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Schedule-Induced Etonitazene Drinking:
Establishment of Etonitazene as a Reinforcer for Rats

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Schedule-Induced Etonitazene Drinking: Establishment of Etonitazene as a Reinforcer for Rats.[1]

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Abstract

Drinking of etonitazene HCl by six rats was studied during daily 4-hr sessions. Four related experiments were sequentially conducted. In the first experiment the sessions were divided into two 2-hr components. During both components each press on the right-hand lever resulted in presentation of a 0.1 ml dipper containing water or an etonitazene solution. During the second 2-hr component presses on the left-hand lever produced 45 mg food pellets under a *chain* DRO 40 sec FR 1 schedule. When water was present, low rates of responding occurred during the first 2 hr and high rates, i.e., schedule-induced polydipsia, occurred during the last 2 hr. Subsequently, etonitazene in an ascending series of concentrations (1.25, 2.5 and 5.0 µg/ml) was presented in place of water. During the first 2 hr all three etonitazene concentrations maintained more responding than water. In the second experiment the concurrent food reinforcement schedule was discontinued. Responses maintained by etonitazene (5 µg/ml) persisted. In the third experiment the number of lever presses required per dipper presentation of etonitazene (5 µg/ml) was increased from one to two and finally to four, i.e., FR 1, 2 and 4. Rate of lever pressing increased directly with the fixed ratio size whereas number of dipper presentations remained constant. In the fourth experiment the 5 µg/ml etonitazene solution was replaced by water. Etonitazene was reintroduced when water maintained responding showed no systematic trend. When water was present, responding declined to low rates, and when etonitazene was reintroduced, responding increased to previous levels. These results demonstrate that etonitazene, 5 µg/ml, was functioning as a positive reinforcer.

Etonitazene is an opioid approximately 1000 times as potent as morphine and has effects qualitatively similar to those of morphine (Wikler, Martin, Pescor & Eades, 1963). For drug naive rats etonitazene methane sulfonate concentrations of 5 and 10 µg/ml are probably not aversive, for when these drug concentrations are substituted for water, the drinking patterns and volumes consumed per 24 hr do not differ from water values (Wikler et al., 1963). Also, during the first 7 hr of drug access, the intake of 5 µg/ml etonitazene is significantly greater (in the statistical sense) than water intake (Wikler et al., 1963). When water and 5 µg/ml etonitazene were concurrently available, volumes consumed did not differ (Wikler & Pescor, 1970). The hydrochloride salt of etonitazene may be more palatable than the methane sulfonate salt, for when rats were restricted to etonitazene HCl concentrations of 3.0 or 10.0 µg/ml, the volumes consumed exceeded water baseline values (McMillan, Leander, Wilson, Wallace, Fix, Redding & Turk, 1976).

Etonitazene drinking is markedly increased if rats are first made physiologically dependent by intraperitoneal injections of increasing doses of morphine. For example, when 5 µg/ml etonitazene was substituted for water during a 24 hr period after the last injection, morphine-dependent rats drank significantly greater volumes of drug solution than did saline-injected control rats (Wikler et al., 1963). Volumes of drug solution consumed by the dependent rats were also significantly larger than either their water intake or water intake by control rats; the rats received only one liquid at a time (Wikler et al., 1963). The greater drinking of 5 µg/ml etonitazene than of water suggests that this drug solution can function as a positive reinforcer for morphine-dependent rats.

Additional data are consistent with the notion that 5 µg/ml etonitazene can function as a reinforcer. For example, during a period 24 to 48 hr since

the last injection, significant differences in liquid intake occurred between morphine-dependent and saline-injected rats: Relative to water drunk by saline--injected rats, morphine-dependent rats drank more of a 5 µg/ml etonitazene solution and less water (Wikler et al., 1963).

In several studies access to etonitazene solutions was contingent upon lever pressing (Leander & McMillan, 1975; Lewis, Margules & Ward, 1975). Fixed-ratio responding by rats was maintained by presentations of 0.1 ml of 5 µg/ml etonitazene flavored with small amounts of anise oil and quinine hydrochloride (Lewis et al., 1975). Maximum ratios maintained were from 40 to 100 lever presses per dipper presentation. Before such responding was established, the rats received morphine intraperitoneally in doses that were gradually increased to 100 mg/kg. Also, when initially exposed to etonitazene, the rats were water deprived.

In another study (Leander & McMillan, 1975) lever pressing was initiated by restricting access to liquid. For 6 days liquid was available only during daily 8-hr sessions. Each lever press delivered 0.3 ml of 3 µg/ml etonitazene either in water or in a quinine sulfate solution of 0.2 mg/ml. Control rats received the vehicle solutions of either water or quinine sulfate. When a water bottle was introduced into the operant-conditioning chamber, the rate of lever pressing maintained by vehicle solutions declined to levels below that maintained by etonitazene solutions. These results also suggest that etonitazene was serving as a reinforcer.

The general purpose of the present series of experiments was to further define conditions under which etonitazene-reinforced lever pressing can be established and maintained. Specific objectives are mentioned in the introduction to each experiment. These experiments differed from some previous ones

in certain respects. The rats were not pretreated by injections of any drug. The etonitazene solutions were not flavored by any additives. Drug access was limited to at most a single daily 4-hr session, and the rats served as their own controls.

Experiment 1. Schedule-induced etonitazene drinking.

When food-deprived rats are intermittently presented with small pellets of food, they drink unusually large volumes of water and certain other liquids. This phenomenon is termed schedule-induced polydipsia (Falk, 1961, 1971). In the present experiment, etonitazene drinking was obtained by substituting etonitazene solutions for water under conditions of schedule-induced polydipsia. In previous studies ethanol has been established as a positive reinforcer for both rats and rhesus monkeys by initially substituting ethanol solutions for water during schedule-induced polydipsia (Freed, Carpenter & Hymowitz, 1970; Meisch, Henningfield & Thompson, 1975; Meisch & Thompson, 1971, 1974).

The purposes of the present study were to determine drug quantity consumed (µg/kg/hr) and number of dipper presentations as a function of etonitazene concentration during schedule-induced polydipsia. An additional purpose was to determine if etonitazene drinking would alter the baseline pattern of food--reinforced responsing. Previous studies of schedule-induced ethanol and pentobarbital drinking noted marked changes in food-reinforced responding when drug solutions were substituted for water (Meisch, 1969; Meisch & Thompson, 1972). It was anticipated that schedule-induced etonitazene intake would provide the rats with sufficient experience with etonitazene such that the drug would be established as a positive reinforcer.

The experimental design used in the present study is similar to a design used in an earlier experiment with ethanol (Meisch & Thompson, 1974). The com-

mon features are an initial 2-hr component during which liquid is presented after each lever press. The first component is followed by a second one during which liquid is again presented after each lever press, and additionally, food pellets are intermittently available. In the earlier study with ethanol, water--reinforced performance was characterized by a very low rate of lever pressing during the first component and a high scheduled-induced rate of lever pressing during the second component (Meisch & Thompson, 1974). The rationale for this design was that if drug-reinforced lever pressing during the first component exceeded water values, then it was likely that the drug was functioning as a reinforcer. The schedule-induced drinking insured that the rat would drink sufficient quantities of the drug to experience the drug effects that occur subsequent to absorption.

Method

Subjects

Six experimentally naive male albino Wistar rats (Bio-Lab Corporation, St. Paul, MN) were housed individually in a continuously illuminated room with the temperature controlled at 24° C. At 4.5 months of age the weighed: N-1, 483 g; N-2, 492 g; N-3, 515 g; N-4, 477 g; N-5, 500 g; and N-6, 490 g. At the beginning of the experiments the rats were approximately 6 months old and were maintained at 70% of their free-feeding weights as determined at age 4.5 months. Water was always available in the rats' home cages except during initial training as explained below.

Apparatus

Three identical operant conditioning chambers were used (Lehigh Valley Electronics model #143-25). The end walls were 25 cm wide and 26.5 cm in height. The chamber length was 30.5 cm. Each chamber was contained in a sound-attenuating cubicle (LVE model #132-02). On one end wall were two levers

(LVE model #121-05), a pellet receptacle connected to a feeder (LVE model #114-20), and opening for a liquid dipper cup attached to a solenoid-driven arm (LVE model #114-02), six cue lights (lever lights), a speaker, a Sonalert (2.9 KHz), and a house light. Figure 1 shows the arrangement of stimuli, levers, and reinforcement mechanisms. The levers were symmetrically centered 8 cm from the midline of the end wall and 4.5 cm above the grid floor. The force requirement for the levers was approximately 0.3 N. Three 1.25 cm diameter jewelled lenses (red, white, and green) were located 5 cm directly above each lever. The jewelled lenses were each illuminated by a #1820 type bulb. The pellet receptacle was 3.5 cm to the left of the midline, and 1.5 cm above the floor. The opening for the dipper cup was 1.25 cm to the right of the midline and 1.5 cm high, and it was recessed 0.75 cm into the end wall. The recessed space in the end wall was 3.0 cm in diameter and 2.5 cm deep. Three cm above the opening for the dipper cup was a circular opening (0.5 cm dia.), and above the opening was a #1820 type bulb. The speaker, Sonalert, and house light were all centered on the midline of the end wall and were 14.5 cm, 20 cm, and 24 cm, respectively, from the floor.

With each operation of the pellet dispenser, a single 45 mg Noyes food pellet was delivered to the receptacle. The 0.1 ml dipper cup was constantly available in the up position, except during the 0.8 sec refilling operation when it was lowered into the reservoir. Liquid was contained in partially covered reservoirs to minimize evaporation. Masking white noise was constantly present, and an exhaust fan provided ventilation. Automatic data recording and programming equipment were located in an adjacent room. The temporal pattern of the lever presses and dipper presentations was continuously recorded by a cumulative recorder and by a counter which printed out every 10 min.

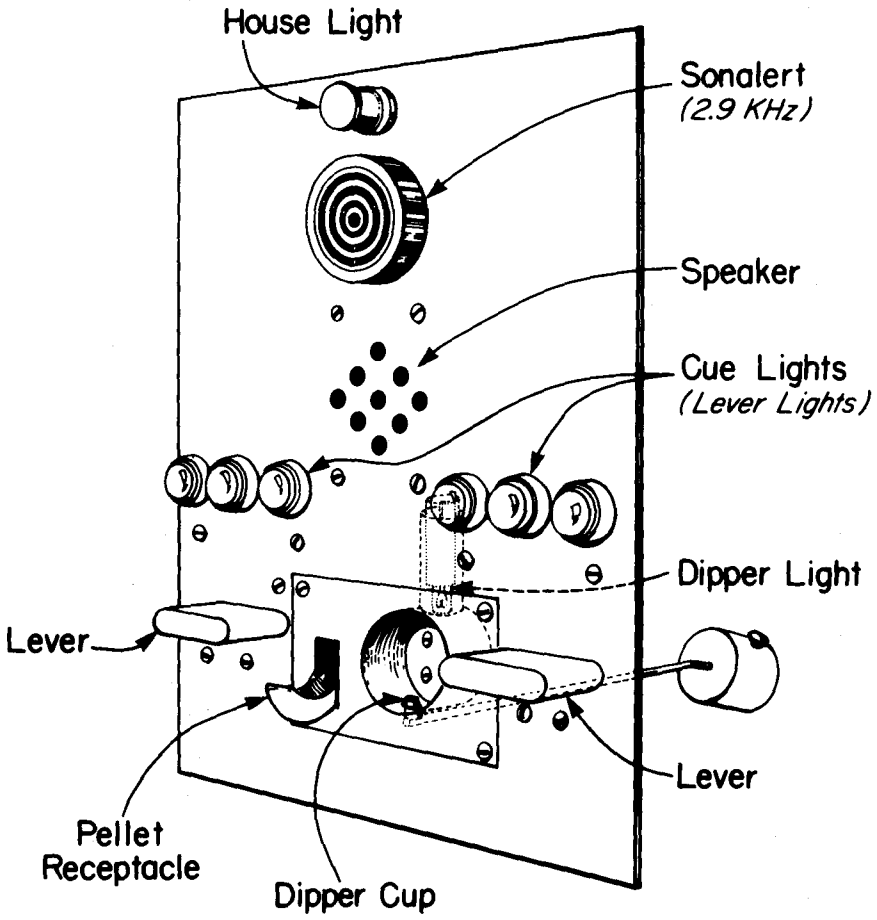

Figure 1. Arrangement of stimuli, levers, pellet receptacle, and dipper cup. The dipper cup is shown in the up-available position.

Procedure

The rats were gradually reduced to 70% of their free-feeding weights by receiving 7 g of Purina Laboratory Chow each day. After reaching 70% of their free-feeding weights, each rat was placed in the operant-conditioning chamber for 4-hr a day at a regular starting time. Initially the rats were deprived of water for 24 hr and the dipper, containing water, was automatically presented on the average once each min, with the time between water presentations varying randomly. During the phase when water was not available in the home cage, the rats' supplementary feedings of Purina Laboratory Chow were placed in the operant-conditioning chamber before the start of the session. This procedure was used to increase the frequency of water responding since the rat usually drinks after eating. Within one to two sessions the rats reliably approached and drank from the dipper. Subsequently, automatic water presentations were discontinued, and the rats were trained to press a lever for water. Each press on the right-hand lever resulted in a refilling operation, during which a tone sounded (Sonalert, 2.9 KHz, Mallory & Co.) and the light above the dipper opening was turned off. The volume delivered per reinforcement was 0.1 ml. After the rats were trained to press the lever and drink from the dipper, three more sessions were conducted before water bottles were restored to their home cages.

Following the initial sessions of water-reinforced lever pressing, the rats were trained to press the left-hand lever for 45 mg Noyes food pellets. Food was never available until 2 hr of the session had elapsed. Availability of food was signalled by illumination of the six lights above the levers and by the simultaneous offset of the house light. Throughout all 4 hr of every training or control session water was concurrently available on a continuous reinforcement schedule (i.e., each press on the right-hand lever produced a refilling operation). Within one to two sessions food-reinforced lever pressing the

rats received from 4 to 17 sessions under a continuous reinforcement schedule of food pellets. Under a continuous reinforcement schedule (abbreviated "CRF" or "FR 1") each response results in some stimulus change. In this case each press of the left-hand lever produced one 45 mg Noyes food pellet. Number of food pellets per session was limited to 200. After each session supplementary feedings of Purina Laboratory Chow were given in the home cages to maintain the rats at 70% of their free-feeding weights. Subsequently the rats were placed on an intermittent schedule of food reinforcement, i.e., a *chain* DRO *n* FR 1 schedule. The DRO *n* component was correlated with illumination of the house light. Each lever press during this component delayed the simultaneous offset of the house light and onset of the lever lights for *n* seconds. After *n* seconds elapsed without a press on the food lever, the house light was extinguished and the lever lights were illuminated. The FR 1 component was correlated with illumination of the lever lights and in this component, the first lever press produced delivery of a 45 mg Noyes food pellet and the offset of the lever lights and illumination of the house light. The abbreviation "DRO" refers to the differential reinforcement of other behavior; in this case not pressing the left-hand lever, while the house light was on, minimized the time between the FR 1 components. The cumulative duration of the FR 1 components was measured each session. Each rat received a single session at DRO values of 5, 10, and 20 sec. Prior to the introduction of etonitazene the rats received from 53 to 105 sessions at DRO 40 sec. See Table 1 for the number of sessions each rat received under each condition.

Etonitazene hydrochloride was presented in an ascending concentration series of 1.25, 2.5 and 5.0 µg/ml. Concentrations are in terms of the salt. Each concentration was present for at least five sessions. Changes were made when there was no trend in the values of the dependent variables as determined.

by visual inspection of the data. In subsequent experiments the same stability criterion was employed. Table 1 lists the number of sessions at each concentration. Drug solutions were prepared using tap water, and all liquids were at room temperature when presented. The volume consumed was determined by subtracting the volume remaining in the reservoir from the volume placed in the reservoir. Corrections were made for volumes lost through evaporation and handling.

Results and Discussion

Dipper presentations during hr 1 and 2

Figure 2 shows that the median number of dipper presentations for the six rats increased as a function of etonitazene concentration up to 2.5 µg/ml. When the concentration was increased to 5 µg/ml, the number of dipper presentations decreased (Figure 2). However, at all etonitazene concentrations, the median number of dipper presentations exceeded the water value. Thus, the data suggests that these etonitazene concentrations were serving as reinforcers. These results were obtained during the first 2 hr of the 4-hr sessions, and during this component food pellets were never available. Under these conditions lever presses that produced water occurred at the low mean rate of 16 per hr. Note that the number of liquid responses is equivalent to the number of dipper presentations, since each lever press resulted in a dipper presentation. Table 2 presents the number of dipper presentations obtained by individual rats at each concentration.

Dipper presentations during hr 3 and 4

Figure 3 shows that during the last 2 hr of the 4-hr sessions the median number of dipper presentations decreased as the etonitazene concentration increased. Studies of schedule-induced pentobarbital and ethanol drinking have

Table 1

Sequence of experimental conditions and number of 4-hr sessions under each condition

Food Schedule DRO (sec)	Liquid Schedule	Drug Conc. µg/ml	Rat N-1	N-2	N-3	N-4	N-5	N-6
FR 1	FR 1	0 (water)	17	14	16	4	7	6
chain DRO 5 FR 1	FR 1	0	1	1	1	1	1	1
chain DRO 10 FR 1	FR 1	0	1	1	1	1	1	1
chain DRO 20 FR 1	FR 1	0	1	1	1	1	1	1
chain DRO 40 FR 1	FR 1	0	53	94	55	82	54	105
chain DRO 40 FR 1	FR 1	1.25	14	12	18	18	15	17
chain DRO 40 FR 1	FR 1	2.50	7	17	24	16	10	14
chain DRO 40 FR 1	FR 1	5.00	13	10	8	10	12	9
EXT	FR 1	5.00	10	8	7	7	7	10
EXT	FR 2	5.00	6	12	6	32	8	23
EXT	FR 4	5.00	10	7	16	11	20	5
EXT	FR 4	0	11	10	23	20	10	--
EXT	FR 4	5.00	17	8	16	30	12	--

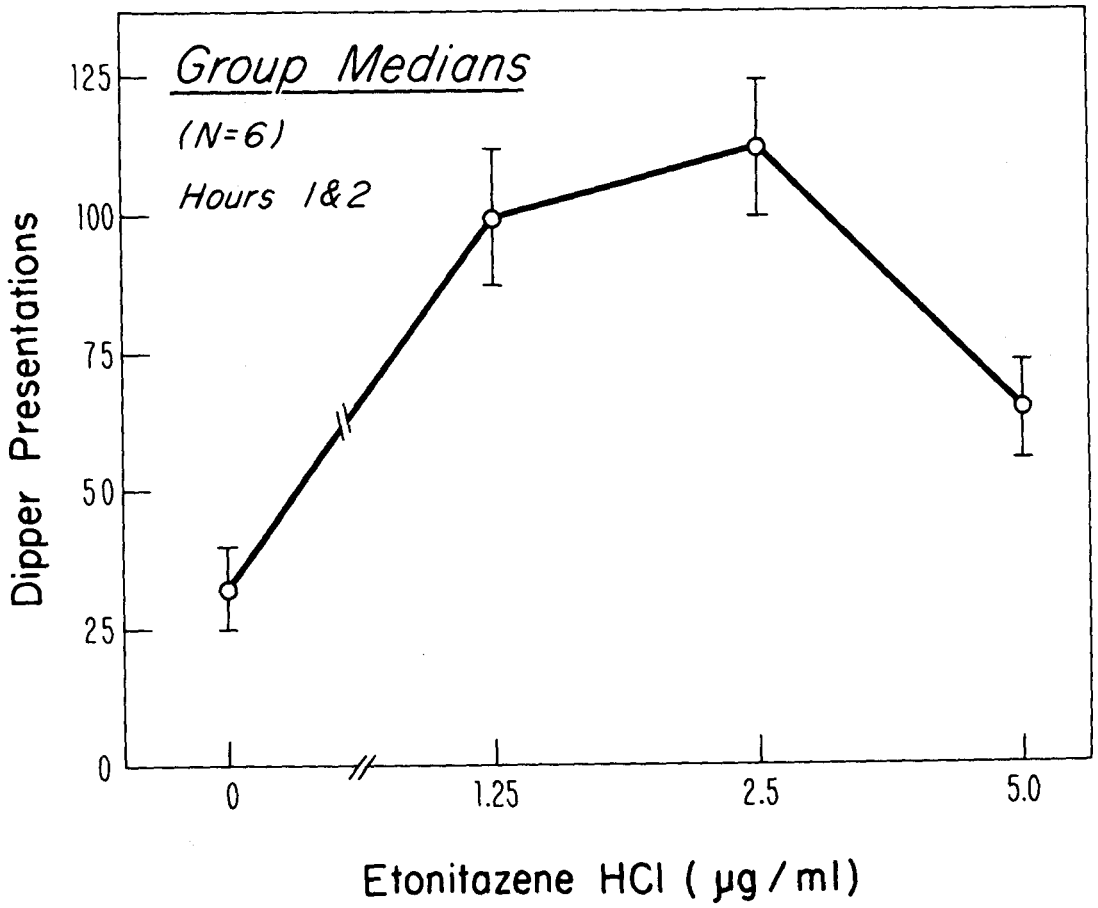

Figure 2. Median number of dipper presentations during the first 2 hr as a function of etonitazene concentration. Each point is a median of six means (six rats X one mean each; each mean is for the last five sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on six standard errors (six rats X one S.E. each). Note that during the first 2 hr the rats never received concurrent food.

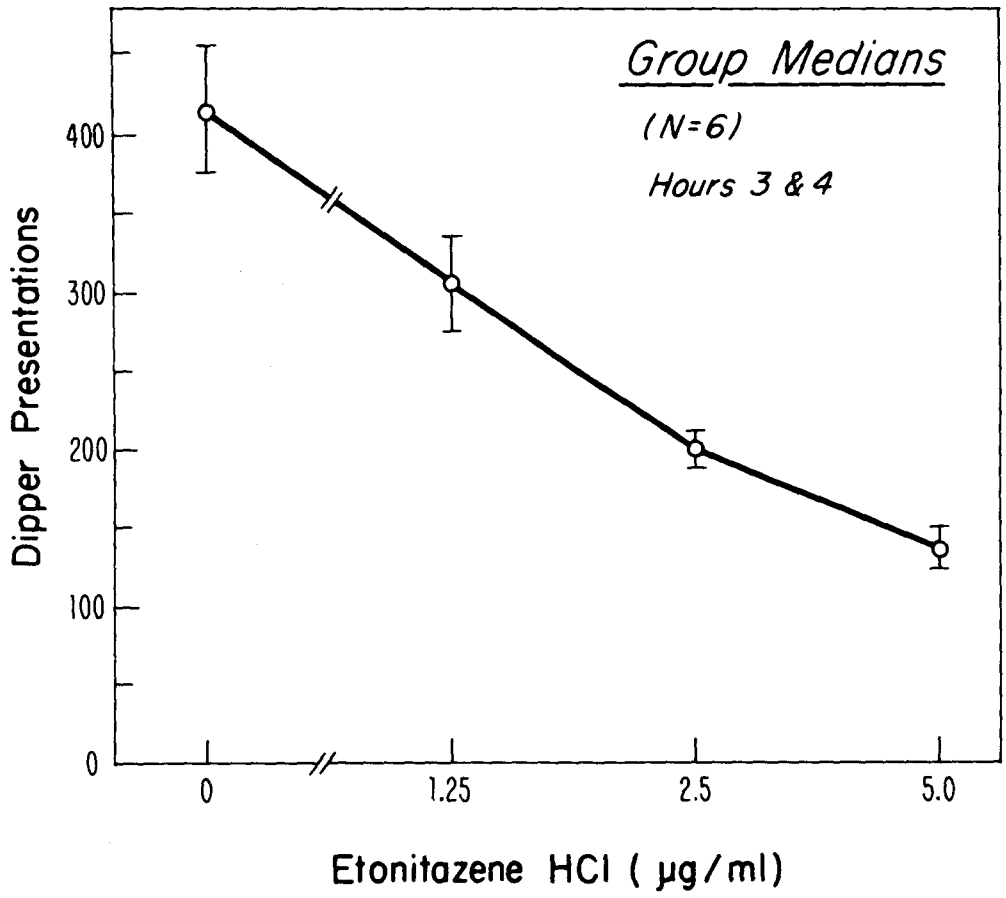

Figure 3. Median number of dipper presentations during the last 2 hr of 4-hr sessions. Values are plotted as a function of etonitazene concentration. Each point is a median of six means (six rats X one mean each; each mean is for the last five sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on six standard errors (six rats X one S.E. each). Note that during the last 2 hr (i.e., hours 3 & 4) the rats concurrently received food and schedule-induced drinking occurred.

Table 2

Dipper presentations (means of five sessions ± S.E.)
during hours 1 and 2 as a function of etonitazene concentration

	Etonitazene (μg/ml)			
Rats	0 (water)	1.25	2.5	5.0
N1	5.1 (2.4)	42.6 (8.7)	57.8 (2.8)	55.4 (1.7)
N2	479.2 (47.3)	648.8 (46.5)	531.8 (31.7)	354.4 (115.2)
N3	1.9 (0.7)	33.6 (3.5)	66.0 (5.4)	58.8 (7.1)
N4	32.4 (10.2)	157.2 (15.6)	787.2 (128.1)	1312.2 (175.9)
N5	33.3 (4.9)	28.0 (3.9)	34.4 (3.7)	34.4 (1.7)
N6	71.8 (28.4)	160.4 (48.9)	159.2 (19.3)	71.8 (10.0)
Median	32.9 (7.6)	99.9 (12.2)	112.6 (12.4)	65.3 (8.6)

also found decreases in dipper presentations with increases in drug concentrations (Meisch, 1969; Meisch & Thompson, 1972, 1974). Data for individual rats are presented in Table 3.

During the last 2 hr of each session food was concurrently available on a *chain* DRO 40 sec FR 1 schedule, and schedule-induced polydipsia occurred. For example, at 0% (water) the median number of dipper presentations was 415, whereas during the first 2 hr the median number was 33 (cf. Figures 2 and 3).

Volume consumed and quantity (µg/kg/hr) of etonitazene intake

The volume consumed during the 4-hr sessions increased over water values when 1.25 µg/ml etonitazene was present (Figure 4). Further increases in the etonitazene concentration produced successive decreases in the volume consumed. (Figure 4). However, the quality consumed (µg/kg/hr) increased as the concentration increased since the volume decreases were to levels above one-half that consumed at the adjacent lower concentration (Figure 4). Tables 4 and 5 present the volumes and quantities consumed, respectively, for individual rats. In other studies of schedule-induced drug drinking, increases in the concentration of morphine, methadone, or ethanol resulted in both systematic decreases in the volume consumed and increases in amount of drug intake (Leander, McMillan & Harris, 1975; Meisch & Thompson, 1972, 1974).

Food-reinforced behavior

Figure 5 shows that as the etonitazene concentration was increased to 2.5 µg/ml, the number of food responses decreased while the cumulative duration in the FR 1 food component increased. A further increase in concentration to 5.0 µg/ml resulted in an increase in the number of responses and a decrease in the cumulative duration spent in the FR 1 component; that is, when the concen-

Table 3

Dipper presentations (means of five sessions ± S.E.)
during hours 3 and 4 as a function of etonitazene concentration

	Etonitazene (μg/ml)			
Rats	0 (water)	1.25	2.5	5.0
N1	160.2 (10.7)	168.6 (18.5)	87.6 (7.4)	55.6 (1.9)
N2	1439.8 (84.4)	807.2 (93.1)	334.0 (48.8)	375.8 (190.2)
N3	588.8 (37.0)	333.8 (52.0)	136.6 (11.5)	80.6 (4.7)
N4	356.6 (115.7)	399.0 (27.7)	546.4 (100.1)	370.6 (78.0)
N5	473.9 (45.6)	280.2 (27.6)	159.4 (9.6)	118.8 (9.9)
N6	384.4 (24.0)	276.6 (28.6)	240.8 (23.7)	157.6 (18.5)
Median	415.3 (41.3)	307.0 (28.1)	200.1 (17.6)	138.2 (14.2)

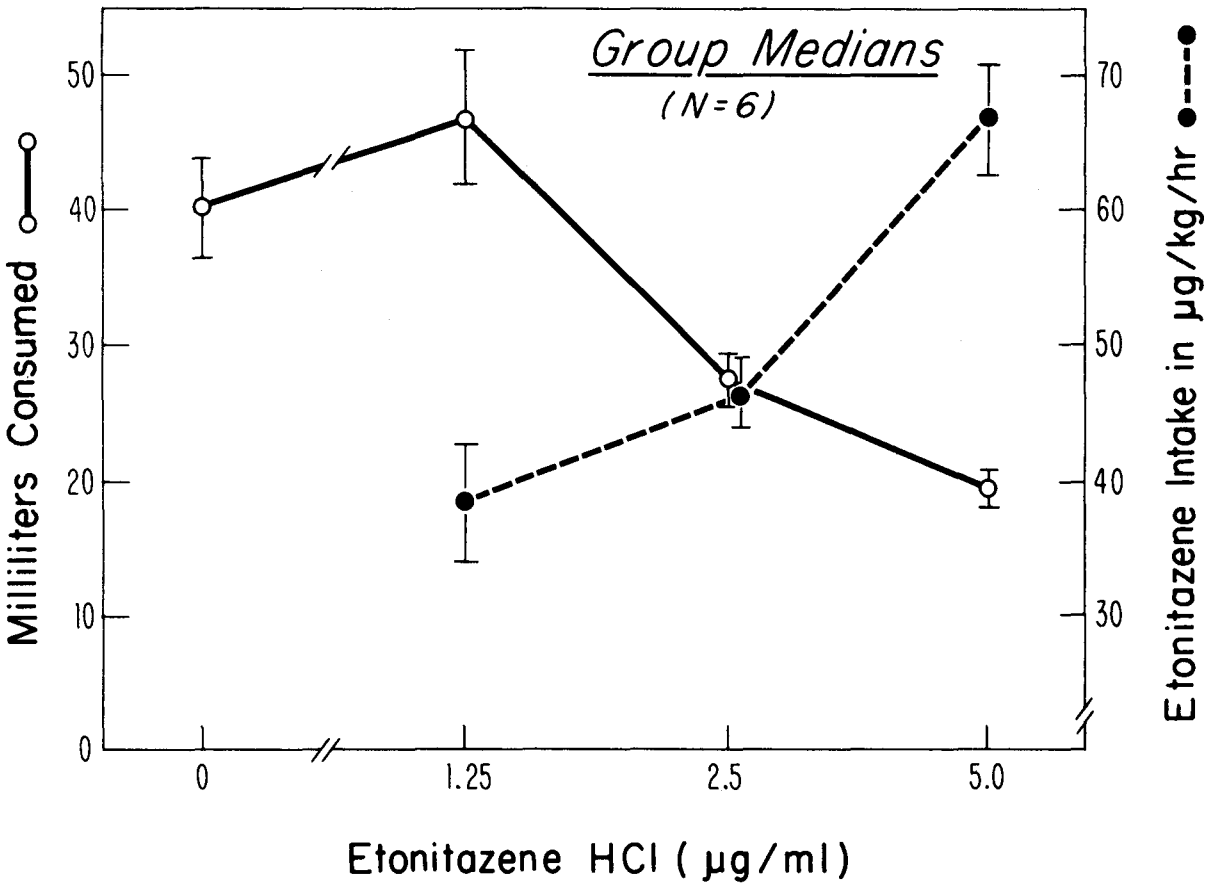

Figure 4. Median ml and quantity (µg/kg/hr) consumed as a function of etonitazene concentration. Values are for 4-hr sessions. Each point is a median of six means (six rats X one mean each; each mean is for the last five sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on six standard errors (six rats X one S.E. each).

Table 4

Volume consumed in milliliters (means of five sessions ± S.E.) during 4-hr sessions as a function of etonitazene concentration

	Etonitazene (μg/ml)			
Rats	0 (water)	1.25	2.5	5.0
N1	16.9 (1.1)	23.5 (2.7)	18.2 (1.0)	14.1 (0.4)
N2	83.5 (3.7)	66.5 (5.6)	36.5 (1.7)	23.3 (1.6)
N3	53.2 (3.5)	38.4 (6.2)	18.2 (1.4)	15.8 (0.7)
N4	39.3 (14.5)	60.5 (2.5)	62.1 (10.1)	34.5 (3.8)
N5	41.0 (3.8)	30.1 (6.2)	16.4 (0.8)	15.9 (0.9)
N6	39.6 (5.8)	55.7 (4.4)	46.5 (1.7)	29.0 (5.8)
Median	40.3 (3.8)	47.1 (5.0)	27.4 (1.6)	19.6 (1.3)

Table 5

Rate of etonitazene intake in μg per kg of body weight per hr (means of five sessions ± S.E.) during 4-hr sessions as a function of etonitazene concentration

	Etonitazene (μg/ml)		
Rats	1.25	2.5	5.0
N1	20.11 (2.28)	31.56 (1.63)	49.29 (1.40)
N2	55.51 (4.71)	61.62 (2.94)	78.67 (5.23)
N3	30.47 (4.90)	29.02 (2.22)	51.25 (2.17)
N4	54.02 (2.26)	114.83 (18.75)	133.93 (14.60)
N5	25.44 (5.25)	27.58 (1.36)	54.60 (3.16)
N6	46.48 (3.70)	78.54 (2.85)	97.00 (19.41)
Median	38.57 (4.20)	46.59 (2.54)	67.00 (4.20)

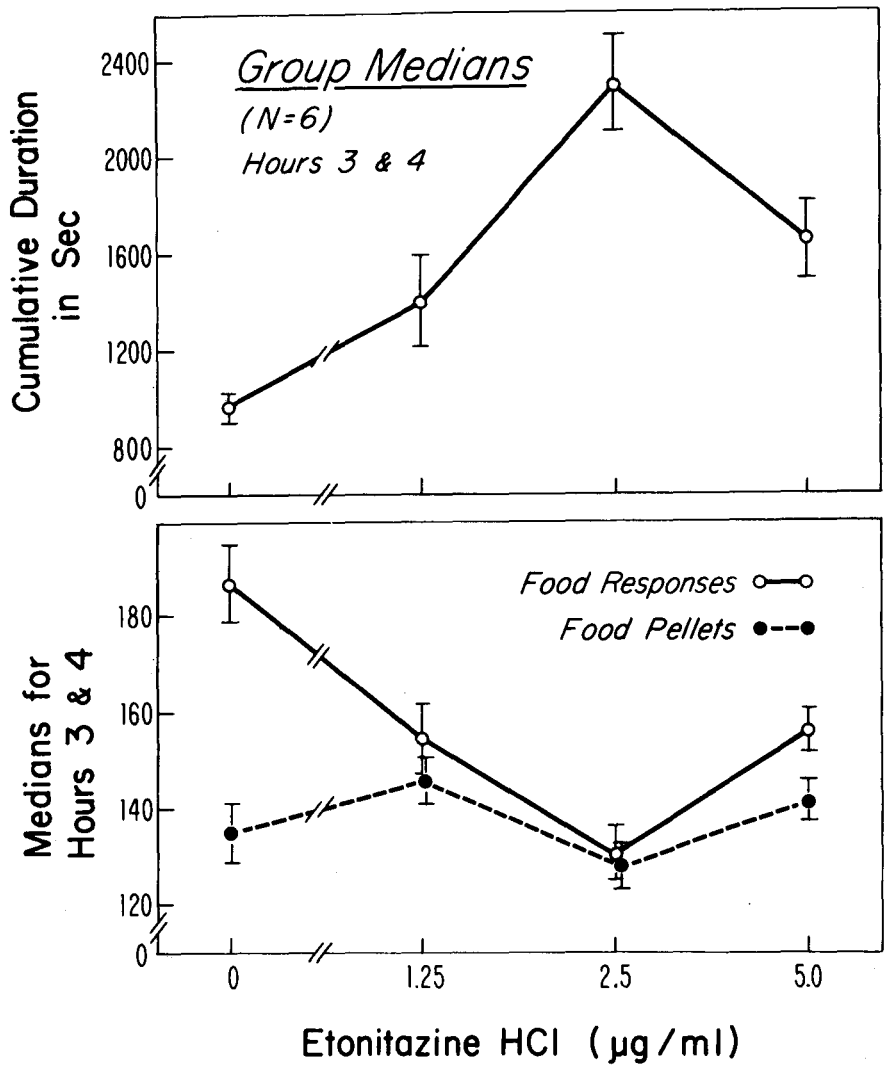

Figure 5. Measures of food-reinforced behavior at each etonitazene concentration. Values are for hours 3 and 4 of the 4-hr sessions. The upper frame shows median cumulative durations (sec) spent in the FR 1 component of the *chain* DRO 40" FR 1 schedule of food reinforcement. The lower frame shows median presses on the lever that produced food and the median number of food pellets obtained. Each point is a median of six means (six rats X one mean each; each mean is for the last five sessions at a particular concentration). Brackets indicate the median standard error of the mean; each median is based on six standard errors (six rats X one S.E. each).

tration was increased to 5.0 µg/ml, food performance shifted back toward that obtained when water was present (Figure 5). Number of food pellets obtained per session did not vary in a systematic manner with etonitazene concentration. Food responses, food pellets obtained, and cumulative durations are presented for individual rats in Tables 6, 7, and 8, respectively.

The changes in food-reinforced behavior are important, in that they indicate the rats were drinking sufficient etonitazene to alter concurrent behavior. Studies of schedule-induced pentobarbital and ethanol drinking have also found concentration related changes in food-reinforced performance (Meisch, 1969; Meisch & Thompson, 1972).

Experiment 2. Termination of schedule-induced polydipsia: Effects on etonitazene drinking.

In some studies of schedule-induced ethanol drinking, the intermittent access to food pellets within sessions was eliminated (Freed et al., 1970; Meisch et al., 1975; Meisch & Thompson, 1971, 1974). Under these conditions ethanol drinking persisted at levels substantially above those that occurred when water was present. Thus ethanol drinking could not be accounted for by the liquid property of the ethanol solutions. These results indicate that ethanol had been established as a positive reinforcer. The purpose of the present experiment was to compare intake of 5 µg/ml etonitazene in the presence and absence of schedule-induced drinking, i.e., in the presence and absence of the concurrent food schedule.

Method

Subjects

Same as in Experiment 1.

Table 6

Food lever presses (means of five sessions ± S.E.)
during hours 3 and 4 as a function of etonitazene concentration

	Etonitazene (µg/ml)			
Rats	0 (water)	1.25	2.5	5.0
N1	232.9 (9.6)	153.4 (4.1)	130.4 (3.4)	117.0 (1.7)
N2	177.6 (5.2)	151.0 (9.0)	171.2 (6.3)	163.6 (11.9)
N3	197.0 (3.2)	143.4 (8.3)	122.6 (2.5)	140.2 (2.2)
N4	130.0 (12.9)	157.8 (3.6)	83.2 (5.1)	168.2 (5.9)
N5	212.8 (6.3)	155.6 (6.5)	131.2 (15.4)	162.8 (45.3)
N6	166.0 (12.2)	172.0 (9.6)	161.4 (7.3)	149.6 (3.0)
Median	187.3 (8.1)	154.5 (7.4)	130.8 (5.7)	156.2 (4.5)

Table 7

Food pellets obtained (means of five sessions ± S.E.) during hours 3 and 4 as a function of etonitazene concentration

	Etonitazene (µg/ml)			
Rats	0 (water)	1.25	2.5	5.0
N1	90.4 (6.7)	147.4 (5.4)	129.6 (3.4)	112.8 (2.6)
N2	159.4 (5.4)	133.4 (7.7)	153.6 (5.5)	144.4 (5.3)
N3	118.1 (4.2)	116.8 (6.6)	111.0 (2.5)	139.0 (2.1)
N4	126.2 (13.1)	156.2 (3.2)	75.0 (6.0)	156.6 (5.3)
N5	186.0 (5.4)	143.8 (4.3)	126.4 (15.7)	106.4 (8.5)
N6	143.8 (9.0)	159.2 (3.6)	147.8 (4.1)	145.2 (3.3)
Median	135.0 (6.1)	145.6 (4.9)	128.0 (4.8)	141.7 (4.3)

Table 8

Cumulative duration (means of five sessions ± S.E.) in seconds of the FR 1 component of the *chain* DRO FR 1 schedule of food reinforcement

	Etonitazene (μg/ml)			
Rats	0 (water)	1.25	2.5	5.0
N1	200.6 (61.8)	1232.6 (188.6)	2061.6 (133.1)	2699.2 (75.2)
N2	1204.2 (54.8)	2181.7 (413.0)	1747.0 (206.6)	1592.2 (297.3)
N3	189.5 (41.0)	1819.5 (360.3)	2547.2 (98.1)	1726.2 (86.6)
N4	2137.4 (521.6)	1011.0 (133.7)	4159.0 (220.1)	869.2 (200.5)
N5		1565.6 (172.9)	4030.2 (501.1)	3060.2 (523.8)
N6	969.2 (223.6)	797.8 (193.2)	1248.6 (187.5)	1419.4 (121.3)
Median	969.2 (61.8)	1399.1 (190.9)	2304.4 (197.1)	1659.2 (160.9)

Apparatus

Same as in Experiment 1.

Procedure

After five stable sessions of schedule-induced drinking of 5 µg/ml etonitazene, the concurrent schedule of food reinforcerment was discontinued and presses on the left (food) lever had no scheduled consequences. The lever lights were no longer used, and illumination was provided by the house light. Thus, the stimulus conditions during the last 2 hr were the same as during the first 2 hr. Other aspects of the procedure such as the session length were the same as in Experiment 1. Values of etonitazene intake in the absence of concurrent food are for five stable sessions.

Results and Discussion

Table 9 shows that for each of the six rats the volume consumed decreases when concurrent food was no longer available. The median value for the group changed from 19.6 to 13.9 ml (Table 9). For four of the rats, number of dipper presentations also decreased (Table 9). Although drinking did decrease, substantial intake of the 5 µg/ml solution persisted. To further evaluate the reinforceing efficacy of the drug solution, presentation of the dipper was made contingent upon more than one lever press. This experiment is described below.

Experiment 3. Etonitazene-reinforced performance as a function of fixed-ratio size.

If a drug is functioning as a positive reinforcer, then presentation of the drug should maintain intermittently reinforced responding. In the present experiment access to a 5 µg/ml etonitazene solution was contingent on one, two,

Table 9

Dipper presentations and milliliters consumed of 5 μg/ml etonitazene (means of five sessions ± S.E.) during 4-hr sessions in the presence and absence of concurrent food

	Dipper presentations		Volume Consumed (ml)	
Rats	Concurrent Food	No Concurrent Food	Concurrent Food	No Concurrent Food
N1	111.0 (3.5)	90.2 (5.7)	14.1 (0.4)	10.7 (1.0)
N2	730.2 (305.2)	668.6 (70.3)	23.3 (1.6)	14.9 (0.7)
N3	139.4 (8.4)	129.0 (3.6)	15.8 (0.7)	12.8 (0.7)
N4	1682.8 (231.5)	3340.8 (373.0)	34.5 (3.8)	29.6 (1.0)
N5	153.2 (9.2)	67.2 (3.8)	15.9 (0.9)	6.2 (0.4)
N6	229.4 (27.1)	255.6 (14.1)	29.0 (5.8)	20.2 (2.2)
Median	191.3 (18.2)	192.3 (9.9)	19.6 (1.3)	13.9 (0.9)

or four lever presses per dipper presentation. That is, etonitazene access was scheduled according to fixed-ratio schedules (FR's) of 1, 2, and 4. It was anticipated that the number of lever presses would increase directly with the FR size while the number of dipper presentations would remain constant.

Method

Subjects

Same as in Experiment 2.

Apparatus

Same is in Experiment 2.

Procedure

Fixed-ratio size was systematically increased from FR 1 to FR 2 to FR 4. Changes from one fixed-ratio value to the next were made after the completion of five stable sessions. Other aspects of the procedure were the same as in Experiment 2.

Results and Discussion

Figure 6 shows that the number of lever presses per session increased directly as a function of fixed-ratio size whereas the number of dipper presentations remained constant. Data for individual rats regarding lever presses, dipper presentations, ml consumed and rate of intake are presented in Tables 10, 11, 12, and 13, respectively. Note that the data for FR 1 are the same data as the "no concurrent food" condition in Experiment 2 (cf. Table 9 with Tables 11 & 12).

The results of this experiment are consistent with the notion that the etonitazene solution is functioning as a positive reinforcer. However, intake of the etonitazend solution could be due to its liquid character and not due to

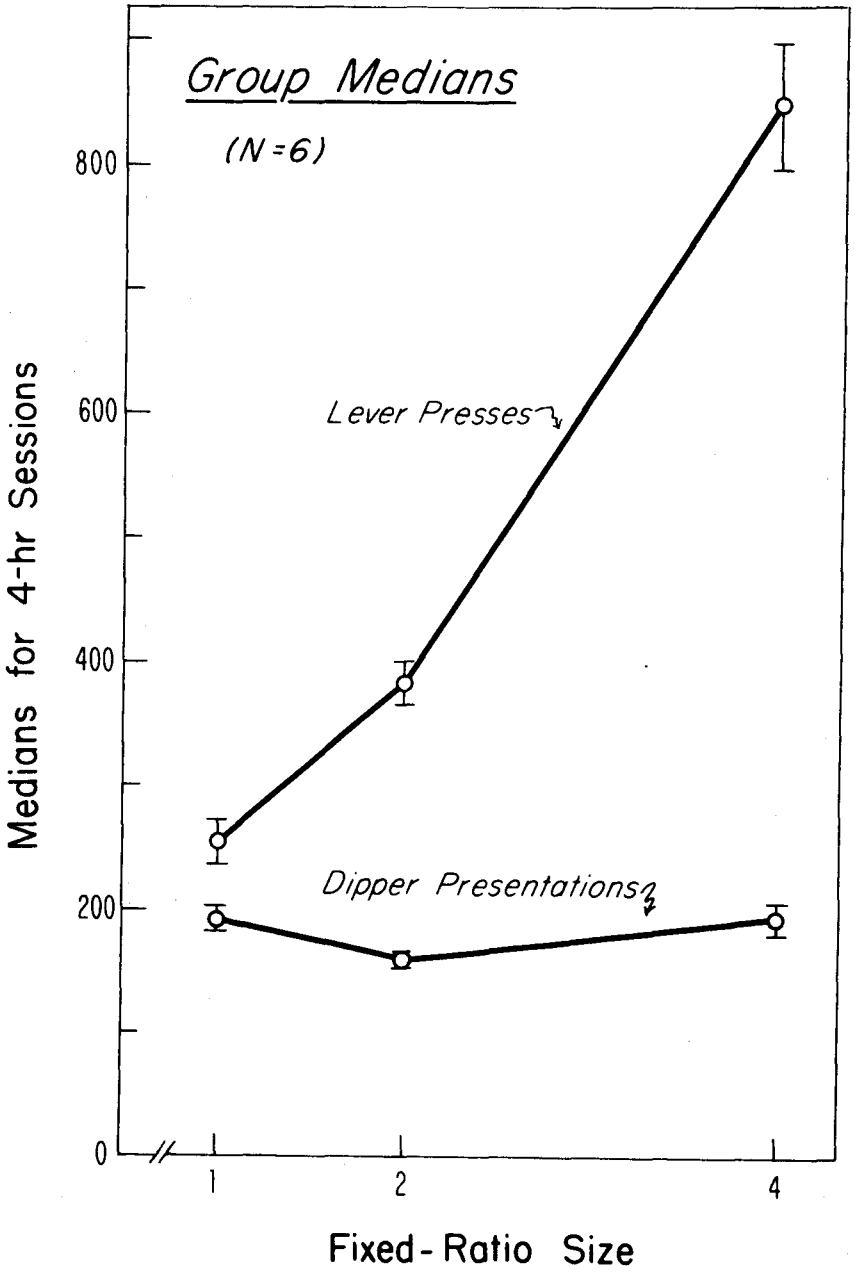

Figure 6. Median number of lever presses and dipper presentations as a function of fixed-ratio size. Each dipper presentation resulted in access to 0.1 ml of etonitazene HCl at a concentration of 5 µg/ml. Each point is a median of six means (six rats x one mean each; each mean is for the last five sessions as a particular concentration). Brackets indicate the median standard error of the mean; each median is based on six standard errors (six rats x one S.E. each).

Table 10

Lever presses (means of five sessions ± S.E.) during 4-hr sessions as a function of fixed-ratio (FR) size. Completion of a ratio produced access to 0.1 ml dipper of 5 µg/ml etonitazene

	Fixed-Ratio Size		
Rats	FR 1	FR 2	FR 4
N1	97.2 (7.1)	159.0 (8.3)	253.6 (24.3)
N2	781.6 (89.3)	830.6 (24.9)	1478.2 (52.7)
N3	171.6 (7.8)	223.2 (9.1)	991.0 (51.3)
N4	6148.6 (546.9)	1137.8 (381.8)	2417.0 (105.1)
N5	88.2 (11.9)	113.6 (7.2)	338.4 (26.8)
N6	337.8 (22.8)	543.0 (52.8)	704.0 (48.3)
Median	254.7 (17.4)	383.1 (17.0)	847.5 (49.8)

Table 11

Dipper presentations (means of five sessions ± S.E.) during 4-hr sessions as a function of fixed-ratio (FR) size. Completion of a ratio produced access to a 0.1 ml dipper containing 5 µg/ml etonitazene

	Fixed-Ratio Size		
Rats	FR 1	FR 2	FR 4
N1	90.2 (5.7)	72.6 (13.6)	56.8 (5.2)
N2	668.6 (70.3)	372.4 (12.1)	345.2 (11.5)
N3	129.0 (3.6)	100.6 (3.0)	221.0 (13.6)
N4	3340.8 (373.0)	500.6 (164.7)	576.2 (23.5)
N5	67.2 (3.8)	57.4 (2.4)	79.4 (6.0)
N6	255.6 (14.1)	240.2 (21.3)	168.6 (10.9)
Median	192.3 (9.9)	170.4 (12.9)	194.8 (11.2)

Table 12

Milliliters consumed of 5 µg/ml etonitazene (means of five sessions ± S.E.) during 4-hr sessions as a function of fixed-ratio size

	Fixed-Ratio Size					
Rats	FR 1		FR 2		FR 4	
N1	10.7	(1.0)	11.0	(0.3)	9.2	(0.3)
N2	14.9	(0.7)	11.8	(0.9)	15.6	(0.3)
N3	12.8	(0.7)	12.5	(0.8)	11.5	(0.4)
N4	29.6	(1.0)	9.0	(1.5)	15.2	(1.4)
N5	6.2	(0.4)	6.1	(1.3)	9.5	(0.8)
N6	20.2	(2.2)	12.8	(0.8)	9.3	(1.5)
Median	13.9	(0.7)	11.4	(0.9)	10.5	(0.6)

Table 13

Rate of etonitazene intake in µg per kg of body weight per hr (means of five sessions ± S.E.) during 4-hr sessions as a function of fixed-ratio (FR) size

	Fixed-Ratio Size					
Rats	FR 1		FR 2		FR 4	
N1	37.2	(3.5)	39.1	(1.0)	32.2	(1.0)
N2	50.3	(2.3)	40.5	(3.0)	53.1	(1.1)
N3	40.4	(2.1)	40.2	(2.5)	36.7	(1.3)
N4	131.1	(4.4)	34.9	(5.7)	53.0	(4.8)
N5	21.4	(1.5)	20.6	(4.3)	32.2	(2.8)
N6	67.7	(7.3)	43.2	(2.7)	30.5	(4.9)
Median	45.8	(2.9)	39.7	(2.9)	34.5	(2.1)

The presence of etonitazene. The next experiment was designed to determine if the drinking of the etonitazene solution was due to the presence of etonitazene.

Experiment 4. Behavior as a function of liquid present: 5 µg/ml etonitazene hydrochloride or water.

In this experiment the drug was initially present in the solution, then absent, and finally reintroduced. These manipulations were done to determine if behavior was being maintained by the liquid or by the presence of the drug.

Method

Subjects

Same as in Experiment 3.

Apparatus

Same as in Experiment 3.

Procedure

Water (the vehicle control) was substituted for etonitazene 5 µg/ml after five stable drug sessions were obtained. Water was present until five stable sessions occurred, and then etonitazene was reintroduced. Again, the data reported are from five stable sessions. Four lever presses resulted in a dipper presentation (FR 4). Other aspects of the procedure were the same as in Experiment 3.

Results and Discussion

Figure 7 shows that when water was substituted for the drug solution, the number of dipper presentations greatly decreased. When the drug solution was again available, the number of dipper presentations returned to the previous drug values. Thus, the 5 µg/ml etonitazene solution was functioning as a

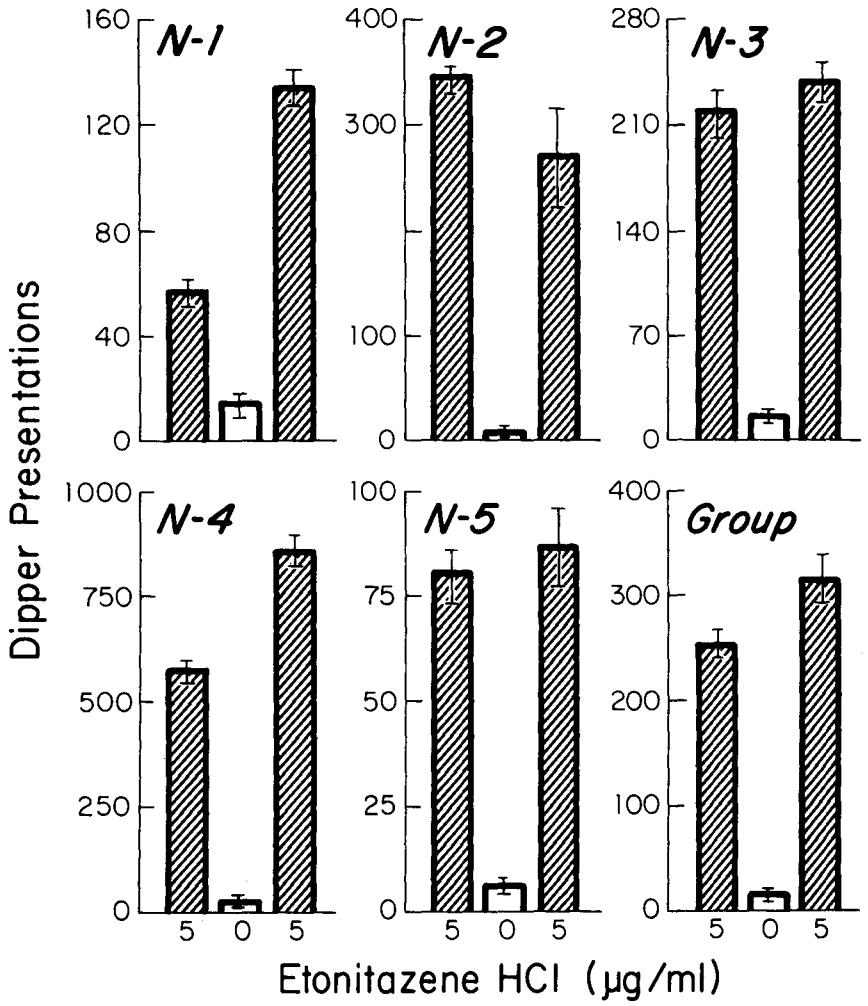

Figure 7. Dipper presentations as a function of liquid present: 5 µg/ml etonitazene or water. Dipper presentations followed every fourth lever press (FR 4). Each bar is a mean of the last five sessions during each phase. Brackets indicate the standard error of the mean. Group bars are means of 25 observations (five rats x five sessions each), and the brackets indicate the mean standard error of the mean (n = 5; five rats x one S.E. each).

positive reinforcer. When water replaced etonitazene the number of dipper presentations gradually declined. Water was present for a median of 11 sessions before etonitazene was reintroduced (see Table 1). The resumption of lever pressing when etonitazene was reintroduced indicates that rats can discriminate the presence of etonitazene without using taste additives or exteroceptive stimuli. With rat N-6 the number of dipper presentations declined when water was introduced. However, when the drug solution was reintroduced, the number of dipper presentations did not increase. No explanation is apparent as to why this rat's behavior differed from the others. For each rat the number of lever presses, dipper presentations, and volume consumed is presented for each liquid condition in Tables 14, 15, and 16, respectively.

Figure 8 shows that when etonitazene was present, the rate of dipper presentations was approximately constant over time. This temporal pattern differs from the negatively accelerated pattern of rats' ethanol intake (cf. Henningfield & Meisch, 1975).

Discussion

This series of experiments demonstrates: (1) that etonitazene can be established as a positive reinforcer for rats following substitution for water during schedule-induced polydipsia; (2) that etonitazene drinking, but not water drinking, persists when schedule-induced polydipsia is terminated; and (3) that access to etonitazene, 5 μg/ml, maintains FR responding; and (4) that responding is maintained by the presence of the drug and not by the liquid properties of the drug solution. Additionally, it was found that (1) during schedule-induced polydipsia food-reinforced performance was altered by the presence of the drug; (2) pretreatment with morphine was not necessary to establish etonitazene as a reinforcer; (3) it was not necessary to add taste

Table 14

Lever-press responses (means of five sessions ± S.E.) as a function of liquid present: Water or etonitazene 5 µg/ml. Liquid presentations were scheduled to occur after every fourth response (FR 4). Values are for 4-hr sessions.

	Etonitazene (µg/ml)					
Rats	5.0		0 (water)		5.0	
N1	253.6	(24.3)	60.4	(21.2)	620.4	(32.9)
N2	1479.2	(52.7)	38.8	(15.9)	1159.8	(195.1)
N3	991.0	(51.3)	78.4	(22.3)	974.0	(87.1)
N4	2417.0	(105.1)	87.6	(45.1)	3775.4	(211.4)
N5	338.4	(26.8)	25.2	(7.2)	375.6	(41.9)
Mean	1095.8	(52.0)	58.1	(22.3)	1381.0	(113.7)

Table 15

Dipper presentations (means of five sessions ± S.E.) as a function of liquid present: Water or etonitazene 5 µg/ml. Dipper presentations were scheduled to occur after every fourth response (FR 4). Values are for 4-hr sessions.

	Etonitazene (µg/ml)					
Rats	5.0		0 (water)		5.0	
N1	56.8	(5.2)	13.4	(4.9)	134.6	(6.6)
N2	345.2	(11.5)	9.0	(3.9)	271.6	(46.3)
N3	221.0	(13.6)	17.8	(4.9)	242.2	(12.3)
N4	576.2	(23.5)	21.0	(11.0)	856.8	(42.7)
N5	79.4	(6.0)	6.0	(1.9)	86.8	(9.4)
Mean	255.7	(12.0)	13.4	(5.3)	318.4	(23.5)

Table 16

Milliliters consumed (means of five sessions ± S.E.) as a function of liquid present: Water or etonitazene 5 µg/ml. Values are for 4-hr sessions.

	Etonitazene (µg/ml)					
Rats	5.0		0 (water)		5.0	
N1	9.2	(0.3)	2.9	(1.0)	11.0	(0.3)
N2	15.6	(0.3)	2.6	(1.2)	13.4	(1.6)
N3	11.5	(0.4)	2.8	(1.0)	12.9	(0.9)
N4	15.2	(1.4)	2.5	(0.4)	21.4	(2.1)
N5	9.5	(0.8)	2.2	(1.6)	7.5	(0.8)
Mean	12.2	(0.6)	2.6	(1.0)	13.2	(1.1)

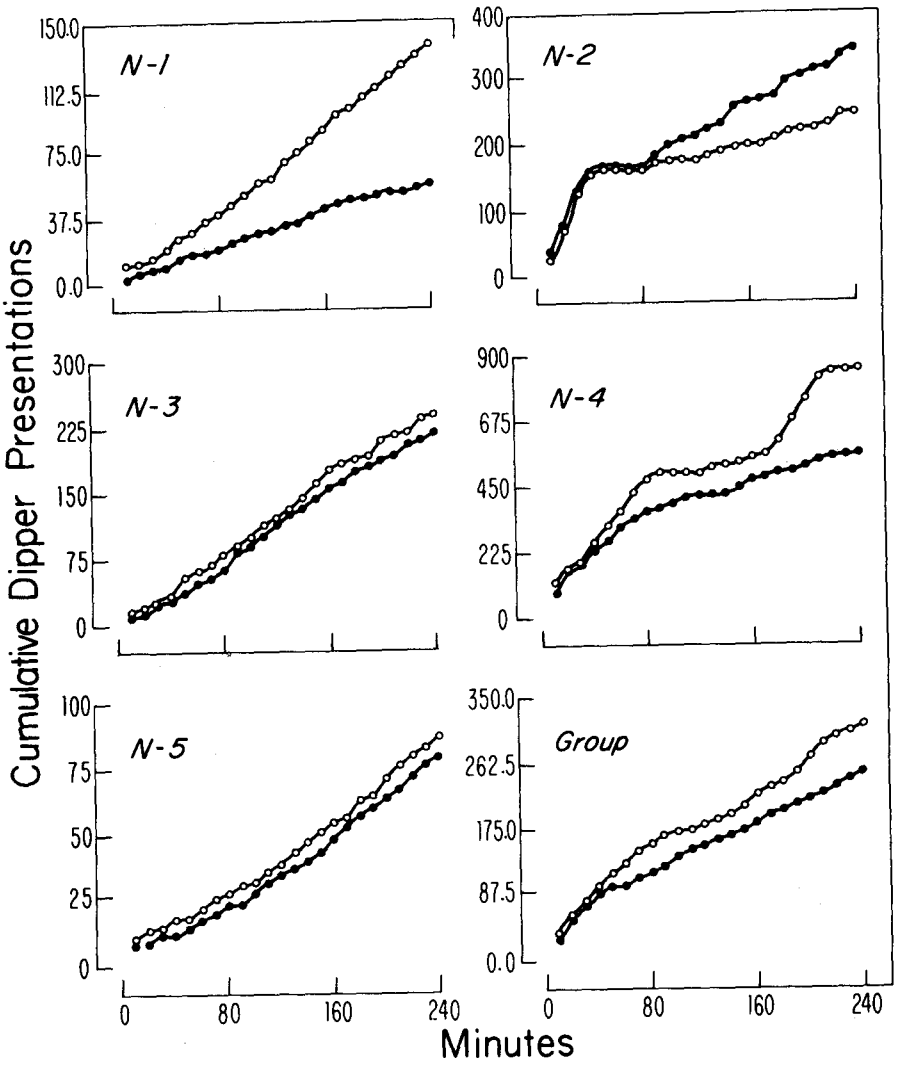

Figure 8. Dipper presentations of 5 µg/ml etonitazene cumulated at 10-min intervals over 4-hr sessions. These temporal patterns of dipper presentations are for the drug sessions whose means are illustrated in Figure 6. Each point is a mean based on observations from five sessions. Filled circles are for etonitazene sessions preceding the series of water sessions, whereas unfilled circles represent etonitazene sessions following the series of water sessions. Group values are means of 25 observations (five rats x values from five sessions each.

cues such as quinine, sodium chloride or anise to the etonitazene solution; (4) water deprivation was not necessary to initiate etonitazene drinking; (5) the time pattern of intake was approximately constant over the 4-hr session; and (6) among rats there were large differences in drug intake. Some of these results extend or confirm the findings of others. However, an interlocking series of experiments that used subjects as their own controls has not been previously reported.

Leander and McMillan (1975; McMillan & Leander, 1976) used schedule-induced polydipsia to obtain drinking of 5 µg/ml etonitazene. They observed changes in behavior such as the collapse of rats on the cage floor. However, they did not report any changes in food-reinforced behavior. Recording changes in food--reinforced performance provides a quantitative objective measure over time of drug-induced alterations in behavior (cf. Meisch, 1969). Other investigators have observed increased muscle tone (Leander & McMillan, 1975), hyperactivity (Lewis et al., 1963), gnawing on the floor and other objects (Lewis et al., 1975: Wikler., 1963), autophagia (Chernov et al., 1972), and loss of hair from the ventral surface of the body (Chernov et al., 1968; Chernov et al., 1972). We also noted form time to time these behaviors occurring during the 4 hr sessions, but our observations of these behaviors were not made systematically. These behaviors were not seen in the home cages immediately prior to sessions, and these observations suggest that these behaviors were not part of an abstinence syndrome.

Leander and McMillan (1975; McMillan & Leander, 1976) concluded that during schedule-induced polydipsia the 5 µg/ml etonitazene solution came to function as a reinforcer since the rats preferred this drug in saline to tap water when both liquids were available within the session. During the initial 2 hr pre-

ceding the subsequent 2 hr of schedule-induced drinking (Experiment 1), we observed more dipper presentations with etonitazene than with water, thus, our data support the notion that etonitazene came to function as a reinforcer within the polydipsia phase, and more generally, these studies confirm the findings of Wikler and co-workers (1963) that etonitazene can serve as a reinforcer for rats.

The maintenance of intermittently reinforcer lever pressing is also consistent with etonitazene serving as a reinforcer. Such meintenance of lever pressing was observed in the present series of experiments and has previously been observed by others (Leander & McMillan, 1975; Lewis et al., 1975). However- in previous studies the control procedure of substitution vehicle (water) for drug solution was not carried out.

In some studies etonitazene solutions were made more discriminable by adding quinine and/or anise (Leander & McMillan, 1975; Lewis et al., 1975). Also, to facilitate etonitazene drinking, the rats were initially water deprived (Leander & McMillan, 1975; Lewis et al., 1975) and/or made physiologically dependent upon morphine (Lewis et al., 1975; Trafton & Marques, 1971; Wikler et al., 1963). In the present series of experiments none of these procedures was necessary to establish etonitazene as a reinforcer.

The effects of a self-administered drug depend in part on the temporal pattern of intake. The temporal pattern observed in the present experiments was a constant rate, and this pattern differs from the negatively accelerated time course seen with ethanol (cf. Meisch, 1975). To our knowledge, the temporal pattern of etonitazene drinking has not been previously reported for experiments wherein drug access was limited to a portion (i.e., several hours) of each day.

Other investigators have noted large intrasubject variability in in etonitazene drinking (Leander & McMillan, 1975), and still other investigators have reported large intersubject variability (Chernov et al., 1968; Chernov et al., 1972; Lewis et al., 1975). We also found large differences between rats in etonitazene intake, and sometimes we saw the same rats show marked variability from session to session. The reasons for such variability are not known.

Many questions concerning etonitazene as a reinforcer remain to be answered. These questions concern in part what variables control etonitazene drinking and how these variables interact. Particular questions of interest are under what conditions is etonitazene most rapidly established as a reinforcer? How does intake change as a function of past history of drug self-administration, and will experience with etonitazene facilitate the establishment of other narcotics as reinforcers? These questions are currently under study in our laboratory.

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