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Taste vs. CNS Effects in Voluntary Oral Opiate Intake: Studies With a Novel Device and Technique

KRISTIN R. CARLSON

Department of Pharmacology, University of Massachusetts Medical Center, Worcester, MA 01655

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CARLSON, K. R. *Taste vs. CNS effects in voluntary oral opiate intake: Studies with a novel device and technique.* PHARMACOL BIOCHEM BEHAV **34**(2) 419–423, 1989.—An apparatus is described which negates the influence of rats' position preferences by presenting alternative solutions at the same location. The licks for both solutions were monitored over consecutive short intervals by lick detectors with computer capture of data. Rats given a choice between water and dilute solutions of the high-potency opiates etonitazene (1.0–5.0 microgram/ml) or fentanyl (10–50 microgram/ml) either licked equally for the two solutions, or gradually developed a preference or aversion regarding the opiate over the course of several days. In contrast, preferential licking for solutions with a definite taste, saccharin or quinine, was established in hours. These data indicate that the taste per se of these opiates is not aversive to rats, and that preferences for or aversions to the opiates have some other base, presumably one or more actions on the central nervous system.

Opiate Etonitazene Fentanyl Taste Position preference Oral self-administration Lick detectors

WHILE rodents will self-administer opiates by many routes, including the intravenous (17) and oral (12), there are important advantages of the latter route over intravenous injection. No surgery is involved, the subjects are not restrained by a catheter harness, no expensive equipment is needed, experiment duration is not limited by catheter patency, and large numbers of animals can be tested conveniently. Nonetheless, the oral paradigm has two technical problems of its own.

First, opiates are bitter in aqueous solution. To overcome this, the taste has been masked with sucrose (2, 9, 10), or, in a paradigm permitting a choice between two solutions, the nonopiate solution has been made equally bitter with quinine (5). Alternatively, with use of a very potent opiate, the solution will be dilute to the point that its taste is apparently not aversive to the animal. Fentanyl (1) and etonitazene (3, 4, 16) are potent enough for the latter strategy and were adopted for the present study.

If oral intake is to reflect accurately the propensity of the animal to self-administer the drug, consumption must not reflect other factors such as the location of alternative choices. Usually these are provided by two bottles side-by-side in the home cage. However, rats quickly develop strong position preferences, and often drink from a single bottle regardless of its contents (3,5). Thus, daily alternation of bottles can measure not the propensity to self-administer, but rather the strength of the rat's position preference. This paper describes a device which, by presenting both solutions at the same location, eliminates the factor of position preference.

It is often assumed that rats accept or reject a drug solution on the basis of the central nervous system (CNS) effects it produces rather than on its taste. Attempts to demonstrate this by blocking the CNS effects of orally-administered opiates with naloxone have produced some equivocal results (11,13), either because adequate blood levels of naloxone were not maintained between injections (8), or because the opiate had acquired secondary reinforcing properties which sustained self-administration in the face of adequate pharmacological blockade (13). The present paper suggests that a noninvasive and simpler procedure is to track drinking in a fine-grained manner over time by means of lick detectors and computer capture of the licks emitted during consecutive short intervals. The effects of taste should be apparent immediately, as the rat can quickly detect and learn to avoid an aversive taste (or perceive and drink more of a pleasant-tasting solution). CNS effects should be much slower in onset, since the opiate must be absorbed, distributed to the CNS, and accumulated there in sufficient concentration. Further, these effects must become associated with some discriminative cue provided by the opiate in order for the animal to learn to seek or avoid the drug, which is also a potentially lengthy process. The present paper provides evidence supporting this reasoning, and suggests that preference for or aversion to high-potency opiates is not based on taste alone.

METHOD

Animals

The subjects were male Sprague-Dawley rats (Harlan Sprague-

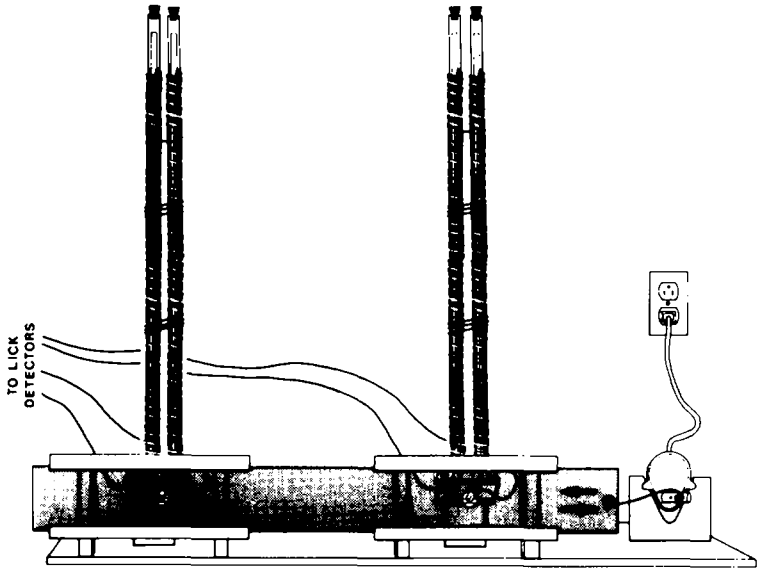

FIG. 1. The switching device. The shutter is drawn stippled to help set it apart from the rest of the apparatus. The operation of the device is described in the text.

Dawley, Indianapolis), weighing 200–250 g at the beginning of experimentation. They were housed individually in $30 \times 34 \times 16$ cm high Plexiglas cages with stainless steel rod tops (containing rat chow). A hardware cloth floor, covering a layer of sawdust bedding, was connected to ground of the lick detector circuit. A 30×50 mm wide hole was located centrally in one end wall.

The cages and switching devices were located in a room maintained at 20 ± 2°C. In order to encourage a more even distribution of drinking over each 24-hr period, a constant dim illumination was maintained by a 15-W bulb.

Apparatus

The switching device, which services two subjects, is shown in Fig. 1. For each rat, two metal drinking spouts are mounted 20 mm apart, 50 mm above cage floor level. They are connected by flexible tubing to glass burettes taped to a vertical rod behind the spouts. Approximately 2 mm in front of the ends of the spouts is a plastic shutter with a 12-mm diameter hole through it. A 1/4 rpm timing motor turns an eccentric cam which is linked to the shutter. As the motor turns, the shutter slides back and forth in its mounting rails, placing the hole alternately in front of each spout every 2 min. The rat's cage is placed against the shutter rails, with the rectangular hole in the cage wall centered on the ends of the spouts. Through it the rat can reach the shutter hole and spout behind it. The lower part of the burettes and the tubing are wrapped with black tape, and the top of the burettes covered with an aluminum foil cap when light-sensitive drugs are in use. The devices are quickly and inexpensively constructed from plastic and other easily obtainable materials.

Each metal spout is connected by a wire to the input of a lick detector circuit shown in Fig. 2. When the rat drinks, each lick closes an electric circuit to ground. The resulting current pulse triggers a "one-shot" (LM556 timer) which sends a +5-V, 1.7-msec pulse to the input of a Metrabyte PI012 digital I/O board, and also to its interrupt input through a diode OR gate. The lick

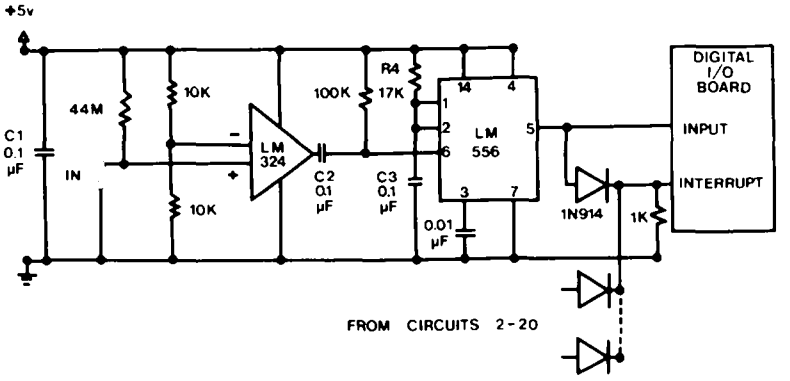

FIG. 2. The lick detector circuit. The LM324 is an operational amplifier and the LM556 a dual timer "one-shot" (only one half is shown; the other is used in a second circuit). R4 and C3 control the duration of the output pulse (1.7 msec with these values). C1 filters the 5-V supply, and C2 makes the one-shot respond to level changes. The 1N914s constitute OR gates.

detector circuits can be built from readily available (e.g., Radio Shack) components, with four circuits mounted on each "experimenter's PC board." The Metrabyte I/O board was installed in an AST Premium 286 computer running at 10 MHz.

It is important to keep the electrical lines related to the lick detector circuits, and the circuits themselves, shielded and out of close proximity to the AC cords running to the motors or AC power lines in general; otherwise, electrical interference produces spurious counts. It is also desirable to use a line power conditioner for the computer.

A program written in Turbo Pascal with inline assembly code for critical functions handles the hardware interrupts, accumulates the licks on each spout, prints out each hour for each rat the number of licks on each spout and the percentage of the total which was for the drug solution, and stores the data for subsequent analysis.

Drugs

The chemicals employed were etonitazene base (NIDA), fentanyl citrate (Sigma), quinine HCl (Sigma), and sodium saccharin (Sigma); all were prepared in tap water.

Procedure

The rats received all their fluid from the apparatus, and were housed continuously with it, except for 0900–1100 daily when they were weighed and routine maintenance was done. The fluid consumed from each burette was measured and corrected for evaporation (less than 0.3 ml) from two control burettes before the burettes were refilled each day.

Rats were shaped to the device by having the shutter stationary with its hole positioned directly in front of a spout containing water for 24 hr. For the next 24 hr the shutter was moving and rats were presented with a water-water choice. All animals quickly learned to drink from both spouts and to switch smoothly between them. Since the rats did not prefer one spout over the other when both contained water (see the Results section), the side of the drug burette was not counterbalanced during the experimental days when the right burette contained the drug solution and the left burette tap water. On the final day a water-water choice was reinstituted.

Cumulative concentration-effect curves were determined with etonitazene and fentanyl. During the experimental phase 10 rats were given three days each of a choice between water and increasing concentrations of etonitazene (1.0, 3.0, and 5.0 micrograms/ml). In the same fashion 10 other rats were presented with a choice between water and 10, 30, and 50 micrograms/ml fentanyl.

To test whether rats given the opportunity to drink solutions with a pronounced taste would accept or reject them immediately, a different set of 10 rats was offered a choice between water and saccharin (0.05%) for 24 hr, followed by a choice between water and quinine (0.16 mg/ml) for 24 hr.

Data Analysis

In the cumulative concentration-effect experiments the daily percentage licks for the right-hand spout and body weight were subjected to one-way analyses of variance with repeated measures, and in all experiments the relation between total licks and volume consumed was tested with a linear regression analysis (ABstat program from Anderson-Bell). Running averages of the percentage licks for the drug spout on an hourly basis for individual subjects were calculated by a program in BASIC.

RESULTS

One or more rats from each group became ill with respiratory congestion and had to be dropped from experimentation, providing the final group sizes shown in Table 1. The remaining subjects were healthy and gained weight steadily, $F(10,60$ and $10,80) = 18.6$ and 99.9, $p<0.0001$, indicating that they were able to obtain sufficient fluid from the apparatus.

TABLE 1

MEAN (±SEM) PERCENTAGE OF TOTAL LICKS WHICH WERE ON THE RIGHT-HAND SPOUT DURING THE WATER-WATER CHOICE DAY BEFORE AND AFTER WATER-DRUG CHOICE DAYS

Drug	N	Before	After
Etonitazene	7	51.0 ± 8.0	60.2 ± 2.4
Fentanyl	9	56.7 ± 3.1	46.7 ± 4.9
Saccharin and Quinine	9	41.2 ± 5.3	28.7 ± 7.9

The correlation between total licks/24 hr and volume of fluid consumed was very good in all experiments ($r = .87–.97$, $p<0.0001$, with the regression lines passing through the origin (y intercepts = −0.27–1.37 ml). In earlier work a similar lick detector circuit was used with cumulative recorders (1) and generated comparable correlation coefficients. Repeated observation of the rats licking confirmed that each lick was accompanied by an audible closure of the cumulative recorder stepper solenoid. Thus, deviations from the regression line are more likely due to small inaccuracies in measuring the volume of fluid consumed than in counting licks.

The rats licked about equally on the two spouts when presented with a water-water choice, as shown in Table 1. The low SEMs indicate that the means are not the result of offsetting extreme values. In the 24 hr following a water-quinine choice, the percentage licks on the right spout remained rather low; even though the burettes were flushed with water repeatedly, it is possible that some quinine had been adsorbed by the tubing.

Providing a constant dim illumination seemed to encourage drinking around the clock. In spite of the fact that the rats received a daily time reference when the apparatuses were serviced, no clear diurnal rhythm was apparent in number of licks or number of rats drinking.

During the last three days of opiate self-administration (the highest concentration) the rats were consuming 324.1 ± 32.9 microgram/kg etonitazene and 2.13 ± 0.2 mg/kg fentanyl. The dosage schedules used in these experiments were not sufficient to induce strong physical dependence, since no rat lost weight (14,15) during the 24 hr after the drug was withdrawn.

The rats exposed to increasing concentrations of etonitazene varied greatly in their acceptance of the drug, such that the ANOVA for percentage licks for the drug spout showed no effect of days, $F(10,60) = 1.4$, ns. The same results were obtained with fentanyl, $F(10,80) = 1.8$, ns.

More illuminating is the fine-grained analysis of the licks/hr of individual rats. The hour to hour percentages of licks on the drug spout were extremely erratic, varying in many cases between 0 and 100%. As a consequence, trends over time were difficult to visualize when the data were plotted as the raw hourly percentage licks for the drug spout. Accordingly, the hourly percentages were transformed into five-point running averages (each time point is the mean of itself and the two adjacent time points on either side) to smooth the plots. Figure 3 shows running averages from two subjects during the three days of exposure to the lowest concentration of each drug. In the case of etonitazene, a preferring and an avoiding rat are shown in the top panel, illustrating that the onset of overall preference or aversion was rather gradual. Regarding fentanyl, a rat with no preference and an avoiding rat are

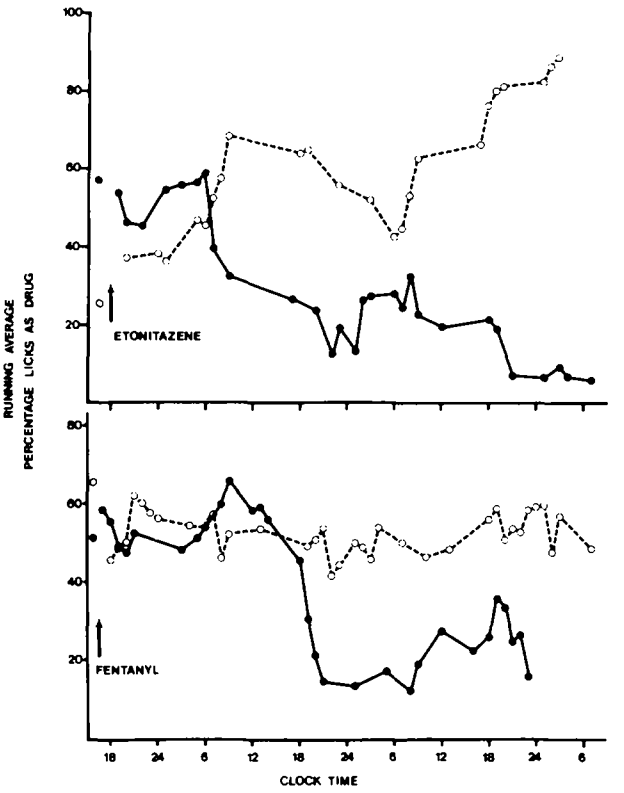

FIG. 3. Running average of the percentage of the total licks in one-hr blocks which were for etonitazene (1.0 micrograms/ml) or fentanyl (10 micrograms/ml). Each graph shows data from two representative rats. The unattached points at the far left of each graph indicate the percentage of total licks which were on the right-hand spout (to become the drug spout) during the previous 24 hr of a water-water choice. A drug-water choice was instituted at the arrows. The absence of a data point indicates that the rat did not drink during that hour.

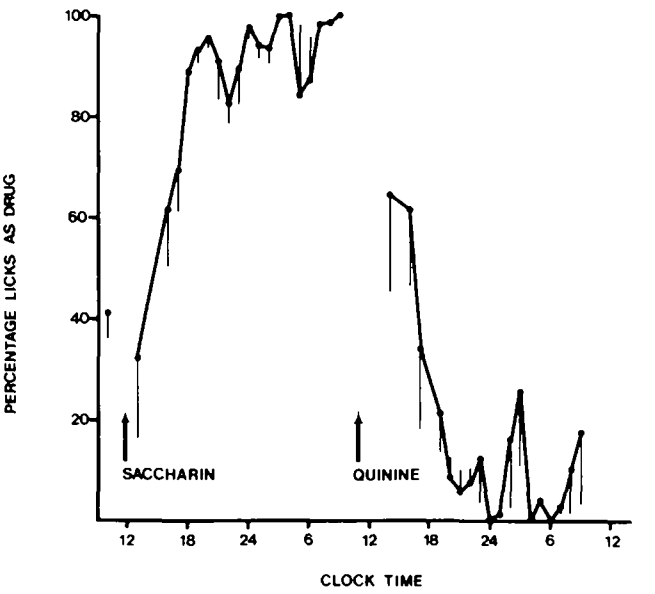

FIG. 4. Mean (± SEM) percentage of the total licks in one-hr blocks which were for saccharin (0.05%) or quinine (0.16 mg/ml). Choices between water and the chemicals were begun at the arrows. The absence of a data point indicates that none of the nine rats drank during that hour.

illustrated in the bottom panel.

Development of a preference or aversion was defined as a 25% or greater change in the percentage licks for the drug from the first to the last (third) day at a given concentration. In general, the number of rats developing a preference or aversion did not increase as the drug concentration increased. For etonitazene, at 1.0 micrograms/ml two rats developed a preference and one an aversion, at 3.0 micrograms/ml one rat developed a preference, and at 5.0 micrograms/ml one developed an aversion. No rat came to prefer fentanyl; two rats developed an aversion at 10 micrograms/ml, one at 30 micrograms/ml, and none at 50 micrograms/ml. With the exception of one rat which developed an aversion to 1.0 micrograms/ml etonitazene but subsequently drank equally of water and the higher concentrations, when a preference or aversion had developed at a particular concentration it was maintained at higher concentrations.

In contrast to the gradual onset of preference or aversion seen with the opiates, Fig. 4 shows that exposure to a solution with a definite taste rapidly induced a pronounced and consistent preference or aversion. The means (± SEMs) of nine rats are illustrated, since all behaved approximately the same.

DISCUSSION

The switching apparatus described here enables one to study self-administration in a paradigm involving a choice which is uncontaminated by rats' position preferences. The results showed that rats licked approximately equally from the two spouts when both contained water. When given an opiate-water choice, some rats developed a preference for one spout over the other. This was a function of the contents of the burettes, not some characteristic of the spouts other than their positions, because when a water-water choice was reinstituted the preference was lost.

The use of lick detectors and computerized data collection allows one to monitor consumption over time in sequential intervals of any duration. The unit employed in the present study, one hour, is sufficiently short to provide an adequately fine-grained analysis over 24 hr. If drinking were limited to several hours per day, a shorter unit might be more appropriate.

Analyses of the licking patterns support the conclusion that the rats did not find the taste of the opiate solutions aversive. First, the percentage licks for the drugs showed great variability from hour to hour, which is inconsistent with observations using quinine. Second, many rats drank drug and water equally, and some rats drank more opiate than water. Third, with the opiates it required several days for those rats which showed a preference or aversion to develop them, in contrast to saccharin and quinine, where preferential drinking was established in all subjects within hours. Thus, the tactic of using a dilute solution of a high-potency opiate to overcome the influence of taste is justifiable.

Nonetheless, it is clear that at least some of the rats received a discriminative cue by which they could identify the opiate solution, or they would not have been able to preferentially seek or avoid it. Whether this cue was a weak, motivationally neutral taste, or some other feature of the solutions or the spouts, is not known.

It is commonly assumed that the development of preferences or aversions depends on the rats receiving another cue, the interior "narcotic cue" (6). There is good evidence that this cue arises from opiate effects on the CNS rather than on the periphery. As one example, rats trained to discriminate parenteral opiate from saline act in the drug-appropriate manner when opiates are injected intracerebrally (6).

The slow onset of preferences or aversions is probably attributable to two factors. First, the drug must accumulate in sufficient concentration to produce the "narcotic cue." Second, this cue must become associated with whatever subtle discriminative cue, taste or otherwise, is also being received. These are lengthy processes, and the second is made more difficult by the concurrent water intake of the rats. The complexity of the task may account for the fact that not all rats acquired preferences. Alternatively, some of these rats may have been relatively insensitive to the CNS actions of opiates, as considerable variability in this trait is seen in a drug discrimination paradigm (7).

Finally, the present report agrees with others [for review see (12)] that etonitazene can serve as a positive reinforcer, i.e., its ingestion supports self-administration behavior which is lost when water is substituted for the drug.

In summary, this is the first report in which the choice that rats make between water and an opiate solution was assessed, under conditions of continuous access in their home cages, in a fashion which allowed one to rule out the influences of both taste and position preferences. Under these circumstances, many rats drank equal amounts of the two solutions, but others preferentially sought or avoided the opiate. By tracking consumption across relatively short consecutive intervals, it was shown that the basis for preferences is not taste alone.

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