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Secondary Reinforcement Property of a Stimulus Paired with Morphine Administration in the Rat[1]

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NUMAN, R., U. BANERJEE, N. SMITH AND H. LAL. *Secondary reinforcement property of a stimulus paired with morphine administration in the rat.* PHARMAC. BIOCHEM. BEHAV. 5(4) 395–399, 1976. – Rats learned to run to the correct arm of a Y-maze. Correct responses were reinforced with morphine injection paired with a conditional tone stimulus. After the maze response was well established, extinction trials were run. During extinction half of the animals received neither morphine nor tone as a consequence of a correct response, while the other half received the tone but no morphine. Rats receiving the tone during extinction required significantly more trials to reach the extinction criteria than rats not receiving tone presentations. Extinction with the tone also facilitated relearning of the maze response. The results support the view that morphine is a potent reinforcer, and that stimuli paired with morphine administration acquire the properties of a secondary reinforcer.

Morphine Conditioning Secondary (conditioned) reinforcement Maze learning Resistance to extinction Drug abuse

A NUMBER of recent studies have shown that the effects of chronically administered narcotic drugs can be classically conditioned to environmental stimuli. Stimuli repeatedly paired with narcotic abstinence acquire the ability of reinstating withdrawal signs in animals already recovered from primary abstinence [3,13]. In contrast, a treatment-oriented approach investigated in our laboratory, and elsewhere, has consistently shown that stimuli paired with morphine administration during the period of addiction come to act like morphine, and reduce abstinence signs when presented alone during withdrawal. In those studies, previously neutral stimuli were shown, by themselves, to reduce withdrawal hypothermia [1, 2, 7], to suppress withdrawal body shakes [6], and to reinstate efficient performance on operant tasks [9,10], after being repeatedly paired with morphine administration. These findings, taken together, stress the importance of the role played by environmental stimuli in the acquisition, maintenance and relapse of drug-abuse behaviors.

Morphine administration has been shown to be a primary reinforcer for both morphine dependent [11], and drug naive animals [8,12]. It is also known that conditional stimuli, when repeatedly paired with a primary reinforcer, acquire the properties of that reinforcer in controlling behavior as a secondary or conditioned reinforcer [4,5]. We have already shown [1, 2, 6, 7] that stimuli paired with morphine administration acquire conditional properties of morphine, in that such stimuli are able to reduce withdrawal signs. Moreover, like morphine, the effects of these conditional stimuli are antagonized by naloxone [1]. In the present experiment we undertook to establish whether or not such conditional stimuli would serve as secondary reinforcers by increasing the resistance to extinction of an instrumental response.

METHOD

Animals

Male hooded rats of the Long-Evans strain (Charles River, Wilmington, Ma.) weighing between 250–300 g at the beginning of the experiment were used. They were housed individually with food and water freely available. House lighting was alternated on a 12-hr day–night cycle (0800–2000 hr).

Apparatus and Drugs

A Plexiglas Y-maze with symmetrical arms (61 × 11 × 15 cm), and grid floor was used. Sliding doors could be lowered at the entrance point of each arm of the maze. One arm of the maze, with blackened walls, was designated as the start arm. The two remaining arms (left and right) were white. The maze was housed in a sound-shielded room, and was diffusely illuminated from above with fluorescent lighting. A tone stimulus (5,000 Hz, generated by an audio-oscillator) was used as the conditional stimulus. The tone sound was generated at the point of intersection of the left and right arms of the maze. Morphine sulphate was dissolved in distilled water and administered intraperitoneally. Morphine doses are expressed as total salt, and the injection volume ranged between 0.25–0.35 ml/rat.

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Procedure

In order to provide evidence for secondary reinforcement we measured the degree of resistance to extinction of a Y-maze response in the presence or absence of the conditioned reinforcer [4,5].

Prior to any drug administration, or conditioning, each animal was placed in the start arm of the Y-maze and allowed to make a response to either the left or right arm. The arm chosen was designated as the preferred side for the animal, and subsequent reinforcement was administered at the opposite, nonpreferred side.

Conditioning. On the day following preference testing, animals were administered 3 daily injections of morphine, in an escalating dosage regimen, for 8 days. The injections were given at 0900, 1500, and 2100 hr. The dosage on Day 1 was 10 mg/kg/injection; on Day 2, 20 mg/kg/injection; and from Day 3 onward, 40 mg/kg/injection. Throughout this 8-day period, all injections were administered in the Y-maze. During this phase of the experiment, all doors at the entrances to the goal arms were lowered, preventing the rat from exiting after placement in a particular arm. First, each rat was placed in the start arm for 30 sec. The rat was then picked up, and placed in its nonpreferred goal arm for 5 min during which time the tone was activated. Morphine was administered intraperitoneally, at one minute following tone onset (total injection time did not exceed 15 sec). A 5 min tone CS was used to assure that the CS would overlap with the central effects of morphine. Twenty-four morphine-tone pairings were administered in this manner prior to the initiation of acquisition trials.

Acquisition. From Day 9 onwards the rats were required to run to the correct arm (nonpreferred side) of the maze for their morphine injections. Two maze trials were administered daily at 0900 and 2100 hr. All doors to the maze arms were open at the beginning of each trial. Each rat was placed in the start arm and allowed to run freely in the maze. When the correct arm was entered, a door was lowered preventing return, and the rat was retained there for 5 min during which time the tone was activated. Morphine (40 mg/kg) was injected 1 min following tone onset. A correction procedure was used for all trials (i.e., on any given trial the rat could exit from an incorrect alley and enter the correct goal arm). However, if a correct response was not made within 4 min, the rat was placed in the correct arm of the maze, the tone was turned on, and morphine administered. The criterion for maze acquisition was 9 errorless responses in 10 consecutive trials. Trials to criteria, errors, and latency were recorded. Errors were counted when the rat placed both fore and hind paws into the incorrect goal arm. Since a correction procedure was used, it was possible for a rat to make more than one error on each trial, since the rat could exit from and then re-enter the incorrect arm. Latency was recorded by activating an electronic timer when the rat was placed in the start arm. The timer was stopped when the rat placed both fore and hind paws into the correct goal arm. If a rat remained in the start arm for 4 min, without making any responses, the trial was recorded as one error with a latency of 4 min.

Extinction. On the day following acquisition, the extinction trials were run. During extinction the rats were divided into 2 groups. In one group, the rats received no treatment (no tone, no morphine) upon emitting a correct response, but were retained in the correct arm for 5 min. In the other group, rats were also retained in the goal arm for 5 min following a correct response, but in this case the 5-min tone (but no morphine) was presented. Extinction was completed in one day of massed trials with a 5-min intertrial interval. The criteria for extinction was 5 consecutive trials without a correct response, or 3 consecutive trials during which no response was made within 4 min of placement in the start arm. Trials to extinction, errors, and latency were recorded.

Relearning and reextinction. On the day following extinction trials, all rats were required to relearn the maze task. Morphine (40 mg/kg) tone pairings were administered for a correct response, and the procedure was identical to that used during acquisition. On the day following completion of relearning, reextinction trials were run in a crossover design. Therefore the group which previously received no treatment for a correct response now received the 5-min tone, while the other group of animals now received no treatment. The procedure and criteria for reextinction were identical to that used for original extinction.

The experiment was conducted in two replications. Since the results of these replications did not differ, the data were pooled. A total of 20 rats were used, 10 in each group. A third group of 5 rats was used as an additional control. These control rats were treated in the same manner as the other animals, except that saline injections, instead of morphine, were paired with the tone. The significance of the results was determined with Student's t-test, two-tailed.

RESULTS

Acquisition and Extinction

All rats readily acquired the maze response, learning to run to the correct arm of the Y-maze for their daily morphine injections paired with a tone. There were no differences between the two groups on any parameter of performance during acquisition (Table 1). (During acquisition, all experimental animals received the same treatment. These animals are designated as two groups of 10 rats each on the basis of their subsequent treatment during extinction; i.e., 10 rats are subsequently extinguished with tone, and 10 rats without tone.) In contrast, during extinction trials, rats receiving the tone following a correct response required significantly ($p<0.05$) more trials to reach the extinction criteria than rats which did not receive the tone upon entering the correct arm (Table 2).

Relearning and Reextinction

The data in Table 1 show that extinction with the tone present facilitated relearning of the maze response. Rats which received the tone during extinction required significantly fewer trials ($p<0.01$) and made significantly fewer errors ($p<0.01$) during relearning in comparison to the rats which did not receive tone exposure during extinction. The latency data for the two groups did not differ. During reextinction, the results are essentially the same as for the original extinction. Animals which received the tone following a correct response required significantly ($p<0.01$) more trials to reach the extinction criteria in comparison to the rats which did not receive tone presentations (Table 2). However, it should be noted, in Table 2, that rats reextinguised without tone did achieve smaller latencies than rats reextinguished with tone ($p<0.05$).

It can also be seen (Table 2) that the order of tone presentation in the extinction phases did not influence the

TABLE 1

ACQUISITION OF A MAZE RESPONSE FOR MORPHINE INJECTIONS PAIRED WITH TONE AND THE EFFECT OF CONDITIONAL TONE PRESENTATIONS DURING EXTINCTION ON RELEARNING

Measure of Maze Performance	Maze Performance, Mean ± SE			
	Acquisition	Relearning Following Extinction with Tone	Relearning Following Extinction without Tone	p*
Trials†	16 ± 0.7	12 ± 0.5‖	—	0.001
% Errors‡	31 ± 2.2	17 ± 1.3‖	—	0.001
Latency§	54 ± 11.6	43 ± 5.5	—	NS
Trials†	18 ± 1.2	—	19 ± 2.2‖	NS
% Errors‡	33 ± 2.3	—	37 ± 6.6‖	NS
Latency§	51 ± 8.7	—	62 ±11.0	NS

*Level of significance, t-test, two-tailed (NS, not significant, $p>0.05$).
†Number of trials to reach criteria.
‡Percent of trials on which one or more errors occurred.
§Time in seconds to enter the correct goal arm.
‖Between group comparisons for both trials and errors is significant ($p<0.01$).

TABLE 2

EFFECT OF CONDITIONAL TONE PRESENTATIONS ON RESISTANCE TO EXTINCTION OF A MORPHINE MOTIVATED MAZE RESPONSE

	Tone Condition During Extinction	Maze Performance, Mean ± S.E. Trials*	% Errors†	Latency‡
Extinction	Tone Presented	17 ± 1.6	70 ± 4.3	66 ± 11.8
	Tone Not Presented	12 ± 1.4	75 ± 3.9	81 ± 9.3
	p§	0.05	NS	NS
Re-Extinction	Tone Presented	18 ± 1.5	63 ± 2.5	63 ± 6.2
	Tone Not Presented	12 ± 1.3	72 ± 4.2	41 ± 7.1
	p§	0.01	NS	0.05

*Number of trials to reach extinction criteria.
†Percent of trials on which one or more errors occurred.
‡Time in seconds to enter the correct goal arm.
§Level of significance, t-test, two-tailed (NS, not significant, $p>0.05$).

results, but rather that resistance to extinction was determined by whether or not the tone was presented as a consequence of a correct response. The number of trials necessary to reach the extinction criteria did not differ between the two groups when they were compared under the same conditions. In contrast, rats in either group required significantly more trials to reach extinction when the tone was present, than when it was absent ($p<0.02$ for each group). (This p value was computed for a within group comparison, t-statistic for paired data. In Table 2, the numbers compared are 17 ± 1.6 vs 12 ± 1.3 and 12 ± 1.4 vs 18 ± 1.5 of the trials column.)

Savings During Relearning

The above results suggested that animals originally extinguished with the tone relearned the maze response faster than rats extinguished initially without tone. Within group comparisons substantiate this finding (see Table 1). Rats extinguished initially with the tone required 16 trials for acquisition, but only 12 trials for relearning. The savings is significant ($p<0.001$). This group also made significantly ($p<0.001$) fewer errors during relearning. In contrast, the group extinguished initially without the tone did not show any savings, requiring 18 trials for acquisition and 19 trials for relearning.

Finally, animals receiving saline injections paired with tone did not acquire the maze task (data not shown). This failure indicates that the tone was, in itself, a neutral stimulus and did not guide the animals' response in the maze. The tone therefore, cannot be regarded as a facilitating or eliciting stimulus [4] for the response in question.

Throughout all phases of this experiment, animals were grossly observed, periodically, for signs of withdrawal distress. While mild withdrawal discomfort probably did occur prior to the administration of the daily injections, gross withdrawal signs did not occur. The animals appeared healthy, and body weights remained stable.

Following termination of the experiment, all animals were again observed, at 24-hr intervals, for withdrawal symptoms. During the period of primary abstinence [13]

all rats showed moderate signs of withdrawal distress including weight loss and body shakes, with peak effects occurring between 48–72 hr after withdrawal.

DISCUSSION

Previous work in our laboratory [1, 2, 6, 7] and other laboratories [9,10] have shown that stimuli, paired with morphine administration, acquire some of the pharmacological actions of morphine, and are able to reduce withdrawal distress. The results of the present experiment further augment the previous conclusions by showing that these conditional stimuli also acquire some of the reinforcing properties of morphine, and can serve as secondary reinforcers.

The criteria used for establishing the conditional stimulus as a secondary reinforcer (conditioned reinforcer) was the resistance to extinction of a Y-maze response. An increase in resistance to extinction, when a conditional stimulus is present, is a commonly accepted measure of the extent of secondary reinforcement [4, 5, 14]. Both within and between group comparisons, in the present experiment, support the view that the tone, after repeated pairings with morphine, served as a secondary reinforcer by increasing resistance to extinction of the maze response. It is unfortunate that, in most cases, the error and latency data did not strengthen these findings, and in one instance (reextinction, Table 2) the latency data contradicted our hypothesis. This was not surprising, since the weak nature of conditioned reinforcers is known [4,5] and therefore conditioned reinforcement effects may not be equally measurable on all response parameters.

The present findings are of further interest in that they clearly establish the reinforcing potency of morphine. Earlier studies have used operant tasks [11,12] to show that rats will work for morphine. Our findings extend these results by demonstrating that rats will learn a complex instrumental discrimination in order to obtain morphine. This reinforcing property of morphine is believed to be due to direct physiological effects of the drug itself [8,12], and to its ability to postpone withdrawal discomfort [6, 11, 13].

A more unexpected finding of the present study was the facilitation of relearning following extinction with the tone, and the absence of savings during relearning in the group extinguished without tone. Since the error data (both % errors, and total actual errors) for the two groups did not differ during the original extinction one cannot explain these results by suggesting that animals extinguished without tone developed incompatible response patterns. One might alternatively suggest that the discriminative cue properties of the tone for the correct goal arm were strengthened by extended presentations during extinction and thereby facilitated relearning when morphine was again available. It should be pointed out here, that the discriminative cue functions of stimuli paired with primary reinforcers may, at least in part, explain the establishment of that stimulus as a secondary reinforcer [4]. The absence of savings in the group extinguished without tone is not clear since one would expect at least some savings due to spontaneous recovery. Perhaps a delay in the onset of morphine's unconditioned effects on reinforced trials could account for this effect.

These results are significant for the further understanding of patterns of human drug abuse and relapse. The conditioning studies previously conducted in our lab, and the present experiment, strongly suggest that environmental stimuli, which have been associated with drug administration, acquire control over drug-seeking behavior. Thus, environmental stimuli may induce a drug-like state, and while temporarily warding off withdrawal discomfort, may eventually lead to a craving for the drug. Such environmental control could feasibly facilitate relapse to drug taking even in cured individuals.

The present study supports this conclusion, indicating that stimuli paired with drug administration strengthen responses directed toward acquiring the drug. Extinction of the response is more difficult in the presence of these stimuli, and moreover, the effects of the stimuli, themselves, may be extremely difficult to extinguish. These results would imply, that in the treatment of human narcotic abusers, stimuli associated with drug abuse should be completely extinguished, and that counterconditioning methods should be employed in an attempt to discourage relapse tendencies.

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