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FAILURE TO REPLICATE AN ENVIRONMENTAL EFFECT OF MORPHINE HYDROCHLORIDE CONSUMPTION: A POSSIBLE PSYCHOPHARMACOGENETIC LINK

by

Bruce Fraser Petrie

B.A., Brock University, 1977

M.A., University of Guelph, 1979

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ABSTRACT

The role of differential housing on sucrose-morphine consumption in Wistar rats was investigated in two studies. The results of earlier studies indicating rats housed in a quasi-natural colony drank significantly less sucrose-morphine than rats isolated in standard laboratory cages could not be replicated. The reason for the nonreplications is the reduced consumption of sucrose-morphine by the isolated animals in the present two studies, a phenomenon noted by other Canadian psychopharmacologists using the same outbred rat strain. The possibility exists that during a colony conversion the supplier inadvertently introduced strain differences making the present rats more resistant to xenobiotic consumption. Discussion documents the role of genetics in morphine consumption, and suggests future basic psychopharmacological work be conducted with inbred animals.

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A. Introduction

Rodent self-administration opioid research has not systematically investigated the role of genetics as a variable in drug consumption. Rat research has typically used outbred animals, the rationale being that the large gene pool from which these rats are drawn approximates the human condition. The research reported in this dissertation indicates that genotype may be a more important variable in drug consumption than has been previously suspected. It is probable therefore that the use of outbred animals in pharmacological research may not be advantageous if parsimonious data interpretations are required.

In recent years self injection and oral ingestion procedures have been developed for investigating opiate self-administration in laboratory rats.

Nichols (1965) attempted to induce individually caged rats to drink a 0.5 mg./ml. morphine solution when offered a plain tap-water alternative. When the rats did not drink the morphine solution, Nichols gave them daily injections of morphine for 25 days. Following these injections, the rats entered withdrawal and simultaneously were deprived of all liquids for 24 hours. They were then offered the morphine solution alone and under the circumstances they drank it.

Nichols (1965) then set up a cyclical pattern in his experimental group. Since withdrawal symptoms reach a peak about

3 days after the last opiate intake, the cyclical training procedure made morphine available every 3rd day for 30 days. To prevent dehydration, Nichols supplied the rats with water for 24 hours after opiate intake. Therefore each 3 day cycle consisted of one day on no liquids, one day on morphine solution (0.5 mg./ml.) and one day on water.

Nichols (1965) measured opiate preference by means of choice tests given after five training cycles (15 days) and again after ten cycles (30 days). He retested his animals 14 and 49 days after the training sessions stopped. During all choice tests the rats increased their consumption of morphine even though they had the option of drinking plain tap water. This preference was maintained even after withdrawal symptoms had subsided, i.e., on trials held 14 and 49 days after the training sessions stopped.

This increase of opiate consumption by animals placed on the 3 day cycle was not found in the control animals that were placed on a 3 day cycle that did not include the aqueous morphine solution. These animals received tap water on two of the three days and no fluid on the third day. They were, however, given injections with the same amount of morphine that arbitrarily paired rats in the experimental group drank. Intake of morphine was therefore identical for the two groups, but the rats in the control group did not act to secure it and for them no opiate-intake response was reinforced (Nichols, 1965).

Nichols believed that the differences in aqueous morphine consumption in the two groups reflected the method in which the morphine was obtained. If an animal drank the fluid and in that way actively reduced its withdrawal symptoms, that behaviour would be reinforced and would increase in frequency when the same situation occurred again. On the other hand, if a rat did not actively reduce its withdrawal symptoms by drinking the morphine solution, but instead had those symptoms alleviated by an injection which it did not control, there would be no reinforcement by the withdrawal alleviating properties of the aqueous morphine solution.

Hence, the key to the increase in morphine drinking behaviour according to Nichols (1965) is the active reinforcement of that behaviour in alleviating withdrawal symptoms.

Another method used to induce rats to self-administer morphine was reported by Khavari, Peters, Baity and Wilson (1975), who found that rats would voluntarily drink large quantites of sucrose-morphine solutions in preference to water. They randomly assigned 60 male Sprague-Dawley rats to six equal groups and housed them in standard laboratory cages. Each group of ten rats was randomly assigned to a particular sucrose-morphine solution. One of the two bottles for each group contained water while the other bottle contained sucrose-morphine. Morphine concentrations were 0.125; 0.25; 0.5; 1.0; 1.5; and 2.0 mg. of morphine hydrochloride per milliliter

of the 10% sucrose solution. All six groups were maintained in this condition, 24 hours per day, for 17 days.

During the 17 day free choice phase none of the three higher morphine concentration (1.0; 1.5; and 2.0 mg./ml.) groups ingested appreciable quantities of their sucrose-morphine solutions.

In the three lower concentration (0.125; 0.25; and 0.5 mg./ml.) groups however, there was an increase in sucrose-morphine intake during the 17 day choice period. The water intake of these three groups declined over the same period.

Khavari et al. (1975) believed the morphine concentrations of 1.0 mg./ml. and higher were too unpalatable for even a 10% sucrose solution to serve as a reinforcing or enticing agent. Khavari et al. (1975) indicated that rats show a clear preference for a 10% sucrose solution containing as much as 0.5 mg./ml. of morphine hydrochloride, over tap water. They concluded that the 10% sucrose solution allowed the rats to voluntarily ingest high quantities of morphine without any premedication or forced consumption.

Weeks and Collins (1979) allowed female rats, of Sprague-Dawley origin, to intravenously inject themselves with morphine sulfate. Eight groups of naive rats, at least ten per group, were offered 24 hour access to morphine doses that ranged from 0.0032 to 10 mg./kg. for six days. On day seven saline was substituted for morphine and the change in weight recorded. Loss

of weight was taken as an indicator of physical dependence. A control group of 28 rats received saline only for seven days.

Weeks and Collins (1979) found that the majority of animals who were self-administering 0.032 mg./kg. or more per injection showed physiological evidence of dependence. The amount of morphine injected averaged from 10.2 to 233 mg./kg./day. Weeks and Collins (1979) noted that there was a negative relationship between the number of injections per day and the amount of morphine sulfate administered per injection. This suggests that the animals were self-monitoring their intake and were adjusting their response rates to maintain their physiological condition and prevent withdrawal.

Therefore, in less than seven days these previously naive rats could be made physiologically dependent on morphine sulfate when allowed to self inject the drug at their own discretion.

The above studies indicate that rats can be induced to self-administer morphine by injection followed by a training procedure that associates oral morphine ingestion with relief of withdrawal symptoms; by presenting morphine in a sucrose vehicle; and by a device that allows the animals to self-inject via an indwelling catheter. The animals show an ability to monitor their drug intake and increase their consumption of the opioid over water as the time spent in the drug consuming condition lengthens.

Numerous other studies have also found that rats self-administer opiates in large quantities and become

physiologically dependent rapidly (Carroll and Meisch, 1979; Khavari and Risner, 1972; Risner and Khavari, 1973; Wikler, Pescor, Miller and Norrell, 1971; Wikler and Pescor, 1967; Wikler, Martin, Pescor, and Eades, 1963).

These findings are often taken to suggest that mammals in general have a natural affinity for opiates. Goldstein asserts "that becoming addicted requires nothing more than availability of the drug, (and) opportunity for its use. Such addicted animals will inject an opiate even in preference to the usual instinctive behaviours that satisfy hunger, thirst, or sexual need" (Goldstein, 1972). "If heroin were universally available and there were no constraints on its use, it is probable that heroin addiction would be very much more prevalent than it is now" (Goldstein, 1976).

The one feature that the previous animal studies have in common is that the rats used as subjects were all housed in standard laboratory cages during their exposure to the opiates. Lore and Flannelly (1977) however, have indicated that rats have highly complex social interactions, are curious, gregarious, wide ranging, and well adapted to group living. These social attributes would appear to be greatly curtailed in an isolated existence, such as the type found in standard laboratory cages. Contrary to Goldstein's natural affinity hypothesis, there is a possibility that this social isolation may account for the opiate consumption found in the previous rodent studies.

This rationale was reponsible for the formation of the Drug Studies Laboratory at Simon Fraser University in 1976. This laboratory is testing the effects of different environments on opioid drug self-administration in animals. To that end, "Rat Park" was developed as a research area in which one room houses rats isolated in metal cages, while a second room holds a colony of animals.

Drinking done by the caged animals is measured by attaching two bottles to the front of each cage - one bottle containing water, the other containing the opioid solution - and weighing each bottle daily. Bottles attached to two empty control cages allow the calculation of the amount of spillage and evaporation that might occur in a 24 hour period.

The measurement of drinking by colony rats is more complex. When "Rat Park" was begun in 1976, a system was developed to measure oral drug consumption by individual rats housed in the colony with a common drinking source. To drink, each animal entered a Plexiglas runway (inside measurements: 4.7 cm. x 5.8 cm. x 24 cm.), triggering a video system that recorded the rats' identifying dye mark, and its consumption of each of two liquids. Data for 24 hours were collected on a one-hour video tape. The rats learned to operate this system rapidly (Coambs, Alexander, Davis, Hadaway, and Tressel, 1980).

The first "Rat Park" study was designed to examine the effect of housing conditions on morphine self-administration in 32 Wistar rats purchased from Charles River Canada, Inc. The

animals were raised from weaning in their respective environments and were 103-107 days of age at the beginning of the experiment. Rats isolated in standard laboratory cages (18 x 25 x 18 cm.) and colony rats living together in an open-topped wooden box (floor area: 8.8 m^2) were given morphine hydrochloride in tap water (0.5 mg./ml.) as their only source of fluid for 57 days. At the conclusion of the 57 day forced consumption period, the animals were exposed to the series of three day cycles (morphine; water; no fluid; etc.) shown by Nichols (1965) to increase self-administration of morphine in caged rats. During morphine/water choice days late in the period of forced consumption and between the Nichols' cycles, the isolated rats drank significantly more morphine solution than the colony rats, and the females drank significantly more than the males. During the four choice days in the Nichols Cycle Period, the isolated rats slightly increased their consumption of morphine but the colony animals decreased theirs (Alexander, Coambs, and Hadaway, 1978).

The next "Rat Park" study used 36 Wistar rats purchased from Charles River Canada, Inc. to compare morphine consumption of rats in the colony versus isolated animals using rats that had no prior exposure to morphine. The rats were raised from weaning in their respective environments. At 85-87 days of age all animals were given a choice between water and progressively more palatable sucrose-morphine solutions.

Once again, the isolated rats drank significantly more of the sucrose-morphine solution, and females drank significantly more than males. In the experimental phase during which sucrose-morphine solution consumption was greatest, the isolated males drank 16 times as much, and the isolated females five times as much sucrose-morphine (mg./kg.) as the colony males and females respectively (Hadaway, Alexander, Coambs, and Beyerstein, 1979).

A third "Rat Park" study used 32 Wistar rats purchased from Charles River. The animals were raised from weaning either in isolation or in the colony. At 65 days of age (ie. 43 days in their condition), half the rats in each environment were moved to the other. At 80 days of age (ie. for the rats who were moved 15 days in their new environment, for the animals who were not moved 58 days in their condition) the animals were given continuous access to water and to a sequence of progressively more palatable sucrose-morphine solutions.

Rats isolated in cages at the time of testing drank more sucrose-morphine solution than colony rats. Colony dwelling rats previously housed in isolation tended to drink more sucrose-morphine solution than those housed in the colony since weaning; however, this effect reached statistical significance only at the lowest concentration of morphine (Alexander, Beyerstein, Hadaway, and Coambs, 1981).

Taken together, these three "Rat Park" studies suggest that consumption of opiates by animals in self-administration studies

may be strongly facilitated by the typical isolated housing conditions present during intake testing. Generalizations from such experiments should be qualified by this possibility. The three "Rat Park" studies indicate that rats that are allowed to pursue a quasi-natural existence consume much less morphine than do isolated animals, even if that morphine is contained in a 10% sucrose vehicle - a vehicle that rats drink in large quantities when it is free of morphine.

During 1981 and 1982 "Rat Park's" colony area was rebuilt and a new computer-controlled system was installed to replace the older, worn out data collecting apparatus.

The first study reported in this dissertation was designed to examine the computerized system's capability for data collection by comparing morphine consumption of rats in the colony versus isolated animals. These rats had no prior exposure to morphine, and all the animals were given the choice between water and progressively more palatable sucrose-morphine solutions.

B. Method

Subjects

There were 10 male and 10 female Wistar rats of Charles River Canada Inc. origin, in both the isolated and colony groups. The animals were raised from weaning (21 days of age) in their respective environments, and were 113 days of age when the experiment started.

Within the colony one male rat died before intake testing began. A female animal was removed prior to intake testing to maintain a one to one gender ratio. A second female rat died after giving birth at the start of the fourth phase of the study. There were no deaths in the isolated group.

Apparatus

Isolated rats were housed from weaning in standard 18 x 25 x 18 cm. rat cages with sheet metal walls that prevented visual contact with adjacent animals. In order to collect wastes, paper was placed on trays under the cages. These rats received fluids through stainless steel drinking tubes from plastic bottles (Girton, Millville, P.A.) fastened on the outside of each cage. Purina Rat Chow was provided ad libitum by means of inside

feeders (11 x 13 x 5.5 cm). Bottles attached to 2 empty control cages allowed for the calculation of spillage and evaporation that might occur in a 24 hour period.

Colony rats lived together from weaning in an open-topped wooden box with a floor area of 8.8 m^2. The box contained a layer of kiln dried cedar shavings (Hyon Bedding) and two large open topped metal cages (40 x 25 x 18 cm.) from which two feeders (24 x 12.5 x 5 cm.) containing Purina Rat Chow were hung. The animals had continuous access to a common drinking source. To drink, each animal climbed a pole (41 cm. long; 4 cm. circumference), triggering a video recording of that rats' identifying hair dye mark (L'Oreal Excellence - Napoli Black. Cosmair Canada, Inc.) and drank from one of two nipples (Edstrom Industries Inc. A 115 Adjustable Flow Valve - #10443). The system noted the weight of each of two fluids consumed to 0.1 gram resolution for each visit to the site by a rat. The time and duration of the visit were also recorded. Rats learned to operate the system within three days of its introduction to the colony.

The Apple II Plus computer controlled apparatus was designed for unattended operation, continually monitoring the performance of several essential components (2 pumps; 2 solenoids; 2 scales; printer status; disk condition-free space; input/output errors; amount of videotape remaining; site and computer AC power; and power and integrity of the interface).

The status of each device in the system is maintained on a three point scale, labelled green, yellow, and red. Fail counts for each device are also logged. Green status signifies normal operation. Yellow status implies performance is below normal levels but within parameters that allow for the continuation of the system in that measures can be accurately taken. Red status indicates that a critical element has failed completely or is performing so inadequately that measures taken would be unreliable. When red status is attained system operation is suspended (Petrie, Gabert, Toms, Tressel, Alexander & Beyerstein, in press).

The white fluorescent lighting in both environments was on a 12 hour light-dark cycle controlled by a single timer (Tork Time Switch Model 7102). Red lights (Sylvania 25 and 60 watt bulbs) were on in both environments at all times.

Procedure

The animals were placed in individual cages or the colony at 21 days of age. At 86 days of age the colony rats were dye marked for identification and at 99 days of age the control animals had a second fluid bottle attached to their cages. Intake testing began at 113 days of age, and all rats were weighed at 114 days of age. Intake testing concluded when the animals were 141 days of age and all rats were weighed again and killed at 143 days of age.

During intake testing all animals were given 24 hour access to tapwater and the experimental fluid alternative. All phases were four days in length. Unlike experiments with the older apparatus, no data were lost due to malfunctioning equipment.

The first and seventh phases provided access to tapwater and to a 10% sucrose solution to determine if housing conditions had any effect on consumption of sucrose.

The second phase compared the intake of tapwater to that of a 0.05 mg. quinine sulfate per millilitre 10% sucrose solution to check for the effects of housing on preference for bitter-sweet solutions. To the palate, this sucrose-quinine solution tasted the same as the sucrose-morphine solution used in the 0.25 morphine hydrochloride (MHCl) phase.

Phases three through six entailed continuous access to water and to progressively decreasing concentrations of MHCl in 10% sucrose. Phase three included 1.0 mg. MHCl per ml. water containing 10% sucrose. Phase four consisted of 0.5 mg. MHCl per ml. water containing 10% sucrose. Phase five involved 0.25 mg. MHCl per ml. water containing 10% sucrose. Phase six comprised 0.125 mg. MHCl per ml. water containing 10% sucrose.

Left-right positions of water and the experimental fluid were reversed every two days in both environments.

C. Results

Two way repeated measures analyses of variance (ANOVA's) were carried out separately for each phase on the proportion of experimental fluid to total fluid consumed; on milligrams of experimental substance ingested per kilogram of body weight; on grams of experimental fluid consumed; and on total fluid consumption (grams). Significant interactions were analyzed using a Newman-Keuls a posteriori comparison (Ferguson, 1971).

For the sake of clarity of exposition, the phase analyses are presented separately.

Phase 1

The analyses of variance source tables for Phase 1 are given in Tables 1-4.

The female rats consumed significantly more mg./kg. of sucrose than did the male rats ($F(1,34) = 15.8$, $p < .001$).

A significant housing x gender interaction was found in the grams of sucrose consumed. A Newman-Keuls test showed that the colony females drank significantly more grams of sucrose than did the colony males (df. 34, $p < .05$).

A significant housing x gender interaction was found in total fluid consumption. A Newman-Keuls test showed that the colony females drank significantly more total fluid than did the colony males (df. 34, $p < .05$).

Table 1

Data Summary and Two Way Analysis of Variance on Proportion of 10% Sucrose-Water to Water Consumed in Phase #1 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	0.071	0.071	2.36	N.S.
Gender	1	0.039	0.039	1.30	N.S.
Housing x Gender	1	0.037	0.037	1.21	N.S.
Error	34	1.024	0.030		
Total	37	1.171			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9 $\bar{x}$=3.9 s=0.03	N=10 $\bar{x}$=3.9 s=0.08
Females	N=9 $\bar{x}$=3.9 s=0.03	N=10 $\bar{x}$=3.8 s=0.31

Table 2

Data Summary and Two Way Analysis of Variance on Milligrams of 10% Sucrose Ingested per Kilogram of Body Weight in Phase #1 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	369.575	369.575	0.19	N.S.
Gender	1	31639.975	31639.975	15.83	.001
Housing x Gender	1	476.688	476.688	0.24	N.S.
Error	34	67961.844	1998.878		
Total	37	100448.082			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=93.0	$\bar{x}$=93.9
	s=20.12	s=18.53
	N=9	N=10
Females	$\bar{x}$=158.2	$\bar{x}$=144.9
	s=70.07	s=40.87

Table 3

Data Summary and Two Way Analysis of Variance on Grams of 10% Sucrose-Water Consumed in Phase #1 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	3884.164	3884.164	0.21	N.S.
Gender	1	7518.165	7518.165	0.40	N.S.
Housing x Gender	1	89812.037	89812.037	4.80	.05
Error	34	635801.703	18700.050		
Total	37	737016.069			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=367.7	$\bar{x}$=444.8
	s=60.65	s=73.89
	N=9	N=10
Females	$\bar{x}$=498.3	$\bar{x}$=380.7
	s=218.34	s=109.11

Table 4

Data Summary and Two Way Analysis of Variance on Grams of Total Fluid Consumption in Phase #1 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	2187.680	2187.680	0.12	N.S.
Gender	1	8746.112	8746.112	0.47	N.S.
Housing x Gender	1	88165.456	88165.456	4.72	.05
Error	34	635678.995	18696.441		
Total	37	734778.243			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=373.1	$\bar{x}$=454.3
	s=61.18	s=73.11
	N=9	N=10
Females	$\bar{x}$=504.9	$\bar{x}$=393.3
	s=219.13	s=107.88

Phase 2

The analyses of variance source tables for Phase 2 are given in Tables 5-8.

Colony animals consumed significantly more quinine sulfate in proportion to water drunk than did the isolated animals ($F(1,34) = 32.6$, $p < .001$).

Colony animals drank significantly more mg./kg. of quinine sulfate than did the isolated animals ($F(1,34) = 14.0$, $p < .001$).

Colony animals drank significantly more grams of quinine sulfate than did the isolated animals ($F(1,34) = 18.8$, $p < .001$).

Colony animals consumed significantly more fluid than did the isolated animals ($F(1,34) = 6.3$, $p < .025$).

There were no significant housing x gender interactions found in this phase of the experiment.

Phase 3

The analyses of variance source tables for Phase 3 are given in Tables 9-12.

Colony animals consumed significantly more of the 1.0 mg. MHCl/ml. water in 10% sucrose solution in proportion to water drunk than did the isolated animals ($F(1,34) = 55.2$, $p <$

Table 5

Data Summary and Two Way Analysis of Variance on Proportion of QSO_4-Water to Water Consumed in Phase #2 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	34.877	34.877	32.57	.001
Gender	1	2.595	2.595	2.42	N.S.
Housing x Gender	1	0.079	0.079	0.07	N.S.
Error	34	36.439	1.072		
Total	37	73.991			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=3.9	$\bar{x}$=2.1
	s=0.05	s=1.44
	N=9	N=10
Females	$\bar{x}$=3.5	$\bar{x}$=1.5
	s=0.74	s=1.04

Table 6

Data Summary and Two Way Analysis of Variance on Milligrams of QSO_4 Ingested per Kilogram of Body Weight in Phase #2 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	5868.014	5868.014	14.04	.001
Gender	1	963.045	963.045	2.30	N.S.
Housing x Gender	1	72.924	72.924	0.17	N.S.
Error	34	14213.696	418.049		
Total	37	21117.678			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=37.8	$\bar{x}$=15.7
	s=6.86	s=12.39
Females	N=9	N=10
	$\bar{x}$=50.8	$\bar{x}$=23.2
	s=24.67	s=26.03

Table 7

Data Summary and Two Way Analysis of Variance on Grams of QSO_4-Water Consumed in Phase #2 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	706.025	706.025	18.77	.001
Gender	1	1.901	1.901	0.05	N.S.
Housing x Gender	1	11.475	11.475	0.31	N.S.
Error	34	1278.845	37.613		
Total	37	1998.246			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=300.2	$\bar{x}$=148.2
	s=47.22	s=118.61
Females	N=9	N=10
	$\bar{x}$=314.2	$\bar{x}$=118.8
	s=141.38	s=130.71

Table 8

Data Summary and Two Way Analysis of Variance on Grams of Total Fluid Consumption in Phase #2 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	77304.414	77304.414	6.31	.025
Gender	1	6502.853	6502.853	0.53	N.S.
Housing x Gender	1	5527.299	5527.299	0.45	N.S.
Error	34	416784.532	12258.369		
Total	37	506119.098			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=303.7	$\bar{x}$=237.5
	s=47.78	s=72.44
	N=9	N=10
Females	$\bar{x}$=355.3	$\bar{x}$=240.8
	s=127.87	s=140.22

Table 9

Data Summary and Two Way Analysis of Variance on Proportion of MHCl-Water to Water Consumed in Phase #3 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	0.511	0.511	55.19	.001
Gender	1	0.017	0.017	1.82	N.S.
Housing x Gender	1	0.019	0.019	2.02	N.S.
Error	34	0.315	0.009		
Total	37	0.862			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=0.19	$\bar{x}$=0.01
	s=0.06	s=0.01
	N=9	N=10
Females	$\bar{x}$=0.29	$\bar{x}$=0.01
	s=0.18	s=0.01

Table 10

Data Summary and Two Way Analysis of Variance on Milligrams of MHCl Ingested per Kilogram of Body Weight in Phase #3 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	10830.339	10830.339	78.80	.001
Gender	1	834.071	834.071	6.07	.025
Housing x Gender	1	817.979	817.979	5.95	.025
Error	34	4672.962	137.440		
Total	37	17155.352			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=25.2	$\bar{x}$=0.7
	s=7.03	s=0.78
	N=9	N=10
Females	$\bar{x}$=44.4	$\bar{x}$=1.3
	s=21.61	s=1.46

Table 11

Data Summary and Two Way Analysis of Variance on Grams of MHCl-Water Consumed in Phase #3 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	1302.648	1302.648	80.98	.001
Gender	1	35.252	35.252	2.19	N.S.
Housing x Gender	1	39.576	39.576	2.46	N.S.
Error	34	546.941	16.087		
Total	37	1924.416			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=10.0	$\bar{x}$=0.3
	s=2.65	s=0.38
	N=9	N=10
Females	$\bar{x}$=14.1	$\bar{x}$=0.3
	s=7.31	s=0.38

Table 12

Data Summary and Two Way Analysis of Variance on Grams of Total Fluid Consumption in Phase #3 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	73338.276	73338.276	11.43	.005
Gender	1	183.920	183.920	0.03	N.S.
Housing x Gender	1	28850.427	28850.427	4.50	.05
Error	34	218107.725	6414.933		
Total	37	320480.348			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9 $\bar{x}$=214.9 s=36.36	N=10 $\bar{x}$=182.1 s=57.98
Females	N=9 $\bar{x}$=277.4 s=130.59	N=10 $\bar{x}$=134.2 s=43.72

.001).

Colony animals consumed significantly more mg./kg. of this solution than did the isolated animals ($F(1,34) = 78.8$, $p < .001$).

Female rats consumed significantly more mg./kg. of this solution than did the male animals ($F(1,34) = 6.1$, $p < .025$).

A significant housing x gender interaction was found in mg./kg. consumption. A Newman-Keuls test showed that the colony females drank significantly more than did isolated males (df. 34, $p < .01$); isolated females (df. 34, $p < .01$) and colony males (df. 34, $p < .01$). In addition colony males drank significantly more than did isolated males (df. 34, $p < .01$) and isolated females (df. 34, $p < .01$).

Colony animals consumed significantly more grams of the 1.0 MHCl sucrose solution than did the isolated animals ($F(1,34) = 80.98$, $p < .001$).

Colony animals consumed significantly more fluid in this phase than did the isolated animals ($F(1,34) = 11.4$, $p < .005$).

A significant housing x gender interaction was found in total fluid consumption. A Newman-Keuls test revealed that the colony females drank significantly more total fluid than did the isolated females (df. 34, $p < .01$) and the isolated males (df. 34, $p < .05$). Colony males consumed significantly more total fluid than did the isolated females (df. 34, $p < .05$).

Phase 4

The analyses of variance source tables for Phase 4 are given in Tables 13-16.

Females consumed significantly more of the 0.5 mg. MHCl/ml. water in 10% sucrose solution in proportion to water drunk than did the males ($F(1,33) = 4.5$, $p < .05$).

Females consumed significantly more mg./kg. of this solution than did the males ($F(1,33) = 12.1$, $p < .005$).

Females consumed significantly more grams of the 0.5 mg. MHCl/ml. water in 10% sucrose solution than did the males ($F(1,33) = 8.4$, $p < .01$).

Colony animals consumed significantly more fluid in this phase than did the isolated animals ($F(1,33) = 17.5$, $p < .001$).

A significant housing x gender interaction was found in total fluid consumption. A Newman-Keuls test showed that colony females consumed significantly more total fluid than did isolated females (df. 33, $p < .01$); isolated males (df. 33, $p < .01$); and colony males (df. 33, $p < .05$). Colony males consumed significantly more total fluid than did isolated females (df. 33, $p < .05$).

Table 13

Data Summary and Two Way Analysis of Variance on Proportion of MHCl-Water to Water Consumed in Phase #4 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	0.001	0.001	0.23	N.S.
Gender	1	0.026	0.026	4.53	.05
Housing x Gender	1	0.004	0.004	0.76	N.S.
Error	33	0.186	0.006		
Total	36	0.218			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=0.04	$\bar{x}$=0.05
	s=0.01	s=0.06
	N=8	N=10
Females	$\bar{x}$=0.12	$\bar{x}$=0.08
	s=0.09	s=0.09

Table 14

Data Summary and Two Way Analysis of Variance on Milligrams of MHCl Ingested per Kilogram of Body Weight in Phase #4 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	92.478	92.478	1.75	N.S.
Gender	1	641.171	641.171	12.12	.005
Housing x Gender	1	111.011	111.011	2.10	N.S.
Error	33	1746.380	52.921		
Total	36	2591.041			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=2.6	$\bar{x}$=2.3
	s=0.82	s=2.82
	N=8	N=10
Females	$\bar{x}$=14.5	$\bar{x}$=7.7
	s=10.24	s=9.07

Table 15

Data Summary and Two Way Analysis of Variance on Grams of MHCl-Water Consumed in Phase #4 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	10.748	10.748	2.16	N.S.
Gender	1	41.756	41.756	8.40	.01
Housing x Gender	1	15.184	15.184	3.06	N.S.
Error	33	164.020	4.970		
Total	36	231.708			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=1.9	$\bar{x}$=2.1
	s=0.72	s=2.59
Females	N=8	N=10
	$\bar{x}$=8.9	$\bar{x}$=4.1
	s=6.42	s=5.01

Table 16

Data Summary and Two Way Analysis of Variance on Grams of Total Fluid Consumption in Phase #4 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	148724.306	148724.306	17.53	.001
Gender	1	8677.679	8677.679	1.02	N.S.
Housing x Gender	1	51566.734	51566.734	6.08	.025
Error	33	280015.774	8485.327		
Total	36	488984.493			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=254.5	$\bar{x}$=197.3
	s=46.03	s=71.40
Females	N=8	N=10
	$\bar{x}$=368.3	$\bar{x}$=164.4
	s=148.52	s=57.87

Phase 5

The analyses of variance source tables for Phase 5 are given in Tables 17-20.

Females consumed significantly more mg./kg. of the 0.25 mg. MHCl/ml. water in 10% sucrose solution than did the males ($F(1,33) = 4.7$, $p < .05$).

No other testings differed significantly from each other in this phase.

Phase 6

The analyses of variance source tables for Phase 6 are given in Tables 21-24.

Females consumed significantly more mg./kg. of the 0.125 mg. MHCl/ml. water in 10% sucrose solution than did the males ($F(1,33) = 5.7$, $p < .025$).

No other testings differed significantly from each other in this phase.

Phase 7

The analyses of variance source tables for Phase 7 are given in Tables 25-28.

Colony animals consumed significantly more of the 10% sucrose solution in proportion to water than did the isolated

Table 17

Data Summary and Two Way Analysis of Variance on Proportion of MHCl-Water to Water Consumed in Phase #5 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	0.183	0.183	0.17	N.S.
Gender	1	1.701	1.701	1.62	N.S.
Housing x Gender	1	3.658	3.658	3.47	N.S.
Error	33	34.772	1.054		
Total	36	40.315			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=0.7	$\bar{x}$=1.1
	s=0.51	s=0.92
	N=8	N=10
Females	$\bar{x}$=1.8	$\bar{x}$=0.9
	s=1.31	s=1.01

Table 18

Data Summary and Two Way Analysis of Variance on Milligrams of MHCl Ingested per Kilogram of Body Weight in Phase #5 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	1176.301	1176.301	0.31	N.S.
Gender	1	17955.381	17955.381	4.74	.05
Housing x Gender	1	7669.746	7669.746	2.03	N.S.
Error	33	124952.334	3786.434		
Total	36	151753.763			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=21.0	$\bar{x}$=35.9
	s=15.72	s=39.89
Females	N=8	N=10
	$\bar{x}$=96.2	$\bar{x}$=54.3
	s=81.47	s=73.29

Table 19

Data Summary and Two Way Analysis of Variance on Grams of MHCl-Water Consumed in Phase #5 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	56.866	56.866	0.15	N.S.
Gender	1	614.841	614.841	1.61	N.S.
Housing x Gender	1	1487.668	1487.668	3.90	N.S.
Error	33	12594.418	381.649		
Total	36	14753.793			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=31.8	$\bar{x}$=70.1
	s=30.50	s=78.99
Females	N=8	N=10
	$\bar{x}$=119.8	$\bar{x}$=56.5
	s=100.01	x=74.48

Table 20

Data Summary and Two Way Analysis of Variance on Grams of Total Fluid Consumption in Phase #5 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	47936.903	47936.903	2.61	N.S.
Gender	1	11.405	11.405	0.01	N.S.
Housing x Gender	1	43173.199	43173.199	2.35	N.S.
Error	33	607062.477	18395.833		
Total	36	698183.984			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=234.7	$\bar{x}$=228.8
	s=37.15	s=200.67
	N=8	N=10
Females	$\bar{x}$=312.2	$\bar{x}$=169.1
	s=141.49	s=56.39

Table 21

Data Summary and Two Way Analysis of Variance on Proportion of MHCl-Water to Water Consumed in Phase #6 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	6.984	6.984	3.94	N.S.
Gender	1	0.191	0.191	0.11	N.S.
Housing x Gender	1	0.122	0.122	0.07	N.S.
Error	33	58.547	1.774		
Total	36	65.843			

Data Summary

	Colony Housing	Isolated Housing
Males	$N=9$ $\bar{x}=2.6$ $s=1.13$	$N=10$ $\bar{x}=1.8$ $s=1.37$
Females	$N=8$ $\bar{x}=2.9$ $s=1.02$	$N=10$ $\bar{x}=1.9$ $s=1.41$

Table 22

Data Summary and Two Way Analysis of Variance on Milligrams of MHCl Ingested per Kilogram of Body Weight in Phase #6 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	2703.211	2703.211	1.32	N.S.
Gender	1	11753.228	11753.228	5.73	.025
Housing x Gender	1	414.853	414.853	0.20	N.S.
Error	33	67730.967	2052.454		
Total	36	82602.259			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=44.3	$\bar{x}$=28.8
	s=26.16	s=24.87
Females	N=8	N=10
	$\bar{x}$=83.5	$\bar{x}$=62.4
	s=44.00	s=63.17

Table 23

Data Summary and Two Way Analysis of Variance on Grams of MHCl-Water Consumed in Phase #6 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	366.560	366.560	1.92	N.S.
Gender	1	286.337	286.337	1.50	N.S.
Housing x Gender	1	114.810	114.810	0.60	N.S.
Error	33	6320.720	191.537		
Total	36	7088.428			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=136.3	$\bar{x}$=109.6
	s=70.09	s=93.38
	N=8	N=10
Females	$\bar{x}$=210.4	$\bar{x}$=131.8
	s=109.19	s=133.36

Table 24

Data Summary and Two Way Analysis of Variance on Grams of Total Fluid Consumption in Phase #6 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	11635.237	11635.237	1.71	N.S.
Gender	1	14778.381	14778.381	2.17	N.S.
Housing x Gender	1	14363.823	14363.823	2.11	N.S.
Error	33	224680.655	6808.504		
Total	36	265458.096			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=210.3	$\bar{x}$=210.8
	s=24.67	s=75.65
Females	N=8	N=10
	$\bar{x}$=293.0	$\bar{x}$=216.5
	s=84.76	s=102.33

Table 25

Data Summary and Two Way Analysis of Variance on Proportion of 10% Sucrose-Water to Water Consumed in Phase #7 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	3.156	3.156	11.66	.005
Gender	1	0.176	0.176	0.65	N.S.
Housing x Gender	1	0.058	0.058	0.21	N.S.
Error	33	8.936	0.271		
Total	36	12.326			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=3.9	$\bar{x}$=3.5
	s=0.01	s=0.71
	N=8	N=10
Females	$\bar{x}$=3.9	$\bar{x}$=3.3
	s=0.02	s=0.63

Table 26

Data Summary and Two Way Analysis of Variance on Milligrams of Sucrose Ingested per Kilogram of Body Weight in Phase #7 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	51.429	51.429	0.05	N.S.
Gender	1	14235.318	14235.318	14.53	.001
Housing x Gender	1	423.191	423.191	0.43	N.S.
Error	33	32326.979	979.606		
Total	36	47036.917			

Data Summary

	Colony Housing	Isolated Housing
Males	N=9	N=10
	$\bar{x}$=68.9	$\bar{x}$=76.9
	s=9.26	s=23.74
Females	N=8	N=10
	$\bar{x}$=115.8	$\bar{x}$=109.7
	s=39.94	s=36.27

Table 27

Data Summary and Two Way Analysis of Variance on Grams of 10% Sucrose-Water Consumed in Phase #7 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	4.963	4.963	0.05	N.S.
Gender	1	0.719	0.719	0.01	N.S.
Housing x Gender	1	734.536	734.536	7.63	.01
Error	33	3117.069	96.275		
Total	36	3917.287			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=273.9	$\bar{x}$=368.1
	s=28.34	s=114.03
	N=8	N=10
Females	$\bar{x}$=367.9	$\bar{x}$=282.9
	s=119.03	s=81.92

Table 28

Data Summary and Two Way Analysis of Variance on Grams of Total Fluid Consumption in Phase #7 - First Sucrose-Morphine Study

Source	df	SS	MS	F	p
Housing	1	17660.885	17660.885	2.15	N.S.
Gender	1	537.964	537.964	0.07	N.S.
Housing x Gender	1	61560.158	61560.158	7.51	.01.
Error	33	270599.314	8199.979		
Total	36	350358.321			

Data Summary

	Colony Housing	Isolated Housing
	N=9	N=10
Males	$\bar{x}$=276.1	$\bar{x}$=401.0
	s=27.81	s=98.93
	N=8	N=10
Females	$\bar{x}$=371.3	$\bar{x}$=330.3
	s=119.68	x=70.41

animals ($F(1,33) = 11.7$, $p < .005$).

Females drank significantly more mg./kg. of this solution than did the males ($F(1,33) = 14.5$, $p < .001$).

A significant housing x gender interaction was found in the grams of sucrose consumed. A Newman-Keuls test showed that colony females drank significantly more grams of sucrose than did colony males (df. 33, $p < .05$). Isolated males drank significantly more grams of sucrose than did colony males (df. 33, $p < .05$).

A significant housing x gender interaction was found in total fluid consumption. A Newman-Keuls test showed that isolated males drank significantly more total fluid than did colony males (df. 33, $p < .01$).

Colony females drank significantly more total fluid than did colony males (df. 33, $p < .05$).

D. Discussion

The data generated from this study indicate a partial refutation of the earlier "Rat Park" work. Females drank significantly more of the experimental fluids (mg./kg.) in all of the four sucrose-morphine phases. This is consistent with Hadaway et al.'s (1979) observation that females generally drank more morphine solution than males.

The major finding of earlier "Rat Park" work that rats housed in the colony at the time of testing drank significantly less morphine than did the isolated rats was not confirmed. In fact, during the 1.0 mg. MHCl/ml. water plus 10% sucrose phase, the colony rats drank significantly more than did the isolated animals on all four measures, although the magnitude of the differences was small. There were no significant differences in morphine consumption between the colony and caged animals in either the 0.5 mg.; 0.25 mg.; or 0.125 mg. MHCl/ml. water in 10% sucrose phases.

The possibility existed that either the results of this study or the three studies conducted with the older technology could be machine artifacts. Therefore the second study reported in this dissertation was designed without any kind of automated equipment measuring the drinking of the colony animals. While this way of measuring fluid intake precluded the gathering of any individual fluid consumption patterns in

the colony rats, the amount of fluid taken from colony reservoirs could be weighed and compared to the amount of fluid being removed from the control cages during the same time interval.

E. Method

Subjects

There were 10 male and 10 female Wistar rats of Charles River Canada Inc. origin, in both the isolated and colony groups. The animals were raised from weaning (21 days of age) in their respective environments, and were 113 days of age when the experiment started.

Within the colony one female rat died before intake testing began. A male animal was removed prior to intake testing to maintain a one to one gender ratio. There were no deaths in the isolated group.

Apparatus

Isolated rats were housed from weaning in standard 18 x 25 x 18 cm. rat cages with sheet metal walls that prevented visual contact with adjacent animals. In order to collect wastes, paper was placed on trays under the cages. These rats received fluids through stainless steel drinking tubes from plastic bottles (Girton, Millville, P.A.) fastened on the outside of each cage. Purina Rat Chow was provided *ad libitum* by means of inside feeders (11 x 13 x 5.5 cm.). Bottles

attached to two empty control cages allowed for the calculation of spillage and evaporation that might occur in a 24 hour period.

Colony rats lived together from weaning in an open-topped wooden box with a floor area of 8.8m^2. The box contained a layer of kiln dried cedar shavings (Hyon Bedding) and two large open topped metal cages (40 x 25 x 18 cm.) from which two feeders (24 x 12.5 x 5 cm.) containing Purina Rat Chow were hung. The animals had continuous access to a common drinking source. The 41 cm. pole present in the first study was removed and the automated drinking system disabled. At the base of the wall on which the drinking system was hung two holes were drilled and two nipples (Edstrom Industries Inc. AL 113 Adjustable Flow Valve - #10441) were positioned in these holes. From each nipple ran a one metre length of plastic tubing (Tygon R-3603) into a one gallon plastic reservoir situated on a 60 cm. high stool. Each reservoir was filled with the assigned fluid and weighed daily. Beside each reservoir there was another identical container filled with the same fluid, with an identical length of hosing leading down to a similar nipple at the end. The nipple for this container was placed against the outside wall of "Rat Park" and was not touched by the rats. These containers served as control reservoirs and allowed for the calculation of any evaporation that might occur in a 24 hour period.

The white fluorescent lighting in both environments was on a 12 hour light-dark cycle controlled by a single timer (Tork Time Switch Model 7102). Red lights (Sylvania 25 and 60 watt bulbs) were on in both environments at all times.

Procedure

Except for the automated drinking system, the experimental protocol followed was identical to the procedure in the previous study.

F. Results

While there could be no inferential analysis of the colony group data since there was no way of identifying individual animals' fluid consumption, the averages obtained indicate that the colony animals outdrank the isolated animals during the 1.0 mg. sucrose-morphine phase ($\bar{x}$ = 4.3 g. - $\bar{x}$ = 0.27 g. per day); while the isolated animals outdrank the colony animals during the 0.5 mg. sucrose-morphine phase ($\bar{x}$ = 9.4 g. - $\bar{x}$ = 3.0 g. per day); the 0.25 mg. sucrose-morphine phase ($\bar{x}$ = 17.4 g. - $\bar{x}$ = 10.9 g. per day); and the 0.125 mg. sucrose-morphine phase ($\bar{x}$ = 44.4 g. - $\bar{x}$ = 33.1 g. per day) (see Table 30).

The differences between the two groups in this study were never close to the differences observed in the eight male and eight female rats that were maintained in their original environments in the Alexander, Beyerstein, Hadaway and Coambs (1981) study. The continuously isolated animals in that study drank up to seven times as much sucrose-morphine as the rats that lived in the colony from weaning to the end of the study (see Table 31).

Table 29

Average Number of Grams of Fluid Consumed Daily

First Sucrose-Morphine Study

PHASE NUMBER	ISOLATED ANIMAL SUMMARY		COLONY ANIMAL SUMMARY	
	WATER	EXPT.	WATER	EXPT.
1: PRE	2.5	103.2	1.3	108.3
2: QSO_4	26.3	33.4	5.5	75.0
3: 1.0 mg. MHCl	39.5	-0.9	58.8	3.6
4: 0.5 mg. MHCl	44.5	0.5	74.6	1.3
5: 0.25 mg. MHCl	33.9	15.7	49.5	18.3
6: 0.125 mg. MHCl	23.2	30.2	19.6	42.8
7: POST	8.8	81.4	0.7	79.6

PRE and POST test EXPT. fluids were water, combined with 10% sucrose. All other EXPT. fluids were blended in an aqueous 10% sucrose solution.

Table 30

Average Number of Grams of Fluid Consumed Daily

Second Sucrose-Morphine Study

PHASE NUMBER	ISOLATED ANIMAL SUMMARY		COLONY ANIMAL SUMMARY	
	WATER	EXPT.	WATER	EXPT.
1: PRE	0.9	116.2	4.2	104.5
2: QSO_4	25.9	26.3	56.3	10.4
3: 1.0 mg. MHCl	33.9	0.3	54.5	4.3
4: 0.5 mg. MHCl	30.9	9.4	60.8	3.0
5: 0.25 mg. MHCl	23.7	17.4	66.6	10.9
6: 0.125 mg. MHCl	11.9	44.4	41.1	33.1
7: POST	5.2	104.2	4.3	94.9

PRE and POST test EXPT. fluids were water, combined with 10% sucrose. All other EXPT. fluids were blended in an aqueous 10% sucrose solution.

Table 31

— Average Number of Grams of Fluid Consumed Daily

Alexander, B.K., Beyerstein, B.L., Hadaway, P.F., and Coambs, R.B., 1981

PHASE NUMBER	ISOLATED ANIMAL SUMMARY		COLONY ANIMAL SUMMARY	
	WATER	EXPT.	WATER	EXPT.
1: PRE	2.7	100.4	3.7	75.0
2: QSO_4	5.9	65.8	18.0	39.4
3: 1.0 mg. MHCl	30.4	4.7	38.4	1.5
4: 0.5 mg. MHCl	13.9	43.1	42.4	9.4
5: 0.3 mg. MHCl	8.2	63.7	36.3	8.9
6: 0.15 mg. MHCl	1.2	121.4	24.5	32.9
7: POST	8.6	70.7	3.6	79.5

PRE and POST test EXPT. fluids were water, combined with 10% sucrose. All other EXPT. fluids were blended in an aqueous 10% sucrose solution.

G. Discussion

The data generated from this study are consistent with the data obtained in the first study using the automated system to monitor colony drinking. It would appear therefore, from the results of the two studies reported in this dissertation that the results of the earlier "Rat Park" work can not be replicated.

H. General Discussion and Conclusions

Alexander, Beyerstein, Hadaway, and Coambs (1981) found that rats that are allowed to pursue a quasi-natural existence consume much less morphine than do isolated animals, even if that morphine is contained in a 10% sucrose vehicle - a solution that rats drink in large quantities when it is free of morphine, regardless of their housing condition.

Both studies reported in this dissertation failed to replicate the results of Alexander et al. (1981). The second study indicated that the cause of the nonreplication in the first study could not be ascribed to automated equipment.

Alexander et al. (1981) hypothesized that colony housed rats avoid morphine because its ingestion interferes with species-specific behaviours such as nest building, mating and fighting. These behaviours can only occur in a colony.

Apart from the hypothesis advanced by Alexander et al. (1981) it would be expected that isolated animals would drink more sucrose-morphine solution than colony animals. Sklar and Amit (1977) investigated the role of aggregation in morphine lethality in rats. They used 160 male Wistars from Charles River Canada, Inc. that weighed between 250-300 grams (60-75 days old). Prior to the experiments the animals were housed individually in stainless steel cages with food and water available ad libitum. During a series of three

experiments all the animals received injections intraperitoneally with morphine sulfate. In all three studies half the animals were isolated in plywood boxes (8" x 8" x 10") and the other animals were grouped in aggregations of 6 or 7 in plastic baskets (12" x 12" x 10"). After injection, the rats became immobile within 20 minutes; the deaths that occurred did so about two hours later. In all three studies, significantly more of the grouped rats died. Sklar and Amit (1977) indicate that grouping rats potentiates the effects of even non-lethal doses of morphine and that the degree of potentiation is a function of the size of the group - the greater the number of animals in the group - the greater the lethality of the drug.

Sklar and Amit (1977) did not speculate on the physiological basis of this phenomenon. It seems reasonable to assume however, that the aggregated male animals were under more stress than were their isolated counterparts, especially when the male dominance phenomenon noted by Calhoun (1962), and also observed in the colony animals in "Rat Park" is taken into account. If that were the case, then one would assume that the aggregated animals would secrete increased amounts of the polypeptide adrenocorticotropin (ACTH). Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale, and Bloom (1977), have shown that ACTH and B-endorphin are secreted concomitantly by the adenohypophysis in response to stress. Furthermore, ACTH and presumably B-endorphin are released for hours after the

initial reaction to the stressor, as part of the body's long-term adaptation to stress (McGeer, Eccles & McGeer, 1978).

The implication of these findings on the Sklar and Amit (1977) results is quite profound. Rats receiving B-endorphin by injection have shown marked, prolonged muscular rigidity and immobility similar to a catatonic state. This condition can be counteracted by the opiate antagonist naloxone (Bloom, Segal, Ling & Guillemin, 1976). In addition the first five amino acids in the B-endorphin molecule (tyr-gly-gly-phe-met) make up methionine enkephalin. Morphine, methionine-enkephalin, and B-endorphin have all been shown to be potent cardiovascular and respiratory depressors when injected intracisternally, applied to the ventral surface of the brain stem, administered to chemosensitive zones in the medulla oblongata, and to the respiratory centres situated close to the floor of the fourth ventricle (McQueen, 1983).

It is therefore quite likely that if the aggregated animals in the Sklar and Amit (1977) study were under more stress than the isolated animals, this could have potentiated the effect of the exogenous opiate by means of increasing the production of endogenous opiates - namely B-endorphin and methionine enkephalin. In effect the stressed animals were getting more opioids than were the isolated animals, and it was this increase in their production of endogenous opioids that was responsible for their deaths.

If this hypothesis is confirmed in empirical investigation it has implications that would extend to many unexplained opioid related deaths. These deaths occur among human addicts who self-administer an amount of heroin that would not be expected to be fatal in these drug-experienced and presumably drug-tolerant individuals (Siegel, 1983; Brecher, 1972). Brecher (1972) indicates that "Syndrome X" - a label he assigns to unexplained opioid related deaths - may be due to polydrug abuse. It is possible that addicts either inject the heroin and one of its typical North American adulterants - quinine - in which case the quinine is suspected of causing the death, or the addict may be injecting heroin into a body that has other central nervous system depressants such as alcohol or a barbiturate in it already. The combination of other drugs act with the heroin to effect a systemic reaction in the addict and this polydrug combination results in his sudden death.

Brecher (1972), however, indicates that in a significant proportion of cases, both quinine and other drugs can be excluded as explanations for these opioid related deaths. The amount of heroin injected is not considered enough to kill an experienced addict. In addition, Siegel (1983) reports that rats injected with heroin at a specific environmental location die at a much higher rate if they are injected with a previously tolerated dose of heroin at a different environmental location, than rats injected with a previously

tolerated dose of heroin at the environmental location they normally receive their injections.

Siegel's (1983) animals did not have any history of either polydrug CNS depressant use or quinine ingestion. This makes the animal deaths consistent with the significant proportion of human addict deaths described by Brecher (1972), where both quinine and other drugs do not appear to be a factor.

It is therefore conceivable that for a proportion of human addicts, and for the rats in Siegel's (1983) study, that a change in the routine associated with drug consumption might lead to apprehension in both humans and rats, and this apprehension could possibly induce a stressful reaction in the affected organism. If this were so, then the endogenous opioids released under conditions of stress might potentiate the injected exogenous opioid and thus contribute to the sudden death of the human or rat. Thus "Syndrome X" might simply be a case of the exogenous and endogenous opioids interacting in a lethal manner to effect the organism's death.

The above discussion pertains to acute stress only. However, other studies (Katz and Steinberg, 1970; Kostowski, Czlonkowski, Rewerski & Piechocki, 1977) have examined the analgesic properties of morphine and found that three weeks or longer of differential housing, using Long Evans rats (Katz & Steinberg, 1970), and Wistar rats (Kostowski, Czlonkowski, Rewerski & Piechocki, 1977), reduces morphine response in

isolated animals, when compared with group housed rats. One possible explanation of this phenomenon would expand the acute stress hypothesis to one that extends over time. It is possible that, in susceptible strains of rats, the results of prolonged aggregation are more stressful than the effects of continued isolation. If this were so then the apprehension level, arousal level and endogenous opioid level of the grouped animals would be elevated when compared to the isolated rats. This elevated endogenous opioid level in the aggregated animals would serve to potentiate the effects of the injected exogenous opioids and would account for the observed behavioural differences in morphine response between the group housed and isolated animals. This hypothesis could be examined by doing a biochemical analysis of central nervous system tissue and looking for differences in endogenous opioid levels between group housed and isolated animals. It would be expected therefore that the group housed animals would have significantly higher levels of endogenous opioids than would the isolated animals.

One could therefore postulate, from the results of some of the above studies, (Sklar & Amit, 1977; Katz & Steinberg, 1970; Kostowski, Czlonkowski, Rewerski & Piechocki, 1977) that physiological mechanisms associated with both short and long term aggregation, might prevent colony rats from consuming as much sucrose-morphine as isolated animals. This is what occurred during the Alexander et al. (1981) study when Charles

River Wistar rats were used. However, in the two studies reported here, the earlier effect was not found.

It is apparent that the difference between the two dissertation studies and the Alexander et al. (1981) study is in the response of the isolated rats. The isolated animals in the two dissertation studies drank much less sucrose-morphine solution than did the isolated rats in the Alexander et al. (1981) study, while there was no appreciable difference in the sucrose-morphine consumption of the colony Wistars in all three studies (see tables 29, 30 & 31). The isolated Wistars in the Alexander et al. (1981) study drank an average of 121.4 grams of the 0.15 mg. sucrose-morphine solution daily. The isolated Wistars in the two dissertation studies drank an average of 37.3 grams of the 0.125 mg. sucrose-morphine solution daily - less than one third the daily average of the Wistars used in the Alexander et al. (1981) study.

In comparison, the colony Wistars in the Alexander et al. (1981) study drank an average of 32.9 grams of the 0.15 mg. sucrose-morphine solution daily. The colony Wistars in the two dissertaton studies drank an average of 37.9 grams of the 0.125 mg. sucrose-morphine solution daily. The increase from 32.9 to 37.9 grams per day could be expected because the sucrose-morphine solution was slightly sweeter - 0.15 mg. MHCl to 0.125 mg. MHCl in 10% sucrose - in the dissertation studies.

These differences in sucrose-morphine consumption are all the more striking when it is realized that the Wistars used in the Alexander et al. (1981) study drank less sucrose, as measured in Phases 1 and 7, than did the Wistars used in the two dissertation studies. Clearly the isolated animals used in the two studies reported here were avoiding the consumption of morphine.

The possibility exists that the Wistar rats used in the two dissertation studies differ from the Wistar rats used in the Alexander et al. (1981) study. While the Alexander et al. study was published in 1981, the research for that publication was done from April to July in 1979. In November 1979, Charles River Canada, Inc. changed Wistar rat colonies. Therefore, the Wistars used in the Alexander et al. (1981) study were Old Colony Wistars, while the rats used in the two dissertation studies were New Colony Wistars.

The reason Charles River changed colonies was that the Wistars used before November 1979 - the Old Colony Wistars - were antibody positive for a number of viruses (H1; Sendai; Sialodacryoadenitis (SDA); Kilham rat virus (KRV); and Pneumonia virus of mice (PVM)). The Wistars sold by Charles River after November 1979 - the New Colony Wistars - were antibody free of these viruses.

Other differences involve the way the animals were housed and bred. The Old Colony Wistars lived on contact bedding and the mating was done on a ratio of 3 females to 1 male. The New

Colony Wistars live in cages that have wire mesh floors, and the breeding is done on a ratio of 20 females to 5 males.

There are no differences between the Old and New Colony Wistars in the handling of the pregnant females from 17 days post conception until the weaning of the offspring at 21 days of age. There are, however, differences in litter size with the New Colony females averaging 12 offspring per litter while the Old Colony females averaged 10 offspring per litter. In addition, the New Colony pups weigh on average 53 grams at 21 days of age while the Old Colony pups weighed on average 50 grams at 21 days of age.

The breeding nucleus of the New Colony Wistars consisted of 500 females and 200 males selected from a pathogen free colony in Portage Michigan and shipped to Charles River's facility in St. Constant, Quebec (J. Goyer, personal communication).

In spite of Charles River Canada Inc.'s assertion that they are not aware of any genetic difference between the Old and New Colony Wistars, a number of differences between the Old and New Colony animals have been noted.

Experimental evidence suggests that the Old and New Colony Wistars from Charles River Canada, Inc. respond differently to equal levels of psychoactive substances. Ton, Blair, Holmes and Amit (1983) compared the effects of chronic naltrexone injection on amphetamine locomotor activity on individually housed Old and New Colony male rats. The 200-250

gram (50-60 day old) animals from the two different colonies were pretreated with naltrexone (10 mg./kg. s.c.) for eight days. After a two day rest period, animals were tested with amphetamine for locomotor activity in the open field with or without white noise. The rats were similarly retested on Day 7 and Day 14. New Colony animals showed a significant attenuation in amphetamine locomotor activity in the absence of noise only. In contrast, chronic naltrexone significantly decreased amphetamine activity in Old Colony animals only under noise conditions.

Ton et al. (1983) believe that the differential effects may reflect predispositional differences across animal populations in the modulation of dopamine function by opioid peptides via opiate receptors.

In addition, changes in temperament have been noticed with the New Colony Wistars being considered much more aggressive and difficult to handle than the Old Colony (F.J. Boland; M. Corcoran; B. Cross; personal communications). These observations of aggressiveness in the New Colony Wistars have been anecdotal, with no operational definition of aggressivity empirically tested. Nevertheless, differences in temperament that include wildness and aggressiveness are considered by Robinson (1965; 1979) to be genetic in nature.

Another difference between Old and New Colony Wistars includes levels of voluntary alcohol ingestion, with consumption by New Colony Wistars being greatly attenuated

when compared to Old Colony intake (F.J. Boland; C. Pang, personal communications).

Nichols and Hsiao (1967) indicate that preference for oral morphine and alcohol intake has a genetic basis. They subjected 223 Sprague-Dawley rats (182 females and 41 males) to ten 3 day training cycles (Nichols, 1965) followed by a choice test administered on the 14th day of abstinence. The animal's test scores were rank ordered and the rats with scores in the highest quartile (the more susceptible animals) were inbred randomly to produce the F_1 generation group. The animals in the lowest quartile (the least susceptible animals) were also inbred randomly to produce the F_1 generation group.

Subjects in the F_1 and succeeding generations were selected for breeding in a manner similar to that used in the parental or F_0 generation.

The more susceptible animals in each generation continued to increase their preference for morphine while the least susceptible animals in each generation decreased their consumption of morphine. The differences between the two groups became significantly greater from generations F_1 to F_2 and from F_2 to F_3.

Nichols and Hsiao (1967) then tested whether susceptibility of each of these two groups to morphine ingestion was unique, or whether it was a specific expression of a more general trait of susceptibility, by running a second experiment on alcohol preference. They used experimentally

inexperienced females from the F_3 generation and found that the animals susceptible to morphine ingestion also drank more alcohol, when compared to the resistant group.

Nichols and Hsiao (1967) did not speculate on the mechanisms responsible for the group differences in opiate and alcohol ingestion, but they felt that the mechanisms were genetic.

It is well known that there are strain differences in responsivity to drugs in both mice and rats (Shearer, Creel & Wilson, 1973; Horowitz, Whitney, Smith & Stephan, 1977; Collins & Whitney, 1978; Oliverio, Castellano, Racagni, Spano, Trabucchi & Cattabeni, 1978; and Bardo & Gunion, 1982). It is therefore quite possible that if a genetic alteration were inadvertently introduced when Charles River changed from Old to New Colony animals, this genetic shift could manifest itself in a New Colony animal that responded in a different way to psychoactive substances than did its Old Colony counterpart.

The fact that Ton et al. (1983) found differences between the two colonies and that Boland; Cross; Corcoran; and Pang (personal communications) indicate temperament and voluntary drug consumption differences between Old and New Colony Wistars, suggests the possibility that the New Colony rats may be genetically different from the Old Colony rats.

Hedrich (1983) maintains that the foundation of new colonies should be limited, as the likelihood of altering the

strain through sample selection errors is quite high. Cryptic alleles so far not detected in the colony may suddenly turn up. In order to maintain the original dispersion of genotypes within an outbred strain, it is necessary that the colony is maintained by a large number of breeding pairs (> 500), without selective forces being applied, to avoid genetic drift (Hedrich, 1983).

Charles River did not follow Hedrich's (1983) criteria when they changed colonies. The New Colony Wistars were not derived from Old Colony Wistars, but were instead a part of another colony altogether. In addition, the number of animals used by Charles River to start the New Colony was 70% of the minimum number thought necessary by Hedrich (1983), to avoid genetic drift. To maximize the possibility of guaranteeing a larger genetic pool, it may have been to Charles River's advantage to use as New Colony breeding stock, offspring of Old Colony animals derived by caesarian section, animals obtained from more than one other colony, or a combination of these two methods (Green, 1981).

In addition, the change from a breeding ratio of 3:1 in the Old Colony to 20:5 in the New Colony could serve to further reduce the genetic pool. If the male dominance phenomenon noted by Calhoun (1962), and also observed in the colony animals in "Rat Park" is present in the Charles River breeding rooms, then it is likely that not all five males would contribute equally to impregnating the 20 females. Thus

the 20:5 breeding ratio could rise as high as 20:1 if one male were dominant to the point of intimidating and deterring from breeding the other four males in the cage. If this were the case, then the gene pool in the New Colony stock would be reduced considerably from the Old Colony animals (Green, 1981).

From the above evidence, it could be inferred, that because of the reduced breeding stock, separate colony transplantation, and probable reduced level of male genetic variance, the animals in the New Colony are likely to be genetically different from the Old Colony Wistars.

Genetic differences that involve oral sucrose-morphine consumption would most probably include the hepatic microsomal enzyme systems. The Ah multigene system in rodents controls, by means of inducing hepatic microsomal enzymes (cytochrome P450's), the ability of those organisms to metabolize foreign compounds (Nebert, 1983). Since morphine is biotransformed by the hepatic microsomal enzyme system, Nebert and Felton (1976) identified the differential opioid consumption in the Sprague-Dawley rats used in Nichols and Hsiao's (1967) study, as an example of how oral opioid intake is limited to how well the hepatic microsomal enzyme (Ah) system can metabolize the drug, and is therefore under genetic control.

Animals such as C57BL/6J mice have been known to ingest large quantities of morphine, whereas the DBA/2J strain consume very little of this drug (Horowitz, Whitney, Smith &

Stephan, 1977). C57BL/6J mice are Ah responsive while the DBA/2J animals are Ah nonresponsive. Since Ah responsiveness is on a continuum, the more Ah responsive the animal is, the more morphine it can metabolize (Nebert & Felton, 1976).

It is therefore possible that the Old Colony Wistars were more Ah responsive and were thus able to metabolize larger quantities of morphine than their New Colony Wistar counterparts. This would make the New Colony Wistars less responsive in metabolizing other foreign compounds - thus providing an explanation for the attenuated drug consumption of the New Colony Wistars used by Pang and Boland.

Another possibility that might account for the differences in sucrose-morphine consumption observed in the Old vs. New Colony isolated Wistars, is that the viruses identified by Charles River Canada, Inc. as being present in the Old Colony Wistars, might themselves play an important role.

The viruses that the Old Colony Wistars tested positive for included: H1; Sendai; Sialodacryoadenitis (SDA); Kilham rat virus (KRV); and Pneumonia virus of mice (PVM). All viruses have a predilection for tissues containing rapidly dividing cells, and therefore could prove deleterious to growth during prenatal development (G. Shkurhan, personal communication).

It is conceivable that viruses could interfere with the prenatal development of opiate receptors as outlined by

Clendeninn, Petraitis and Simon (1976), and with the development of narcotic drug metabolizing enzymes as described by Yeh and Krebs (1980). Possibly these developmental alterations could influence the consumption of sucrose-morphine when the animals reached maturity. For example, H1 - a rat parvovirus that the Old Colony Wistars were antibody positive for - can, in its acute phase, be transmitted both horizontally and vertically; may be excreted in feces, urine, and milk; and will settle in both brain and liver tissue (Jacoby, Bhatt, & Jones, 1979). In addition, the SDA virus, at its peak, appears to both inhibit the rate of implantation of fertilized eggs, and lower the rate of reproduction in affected animals. In practice however, animals would not be paired at the time of peak infection. There is also no evidence to suggest that the surviving offspring are adversely affected by the SDA virus (Heywood & Buist, 1983). Sendai; PVM; and KRV are considered common viruses that do not appear to cause problems in either the rate of reproduction or the health of the offspring (Heywood & Buist, 1983).

An important point to consider is that testing antibody positive for a specific virus does not mean that that organism has suffered the full effects of this pathogen. Genetic makeup is believed to account for the differences observed in the severity of symptoms produced by viral agents, when other factors, such as housing and nutrition are held constant (G. Shkurhan, personal communication).

Viruses that succeed in crossing the placental barrier will act as teratogenic agents during organogenesis. When the embryonic period is complete, the possibility of fetal malformation as a result of viral infection, is remote (Moore, 1977; G. Shkurhan, personal communication). Not all viruses cross the placental barrier; however, the viruses that have that capability, (for example, H1) can often be successfully resisted by the pregnant female. This resistance to viral infection that involves the crossing of the placental barrier is believed to be genetic in nature (G. Shkurhan, personal communication). If a virus crosses the placental barrier, and acts as a teratogenic agent, then the probability exists that a spontaneous abortion or malformed pups will be the result. If premature, abnormally small, or malformed pups are born, they are likely to be abandoned or cannabalized by their mother. Thus pups in the Charles River breeding colonies that were the victims of viral teratogens, would not survive to weaning, and would therefore not be available for use in research. On the other hand, pups that were born into an environment that had viruses present, and had the genetic capacity, by means of antibody production, to resist these agents, would, while being antibody positive for these viral agents, not show any adverse reactions to these potential pathogens. These rats would thus survive and be available for research (G. Shkurhan, personal communication).

It is therefore most likely that the Old Colony animals that were available from Charles River for research purposes were healthy despite being antibody positive for a number of viruses. It appears unlikely that the viruses present in the Old Colony Wistars could have influenced drug ingestion to as great an extent as either environmental or genetic factors. However, in the two dissertation studies, the environmental effect present in the Alexander et al. (1981) study could not be replicated. It seems therefore, that in the case of the New Colony Wistars, the hypothesis that there is a genetic difference between these animals and their Old Colony couterparts, is still the most parsimonious.

To show conclusively however, that this viral hypothesis was not a plausible one, it would be necessary to test it empirically. One approach to this investigation would be to subject the New Colony animals to the viruses the Old Colony animals were antibody positive for. It is important that the viral strains be identical and that the New Colony animals be exposed to all combinations of the viruses the Old Colony animals had. Coupled with this exposure would be the testing of both New and Old Colony animals on their consumption of sucrose-morphine. If, after the testing, using all the viruses, there were still differences between the two colonies in sucrose-morphine consumption, then the results would point to a difference in genetics between the two colonies.

Another possibility that might account for the differences in sucrose-morphine consumption observed in the Old vs. New Colony isolated Wistars is the bedding the animals were exposed to.

The Alexander et al. (1981) study was conducted with the colony animals lying on resaw piling softwood sawdust - sawdust from hemlock and fir that had not been kiln dried. The isolated animals had the sawdust placed on trays under their cages. The two dissertation studies were conducted with the colony animals lying on kiln dried softwood chips. The isolated animals had paper placed on trays under their cages.

Vesell (1967) reported that three drug-metabolizing enzymes occurring in hepatic microsomes of male and female mice and male Sprague-Dawley rats could be induced by letting the animals lie on softwood bedding that had not been heat treated. Vesell (1967) noted that the sleeping times of the rats that were injected with hexobarbital were reduced by 66% of initial values, and hepatic enzyme activity increased correspondingly, if the animals had been subjected to the softwood bedding for as little as 48 hours. The sleeping times of the rats increased, and hepatic enzyme activity decreased, to the values established prior to exposure to softwood bedding, within 48 hours of the animals being placed on hardwood bedding. If the softwood bedding was heat treated with hexane, the sleeping times, in mice exposed to the red cedar bedding for seven days, were decreased by only 25%

compared to an 80% decrease exhibited by mice kept on untreated red cedar bedding.

Vesell (1967) indicates that all the softwood bedding he tested, which included red cedar, white pine, and ponderosa pine, contained the inducing substance or substances. Vesell (1967) was unable, however, to ascertain whether the induction of the hepatic enzymes in the mice and the rats followed ingestion or inhalation of these compounds.

One of the three drug metabolizing enzymes isolated by Vesell (1967) was ethyl morphine N-demethylase, a principal agent in the metabolic biotransformation of morphine (Misra, 1978; Fishman & Hahn, 1978).

It is possible therefore, if Vesell's (1967) findings can be extended to Old Colony Wistars, that those results, coupled with Sklar and Amit's (1977) results, could have biased the Alexander et al. (1981) study. Sklar and Amit's (1977) study indicates that grouping potentiates the lethal effects of even a non lethal dose of morphine, and that the degree of potentiation is a function of the size of the group. It would therefore be expected that the colony animals in "Rat Park" would not drink as much morphine as their isolated counterparts, because of this aggregation phenomenon.

If inhalation of the inducing compounds in the untreated softwood bedding was a factor, one would expect that both the colony and the isolated animals would increase their drinking of sucrose-morphine - the morphine would be metabolized more

efficiently in the first entero-hepatic pass, and the sucrose in the compound would prove very rewarding. However, because of the aggregation phenomenon observed by Sklar and Amit (1977), it would be expected that the amount of sucrose-morphine consumed by the colony animals in "Rat Park" would not match that of their isolated counterparts, and the differences between the two groups would be exaggerated considerably more than what would be expected if the animals were lying on hardwood bedding or softwood bedding that had been kiln dried.

During the two dissertation studies the animals in "Rat Park" lay on kiln dried softwood chips, while the isolated animals had paper placed on trays under their cages. If Vesell's (1967) conclusions can be extended to New Colony Wistars, one would anticipate that the processing of the paper and the softwood chips would mean that there should be no rise in any of the hepatic enzymes involved in drug metabolism, identified by Vesell (1967).

The dissimilarity in bedding might account, in part, for the difference in the results between the Alexander et al. (1981) study and the two dissertation studies. It does not explain however, the differences found in the Ton et al. (1983) study or the work done by Boland and Pang, which fails to replicate the Old Colony results using New Colony animals. The bedding used by the animals in all these studies was not systematically varied - the only known change was in

the animals used (F.J. Boland & C. Pang, personal communications). It would appear therefore that the bedding variable is not a major factor in explaining the differences in drug consumption in the studies cited above.

The above discussion indicates that there is a difference in the way Old and New Colony Wistars from Charles River Canada, Inc. process psychoactive substances. When the variation in bedding between the two dissertation studies is taken into consideration, it is not likely to be of major importance. However, even with no elevation of drug metabolizing hepatic enzymes, the data generated by Sklar and Amit (1977), and confirmed in the earlier Rat Park studies would predict that there still should be a difference in sucrose-morphine consumption, with the isolated animals drinking more of the solution than the colony animals. When the New Colony Wistars were used in the dissertation studies, there was no difference close to the magnitude found with the Old Colony Wistars in the drinking behaviour of the isolated animals. The colony animals of both Old and New Colony Wistars consumed nearly the same amount of sucrose-morphine in all three studies.

The dissertation data therefore indicate that there is a change in sucrose-morphine consumption between Old and New Colony Wistars, and that the isolated animals are differentially affected. One possible, although highly unlikely explanation of these differences, is that they are

due to the elimination of the viruses in the New Colony Wistars that the Old Colony Wistars tested positive for.

The probability is such however, that there are very likely genetic differences that account for the variation in the drinking of sucrose-morphine. These differences are most likely due to genetic alteration of the hepatic microsomal system served by the Ah multigene system, and instrumental in the biotransformation of morphine. It is probable that either the reduced breeding stock, separate colony transplantation, probable reduced level of male genetic variance, or combinations of these variables, made the New Colony Wistars less Ah responsive than their Old Colony counterparts. This lack of responsitivity would manifest itself in a reduced capacity of morphine metabolization, and thus make the New Colony Wistars less likely to drink as much sucrose-morphine solution as the Old Colony Wistars.

It is therefore quite conceivable that using another strain of rat or Wistar rats from another breeder, that the Alexander et al. (1981) hypothesis would still be confirmed. This hypothesis must be coupled with the realization that previous work (Sklar & Amit, 1977; Katz & Steinberg, 1970; Kostowski, Czlonkowski, Rewerski & Piechocki, 1977) indicates that physiological mechanisms associated with both short and long term aggregation, might prevent colony rats from consuming as much sucrose-morphine as isolated animals.

In conclusion, the results of Nichols (1965), Khavari, Peters, Baity and Wilson (1975), Weeks and Collins (1979), and the earlier "Rat Park" research which, taken together, suggest that in the appropriate housing conditions, rats will self administer opiates in large quantities, appear now to be obscured by a factor or factors that must be more fully explored. These variables are most likely genetic in nature and are most probably the reason for the variation in the results obtained in the oral sucrose-morphine consumption of the Old and New Colony Wistars.

The one feature that the above studies have in common is the fact that the animals used were all outbred - rats that were derived from a large gene pool and from which little was understood about their genotypes. It is therefore imperative that future studies involving morphine consumption include the use of inbred rats - animals of which the genetics are more completely understood.

It would be possible with these inbred animals to observe drug ingestion in rats that were known to possess varying levels of Ah responsivity while, at the same time, systematically varying environment and social contact. In this way, more could be learned about the cause and effect of morphine ingestion in a rodent population.

It is therefore important that further animal work be done. Future research in opioid consumption should include the use of other routes of administration, other animal models,

and various environmental paradigms, in an attempt to more closely approximate the human condition. It is important that inbred animals be used in every phase of this work so that the variable of genotype will be more closely controlled. Through this approach more will be learned about the phenomenon of opioid usage, and eventually the results obtained in animal work may prove useful in understanding and treating humans who use these drugs in a detrimental or compulsive manner in a society. It is only through understanding the phenomenon of opioid usage that a more humane way of treating and perhaps preventing opioid abuse can ever be developed.

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