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Reward and Reinforcement Produced by Drinking Sucrose: Two Processes That May Depend on Different Neurotransmitters

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ÅGMO, A., A. GALVAN AND B. TALAMANTES. *Reward and reinforcement produced by drinking sucrose: Two processes that may depend on different neurotransmitters.* PHARMACOL BIOCHEM BEHAV **52**(2) 403-414, 1995. - The capacity of sucrose drinking to produce conditioned place preference (CPP) and the effects of dopamine and opioid receptor blockade on acquisition of this preference and on sucrose consumption were evaluated. The establishment of place preference would reflect the reinforcing consequences of drinking, while the amount of sucrose solution consumed would illustrate its rewarding properties. Male Wistar rats were allowed to drink an 18% sucrose solution. No deprivation was used. Drinking produced place preference, and this was blocked with cis(z)-flupentixol, 0.5 mg/kg. Naloxone, 16 mg/kg, not only blocked place preference, but also reduced sucrose consumption. In fact, animals consumed amounts similar to those consumed by rats given plain water. Lower doses of naloxone, 1 and 4 mg/kg, also decreased sucrose consumption, but 1 mg/kg did not block place preference. As the dopamine antagonist did not affect consumption at a dose that inhibited conditioned place preference, it is suggested that dopamine is critical for the establishment of reinforcement produced by sucrose. Opioids seem to be more important for the rewarding effect because naloxone reduces drinking even at a dose that does not affect place preference.

Reward Reinforcement Dopamine Opioids Place preference

WE HAVE previously reported that sexual reinforcement, as evaluated with the conditioned place preference (CPP) procedure, was completely blocked by the opioid antagonist naloxone, both when the drug was administered systemically or when it was infused into the medial preoptic area (1,3). Sexual behavior was not modified by naloxone. A dopamine antagonist, pimozide, was unable to inhibit CPP and did not affect sexual behavior. These data prompted the hypothesis that sexual reinforcement is an opioid-dependent phenomenon. In a further study, it was reported that CPP produced by drinking water, in water deprived rats, could be inhibited equally well by opioid and dopamine antagonists. Further, both kinds of drugs had similar effects on consumption (5). It was suggested that dopamine is an important transmitter for reward and reinforcement produced by drinking. It appears, then, that the reinforcement-related cerebral processes activated by sexual behavior are different from those activated by drinking water. This observation was the rationale for studying an additional natural reinforcer.

In the present experiments, the ability of sucrose drinking to produce CPP was analyzed. Then, the effects of dopamine or opioid receptor blockade on acquisition of conditioned place preference were evaluated. Drug effects on reward were differentiated from those on reinforcement. Although this distinction may not be conceptually unambiguous, clear operational definitions are provided (see Experiment 2).

Opioid antagonists, like many other drugs, produce taste aversion [(25,47) and references therein]. Because the CPP procedure requires multiple drug administrations, it would be possible that effects of an opioid antagonist on sucrose consumption were a consequence of taste aversion rather than of modified reward value. A procedure had to be found, therefore, where taste aversion was not produced.

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GENERAL METHOD

Subjects

Male Wistar rats (300–400 g) from a local colony were housed individually under a reversed 12 L : 12 D cycle (lights off 0900 h) at a temperature of 22 ± 1°C. Commercial rat pellets and tap water were available ad lib unless stated otherwise.

Apparatus

Licking was registered with an optical lickometer (Coulbourn S23-01) mounted 3 cm above the grid floor of a Plexiglas box (inner dimensions, 35 × 25 × 25 cm high). The box was located in a sound-attenuating cage (Lafayette) and lit with a 5 W white house light. The cage ventilator provided internal masking noise. The latency to the first lick and the number of licks were recorded with a BRS/LVE electromechanical equipment placed in an adjacent room.

It must be noted that there appears to be a close relationship between licking and drinking. In some experiments, the volume drunk was recorded in addition to the number of licks. Independently of drug treatment, a correlation of 0.91–0.96 was obtained between the number of licks made and the volume drunk. We propose, therefore, that the reinforcing event in these studies is sucrose drinking rather than the execution of licking responses.

The place preference apparatus has been described in detail elsewhere (1). Briefly, three-compartment boxes were used. The lateral compartments differed in color, odor, and floor texture. The boxes were located in the same room as the lickometer. The room was illuminated with dim white light and a 60 dB white noise masked sounds.

Drugs

Naloxone HCl (Rhone-Poulenc Farma, Mexico City), cis(z)-flupentixol HCl (Lundbeck, Copenhagen, Denmark), and LiCl (Sigma, St. Louis, MO) were dissolved in physiological saline and injected intraperitoneally (IP) in a volume of 1 ml/kg b.wt.

EXPERIMENT 1

This experiment had two purposes. First, to determine whether our version of the conditioned taste aversion procedure works in a way similar to other such procedures and, second, to determine whether taste aversion could be produced in animals that were habituated to the sucrose solution in a way similar to that used in place preference conditioning (Experiment 2).

Method

Procedure. Water was removed from the subject's home cage at about 0700 h. Seven hours later, the subject was placed in the lickometer and allowed to drink an 18% (w/w) sucrose solution in water during 10 min. This concentration of sucrose was chosen because it has been shown to be the most efficient for promoting learning in nondeprived rats (55). The subjects that made more than 500 licks were given an IP injection of saline or drug 2 min after the end of drinking. The animals were then returned to the colony room and given free access to water. Twenty-four hours later, they were again allowed to drink the sucrose solution in the lickometer. The subjects were deprived of water for 7 h before the 10-min test.

Additional groups of rats were habituated to the sucrose solution before the taste aversion conditioning. These animals had free access to the 18% sucrose solution in their home cage for 48 h. Tap water was also freely available. Twenty-four and 48 h later, they were allowed to drink sucrose in the lickometer at two habituation sessions of 10 min each. The following day the experimental treatment was administered either 2 or 30 min before drinking sucrose for 10 min in the lickometer. The test (10 min) was made the following day. All subjects that made fewer than 500 licks at the second habituation session were eliminated.

Design. Three treatments were used, each given to separate groups of animals: LiCl, 50 mg/kg; naloxone, 16 mg/kg; and flupentixol, 0.5 mg/kg. On the conditioning sessions, half of the animals in each group was treated with drug and the other half with saline. LiCl was used because it is a compound that reliably produces taste aversion (25). The dose of naloxone was the same as in previous studies on sexual and drinking-induced reinforcement (1,5). The flupentixol dose has some effect on ambulatory activity but does not affect motor coordination (2,5).

The rats habituated to the sucrose solution were divided in six groups. Three different groups were used for each of two treatment schedules (drug administration 2 or 30 min before conditioning). The treatments were saline, LiCl, 50 mg/kg, and naloxone, 16 mg/kg.

Two intervals between drug injection and sucrose drinking were used because lengthening the interval between injection and conditioning has been found to reduce aversive effects (13). Thirty minutes was the interval chosen because of naloxone's short half-life (31,48).

Statistical analysis. Data were analyzed by ANOVAs for repeated measures on one factor. The within-groups factor was session and the between-groups factor was treatment.

Results and Discussion

ANOVA of the number of licks showed a significant effect of treatment in the LiCl and naloxone groups, $F(1, 5) = 7.13$, $p = 0.044$, and $F(1, 10) = 6.48$, $p = 0.029$, respectively. There was also a difference between sessions [LiCl, $F(1, 10) = 6.18$, $p = 0.032$; naloxone, $F(1, 20) = 9.46$, $p = 0.006$], and the interaction treatment × session was significant [LiCl, $F(1, 10) = 8.13$, $p = 0.017$; naloxone, $F(1, 20) = 10.31$, $p = 0.004$]. Flupentixol had no effect (all $p > 0.4$). A posteriori comparisons showed that LiCl, 50 mg/kg, and naloxone, 16 mg/kg, reduced the number of licks at the test (Table 1). Naloxone also increased the lick latency at the test.

In the rats habituated to the sucrose solution, no effect was obtained either when the treatments were administered 2 min before drinking, or when they were administered 30 min before (Table 2). The ANOVA results were the following: drug administration 2 min before drinking; treatment, $F(2, 21) = 1.06$, NS, sessions, $F(3, 63) = 0.93$, NS, interaction treatment × session, $F(6, 63) = 0.18$, NS; drug administration 30 min before drinking; treatment, $F(2, 18) = 2.29$, NS, session, $F(3, 54) = 1.21$, NS, interaction treatment × session, $F(6, 54) = 1.03$, NS. There was no statistical significance with regard to lick latency (all $p > 0.2$).

These results show that taste aversion is established when the taste is novel and the drug administered after exposure to the taste. However, when the subjects had been habituated to the taste, and when the drugs were administered before beginning the conditioning session as was later made in the

TABLE 1

LICK LATENCY AND NUMBER OF LICKS IN ANIMALS SUBJECTED TO TASTE AVERSION CONDITIONING: THE TREATMENT WAS GIVEN 2 MIN AFTER DRINKING SUCROSE

	Conditioning		Test	
Treatment	Latency	Licks	Latency	Licks
Saline	112 ± 70.9	1305 ± 247	25 ± 8.7	1689 ± 292
LiCl 50	90 ± 32.4	1879 ± 204	187 ± 107.0	263 ± 132†
Saline	30 ± 32.4	1977 ± 297	72 ± 19.9	1787 ± 269
Naloxone 16	49 ± 16.5	2022 ± 112	296 ± 74.6*	195 ± 139†
Saline	50 ± 39.2	2681 ± 482	6 ± 2.8	1897 ± 428
Flupentixol 0.5	30 ± 2.6	1734 ± 330	32 ± 8.8	1475 ± 473

Doses in mg/kg. Data are means ± SE. Latencies in sec. $n = 6$ (LiCl); 11 (naloxone); 5 (flupentixol). The number of saline-treated animals was always equal to the number of drug treated subjects.

*Different from saline, $p < 0.05$; †$p < 0.001$.

CPP experiments, no taste aversion was obtained. The lack of effect is not due to the temporal relation between drug administration and conditioning. In other experiments, where naloxone, 16 mg/kg, was given 30 min before conditioning to nonhabituated rats, a clear taste aversion was obtained (mean ± SE number of licks at the test was 2255 ± 415 after saline and 766 ± 284 after naloxone, $p < 0.001$, $n = 8$ in both groups). Thus, habituation to the taste seems to be the critical factor. This is in agreement with other studies, where it has been shown that the novelty of the taste stimulus is important [e.g. (24,45)]. Indeed, preexposure to sucrose of less duration than the one employed here completely abolished taste aversion (8).

These data suggest that the procedure used here and in the following CPP studies does not readily produce taste aversion. Any drug effect on sucrose consumption cannot, therefore, be immediately attributed to this mechanism. It is also important to note that naloxone was administered in a large dose. Although this may make conclusions regarding pharmacological specificity difficult, it should be evident that the far lower doses used in Experiment 3 are unlikely to produce direct aversive effects.

EXPERIMENT 2

Place preference conditioning may be considered as a three-stage process (52). First, a stimulus needs to produce hedonic activation (reward). Second, this hedonic activation must be associated with environmental cues. Third, the association has to be remembered at the test. The last two stages are the consequences of reinforcement (learning), whereas the consumption of the stimulus reflects its rewarding capacity (51).

The capacity of sucrose drinking to induce an associable affective state, using the CPP procedure, was evaluated in this experiment. Furthermore, the effects of dopamine and opioid antagonists on the establishment of this CPP and on sucrose consumption were determined. The consumption of sucrose was used as a measure of its reward value, while the establishment of place preference was the indicator of reinforcement.

TABLE 2

LICK LATENCY AND NUMBER OF LICKS IN ANIMALS THAT WERE HABITUATED TO THE SUCROSE SOLUTION AND THEN SUBJECTED TO A VARIANT OF THE CONDITIONED TASTE AVERSION PROCEDURE

	1st Habituation		2nd Habitation		Conditioning		Test	
Treatment	Latency	Licks	Latency	Licks	Latency	Licks	Latency	Licks
				2 Min Before				
Saline	80 ± 30.8	1440 ± 296	28 ± 10.2	1202 ± 226	12 ± 6.8	1380 ± 258	30 ± 9.1	1131 ± 211
LiCl 50	120 ± 46.9	1186 ± 206	57 ± 34.3	1610 ± 225	21 ± 6.3	1086 ± 318	21 ± 8.8	1051 ± 271
Naloxone 16	152 ± 44.4	929 ± 111	55 ± 17.4	1519 ± 252	31 ± 10.5	802 ± 220	63 ± 21.7	1002 ± 354
				30 Min Before				
Saline	91 ± 44.1	2114 ± 243	15 ± 5.8	1534 ± 209	36 ± 7.3	1923 ± 338	29 ± 10.6	1405 ± 262
LiCl 50	95 ± 39.0	1946 ± 146	36 ± 14.8	1281 ± 200	43 ± 12.1	934 ± 116	25 ± 8.3	1166 ± 149
Naloxone 16	99 ± 34.3	1525 ± 574	32 ± 9.1	1622 ± 109	21 ± 7.6	1382 ± 259	32 ± 14.3	1288 ± 179

Drug treatments were administered either 30 or 2 min before the conditioning session. Doses in mg/kg. Data are means ± SE. Latencies in sec. $n = 8$ (all groups given the drugs 2 min before licking) and 7 (all groups treated 30 min before).

Sucrose was consumed immediately before the subjects were placed in the place preference boxes so that the rewarding and/or reinforcing stimulus itself could not be directly associated with the stimuli in the place preference boxes. Only the affective state aroused by the stimulus would be carried over to the place preference conditioning procedure. This, then, would eliminate stimulus-stimulus associations and permit only affective state-stimulus associations. Such a procedure has previously been used successfully (1,3,5).

A biased CPP procedure was used, because we have found it most adequate for studies of naturally reinforcing events (1,3,5). Generally, results obtained with biased procedures do not differ from those obtained with unbiased ones (43). In studies of place aversion, however, the two procedures may yield conflicting results [discussed in (5)]. Nevertheless, it has been suggested that the biased procedure is more conservative than the unbiased, because the rewarding event needs to overcome the subjects' initial tendency (17). This should reduce the likelihood of obtaining false positive effects.

Method

Procedure. All subjects that were to receive the 18% sucrose solution as the reinforcing event were habituated to the solution for 48 h in their home cage. In addition to the ordinary water bottle, a bottle containing 260 ml sucrose solution was available.

Next, the animals were habituated to the lickometer during two sessions of 1 h each, separated by about 1 week. At these sessions, only the 18% sucrose solution was available. All subjects that made less than 1000 licks on the second session were eliminated.

Twenty four to 72 h after the end of habituation, the pretest was performed in the place preference boxes. The subject was placed in the middle compartment, and was allowed to move freely within the box for 10 min. The time spent in each lateral compartment was recorded. During conditioning, the putatively reinforcing event was always associated with the nonpreferred compartment.

At the conditioning sessions, subjects rewarded/reinforced with sucrose were allowed to drink the solution for 15 min or until a preestablished number of licks were made, whatever occurred first. In addition to the number of licks, the latency to lick was recorded. The animal was then immediately transferred to a place preference box, and confined to the reinforced compartment for 30 min. On alternate days, the subject was placed in the nonreinforced compartment for 30 min without exposure to the lickometer.

There were three reinforced and three nonreinforced sessions before the test. This was identical to the pretest. After the test two posttest sessions were conducted, at which the subjects were injected with vehicle before being placed in the lickometer, where they were allowed to drink for 15 min or until 2000 licks were made.

Design. Four groups of eight animals each were used to evaluate the effects of naloxone and flupentixol in the absence of sucrose drinking. The animals were neither habituated to the sucrose solution nor to the lickometer. The putatively reinforcing events were the following: saline, 16 min before being placed in the nonpreferred compartment of the place preference box; naloxone, 16 mg/kg, 16 min before; naloxone, 16 mg/kg, 45 min before; flupentixol, 0.5 mg/kg, 30 min before. At nonreinforced sessions, saline was injected at the times indicated. The putatively reinforcing/rewarding events and the drug treatments given to the seven groups in the main experiment are shown in Table 3.

TABLE 3

SUMMARY OF THE REINFORCING/REWARDING EVENTS AND THE DRUG TREATMENTS GIVEN TO THE SEVEN GROUPS IN THE MAIN EXPERIMENT

Group	Reinforcing Event (Drug Treatment)
A	None (saline)
B	500 licks (saline)
C	2000 licks (saline)
D	2000 licks (naloxone 16 mg/kg−1 min)
E	2000 licks (naloxone 16 mg/kg−30 min)
F	2000 licks (flupentixol 0.5 mg/kg)
G	Plain water (saline)

There were 7–13 animals per group.

Statistical analysis. Two measures of conditioned place preference were analyzed: the time spent in the reinforced compartment, and the preference score [time in the reinforced compartment/(time in the reinforced compartment + time in the nonreinforced compartment)]. Both measures should show a significant increase between pretest and test to consider a treatment-produced place preference. The reasons for using these criteria have been exposed elsewhere (3,5).

All preference data were analyzed with two-factor ANOVAs with repeated measures on one factor. The factors were groups and session (pretest and test). In case of significant interaction, tests for simple main effects were performed.

The number of licks and the lick latency were analyzed for groups C to G with 5 × 5 ANOVAs for repeated measures on one factor. The factors were group and session (the three conditioning sessions + the two posttest sessions). In case of significant interaction, tests for simple main effects of groups within sessions were performed. The Tukey HSD procedure was used for a posteriori comparisons.

Results and Discussion

Licking. When the number of licks over sessions in groups C to G was evaluated, the group effect was significant, $F(4, 39) = 18.59$, $p < 0.001$. There was also a difference between sessions, $F(4, 39) = 37.80$, $p < 0.001$, and the group × session interaction was significant, $F(16, 156) = 6.91$, $p < 0.001$. Tests for simple main effects of groups showed that the groups differed at all sessions A posteriori comparisons revealed that the group treated with naloxone 1 min before drinking licked less than the saline treated group at all conditioning sessions and at posttest session 1. Indeed, this group did not differ from the one given plain water instead of sucrose. The group given naloxone 30 min before drinking drank less than the saline group at sessions 2 and 3. Flupentixol had no effect on licking. Data are shown in Fig. 1A.

The group, $F(4, 39) = 5.68$, $p = 0.001$, session, $F(4, 156) = 6.83$, $p < 0.001$, and interaction group × session effects, $F(10, 156) = 3.95$, $p < 0.001$, were all significant when the lick latency was analyzed. Tests for simple main effects of groups within session revealed no difference at conditioning sessions 1 and 2. At session 3, the groups differed. A posteriori comparisons showed that the subjects given naloxone 1 min before drinking and plain water instead of sucrose had longer latencies than the animals treated with saline. No group was different from saline at the posttest sessions. The data are illustrated in Fig. 1B.

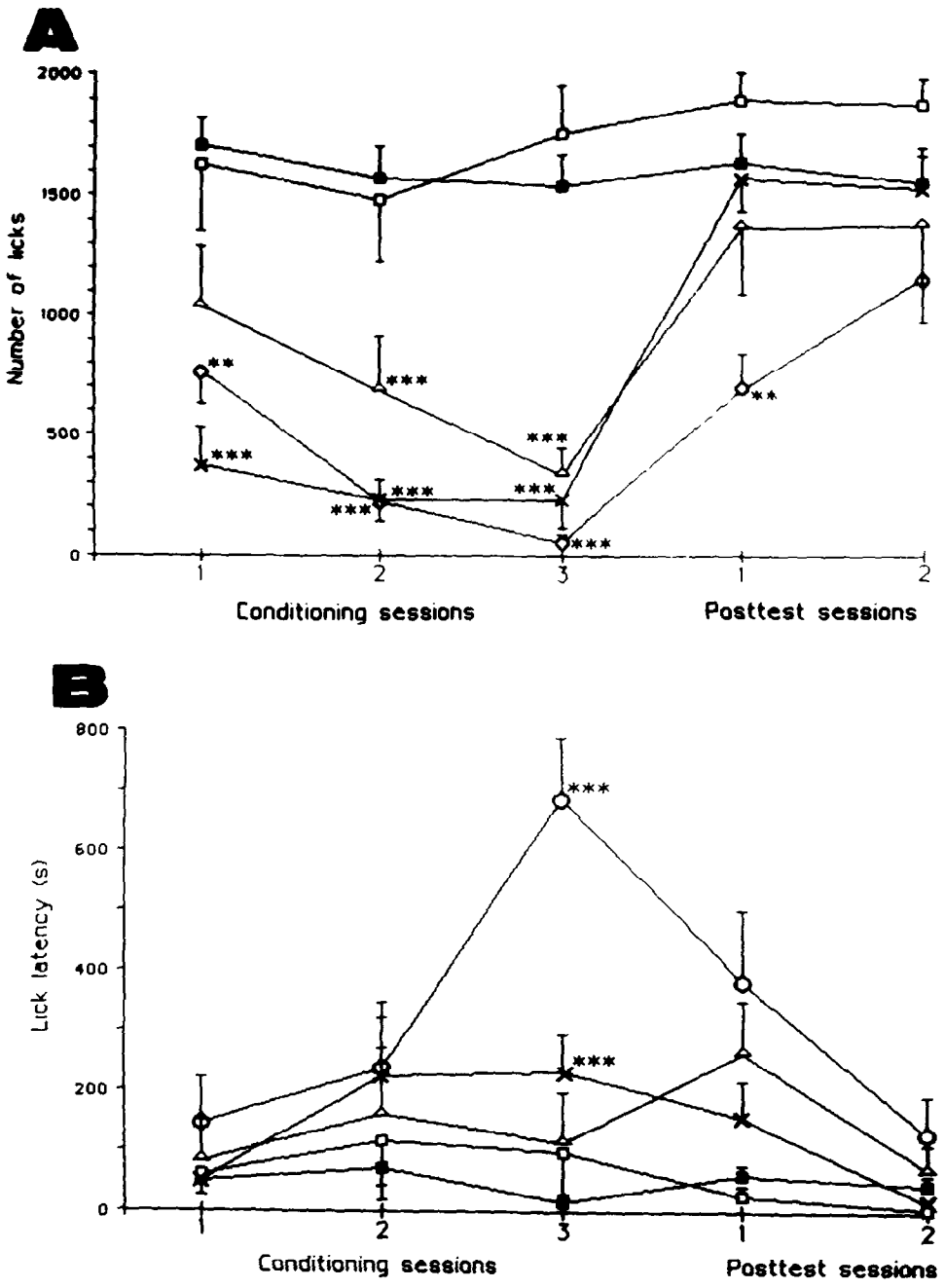

FIG. 1. Number of licks (A) and lick latency (B) in subjects allowed to drink an 18% sucrose solution in three place preference conditioning sessions and two posttest sessions. ■, saline; ◇, naloxone, 16 mg/kg, 1 min before drinking; △, naloxone, 16 mg/kg, 30 min before drinking; □, flupentixol, 0.5 mg/kg; X, water instead of sucrose. Data are means ± SE. **Different from pretest, $p < 0.01$; ***$p < 0.001$.

To summarize, naloxone given 1 min before drinking had effects very similar to those obtained when sucrose was replaced with plain water, i.e., a reduced number of licks at all conditioning sessions and increased latency to drink at conditioning session 3. In the group given plain water, drinking was immediately reestablished when sucrose was reinstated (posttest session 1). This was not the case in the naloxone group, which did not recuperate at the first drug free session. Flupentixol had no effect on drinking.

Place preference. When the effects of naloxone, 16 mg/kg, injected 16 or 45 min before conditioning and of flupentixol, 0.5 mg/kg, were evaluated in the absence of drinking, a significant difference between groups were obtained with regard to the time spent in the reinforced compartment, $F(3, 27) =$

4.35, $p = 0.013$. There was no difference between sessions and no interaction group × session. A posteriori comparisons of the interaction means revealed that no two groups differed at the pretest or test (data not shown).

Similar results were obtained for the preference score. The groups differed, $F(3, 27) = 4.77$, $p = 0.009$, but neither the effect of session nor the interaction group × session were significant. Again, a posteriori comparisons of the interaction means failed to reveal group differences. It is important to observe that the time spent in the reinforced compartment and the preference score remained stable between pretest and test in the saline group. This shows that spontaneous preference shifts do not occur to any significant degree.

When the time spent in the reinforced compartment was analyzed for the groups exposed to the lickometer, effects of group, $F(1, 6) = 7.72$, $p < 0.001$, session, $F(1, 62) = 4.45$, $p = 0.039$, and interaction group × session, $F(6, 62) = 10.87$, $p < 0.001$, were found. Tests for simple main effects of groups within sessions showed that there was a difference at the test. Analysis of sessions within groups showed that exposure to the lickometer without drinking was ineffective. Five hundred and 2000 licks increased the time spent in the reinforced compartment, $F(1, 62) = 51.59$, $p < 0.001$, and $F(1, 62) = 7.56$, $p = 0.008$, respectively. Naloxone given 1 min before drinking reduced the time spent in the reinforced compartment, $F(1, 62) = 10.48$, $p < 0.002$. Naloxone given 30 min before drinking and flupentixol blocked the effect of drinking sucrose, i.e., the time spent in the reinforced compartment did not change between pretest and test. Drinking plain water had no effect. Data are summarized in Fig. 2A.

Analysis of the preference score also revealed a difference between groups, $F(6, 62) = 6.55$, $p < 0.001$, sessions, $F(1, 62) = 6.71$, $p < 0.001$, and a significant interaction group × session, $F(6, 62) = 12.26$, $p < 0.001$. Tests for simple main effects of group within session indicated a difference at the test. Evaluation of sessions within groups showed that the preference score did not change in the group that spent 15 min in the lickometer without licking. Both 500 and 2000 licks were effective, $F(1, 62) = 26.25$, $p < 0.001$ and $F(1, 62) = 30.30$, $p < 0.001$, respectively. Naloxone given 1 or 30 min before drinking reduced the preference score between pretest and test, $F(1, 62) = 15.61$, $p < 0.001$, and $F(1, 62) = 6.77$, $p = 0.012$, respectively. Flupentixol blocked the effect of drinking sucrose, and drinking plain water had no effect. Data are shown in Fig. 2B.

These data show that exposure to the lickometer without drinking sucrose is without effect. Five hundred and 2000 licks are effective in producing place preference. Naloxone given 1 min before drinking inhibits the establishment of place preference and produces place aversion. Naloxone given 30 min before reduce the preference score but not the time spent in the reinforced compartment. Flupentixol blocks place preference, but does not show any tendency to produce place aversion. Drinking plain water is ineffective.

It could be argued that the absence of place preference after treatment with naloxone or flupentixol is a consequence of state-dependent learning, because the drugs were not administered at the test. There is, however, no clear evidence for state dependency in place preference conditioning (54). The possibility of state dependence cannot be definitively discarded until further experiments have been performed, though.

We have previously proposed that a rewarding and/or reinforcing event produces some physiological state that activates an affective reaction (3,5). Because place preference conditioning usually is considered as a kind of pavlovian conditioning (43), this physiological state can be regarded as the unconditioned stimulus, and the ensuing affective reaction as the unconditioned response. The conditioned stimuli would then be environmental cues in the reinforced compartment, and the conditioned response a fraction of the initial affective reaction (3,5). That fraction would then produce the instrumental response of approach, shown as preference for the reinforced compartment at the test. The relationship between a conditioned emotional response and an instrumental response has been analyzed within the context of learning theory (32,38).

The supposition that naturally rewarding events are able to induce an affective reaction outlasting the event itself is fundamental to the present experiments. There is no direct evidence for such a state besides the effects observed in the CPP procedure in the present and previous studies (1,5). However, this procedure was designed to measure positive affect produced by drugs of abuse (37,40), and by extension, we and others [see (43) for references] have applied it to natural reinforces. It seems reasonable to consider that a place preference is indicative of an otherwise unobservable affective reaction.

Processes other than classical conditioning have been suggested to intervene in the place preference procedure. For example, increase in the time spent in the nonpreferred (reinforced) compartment could be interpreted as a consequence of reduced aversion, secondary to anxiolysis, or as being a result of enhanced locomotor activity or exploration. There is, though, considerable evidence showing that these alternative explanations are unlikely (17,39,43).

The fact that flupentixol abolished place preference without affecting sucrose consumption would suggest that enhanced dopaminergic activity is necessary for the establishment of the association between the physiological state and environmental cues. Naloxone also inhibited place preference, but this inhibition was associated with reduced sucrose consumption. Indeed, sucrose was not consumed more than plain water. This suggests that naloxone blocked the rewarding effects of sucrose, i.e., the hedonic activation necessary for the establishment of place preference.

It has been reported that pharmacological treatments that reduce the reward value of intracranial self-stimulation or drug self-administration initially leaves responding unaffected or even enhance it [reviewed in (53,54)]. If naloxone reduced the reward value of sucrose, then it would be expected that licking would be unaffected or enhanced at the beginning of the session. Additional analysis of the licking data from Experiment 1 showed that the number of licks per minute increased during the first min in the animals treated with naloxone, 16 mg/kg. Thereafter licking was gradually reduced (data not shown).

The place aversion observed in the group that was treated with naloxone 1 min before drinking sucrose could suggest that naloxone produced an aversive state. However, the group given naloxone 30 min before drinking did not display reliable place aversion. Furthermore, naloxone was ineffective in the control experiments and in the taste aversion experiments when administered to rats habituated to the sucrose solution. Moreover, place aversion following naloxone treatment was found when the drug was administered before conditioning using sexual activity or drinking water as rewarding events (1,5). The proposition that the drug reduces the reward value of sucrose seems to better coincide with available data. A reduced reward value has been reported to induce frustrative nonreward, which is an aversive state (6,20). We suggest that the frustrative nonreward, rather than any direct aversive effects of naloxone, becomes conditioned.

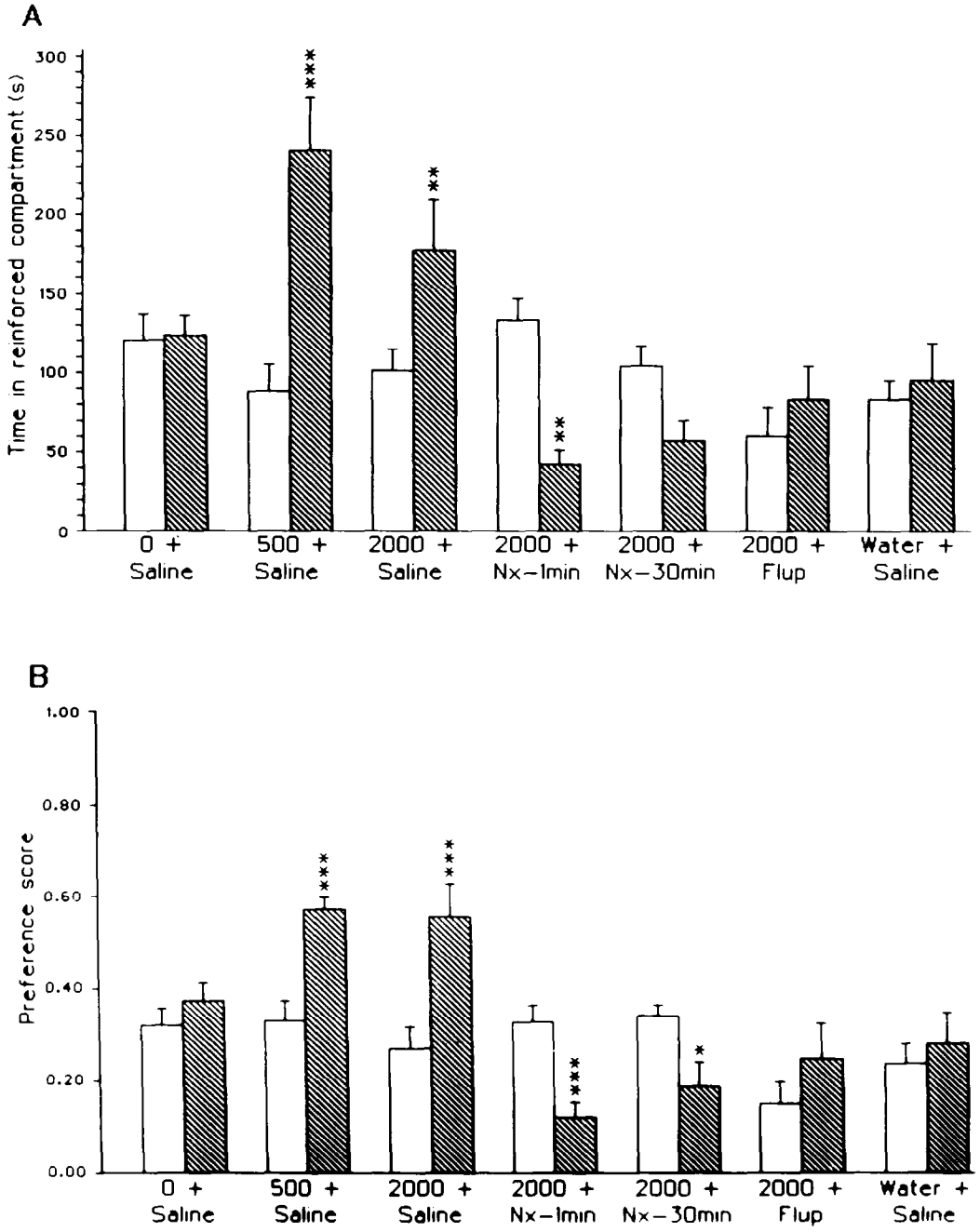

FIG. 2. Time spent in the reinforced compartment (A) and preference score (B) at the pretest (open bars) and at the test (striped bars) in animals exposed to the lickometer but without access to the sucrose solution (0 + saline), in subjects that were allowed to make 500 licks of the 18% sucrose solution (500 + saline), and in subjects that were allowed to make 2000 licks or drink for 15 min (2000 + . . .) and treated with saline, naloxone (Nx), or flupentixol (Flup) before each conditioning session. Naloxone was injected either 1 min (−1) or 30 min (−30) before drinking. An additional group was given water instead of sucrose (water + saline). Data are means + SE. *Different from pretest, $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Present data suggest that opioid systems are important for sucrose-induced reward. Because the naloxone dose used in this experiment was rather large, it was decided to evaluate the effects of lower doses. This would allow for a better understanding of the role of opioids in reward processes.

EXPERIMENT 3

Sweet substances stimulate opioid release in the hypothalamus (22), and intraoral infusion of sucrose produces naloxone-reversible hypoalgesia, indicative of opioid release (15,

16). Such a release could be responsible for the rewarding effects of sweet taste. Thus, the reduced sucrose consumption observed after naloxone treatment may be a consequence of reduced reward, as suggested above. If naloxone indeed inhibits sucrose-induced place preference because it reduces the reward value of sucrose drinking, then the drug should not inhibit place preference at a dose that has slight or no effects on drinking. This hypothesis was evaluated in this experiment.

Method

Procedure. Identical to the one used in Experiment 2, with the exception that the animals were allowed to drink sucrose for 15 min independently of the number of licks made. This change was introduced to better observe effects on consumption.

Design. Three groups were used. At the reinforced conditioning sessions, all subjects were allowed to drink sucrose in the lickometer immediately before being placed in the nonpreferred compartment of the place preference cage. Naloxone was injected 30 min before drinking in doses of 1 and 4 mg/kg to different groups of animals. A third group was given saline. At nonreinforced sessions, saline was administered to all three groups 45 min before the subjects were placed in the preferred compartment of the place preference cage.

Statistical analysis. Place preference data were analyzed with 3 × 2 ANOVAs and licking with 3 × 5 ANOVAs. Otherwise, the analyses were identical to those of Experiment 2.

Results and Discussion

Licking. A significant effect of group was found, $F(2, 31) = 11.06$, $p < 0.001$. There were also significant effects of session, $F(4, 124) = 11.29$, $p < 0.001$, and of the interaction session × group, $F(8, 124) = 3.24$, $p = 0.002$. Tests for simple main effects of group within sessions showed group differences at session 1 to 3 and at posttest session 2.

A posteriori comparisons showed that both groups treated with naloxone licked less than the saline treated group at all conditioning sessions. There was no difference between the groups given naloxone 1 or 4 mg/kg. At posttest session 2, the group given naloxone, 4 mg/kg, licked less than the group treated with saline. Data are summarized in Fig. 3A.

There was no effect of group and no interaction group × session on lick latency. A significant difference between sessions was found, $F(4, 124) = 3.30$, $p = 0.013$. A posteriori comparisons of the interaction means showed that the group treated with naloxone, 4 mg/kg, had longer latencies than the saline treated group at posttest sessions 1 and 2 (Fig. 3B).

Place preference. ANOVA of the time spent in the reinforced compartment showed a significant effect of group, $F(2, 31) = 5.31$, $p = 0.01$. There was no difference between pretest and test, but the interaction group × session was significant, $F(2, 31) = 8.55$, $p = 0.001$. Tests of simple main effects of groups showed that there was a group difference at the test. Tests of simple main effects of sessions within each group showed that the time spent in the reinforced compartment increased in the group treated with saline, $F(1, 31) = 4.49$, $p = 0.042$, and naloxone, 1 mg/kg, $F(1, 31) = 6.32$, $p = 0.017$. In the group treated with naloxone, 4 mg/kg, the time spent in the reinforced compartment was reduced between pretest and test, $F(1, 31) = 7.32$, $p = 0.011$. Data are found in Fig. 4A.

ANOVA of the preference score showed a significant effect of group, $F(2, 31) = 6.81$, $p = 0.004$, session, $F(1, 31) = 9.36$, $p = 0.005$, and of the interaction group × session, $F(2, 31) = 9.72$, $p = 0.001$. When analysis of simple main effects were performed on the factor session, a group difference was found at the test. Tests of sessions within each group showed that the preference score increased in the groups treated with saline and naloxone, 1 mg/kg ($F(1, 31) = 12.72$, $p < 0.001$ and $F(1, 31) = 11.66$, $p = 0.002$, respectively). No difference between pretest and test was found in the group treated with naloxone, 4 mg/kg. Data are shown in Fig. 4B.

These data confirm that drinking sucrose immediately before conditioning produces place preference. This effect was not blocked by naloxone, 1 mg/kg, although this dose reduced sucrose consumption. A naloxone dose of 4 mg/kg blocked the establishment of place preference.

Naloxone reduces intake of sweet solutions as well as of food and water (27-29,36,42). Detailed analyses of the effect of opioids on feeding behavior have shown that these drugs modify the palatability of food, and this has been associated with the affective impact of tastants (21,29,34,44). This coincides with the hypothesis that naloxone reduces the reward value of the sucrose solution. The fact that naloxone is capable of producing taste aversion (see introductory paragraphs and Experiment 1) does not necessarily mean that the drug is aversive. Rewarding drugs like amphetamine and morphine also produce taste aversion [for a review see (25)].

It has been proposed that opioid antagonists accelerate satiety (26). However, naltrexone has been found to reduce consumption of a preferred food in a choice situation, but ingestion of nonpreferred food was increased (18). The deleterious effects of naloxone on sweet consumption can be blocked or reduced by increasing the concentration of the sweetener (27). If satiety were accelerated, this should rather reinforce the effects of naloxone. It seems, then, that available data are more consistent with reward reduction or aversive effects associated with reduced reward than with satiety acceleration.

GENERAL DISCUSSION

Place preference produced by sucrose drinking was effectively blocked by some doses of naloxone in the present experiments. Naloxone also reduced sucrose consumption. Even a dose of 1 mg/kg, unable to block the establishment of place preference, had deleterious effects on drinking. This would suggest that the rewarding properties of sucrose are more sensitive to naloxone than its reinforcing properties. It is proposed, therefore, that endogenous opioid systems are activated by sucrose drinking, and that these systems are responsible for the ensuing affective state (Fig. 5). The induction of this affective state would be equal to reward; hence, naloxone inhibits this process. There is no compelling reason to believe that naloxone directly blocks the physiological consequences of sucrose drinking, such as taste perception or activation of the hepatic branch of the vagus nerve.

The dopamine antagonist cis(z)-flupentixol blocked place preference without affecting sucrose consumption. Lack of effect of a slightly larger dose of flupentixol on consumption has also been reported when sucrose is administered intraorally (11). This suggests that dopamine is not important for the rewarding effects of sucrose. Moreover, haloperidol has been found to block CPP produced by food reward, but the amount of food consumed was not modified by the drug (46). Similar observations have been made when drinking water was the rewarding event (5). This would indicate that the reward value of food or water was not reduced, although the subjects were unable to associate or remember the association between environmental stimuli and reward. On the other hand, it has

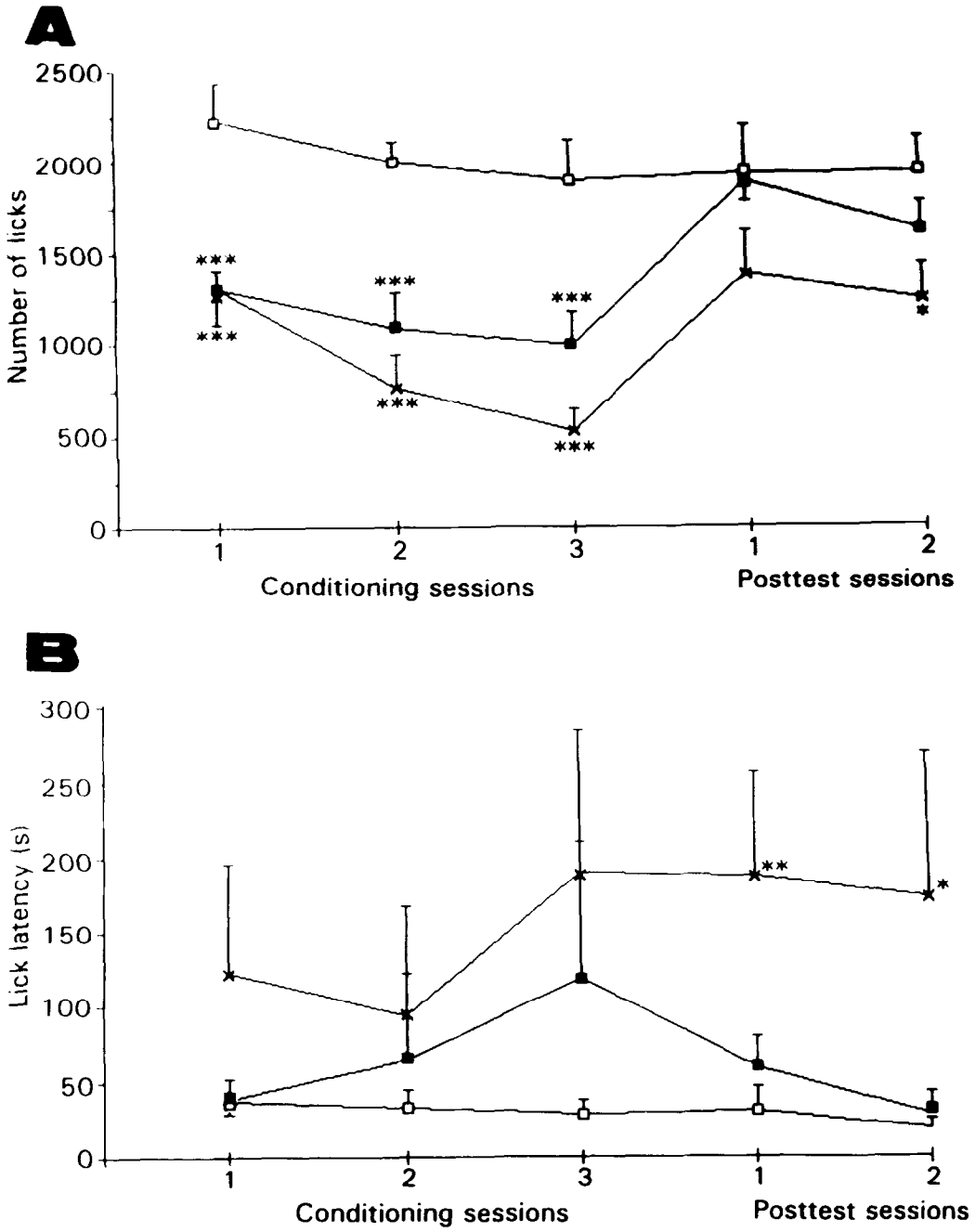

FIG. 3. Number of licks (A) and lick latency (B) in animals allowed to drink an 18% sucrose solution in three place preference conditioning sessions and two posttest sessions. □, saline; ■, naloxone, 1 mg/kg, 30 min before drinking; ×, naloxone, 4 mg/kg, 30 min before drinking. Data are means ± SE. *Different from saline, $p < 0.05$; **$p < 0.01$;***$p < 0.001$.

been reported that intraaccumbens infusions of amphetamine or haloperidol reduces drinking. However, these effects were interpreted as being a consequence of altered attentional mechanisms (9,10). It is well known that reduced dopaminergic activity inhibits lever pressing for food or water [see, e.g., (53,54)], but doses of haloperidol that reduces lever pressing do not modify free feeding (41). Similar results have been obtained with regard to sexual behavior, where a dopamine antagonist reduce operant responding for access to a receptive female without modifying consummatory sexual behavior (23). There are also data showing that enhanced dopaminergic activity reduces sham feeding (19) and ingestion of intraorally administered sucrose (12). It seems that the role of dopamine in the control of consummatory behaviors is far from clear.

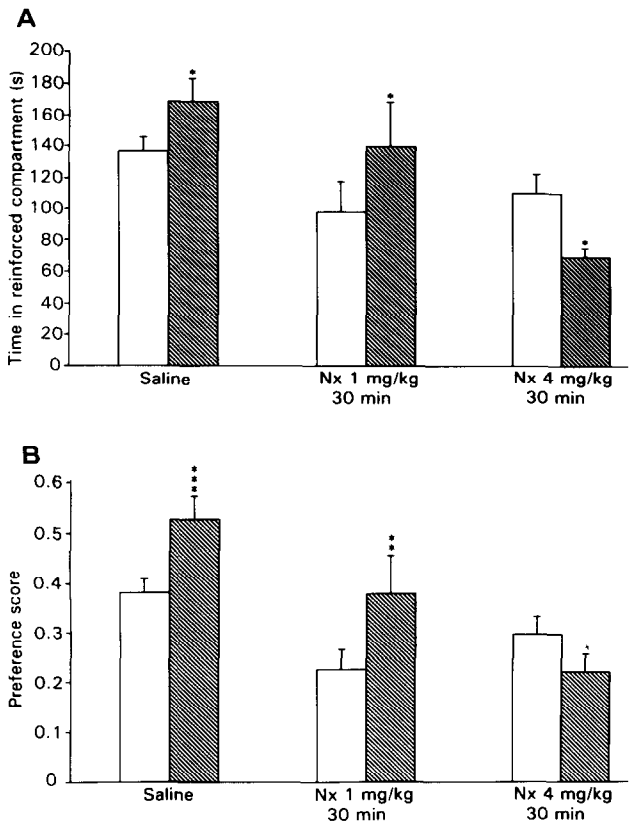

FIG. 4. Time in the reinforced compartment (A) and the preference score (B) at the pretest (open bars) and at the test (striped bars) in animals allowed to drink an 18% sucrose solution and treated with saline or naloxone, 1 or 4 mg/kg, before each conditioning session. Values are mean + SE. *Different from pretest, $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Dopamine antagonists not only interfere with positively reinforced instrumental responses, but also block the performance and acquisition of active avoidance responses (7,14, 35,49,50). Available data, thus, suggest that dopamine is equally important for behaviors maintained by positive and negative reinforcers. It is proposed that dopamine release is not the physiological state producing an affective reaction equivalent to reward. Rather, it may be involved in the formation or retrieval of associations between the physiological state and environmental cues (Fig. 5).

It appears that reward/reinforcement produced by sexual activity, drinking sucrose, or drinking water are differently affected by dopamine and opioid antagonists. Sexual behavior is not modified by any of them, but sexual reinforcement is abolished by naloxone when our procedure is used. When the males are allowed to copulate in the place preference cage, naloxone is ineffective (23,30). This suggests that there are important differences between the reinforcement produced

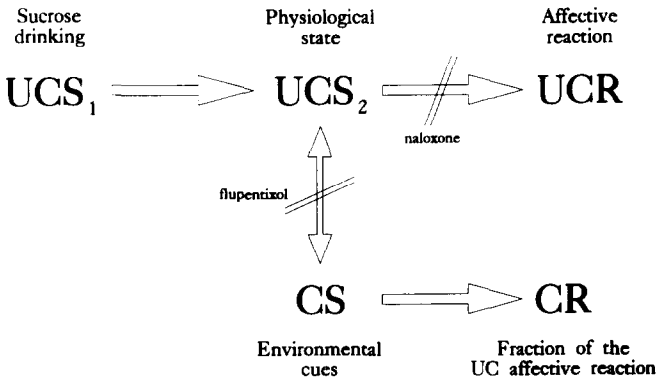

FIG. 5. Schematic description of the place preference conditioning supposed to occur after drinking sucrose. The proposed sites of action of an opioid antagonist and of a dopamine antagonist are shown. UCS, unconditional stimulus; CS, conditional stimulus; UCR, unconditional response; CR, conditional response. For further details, see text.

by the execution of a behavior and the reinforcement associated with the affective state activated by the behavior. Further, sucrose drinking is not modified by a dopamine antagonist, but much reduced by naloxone. Sucrose-induced reinforcement is blocked by flupentixol but not by a low dose of naloxone. Finally, drinking water is not modified either by dopamine or opioid antagonists at doses that inhibit place preference.

The reward value of sex, drinking water, or sucrose does not seem to be dependent on dopaminergic mechanisms. Reinforcement (learning), on the other hand, seem to depend on dopamine for the ingestive behaviors but not for sexual behavior. Further, sexual reward (execution of sexual behavior) is not modified by naloxone, whereas ingestive reward (consumption of water and sucrose) is. Whether these differences are related to the biological urgency of the rewarding event can, at present, only be a matter of speculation. Nevertheless, these observations show that it may be useful to distinguish reward from reinforcement, and that different reinforcers are acting in different ways. This makes it doubtful that there exists a general reinforcement mechanism, applicable to all reinforcers.

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