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An Examination of Heroin Conditioning in Preferred and Nonpreferred Environments and in Differentially Housed Mature and Immature Rats

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SCHENK, S., F. ELLISON, T. HUNT AND Z. AMIT. *An examination of heroin conditioning in preferred and non-preferred environments and in differentially housed mature and immature rats.* PHARMACOL BIOCHEM BEHAV 22(2) 215–220, 1985.—The study addressed two issues. First, we examined the effectiveness of heroin as a conditioning agent in a preferred environment using a place preference paradigm. Four daily injections of 80 μg/kg (SC) of heroin HCl were paired with environments that rats initially found to be either preferred or non-preferred. In subsequent tests, only those that had experienced the drug effects in the non-preferred environment increased the percentage of time spent in that environment. Rats conditioned in the test chamber that was initially preferred failed to increase the amount of time spent in that chamber post-conditioning. These results suggest that the conditioned place preference paradigm does not simply assess the rewarding consequence of heroin injections. We also examined the effects of grouped and isolation housing conditions on the heroin-produced conditioned place preference. Rats were housed under these conditions either immediately post weaning or at 120 days of age. There was a difference between the magnitude of the place preference produced by 20 μg/kg heroin in the isolated but not in the group housed rats. When isolated at weaning the rats were less sensitive to the drug than were rats isolated at maturity. These data are discussed with particular reference to the development of the endogenous opioid system.

Housing Heroin Place preference Developmental Opiate receptor

MANY of the behavioral effects of opiates are characterized by large across subject variability. For example, only a proportion of drug-naive subjects will self-administer morphine [7]. The effects of morphine on electrical self-stimulation of the brain are also highly variable with only 50% or so of the subjects showing a facilitatory effect [13]. One possible explanation for the variability is that there is some inherent difference between subjects that makes some rats more susceptible to the effects of opiates than others. One of the factors that may contribute to the differential magnitude of the individual response configuration is the degree of opiate receptor binding which has been shown to be modified by both pharmacological and environmental factors [4, 10, 12]. For example, rats that were chronically treated with the opiate antagonist naloxone were subsequently more sensitive to the analgesic properties of morphine compared to rats treated with a control substance [18]. Further, the naloxone-treated subjects had more central opiate receptor binding sites [10].

Another manipulation that can modify opiate receptor binding is the post-weaning housing condition. Rats housed in isolation for 6 weeks have fewer opiate receptor binding sites than rats reared in groups [12]. Rats housed in isolation are also less analgesic [9], show a less severe opiate withdrawal syndrome [1] and tend to self-administer more morphine solution through the oral route [3] than rats housed in groups. We have suggested that these differences may be the result of differential development of the endogenous opioid system resulting from the housing manipulation. The proliferation of opiate receptors in rat brain is most rapid between mid-fetal life and 3 weeks post-natally, tapering off at approximately 20 weeks of age [6]. All studies of the effects of housing on the behavioral efficacy of opiates take place during the first 20 weeks of age (e.g. [2, 3, 4, 9]) presumably since younger rats are easier to obtain from the breeding farms and are considerably less expensive than mature rats. If the differential effects of housing on opiate-produced behaviors are due to differential opiate receptor development (i.e., isolation housing inhibits the development) then one might expect the housing manipulation to be

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less effective in mature rats. That is, the effects may be an interaction of rearing and housing rather than strictly a housing effect.

In the present study we examine this possibility by testing the effectiveness of heroin in mature rats that are differentially housed. To assess the effect of heroin we chose to use the conditioned place preference paradigm. This paradigm has been suggested as a viable alternative to the self-administration paradigm for assessing the reinforcing properties of dependence-inducing drugs [8,11]. It is based on the associative notion that rats will return to a place in which they experienced the reinforcing consequences of a drug infusion. In most studies of this nature, drug injections are paired with an environment that is initially non-preferred. Following a series of such conditioning trials, more time will be spent in the environment in which the rats received the drug infusion. This has been taken to reflect the positively reinforcing properties of the injected drug.

We have used the paradigm to show that isolation reared rats are less sensitive to heroin than group reared rats [14]. Further, we have argued against the interpretation that the paradigm provides an index of opiate produced reward. If it does, one should be able to observe a conditioned place preference when rats are conditioned in a preferred as well as in a non-preferred environment. In the first and second experiments, we show that heroin is ineffective in producing a conditioning effect when paired with a preferred environment. In the second and third experiments, we demonstrate that the housing manipulation is only effective in altering the sensitivity of young rats to heroin; mature rats (120 days of age) are equally sensitive to the drug regardless of housing conditions.

EXPERIMENT 1

METHOD

Subjects

Subjects were 18 male Long Evans rats obtained at 42 days of age (Canadian Breeding Farms Ltd., St. Constant, Quebec). They were housed one to a cage for 1 week prior to the experiment to allow them to habituate to laboratory conditions. Food and water were freely available at all times.

Apparatus

Six testing chambers were use. Each consisted of a plywood box (56.5×12.5×30 cm) with a removeable lucite top. Each box was divided into two distinct sections. In one, the floor was made of plywood wrapped in wire mesh and the walls were constructed from sheet metal. In the other, the floor was made of plywood and the walls were plywood painted with black stripes. The floor of the apparatus was balanced on a central dowel such that uneven weight distribution caused the depression of a microswitch which activated a timing mechanism. In this manner, the amount of time per session spent on either side of the testing chamber could be recorded.

Procedure

For all subjects the general procedure was the same. The experiment consisted of five phases. During all phases, testing was carried out between 1600 and 2000 hr under low level lighting conditions.

(1) Habituation (4 days) During this phase, the rats were permitted access to the entire testing chamber for 15 min per day. The proportion of time spent on each side of the chamber was recorded. The average time spent on each side during the last two days served as the pre-conditioning baseline score.

(2) Conditioning 1 (4 days). During this phase the rats were injected with either a vehicle solution or 80 μg/kg heroin HCl (SC) and confined to one side of the testing chamber for 15 min per day. Half the rats were confined to the side of the box for which they demonstrated a preference during habituation while the other half were confined to the non-preferred side.

(3) Test 1 (1 day). On this day, the rats were again permitted free access to the entire testing chamber following an injection of vehicle solution. The amount of time spent on each side of the box was recorded and compared to that observed during habituation days.

(4) Conditioning 2 (4 days). The rats were again given daily injections of either vehicle or 80 μg/kg heroin but this time they were confined to the opposite side of the test chamber than in the first conditioning phase.

(5) Test 2 (1 day). The rats were given an injection of vehicle solution and given free access to the entire testing chamber. The amount of time spent on each side of the chamber was again recorded and compared to that during Test 1.

The order of conditioning was counter-balanced such that half the rats were first conditioned on the preferred side (Conditioning 1) followed by the non-preferred side (Conditioning 2) whereas the other half was conditioned first on the non-preferred side followed by the preferred side. There were four rats in each control (0 μg/kg) group and 5 rats in each drug (80 μg/kg) condition. Accordingly, the design was a 2×2 factorial with one factor being the method of conditioning and the other factor being drug dosage. The dose of 80 μg/kg was chosen since we have previously found this dose to produce maximal place preference effects [14].

The drug was dissolved in a Ringer's solution and 0.1% sodium meta bisulphite to prevent oxidation of the salt.

RESULTS

Figure 1 presents the mean percentage of time spent on the conditioned side of the test chamber for the rats conditioned on their initially preferred side (panel A) and for those conditioned on their initially non-preferred side (panel B). When conditioned on the preferred side, rats that initially spent about 80% of the session time in the conditioned environment spent approximately 70% after four conditioning days with 80 μg/kg heroin. That is, they failed to increase the amount of time spent in the conditioned environment. In contrast, when rats are conditioned in the environment in which they initially spent 20–30% of the session time, they increased the amount of time spent in that environment post-conditioning to approximately 55 to 65% of the session time. The effect observed following conditioning in the non-preferred environment was not influenced by conditioning method ($t(8)=0.971$, N.S.); it did not make a difference whether the rats had drug experience by first being conditioned on the preferred side of the test chamber. The pooled results from the non-preferred side, collapsed across conditioning method yielded a significant increase in the percentage of time (27%) spent in the initially non-preferred environment for the 80 μg/kg group ($t(8)=2.63$, $p<0.025$).

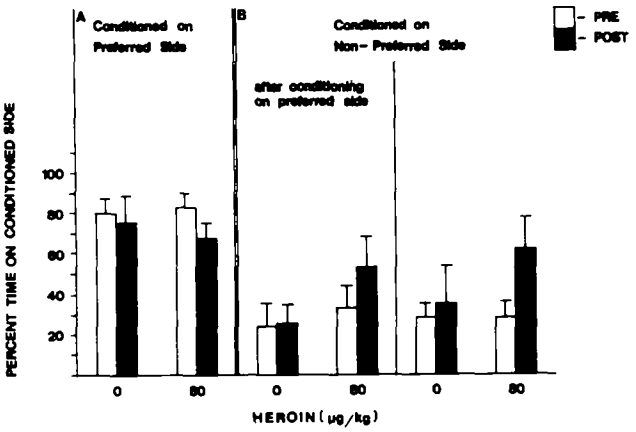

FIG. 1. Percentage of time spent on the conditioned side of the test chamber for rats conditioned on the preferred side of the chamber (panel A) and on the non-preferred side of the chamber (panel B). Open Squares refer to the percentage of time during last 2 habituation days. Closed squares refer to the percentage of time spent on test day.

DISCUSSION

The results of this experiment show differential effects of heroin in the place preference paradigm as a function of degree of initial preference. In our apparatus, we observe that most of our rats initially show a clear preference for one environment. After experiencing heroin in the initially non-preferred chamber the rats will show an indifference for either side of the box, spending approximately equal time in the conditioned and non-conditioned environments. This finding is consistent with other studies using morphine [8,11] amphetamine [17] cocaine [11,16] and heroin [5,15] as the conditioning agents. We have questioned whether this shift to indifference following heroin conditioning reflects the reinforcing properties of the drug or whether this small increase in the percentage of time spent in the conditioned environment represents some other component of drug-induced effects [14]. Recently, Spyraki and colleagues [16] have suggested that the effects of cocaine in the place preference paradigm may, in fact, reflect something quite distinct from the reinforcing properties of this drug. Our failure to observe an increase in the percentage of time rats spent in the initially preferred environment following conditioning with heroin at a dose that produced a clear increase in percentage of time spent in the initially non-preferred environment suggests that the effects of this drug in the place preference paradigm may not reflect the activation of simple reinforcement processes.

In the second experiment we focussed on two issues. We again examined the differential effects of conditioning method on the heroin induced conditioned place preference with increased sample sizes and a wider range of doses. We also compared the ability of heroin to produce conditioning effects in mature rats that had been differentially housed. Alexander [2] has suggested that the effectiveness of the housing manipulation in modifying the adult rat's sensitivity to opiates is dependent on the contemporaneous rather than the early housing conditions. However, in his study rats were tested during a period of rapid proliferation of the opiate receptor system [6]. One may therefore expect the effects of an earlier housing manipulation to be masked by the more recent housing condition. This may explain the failure of the early housing manipulation to have long-term consequences for the behavioral efficacy of the opiate.

In this study, we obtained rats at 120 days of age and then introduced the housing manipulation. At this stage, the endogenous opioid system is reported to be nearly fully developed [6]. We therefore hypothesized that the housing manipulation would be relatively ineffective in these mature rats as compared to the effects observed when young rats (immediately post-weaning) are housed in isolation.

EXPERIMENT 2

METHOD

Subjects

Subjects were 66 male Long Evans rats obtained at 120 days of age (Charles River Laboratories, Mississauga, Ontario). The subjects were housed either singly in metal cages (20×25×18 cm) or in groups of three per metal cage (41×25×18 cm). These housing conditions were maintained for six weeks. Food and water were freely available at all times.

Procedure

Since we found no difference in the results of the conditioning method in the first experiment, all rats were conditioned first on the preferred side of the test chamber followed by conditioning on the initially non-preferred side. The procedure was the same as that used in Experiment 1 except that a larger range of doses was used. Grouped and isolated rats were randomly divided into 0, 20, 40 and 80 µg/kg dosage groups. There were 9 rats in each control group and 8 in each drug condition. All testing was carried out at the same time each evening (1600–2000 hr) under low level indirect lighting conditions.

RESULTS

Table 1 presents the percentage of time spent in the initially preferred environment before and after the first conditioning session for the grouped and isolated rats. As in the first experiment, there is a tendency for the rats in both groups to spend less time in the environment in which they received drug injections under these conditions. These effects are not statistically significant, but demonstrate that place preference conditioning is not effective when heroin is paired with a preferred environment. Four control rats (2 grouped and 2 isolated) and 6 rats from the drug conditions actually reversed their initial preference folowing this conditioning procedure.

All rats were subsequently conditioned on the other side of the test chamber. For the 10 rats in which the preference had shifted following the first set of conditioning trials, the pre-conditioning times were now greater than 67% of the total session time. That is, they were conditioned on the preferred rather than on the non-preferred side of the test chamber during the second set of conditioning trials. We therefore eliminated the results from these subjects from any analysis. The results from the remaining subjects are presented in Fig. 2.

The pattern of effects for the grouped and isolated rats is similar. Maximal effects for both conditions are attained at the 20 µg/kg dose (grouped=38% shift; isolated=30% shift). A 3-way ANOVA (group × dose × time) was performed on the percentage scores. A significant main effect of time (pre/

TABLE 1

PERCENTAGE TIME SPENT IN THE INITIALLY PREFERRED ENVIRONMENT DURING HABITUATION AND ON TEST DAY FOLLOWING THE PAIRING OF HEROIN WITH THE PREFERRED ENVIRONMENT

	Dose Heroin (μg/kg)							
	0		20		40		80	
	hab	test	hab	test	hab	test	hab	test
Mean Grouped	74.58	65.39	86.33	71.03	77.20	78.22	73.35	68.38
SEM	5.64	11.00	4.05	10.77	5.58	6.63	7.21	10.49
Mean Isolated	80.32	62.38	88.15	83.66	82.73	68.69	80.97	70.79
SEM	6.06	10.88	2.99	7.28	5.40	10.49	3.18	9.22

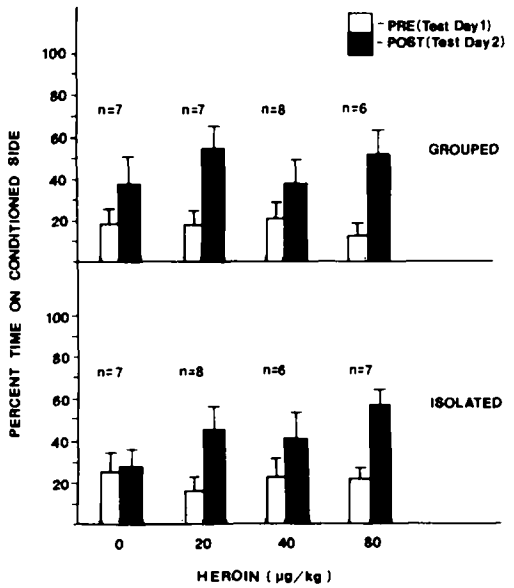

FIG. 2. Percentage of time spent in the initially non-preferred environment as a function of drug dosage for grouped and isolation housed mature rats.

post conditioning) was observed, $F(1,48)=34.17$, $p<0.0001$, but no significant interaction effects were found. These findings confirm that the effects of heroin in the place preference paradigm are not influenced by the housing manipulation in older rats.

EXPERIMENT 3

There was no difference between the effects of heroin in the rats that were grouped or isolated at maturity. This finding is in contrast to the differences observed when rats were housed under these conditions immediately after weaning (21 days) [14].

In this final experiment we replicated our earlier findings with young rats using the same procedure as in Experiment 2. Only a single drug dose was used (20 μg/kg) since this was the dose at which we had previously found the largest differences between rats that were grouped and isolated at weaning.

METHOD

Subjects

Subjects were 36 male Long Evans rats obtained at 21 days of age, immediately post-weaning (Canadian Breeding Farms Ltd., St. Constant, Quebec). The apparatus and procedure were the same as in Experiment 2 except that only the 20 μg/kg dose was used. There were 9 subjects in each group. As in Experiment 2, some rats (6 control and 2 drug) changed their preference following the first conditioning phase and hence were conditioned in the preferred environment during the second conditioning phase. They were therefore eliminated from any data analysis. As a result, data from 28 rats (6 in each control group, 7 in the grouped 20 μg/kg group, and 9 in the isolated 20 μg/kg group) contributed to our analysis.

RESULTS

Table 2 shows the effects of conditioning with 20 μg/kg of heroin for the young (from this experiment) and mature rats (from Experiment 2) under the two housing conditions. We used *t*-tests to compare the differences in percentage time spent in the conditioned environment before and after conditioning. For the isolation housed subjects given 20 μg/kg heroin, the difference in perecentage time for the mature and young rats was 30.38% and 7.18% respectively. The differences between the average percentage change in these rats was statistically reliable ($t(15)=1.847$, $p<0.05$). The shift for the young group-housed rats given 20 μg/kg heroin (15.5%) was smaller than for their mature counterparts (37.96%) although not statistically so ($t(12)=1.39$, $p>0.05$).

GENERAL DISCUSSION

At least two general findings emerge from the present study. First, we addressed the issue of whether the place preference paradigm measures the reinforcing properties of heroin.

The results of Experiments 1 and 2 show that conditioning to a preferred environment is not effective. The percentage change observed in experiments of this kind when rats are conditioned in the non-preferred environment [5,15] is generally in the range of 20–30%. We expected that rats initially spending 70–80% of the session time in the conditioned environment would also shift 20–30% following conditioning. This was not observed. In fact, rats con-

TABLE 2

MEAN PERCENTAGE CHANGE (POST-PRE CONDITIONING) IN PERCENTAGE TIME SPENT IN THE CONDITIONED ENVIRONMENT FOR MATURE (120 DAYS OLD) AND YOUNG (21 DAYS OLD) GROUPED AND ISOLATION HOUSED RATS

		Dose Heroin (μg/kg)			
		0		20	
		grouped	isolated	grouped	isolated*
mature	Mean	19.51	2.06	37.96	30.38
	SEM	11.18	10.17	10.37	11.42
young	Mean	5.09	17.3	15.55	7.18
	SEM	5.88	14.92	12.25	6.14

*$p<0.05$ between groups.

ditioned in the preferred environment tend to decrease the time spent there to 60–80% of the session time. This finding can be interpreted to indicate that either the "attractiveness" of one environment or the "aversiveness" of the other has decreased. It is possible that the heroin effects observed when conditioning is performed in the non-preferred environment reflects a decrease in the aversive properties that are initially associated with that environment. Another possibility is that heroin causes the rats to become "indifferent" to either environment resulting in a decrease in the amount of time spent in the initially preferred place and an increase in the time spent in the initially non-preferred place. If so, then it is not clear that the paradigm assesses the reinforcing properties of opiates.

The second aspect of the study concerns the ineffectiveness of our housing manipulation to modify the mature rat's reaction to heroin in the place preference paradigm. The results of Experiments 2 and 3 show that rats isolated at weaning are less sensitive to heroin in the place preference paradigm than rats isolated at maturity. We have suggested that the sensitivity of the adult rat to heroin may, in part, be a reflection of the extent of development of the endogenous opioid system which is somewhat inhibited by social isolation in the young rat [14]. At 120 days of age, the development of this endogenous system reaches a maximum [6] and we therefore hypothesized that the housing manipulation would be ineffective in rats of this age. The results of Experiment 2 support this notion. It would be of interest to compare the amount of opiate binding in the brains of rats that are differentially housed at this age.

In summary, we have laid out the extreme boundaries for the effectiveness of the housing manipulation on the sensitivity to heroin. These boundaries are between 21 and 120 days of age. In the next series of experiments (in preparation) we hope to better define these limits and to reduce the length of the housing manipulation to a minimum. We have also questioned the interpretation of data from place preference experiments. Since conditioning in a preferred environment fails to increase the amount of time a subject spends in that environment, we suggest that the paradigm may not simply assess the rewarding properties of heroin but, rather, may reflect a more complicated interaction of the behavioral effects of opiates.

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