., Alleva, E & Oliverio, A. (1980). An analysis of neurophysiological and behavioral arousal in the mouse. Physiology and Behavior, 25, 421-424.

Schenk, S. & Nawiesniak, E. (1985). Chronic naltrexone treatment increases the heroin-produced facilitation of self-stimulation. Pharmacology, Biochemistry & Behavior, 22, 175-177.

Schneider, C., Trizil, P. & D' Andrea (1974). Neural tolerance in high and low ethanol selecting mouse strains. Pharmacology, Biochemistry & Behavior, 2, 549-551.

Schwab, C., Brückner, G, Castellano, C., Oliverio, A. & Biesold, D. (1990). Regional cholinergic differences in the brain of DBA/2 and C57BL/6 mice: A Morphological study during ontogeny. Dementia, 1, 74-81.

Shanks, N., Zalcman, S., Zacharko, R. M. & Anisman, H. (1991). Alterations of central norpinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. Pharmacology, Biochemistry & Behavior, 38, 69-75.

Shannon, H. E., Bemis, K. G. & Peters, S. C. (1991). Potency and efficacy of dopamine agonists in mouse strains differing in dopamine cell and receptor number. Pharmacology, Biochemistry & Behavior, 40, 103-107.

Stapleton, J. M., Ostrowski, N. L., Merriman, V. J., Lind, M. D. & Reid, L. D. (1979). Naloxone reduces fluid consumption in water-deprived and non-deprived rats. Bulletin of the Psychonomic Society, 13(4), 237-239.

Stolerman, I. P. (1985). Motivational effects of opioids: Evidence on the role of endorphins in mediating reward or aversion. Pharmacology, Biochemistry & Behavior, 23, 877-881.

Stolerman, I. P. & Kumar, R. (1970). Preferences for morphine in rats: validation of an experimental model of dependence. Psychopharmacologia, 17, 137-144.

Tabachnick, B. G. & Fidell, L. S. (1989). Using Multivariate Statistics. New York: Harper & Row.

Tabakoff, B. & Ritzman, R. F. (1979). Acute tolerance in inbred and selected lines of mice. Drug & Alcohol Dependence, 4(1-2), 87-90.

Tempel, A., Gardner, E. L. & Zukin, R. S. (1984). Neurochemical and functional correlates of naltrexone-induced opiate receptor up-regulation. Journal of Pharmacology and Experimental Therapeutics, 232(2), 439-444.

Tunnicliff, G., Wimer, C. C. & Wimer, R. E. (1973). Relationship between neurotransmitter metabolism and behavior in seven inbred strain of mice, Brain Research, 61, 428-434.

Valenstein, E. S., Kakolewski, J. W. & Cox, V. C. (1967). Sex differences in taste preference for glucose and saccharin solution. Science, 156, 642-943.

Vetulani, J., Sansone, M. & Oliverio, A. (1982). Analysis of the difference in the behavioral effects of apomorphine in C57/6 and DBA/2 mice. Pharmacology, Biochemistry & Behavior, 17, 967-971.

Way , E. L. & Glasgow, C. (1978). Recent developments in MHCl analgesia: Tolerance and dependence. In M. A. Lipton, A. DiMascio & K. F. Killam (Eds.). Psychopharmacology: A Generation of Progress. New York: Raven Press.

Whitney, G. & Horowitz, G. P. (1978). Morphine preference of alcohol-avoiding and alcohol-preferring C57BL mice. Behavior Genetics, 8(2), 177-182.

Whitney, G., McClearn, G. E. & DeFries, J. C. (1970). heritability of alcohol preference in laboratory mice and rats. Journal of Heredity, 61, 165-169.

Wimer, R. E., Norman R. & Eleftheriou, B. (1973). Serotonin levels in hippocampus: Striking variations associated with mouse strain and treatment. Brain Research, 63, 397-401.

Yanagita, T. (1970). Self administration studies on various dependence-producing agents in monkeys. University of Michigan Medical Center Journal, 36 (pt. 2) 216-224.

Yirmiya, R. & Taylor, A. (1989). Genetic differences in opiate receptor concentration and sensitivity to ethanol's effects. Pharmacology, Biochemistry & Behavior, 33, 793-796.

Yirmiya, R., Lieblich, I. & Liebeskind, J. C. (1988). Reduced saccharin preference in CXBK (opioid receptor-deficient) mice. Brain Research, 438, 339-342.

Yirmiya, R., Lieblich, I., Liebeskind, J. C. & Garcia, J. (1988). Lithium chloride produces illness-induced analgesia. Bulletin of the Psychonomic Society, 26(3), 261-262.

Zellner, D. A. & Berridge, K. C. (1985). Rats learn to like the taste of morphine. Behavioral Neuroscience, 99(2), 290-300.

Zukin, S. R., Sugarman, J. R., Fitz-Syage, M. L., Gardner, E. L., Zukin, S. R. & Gintzler, A. R. (1982). Naltrexone-induced opiate receptor supersensitivity. Brain Research, 245, 285-292.