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Ethanol Intake During Scheduled and Non-scheduled Food Presentations[1]

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BARTNESS, T. J. AND L. A. ALFERINK. *Ethanol intake during scheduled and non-scheduled food presentations.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 637–642, 1979.—Ethanol (ETOH) intake baselines were obtained using a continuous, two-choice presentation of one of a series of increasing ETOH concentrations and water. Following the development of stable water intakes using a schedule-induced polydipsia (SIP) procedure, increasing concentrations of ETOH were repeated in the operant chamber. This was followed by a reinstatement of the pre-polydipsia home cage procedures. It was found that: 1) scheduled delivery of food slightly lowered or did not change ETOH intake in contrast to non-scheduled presentations, 2) during non-scheduled food presentations, maximum ETOH intakes occurred at higher concentrations than during scheduled presentations, and 3) post polydipsia water intakes were increased over pre-polydipsia baseline intakes. Because of these findings it was concluded that the use of SIP procedures to generate increased ETOH intakes appears to be questionable and comparisons between home cage (non-scheduled food) and SIP (scheduled food) ETOH intakes are not appropriate due to the different self-selection functions for ETOH. These findings may be due to the different hydrational states of the animals when water is available in addition to ETOH (home cage) versus forced ETOH presentation (SIP).

Ethanol Schedule-induced polydipsia Ethanol preference

FOR nearly forty years researchers have attempted to develop an animal model of human alcoholism with little success [5]. One of the most promising of these attempts has been generated by Falk and his colleagues [7]. This model was derived from Falk's earlier discovery that unusually high water consumption occurs when food-deprived rats are delivered food pellets on an intermittent basis [4]. This high fluid intake, termed schedule-induced polydipsia (SIP), also persists when ethanol (ETOH) is substituted for the water available in the operant chamber [12]. By evenly distributing the pellet delivery sessions throughout 24 hour periods, uniformly high blood ethanol levels resulted. Uniformly high blood ethanol levels have been shown to be necessary for the demonstration of physical dependence to ETOH as is evidenced by the production of sound-induced, tonic-clonic convulsions after a period of restricted access to ETOH [5, 6, 7]. Systematic replications of this procedure have also reported similarly high ETOH intakes although the incidence of and the necessary conditions for the production of sound-induced seizures has been less substantiated [9,15].

A major criticism of this evenly-spaced pellet distribution procedure has been that while physical dependency *may* have been demonstrated, "psychological dependency" has not been satisfactorily shown [9]. Although the utility of the term "psychological dependency" has been questioned and varied definitions offered [19,22], the evaluators of the SIP model have defined the term as the consumption of "supranormal" ETOH intakes in order to prevent withdrawal symptoms. Using this definition it has been concluded that this model has failed to produce psychological dependency. That is, when SIP procedures are terminated followed by a period without access to ETOH, ETOH consumption is greatly lowered when ETOH and tap water are subsequently offered to the animals in their home cages [9].

The above determination of "psychological dependency" contains some potential procedural problems which could alter conclusions concerning the demonstration of this dependency and the efficacy of the SIP model. The primary problem of the comparison of ETOH consumption using a spaced versus a non-spaced food presentation procedure is that the two conditions are clearly different. One might wonder why it would be expected that animals consuming ETOH as a by-product of a schedule of reinforcement should, when the schedule of reinforcement is terminated, continue to drink the same or greater amounts of ETOH. It has been shown that when the total allotment of food pellets is presented non-intermittently, low levels of water consumption are observed, where water is the only fluid available to the

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animals [8]. Therefore, the spacing of food pellets might seem to be necessary for the production of high fluid intake whether the fluid available is water or ETOH.

A more appropriate determination of the efficacy of the SIP model in creating relatively permanent enhancement of ETOH consumption would involve the use of prepolydipsia home cage intake baselines with which polydipsia and subsequent post-polydipsia intakes can be compared. If post-polydipsia ETOH consumption is higher than prepolydipsia ETOH consumption, the use of a SIP paradigm for creating relatively permanent enhanced ETOH intakes could be substantiated. If pre- and post-SIP consumption of ETOH did not differ, then the SIP paradigm should be evaluated as a means of creating *temporary* increases in ETOH consumption. Unfortunately, such baselines have been absent from even the most extensive of the SIP-ETOH studies to date [7, 9, 15].

It has also not been established whether ETOH self-selection curves are the same in the home cage and operant chamber (i.e., the ETOH concentration that yields the highest mean daily ETOH intake in g per kg is the same with non-spaced and spaced food presentations, respectively). If a difference does exist in self-selection curves then previous home cage and operant chamber ETOH consumption comparisons may not have been valid [9].

The purpose of the present investigation was to obtain prepolydipsia home cage ETOH self-selection curves and intake baselines with which polydipsia and post-polydipsia measures could be compared using a within-subject design. Such a design should enable, not only a test of the validity of previously performed measures of "psychological dependence" to ETOH [8], but also to evaluate the usefulness of the SIP paradigm in creating relatively permanent increases in ETOH consumption.

METHOD

Animals

Six male hooded rats (Blue Spruce Farms) approximately 175 days old, with a mean free-feed weight of 253 g at the beginning of the experiment were used. The animals were reduced to 80% of their free-feed weights over a two week period and remained at this weight one month prior to any experimental manipulations. Rats Z-1, Z-2, Z-4 and Z-5 were randomly selected to serve as the experimental animals while Rats Z-6 and Z-7 were selected to serve as the controls.

Apparatus

Two standard operant chambers measuring 24.5×22.5 ×27.5 cm were employed. These chambers were individually enclosed in sound-attenuated shells and contained a houselight, a speaker for white masking noise, a pellet dispenser, an exhaust fan and a food cup. Fluid was made available in each chamber through unrestricted access to a stainless-steel watering tube. The tube protruded into the chamber 2 cm through the back wall. The chambers were constantly illuminated.

When not housed in the operant chambers, all rats were housed singularly in stainless steel group cages (40.6×25.4 ×17.9 cm) which had three 2.5 cm×2.5 cm apertures cut into the front side of each cage. Through each of these three apertures, a calibrated, 250 ml glass Richter tube (Wahmann Mfg. Co.) was inserted. A stainless steel food cup 10.2×6.4×5.1 cm was positioned in the center of the 25.4 cm interior wall. The food cup was used to hold 240 (45 mg) food pellets (P. J. Noyes Co.) needed to maintain the animals at 80% of their free-feed weights. The animal colony was constantly illuminated and kept at 24°C.

Procedure

Home cage. After remaining at their 80% free-feed weights for one month, the animal's home cage water consumption was monitored. One of the three Richter tubes was filled daily with room temperature distilled water; the other tubes remained empty. The position of the tube filled with distilled water was randomly determined on a daily basis to control for a possible position bias [16].

A two-choice presentation of fluids occurred next, where one of the three tubes was filled with distilled water, the second with an ETOH solution, with the third remaining empty. One of a series of increasing concentrations of ETOH in solution with distilled water was prepared volumetrically [21], and presented for four days before changing to the next solution concentration. The ETOH concentrations were increased by one percent increments in an ascending order until a decrease occurred in the mean daily ETOH intake (g/kg) for two successive concentration values. The positions of the three tubes were randomly determined on a daily basis. The ETOH and water tubes were filled daily with fresh solutions.

After the series of ascending ETOH concentrations were terminated, the concentration of ETOH that yielded the highest mean daily intake of ETOH (g/kg) was presented for a second time. All conditions remained the same as had occurred during the first presentation of this concentration except that each animal was exposed to this solution for a minimum of eight days and until intake stabilized. Data collected on ETOH consumption provided a baseline for comparison with the data obtained in the operant chamber and in the subsequent return to the home cage condition. The control animals remained in the home cage condition for the duration of the experiment to facilitate in the detection of the development of tolerance due to continued ETOH exposure.

Operant chamber. Upon completion of the home cage conditions, the animals were housed in the operant chambers where 45 mg food pellets were delivered on a fixed time 90 sec (FT90) schedule of reinforcement programmed by solid state logic (BRS-LVE). The pellets were delivered for one hour sessions that were programmed to occur every three hours. Thus, six one-hour sessions occurred daily during which a cumulative total of 240 pellets were delivered. White noise was programmed to occur during the pellet delivery sessions. Distilled water was constantly accessible via a stainless steel watering tube. Every day, upon completion of the 1800 hr delivery session, fluid intakes and body weights were recorded and fresh distilled water replaced the remaining unconsumed fluid. Due to equipment restrictions, Rats Z-2 and Z-4 entered the operant chambers first while Rats Z-1 and Z-5 remained in the home cages with their respective ETOH solution concentrations and water.

Following stabilization of water consumption, the water was replaced with a 2% ETOH solution. The criteria for stable water consumption was no systematic trend in daily water intakes for at least eight consecutive days. After a minimum of four days exposure to the ETOH solution and until no systematic trend in consumption was apparent (usually 8 to 12 days), the ETOH concentrations were in-

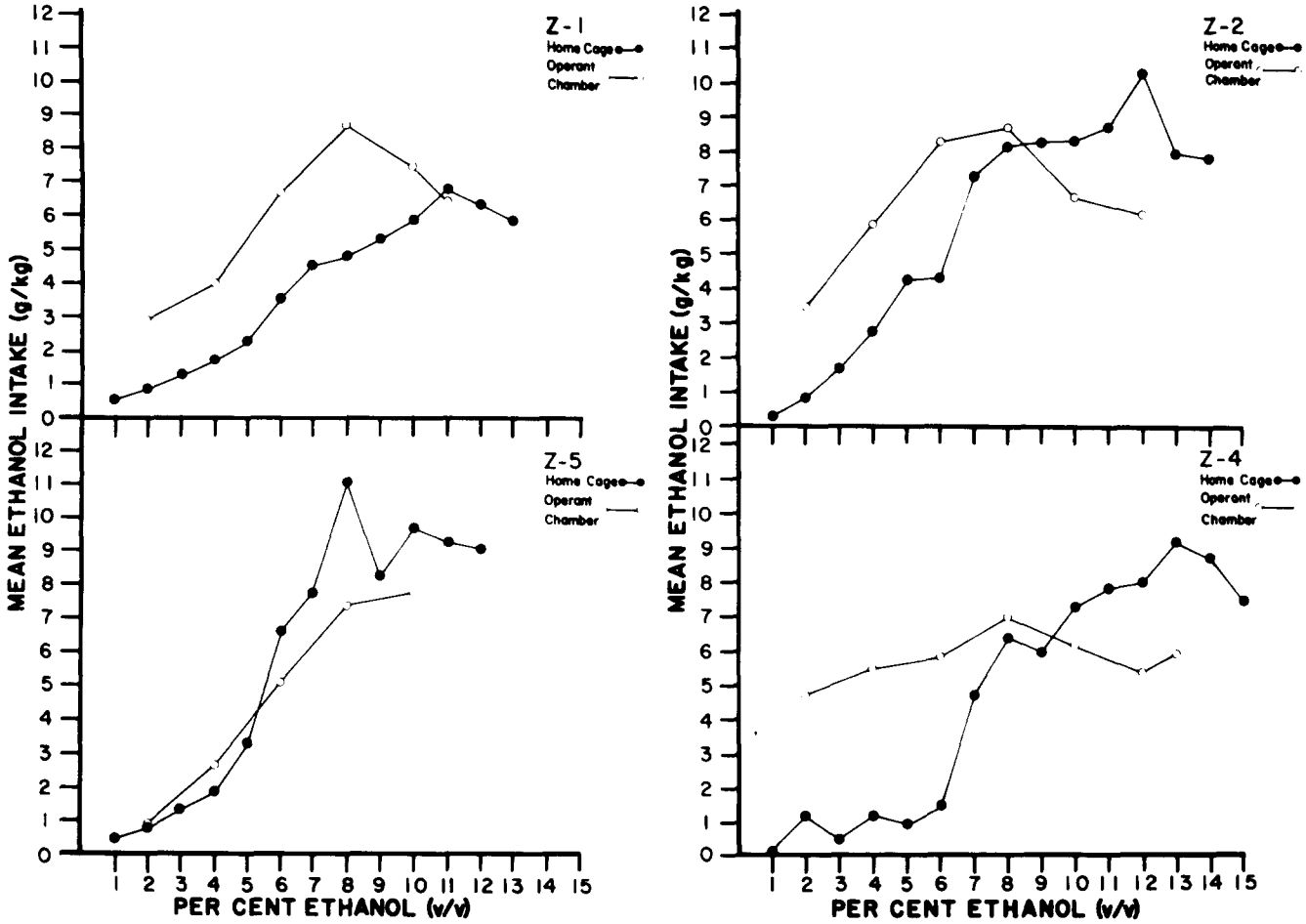

FIG. 1. Mean daily ethanol intakes (g/kg) in the initial home cage condition and during operant chamber (SIP) conditions during the presentation of ascending ethanol concentrations.

creased by two percent increments in an ascending order until the previously determined ETOH solution concentration that yielded the highest mean g of ETOH per kg of body weight was reached. At that point, the animals remained at their respective ETOH concentrations for a minimum of eight days and until ETOH consumptions had stabilized.

Once ETOH consumption had stabilized, blood samples were taken from each animal's tail in order to determine the blood ethanol levels for a given 24 hr period. The blood samples were analyzed using gas chromatograph techniques [11]. Two samples were taken per day, one hour after the termination of a pellet delivery session. Because the sampling of blood elevated ETOH intakes during the three-day sampling period, Rats Z-2 and Z-4 remained in the operant chamber with their respective ETOH solutions for eleven more days before being returned to their home cages. The data from the last eight days of these eleven days served as the mean intake of ETOH for the polydipsia condition. Once Rats Z-2 and Z-4 were returned to their home cages, Rats Z-1 and Z-5 were then housed in the operant chambers and were subjected to the same experimental manipulations that occurred for Rats Z-2 and Z-4, except that no blood samples were taken.

Return to home cage. The animals were then returned to their home cages, deprived of access to any fluid for eight hr (as is typical of most tests for withdrawal symptoms) and then exposed to the two-choice presentation of distilled water and their respective ETOH solution concentration determined in the original home cage condition. No withdrawal symptoms were observed such as body tremor or obvious hyperactivity. Because of the failure to observe withdrawal symptoms, and the blood ethanol concentrations and ETOH intakes that were lower than those reported in procedurally similar experiments [7, 9, 15], no test for physical dependency (e.g., the shaking of keys) was performed. Each day during this condition, 240 food pellets were given once a day. This condition remained in effect for eight days.

RESULTS

During the exposure to the ascending series of ETOH solution concentrations in the home cage, it was found that Rat Z-5 initially consumed the highest mean daily g of ETOH per kg of body weight at an eight percent concentration as is shown in Fig. 1. This was followed by a reduction in intake at concentrations of nine and ten percent. Since the g/kg ingested at the ten percent concentration was above that of the nine percent concentration, yet was lower than that at the eight percent concentration, additional increasing ETOH concentrations were presented. When the recovery of the

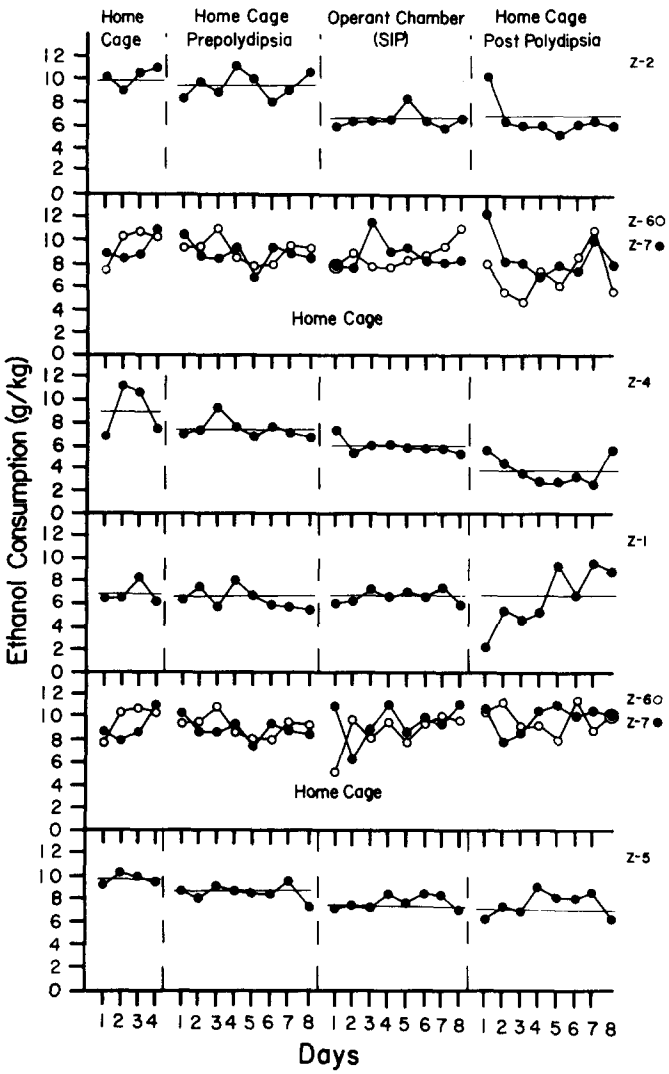

FIG. 2. Mean daily ethanol intakes for experimental (Z-1, Z-2, Z-4, and Z-5) and control (Z-6, Z-7) rats at the ethanol concentrations that yielded the highest consumption (g/kg) initially in the home cage during the ascending ethanol series presentation (Home Cage), recovery of that intake in the home cage prior to SIP procedures (Home Cage Prepolydipsia), during the SIP procedures (Operant Chamber—SIP) and following the return to the home cages (Home Cage Post Polydipsia). Except for the initial Home Cage condition, data are from the last eight days for each condition.

eight percent concentration was attempted, a much lower intake was recorded than previously exhibited. There was then an attempt to recover the next highest intake, the ten percent concentration. Although this intake was also lower than seen previously, it served as the home cage ETOH intake baseline for Rat Z-5. It can be seen in Fig. 1 that Rat Z-1's highest mean daily ETOH intake occurred at the eleven percent concentration while Rats Z-2 and Z-4 had their highest intakes at twelve and thirteen percent in the home cages respectively.

When the animals were presented with the ascending series of ETOH concentrations in the operant chamber it was found that Rats Z-1, Z-2 and Z-4 all had their highest daily mean ETOH consumption at ETOH concentrations of three to five percent lower than in the home cage condition. For Rat Z-5, the ETOH concentration that yielded the highest intake in the home cage also engendered the highest intake in the operant chamber under the SIP paradigm.

Figure 2 depicts the mean ETOH intakes for both the control and experimental animals under the home cage and operant chamber (i.e., SIP) conditions with each rat's ETOH concentration that yielded the highest mean daily ETOH intake (g/kg) during the original home cage condition. It can be seen that three of the four experimental animals had slightly lower ETOH intakes during the SIP condition than their respective home cage intake baselines. Rat Z-1 exhibited essentially no change across all conditions. The control animals, Z-6 and Z-7, exhibited little change in their ETOH intakes throughout the duration of the experiment. These two animals also did not change their fluid preference during the course of the experiment.

Upon return to the home cage conditions after the SIP condition, all animals either had ETOH intakes that were not different from their SIP intakes (Rats Z-2, Z-1, and Z-5) or exhibited slight decreases in contrast to their SIP intakes (Rat Z-4).

The fluid intakes recorded for the home cage prepolydipsia two-choice presentation condition (i.e., home cage ETOH intake baseline) and the procedurally identical post-polydipsia home cage condition were converted to preferences for ETOH and plotted in Fig. 3. Preference for ETOH was computed by dividing the ETOH intakes by the total volume of fluids ingested (both ETOH and water) multiplied by one hundred. A statistical analysis of the ETOH preferences in the pre- versus post-polydipsia condition revealed a significant decrease in preference for ETOH from the pre-polydipsia to the post-polydipsia home cage conditions ($t=3.38$, $df=3$, $p=0.05$). A further analysis of the ETOH and water intakes revealed that the significant decrease in ETOH preference was primarily due to an increase in water consumption, not a decrease in ETOH consumption (pre-polydipsia mean daily water intake across all animals=4.88, post-polydipsia=9.18).

The analysis of Rat Z-2 and Z-4's blood revealed blood ethanol concentrations which ranged from 105 to 0 mg of ETOH per 100 ml of blood for Z-2 across the 24 hr representative sample (mean=43.4 mg/100 ml) and from 75 to 0 mg of ETOH per 100 ml of blood for Rat Z-4 (mean=42 mg/100 ml). Both animals showed their lowest blood ethanol concentrations between 1200 and 1600 hrs.

It was observed that ETOH consumption for all animals occurred almost exclusively during the pellet delivery periods and not during the intervening periods of non-pellet delivery. It was also observed that these animals showed slight ataxia during the daily weighing sessions in the operant chamber phase of this experiment. Motor ataxia was not apparent at any time for the control animals, Z-6 and Z-7.

DISCUSSION

The use of prepolydipsia home cage ETOH intake baselines has been absent from even the most extensive schedule-induced polydipsia investigations employing ETOH [7, 9, 15]. Without such baselines there can be no thorough evaluation of the SIP paradigm as a means of increasing ETOH consumption during intermittent pellet delivery or of the ability of exposure to the SIP procedures to create persisting high ETOH intakes even after such procedures are terminated. The use of such baselines in the present investigation revealed a slight decrease or no change in

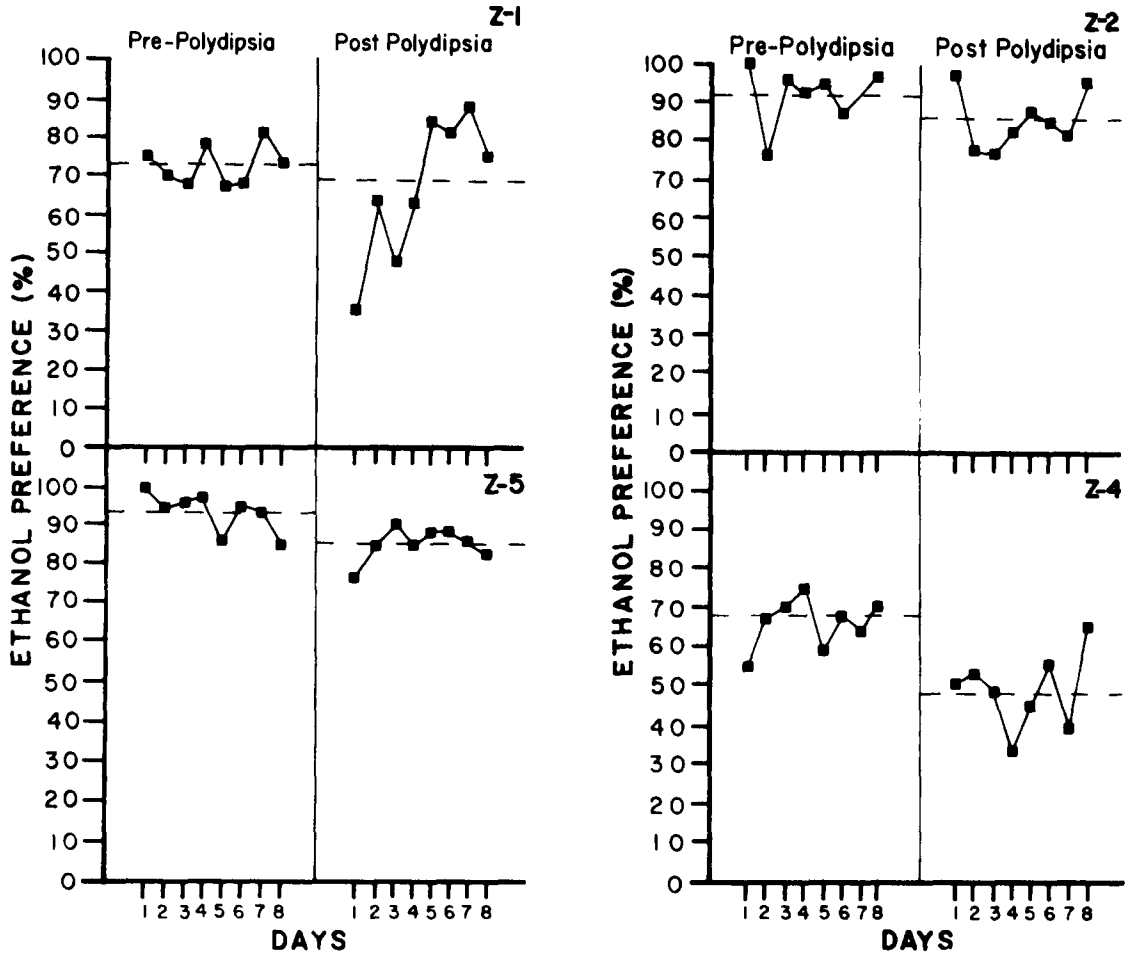

FIG. 3. Home cage ethanol preferences (ethanol intake/ethanol+water intake)×100 for the last 8 days prior to the SIP procedures and the first 8 days after the termination of the SIP procedures and the return to the home cage condition.

ETOH intake during and subsequent to the SIP procedures. These results appear to question the use of the SIP paradigm as an effective means of producing high ETOH consumption. These findings are consistent with those of other investigators [15] who found that nearly equivalent ETOH intakes could be established if the spacing of food pellets was eliminated and, instead, all pellets were presented non-intermittenly.

The development of tolerance to the ETOH solutions was not exhibited by the control animals, Z-6 and Z-7, even though they had constant access to ETOH and distilled water through the duration of the experiment. They also did not exhibit any fluid preference changes, always consuming a larger volume of their respective ETOH solution than water. Additionally, no evidence of motor ataxia was apparent in these animals as was true for the experimental animals when they were in the home cage. In contrast, the experimental animals exhibited motor ataxia under the ETOH-SIP phases of this experiment. The presence or absence of motor ataxia may be explained by the distribution of ETOH consumption creating a more sustained level of high blood ETOH during the SIP phases relative to the Home Cage conditions (see below for further discussion of possible differences in ETOH consumption between the SIP and Home Cage conditions).

It is possible that the failure to see enhanced ETOH intakes during the SIP condition and subsequent home cage intakes was due to the inability of the present schedule of food reinforcement (FT 90) to generate ETOH intakes that resulted in high blood ethanol concentrations. McMillan *et al.* [15] using the same schedule of reinforcement reported ETOH intakes of 9.5 g/kg/day in contrast to the 7.0 g/kg/day average ETOH intake in the present investigation. Other investigators, using a FT 120 sec schedule have reported mean intakes of 11.2 and 13.1 g/kg/day [7,9].

It is also possible that the difference in ETOH intakes between the present investigation and those cited above is due to strain differences in the rats used. ETOH self-selection can be genetically selected for [2], and varies from strain to strain [2, 13, 14].

The absence of ETOH in the blood during the 1200 to 1500 hr samplings is consistent with the procedurally similar SIP studies employing ETOH [7,9]. Blood ethanol levels were detectable at all other times, indicating that the animals were drinking the ETOH solutions.

While the comparison between home cage and pre- and post-polydipsia preferences for ETOH revealed a statistically significant decrease in ETOH preference in the post-polydipsia condition, analysis of the fluid consumptions indicated an increase in water consumption during the post-polydipsia phase with a concomitant maintenance of the volume of ETOH consumed. This increase in water con-

sumption is not surprising since one of the effects of forced ETOH consumption (as occurred in the SIP condition) is dehydration and subsequent water-electrolyte imbalance [3]. This dehydrating effect of ETOH is especially powerful at relatively high ETOH concentrations (e.g., 8–16% v/v) and has been substantiated in a variety of experimental paradigms [3,20]. The results of the present investigation suggest that if home cage fluid monitoring is to occur after the termination of SIP procedures to test for maintenance or change in alcohol intake, water should be available in addition to ETOH or ETOH should be the only fluid available in all conditions.

Maximum ETOH intakes generally occurred at lower concentrations in the SIP condition than in the home cage condition. This finding suggests that the proposed and performed test of "psychological dependency," where SIP procedures are terminated, access to ETOH is restricted, and the animals are returned to their home cages where ETOH and water are made available, were inappropriate [9]. Clearly, if ETOH intake functions (g/kg) are condition dependent (i.e., high ETOH concentrations are consumed in larger quantities in the home cage than in the operant chamber under SIP procedures) then there is no valid basis for polydipsia and home cage intake comparisons.

It is possible that the two distinct ETOH self-selection curves observed during schedule and non-scheduled food presentations may have been due to the presence of water in the home cage but not the operant chamber conditions and/or the "time restrictions" placed on ETOH consumption. Regarding the latter possibility, it has been shown [10] that when a 3.5 hr pellet delivery session (with ETOH concentrations ranging from 3 to 20% as the only available fluid) were compared to home cage intake during the remaining 20.5 hr, most of the total daily ETOH intake occurred in the home cage. One explanation suggested by these investigators for their findings was that higher concentrations of ETOH were more readily consumed in the home cage because "noxious" ETOH concentrations could be consumed in small distributed bouts of drinking during the 20.5 hr home cage period. In reference to the present investigation, it is possible that the consumption of ETOH was also distributed throughout the 24 hr period in the home cage. This would be in contrast to the "restricted" drinking of ETOH seen primarily during the pellet delivery sessions. It is also possible that the presence of water in the home cage with which dilution of high ETOH concentrations could occur, may account for the increased intake of ETOH in the home cage in contrast to the operant chamber where water was not available.

Because of the above findings, it was concluded that the use of the SIP paradigm employing ETOH solutions appears to be questionable as a means of creating elevated ETOH intakes and as an animal model of human alcoholism [7]. It was found that ETOH intakes were not elevated during SIP procedures but were slightly decreased or unchanged. It was demonstrated that the test of "psychological dependency" [9] that has been conducted may have been inappropriate due to the different ETOH self-selection intake functions observed during scheduled and non-scheduled food presentations. The difference in self-selection intake functions was hypothesized to be due to the absence of water during the SIP condition and its presence in the home cage conditions. Therefore, care must be taken in further examinations of SIP using ETOH solutions such that the hydrational states of the animals are equated in all conditions.

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