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Oral Self-Administration of Etonitazene in Rhesus Monkeys: Use of a Fading Procedure to Establish Etonitazene as a Reinforcer

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MEISCH, R. A. *Oral self-administration of etonitazene in rhesus monkeys: Use of a fading procedure to establish etonitazene as a reinforcer.* PHARMACOL BIOCHEM BEHAV **50**(4) 571-580, 1995.—The establishment of orally delivered etonitazene (a potent opioid) as a reinforcer, was studied in eight rhesus monkeys. Initially, when given concurrent access to 2.5 μg/ml etonitazene and the water vehicle, five of the monkeys rejected the drug, whereas the other three monkeys consumed more drug solution than water. The five monkeys that rejected the drug solution underwent an acquisition phase to establish the drug as a reinforcer. A fading procedure was used to transfer control of responding from a 2% (wt/vol) ethanol solution to a 2.5 μg/ml etonitazene solution. Initially, responding was maintained by contingent deliveries of 2% ethanol. Next, across blocks of six or more sessions, increasing amounts of etonitazene were added in steps to the 2% ethanol solution. Subsequently, the 2% ethanol solution was decreased in steps to zero, leaving only the 2.5 μg/ml etonitazene present. When the fading procedure was completed, dose of etonitazene was varied by increasing the volume delivered, first under fixed ratio (FR 4) and then under an FR 8 reinforcement schedule. The same dose manipulations were made with the three monkeys who did not undergo the fading procedure because they preferred etonitazene over water when first tested. Etonitazene was established as a reinforcer for six of the eight monkeys because drug deliveries exceeded vehicle deliveries across a range of drug doses.

Drug self-administration Oral route Etonitazene Opioids Choice Stimulus fading
Drug reinforcement Acquisition Rhesus monkeys

THE ACQUISITION or development of drug reinforced behavior is of interest for multiple reasons. For example, an understanding of the processes that occur as behavior comes under the control of drug delivery may lead to a rational approach to preventing the development of drug abuse. In the last 5 years several research groups have studied factors that alter the development of intravenous drug reinforcement in rats [e.g., (2,18,19,21)]. Most of these studies examined the effects of drug pretreatment on the subsequent development of drug reinforced responding [e.g., (9)]. Common characteristics of these studies are the use of rats, group designs, the intravenous route, and psychomotor stimulant drugs. Few acquisition studies have used either nonhuman primates or within-subject designs.

It is difficult to use within-subject designs to analyze the development of drug reinforcement because the acquisition of a behavior is usually an irreversible process; that is, once acquisition has occurred, it is not possible to return to the naive state that preceded acquisition [see (22)]. However, innovative experimental designs make within-subject analysis more feasible. One such design is the procedure for studying the repeated acquisition of behavioral chains (1). Another development is the use of alternating blocks of training and testing sessions during acquisition to assess the control of behavior exerted by the new drug at each step of the acquisition procedure (12). This alternation procedure was used in a study of the development of orally delivered cocaine as a reinforcer for rhesus monkeys (12). Another development has been the use of fading procedures to establish new drugs as reinforcers (6,10,13,14,20). For example, orally delivered cocaine has been established as a reinforcer for rhesus monkeys by placing increasing cocaine concentrations in an 8% ethanol solution, and in the next phase gradually decreasing the ethanol concentration to zero. Prior to the introduction of cocaine, 8% ethanol was established as a reinforcer (13). In a previous report, these two procedures of fading and of alternating training and testing sessions were combined to study the development of orally delivered cocaine as a reinforcer (12). In the training sessions, the two liquids were water and the combination cocaine-ethanol solution. In the test sessions, the two liquids

were the ethanol solution and the combination cocaine-ethanol solution. The only difference between the two solutions was the presence of cocaine in one of them and, thus, any differences in responding could be attributed to the cocaine.

The same strategy of combining two procedures, namely fading and interpolating test sessions between training steps, was used in the present study. The objective was to examine the development of the reinforcing effects of etonitazene at each step of the acquisition procedure. The value of only one variable at a time was changed. At each step, the experimental design permitted assessment of the degree to which etonitazene controlled behavior. One advantage of using the oral route is that the acquisition of drug-reinforced responding is less rapid than with the intravenous route. Therefore, the transition that results in behavior coming to be controlled by delivery of the novel drug (i.e., by the novel drug's reinforcing effects) may be easier to study with the oral route.

METHOD

Subjects

Subjects were eight adult male rhesus monkeys (*Macaca mulatta*). All eight had participated in studies concerning the establishment of 2% ethanol (11) and then orally delivered cocaine (12) as reinforcers. All monkeys were maintained at reduced body weights to increase the probability of establishing drug-reinforced behavior [for a review of the effects of food deprivation on drug self-administration see (4)]. Monkeys AL, CM, ED, EG, JS, LZ, NL, QL, and RK were maintained at 6.9, 8.0, 9.4, 8.4, 8.2, 8.7, 8.5, and 8.1 kg, respectively, which were 77, 90, 85, 65, 82, 95, 81, and 81% of their free-feeding weights. The monkey (EG) that was maintained at the lowest percent of free-feeding weight had the highest free-feeding weight. Individual weights varied across a narrow range. It is important to note that free-feeding weights may be substantially higher than normal weights, because monkeys can become obese when housed one to a cage with unlimited access to food [see (16)]. Thus, free-feeding weights obtained under these laboratory conditions are not necessarily representative of normal weights obtained under more natural conditions. The monkeys' maintenance weights in the present experiments do not reflect a marked degree of food deprivation, and the monkeys' health and appearance were good. Animal care was in accordance with the regulations of the Committee on Care and Use of Laboratory Animal Resources, National Research Council (5).

Apparatus

Each subject was individually housed 24 h a day in a stainless steel primate cage (Lab Products) that also served as the experimental chamber. Each cage had three solid walls and one barred wall. Cage dimensions (76 × 102 × 81 cm) provided adequate housing space for the rhesus monkeys (5). A liquid-delivery apparatus panel was attached to the outside of one side wall, and spouts and stimulus lights protruded into the cage through holes cut in that wall. A T-shaped bar was attached to the back of the apparatus panel; a stainless steel reservoir covered with a lid was fastened on each limb of this bar. Liquids contained in each reservoir passed through polyethylene tubing to a solenoid-operated valve at the rear of one of the two brass spouts. These spouts (1.2 cm o.d., 0.2 cm i.d.) protruded 2 cm into the cage, 64 cm above the floor, and 15.5 cm either side of the midline. The spouts served as manipulanda for operant responses (mouth contacts with either spout), which were reinforced according to contingencies programmed for the liquid-delivery reinforcement schedules. Mouth contacts on the spout completed a drinkometer circuit and resulted in the illumination of a pair of spout lights for the duration of the contact (see below). The electronic components for the drinkometer circuit were housed in an enclosure at the rear of the spout. With each liquid delivery, a solenoid-operated valve at the rear of a spout was activated for approximately 150 ms, allowing approximately 0.67 ml of liquid to pass through the spout and into the monkey's mouth. To minimize spillage, solenoid activation terminated short of 150 ms if mouth contact with the spout was broken before this interval had elapsed. The liquid-delivery apparatus has been described extensively elsewhere (7,8).

Spouts were embedded in Plexiglas disks that covered the 7 cm diameter holes in the cage wall through which the spouts entered. At each spout, two 1.1 W lights, one located 2.5 cm on either side of the spout and visible through the Plexiglas, were aligned diagonally; these spout lights were capped with green translucent lenses. Another two 1.1 W spout lights, one located 2.5 cm on either side of the spout, were aligned on the opposite diagonal, and were capped with white translucent lenses. Thus, each spout was in the center of a square pattern of four spout lights, two green and two white. The spout lights provided a stimulus change with each response. A green jewel-capped stimulus light (2.8 W), extending 2 cm into the cage, was located 12 cm directly above each spout. These stimulus lights served as discriminative stimuli for liquid-delivery reinforcement schedules, as described in the Procedure section.

A DEC PDP-11 computer and SKED® software were used to program experimental events and to record behavior. This equipment was located in a room near the rooms containing the experimental chambers.

Drugs

Monkeys' daily ethanol solutions were mixed by adding appropriate amounts of tap water to a measured amount of 95% (v/v) ethanol approximately 20 h prior to each session. A concentrated stock solution (10 μg/ml) of etonitazene hydrochloride (National Institute on Drug Abuse, Rockville, MD) was prepared twice a week and stored at 3°C. Monkeys' daily etonitazene solutions were mixed by adding appropriate amounts of tap water to a measured amount of stock solution approximately 2 h prior to each session. Etonitazene concentrations are expressed in terms of the salt. All drug solutions were at room temperature at the start of the sessions.

Procedure

Experimental sessions were 3 h in length (from 1100–1400 h) and were conducted 7 days per week. A time-out period was in effect during the hour immediately before the session (1000–1100 h). During this period, in which the equipment was not operative, the number of water deliveries and the volume of water consumed since the last experimental sessions were recorded, and liquids appropriate for the sessions were placed in the monkeys' reservoirs. Some of each solution was drained through the respective tubing leading from the reservoir to the solenoid-operated spout. This ensured that the appropriate solution was present on the first liquid delivery of the session. Liquids were measured after this flushing procedure, to obtain the exact volume in the reservoirs at each session's outset. For 1 h immediately following the session

(1400–1500 h), another time-out period was in effect. During this period, numbers of liquid deliveries and volumes of liquid consumed were recorded, and water was placed in one of each monkey's reservoirs and flushed through the tubing to the spout. Water was then available under a fixed ratio (FR) 1 schedule from one spout from 1500 until 1600 h. The spout from which water was available between sessions alternated from day to day. A final time-out period was in effect from 1600 until 1700 h, at the beginning of which the monkeys' maintenance feeding (Harlan Teklad® plus half an apple) was placed in the food hopper attached to the cage. Finally, from 1700 h until 1000 h on the next day water was available under an FR 1 schedule from one spout.

When water was available from a spout between sessions, the jewel-capped stimulus light above the spout was illuminated. Each mouth-contact response on that spout resulted in delivery of water and illumination of the white-lensed pair of spout lights for the duration of the mouth contact. Responses on the spout at which liquid was not available were recorded but had no programmed consequences; the jeweled stimulus light over this spout was not illuminated. A 12 L : 12 D cycle was in effect with lights on at 0600 h.

During experimental sessions, the jeweled stimulus lights above each spout blinked at a rate of 10 Hz. Identical discriminative stimuli were used for both spouts to control for differential responding that might otherwise result from the presence of dissimilar exteroceptive visual stimuli. Each mouth contact with a spout illuminated the green-lensed pair of spout lights for the duration of the response.

During experimental sessions, deliveries of liquids (approximately 0.67 ml per delivery) were contingent upon a subject making four mouth contacts with a drinking spout (FR 4 reinforcement schedule). The schedules for each of the spouts operated concurrently and independently, that is, responses on one spout did not alter the number of responses required at the opposite spout, and vice versa. An FR 4 schedule was used rather than an FR 1 schedule because moderate-sized fixed-ratio schedules decrease the effects on drug-maintained behavior of extraneous variables such as those that produce side preferences and nonspecific responding. Moderate size fixed-ratio schedules can also increase differences in response rates maintained by two events that produce unequal reinforcing effects (15,16). The side positions of liquids were alternated from session to session, thereby equalizing effects of any side preferences that might have been present.

Changes from one experimental condition to another were made after obtaining six consecutive sessions with no increasing or decreasing trend in the number of deliveries of either available liquid. This stability criterion was adhered to throughout the study.

Substitution test. A substitution test was conducted to determine whether a 2.5 μg/ml etonitazene concentration would function as a reinforcer in the absence of a training procedure. This concentration is an intermediate one in the range of concentrations that monkeys may come to self-administer (3). Six sessions of stable behavior were obtained with 2% ethanol and water concurrently available. In the next phase, the 2% ethanol was replaced by water so that water was available from both spouts until behavior was stable for six consecutive sessions. In the substitution phase, the 2.5 μg/ml etonitazene solution replaced water in one of the reservoirs so that a drug solution was present in one reservoir and water in the other. If the monkey consistently consumed either similar volumes of the two liquids or less drug solution than water, this phase continued with the next two conditions: water vs. water, and then 2% ethanol vs. water. Table 1 shows the sequence of these conditions. However, if the monkey consistently consumed more drug solution than water during the substitution probe, the next two tests and the subsequent training procedure were not conducted, and the monkey immediately entered the dose-response test, as described below.

Training and testing components of the acquisition procedure. After completion of the substitution test, the monkeys received alternating blocks of training and testing sessions. The general strategy was to fade in etonitazene by adding gradually increasing concentrations of etonitazene to a 2% ethanol solution and then to fade out ethanol from the solution. The sequence of conditions is listed in Table 2.

During training sessions, the drug combination solution was available concurrently with water. During test sessions, the combination ethanol-etonitazene solution was available concurrently with an ethanol solution. The ethanol solution was identical in concentration to the ethanol concentration in the combination solution. Thus, during test sessions, the only difference between the two solutions was that one contained etonitazene and the other did not. Therefore, it was possible during test sessions to evaluate the degree to which etonitazene controlled behavior. Following each block of test sessions, the subject was returned to the preceding training condition for two sessions. No attempt was made to obtain stable behavior at these 2-day transition conditions, and the data are not reported here. The rationale was simply to provide a transition step so that only one change at a time was made in an independent variable. Following these two sessions, the next training condition was instituted.

Testing after completion of the acquisition procedure. After completion of the acquisition procedure, several variables were manipulated to confirm (or not confirm) that behavior was reinforced by etonitazene. At FR 4 ,the dose was changed by varying volume (one, two, or four liquid deliveries per completed fixed ratio) and holding concentration constant at 2.5 μg/ml. Each of these multiple deliveries was contingent upon the monkey making a separate mouth contact with the spout. The sequence of conditions is listed in Table 3. Different volumes were first tested at FR 4 and then at FR 8.

Several monkeys (AL, QL, and CM) showed high levels of water-maintained responding. Such responding might have been due to poor discriminative stimulus control by the taste of the etonitazene solution. Therefore, for these three monkeys (AL, QL, CM) plus two additional monkeys (NL and EG) a sequence of etonitazene volumes was tested under conditions where the discriminative stimulus conditions for the etonitazene solution and the water vehicle differed. Stimulus conditions for etonitazene remained unchanged (the jewel-capped light blinked at a rate of 10 Hz), whereas for water the jewel-capped light above the spout was steadily illuminated, and, for the duration of each response, white rather than green spout lights were illuminated.

TABLE 1

SEQUENCE OF PHASES DURING THE SUBSTITUTION TEST

Phase 1. 2% ethanol	vs. water
Phase 2. Water	vs. water
Phase 3. 2.5 μg/ml etonitazene	vs. water
Phase 4. Water	vs. water (retest)
Phase 5. 2% ethanol	vs. water (retest)

RESULTS

Substitution Test

Figure 1 shows that 2% percent ethanol served as a reinforcer for all eight monkeys because ethanol deliveries substantially exceeded water deliveries. In the next condition, the 2% ethanol solution was replaced by water, which resulted in water being available from both spouts. Under this condition, liquid deliveries were more equally distributed across both spouts, and for some monkeys the total number of liquid deliveries (i.e., sum of liquid deliveries from both spouts) declined. After responding stabilized with water available from both spouts, conditions were changed by making 2.5 μg/ml etonitazene concurrently available with water. Two patterns of behavior resulted: for five monkeys drug deliveries were very low (QL) or less than water vehicle deliveries (NL, RK, ED, EG). In contrast, for three monkeys (JS, AL, and CM), the opposite results were obtained in that the drug deliveries were greater than water deliveries. These latter three monkeys (JS, AL, and CM), therefore, proceeded directly to the experimental manipulations in which etonitazene dose was varied (see below), and did not undergo the acquisition procedure (i.e., the training and testing). The five monkeys for whom the 2.5 μg/ml etonitazene did not initially serve as a reinforcer were retested first under the condition in which water was available from both spouts and second under the condition in which a 2% ethanol solution and water were concurrently available. Figure 1 shows that the results of these retests were similar to the original results.

Training and Testing

Blocks of training sessions alternated with blocks of testing sessions. For clarity, the training and testing results are presented in different figures (Figs. 2 and 3, respectively). Figure 2 shows that deliveries of the solution containing combinations of etonitazene and ethanol tended to decrease across blocks of training sessions. The decrease in the number of deliveries was most consistent during the phase where the etonitazene concentration was progressively increased. A general finding was that the etonitazene-ethanol solution was consumed in greater volumes than the concurrently available water vehicle, with one exception: for monkey EG, as the ethanol concentration decreased, water deliveries increased, and at the

TABLE 2

SEQUENCE OF EXPERIMENTAL CONDITIONS DURING ACQUISITION

Condition	Combination		Concurrent solution	Number of Sessions at Each Condition				
	Ethanol	+Etionitazene		M-NL	M-ED	M-RK	M-QL	M-EG
Testing	0	0	water	8	7	13	6	6
Training	2	0.312	water	7	8	14	7	12
Testing	2	0.312	2% ethanol	7	7	14	15	9
Transition	2	0.312	water	2	2	2	2	2
Training	2	0.625	water	8	10	6	6	7
Testing	2	0.625	2% ethanol	8	11	11	6	10
Transition	2	0.625	water	2	2	2	2	2
Training	2	1.25	water	9	9	7	10	8
Testing	2	1.25	2% ethanol	7	10	6	15	17
Transition	2	1.25	water	2	2	2	2	2
Training	2	2.5	water	8	9	6	7	8
Testing	2	2.5	2% ethanol	9	13	10	8	6
Transition	2	2.5	water	2	2	2	2	2
Training	1	2.5	water	6	7	7	8	9
Testing	1	2.5	1% ethanol	8	11	11	6	10
Transition	1	2.5	water	2	2	2	2	2
Training	0.5	2.5	water	6	8	6	11	7
Testing	0.5	2.5	0.5% ethanol	12	9	10	8	9
Transition	0.5	2.5	water	2	2	2	2	2
Training	0.25	2.5	water	17	9	8	10	6
Testing	0.25	2.5	0.25% ethanol	11	7	9	6	7
Transition	0.25	2.5	water	2	2	2	2	2
Training	0.125	2.5	water	9	8	7	8	10
Testing	0.125	2.5	0.125% ethanol	12	11	7	8	11
Transition	0.125	2.5	water	2	2	2	2	2
Training	0.0625	2.5	water	11	6	8	6	11
Testing	0.0625	2.5	0.0625% ethanol	11	8	9	8	16
Transition	0.0625	2.5	water	2	2	2	2	2
Testing	0	2.5	water	7	10	14	8	10
			Total days in acquisition	199	196	201	185	207

*Minimum of six sessions for training and testing and two sessions for transition.

TABLE 3

SEQUENCE OF TEST CONDITIONS THAT FOLLOWED THE ACQUISITION PHASE AND MEAN ETONITAZENE INTAKE (μg OF DRUG/kg OF BODY WEIGHT/3-H SESSION) AT EACH TEST CONDITION

Conditions			Subjects							
FR Size	Deliveries per FR	Stimulus Condition for E and W	M-NL	M-ED	M-RK	M-QL	M-EG	M-CM	M-JS	M-AL
4	1	Same	63.1	32.7	24.2	15.1	8.2	20.7	–	43.6
4	2	Same	77.4	39.7	24.0	19.1	19.8	16.2	–	28.4
4	4	Same	74.5	47.7	34.7	20.1	19.0	17.2	–	58.5
4	1	Same	62.4	31.4	17.8	15.7	17.6	14.7	–	45.1
8	1	Same	46.7	23.9	16.2	12.7	6.5	8.3	35.2	24.7
8	2	Same	80.0	24.7	23.5	18.2	8.4	6.0	43.6	38.8
8	4	Same	92.5	45.6	28.6	20.2	14.0	15.5	68.7	40.3
8	8	Same	94.6	54.8	22.4	27.5	20.2	21.0	–	50.3
8	1 [8]	Same	47.9	21.1	14.9	17.1	5.1	2.6	41.4	46.8
8	1	Different	57.4	–	13.4	14.1	5.1	2.6	–	12.5
8	2	Different	84.6	–	21.6	19.6	6.9	5.1	–	23.3
8	4	Different	99.9	–	20.9	32.0	8.6	5.3	M	43.2
8	8	Different	115.8	–	32.3	44.7	12.6	8.7	–	36.4
8	1 [8]	Different	57.5	–	16.9	17.9	2.1	2.3	–	36.9

When the FR size was eight, subject M-AL was tested by varying the number of deliveries per completed fixed ratio in decreasing (i.e., eight, four,) rather than increasing (i.e., one, two, four, eight) order. Therefore, during the retest, eight deliveries occurred per FR schedule unit rather than one.

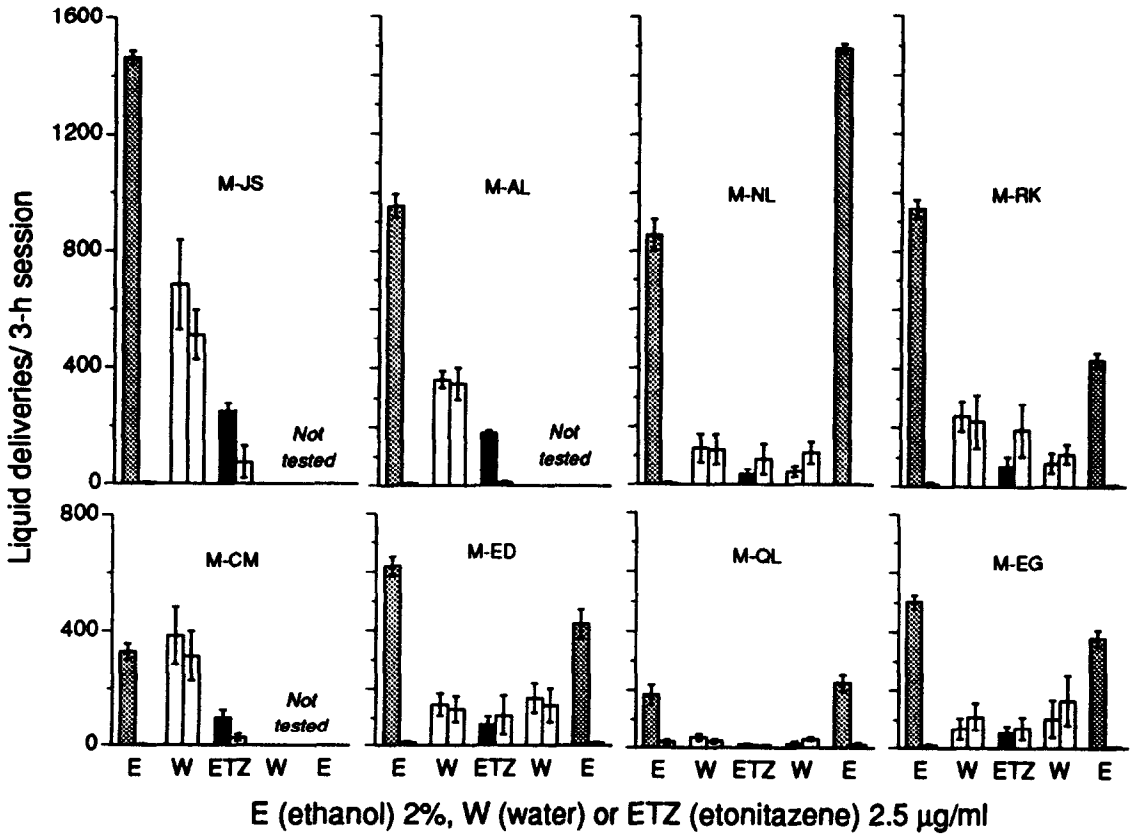

FIG. 1. Etonitazene drinking prior to the acquisition procedure. Mean liquid deliveries (n = 6 sessions) are shown as a function of different liquids. Brackets depict the SEM. Absence of brackets indicates that they fell within the area of the plotted point. Gray bars: 2% ethanol deliveries. White bars: water deliveries. Black bars: 2.5 μg/ml etonitazene deliveries.

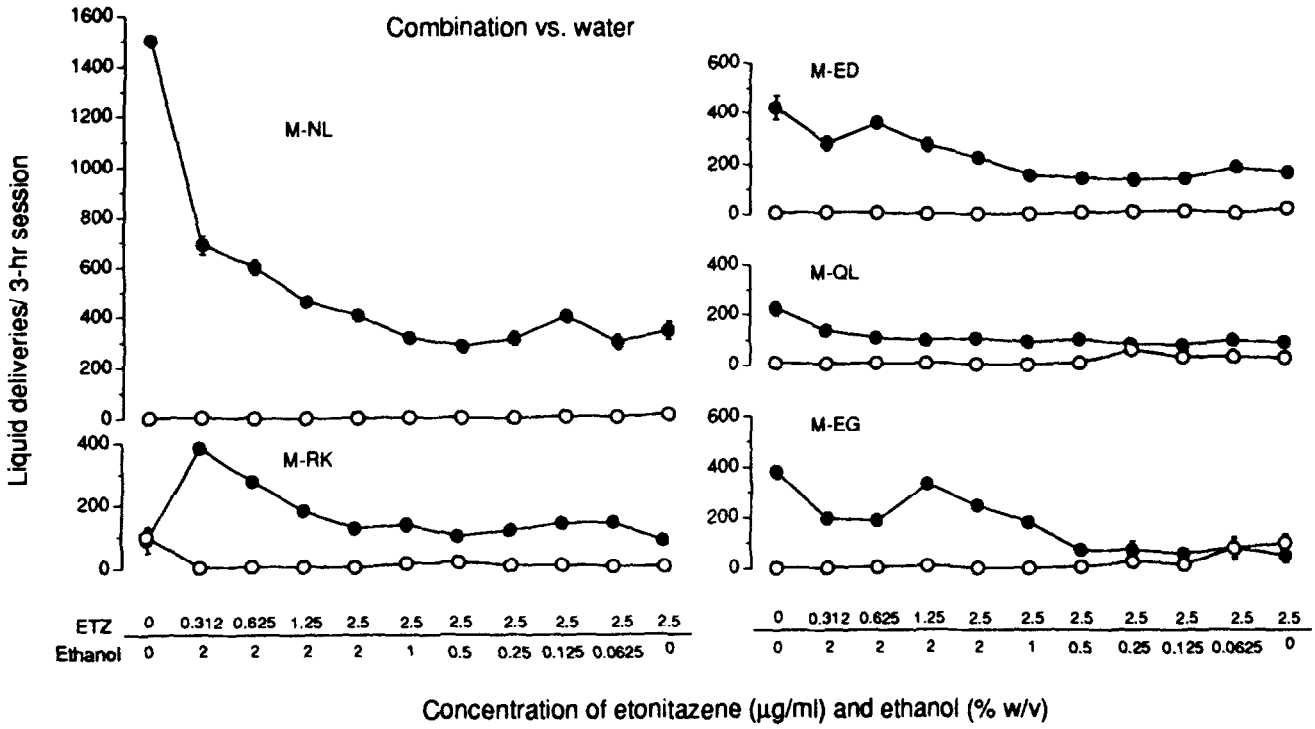

FIG. 2. Mean liquid deliveries (n = 6) as a function of the ethanol and etonitazene concentrations. Values along the ordinate specify the concentrations of etonitazene and ethanol. Brackets depict the SEM. Absence of brackets indicates that they fell within the area of the plotted point. Filled circles: drug values. Unfilled circles: water values.

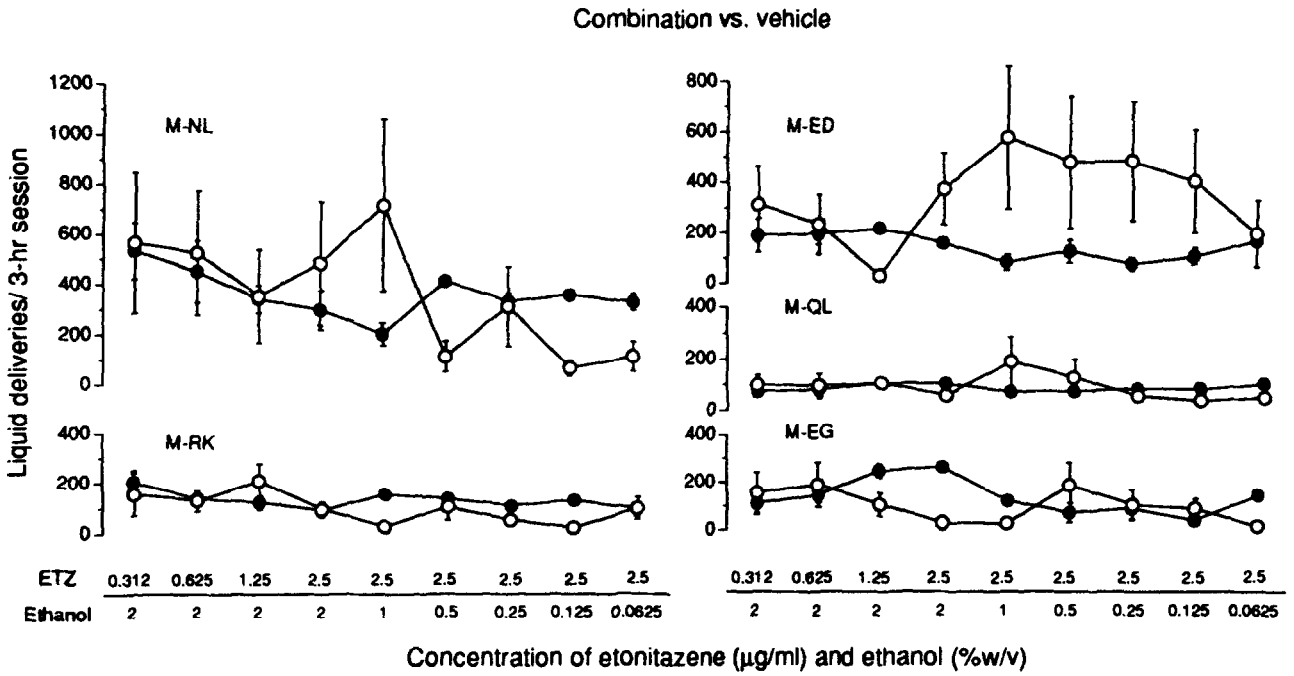

FIG. 3. Mean liquid deliveries (n = 6) as a function of the ethanol and etonitazene concentrations. Values along the ordinate specify the concentrations of etonitazene and ethanol. Brackets depict the SEM. Absence of brackets indicates that they fell within the area of the plotted point. Filled circles: etonitazene-ethanol combination deliveries. Unfilled circles: ethanol deliveries.

last test point, when ethanol was no longer present, water deliveries exceeded etonitazene deliveries.

Figure 3 shows deliveries of the etonitazene-ethanol solution and of the corresponding ethanol vehicle as a function of the combination of etonitazene and ethanol concentrations. The vehicle solution at each test condition contained the same concentration of ethanol as the etonitazene-ethanol solution; thus, the only difference between pairs of solutions was that one contained etonitazene. Consequently, differences in the number of liquid deliveries between the two solutions, when they occurred, were due to the presence of etonitazene in one of the solutions. During the phase when increasing amounts of etonitazene were presented in combination with the 2% ethanol solution, the combination and vehicle solutions generally maintained equal rates of responding. Monkey EG's performance was an exception, in that across three consecutive test points, the drug combination maintained greater rates. During the phase when concentrations of ethanol were progressively decreased, two patterns emerged: for three of the monkeys, the combination solutions were consumed in approximately the same quantity as the ethanol vehicle except for the last two test points (QL and NL) or the last test point (EG). For the other two monkeys, either the drug combination (RK) or the ethanol vehicle (ED) maintained higher rates; however, for both monkeys, at the final test point rates maintained by the drug and vehicle were equal.

Testing After Completion of the Acquisition Procedure

Figure 4 shows response rate as a function of three etonitazene doses under an FR 4 schedule. Etonitazene dose was changed by varying the volume of drug solution delivered: across blocks of sessions the monkeys received one, two, or four deliveries of drug solution upon completion of each fixed ratio; the concentration of etonitazene in the solution was held constant at 2.5 μg/ml. Response rate decreased with dose increases. For three monkeys (RK, ED, and NL) rates of etonitazene-maintained responding always exceeded rates of vehicle (water)-maintained responding, and in most comparisons, the standard errors of drug values did not overlap the standard errors of vehicle values. For three other monkeys (EG, QL, and AL), etonitazene-maintained behavior was substantially greater than water values at two of the four test points, and for monkey CM, there were no consistent differences between drug and water values. Monkey JS had very high drug intake and could not be systematically tested at FR 4 but was tested under an FR 8 schedule. Etonitazene intake (μg of drug/kg of body weight/3-h session) increased with increases in drug dose (Table 3). Retest values at the condition where one delivery followed each competed fixed ratio were generally similar to initial values with the exception that for three of the monkeys (ED, CM, and EG) water deliveries were decreased on retest.

Dose manipulations were then repeated, but at FR 8 instead of FR 4, and four delivery (dose) values were studied. Figure 5 shows that response rate was again an inverse function of dose. For five monkeys, at all test points, response rates maintained by etonitazene deliveries exceeded rates maintained by water deliveries. However, three monkeys had equivalent rates (AL, CM, and EG). Etonitazene intake increased with increases in dose (Table 3).

The three monkeys that had, relative to drug, high rates of water responding, (AL, CM, and QL) were further tested along with several other monkeys. Different rather than identical stimulus lights were paired with drug and vehicle. Figure 6 shows that for two monkeys (AL and QL), water-maintained

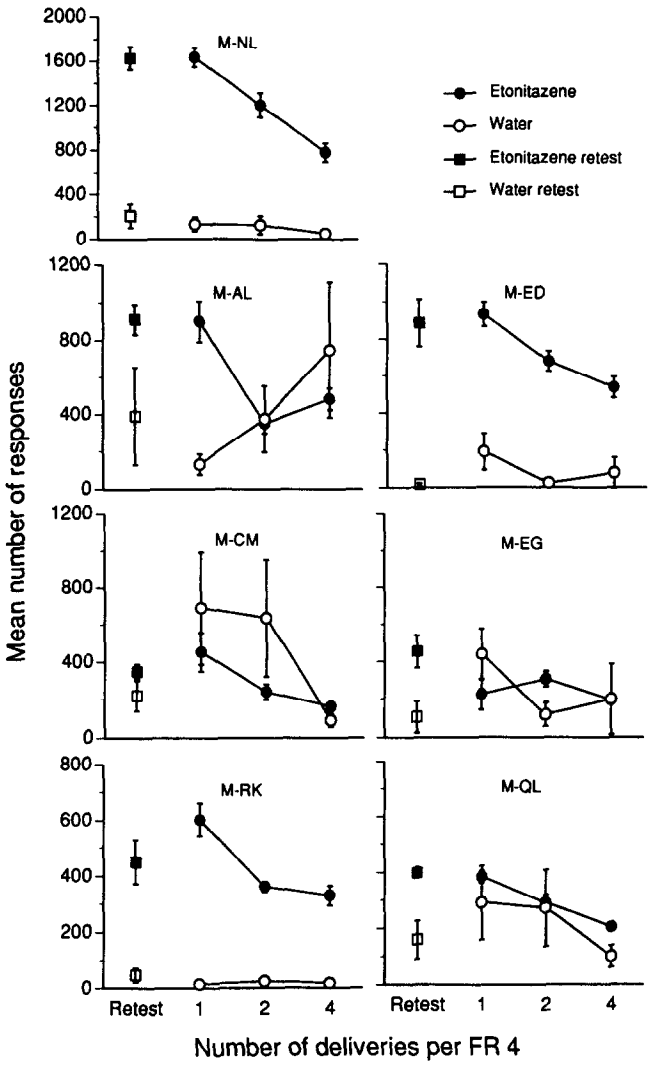

FIG. 4. Mean number of responses (n = 6) as a function of etonitazene dose. Etonitazene dose was changed by altering the volume and holding the concentration constant at 2.5 μg/ml. The volume was varied by scheduling one, two, or four liquid deliveries per each FR 4 completed. Brackets depict the SEM. Absence of brackets indicates that they fell within the area of the plotted point. Retest values were obtained at FR 4 one delivery.

responding occurred at lower rates with the different discriminative stimuli, thereby resulting in greater differences between drug- and vehicle-maintained response rates. For monkey NL, who displayed high rates of drug responding and low rates of water responding when stimuli were identical, the change to different stimuli did not alter the large differences in drug and water-maintained responding. However, for two monkeys, etonitazene did not maintain responding above vehicle levels. For monkey CM, both water and drug responding remained low and did not differ, and for monkey EG, drug-maintained responding was low and equal to or less than water vehicle values. With the monkeys for whom etonitazene served as a reinforcer, the findings were similar to those obtained when identical stimuli were used: in general, response rate was an

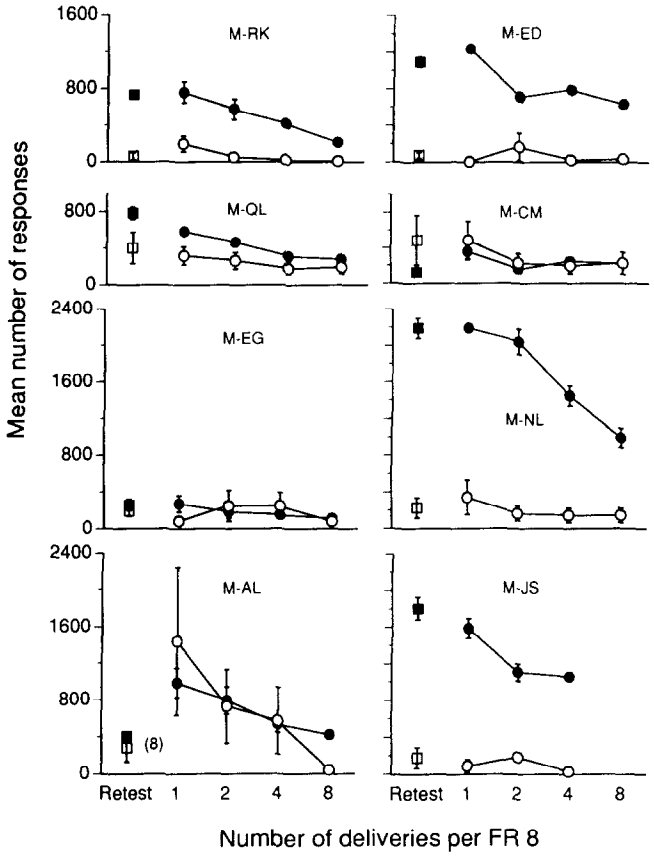

FIG. 5. Mean number of responses (n = 6) as a function of etonitazene dose. Etonitazene dose was changed by altering the volume and holding the concentration constant at 2.5 μg/ml. The volume was varied by scheduling one, two, four, or eight liquid deliveries per each FR 8 completed. Brackets depict the SEM. Absence of brackets indicates that they fell within the area of the plotted point. Retest values were obtained were obtained at FR 8 one delivery.

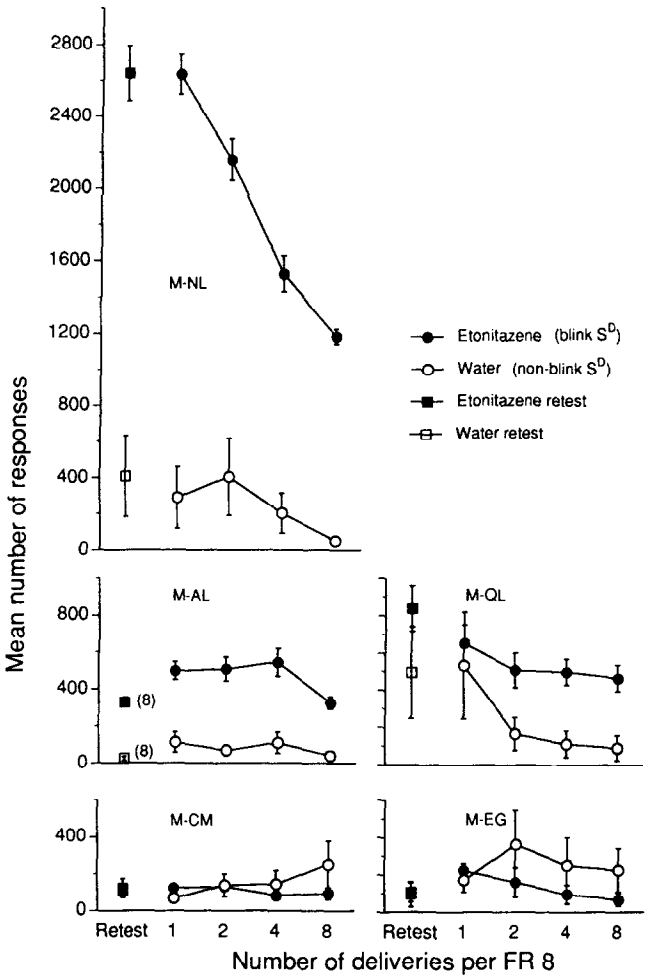

FIG. 6. Mean number of responses (n = 6) as a function of etonitazene dose under conditions where etonitazene and water spouts were correlated with different discriminative stimuli. Etonitazene dose was changed by altering the volume and holding the concentration constant at 2.5 μg/ml. The volume was varied by scheduling one, two, four, or eight liquid deliveries per each FR 8 completed. Brackets depict the SEM. Absence of brackets indicates that they fell within the area of the plotted point. Retest values were obtained at FR 8 one delivery.

inverse function of etonitazene dose, except for monkey AL. Table 3 shows that etonitazene intake increased with etonitazene dose.

DISCUSSION

Etonitazene was successfully established as a reinforcer for six of eight monkeys. The reinforcing effects were demonstrated by higher rates of drug than vehicle (i.e., water)-maintained behavior. Additionally, when dose was systematically varied, orderly dose-response functions were obtained. These findings replicate and extend those of an earlier study in which orally delivered etonitazene was established as a reinforcer for rhesus monkeys (3). In that study, it was not possible to maintain responding at fixed ratios greater than four, and although the etonitazene clearly served as a reinforcer, it was only moderately effective in reinforcing behavior. In contrast to the findings of the earlier study, the present results indicate that etonitazene can be a very effective reinforcer, in that it can maintain responding with relatively little variability over a range of doses and FR values.

For three monkeys an acquisition procedure was not conducted because high rates of drug responding relative to vehicle responding occurred during the initial substitution test. In subsequent dose-response determinations, two of the monkeys (M-JS, M-AL) had higher drug than vehicle response rates. However, subsequent testing with the third monkey (CM) revealed that the drug was not consistently functioning as a reinforcer. These findings suggest that a more stringent requirement is necessary during the probe test to decrease the probability of false positive results.

Five of the monkeys completed an acquisition procedure that used the technique of fading to transfer control from one reinforcing stimulus event to another stimulus event that

initially did not produce reinforcing effects. In earlier studies in our laboratory a fading procedure was used to establish pentobarbital (10,14) and cocaine (12,13) as reinforcers of monkeys' behavior. A fading procedure has also been used to establish cocaine-reinforced behavior in mice (6) and ethanol-reinforced behavior in rats (20). Fading procedures have usually been employed to transfer control of behavior from one discriminative stimulus to another stimulus. However, the findings of the present and earlier studies show that the effectiveness of fading procedures is not limited to transferring control between discriminative stimuli.

Fading procedures have features that make them especially suitable for establishing drugs as reinforcers, particularly orally delivered drugs. A major problem in establishing drugs as reinforcers is that drug ingestion is a necessary step, and because operant levels of oral drug intake are often low, such ingestion must often be contrived [for a review of establishing procedures see (17)]. One method to obtain drug intake is to place the drug in a liquid that is reliably consumed in high volumes. One such liquid is 2% ethanol (w/v), which rapidly comes to serve as a reinforcer for rhesus monkeys (11). A liquid that is reliably ingested in high volumes such as 2% ethanol has the advantage that when low concentrations of a new drug are added to it, the new drug may produce CNS effects due to the cumulative amount consumed over many drug deliveries (i.e., volume × concentration = drug amount). Moreover, the pharmacological effects of the new drug may occur at low concentrations that are less aversive in taste than higher concentrations. Reliable daily intake permits the repeated ingestion of each concentration and thereby repeated drinking of a distinctively tasting liquid that is followed by onset of pharmacological effects. Subsequently, in controlled steps, higher concentrations of the new drug can be introduced without disrupting responding. Once an appropriate concentration of a new drug is reached, the concentration of the original drug can be decreased in steps until it is no longer present. The use of small steps probably increases the likelihood that behavior will not be disrupted when the concentration of the original drug is diminished.

There are probably multiple factors involved in the effectiveness of 2% ethanol as the vehicle into which new drug is added. One of these is the reliable consumption of large liquid volumes, as mentioned above. Increases in the absorption of drugs may be another factor. The pharmacological effects of the ethanol may be important, but it is also possible that a nondrug liquid (such as a dilute saccharin solution) would work equally well. A further understanding of appropriate liquids to use in fading procedures should lead to procedures that are more efficient in establishing new drugs as reinforcers. Between each of the training steps in the present study a block of test sessions was inserted. In these test sessions, equal concentrations of ethanol were available from both spouts, and in addition, etonitazene was present in one of the ethanol solutions at a concentration that was used in the preceding block of training sessions. Thus, the only difference between the two solutions was the presence of etonitazene in one of them. Therefore, any differences in the rates of responding maintained by the two solutions could be attributed to the presence of etonitazene in one of them. In an earlier study of the development of cocaine-reinforcing effects with these same monkeys, behavior during the test sessions was predictive of behavior exhibited at the conclusion of the acquisition procedure: during test sessions the monkeys for whom cocaine was eventually established as a reinforcer had higher rates of responding maintained by the combination of ethanol and cocaine than by the equal concentration ethanol solution, and monkeys for whom cocaine was not established as a reinforcer had lower response rates maintained by the combination of ethanol and cocaine than by the equal concentration ethanol solution. In the present study, such a relationship was not observed. The reasons for this lack of correspondence between studies will have to be clarified in future studies.

In the earlier study that examined the establishment of orally delivered cocaine as a reinforcer, orally delivered cocaine came to function as a reinforcer for all monkeys expect CM and EG (12). In the present study, these same two monkeys did not develop etonitazene reinforced responding. However, 2% ethanol rapidly came to function as a reinforcer for all eight monkeys. An objective for future studies is to identify the optimal conditions for the establishment of oral drug reinforcement. To identify optimal conditions it may be necessary to analyze procedural details such as the number of concentrations, the rate of change in concentration, and size of each change. Once optimal conditions are identified, comparisons could be made among drugs in the rate of acquisition and the proportion of subjects that acquire drug-reinforced behavior.

In an ongoing study, orally delivered methadone has been established as a reinforcer for several monkeys that were subjects in the present study (unpublished data). The monkey that has self-administered methadone over the widest range of conditions and in the largest amounts is JS. In the present study, this monkey also took large amounts of etonitazene across a range of conditions. Persisting individual differences in drug-reinforced behavior of rhesus monkeys are potentially important in that identification of the determinants of such differences should aid in understanding differences among humans in drug self-administration. A related goal is increased knowledge of acquisition processes. Such knowledge provides a scientific foundation for developing prevention programs, and prevention is the least expensive way to promote health.

Etonitazene also was safe in that no toxicity or other untoward effects were noted, with one exception: during the initial probe tests, some monkeys (especially JS) rapidly consumed large amounts of the drug. Later, in the 3-h session, the monkeys' motor behavior was markedly changed. Motor behavior was decreased greatly and the monkeys remained in a sitting posture, swayed back and forth, and placed their hands wide apart in an apparent attempt to keep their balance. These changes were noted only during the initial sessions of the probe series. Tolerance probably developed because such signs were not seen later in the study.

In summary, orally delivered etonitazene was established as a reinforcer for six of eight rhesus monkeys. Use of the drug concentrations and training sequences employed in the present study will be helpful for other investigators in establishing etonitazene-reinforced behavior. Etonitazene served as an effective and safe reinforcer and, thus, it should be feasible to conduct extended studies in rhesus monkeys with other orally delivered opioids.

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