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Schedule-Induced Oral Self Administration of Etonitazene

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MCMILLAN, D. E. AND LEANDER, J. D. *Schedule-induced oral self administration of etonitazene.* PHARMAC. BIOCHEM. BEHAV. 4(2) 137–141, 1976. – Rats were induced to drink either a saline-etonitazene solution or a saline solution with a schedule-induced polydipsia paradigm. When water was freely available, the rats continued to drink the saline solution or the saline-etonitazene solution, rather than the water. When the locations of the solutions were switched, the rats that were drinking saline switched to water (drank at the usual location), but the rats that were drinking saline-etonitazene continued to drink the saline-etonitazene solution (drank from the bottle at the other location). Naloxone administration temporarily eliminated the drinking of saline-etonitazene solution, but not that of saline solution.

Oral self administration Schedule-induced polydipsia Etonitazene

WE have been studying the oral ingestion of opiates and opiate-like drugs to develop a model to study drug abuse patterns [8, 9, 10, 11, 16]. Among the difficulties encountered in these experiments is the aversive taste of some of these drugs. For example, morphine sulfate in solution is a weak base with a bitter taste that laboratory animals often do not accept [14].

Etonitazene [5,6], an extremely potent opiate-like drug, is well absorbed by the oral route, and appears to have little if any taste at concentrations that produce marked behavioral effects upon ingestion [2,15]. Because of these characteristics we chose to study its oral self administration. The etonitazene was given in a saline solution so that the rats could discriminate between the drug solution and water on the basis of taste and because the addition of saline to opioid solutions has been shown to increase their ingestion [7,9].

The schedule-induced polydipsia phenomenon described by Falk [3] was used to induce animals to drink the saline solution containing etonitazene. This procedure was used becauses the correlation between pellet delivery and drinking has been shown to rapidly induce the ingestion of other narcotic analgesics [8, 9, 10]. After the daily pattern of drug intake stabilized the rats were offered a choice between saline-etonitazene solutions and water. Finally, the effects of naloxone on the drug ingestion pattern were determined.

METHOD

Animals

Six Sprague Dawley male rats with no previous training were deprived of food for 48 hr and of water for 24 hr, at which time they weighed approximately 200 g. Subsequently, the rats were maintained at approximately this weight by the pellets delivered under the schedule.

Apparatus

The test chambers were 2 Gerbrands rat chambers and 1 Lehigh Valley rat chamber enclosed in sound attenuating enclosures. A bottle was mounted outside the test chamber with its metal drinking spout remaining just outside one of the holes in the cage normally used to house stimulus lights. The rat could drink by putting his tongue through the hole and licking the tip of the metal drinking spout. The hole was located within a few inches of the hopper into which food pellets could be delivered. At the opposite end of the test chamber, about 10 in. from the front panel, a second bottle was mounted outside the test chamber in a similar manner with its metal drinking spout protruding slightly into the test chamber. The chamber was illuminated by a 28 V bulb when pellets were being delivered under the schedule. No illumination of the test cage was provided at other times.

Licks on the metal drinking spout at the front of the test chambers were detected by a Gerbrands Drinkometer. The licks were recorded on response counters and on a Gerbrands Event Recorder located in another room. From these data, average rates of licking were calculated. In some experiments the licks that occurred after pellet delivery (postpellet licks) were recorded, as well as the licks that occurred when no pellets were delivered (intersession licks). In other experiments, the intersession licks and the postpellet licks were cumulated every 3 hr.

Procedure

The schedule was a fixed-time 90 sec schedule of food pellet delivery (FT 90). Under the FT 90 schedule, a 97 mg Noyes rat food pellet was delivered to the rat every 90 sec, regardless of the rat's behavior. Thirty pellets were delivered to the rat 4 times daily at equally spaced intervals

TABLE 1

OUTLINE OF EXPERIMENTAL CONDITIONS

Phase	Rats B-1, B-2, B-3	Rats BB-1, BB-2, BB-3
1	5 sessions with water in the front bottle and no rear bottle	5 sessions with water in the front bottle and no rear bottle
2	15 sessions with etonitazene and saline in the front bottle and no rear bottle	15 sessions with saline in the front bottle and no rear bottle
3	39 sessions with etonitazene and saline in the front bottle and water in the rear bottle	39 sessions with saline in the front bottle and water in the rear bottle
4	2 sessions with bottle positions reversed, separated by a session with bottles in the usual location	2 sessions with bottle positions reversed, separated by a session with bottles in the usual location
5	2 sessions as in phase 3	2 sessions as in phase 3
6	1 session with 2 mg/kg naloxone	1 session with 2 mg/kg naloxone

(12 p.m., 6 p.m., 12 a.m., 6 a.m.), so that the rat received 30 pellets every 6 hr for a daily total of 120 pellets.

Initially, the food- and water-deprived rats were placed in the test chamber at about noon and the FT 90 schedule began. Thereafter, the rats were removed from the test cages for about 5 min at noon each day, so that the cages could be cleaned, the data recorded, and the bottles weighed. Subsequently, the rats were returned to the test chambers and the delivery of the first 30 pellets under the FT 90 schedule was started immediately. During initial sessions, water was in the bottle at the front of the cage and the bottle at the rear of the cage was absent. After postpellet drinking was established, the water bottle was added at the rear of the cage. Table 1 outlines the experiments that followed.

Drugs

Etonitazene was used as the hydrochloride at a concentration of 5.0 μg/ml. All sodium chloride solutions were 0.5% solutions. Tap water was used in the water bottle and was also the solvent for the saline solutions and the saline-etonitazene solutions.

Naloxone hydrochloride was dissolved in distilled water and the injection volume was 1 ml/kg. The intraperitoneal injections of naloxone (2 mg/kg) were given 1 min before the rat was returned to the test chamber and the delivery of the pellets started.

RESULTS

All of the rats licked the water spout in the front panel of the chamber during the first session. By the second session, the usual pattern of postpellet licking as described by Falk [3] was obvious in all rats (a sustained burst of licking beginning within a few seconds after the pellet was consumed and usually terminating before the delivery of the next pellet).

Figure 1 shows changes in licking parameters when saline replaced water in the drinking bottle (top frames). With water in the bottle the 3 rats averaged about 20,000 licks/day with about 80% of these following pellet deliveries (postpellet licks) and the remaining 20% of the licks occurred during the 5 hr and 15 min periods when no pellets were being delivered (intersession licks). Rats BB 1 and BB 2 drank between 25 and 50 mls on the final day of exposure to water and rat BB 3 drank almost 100 mls. On the day when saline was substituted for water, the number of licks/day and the mls of fluid consumed increased for all 3 rats, but there was little change in the percentage of postpellet and intersession licks.

The lower frames of Fig. 1 show changes in the licking pattern when saline-etonitazene solution replaced the water in the drinking bottle. The total number of licks decreased for all 3 rats, but the daily amount of fluid consumed decreased only for Rat B-1, the rat with the highest daily water intake before substitution of the saline-etonitazene

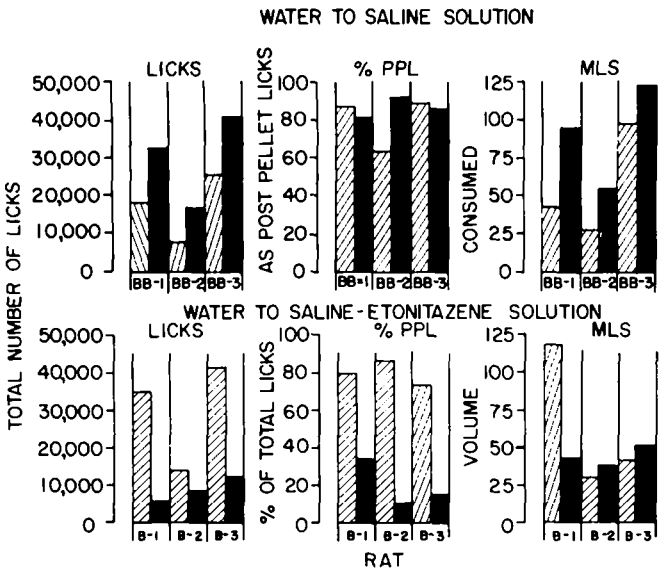

FIG. 1. Effects of changing from water (striped bars) to saline solution (filled bars, upper frame) or to saline-etonitazene solution (filled bars, lower frame) on total number of licks, % of total licks as post pellet licks (% PPL) and ml consumed. The striped bars are for the final day of water drinking and the filled bars are for the first day of saline or saline-etonitazene drinking. The BB and B designations are the rat identification numbers.

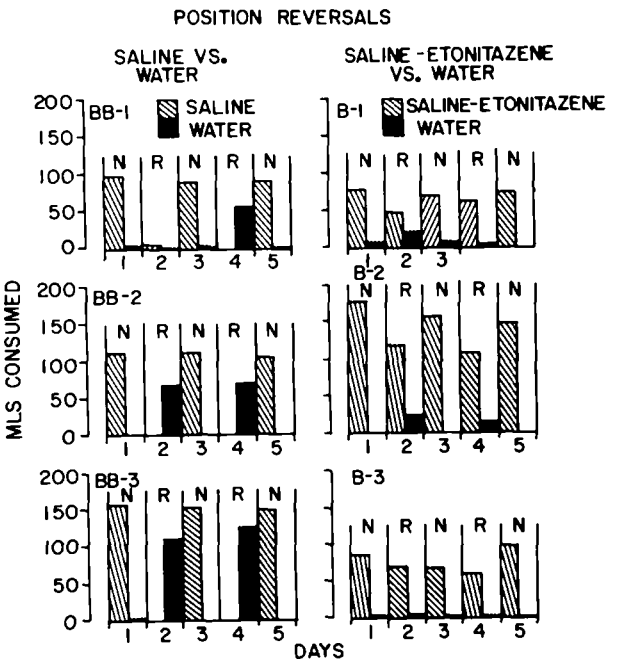

FIG. 2. Effects of reversing the location of water and saline solution or water and saline-etonitazene solution on fluid intake. N refers to bottles in the normal position (water in the rear bottle and saline or saline-etonitazene solution in the front bottle) and R refers to bottles in the reversed position (water in the front bottle and saline or saline-etonitazene solution in the rear bottle).

solution. In addition to the decrease in licking when the saline-etonitazene solution was introduced, the pattern of licking by these rats also changed. The percentage of postpellet licks (about 80% when water was in the drinking bottle) decreased to only about 20% during the first session that etonitazene was in the drinking bottle.

All of the rats drank sufficient etonitazene solution to produce a coma within the first few minutes of postpellet licking when etonitazene was introduced. In fact 5 rats died from respiratory depression during the first session of drinking the saline-etonitazene solution, so that it was necessary to test 8 rats to obtain the 3 survivors shown in the bottom frames of Fig. 1. The range of doses that produced death in these animals was 237–424 μg/kg.

During the next few sessions, the volume of saline-etonitazene solution that was consumed generally increased for all rats, but the daily intake varied greatly. For example, Rat B-2 drank more than 100 ml of saline-etonitazene solution on Days 2, 3, 5 and 6 of the exposure to this solution, but Rat B-2 did not drink any saline-etonitazene solution on Day 4 of exposure. Although this was the most extreme degree of variability observed, occasionally the other two rats would drink smaller than usual volumes of etonitazene solution. When the consumption of etonitazene solution was unusually low the animals lost weight and exhibited loose stool.

After 15 sessions during which the rats drank saline or saline-etonitazene solutions, a water bottle was introduced at the back of all test chambers for the next 39 sessions (Table 1). The rats drinking the saline solution averaged over 120 ml/day of saline solution and less than 1 ml/day of water. The rats drinking the saline-etonitazene solution averaged more than 100 ml/day of saline-etonitazene solution and less than 2 ml/day of water. This resulted in an average daily dose of approximately 2500 μg/kg of etonitazene per day.

During 2 of the next 5 sessions the position of the bottles in the cages were reversed, so that the saline and the saline-etonitazene solutions were in bottles located at the rear of the cages and the water bottles were at the front of the cages near the pellet hopper. Figure 2 shows the effects of reversing the position of the fluids on the volumes of fluids consumed. For the rats drinking saline solution, in 5 of the 6 cases where the reversal in position occurred, the rats switched to water from saline. In other words, the rats continued to drink at the same position even though water had replaced the saline solution. The only exception to this was the first reversal for Rat BB-1 where very little drinking of either fluid occurred. The amount of water consumed on reversal days (Fig. 2) was as much or greater than the amount consumed when water was the only solution available (Fig. 1). In contrast, when the position of the water bottle and the saline-etonitazene bottle were reversed, in all cases the rats continued to drink primarily the saline-etonitazene solution. In other words, the rats changed their drinking position to the rear of the cage at the place to which the saline-etonitazene bottle had been moved (Fig. 2, right frames).

After 2 sessions with the bottles in their usual positions, a dose of 2 mg/kg of naloxone was administered 1 min before the delivery of the first series of pellets was started. The effects of naloxone on the volume of fluid consumed are shown in Fig. 3. In the rats that drank saline solution, for which data on the effects of naloxone are available, the naloxone had very little effect on the volume of either water or saline solution consumed. For the rats drinking the saline-etonitazene solution, naloxone had litte effect on the

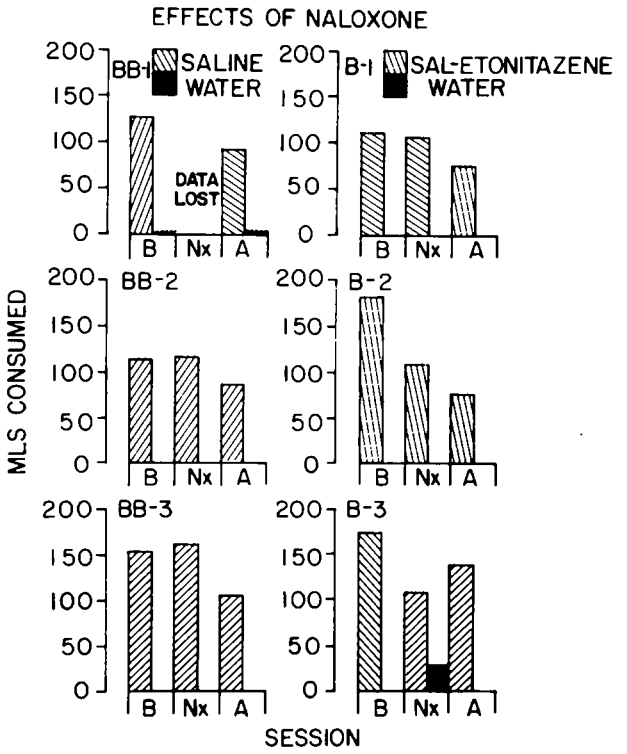

FIG. 3. Effects of naloxone administration on fluid intake of rats drinking saline solution or saline-etonitazene solution. B refers to the day before naloxone administration, Nx refers to the day of naloxone administration, Nx refers to the day of naloxone administration and A refers to the day after naloxone administration. The stopper became dislodged from the bottle during the day that naloxone was administered to Rat BB-1.

drinking of saline-etonitazene solution in Rat B-1, but it reduced the amount of saline-etonitazene solution consumed by Rat B-2 without affecting the negligible water intake. In Rat B-3, naloxone reduced the amount of saline-etonitazene solution consumed and increased the amount of water consumed.

A factor contributing to the variable effects of naloxone in the rats drinking the saline-etonitazene solution is shown in Fig. 4 which shows the number of licks by each rat, cumulated every 3 hr for 24 hr before naloxone administration and for 24 hr after naloxone administration. The rats drinking saline showed very similar distributions of licking before and after naloxone. Particularly striking are the high rates of licking the saline tube during the 3 hr periods immediately after naloxone administration. In contrast, naloxone changed the usual licking pattern of the rats drinking the saline-etonitazene solution. The high rates of licking the saline-etonitazene tube during the first 3 hr of the session almost completely disappeared. Rat B-1 started to lick at a high rate after 6 hr of decreased licking. This was the rat whose daily volume of saline-etonitazene solution was not decreased by naloxone. Rats B-2 and B-3 were slower to resume licking, taking 9 to 12 hr. The total daily intake of saline-etonitazene solution was decreased in these rats because of the longer suppression of licking after the naloxone injection.

DISCUSSION

These experiments showed that rats induced to drink

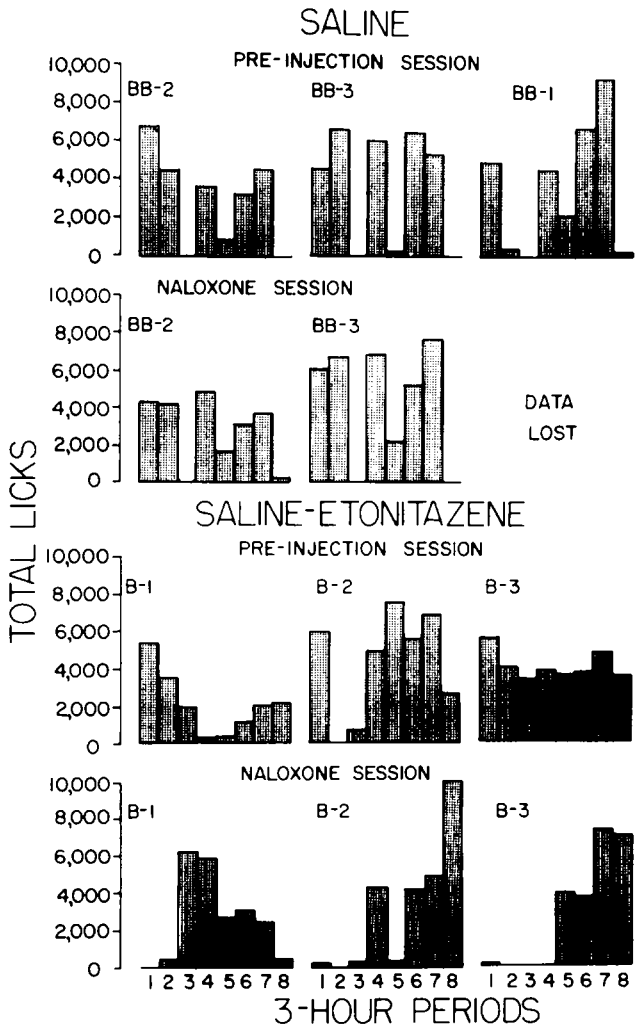

FIG. 4. Effects of naloxone on the daily pattern of licking. The 24 hr session is divided into eight 3 hr periods and the total number of licks is shown for each rat during each 3 hr period. The stopper was dislodged from the bottle during the day that naloxone was administered to Rat BB-1.

large amounts of water under a schedule-induced polydipsia technique also drink large amounts of a saline-etonitazene solution if it is substituted for water. Further, the rats continue to drink this solution when a water bottle is placed at the back of the test cage, suggesting that the rats were not drinking the saline-etonitazene solution merely because they were deprived of other fluids. Unfortunately, rats also chose to drink saline solutions that did not contain any etonitazene when presented with a similar choice between saline solution and water.

There are at least two possible explanations as to why the rats drank more of both the saline-etonitazene solution and the saline solution than water. The first is that the rats may simply prefer saline solutions to water. There is considerable evidence that rats will drink more saline solution than water under schedule-induced polydipsia [4], a finding that is supported by our data in Fig. 1. A second possibility is that the rats adopted a position preference, preferring to drink from the spout in close spatial pro-

ximity to the food hopper, rather than from the water spout at the back of the cage. Clark [1] has shown that it is important to locate the water close to the food hopper if schedule-induced polydipsia is to be maintained.

The importance of the location of the bottles was tested by reversing the position of the solutions in the cage. Water was placed in the bottle at the front of the cage near the food hopper and saline or saline-etonitazene solutions were placed in the bottle at the rear of the cage. The rats choosing between saline solution and water stopped drinking saline solution and began drinking water, indicating that the position of the bottle was a very important variable for these rats. In contrast, when the bottle positions were reversed, the rats choosing between the saline-etonitazene solution and water drank primarily the saline-etonitazene solution from the bottle at the rear of the cage, rather than drinking water from the bottle in the front of the cage. For these rats the position of the bottle was a much less important variable than it was for the rats drinking the saline solution. Since the only difference was the presence of the etonitazene in the saline solution, it is reasonable to conclude that the rats drinking the saline-etonitazene solution continued to drink this solution when its location was changed, because the etonitazene acted as a positive reinforcer.

The disruption of the drinking of saline-etonitazene solution for 6 to 12 hr after a dose of naloxone that had no effect on the drinking pattern of rats drinking saline solution, suggests that these rats were physically dependent on etonitazene. A similar disruption of the drinking of morphine after naloxone administration has been observed with rats induced to drink morphine under the schedule-induced polydipsia procedure [10]. Further indication that these rats were physically dependent was obtained from the causal observation of abstinence signs (wet dog shakes, diarrhea, weight loss, etc.) in some rats on days when little etonitazene drinking occurred.

These data should not imply that the schedule-induced drinking of etonitazene is the only technique whereby rats can be induced to drink etonitazene solutions and become physically dependent on them. We have shown previously that when 3 or 10 μg/ml of etonitazene replaces water as the only available drinking fluid there is a transient increase in fluid intake that lasts for about a week [13]. Subsequently, these rats were shown to be physically dependent on etonitazene after 12 days of drinking it, as demonstrated by the elicitation of withdrawal symptoms following naloxone injection.

The replacement of water by saline-etonitazene solution disrupted the usual pattern of postpellet drinking so that a much higher proportion of licking occurred during periods when pellets were not being delivered. Similar effects have been observed with morphine [9] and with ethanol [12]. Whether this change in the licking pattern occurs with all drugs, only drugs that act on the central nervous system, or only with those with abuse potential, is an interesting question deserving further study.

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