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CONDITIONED SUPPRESSION BY A STIMULUS ASSOCIATED WITH NALORPHINE IN MORPHINE-DEPENDENT MONKEYS[1]

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Three rhesus monkeys, physically dependent on morphine, were trained to press a lever for food on a fixed ratio of 10 responses. A tone, initially a neutral stimulus, was aperiodically presented every third or fourth session, 5 min before and after the intravenous injection of nalorphine, a morphine antagonist which produces an immediate withdrawal syndrome in morphine-dependent monkeys. After several sessions, conditioned suppression of food-lever response rate was observed. Conditioned bradycardia, emesis, and excessive salivation also occurred. In 40 to 45 sessions the conditioned suppression of food-lever response rate and the conditioned autonomic changes were extinguished by presenting pairings of a tone and saline injection. The monkeys were then reconditioned by presenting the tone aperiodically, every third or fourth session, 5 min before and after the intravenous injection of nalorphine. Results were similar to the initial conditioning sessions. Two rhesus monkeys not dependent on morphine were stabilized on a food schedule similar to that used for the first three monkeys. These monkeys showed no change in food-lever response rate during or after nalorphine injections.

In morphine addicts, an abrupt and complete withdrawal of morphine is followed by an abstinence syndrome which is an indication of the addict's physical dependence on the drug. Morphine-abstinence symptoms have been described as both "non-purposive" (physiological) and "purposive" (behavioral) by Wikler (1955). Physiological changes, such as excessive salivation, body temperature changes, piloerection, muscle aching and twitching, emesis and tachycardia, involve the neuromuscular, autonomic, and endocrine systems. Behavioral changes consist of a disruption of normal ongoing behavior and a reorientation of behavior toward drug acquisition. In morphine addicts an injection of nalorphine, a potent antagonist of morphine, immediately elicits the abstinence syndrome normally associated with abrupt withdrawal of morphine. The effects of intravenously administered nalorphine are seen within seconds and last for several hours. In minimal doses, capable of producing the abstinence syndrome in morphine addicts, nalorphine has no noticeable effects on normal subjects.

Human addicts, who have been withdrawn from morphine and are no longer physically dependent upon the drug, have described the recurrence of certain withdrawal responses when they return to an environment previously associated with drug-taking behavior. Wikler (1961) has interpreted these observations as an indication that the withdrawal syndrome can be classically conditioned and that this conditioning may be a major factor in post-addicts' relapse to drug-taking. Using rats as subjects, Wikler (1965) has obtained results which indicate the occurrence of "conditioned withdrawal" produced by returning the rats to an environment previously associated with drug withdrawal.

Irwin and Seevers (1956) have provided experimental results suggesting that nalorphine-induced withdrawal can be classically conditioned. Morphine-dependent monkeys which had undergone repeated nalorphine-induced withdrawal continued to show a "withdrawal-like" response to both nalorphine and saline injections several months after they had been withdrawn from morphine. After repeated injections of saline the "withdrawal-like" response was no longer elicited.

The present experiment is the first in a series, using a procedure developed to study the conditioning of both the behavioral and

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physiological aspects of the morphine-withdrawal syndrome.

METHOD

Subjects

Experiment 1 studied an adult, male rhesus monkey weighing 5 kg and physically dependent on morphine; in Exp. 2, four adult, female rhesus monkeys weighing between 3.8 and 4.8 kg were used. The monkey in Exp. 1 and two of the monkeys (M474 and M574) in Exp. 2 had been physically dependent on morphine for approximately 18 months before these experiments. During this time they were maintained on 12 mg/kg a day of morphine sulfate, given as a subcutaneous injection of 3 mg/kg every 6 hr. The dosage for the monkey in Exp. 1 was gradually increased over a period of several months before the experiment to a final dosage of 42 mg/kg a day. The other two subjects in Exp. 2 (M2018 and M2037) were not physically dependent on morphine.

All monkeys were surgically prepared with chronic indwelling jugular catheters (Schuster and Brady, 1964). Immediately before the experiments the monkeys were reduced to 85% of free-feeding weight. They were then trained to press a lever for food reinforcement on a fixed-ratio 10 schedule (FR 10); that is, a pellet of food was delivered for every tenth response.

Apparatus

The monkeys were restrained during the experimental sessions in Foringer Primate Cockpits (Cat. #1206 M1) enclosed in isolation booths (Cat. #3011 M1). At other times they were maintained in separate cages with water available, but no food. The cockpits were equipped with mouth-operated food and water operanda (Thompson, Schuster, Dockens, and Lee, 1964). A Foringer pellet dispenser provided 0.7 g Dietrich and Gambrill monkey food pellets (Foringer Cat. #1281). Each depression of the water operandum produced 1.0 ml of water. A stimulus light panel was mounted at eye level on the door of the isolation booth. A wide-angle viewing lens allowed observation of the monkey in the booth. To mask the sounds of programming and recording equipment a white noise generator was operated continuously during all sessions. Injections were administered from outside the isolation booth by syringes connected by a polyethylene catheter (PE-100) to the implanted jugular catheter in the monkey. A 0.9% physiological saline solution was used for saline injections. Nalorphine injection solutions were prepared daily by adding the desired amount of nalorphine HCl to 0.9% physiological saline. Wound clips attached to the area of the right shoulder and left waist served as electrocardiogram leads which led from the isolation booth to an Offner Electroencephalograph. Cables connected apparatus in the isolation booth to automatic programming and recording apparatus.

General Procedure

The monkey in Exp. 1 and monkeys M474 and M574 in Exp. 2 were tested for 2 hr a day, 1 to 2 hr after a morphine injection. They were conditioned initially to depress the food lever on the FR 10 schedule of reinforcement and the water operandum on an FR 1 schedule of reinforcement. After the jugular catheters were surgically implanted, the monkeys were placed on a 2-hr, three-component, chain schedule of reinforcement, which continued unchanged during all conditioning, extinction, and reconditioning sessions. The sequence of components within each 2-hr session was: 30-min FI component; 1-hr FR 10 food component; 30-min S^{Δ} component. Thus, the monkeys' first response on the food lever after 30 min produced a stimulus in the presence of which every tenth response was reinforced with a pellet of food. At the end of 1 hr in the FR 10 period, or after 100 reinforcements, they were advanced into a 30-min S^{Δ} period, in which responses had no consequences, after which they were removed from the situation. A house light was illuminated throughout the session. The discriminative stimuli during the FI period were two 6-w, 110-v blue lights; during the FR 10 food period they were two 6-w, 110-v white lights. During the 30-min S^{Δ} period only the house lights remained on. Water reinforcement was continuously available during the session.

After the food-lever response rate was stabilized on this schedule, an auditory stimulus (tone) was aperiodically presented every third or fourth session. The tone was presented approximately 10 min after the start of the FR 10 food component for 5 min before and after an intravenous injection of 1 cc of

saline. After several sessions, neither the tone nor the injection procedure disrupted the monkeys' food-lever response rate or heart rate, thus establishing them as neutral stimuli. Following this, the tone was presented aperiodically every third or fourth session, 5 min before and after an intravenous dose of nalorphine. The subjects were intermittently observed during the session through the wide-angle viewing lens.

The two non-dependent monkeys in Exp. 2 (M2018 and M2037) were surgically prepared with chronic indwelling jugular catheters. They worked on a schedule similar to that described above, except that the present one was terminated after 80 food reinforcements or 1 hr. No tone was presented during the sessions.

For purposes of this report only the FR 10 food component is discussed.

EXPERIMENT 1

The monkey (M4) was stabilized on the schedule without the presentation of tone-injection pairings. Several doses of nalorphine were administered during different sessions to determine a dose of the drug that immediately terminated food-lever responding for the remainder of the session. A total dose of 2 mg of nalorphine was found reliably to produce this effect. Tone-saline injection pairings (T + S) were then presented for several sessions during the FR 10 food component to establish these as neutral stimuli. After these sessions a tone-nalorphine pairing (T + N) was presented aperiodically, once every third or fourth session, during the FR 10 food component. Electrocardiogram samples were taken during all sessions. Several sessions after the third T + N session, the monkey's health declined and it was removed from the experiment.

Results

Figure 1 shows the cumulative response records for monkey M4. Session 1 was a control session before conditioning began. It demonstrated that the stimuli associated with the injection procedure were initially neutral and produced no change in the monkey's food-lever response rate. Sessions 2, 5, and 8 were conditioning sessions in which an injection of 2 mg of nalorphine was given. After injections of nalorphine the monkey's food-lever responding was completely suppressed for the remainder of the session. In addition, emesis and excessive salivation were observed and heart rate increased from an average FR 10 food period level of 180 to 190 beats per min to 240 to 250 beats per min. During control sessions 3, 4, 6, and 7 response rate and heart rate did not change after saline injections. During saline control session 9, following the third conditioning session, the saline injection completely suppressed food-lever responses and decreased heart rate to 140 to 150 beats per min. These effects lasted approximately 15 min; then, both food-lever response rate and heart rate returned to approximately the level observed before the saline injection. The monkey also showed emesis and excessive salivation, which had not occurred after the previous saline injections. In Session 10, the second control session following the third conditioning session, the saline injection no longer produced a change

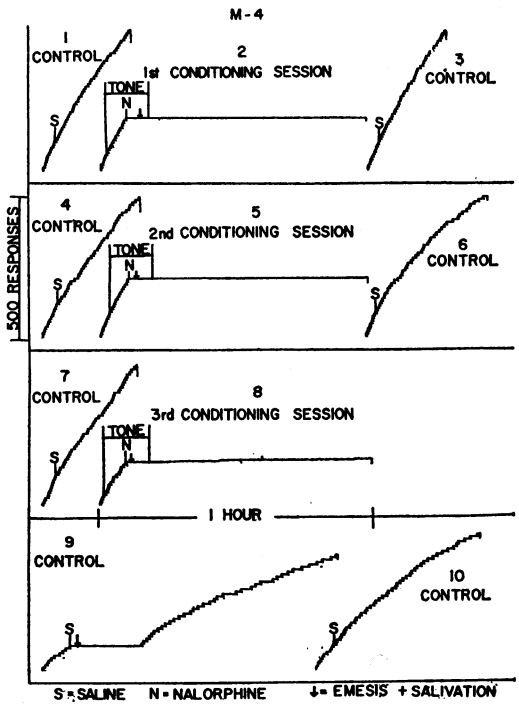

Fig. 1. Cumulative response curves from M4. Each segment shown is the initial portion of an FR 10 food component record extracted from a 2-hr session. The numeral 1 designates a control session which established that the saline injection was a neutral stimulus; 2, 5, and 8 were conditioning sessions, and 3, 4, 6, 7, 9, and 10 were control sessions before and after conditioning sessions.

in food-lever response rate or heart rate. The monkey was removed from the experiment after control session 10.

Discussion

During the sessions before the third T + N pairing, saline injections produced no change in food-lever response rate or heart rate. After the third pairing, the saline injection suppressed responses on the food lever and elicited emesis, excessive salivation, and a fall in heart rate. It should be noted that heart rate decreased after the saline injection in contrast to the increase produced by the nalorphine injections. Similar findings have been reported in studies using the conditioned emotional response paradigm with shock. The stimulus preceding shock produced a decrease in heart rate in contrast to the presentation of shock, which elicited an increase (Wilson, 1964; deToledo and Black, 1966). It appears that the stimuli associated with the injection procedure, previously neutral interoceptive stimuli, became conditioned stimuli after their association with nalorphine. This is not surprising, since the infusion of solutions into the internal jugular vein is known to have stimulus properties (Schuster and Brady, 1964). A second series of experiments was carried out to determine whether the tone preceding the injection of nalorphine could acquire the ability to suppress response rate for food and elicit salivation, emesis, and heart rate changes.

EXPERIMENT 2

Monkeys M474 and M574 were maintained on 12 mg/kg of morphine a day. They were conditioned using 1-mg total dose of nalorphine in sessions with T + N pairings. Lower dosages of morphine and nalorphine were chosen to prevent a decline in the health of these monkeys, as occurred in M4 in Exp. 1. No saline injection was given on control days. In all other respects the schedule was the same as in Exp. 1. After the tone and injection of saline were established as neutral stimuli, five more sessions with T + S pairings were conducted. Then, 10 conditioning sessions with T + N pairings were given. The conditioning sessions were conducted every third or fourth day with control sessions interspersed. After the tenth conditioning session, extinction sessions with T + S pairings were conducted each day. Following extinction, the monkeys were reconditioned in sessions with T + N pairings. Monkeys were intermittently observed and samples of electrocardiogram records were taken during all sessions.

In the control sessions following the sixth and ninth conditioning sessions, monkey M574 exhibited what could be a trace conditioned response. At approximately the same time that the tone would have been presented, food-lever responses stopped, heart rate fell, and emesis and excessive salivation were seen. This effect lasted approximately 10 min, but disappeared by the control session on the following day and was not observed at any other time.

A control experiment was conducted with the two monkeys (M2018 and M2037) not dependent on morphine. They worked on a schedule identical to that in Exp. 2, except that it was terminated after 80 food reinforcements or 1 hr. No injections were given on control days. On test days, an injection of 1 mg of nalorphine was given. No auditory stimulus was presented.

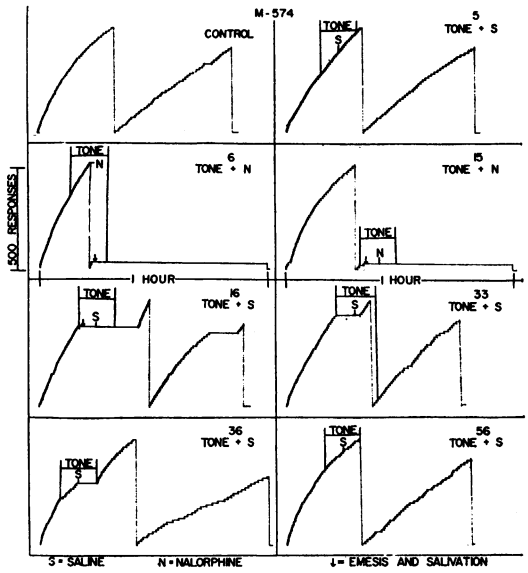

Fig. 2. Cumulative response curves from M574. Each segment shown is a complete FR 10 food component record extracted from a 2-hr session. A control session before tone-injection pairings is shown first; 5 was a session establishing tone and saline injection as neutral stimuli; 6 was the first conditioning session, 15 was the tenth conditioning session; 16 was the first extinction session, 33 the eighteenth, 36 the twenty-first, and 56 the forty-first extinction session. Arrows indicate observation of emesis and excessive salivation.

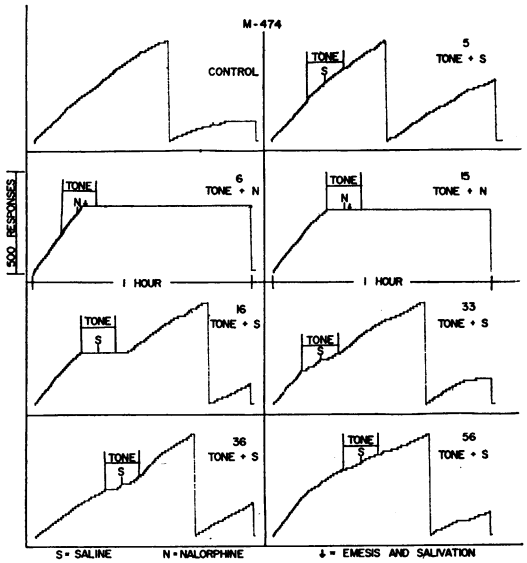

Fig. 3. Cumulative response curves from M474. Each segment shown is a complete FR 10 food component record extracted from a 2-hr session. A control session before tone-injection pairings is shown first; 5 was a session establishing tone and saline injection as neutral stimuli; 6 was the first conditioning session, 15 was the tenth conditioning session; 16 was the first extinction session, 33 the eighteenth, 36 the twenty-first, and 56 the forty-first extinction session. Arrows indicate observation of emesis and excessive salivation.

Results

Figure 2 shows cumulative response records of selected sessions for M574. Figure 3 shows cumulative response records of the same sessions for M474. In the control sessions before the start of conditioning, more prolonged pausing after reinforcements was exhibited than is usually seen on this type of schedule. The monkeys' performance, however, was quite stable over sessions. Session 5 was the last day of the five sessions establishing the tone and injection as neutral stimuli. The onset of the tone and the injection of saline did not disrupt the monkeys' food-lever response rate. Session 6 was the first conditioning session with a T + N pairing. The monkeys responded normally during the tone period before the injection and continued to respond for 3 to 4 food reinforcements after the nalorphine injection. Responding was then completely suppressed for the rest of the session. After nalorphine injections, emesis and excessive salivation were observed in both monkeys. Session 15 was the tenth conditioning session with a T + N pairing. This session illustrates the conditioned suppression observed in T + N sessions, 9 through 15. Before the tone in Session 15, the monkeys' performance was comparable to that on control days. With the onset of the tone, food-lever responding was immediately suppressed for the rest of the session. Emesis and excessive salivation were observed in M574 during the tone period before the injection of nalorphine and in M474 after the nalorphine injection. Session 16 was the first extinction session with a T + S pairing. The monkeys responded normally for food until the onset of the tone. Food-lever responding was then completely suppressed during the entire tone period. After the onset of the tone, emesis and excessive salivation were observed in M574. After the tone terminated, food-lever response rate was completely suppressed for 10 min in M574 and for 5 min in M474. Food-lever response rates then returned to normal. The post-tone pausing gradually disappeared during the following four extinction sessions. Emesis and excessive salivation were no longer observed in M574 during extinction after Session 25. Session 33 was the eighteenth extinction session with a T + S pairing. The monkeys responded normally for food until the onset of the tone. Food-lever response rate of M574 was completely suppressed during the tone period before the saline injection, but returned to normal in the tone period after the injection. Food-lever response rate of M474 was partially suppressed during the tone period before and after the saline injection. Session 36 was the twenty-first extinction session. The monkeys responded normally for food until the onset of the tone. After the onset of the tone, response rate of M574 was partially suppressed during the tone period until the injection of saline. Immediately after the saline injection, response rate was completely suppressed until the end of the tone period. Responding by Monkey M474 was completely suppressed for about the first 3 min of the 5-min tone period before the injection. Response rate then returned to a near normal rate before the injection but was completely suppressed immediately after it and continued so for about 2 min. Response rate then returned to normal for the remainder of the tone period. Food response rate for both monkeys was normal after the tone stopped. Session

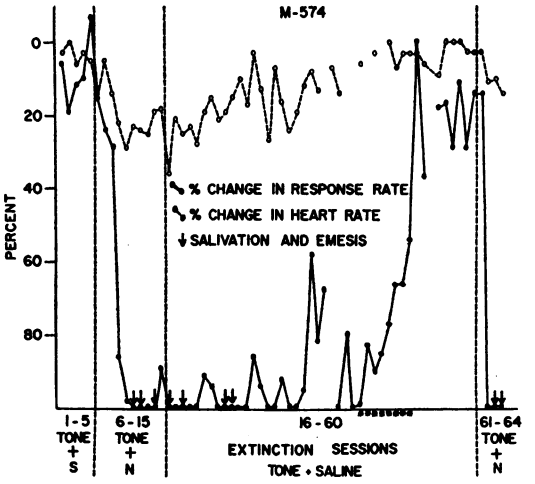

Fig. 4. M574. Percentage change in heart rate and response rate from the 5-min period preceding the tone onset to the 5-min period during tone presentation and before injection. Numerals 1 to 5 designate sessions establishing tone and saline (S) injection as neutral stimuli; 6 to 15 were the conditioning sessions during which nalorphine (N) was administered; 16 to 60 were extinction sessions with tone + saline (S) presentations. Those marked by asterisks were sessions in which the FR requirements were reduced during the tone period; 61 to 64 were reconditioning sessions with tone + nalorphine (N) presentations. Arrows mark sessions when emesis and excessive salivation were observed in the tone period before the injection of saline or nalorphine. Breaks in the curve indicate sessions where mechanical problems prevented data collection. Each conditioning and reconditioning session was followed by 3 to 4 control sessions not indicated on the graph.

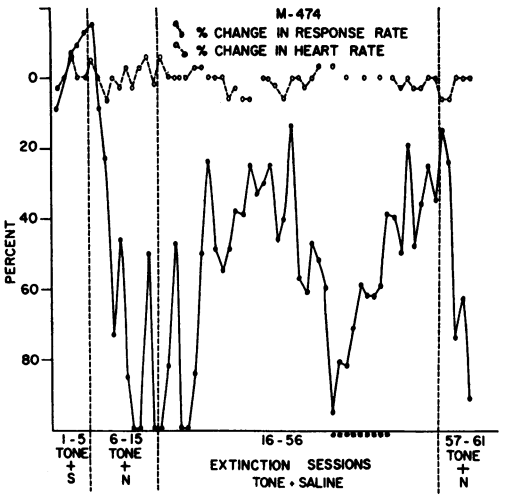

Fig. 5. M474. Percentage change in heart rate and response rate from the 5-min period preceding the tone onset to the 5-min period during tone presentation and before injection. Sessions 1 through 5 established tone and saline (S) injections as neutral stimuli; 6 to 15 were the conditioning sessions during which nalorphine (N) was administered; 16 to 56 were extinction sessions with tone and saline (S) presentations. Those marked by asterisks were sessions in which the FR requirements were reduced during the tone period. Sessions 57 to 61 were reconditioning sessions with tone + nalorphine (N) presentations. No emesis or excessive salivation was observed in the tone period before injection of saline or nalorphine. Breaks in the curve indicate sessions where mechanical problems prevented data collection. Each conditioning and reconditioning session was followed by 3 to 4 control sessions not indicated on the graph.

56 was one of the final extinction sessions. Both monkeys' food-lever response rate during the tone approached normal response levels observed in the initial T + S sessions. This indicates that the conditioning and extinction procedure had not interfered with the monkeys' baseline FR performance.

The percentage change in heart rate and food-lever response rate from the 5-min period preceding the tone onset to the 5-min period during the tone presentation, before the injection of nalorphine or saline, is shown in Fig. 4 for M574 and in Fig. 5 for M474.

The first five sessions established the tone and injection of saline as neutral stimuli. After Session 6, the first conditioning session, food-lever response rate during the tone decreased and was almost completely suppressed after four conditioning sessions for M574 and after six sessions for M474. Food-lever response rate remained suppressed throughout the tone period during the initial extinction sessions for both monkeys.

In conditioning sessions 9 to 15, heart rate for M574 declined during the 5-min tone period before the nalorphine injection. After the injection of nalorphine, heart rate in both monkeys rose from an average FR 10 food period level of 160 to 190 beats per min to 240 to 260 beats per min. During the initial extinction sessions, M574's heart rate continued to decline during the tone period before and after the saline injection. Emesis and excessive salivation were observed in M574 during the tone period preceding the injection. This is marked on the graph with arrows. No heart rate changes, emesis or excessive salivation were observed in M474 during the tone period before the injections of nalorphine or saline.

The heart rate response of M574 during the tone was partially extinguished after 15 sessions of T + S pairings. At this time, however,

the food-lever response rate remained completely suppressed during the tone and continued to be suppressed for another 13 sessions. After 10 extinction sessions, food-lever response rate of M474 approached normal baseline levels during the tone but this was not consistent and occasional suppression continued. To hasten extinction, the FR requirements were reduced during the tone period and then gradually returned to FR 10 over the next 8 to 9 sessions. These sessions are marked in Fig. 4 and 5 with asterisks. Response rates during the tone increased over these sessions to a value approximating that observed in the initial T + S sessions (1 to 5). Reconditioning sessions with T + N pairings were then conducted and results closely paralleled those in the initial conditioning sessions. Additional reconditioning sessions were not conducted because the health of both animals declined.

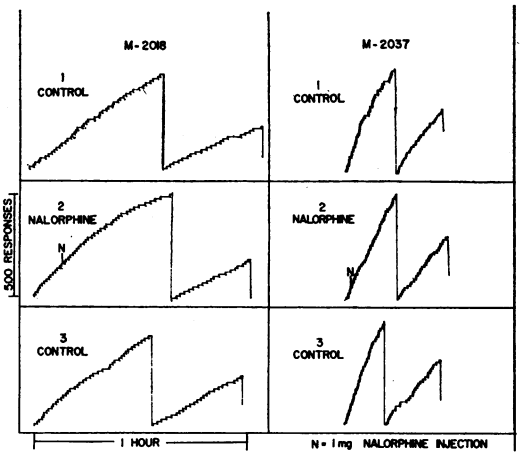

Fig. 6. Cumulative response curves from M2037 and M2018. Each session shown is a complete FR 10 food component record. Sessions 1 and 3 are control sessions, before and after Session 2 during which nalorphine was administered.

Figure 6 shows cumulative response records for the two monkeys (M2018 and M2037) not dependent on morphine. Sessions 1 and 3 were control sessions before and after the sessions in which nalorphine was administered. During Session 2 an injection of 1 mg of nalorphine was given after approximately 10 reinforcements. The nalorphine injections produced no change in the monkeys' FR food responding, compared to control sessions. Observation of these animals after the nalorphine injection failed to reveal any emesis or excessive salivation. It was not possible to record heart rate.

GENERAL DISCUSSION

The results of Exp. 1 and 2 demonstrate that the intravenous administration of nalorphine to morphine-dependent monkeys abruptly terminated food-reinforced, fixed-ratio behavior for the remainder of the session. Further, these subjects showed a marked increment in heart rate, excessive salivation, and emesis within several minutes after the injection of nalorphine. In contrast, monkeys M2018 and M2037, which were not dependent upon morphine, showed no disruption in their fixed-ratio behavior or any observable salivation or emesis after nalorphine was administered. This indicates that the behavioral disruption and physiological changes induced by nalorphine do not occur in non-dependent monkeys, but rather are effects based upon nalorphine's ability to antagonize certain actions of morphine. This is in accord with the well established fact that nalorphine can produce the withdrawal syndrome in morphine-dependent organisms at doses which have little pharmacological action in normals.

The results of Exp. 1 suggest that stimuli associated with the injection of nalorphine could acquire the ability to produce both physiological and behavioral changes. This was more definitely established in Exp. 2 where the tone preceding nalorphine administration acquired the ability to produce a complete suppression of the monkeys' fixed-ratio behavior. This suppression was rapidly established for both monkeys in both the initial conditioning sessions and in reconditioning following the extinction sessions. For M574 the tone also acquired the ability to produce emesis and excessive salivation and a marked decrement in heart rate. One possible explanation for this decrease in heart rate would be that it is due to the suppression of food-lever response rate. This explanation is not tenable, however, since we have observed sessions, before test sessions with saline or nalorphine injections, during which a monkey would not respond for food in the FR 10 food component, yet the heart rate remained at a level of 170 to 190 beats per min. Also, if a decrement in response rate were the explanation for the fall in heart rate, M474 would

also have shown this effect during conditioning and extinction sessions when food responding was suppressed. This would suggest that the tone had become a conditioned stimulus capable of eliciting some of the physiological changes elicited by nalorphine in physically dependent monkeys. This may well be the explanation for the suppression of the animals' fixed-ratio behavior during the tone. That is, if the tone acquires the ability to elicit certain aspects of the withdrawal syndrome these physiological changes may in turn disrupt the animals' food-reinforced, fixed-ratio behavior. This analysis of the mechanism underlying the conditioned suppression is weakened by the fact that the conditioned physiological changes were not observed in M474. It should be noted, however, that the conditioned suppression in this subject was more variable and less resistant to extinction. Further, the failure to observe physiological changes in M474 may be a reflection of the crudeness of the present observations rather than an absence of these changes. We are currently adapting for use in this procedure certain physiological techniques which measure gastro-intestinal motility, salivation, and respiration rate. This will allow the detection of more subtle changes elicited by nalorphine and their possible conditioning to stimuli associated with nalorphine administration. In this way it may be possible to determine whether the classical conditioning of these physiological changes is a necessary prerequisite for the conditioned suppression of the ongoing food-reinforced operant behavior.

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