GENETIC STRAIN DIFFERENCES IN THE OPIATE ABSTINENCE SYNDROME

A Thesis

Presented to

the Faculty of the Department of Psychology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

By

Larry P. Gonzalez

August, 1975

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ABSTRACT

Five strains of rats, two outbred (Sprague-Dawley and Holtzman) and three inbred strains (Buffalo, Lewis, and Fisher), received subcutaneous implants of either morphine or placebo pellets. The naloxone-precipitated opiate abstinence syndrome was observed after one, two, or three days of exposure to the implanted pellet. The presence or frequency of occurrence of fourteen abstinence signs was monitored both before and after a subcutaneous injection of naloxone hydrochloride (.4 mg/Kg). Inter-strain differences were present both pre- and post-naloxone injection in animals receiving either morphine or placebo. Differences were most evident in the frequency of occurrence of escape attempts, wet dog shakes, teeth chattering episodes, and activity counts.

Following injection of naloxone, the profile of abstinence responses changed depending on the length of exposure to the implanted morphine pellet. This change was statistically significant in four of the strains observed; no significant effect of length of exposure was found in the response profile of Holtzman rats.

For those strains showing a significant length-of-exposure effect, a discriminant function based on the abstinence syndrome profile was obtained. This function describes differences along the length-of-exposure dimension and may prove useful as a quantitative assessment of the abstinence syndrome.

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CHAPTER I

STATEMENT OF THE PROBLEM

The technique of subcutaneous implantation of morphine pellets as a means of producing physical dependence on morphine has been used widely in studies of the mechanisms of morphine action (Cicero and Meyer, 1973). Several investigators have assessed the degree of physical dependence established with this method by precipitating the withdrawal syndrome with morphine antagonists (Blasig, Herz, Reinhold, and Zieglgansberger, 1973; Cicero and Meyer, 1973; and, Wei, 1973).

Comparisons between studies have been difficult because of the many different withdrawal signs observed by various investigators. Attempts to develop rating systems to quantify the severity of abstinence have had only limited success. In addition, large species differences are apparent and some strain differences have been noted (Way, Loh, and Shen, 1969; and, Tilson and Rech, 1974).

In an attempt to compensate for such inter-study variability, Blasig *et al.* (1973) argued that the abstinence syndrome should be characterized by a profile of withdrawal signs to optimize quantification, and reported such a profile in the laboratory rat. They have not, however, suggested a means of quantifying changes in this multivariate profile. Even with such extensive profiles, inter-study comparisons are also complicated by the use of different rat strains.

Since large differences in growth rates, spontaneous activity

levels, and metabolic rates have been reported for various strains of rats (Segal, Kuczenski, and Mandell, 1972; and, Morrison, 1973), important strain differences might be expected in the observed withdrawal syndrome.

This study was designed to examine differences in the profile of withdrawal from morphine in several strains of rats and in rats exposed to implanted morphine pellets for different time periods. Because the opiate abstinence syndrome is best represented as a profile of responses, multivariate statistical techniques were selected as most appropriate for the analysis of differences in the abstinence syndrome.

A further objective of this thesis was the determination of a rating system such that the abstinence score obtained by applying the system to a multivariate profile of withdrawal responses would serve as a significant indicator of the length of morphine exposure prior to withdrawal. Multivariate discriminant function analysis was chosen to describe differences in the response profiles of animals receiving morphine-pellet implants for different lengths of time. This analysis enabled determination of the relative statistical contribution of each variable to discrimination along the "length-of-exposure" dimension.

CHAPTER 2

REVIEW OF THE LITERATURE

Following chronic administration of morphine, abrupt withdrawal from the drug regimen or administration of a narcotic antagonist results in a variety of physiological and behavioral responses. These responses are referred to collectively as the opiate abstinence syndrome, and their occurrence defines a condition of physical dependence upon morphine.

Martin, Wikler, Eades, and Pescor (1963) have studied the development of physical dependence on opiates and the abstinence syndrome following abrupt withdrawal of morphine in the rat. Others (Kaymakcalan and Woods, 1956; Hanna, 1960; Buckett, 1964; and, Blasig et al., 1973) have studied withdrawal precipitated by the administration of a narcotic antagonist.

A variety of responses have been reported to occur during withdrawal in rats. Himmelsbach, Gerlach, and Stanton (1935) found that "temperamental hyperirritability," defined as the number of struggling episodes in restrained rats, increased during withdrawal from morphine. Fichtenberg (1951) reported increased aggression and loss in body weight after abrupt withdrawal from a regimen of morphine sulphate injections. Mercier and Sestier (1954) found a decrease in the performance of a trained, discriminatory-drinking test in rats during withdrawal. These animals

also showed increased motor activity, aggressiveness, vocalizations, and piloerection.

Following abrupt withdrawal of morphine in rats, Martin et al. (1963) observed an increase in the frequency of spontaneously occurring episodes of repetitive shaking of the head and trunk ("wet dog shakes"), loss in body weight, decrease in body temperature and metabolic rate, diarrhea, increase in exploring activities, and hostile behavior when handled. Halbach and Eddy (1963), reviewing several studies of opiate abstinence in rats, noted reports of increased intestinal activity (increased defecation and diarrhea), increased motor activity followed by sedation, vocalization, teeth chattering and chewing, hypersensitivity to noise, ptosis, stretching (writhing), and scratching.

Other responses observed in rats during morphine withdrawal include attempts to escape from the observation chamber (Francis and Schneider, 1971), eye twitching, rhinorrhea, lacrimation, and penile erections (Blasig et al., 1973). Adler, Lin, Smith, Tresky, and Gildenberg (1974) report a lowered seizure threshold in response to flurothyl administration during morphine withdrawal. Puri and Lal (1974) report a lowering of the threshold for pain-induced aggression in morphine-withdrawn rats.

Many attempts have been made to quantify the intensity of the abstinence syndrome and thus, the degree of physical dependence. Himmelsbach et al. (1935) found that the increase in "temperamental hyperirritability" during withdrawal from morphine was proportional

to the length of exposure to morphine.

Investigators have reported correlations between amount of morphine exposure (either as the length of time during which animals remained on a drug regimen or as the maximum dose tolerated) and resulting loss in body weight during withdrawal (Hosoya, 1959; Akera and Brody, 1968; Goode, 1971; and Adler et al., 1974). Similar correlations of morphine exposure have been found with wet dog shakes (Wikler and Pescor, 1967; Lorenzetti and Sancilio, 1970; and, Cicero and Myer, 1973), withdrawal jumping (Francis and Schneider, 1971), and writhing (Lorenzetti and Sancilio, 1970). Tilson, Rech, and Stolman (1973) suggest the use of an hyperalgesia response as a quantitative measure of morphine dependence.

The use of single response measures in the assessment of physical dependence has been questioned because of the possible dissociation of a single response from the total abstinence syndrome (Collier, Francis, and Schneider, 1972; Blasig et al., 1973; Wei, 1973; and, Wei, Loh, and Way, 1973). For example, Grumbach (1969) reports that atrophine intensifies abstinence. Observing several abstinence signs, Collier et al. (1972) found that atropine increased the incidence of some abstinence signs, decreased some, and had no effect on others. Diethyldithiocarbamate has been reported to decrease the incidence of wet dog shakes and diarrhea during morphine withdrawal, but it increases hypothermia and has no effect upon other behavioral measures of the abstinence syndrome (Schwartz and Eidelberg, 1970).

Wei (1973) reports that food and water deprivation can prevent the loss of body weight during withdrawal while not affecting other behavioral signs of abstinence.

Recent attempts to quantify the severity of the abstinence syndrome use rating systems which grade the occurrence of several responses. These rating systems have not been uniform and there is disagreement over the importance of different abstinence signs.

Wet dog shakes, for example, receive a low score in Buckett's rating system (Buckett, 1964) and in a rating system used by Lorenzetti and Sancilio (1970), but a high score in that reported by Wei (1973). Kerr and Pozuelo (1971) found wet dog shakes too unreliable a measure to include in their grading system. Writhing, the highest rating response in both Buckett's system (1964) and that of Lorenzetti and Sancilio (1970), is not mentioned at all in reports by Wei (Wei, 1973; and, Wei, Loh, and Way, 1973).

In addition to the differences between investigators in the responses used to assess the abstinence syndrome, inter-study comparisons are further complicated by the use of different rat strains. Strain differences in response to various drugs have been noted in several animal species (Brown, 1964; and, Oliverio, 1974).

Several investigators have reported differences in inbred strains of mice in the analgesic response to morphine and in single measures of the abstinence syndrome (Way, Loh, and Shen, 1969; Maruyama, Hayashi, Smits, and Takemori, 1971; and, Gebhart and Mitchell, 1973). There are few reports of differences in the responses of various rat strains

to psychotropic drugs.

Different rat strains are reported to differ in growth rate, feed utilization efficiency, and metabolic rate (Kleiber and Cole, 1950; Schemmel, Mickelsen, and Motawi, 1972; and, Morrison, 1973). Several investigators report rat strain differences in the activity of hepatic microsomal enzymes responsible for drug metabolism in Wistar, Holtzman, Sprague-Dawley, and Long-Evans rats (Furner, Gram, and Stitzel, 1969; and, Jori, Pescador, and Pugliatti, 1971). Rosecrans and Schechter (1972) report rat strain differences in brain serotonin metabolism in Sprague-Dawley and Fisher rats. Segal et al. (1972) found strain differences between BUF, LEW, F-344, ACI, BN, and Sprague-Dawley rats in brain levels of tyrosine hydroxylase, the rate limiting enzyme in the catecholamine biosynthetic pathway (Levitt, Spector, Sjoerdsma, and Udenfriend, 1965). Differences in brain concentrations of serotonin and norepinephrine have been found in a single strain of rats obtained from different suppliers (Miller, Cox, and Maickel, 1968).

Tilson and Rech (1974) report differences in morphine-produced analgesia in Sprague-Dawley and Fisher strains of rats and in the effect of p-chlorophenylalanine, an inhibitor of serotonin synthesis, on morphine analgesia and withdrawal in these rat strains.

This thesis reports differences between rat strains in response to withdrawal from morphine. Multivariate data analysis is used to determine strain differences in the profile of withdrawal responses. This analysis is also used in the formulation of a rating system for quantitative assessment of the abstinence syndrome.

CHAPTER III

METHOD

Subjects

Five strains of rats were used in this study. These consisted of three inbred strains (BUF, Buffalo; LEW, Lewis; and F-344, Fisher) and two outbred strains (Holtzman and Sprague-Dawley).

The inbred strains were obtained from Microbiological Associates, Inc., Walkersville, Maryland. Holtzman outbred rats were obtained from Holtzman, Inc., Madison, Wisconsin. The Sprague-Dawley rats used in this study were obtained from Simonsen Laboratories in Gilroy, California.

All subjects were males, 90 to 120 days old, weighing 200 to 300 g. Animals were maintained for at least five days in the same conditions of environment, diet, and daily handling before any experimental treatment. They were housed in indivdual cages with free access to food and water for the duration of the experiment.

Procedure

Pellet preparation and implantation. Morphine pellets were prepared according to a procedure similar to that reported by Gibson and Tinstad (1970). Morphine pellets contained 75 mg. morphine base, 75 mg. microcrystalline cellulose (Avicel), .75 mg. fumed silicon dioxide (Cab-I-Sil) and 1.5 mg. of magnesium stearate. Tablets were cylindrical, 3 mm. thick, with a diameter of 5 mm.

Placebo pellets were prepared to the same weight by replacing the morphine base with an equivalent amount of the excipient.

For implantation, the animals were lightly anesthetized with ether for 30 to 45 sec. A two cm. incision was made on the dorsal surface of the neck just posterior to the ears on the midline. A pellet (morphine or placebo) was inserted subcutaneously one or two cm. from the incision and the incision closed with surgical clips.

Precipitation of withdrawal. The development of physical dependence was assessed in terms of the withdrawal syndrome precipitated by injection of the morphine antagonist naloxone. Animals received .4 mg/Kg of naloxone hydrochloride, subcutaneously.

Subjects from each strain of rats received implants of either morphine or placebo. A factorial design was used in the collection of data so that tests could be made of the effects of strain (BUF, LEW, F-344, Sprague-Dawley, and Holtzman), pellet type (morphine and placebo), length of pellet exposure (one, two, or three days), and their interactions. For each strain of rats, twelve Ss with morphine pellet implants and twelve with placebo pellet implants were observed for the withdrawal signs described below, after different lengths of exposure to the pellet. Withdrawal was precipitated only once in each animal without the removal of the implanted pellet. After exposure to a pellet for either one, two, or three days, animals were observed for ten minutes prior to an injection of naloxone and for 30 minutes following injection. The 30 minute period post-injection was divided into three ten-minute segments. Subjects

were observed individually in observation cages of stainless steel wire mesh, open at the top, measuring 43 cm. x 25 cm. x 18 cm.

Observed withdrawal responses. During each ten-minute observation period, the frequency of occurrence of the following signs was recorded: activity (described as number of crossings of the animal from one quadrant of the observation chamber to another), escape attempts (climbing onto the edge of the observation chamber, jumping onto the edge, or jumping out of the chamber; in either case the S was returned to the chamber by the observer), wet dog shakes (episodes of rapid repetitive shaking of the entire trunk), teeth chattering (number of episodes), writhing (number of episodes), yawning, and penile erections (with seminal emission and licking the penis). These responses are referred to as Type I signs for the remainder of this paper.

During each ten-minute interval, the presence or absence of the following signs was checked: vocalization or hostility on handling, ptosis, eye twitching, lacrimation, rhinorrhea, and diarrhea. These are referred to as Type II signs.

Morphine absorption. After observation of the withdrawal syndrome, the residue of pellets was collected from the subcutaneous sites of implant, and the absorption of morphine from these pellets was determined. Morphine was extracted from the pellet residue in 1N HCl. The amount of morphine present was determined spectrophotometrically, comparing the amount of UV light (wavelength 285 nm.) absorbed by this sample to an absorption curve of known morphine standards (Taylor, 1971).

Data analysis. The raw data collected consist of the frequency of occurrence of Type I responses and the presence or absence of Type II responses, within each ten-minute observation period.

Multivariate analysis of variance (MANOVA) provides a test of the effects of several factors (strain, pellet type, and length of pellet exposure) simultaneously on the total set of Type I and II response variables. This is accomplished by multiplying each response measure in the set by a weighting coefficient and adding the products, to form a single combined measure. The set of weighting coefficients is selected to maximize the univariate F-ratio, computed on the combined variable. The largest F-ratio obtained provides a test of the overall null hypothesis of no experimental effect on the response profile (Harris, 1975).

Comparison of the F-ratios from univariate analysis of variance (ANOVA) tests of the individual response variables are used as a measure of the degree to which effects on each variable contribute to the test of effects on the total response profile. Since intercorrelations between the response variables differ, however, interpretation of univariate F-ratio comparisons is difficult (Wilkinson, 1975). A second measure of individual response-variable contribution is obtained by comparing the magnitude of the standardized weighting coefficients. Standardized weighting coefficients are obtained by multiplying each raw coefficient by the variance of the corresponding response variable.

The weighting coefficients also provide a means of describing

group differences. Each linear combination of the response variables (multiplied by the corresponding raw weighting coefficient) is called a discriminant function. The combination for which the F-ratio is maximized, is the first or primary discriminant function; other, less optimal, functions, may be obtained within certain restrictions. Each discriminant function gives a description of group differences, although all may not contribute significantly to the discrimination between groups.

Discriminant function analysis and MANOVA are used in this study to examine differences in the overall profile of withdrawal responses. Discriminant functions are used to describe differences within a strain resulting from differences in length of morphine exposure.

These analyses were performed with the computer program NYEMUL, obtained from the Computing Center of the State University of New York at Buffalo. The analyses were done at the University of Houston Computing Center on a Univac 1108 computer.

CHAPTER IV

RESULTS

Pre-Naloxone Measurements

The strains were found to differ significantly ($F = 21.49$, $df = 4.0$ and 330.0, $p < .0001$) in their original weight prior to pellet implant. These weights are presented in Table 1. For this reason, all following analyses were performed with original subject weight as a covariate. This did not change the significance of any effect in the situations examined. Lacrimation, writhing, and yawning were never observed in any of the animals, so these responses were removed from analyses.

Strains were found to differ significantly on the pre-naloxone measurements with adjustment for the covariance of original weight (multivariate $F = 6.53$, $df = 44.0$ and 1222.37, $p < 0001$). Univariate analysis of the measures observed and consideration of the standardized coefficients from a discriminant function analysis (see Table 2) indicate that the strains differed primarily on the following measures: weight change after pellet implant to the day of testing, vocalization on handling, escape attempts, wet dog shakes, teeth chattering, and activity rate. The mean responses for each strain on these variables are presented in Appendix A.

The type of pellet implanted also had a significant effect upon the response profile as observed in the ten minutes prior to naloxone

TABLE 1

Mean Subject Weight by Strain
Prior to Pellet Implantation

Strain (n = 72 per strain)	Mean Weight (Grams)	
	$\bar{X}$	SD
BUF	244.236	21.616
LEW	251.431	23.318
F-344	230.222	19.913
Holtzman	243.277	19.101
Sprague-Dawley	223.458	14.721

TABLE 2

Univariate ANOVA and Multivariate Discriminant Function Analysis Of Strain Effects on Pre-Naloxone Response Measures

Variable	Univariate F (df-4.0 and 329.0)	Discriminant Function Coefficients[a] (Standardized)		
		1	2	3
Weight Change[b]	10.9822**	-.3640	-.4226	.1615
Vocalization on Handling	2.8668*	-.2960	-.0231	-.2001
Ptosis	1.3187	.0191	-.3237	.0330
Eye Twitching	1.5768	.0640	.1770	.3765
Rhinorrhea	1.2719	.0882	-.1442	-.2806
Diarrhea	.7904	-.1857	.1828	.1798
Penile Erections	1.6877	.0887	.0521	-.2029
Escape Attempts	15.2330**	.0646	-.6020	.3388
Wet Dog Shakes	7.0839**	-.0020	-.7379	-.2013
Teeth Chattering	2.5702*	-.0300	.0476	-.6998
Activity	44.8091**	.8941	.2674	-.0180

Note—Measures have been adjusted for the covariance of original subject weight.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
*$p < .05$
**$p < .01$

injection (multivariate F = 3.47, df = 11.0 and 319.0, $p < .0002$).

Table 3 presents the results of ANOVA tests of the individual response variables; standardized coefficients are also listed for the discriminant function describing differences between animals implanted with different types of pellets (morphine or placebo). Escape attempts, wet dog shakes, teeth chattering, and activity are the only variables which had significant univariate F-ratios, and these variables contribute most to the discriminant function.

The number of days of pellet implantation had a significant effect (multivariate F = 2.13, df = 22 and 6.38, $p < .002$) on the response profile. Wet dog shakes and activity rate showed univariate significance and only activity rate received a high weighting in the single significant discriminant function (see Table 4).

The effects of strain and of pellet type on the response profile interacted significantly (multivariate F = 1.87, df = 44.0 and 1222.37, $p < .0006$). Interpretation of this finding is difficult for the total response profile. Weight change to the day of testing, escape attempts, wet dog shakes, and activity rate show evidence of this interaction with significant F-ratios in the univariate ANOVA tests (see Table 5). These variables with rhinorrhea and penile erections contributed heavily to the one significant discriminant function (see Table 5). The interaction of pellet type and days of exposure (see Table 6) also had a significant effect on the pre-naloxone measures (multivariate F = 1.58, df = 22.0 and 638.0, $p < .05$). No other factors interacted significantly ($p > .05$) in their effects on the pre-naloxone response variables.

TABLE 3

Univariate ANOVA and Multivariate Discriminant Function Analysis Of the Effects of Pellet Type on Pre-Naloxone Response Measures

Variable	Univariate F (df=1.0 and 329.0)	Discriminant Function Coefficients (Standardized)
Weight Change[a]	.7102	-.1474
Vocalization on Handling	.7855	-.1893
Ptosis	.0007	-.0035
Eye Twitching	.3831	-.0443
Rhinorrhea	.1363	.0520
Diarrhea	.0020	-.0227
Penile Erections	.0016	.1551
Escape Attempts	7.6220**	.2334
Wet Dog Shakes	8.6905**	-.5975
Teeth Chattering	11.2246**	-.5950
Activity	8.0455**	.5452

Note.--Measures have been adjusted for the covariance of original subject weight.
[a]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
**$p < .01$

TABLE 4

Univariate ANOVA and Multivariate Discriminant Function Analysis Of the Effects of Days of Pellet Exposure On Pre-Naloxone Response Measures

Variable	Univariate F (df=2.0 and 329.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	1.5568	-.2314
Vocalization on Handling	2.5400	.2737
Ptosis	.4696	-.1133
Eye Twitching	1.0460	.1051
Rhinorrhea	.6650	-.0978
Diarrhea	1.8439	.1568
Penile Erections	.4727	-.0756
Escape Attempts	.4955	-.4024
Wet Dog Shakes	3.0736*	-.1900
Teeth Chattering	.3883	-.0864
Activity	11.7864**	.9474

Note.--Measures have been adjusted for the covariance of original subject weight.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
*$p < .05$
**$p < .01$

TABLE 5

Univariate ANOVA and Discriminant Function Analysis Of the Strain x Pellet Type Interaction Effects On Pre-Naloxone Response Measures

Variable	Univariate F (df=4.0 and 329.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	4.1353**	.4757
Vocalization on Handling	.6601	-.0678
Ptosis	.4128	.0441
Eye Twitching	.3543	.0525
Rhinorrhea	2.1192	.3933
Diarrhea	1.2796	-.0451
Penile Erections	1.3464	-.2993
Escape Attempts	3.1459*	.3044
Wet Dog Shakes	4.3761**	-.4599
Teeth Chattering	1.6761	.2039
Activity	2.9421*	.2777

Note.--Measures have been adjusted for the covariance of original subject weight.
[a] Only statistically significant ($p < .05$) functions are presented.
[b] Weight Change = (Weight on test day pre-naloxone) - (Original weight).
*$p < .05$
**$p < .01$

TABLE 6

Univariate ANOVA and Discriminant Function Analysis Of the Pellet Type x Days of Exposure Interaction Effects On Pre-Naloxone Response Measures

Variables	Univariate F (df=2.0 and 329.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	4.2032*	.5930
Vocalization on Handling	1.3717	.0330
Ptosis	1.5644	.3970
Eye Twitching	.3107	.0006
Rhinorrhea	.7323	-.1115
Diarrhea	.0023	-.0876
Penile Erections	3.7157*	.4778
Escape Attempts	.3839	-.4116
Wet Dog Shakes	1.0355	.0037
Teeth Chattering	.3046	-.2433
Activity	1.7349	.5979

Note.--Measures have been adjusted for the covariance of original subject weight.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
*$p < .05$

Further analysis of pre-naloxone measures was performed with the data subdivided as to pellet type. Animals receiving either morphine pellets (see Tables 7 and 8) or placebo pellets (see Tables 9 and 10) showed significant effects of strain ($p < .0001$) and day-of-testing-after-implant ($p < .05$); but, no significant interaction was present.

Post-Naloxone Measurements

For purposes of analysis, data were summed over the three ten-minute observation intervals after the injection of naloxone. The frequency of occurrence of each Type I response was recorded during the 30 minute post-naloxone period, and the presence of each Type II response is the number of post-naloxone periods (with a maximum of three) during which the sign was present.

Because writhing and yawning were never observed in any of the animals, these measures were dropped from the analyses.

Placebo-implanted animals exhibited very few of the observed withdrawal signs. The morphine-implanted rats of all five strains showed significantly more withdrawal responses (multivariate $F = 428.35$, $df = 12.0$ and 318.0, $p < .001$) than did the placebo rats. The figures in Appendix B present the mean frequency of occurrence of counted (Type I) signs for each of the strains. The presence of observed (Type II) signs for each strain is presented in the tables in Appendix C.

Significant strain differences (multivariate $F = 11.66$, $df = 48.0$ and 1227.01, $p < .001$) and a significant effect of days of exposure to the implanted pellet (multivariate $F = 5.75$, $df = 24.0$ and 636.0,

TABLE 7

Multivariate and Univariate ANOVA and Discriminant Function Analysis
Of Strain Effects on Pre-Naloxone Response Measures
(Morphine-Implanted Animals)

Multivariate F = 2.94, df = 44.0 and 591.12, $p < .0001$

Variable	Univariate F (df=4.0 and 164.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	3.2365*	.2337
Vocalization on Handling	.9723	-.1431
Ptosis	1.0612	-.0990
Eye Twitching	.7796	.0686
Rhinorrhea	1.1806	.0432
Diarrhea	.9634	-.2243
Penile Erections	1.5497	.0659
Escape Attempts	7.7379**	-.0802
Wet Dog Shakes	.7629	-.3734
Teeth Chattering	.7406	-.0413
Activity	20.8328**	1.1120

Note.--Measures have been adjusted for the covariance of original subject weight.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
* $p < .05$
** $p < .01$

TABLE 8

Multivariate and Univariate ANOVA and Discriminant Function Analysis Of the Effects of Days of Exposure on Pre-Naloxone Response Measures (Morphine-Implanted Animals)

Multivariate F = 1.66, df = 22.0 and 308.0, $p < .0336$

Variable	Univariate F (df=2.0 and 164.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	.3947	.2575
Vocalization on Handling	1.0237	-.1390
Ptosis	1.1239	-.2625
Eye Twitching	.9881	-.1238
Rhinorrhea	.9311	.1731
Diarrhea	1.0390	.0093
Penile Erections	3.2415*	-.1313
Escape Attempts	.0038	.4185
Wet Dog Shakes	4.2584*	-.2580
Teeth Chattering	.1950	-.0987
Activity	8.0431**	-.8547

Note.--Measures have been adjusted for the covariance of original subject weight.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
*$p < .05$
**$p < .01$

TABLE 9

Multivariate and Univariate ANOVA and Discriminant Function Analysis Of Strain Effects on Pre-Naloxone Response Measures (Placebo-Implanted Animals)

Multivariate F = 5.76, df = 44.0 and 591.12, p<.0001

Variable	Univariate F (df=4.0 and 164.0)	Discriminant Function Coefficients[a] (Standardized)			
		1	2	3	4
Weight Change[b]	11.4548**	.3493	.5320	.0966	-.2539
Vocalization on Handling	2.2843	-.3293	-.1833	.1894	-.2181
Ptosis	.5001	.1323	-.2168	-.1744	-.0734
Eye Twitching	.9390	.1113	.0397	-.0797	.5131
Rhinorrhea	2.7984*	.1348	.0850	-.7626	-.1103
Diarrhea	1.5534	-.2775	.1514	.2859	-.3201
Penile Erections	1.3962	.2548	-.2205	-.1225	.0424
Escape Attempts	16.8028**	.3702	-.5817	.1090	.1088
Wet Dog Shakes	7.6967**	.1934	-.6487	.1015	-.4190
Teeth Chattering	2.0831	-.1784	.2441	-.3403	-.6330
Activity	30.3922**	.7404	.3667	-.1725	.1335

Note.--Measures have been adjusted for the covariance of original subject weight.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
*$p < .05$
**$p < .01$

TABLE 10

Multivariate and Univariate ANOVA and Discriminant Function Analysis Of the Effects of Days of Exposure on Pre-Naloxone Response Measures (Placebo-Implanted Animals)

Multivariate F = 1.75, df = 22.0 and 308.0, p < .0210

Variable	Univariate F (df=1.0 and 164.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	5.3279**	.4619
Vocalization on Handling	2.6262	.3311
Ptosis	.9903	-.2180
Eye Twitching	.5016	.0915
Rhinorrhea	.4313	-.1337
Diarrhea	.9257	.2463
Penile Erections	1.0167	.0146
Escape Attempts	3.3908*	-.2845
Wet Dog Shakes	1.7191	-.3867
Teeth Chattering	.4072	-.0755
Activity	4.4018*	.5882

Note.--Measures have been adjusted for the covariance of original subject weight.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day pre-naloxone) - (Original weight).
*$p < .05$
**$p < .01$

$p < .001$) were also present. In addition, all interactions of effects (Strain x Days of Exposure, Strain x Pellet Type, Pellet Type x Days, and Strain x Days x Type) were significant ($p < .05$).

In order to better characterize the effect of precipitated morphine withdrawal in the strains observed, the following analyses were performed with that data obtained only from animals receiving morphine-pellet implants.

A regression analysis indicated a significant association between pre- and post-naloxone measures ($F = 1.33$, $df = 132$ and 1167.36, $p < .01$). For this reason, responses on the pre-naloxone measures were treated as covariates in the analyses which follow.

In animals receiving morphine pellets, strain differences were significant (multivariate $F = 7.97$, $df = 48$ and 552.89, $p < .0001$). All of the post-naloxone measures showed significant ($p < .05$) strain effects on univariate analysis of variance tests (see Table 11). A discriminant function analysis revealed that each of the four theoretically allowable discriminant functions contributed significantly to the discrimination of strains. The standardized weighting coefficients for these functions are presented in Table 11. Comparison of the magnitudes of these coefficients suggests that responding on the following variables contributes most to the discrimination of strains: wet dog shakes, rhinorrhea, eye twitching, ptosis, teeth chattering, escape attempts, vocalization on handling, and activity rate.

Days of exposure to the implanted morphine was also found to be significant ($p < .0001$). This effect was significantly represented in a

TABLE 11

Multivariate and Univariate ANOVA and Discriminant Function Analysis Of Strain Effects on Post-Naloxone Response Measures (Morphine-Implanted Animals)

Variable	Univariate F (df=2.0 and 154.0)	Discriminant Function Coefficients[a] (Standardized)			
		1	2	3	4
Weight Change[b]	9.9422**	.1894	.2401	.2645	-.0601
Vocalization on Handling	6.4083**	.0125	-.1157	-.5518	.3965
Ptosis	7.9777**	.1869	-.7198	.5421	.3459
Eye Twitching	6.6780**	-.4172	.4996	-.2818	.2110
Lacrimation	2.3093*	-.1091	.2254	.3698	-.1282
Rhinorrhea	10.7288**	-.4326	-.2816	-.0862	.4263
Diarrhea	5.2109**	-.0307	-.2882	-.2118	.2177
Penile Erections	3.6436**	-.2894	.1354	.0401	.0321
Escape Attempts	8.6332**	-.0485	-.1953	.6483	.1772
Wet Dog Shakes	33.2657**	-.7690	.0645	.0273	-.1531
Teeth Chattering	12.0145**	-.0429	.5279	-.0769	.6478
Activity	5.9398**	-.1521	-.0524	-.3689	-.5426

Note.--Measures have been adjusted for the covariance of pre-naloxone measures.

[a]Only statistically significant ($p < .05$) functions are presented.

[b]Weight Change = (Weight on test day post-naloxone) - (Weight on test day pre-naloxone).

*$p < .05$

**$p < .01$

single discriminant function. The standardized coefficients for this function and univariate analysis of variance tests of each post-naloxone measure are presented in Table 12. Wet dog shakes and vocalization on handling contribute most to this discrimination. Vocalization on handling, eye twitching, diarrhea, penile erections, and wet dog shakes showed significance on univariate tests. The Strain x Days of Exposure interaction was also found to be significant, $p < .02$ (see Table 13).

With further subdivision of subjects it was found that strain differences are significant within inbred strains ($p < .0001$), within outbred strains ($p < .0001$), and between inbred and outbred strains ($p < .0001$).

Several of the response measures are presented in Figures 1 through 7 to allow strain comparisons. Strain differences are evident in these figures as is the effect of length of morphine exposure.

Morphine-implanted animals of each strain were examined for the effect of length of morphine exposure on withdrawal responses. All of the strains examined, with the exception of the Holtzman strain, showed a significant effect of length of morphine exposure.

Length of exposure was not a significant effect for the Holtzman strain as determined by a multivariate analysis of variance ($F = 1.26$, $df = 24$ and 34, $p > .25$). In contrast, one variable, wet dog shakes, showed significant effects in Holtzman rats, in a univariate test ($F = 7.52$, $df = 2$ and 28, $p < .003$).

Discriminant functions were obtained for the four strains which did

TABLE 12

Multivariate and Univariate ANOVA and Discriminant Function Analysis Of the Effects of Days of Exposure on Post-Naloxone Response Measures (Morphine-Implanted Animals)

Multivariate F = 4.05, df = 24.0 and 286.0, $p < .0001$

Variable	Univariate F (df=2.0 and 154.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	1.9872	.0240
Vocalization on Handling	11.9209**	.4833
Ptosis	.6344	-.0671
Eye Twitching	3.5623*	.3116
Lacrimation	1.9039	.0075
Rhinorrhea	.2304	.0425
Diarrhea	5.6338**	.3191
Penile Erections	3.6955*	-.2231
Escape Attempts	1.7024	-.0994
Wet Dog Shakes	25.8450**	.7276
Teeth Chattering	2.1913	-.1180
Activity	2.3692	.2226

Note.--Measures have been adjusted for the covariance of pre-naloxone measures.
[a]Only statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day post-naloxone) - (Weight on test day pre-naloxone).
*$p < .05$
**$p < .01$

TABLE 13

Multivariate and Univariate ANOVA and Discriminant Function Analysis Of Strain x Days of Exposure Interaction Effects on Post-Naloxone Response Measures (Morphine-Implanted Animals)

Multivariate F = 1.3470, df = 06.0 and 973.57, p <.02

Variable	Univariate F (df=6.0 and 154.0)	Discriminant Function Coefficients[a] (Standardized)
Weight Change[b]	.9086	.5287
Vocalization on Handling	1.8847	-.2433
Ptosis	.8435	.0358
Eye Twitching	1.8875	-.1876
Lacrimation	1.0944	.2265
Rhinorrhea	.4246	.3279
Diarrhea	1.4581	-.1184
Penile Erections	.6317	-.0851
Escape Attempts	1.1614	.3985
Wet Dog Shakes	3.3748**	.6892
Teeth Chattering	.6204	-.0852
Activity	.8191	-.4753

Note.--Measures have been adjusted for the covariance of pre-naloxone measures.
[a]Only Statistically significant ($p < .05$) functions are presented.
[b]Weight Change = (Weight on test day post-naloxone) - (Weight on test day pre-naloxone).
**$p < .01$

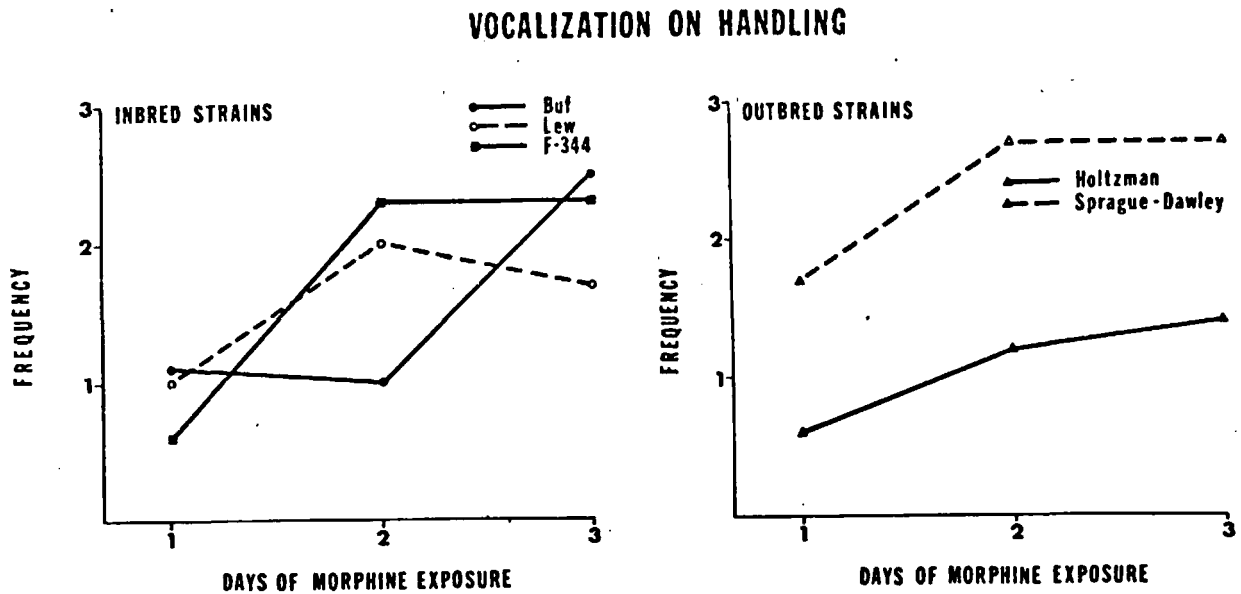


FIGURE 1

Mean Frequency of Occurrence of Vocalization on Handling
In Inbred and Outbred Rat Strains

Ordinate presents the frequency of occurrence of responses during the three ten-minute periods after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

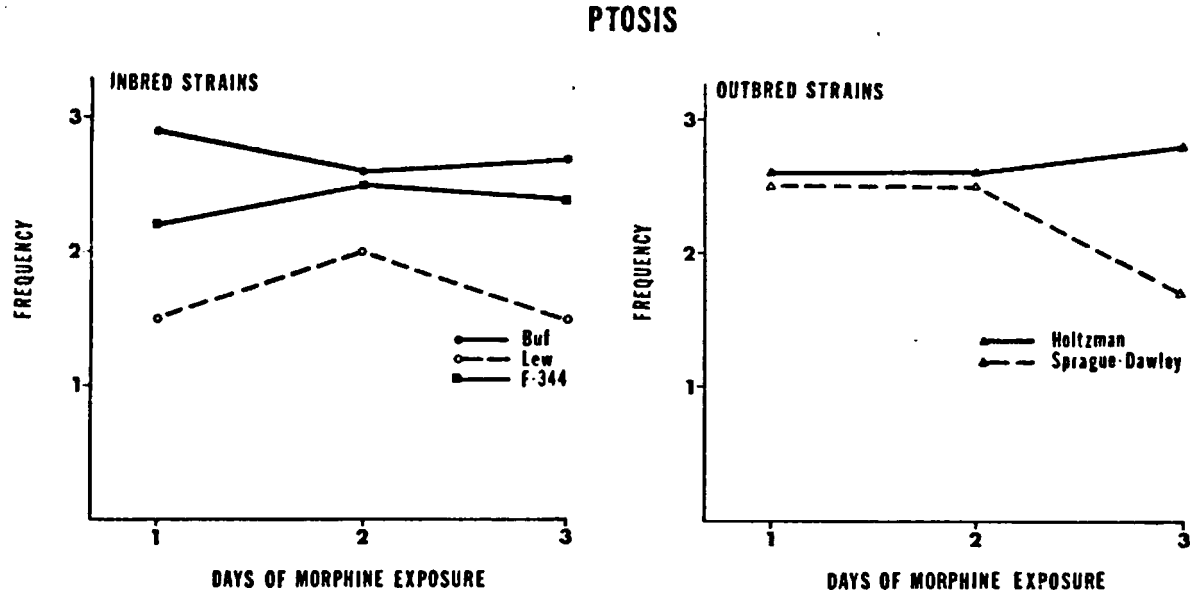


FIGURE 2

Mean Frequency of Occurrence of Ptosis
In Inbred and Outbred Rat Strains

Ordinate presents the frequency of occurrence of responses during the three ten-minute periods after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

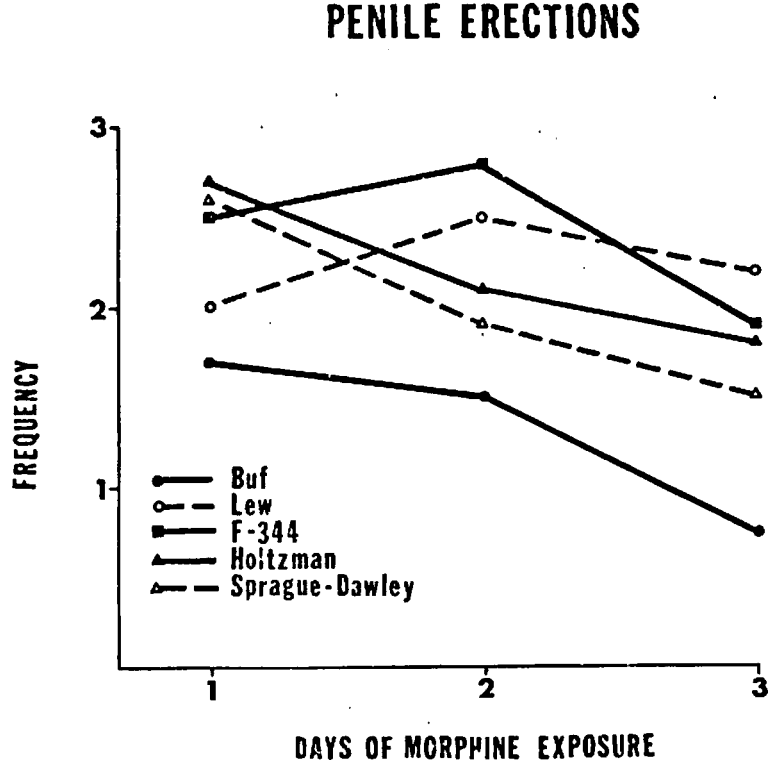


FIGURE 3

Mean Frequency of Occurrence of Penile Erections
In Inbred and Outbred Rat Strains

Ordinate presents the frequency of occurrence of responses during the three ten-minute periods after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

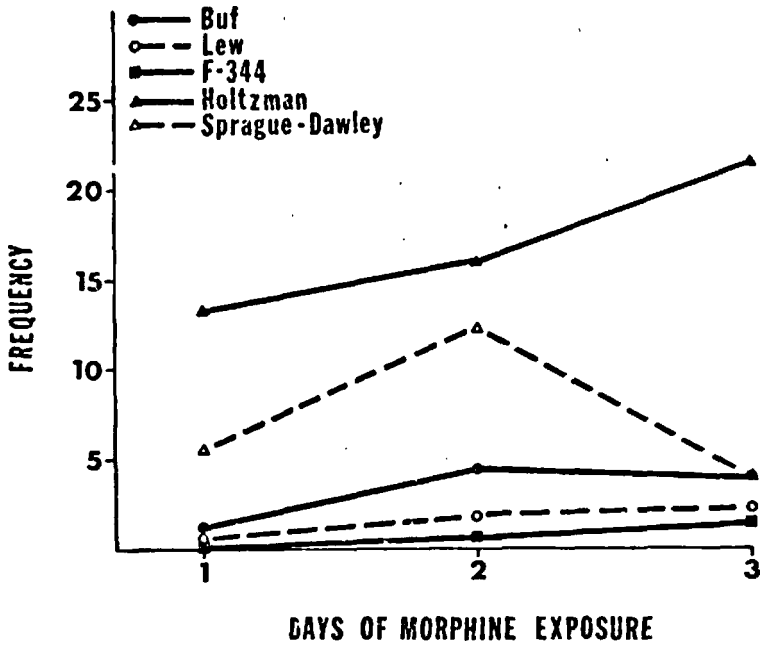


FIGURE 4

Mean Frequency of Occurrence of Escape Attempts
In Inbred and Outbred Rat Strains

Ordinate presents the frequency of occurrence of responses during the three ten-minute periods after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

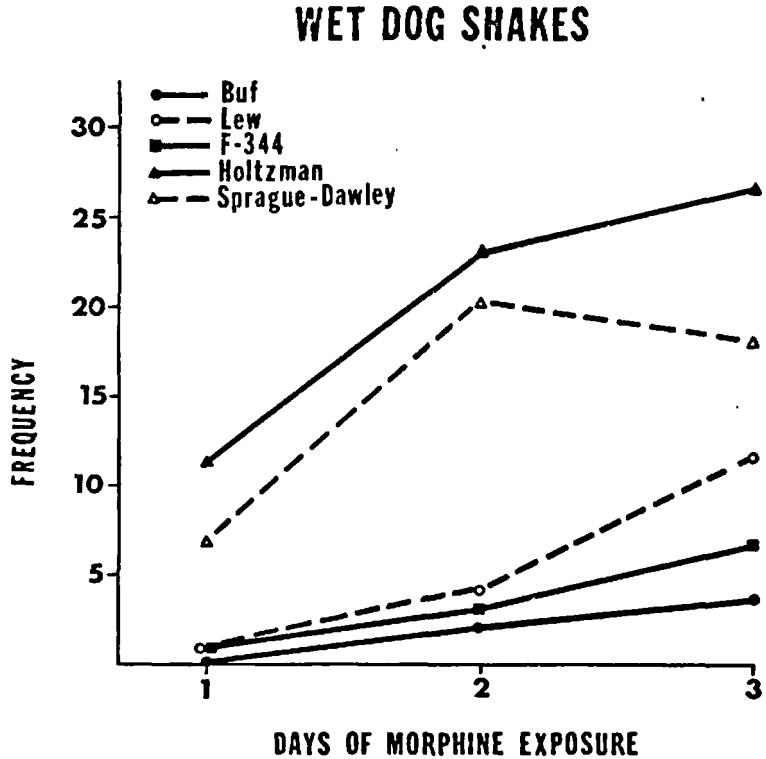


FIGURE 5

Mean Frequency of Occurrence of Wet Dog Shakes
In Inbred and Outbred Rat Strains

Ordinate presents the frequency of occurrence of responses during the three ten-minute periods after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

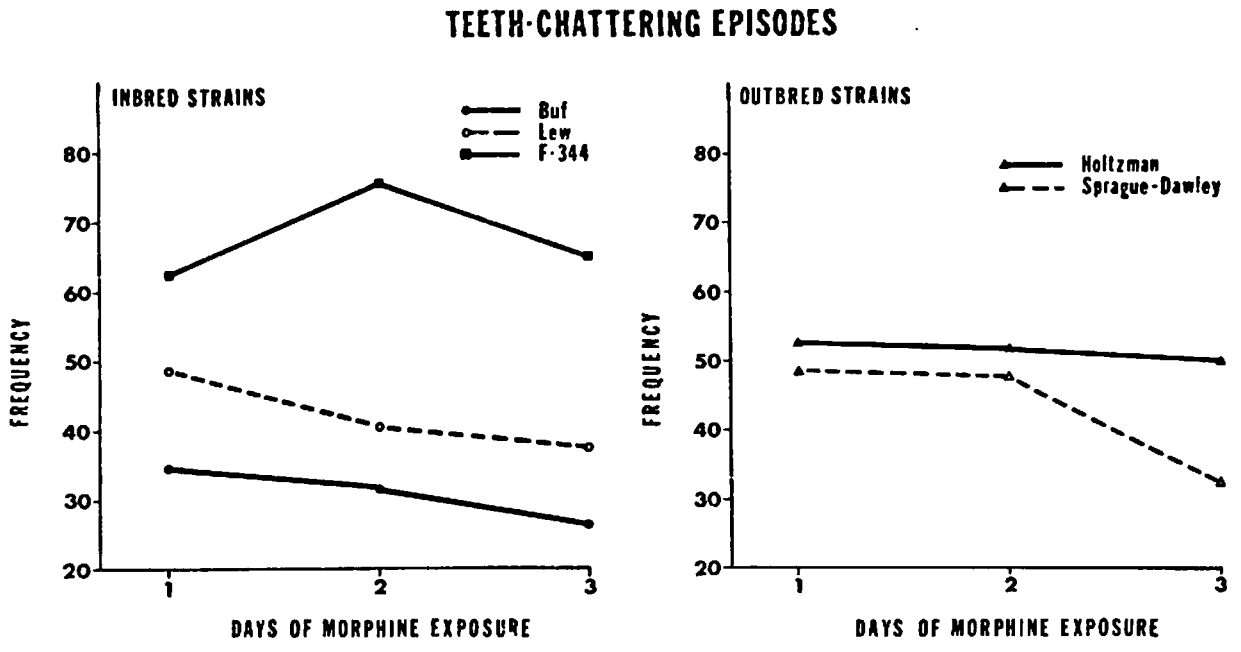


FIGURE 6

Mean Frequency of Occurrence of Teeth Chattering Episodes
In Inbred and Outbred Rat Strains

Ordinate presents the frequency of occurrence of responses during the three ten-minute periods after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

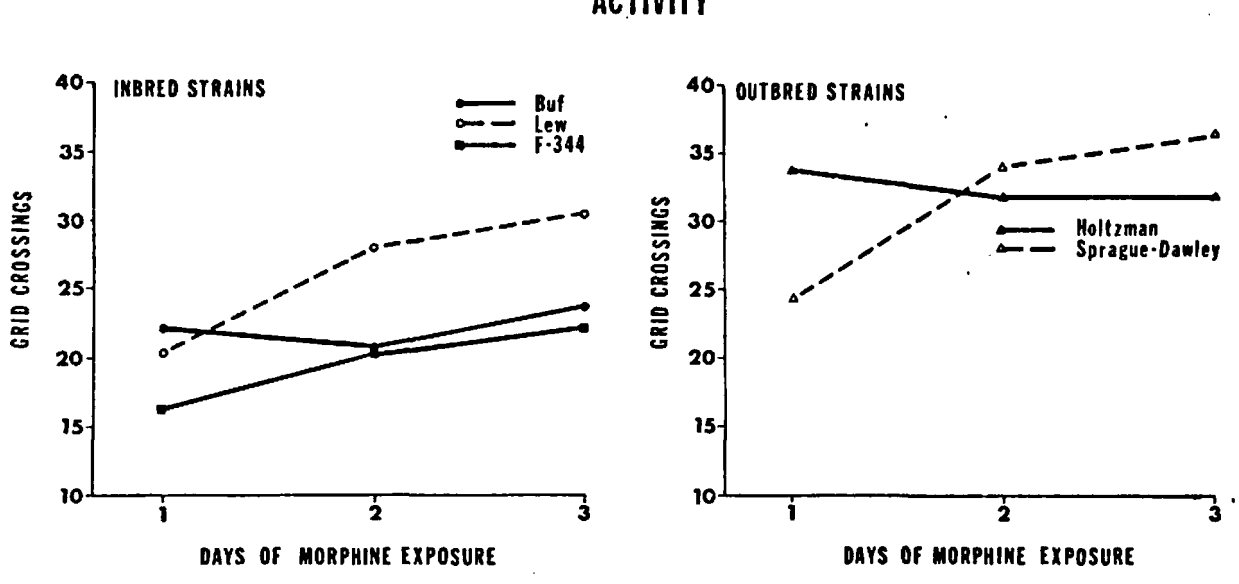


FIGURE 7

Mean Frequency of Occurrence of Activity
In Inbred and Outbred Rat Strains

Ordinate presents the frequency of occurrence of responses during the three ten-minute periods after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

show a significant effect of length of exposure on the profile of withdrawal responses. Both raw and standardized weighting coefficients, after adjustment for the covariance of pre-naloxone measures are presented (see Tables 14 and 15).

To illustrate the change in the distribution of responses within the 30 minutes post-naloxone injection, responding within each ten-minute interval is presented for select variables in Table 16 for the Sprague-Dawley strain. Most of the observed responding is seen to occur in the first ten minutes after naloxone injection.

Morphine Absorption

Figure 8 summarizes the analysis of the amount of morphine absorbed from the implanted pellet after one, two, or three days of exposure. It can be seen that rapid absorption during the first day after pellet implant is followed by a decreasing rate during the next two days.

TABLE 14

Discriminant Function Analysis of Post-Naloxone Response Measures For the Effects of Days of Morphine Exposure: Raw Coefficients (Morphine-Implanted Animals)

Variable	Raw Coefficients			
	BUF	LEW	F-344	Sprague-Dawley
Weight Change[a]	.0228	-.2204	.0431	-.1276
Vocalization on Handling	-1.0401	-.2053	-.3890	.2076
Ptosis	.4594	-.8715	.1089	-.2568
Eye Twitching	-.1526	-.0218	-.6289	.6812
Lacrimation[b]	-----	2.0762	.2290	-.2045
Rhinorrhea	.1494	.5109	.9167	.2942
Diarrhea	.0474	-.5228	-.4446	-.0871
Penile Erections	.8147	-.1176	.3469	-.0136
Escape Attempts	-.1457	.1441	-.3678	-.0588
Wet Dog Shakes	-.3647	-.4598	-.2507	.1849
Teeth Chattering	.0151	.0138	-.0397	.0218
Activity	.0596	.1148	.0029	.0344

Note.--Measures have been adjusted for the covariance of pre-naloxone measures.
[a]Weight Change = (Weight on test day post-naloxone) - (Weight on test day pre-naloxone).
[b]Lacrimation did not occur in BUF rats, and therefore was eliminated from this analysis.

TABLE 15

Discriminant Function Analysis of Post-Naloxone Response Measures For the Effects of Days of Morphine Exposure: Standardized Coefficients (Morphine-Impalnted Animals)

Variable	Standardized Coefficients			
	B6F	LE	F-344	Sprague-Dawley
Weight Change[a]	.0787	-.6565	.0850	-.7222
Vocalization on Handling	-1.0102	-.2323	-.8102	.2147
Ptosis	.2872	-1.0145	.0990	-.1938
Eye Twitching	-.1500	-.0277	-.4916	.4483
Lacrimation[b]	-----	.5248	.1096	-.1298
Rhinorrhea	.1599	.1394	.4676	.3669
Diarrhea	.0368	-.5603	-.4185	-.0420
Penile Erections	.9053	-.1638	.4257	-.0172
Escape Attempts	-.5667	.2261	-.4399	-.6366
Wet Dog Shakes	-.5757	-1.6548	-.6519	1.3586
Teeth Chattering	.3285	.2723	-.8617	.3916
Activity	.4216	1.0586	.0211	1.0620

Note.--Measures have been adjusted for the covariance of pre-naloxone measures.
[a]Weight Change = (Weight on test day post-naloxone) - (Weight on test day pre-naloxone).
[b]Lacrimation did not occur in B6F rats, and therefore was eliminated from this analysis.

TABLE 16

Distribution of the Frequency of Occurrence of Responses Throughout the Thirty Minutes Post-Naloxone Injection (Sprague-Dawley, Morphine-Implanted Animals)

Time Post-Naloxone (Minutes)	Variables			
	Escapes	Wet Dog Shakes	Teeth Chattering	Activity
	Days of Morphine Exposure: One			
10	5.17	5.42	20.33	21.17
20	.33	1.08	15.58	2.42
30	.00	.33	12.33	.75
	Days of Morphine Exposure: Two			
10	8.50	12.08	16.50	23.17
20	3.00	4.83	15.58	6.17
30	.75	3.25	15.17	4.67
	Days of Morphine Exposure: Three			
10	3.00	12.42	13.83	27.75
20	1.00	3.42	9.25	6.00
30	.00	2.17	9.00	2.58

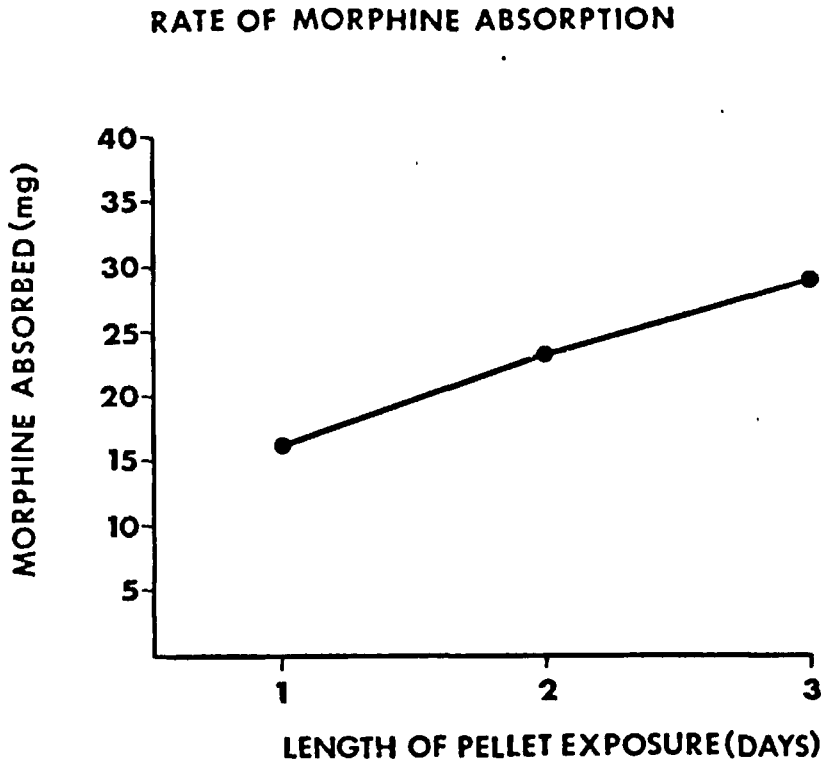


FIGURE 8

Amount of Morphine Absorbed from the Subcutaneous Pellet Depot After Different Time Periods of Exposure

Ordinate presents amount of morphine absorbed in mg. Abscissa presents duration of pellet implantation in days.

CHAPTER V

DISCUSSION AND CONCLUSIONS

This study has determined that there are substantial differences in the characterization of the opiate abstinence syndrome between different rat strains. Quantitative and qualitative differences in the profile of withdrawal responses are evident between both inbred and outbred strains of rats. In addition, changes occur in the abstinence syndrome with differences in the length of exposure to morphine, and these changes interact with the effects of strain. Discriminant function analysis of the length of exposure effect provides a function which can be used as a method for rating and quantitative assessment of the opiate abstinence syndrome.

Both morphine and placebo implanted rats exhibit strain differences in the profile of responses observed prior to the precipitation of abstinence with naloxone. The differences in activity rate observed prior to naloxone injection are similar to those reported by Rosecrans and Schechter (1972) for Sprague-Dawley and Fisher rats, and by Segal *et al.* (1972) for the BUF, LEW, F-344, and Sprague-Dawley strains. As might be expected from studies of genetic differences in drug effects on mice (Oliverio, 1974), the responses of animals from inbred strains were much less variable, both before and after precipitated withdrawal, than were responses of rats from random, outbred strains. Also significant is the effect of the type of pellet implanted on the response pro-

file prior to naloxone injection as well as after injection.

Strain differences in weight loss after naloxone between Sprague-Dawley and Fisher rats are similar to those reported by Tilson and Rech (1974). The finding here that strains vary significantly in wet dog shakes is contrary to a suggestion by Lorenzetti and Sancilio (1970) of no difference between Holtzman and Sprague-Dawley rat strains. They do not, however, present data to support this contention.

In addition to the significant strain differences in weight loss and other measures, a significant effect of length of exposure to morphine was obtained. The various withdrawal signs do not, however, change in the same way with changes in length of morphine exposure for the different strains. Teeth chattering, for example, either decreased, after two or three days of exposure, or increased after two days and then decreased (see Figure 6). Wet dog shakes (Figure 5) showed a similar increase after two days of morphine exposure and then decreased with three days of morphine in the Sprague-Dawley rats. This finding is in agreement with the abstinence profiles reported by Blasig et al. (1973) for Sprague-Dawley rats.

Of the five strains used in this study only the Holtzman strain did not show significant changes in the total abstinence profile across days of morphine exposure. This finding questions the generalization of results obtained with this strain to other rat strains. The studies of single-dose tolerance to morphine by Kornetsky and Bain (1968) and by Kayan and Mitchell (1972), for example, may not be applicable to other rat strains. While the overall response profile of Holtzman

rats in this study did not change significantly, the changes in wet dog shakes for this strain are comparable to those reported by Cicero and Meyer (1973) for the same strain.

The discriminant analysis of length of morphine exposure for each strain, with the exception of the Holtzman strain, is presented as a means of quantitatively assessing the abstinence profile. The resulting discriminant function can serve to transform the total array of withdrawal signs in the abstinence profile into a single score along the length-of-exposure dimension. The result of transforming the mean response profiles for each strain with their respective discriminant function, obtained from Table 14, is presented in Figure 9. Each strain shows an orderly change in the discriminant function score with increasing length of exposure to morphine. The specific functions presented in this study can serve as a measure of the abstinence profile in each of the strains examined, for dependence established with morphine-pellet implants of one to three days of pellet exposure.

The use of discriminant function analysis as a means of assessing the abstinence syndrome is suggested as a more appropriate method than has been presented by other investigators (Halbach and Eddy, 1963; and, Wei, 1973). In previous attempts to develop rating systems, responses have been ranked along an ordinal scale. Those responses which occurred only after longer exposure to or higher doses of morphine received higher ranks. The abstinence syndrome was then scored either as to the highest ranking response observed, as to the sum of the ranks of all observed responses, or as to the sum of the frequency of occurrence of

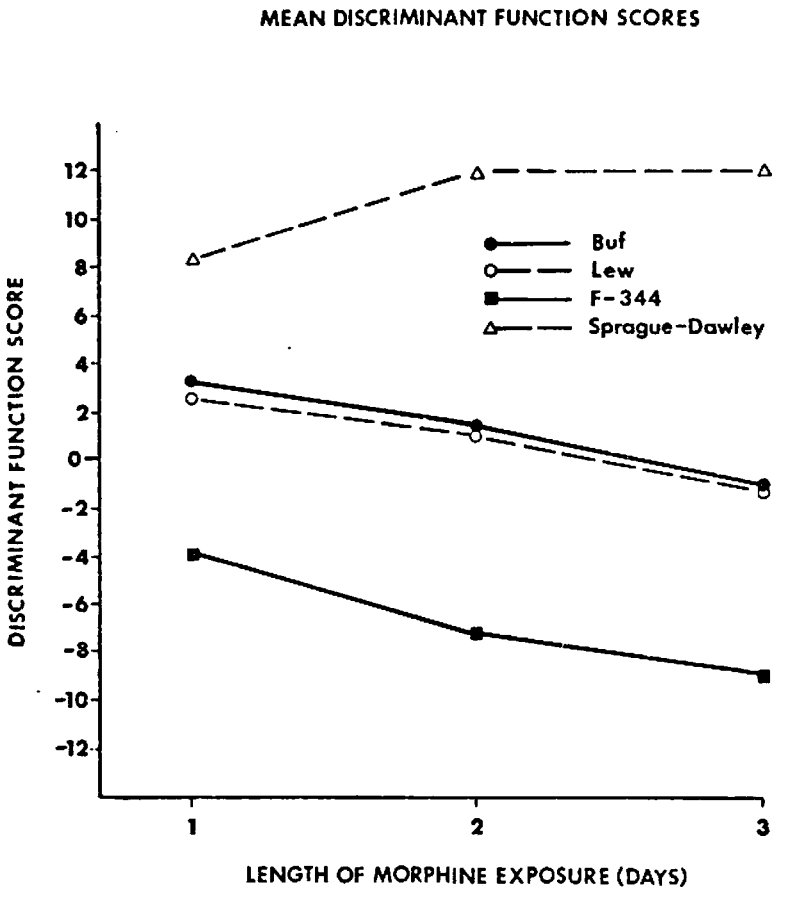


FIGURE 9

Mean Discriminant Function Scores
For Strains Showing a Significant Length of Exposure Effect

Ordinate presents scores for individaul strains on a discriminant function which rates the withdrawal response profile along a length-of-exposure dimension. Abscissa presents duration of exposure to morphine pellets in days.

all observed responses weighted by the rank of each response. Since these response ranks are measures along an ordinal scale, the above manipulations may not be appropriate and the use of parametric statistical tests of differences in such scores is questionable (Siegel, 1956).

The rating system developed in this thesis by means of discriminant function analysis is selected to statistically maximize differences between animals receiving different lengths of morphine exposure. The weighting coefficients applied to the frequency of occurrence of responses have the properties of an interval scale of measurement, so that parametric tests can be applied to the resulting length-of-exposure score. Application of this technique to other methods of establishing physical dependence and to more extensive examination of the length-of-exposure dimension would surely extend its usefulness.

Several investigators have suggested the involvement of brain biogenic amines in the development of tolerance and physical dependence to morphine (Bhargava and Way, 1972; Shen, Loh, and Way, 1970; Schwartz and Eidelberg, 1970; and, Sloan, Brooks, Eisenman, and Martin, 1963). Tilson and Rech (1974) observed differences in morphine analgesia and tolerance development in rat strains (Sprague-Dawley and Fisher) selected because they differ in brain levels of 5-hydroxytryptamine (Rosecrans and Schechter, 1972). The BUF, LEW, F-344, and Sprague-Dawley strains observed in this study have been reported to differ in the activity of brain tyrosine hydroxylase (Segal et al., 1972), the rate-limiting enzyme in the catecholamine biosynthetic pathway (Levitt, Spector, Sjoerdsma, and Udenfriend, 1965). The strain differences in the opiate abstinence syndrome reported in this

thesis could, therefore, be the result of initial differences between strains in brain levels or in the activity of several biogenic amines.

The discriminant function analysis of strain differences indicates a complex relationship between strains. No simple combination of the variables will uniquely discriminate the strains from one another. This result suggests that comparisons between strains, and thus comparisons between many previous research studies, are very difficult. The assumption that random-bred strains are equivalent for experimental use (Brown, 1964) is not supported.

These findings suggest that researchers investigating the mechanisms of morphine action should select a single strain of animals for study to facilitate inter-study comparisons. In this study, the abstinence syndrome was, in general, less severe in the inbred strains than in the outbred strains. Since the Holtzman strain did not show changes in the withdrawal response profile with changes in the duration of pellet exposure, the Sprague-Dawley strain is probably the most sensitive of the strains examined in this study to the effects of morphine when the severity of the abstinence syndrome is used as a measure. Therefore, this strain may be the most appropriate subject for investigations of the opiate abstinence syndrome.

Castellano and Oliverio (1975) have reported that the acute effects of morphine in mice are genetically determined. Nichols and Hsiao (1967) have shown the possibility of breeding rats for differences in their sensitivity to morphine addiction. Similar breeding to develop a strain highly sensitive to the effects of morphine on analgesia, tolerance,

and physical dependence could result in a better understanding of the mechanisms of action of this drug.

In summary, this thesis reports strain differences in the opiate abstinence syndrome and, through multivariate discriminant function analysis, characterizes changes in the profile of withdrawal responses which accompany changes in the duration of exposure to morphine.

REFERENCES

REFERENCES

Adler, M.W., Lin, C., Smith, K.P. Tresky, R., and Gildenberg, P.L. Lowered seizure threshold as a part of the narcotic abstinence syndrome in rats. Psychopharmacologia, 1974, 35, 243-247.

Akera, T., and Brody, T.M. The addiction cycle to narcotics in the rat and its relation to catecholamines. Biochem. Pharmacol., 1968, 17, 675-688.

Bhargava, H.N., and Way, E.L. Acetylcholinesterase inhibition and morphine effects in morphine tolerant and dependent mice. J. Pharm. exp. Ther., 1972, 183, 31-40.

Blasig, J., Herz, A., Reinhold, K., and Zieglgansberger, S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia, 1973, 33, 19-38.

Brown, A.M. Strain and sex differences in response to drugs. In D.R. Laurence and A.L. Bacharach (Eds.), Evaluation of drug activities: pharmacometrics. Vol. 1. New York: Academic Press, 1964. Pp. 111-123.

Buckett, W.R. A new test for morphine-like physical dependence (addiction liability) in rats. Psychopharmacologia, 1964, 6, 410-416.

Castellano, C., and Oliverio, A. A genetic analysis of morphine-induced running and analgesia in the mouse. Psychopharmacologia, 1975, 41, 197-200.

Cicero, T.J., and Meyer, E.R. Morphine pellet implantation in rats: quantitative assessment of tolerance and dependence. J. Pharmacol. exp. Ther., 1973, 184(2), 404-408.

Colasanti, B., and Khazan, N. Antagonism of the acute electroencephalographic and behavioral effects of morphine in the rat by depletion of brain biogenic amines. Neuropharmacol., 1973, 12, 463-469.

Collier, H.O., Francis, D.L., and Schneider, C. Modification of morphine withdrawal by drugs interacting with humoral mechanisms: some contradictions and their interpretation. Nature, 1972, 237, 220-223.

Davis, W.M., and Nichols, J.R. Physical dependence and sustained opiate-directed behavior in the rat (a preliminary report). Psychopharmacologia, 1962, 3, 139-145.

Fichtenberg, D.G. Study of experimental habituation to morphine. Bull. Narcot., 1951, 3(3), 19-42.

Francis, D.L., and Schneider, C. Jumping after naloxone precipitated withdrawal of chronic morphine in the rat. Brit. J. Pharmacol., 1971, 41, 424P-425P.

Furner, R.L., Gram, T.E., and Stitzel, R.E. The influence of age, sex, and drug treatment on microsomal drug metabolism in four rat strains. Biochem. Pharmacol., 1969, 18, 1135-1641.

Gebhart, G.F., and Mitchell, C.L. Strain differences in the analgesic response to morphine as measured on the hot plate. Arch. int. Pharmacodyn., 1973, 201, 128-140.

Gibson, R.D., and Tingstad, J.E. Formulation of a morphine implantation pellet suitable for tolerance-physical dependence studies in mice. J. Pharm. Sci., 1970, 59(3), 426-427.

Goode, P.G. An implanted reservoir of morphine solution for rapid induction of physical dependence in rats. Brit. J. Pharmacol., 1971, 41, 558-566.

Grumbach, L. The effect of cholinergic and cholinergic-blocking drugs on the abstinence syndrome in the rat. Fed. Proc., 1969, 28, 262.

Halbach, H., and Eddy, N.B. Tests for addiction (chronic intoxication) of morphine type. Bull. Wld. Hlth. Org., 1963, 28, 139-173.

Hanna, C. A demonstration of morphine tolerance and physical dependence in the rat. Arch. int. Pharmacodyn., 1960, 124, 326-329.

Harris, R.J. A primer of multivariate statistics. New York: Academic Press, 1975.

Himmelsbach, C.K., Gerlach, G.H., and Stanton, E.J. A method for testing addiction, tolerance, and abstinence in the rat. J. Pharmacol. exp. Ther., 1935, 53, 179-188.

Hosoya, E. Some withdrawal symptoms of rats to morphine. Pharmacologist, 1959, 1, 77.

Jori, A., Pescador, R., and Pugliatti, C. Rat strain differences in the activity of hepatic microsomal enzymes. Biochem. Pharmacol., 1971, 20, 2695-2701.

Kayan, S., and Mitchell, C.L. Studies on tolerance development to morphine: effect of the dose-interval on the development of single dose tolerance. Arch. int. Pharmacodyn., 1972, 199, 407-414.

Kaymakcalan, S., and Woods, L.A. Nalorphine-induced "Abstinence Syndrome" in morphine-tolerant albino rats. J. Pharmacol. exp. Ther., 1956, 117, 112-116.

Kerr, F.W.L., and Pozuelo, J. Suppression of physical dependence and induction of hypersensitivity to morphine by stereotaxic hypothalamic lesions in addicted rats. Proc. Mayo Clin., 1971, 46, 653-665.

Kleiber, M., and Cole, H.H. Body growth rate and metabolic rate in two inbred strains of rats. Am. J. Physiol., 1950, 161, 294-299.

Kornetsky, C., and Bain, G. Morphine: single-dose tolerance. Science, 1968, 162, 1011-1012.

Levitt, M., Spector, S., Sjoerdsma, A., and Udenfriend, S. Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea pig heart. J. Pharmacol. exp. Ther., 1965, 148, 1-8.

Lorenzetti, O.J., and, Sancilio, L.F. Morphine dependent rats as a model for evaluating potential addiction liability of analgesic compounds. Arch. int. Pharmacodyn., 1970, 183, 391-402.

Martin, W.R., Wikler, A., Eades, C.G., and Pescor, F.T. Tolerance to and physical dependence on morphine in rats. Psychopharmacologia, 1963, 4, 247-260.

Maruyama, Y., Hayashi, G., Smits, S.E., and Takemori, A.E. Studies on the relationship between 5-hydroxytryptamine turnover in brain and tolerance and physical dependence in mice. J. Pharmacol. exp. Ther., 1971, 178, 20-29.

Mercier, J., and Sestier, M.R. Determinations de L'Accoutumance a la Morphine et de L'Insulence du Sevrage en Toxique par une Methode Psychophysiologique. Ann. pharm. franc., 1954, 12, 458-465.

Miller, F.P., Cox, R.H., and Maiekel, R.P. Intrastrain differences in serotonin and norepinephrine in discrete areas of rat brain. Science, 1968, 162, 463-464.

Morrison, S.D. Differences between rat strains in metabolic activity and in control systems. Am. J. Physiol., 1973, 224, 1305-1308.

Nichols, J.R., and Hsiao, S. Addiction liability of albino rats: breeding for quantitative differences in morphine drinking. Science, 1967, 157, 561-563.

Oliverio, A. Genetic factors in the control of drug effects on the behaviour of mice. In J.H.F. van Abeelen (Ed.), The Genetics of Behaviour. New York: American Elsevier Publishing Company, Inc., 1974. Pp. 375-395.

Puri, S.K., and Lal, H. Reduced threshold to pain induced aggression specifically related to morphine dependence. Psychopharmacologia, 1974, 35, 237-241.

Rosecrans, J.A., and Schechter, M.D. Brain 5-hydroxytryptamine correlates of behavior in rats: strain and sex variability. Physiol. Behav., 1972, 8, 503-510.

Schemmel, R., Mickelsen, O., and Motawi, K. Conversion of dietary to body energy in rats as affected by strain, sex, and ration. J. Nutr., 1972, 102, 1187-1198.

Schwartz, A.S., and Eidelberg, E. Role of biogenic amines in morphine dependence. Life Sci., 1970, 9, 613-624.

Segal, D.S., Kuczenski, R.T., and Mandell, A.J. Strain differences in behavior and brain tyrosine hydroxylase activity. Behav. Biol., 1972, 7(1), 75-81.

Shen, F., Loh, H.H., and Way, E.L. Brain serotonin turnover in morphine tolerant and dependent mice. J. Pharm. exp. Ther., 1970, 175, 427-434.

Siegel, S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill Book Company, 1956.

Sloan, J.W., Brooks, J.W., Eisenman, A., and Martin, W.R. The effect of addiction to and abstinence from morphine on rat tissue catecholamine and serotonin levels. Psychopharmacologia, 1963, 4, 261-270.

Taylor, J.F. Methods of chemical analysis. In D.H. Clouet (Ed.), Narcotic drugs: biochemical pharmacology. New York: Plenum Press, 1971. Pp. 17-88.

Tilson, H.A., and Rech, R.H. The effects of p-Chlorophenylalanine on Morphine Analgesia, tolerance, and dependence development in two strains of rats. Psychopharmacologia, 1974, 35, 45-60.

Tilson, H., Rech, R., and Stolman, S. Hyperalgesia during withdrawal as a means of measuring the degree of dependence in morphine dependent rats. Psychopharmacologia, 1973, 28, 287-300.

Way, E.L., Loh, H.H., and Shen, F. Simultaneous quantitative assessment of morphine tolerance and physical dependence. J. Pharmacol. exp. Ther., 1969, 167(1), 1-8.

Wei, E. Assessment of precipitated abstinence in morphine-dependent rats. Psychopharmacologia, 1973, 28, 35-44.

Wei, E., Loh, H.H., and Way, E.L. Quantitative aspects of precipitated abstinence in morphine-dependent rats. J. Pharmacol. exp. Ther., 1973, 184, 398-403.

Wei, E., and Loh, H.H. Morphine physical dependence unaltered by previous dependence on morphine. Nature, 1972, 238, 396-397.

Wikler, A., and Pescor, F.T. Classical conditioning of a morphine abstinence phenomenon, reinforcement of opioid-drinking behavior and "relapse" in morphine-addicted rats. Psychopharmacologia, 1967, 10, 255-284.

Wilkinson, L. Response variable hypotheses in the multivariate analysis of variance. Psychol. Bull., 1975, 82, 408-412.

APPENDIX A

Mean Occurrence of Responses Showing Significant Strain Differences Prior to Naloxone Injection

Mean Weight Change (Grams) from Time of Pellet Implantation To Day of Testing (Prior to Naloxone Injection)

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	10.417	4.033	7.583	2.999	9.083	10.282
Placebo	.083	7.166	-2.333	10.500	4.500	13.695
LEW STRAIN						
Morphine	3.750	6.269	7.667	5.789	5.000	7.274
Placebo	1.917	8.785	12.750	12.969	5.417	13.734
F-344 STRAIN						
Morphine	.500	4.400	1.667	5.449	2.500	5.776
Placebo	-8.583	13.708	-2.333	2.708	-.833	8.685
HOLTZMAN STRAIN						
Morphine	10.667	13.700	6.417	21.318	2.500	23.570
Placebo	9.750	11.616	10.083	9.558	11.167	14.547
SPRAGUE-DAWLEY STRAIN						
Morphine	12.167	4.783	4.250	7.956	6.917	14.068
Placebo	3.417	11.912	10.417	13.283	17.917	12.638

Note.--Weight Change = (Weight on test day pre-naloxone) - (Original weight).

Mean Frequency of Occurrence of Vocalization on Handling
In the Ten-Minute Period Prior to Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	.000	.000	.167	.389	.083	.289
Placebo	.000	.000	.000	.000	.167	.389
LEW STRAIN						
Morphine	.000	.000	.083	.289	.000	.000
Placebo	.000	.000	.000	.000	.083	.289
F-344 STRAIN						
Morphine	.083	.289	.000	.000	.167	.389
Placebo	.083	.289	.167	.389	.250	.452
HOLTZMAN STRAIN						
Morphine	.000	.000	.000	.000	.000	.000
Placebo	.000	.000	.000	.000	.000	.000
SPRAGUE-DAWLEY STRAIN						
Morphine	.000	.000	.083	.289	.083	.289
Placebo	.167	.389	.000	.000	.167	.389

Mean Frequency of Occurrence of Escape Attempts
During the Ten-Minute Period Prior to Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	.000	.000	.000	.000	.000	.000
Placebo	.000	.000	.833	2.887	.167	.577
LEW STRAIN						
Morphine	.417	.900	.750	1.603	2.250	3.388
Placebo	.167	.577	.750	1.765	1.583	2.644
F-344 STRAIN						
Morphine	.000	.000	.000	.000	.000	.000
Placebo	.000	.000	.000	.000	.000	.000
HOLTZMAN STRAIN						
Morphine	10.500	17.794	8.167	11.456	11.333	11.972
Placebo	2.917	3.118	9.833	11.191	3.583	5.282
SPRAGUE-DAWLEY STRAIN						
Morphine	6.333	19.523	8.167	12.848	2.500	7.489
Placebo	.250	.866	.000	.000	.000	.000

Mean Frequency of Occurrence of Wet Dog Shakes
During the Ten-Minute Period Prior to Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	.000	.000	.250	.866	.417	.900
Placebo	.083	.289	.417	.900	.333	.651
LEW STRAIN						
Morphine	.000	.000	.083	.289	.000	.000
Placebo	.000	.000	.083	.289	.000	.000
F-344 STRAIN						
Morphine	.000	.000	.083	.289	.250	.622
Placebo	.083	.289	.500	.522	.167	.389
HOLTZMAN STRAIN						
Morphine	.083	.289	.417	.793	.083	.289
Placebo	1.167	1.992	1.250	1.545	.583	.793
SPRAGUE-DAWLEY STRAIN						
Morphine	.000	.000	.250	.452	.333	.492
Placebo	.083	.289	.333	.651	.333	.888

Mean Frequency of Occurrence of Teeth Chattering Episodes During the Ten-Minute Period Prior to Naloxone Injection

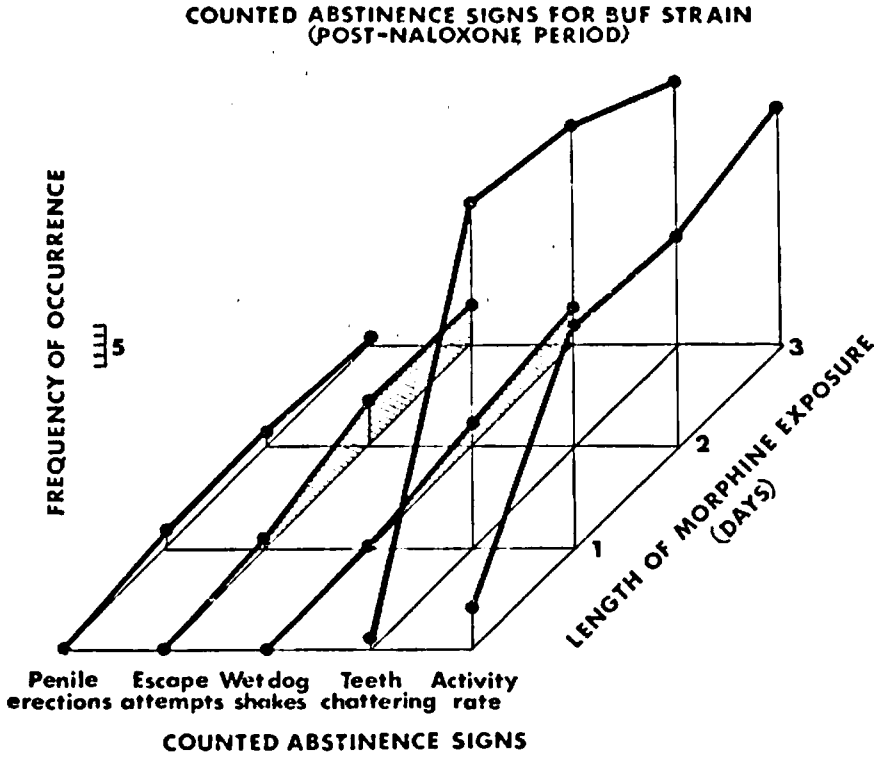
Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	.000	.000	.167	.389	.167	.577
Placebo	.833	2.329	1.417	2.968	.583	1.084
LEW STRAIN						
Morphine	.000	.000	.000	.000	.000	.000
Placebo	.333	.778	.083	.289	.333	.888
F-344 STRAIN						
Morphine	.083	.289	.000	.000	.000	.000
Placebo	.083	.289	.000	.000	.250	.622
HOLTZMAN STRAIN						
Morphine	.000	.000	.250	.866	.000	.000
Placebo	.167	.389	.833	1.403	.083	.289
SPRAGUE-DAWLEY STRAIN						
Morphine	.167	.577	.000	.000	.250	.622
Placebo	.500	1.446	.250	.866	.417	1.443

Mean Activity Rate
During the Ten-Minute Period Prior to Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	13.167	7.554	16.750	6.982	20.583	9.577
Placebo	14.083	5.299	11.833	7.650	17.167	6.631
LEW STRAIN						
Morphine	11.917	4.852	13.917	8.140	25.917	9.634
Placebo	12.083	6.288	17.083	5.977	23.833	5.750
F-344 STRAIN						
Morphine	6.500	4.719	6.167	5.340	8.417	5.567
Placebo	8.167	3.538	7.750	4.434	7.333	3.846
HOLTZMAN STRAIN						
Morphine	26.583	8.969	27.917	18.540	27.667	8.316
Placebo	22.667	3.367	24.500	11.107	23.000	5.009
SPRAGUE-DAWLEY STRAIN						
Morphine	14.083	11.813	24.417	14.406	23.667	11.130
Placebo	13.333	5.433	12.583	5.992	14.917	8.039

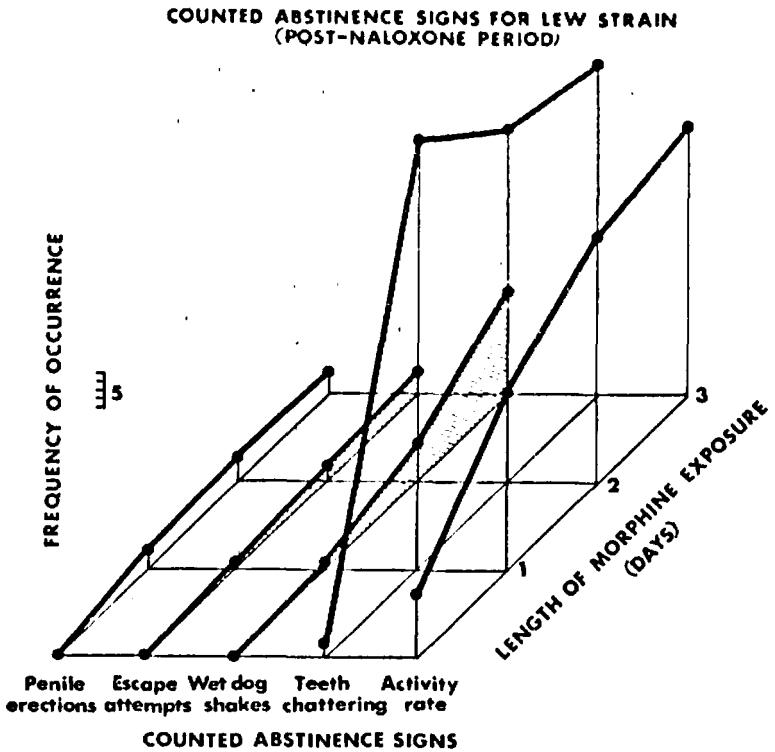
APPENDIX B

Mean Frequency of Occurrence of Counted (Type I) Withdrawal Signs During the 30-Minute Period After Injection of Naloxone



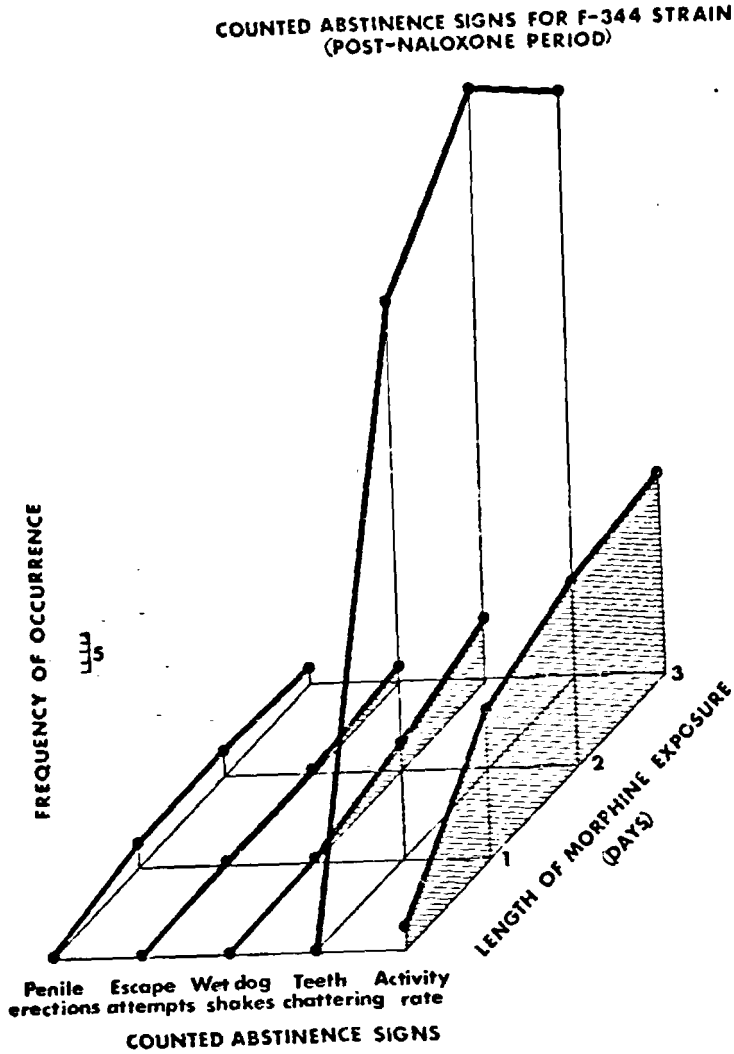
Mean Frequency of Occurrence of Counted (Type I) Abstinence Signs For BUF Strain

Ordinate presents the incidence of individual signs during the 30-minute period after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.



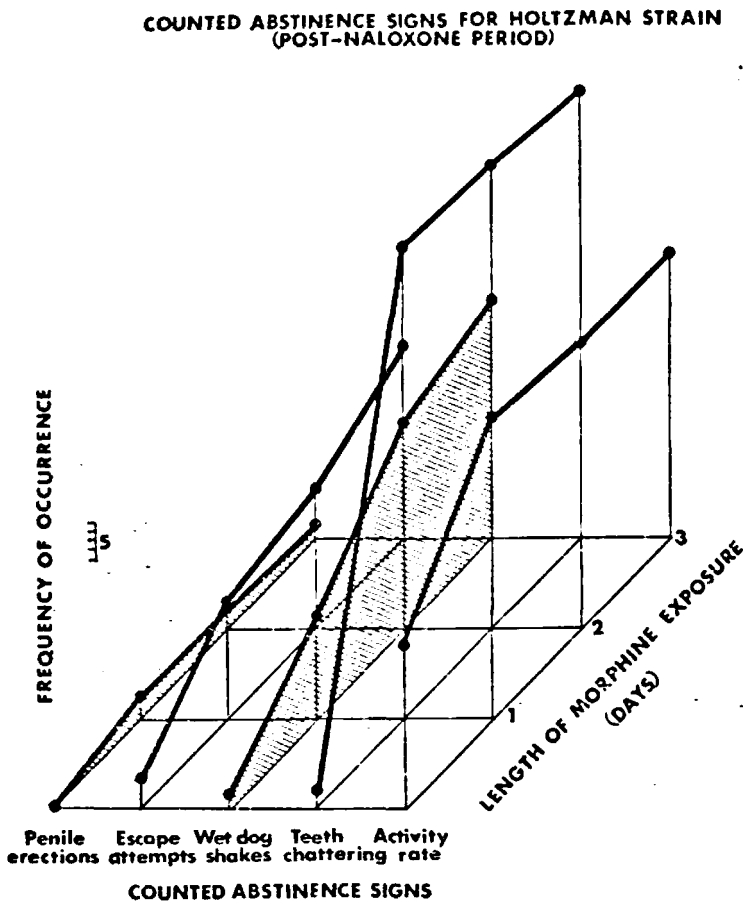
Mean Frequency of Occurrence of Counted (Type I) Abstinence Signs For LEW Strain

Ordinate presents the incidence of individual signs during the 30-minute period after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.



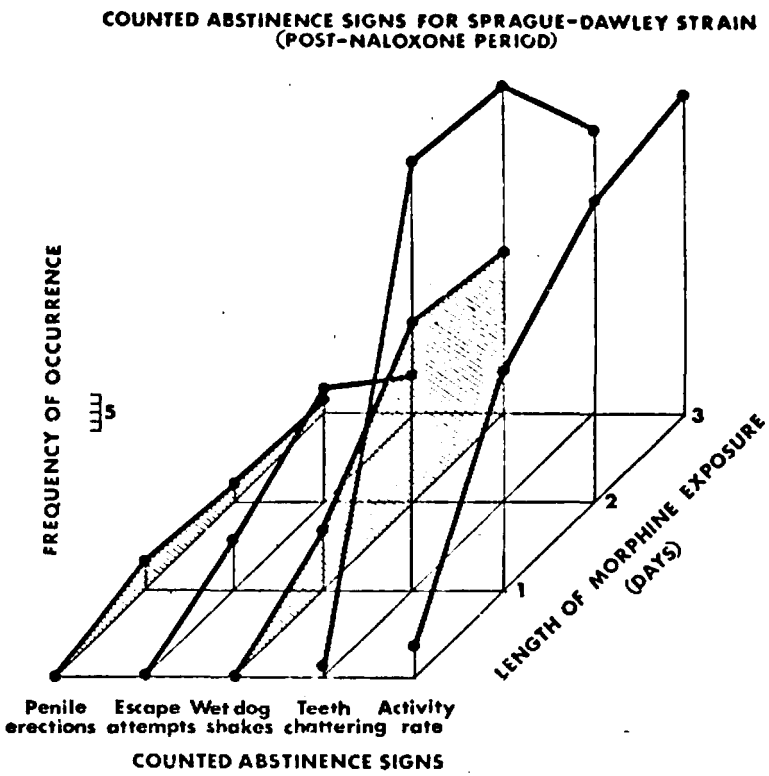
Mean Frequency of Occurrence of Counted (Type I) Abstinence Signs For F-344 Strain

Ordinate presents the incidence of individual signs during the 30-minute period after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.



Mean Frequency of Occurrence of Counted (Type I) Abstinence Signs For Holtzman Strain

Ordinate presents the incidence of individual signs during the 30-minute period after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.



Mean Frequency of Occurrence of Counted (Type I) Abstinence Signs For Sprague-Dawley Strain

Ordinate presents the incidence of individual signs during the 30-minute period after naloxone injection. Abscissa presents duration of exposure to morphine pellets in days.

APPENDIX C

Mean Frequency of Occurrence of Observed (Type II) Withdrawal Signs During the 30-Minute Period After Injection of Naloxone

Mean Weight Change (Grams) from Pre-Naloxone Period
To 30 Minutes Post-Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	-11.167	3.040	-12.333	2.570	-12.083	4.420
Placebo	-3.5	2.236	-3.167	1.850	-2.917	2.314
LEW STRAIN						
Morphine	-9.333	3.774	-11.167	1.528	-11.667	4.075
Placebo	-4.500	2.195	-3.833	2.918	-3.250	2.491
F-344 STRAIN						
Morphine	-5.917	1.782	-8.667	2.103	-9.917	2.065
Placebo	1.500	13.561	-2.833	3.040	-3.250	2.221
HOLTZMAN STRAIN						
Morphine	-11.667	4.774	-11.917	4.461	-10.750	4.413
Placebo	-4.167	2.038	-1.917	1.832	-1.750	3.137
SPRAGUE-DAWLEY STRAIN						
Morphine	-11.750	3.745	-12.583	2.234	-14.167	3.810
Placebo	-3.083	1.929	-4.000	1.809	-5.583	3.753

Note.--Weight Change = (Weight on test day post-naloxone) - (Weight on test day pre-naloxone).

Mean Frequency of Occurrence of Vocalization on Handling
During the Three Ten-Minute Periods After Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	1.083	1.084	1.000	1.128	2.500	.674
Placebo	.167	.389	.000	.000	.250	.866
LEW STRAIN						
Morphine	1.000	1.206	2.000	.953	1.667	1.155
Placebo	.000	.000	.000	.000	.000	.000
F-344 STRAIN						
Morphine	.583	.793	2.333	1.073	2.333	.985
Placebo	.333	.888	.000	.000	.583	.996
HOLTZMAN STRAIN						
Morphine	.583	.900	1.167	1.403	1.417	1.505
Placebo	.000	.000	.000	.000	.000	.000
SPRAGUE-DAWLEY STRAIN						
Morphine	1.667	1.371	2.667	.651	2.667	.888
Placebo	.083	289	.000	.000	.000	.000

Mean Frequency of Occurrence of Ptosis
During the Three Ten-Minute Periods After Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	2.917	.289	2.667	.888	2.750	.452
Placebo	.500	1.000	.417	.996	.083	.289
LEW STRAIN						
Morphine	1.500	1.446	2.000	1.044	1.500	1.168
Placebo	.083	.289	.083	.289	.000	.000
F-344 STRAIN						
Morphine	2.250	.965	2.583	.515	2.417	.996
Placebo	.083	.289	.417	.996	.333	.888
HOLTZMAN STRAIN						
Morphine	2.667	.778	2.667	.492	2.833	.389
Placebo	.083	.289	.000	.000	.000	.000
SPRAGUE-DAWLEY STRAIN						
Morphine	2.500	.522	2.583	.669	1.750	1.055
Placebo	.000	.000	.000	.000	.000	.000

Mean Frequency of Occurrence of Eye Twitching
During the Three Ten-Minute Periods After Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	1.333	.985	1.667	1.073	2.583	.669
Placebo	.000	.000	.083	.289	.250	.866
LEW STRAIN						
Morphine	1.500	1.446	2.250	1.055	1.500	1.243
Placebo	.000	.000	.167	.389	.167	.577
F-344 STRAIN						
Morphine	2.250	.754	2.417	.900	2.583	.515
Placebo	.083	.289	.083	.289	.167	.577
HOLTZMAN STRAIN						
Morphine	2.583	.900	3.000	.000	3.000	.000
Placebo	.000	.000	.000	.000	.000	.000
SPRAGUE-DAWLEY STRAIN						
Morphine	2.583	.669	3.000	.000	2.333	.985
Placebo	.000	.000	.000	.000	.000	.000

Mean Frequency of Occurrence of Lacrimation
During the Three Ten-Minute Periods After Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	.000	.000	.000	.000	.000	.000
Placebo	.000	.000	.000	.000	.000	.000
LEW STRAIN						
Morphine	.083	.289	.083	.289	.000	.000
Placebo	.000	.000	.000	.000	.000	.000
F-344 STRAIN						
Morphine	.000	.000	.333	.778	.000	.000
Placebo	.000	.000	.000	.000	.000	.000
HOLTZMAN STRAIN						
Morphine	.083	.289	.417	.900	.583	1.084
Placebo	.000	.000	.000	.000	.000	.000
SPRAGUE-DAWLEY STRAIN						
Morphine	.000	.000	.167	.577	.333	.888
Placebo	.000	.000	.000	.000	.000	.000

Mean Frequency of Occurrence of Rhinorrhea
During the Three Ten-Minute Periods After Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	1.000	1.348	.500	.798	.583	.900
Placebo	.333	.888	.167	.577	.000	.000
LEW STRAIN						
Morphine	.250	.452	.000	.000	.000	.000
Placebo	.000	.000	.000	.000	.000	.000
F-344 STRAIN						
Morphine	.167	.577	.167	.577	.083	.289
Placebo	.000	.000	.083	.289	.250	.866
HOLTZMAN STRAIN						
Morphine	.667	.888	.750	.965	1.083	1.165
Placebo	.083	.289	.000	.000	.000	.000
SPRAGUE-DAWLEY STRAIN						
Morphine	1.083	1.165	1.167	1.193	1.250	1.357
Placebo	.083	.289	.083	.289	.000	.000

Mean Frequency of Occurrence of Diarrhea
During the Three Ten-Minute Periods After Naloxone Injection

Pellet Type	Length of Pellet Exposure (Days)					
	1		2		3	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
BUF STRAIN						
Morphine	2.500	.674	2.167	1.030	2.750	.452
Placebo	.000	.000	.000	.000	.000	.000
LEW STRAIN						
Morphine	1.417	1.240	2.333	.985	2.083	.996
Placebo	.000	.000	.000	.000	.167	.577
F-344 STRAIN						
Morphine	1.167	.835	1.833	1.030	2.500	.798
Placebo	.000	.000	.000	.000	.000	.000
HOLTZMAN STRAIN						
Morphine	1.583	1.084	1.750	.866	2.250	.866
Placebo	.167	.389	.000	.000	.000	.000
SPRAGUE-DAWLEY STRAIN						
Morphine	2.417	.515	2.417	.669	2.583	.515
Placebo	.167	.389	.083	.289	.000	.000