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Effect of Early and Later Colony Housing on Oral Ingestion of Morphine in Rats

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ALEXANDER, B. K., B. L. BEYERSTEIN, P. F. HADAWAY AND R. B. COAMBS. *Effects of early and later colony housing on oral ingestion of morphine in rats.* PHARMAC. BIOCHEM. BEHAV. 15(4) 571–576, 1981.—Male and female rats were raised from weaning either in isolation or in a large colony. At 65 days of age, half the rats in each environment were moved to the other. At 80 days, the animals were given continuous access to water and to a sequence of 7 solutions: 3 sweet or bitter-sweet control solutions and 4 different concentrations of morphine hydrochloride (MHCl) in 10% sucrose solution. Rats housed in the colony at the time of testing drank less MHCl solution than isolated rats, but no less of the control solutions. Colony-dwelling rats previously housed in isolation tended to drink more MHCl solution than those housed in the colony since weaning, but this effect reached statistical significance only at the lowest concentration of MHCl. These data were related to the hypothesis that colony rats avoid morphine because it interferes with complex, species-specific behavior.

Morphine Self-administration Environment Isolation

UNDER appropriate conditions, laboratory animals drink opiate drug solutions in preference to water [3, 14, 16], and self-inject opiates through indwelling catheters [19,20]. These findings are sometimes taken to suggest that mammals, in general, have a natural affinity for opiates [2, 7, 8]. However, recent data indicate that laboratory housing may itself increase opiate intake. Rats housed in a quasi-natural colony drank much less morphine hydrochloride (MHCl) solution than rats isolated in standard laboratory cages. This was found both in rats which had been pre-treated with morphine [1] and in untreated rats [10].

The present experiment is designed to analyse this housing effect more fully by separating the effect of early housing from that of housing contemporaneous with intake testing. We have proposed [10] that colony housed rats avoid morphine because its ingestion interferes with species-specific behaviors which can occur only in a colony, such as nest building, mating, and fighting. This speculation implicates housing contemporaneous with testing as the cause of the housing effect, and is compatable with recent demonstrations that relatively small doses of morphine significantly reduce sexual behavior and "social cohesion" in rats [15,17], and with the evidence that species-specific behaviors are self-reinforcing [6]. Another plausible explanation for the housing effect, that morphine reinforces isolated rats because it relieves the stress of isolation, also would implicate the contemporaneous environment.

On the other hand, complexity of the very early post-weaning environment has major effects on development of the central nervous system (e.g., [9,11]), some of which have been related to drug use [18]. Many of the widely accepted personality theories of human addiction (e.g., [13]) also stress very early experience. Early rather than contemporaneous housing could clearly be responsible for the housing effect observed in our previous experiments. [1,10].

METHOD

Subjects

Sixteen male and sixteen female albino rats of Wistar origin (University of British Columbia Breeding Stock) were obtained at weaning (21±2 days of age). Eight males and eight females were placed in individual housing; eight males and eight females were housed in a colony.

Apparatus and Procedure

Individual housing was in standard wire mesh cages (18×25×18 cm). During intake testing, fluid consumption was monitored by weighing the two drinking bottles affixed to each cage daily. An approximate correction for leakage and evaporation was made by subtracting the mean weight loss from two similar bottles mounted on empty cages in the same rack.

Colony housing was in a large (8.8 m^2), open-topped plywood enclosure containing cedar shavings, empty cannisters, and small boxes for hiding and nesting. Fluids were available at the end of a short transparent tunnel attached to an opening in the wall. Inside dimensions of the tunnel were just sufficient to accomodate one adult rat at a time (4.4×5.8 cm). At the far end of the tunnel were two fluid dispensers (Lafayette Instruments, catalogue no. 80201), each posi-

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TABLE 1

HOUSING CONDITIONS AND SEQUENCE OF DRUG-SUCROSE SOLUTIONS PRESENTED IN EXPERIMENTAL PHASES

Designation	Housing Conditions: Early (22–65 Days)	Housing Conditions: Contemporaneous (65 Days Onwards)	N
II	Isolated	Isolated	4M,4F
CI	Colony	Isolated	4M,4F
IC	Isolated	Colony	4M,4F
CC	Colony	Colony	4M,4F

Phase Name	Phases: Morphine or Quinine/Sucrose Solution	Days	Usable Days
PRE	water+10% sucrose	3	3
Q	0.06 mg QSO_4/ml water+10% sucrose	5	2
1	1.00 mg MHCl/ml water+10% sucrose	5	4
0.5	0.50 mg MHCl/ml water+10% sucrose	5	2
0.3	0.30 mg MHCl/ml water+10% sucrose	5	5
0.15	0.15 mg MHCl/ml water+10% sucrose	5	5
POST	water+10% sucrose	3	2

tioned over a shallow well, with a photoelectric beam running across each well. Drinking from a well required a rat to break a light beam with its head. Withdrawing its head re-instated the beam, automatically releasing another drop of fluid into the well. The number of drops released from each dispenser was displayed on digital counters. This device is described in greater detail elsewhere [5].

When a rat entered the tunnel, a photoelectrically activated video camera recorded the dye mark on its back and the number of drops released by the previous rat as displayed on the digital counters. Any overflow from a well was drawn off by a vacuum pump into separate containers for each fluid. This loss (usually less than 10% of total intake) was subtracted proportionately to each rat's intake for the day.

Other environmental variables were controlled by situating both housing environments in the same large room, with a 12 hr light-dark cycle. Food was available ad lib throughout.

At 60 days of age all rats were weighed and dye marked for identification. At 65 days of age, half the colony rats were transferred to individual cages and half the isolated rats were placed in the colony, producing four housing conditions each containing four rats of each sex (see Table 1). The rats were then left undisturbed for two weeks. During this period colony rats drank their water from the dispensing device, a skill which each rat learned readily.

Intake testing began at 80 days of age. In each phase rats were given 24 hr access to a drug-sucrose solution and a tap water alternative. The concentrations of the drug-sucrose solutions, sequence and length of the seven phases, and phase abbreviations appear in Table 1. Phase PRE provided access to tap water and to a 10% sucrose solution to determine if housing conditions had affected preference for sweet solutions. Next, Phase Q entailed access to water and to a solution of 0.06 mg quinine sulfate/ml 10% sucrose solution to check for effects of housing on preference for bitter-sweet solutions. To the human palate, this quinine-sucrose solution tasted the same as the morphine-sucrose solution used later in Phase 0.3. In a pilot experiment, rats given 8 hours exposure to these two solutions ingested approximately equal amounts.

The next 4 phases each entailed continuous access to water and to progressively decreasing concentrations of MHCl in 10% sucrose. In Phase 1, the drug solution contained 1 mg MHCl/ml of vehicle; in Phase 0.5, 0.5 mg MHCl/ml vehicle; in Phase 0.3, 0.3 mg MHCl/ml vehicle, and in Phase 0.15, 0.15 mg MHCl/ml vehicle. Finally, Phase POST entailed the same water vs sugar-water alternatives as Phase PRE. Left-right positions of water and drug-sucrose solution were reversed after each phase in both environments.

The "days" column in Table 1 indicates the number of days in each phase. A persistent electronic malfunction caused the loss of several days' data from the colony rats. On these days, data for isolated rats was also dropped from the ANOVA. The "usable days" column indicates the number of days actually analysed for each phase. The lost days were 1, 2, and 4 from Phase Q; 2 from Phase 1; 1, 3, and 4 from Phase 0.5; and 1 from Phase POST. Fortunately, no days were lost from 2 of the 3 phases which provided the critical test of the housing effect, but the lost data from the Q Phase may have contributed some ambiguity to the results for females, as reported below.

Repeated measures analyses of variance (ANOVAs) were run separately for each phase for grams drug-sucrose solution ingested, mg quinine sulphate or MHCl/kg body weight, and proportion of drug-sucrose solution to total fluid intake. Death of a female in group CC after completion of Phase 0.3 and a female in group IC after Phase 0.15 reduced the number to 3 for these groups in the analyses for the final phases. ANOVAs were run separately for males and females and for all animals together. Because of differences in outcome, the data relating to housing effects are reported below separately for males and females.

RESULTS

Housing Effects: Males

Males living in the colony at the time of testing ingested much less MHCl solution than isolated males, but no less of the control solutions. Early isolation appeared to increase morphine intake in the colony-dwelling males, but the effect reached significance only in Phase 0.15.

No males drank much of the extremely bitter MHCl solution in Phase 1, however, in Phases 0.5, 0.3, and 0.15, colony males (conditions CC and IC) drank less of the morphine-sucrose solution than isolated males (conditions II and CI) on all three measures (see Fig. 1). Eight of nine Fs for contemporaneous environment in these three phases were statistically significant, five beyond the 0.0001 level. Significance levels for each phase appear in Fig. 1. The effect was greatest in Phases 0.5 and 0.3, in which the isolated males drank 19 times and 6 times as much MHCl solution as the colony males respectively (based on mg/kg data).

There were no significant Fs due to early environment alone, but the early by contemporaneous environment interactions in Phase 0.15 were significant for g MHCl solution and mg MHCl/kg body weight. On both measures, colony males which had been isolated early in life (IC condition)

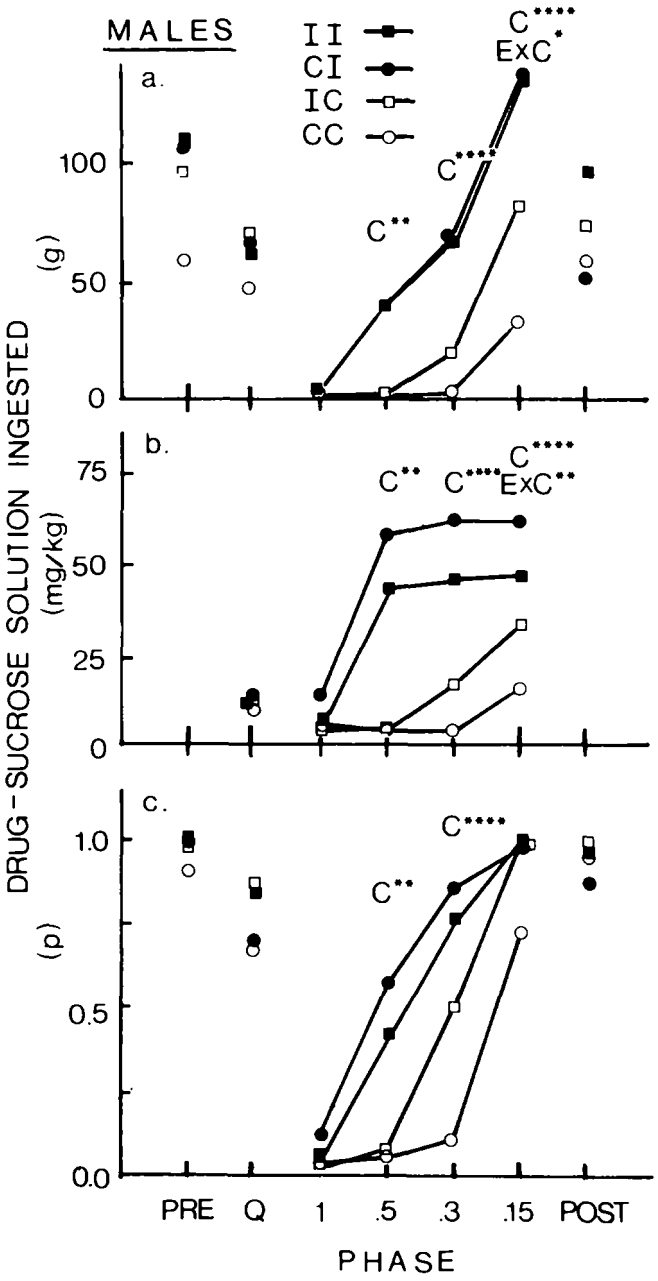

FIG. 1. Ingestion of drug-sucrose solutions by male rats measured as g drug-sucrose solution, mg MHCl or quinine sulfate/kg body weight, and proportion of drug-sucrose solution to total fluid intake. C signifies that the F for the effect of contemporaneous environment was significant, E indicates that early environment was significant, and E×C indicates a significant interaction. *, **, *** and **** signify significance at the 0.05, 0.01 0.001 and 0.0001 levels, respectively.

drank more MHCl solution than males reared from weaning in the colony (CC). Differences between II and CI males, however, were not consistent between measures (see Fig. 1).

The variance was large, both between individuals and between the same individual's scores on different days. The influence of the large variance on the significance tests was reduced by basing inter-condition comparisons on 5-day totals. To illustrate the nature of the variability, Table 2 presents day-by-day individual data for a representative phase (Phase 0.3, males, mg/kg) and an abbreviated ANOVA summary table.

Housing Effects: Females

Results were similar for females, but less conclusive. Females housed in the colony consumed less MHCl in Phases 0.5, 0.3, and 0.15 on all three measures. Fs for contemporaneous environment were significant for g MHCl solution and mg MHCl/kg in Phase 0.3 and for proportion of MHCl solution to total fluid intake in Phases 0.5, 0.3, and 0.15 (see Fig. 2 for significance levels). IC females appeared to consume much more MHCl than CC females in Phase 0.15 in all three measures, but the only significant F was for early environment for the proportion measure in Phase 0.15.

Although no differences reached statistical significance in control Phases PRE, Q, or POST, the colony females (CC and IC) tended to drink less quinine-sucrose solution in Phase Q than the isolated (II and CI) females (Fig. 2a–c). The F values for contemporaneous environment approached significance for g MHCl solution and mg MHCl/kg, $F(1,12)=2.45$, $p=0.143$, and $F(1,12)=2.58$, $p=0.134$, respectively. The loss of three days data from the Q Phase may have increased the intra-group variance, thus reducing the likelihood of statistical significance in this phase.

Because of the possibility of an effect of housing on preference for bitter-sweet solutions in females, which could have affected their intake of the drug-bearing solutions, the female data from Phases 0.5, 0.3, and 0.15 were subjected to analysis of covariance. For each measure, the covariate was mean consumption by the same measure in Phase Q. Of the 5 significant Fs for contemporaneous environment, 3 were also significant in the analysis of covariance, all 3 for the proportion measure (Phase 0.5; $F(1,11)=5.53$, $p<0.05$: Phase 0.3; $F(1,11)=8.91$, $p<0.05$: Phase 0.15; $F(1,10)=6.52$, $p<0.05$). The Fs for g MHCl solution and mg MHCl/kg in Phase 0.3, both significant in the original ANOVA, narrowly missed significance, $F(1,11)=3.71$, $p=0.08$, and $F(1,11)=3.65$, $p=0.08$, respectively.

No Fs for early housing conditions reached significance in the analysis of covariance, but three early by contemporaneous interactions did. For the proportion measure in Phase 0.15, the adjusted cell means were similar in the II, CI, and IC females and much lower in the CC females, indicating that early isolation had increased MHCl consumption in the colony females, but not in the isolated females. For g MHCl solution in Phase 0.15, and for mg MHCl/kg in Phase 0.5, the adjusted cell means were highest in the II females followed by the CC, CI, and IC females, in that order. Overall, the adjusted means for the isolation housed females were higher than those of the colony housed females, in spite of the relatively high adjusted means of the CC group.

Gender Differences

Analyses of variance performed on data for both sexes revealed that neither the ingestion of morphine nor of control solutions was affected by gender. Of 76 F tests for the gender main effect plus all its interactions with early and contemporaneous environment in the 7 phases, only 1 was significant at the 0.05 level.

DISCUSSION

Rats living in a colony at the time of testing consumed less

TABLE 2

SAMPLE DATA AND ANOVA SUMMARY. DATA FROM PHASE 0.3, MALES, mg MHCl CONSUMED /kg BODY WEIGHT

Condition	Day 1	2	3	4	5	Mean
IC	8.862	15.821	29.077	13.368	27.601	18.946
	2.679	2.673	7.401	25.189	33.615	14.311
	0.0	0.728	1.436	1.239	3.650	1.411
	3.238	22.107	22.553	35.376	36.964	24.048
CC	0.000	0.000	0.793	6.268	0.000	1.412
	1.070	4.026	2.825	4.644	5.276	3.568
	2.857	1.695	0.767	2.066	1.133	1.704
	0.0	2.742	3.575	4.160	5.096	3.115
I I	66.414	59.085	68.021	79.144	61.335	66.800
	77.167	3.565	82.269	34.320	20.270	43.518
	56.932	28.466	5.790	17.550	19.782	25.704
	87.166	12.612	2.233	14.714	25.815	28.508
CI	93.244	64.880	21.740	28.109	78.723	57.335
	68.999	84.104	24.489	41.918	47.102	53.322
	88.335	3.198	5.356	26.860	70.668	38.883
	111.561	131.539	78.699	37.156	62.549	84.301

Analysis of Variance for Housing Conditions

	df	MS	F	*p*
Contemporaneous Environment (C)	1	34002	33.64	<0.0001
Early Environment (E)	1	130	0.13	N.S.
C × E	1	4368	4.32	0.06
Error	12	1011		

MHCl than isolated rats, whatever their early housing condition, even though they had been exposed to the early environment for 44 days and to the contemporaneous environment for only 15 days prior to the start of the experiment. Early isolation appeared to increase morphine consumption in Phase 0.15 for colony-dwelling rats, but the statistical support for this observation was weaker.

The apparently large effect of living in a colony on morphine consumption must be qualified by recognition of two facts. First, the loss of 3 day's data on Phase 0.5 reduces the confidence with which that phase's results can be accepted. This problem, however, does not apply to Phases 0.3 in which the housing effect was also large, since no data were lost. Second, there was a trend toward increasing consumption of MHCl in the IC rats and, to a lesser extent, the CC rats across the 5 days of Phases 0.3 and 0.15, suggesting that a longer period of exposure might reduce the magnitude of the housing effect. In spite of these qualifications, however, the present results and those of two previous experiments [1,10] suggest that the housing effect is both large and robust.

Two broad conclusions are suggested. First, the consumption of opiates by animals in self-administration experiments may be strongly facilitated by the typically isolated housing conditions during intake testing. Generalizations from such experiments should be qualified by this possibility. Second, some attributes which differ between the two housing environments in this experiment must affect a powerful control mechanism for opiate self-administration. Full analysis of the effect requires determining which attributes of the two environments are most critical and how their effect on opiate consumption is mediated. In addition to space and social contact, the colony environment contained cedar shavings, empty cans and boxes, and a high ceiling which allowed three-dimensional movement. Experiments underway in our laboratory should reveal the relative contributions of such factors.

The fact that housing at the time of intake testing accounts for most of the housing effect is compatible with our speculation [10] that colony rats avoid opiates because opiate consumption interferes with the performance of complex, species-specific behaviors. This speculation grew from evidence that colony rats forced to consume MHCl engage in significantly less fighting and sexual behavior [1], that relatively small doses of morphine significantly reduce sexual behavior and "social cohesion" in group caged rats [15,17], and that species-specific behaviors are self reinforcing [6].

There are, however, several other possible explanations for the housing effect. One of the most plausible is that morphine may reinforce isolated rats by relieving stress resulting from social and sensory isolation. This possibility, however, is contradicted by demonstrations that isolated, non-

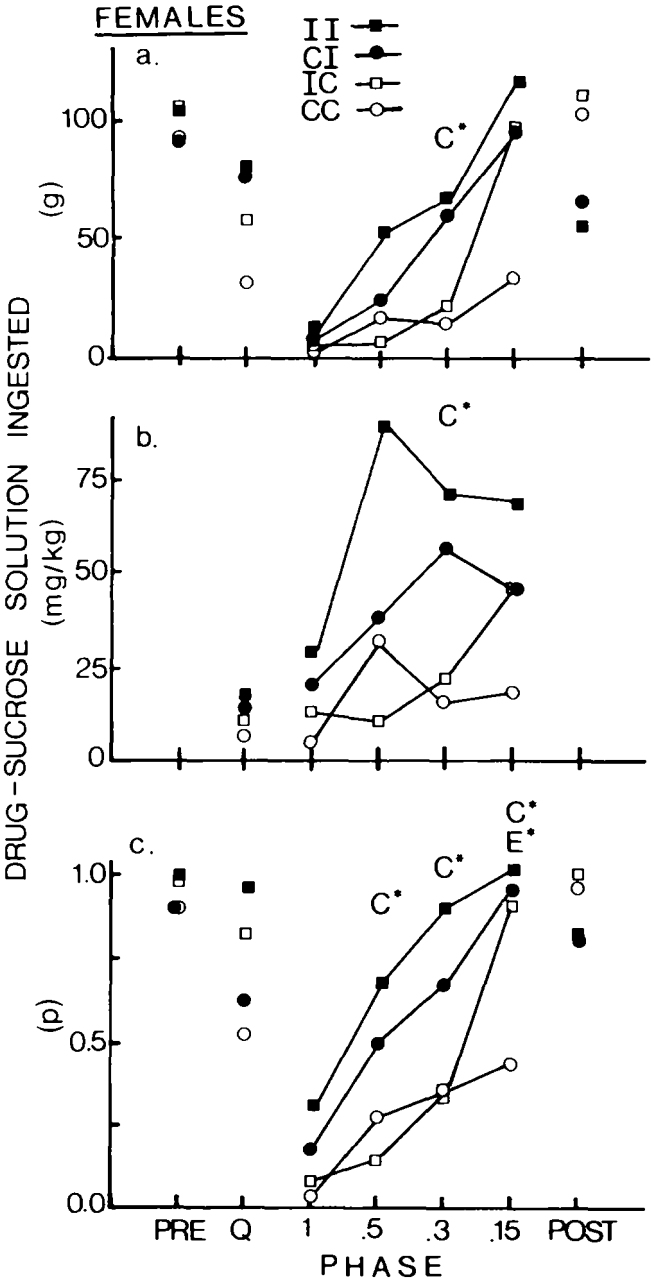

FIG. 2. Consumption of drug-sucrose solutions by female rats. Notation is the same as for Fig. 1.

physically-dependent rats avoid drinking opiates, unless they are induced to by sweetening the opiate solution. Isolated, non-physically-dependent rats with sectioned lingual and glossopharyngeal nerves reject morphine solution [12], indicating an aversion to its effects rather than to its bitter taste. Isolated non-physically-dependent rats also reject presumably tasteless solutions of etonitazene [21]. It has recently been reported [4] that rats rejected methadone when the alternative was an equally bitter quinine solution. However, when given naltrexone injections, eliminating the pharmacological effects of methadone, the rats drank equal amounts of both solutions. Therefore the initial avoidance was not to the taste or odor of the opiate, but to its effects. These findings suggest that oral opiates are not reinforcing to isolate rats. The apparent contradiction between these findings and observations of spontaneous opiate self-administration by isolated rats in self-injection experiments remains to be explained.

The present data comprise a better controlled replication of the previously reported housing effect. Colony males ingested much less morphine solution than isolated males though there was no difference in preference for sweet or bitter-sweet solutions. The data were not as conclusive for females, since there was a trend (short of statistical significance) toward less intake of bitter-sweet control solution in colony females. Analysis of covariance generally indicated a housing effect, but a more precise measure of it for females would require ruling out taste factors, perhaps by using etonitazene.

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