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Effects of Food Deprivation on Etonitazene Consumption in Rats[1]

MARILYN E. CARROLL[2] AND RICHARD A. MEISCH[3]

Psychiatry Research Unit, Mayo Box 392, University of Minnesota, Minneapolis, MN 55455

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CARROLL, M. E. AND R. A. MEISCH. *Effects of food deprivation on etonitazene consumption in rats.* PHARMAC. BIOCHEM. BEHAV. **10**(1) 155–159, 1979.—One group of free-feeding rats was given a 5 μg/ml etonitazene HCl solution as their sole liquid. This group increased their drug intake by 100% when they were partially food-deprived during a 23-day period. Another group that remained food-satiated and received etonitazene for an equal number of days did not show similar increases in drug intake. However, this group drank greater volumes of the etonitazene solution than a food-satiated control group drank of water. These results are contrasted with a fourth group showing a 50% decrement in water intake during similar food-deprived conditions. The food-deprived group drinking etonitazene showed highly erratic drinking patterns compared to all the other groups. Daily liquid intake ranged from 30 to 250 ml in this group, and volumes oscillated from high to low on alternating days. When the food-deprived/food-satiated conditions were replicated in this experimental group, corresponding increases and decreases in drinking reliably occurred. However, during the second food-deprived phase, the large increases occurred almost immediately as contrasted with a gradual increase over 17 days during the first food-deprived phase. This would suggest a learning mechanism may be involved. Self-mutilation and other forms of stereotypy were noted only in food-deprived rats consuming etonitazene.

Etonitazene intake Food deprivation Rats Physical dependence Self-mutilation Stereotypy Tolerance

FOOD deprivation has a variety of effects upon the behavior of laboratory rats. For instance, it is well known that food deprivation results in decreases in water intake [18,26]. However, other consummatory behaviors have been reported to increase with food deprivation, consumption of saccharin solutions [6, 23, 24], drinking produced by injections of hypertonic saline [19], and hypothalamic drinking [17]. Intracranial self-stimulation [3,20] and muricidal behavior [14,21] also increase during conditions of food deprivation.

The effect of food deprivation on drug effects and drug self-administration behavior is not well known, especially regarding drugs other than ethanol. Previous reports comparing food deprivation and food satiation have shown that food deprivation increases ethanol intake in rats receiving daily access to the drug [1, 15, 16, 27, 32]. In many areas of research concerning drug effects, food deprivation is commonly used. For instance, it is used in polydipsia procedures used for oral administration (e.g., [5,11]), studies of the effects of drugs on schedule-controlled behavior (e.g., [9]), and drugs as discriminative stimuli (e.g., [2]).

The purpose of the present research was to investigate the effects of food deprivation on etonitazene intake in rats given continuous access to the drug in their drinking water. Etonitazene is an opioid approximately 1,000 times as potent as morphine, non-caloric, and apparently low in aversive taste properties [12,28]. Etonitazene has been used in several studies of oral drug intake [10, 12, 13].

METHOD

Animals

Thirty-four naive, male Wistar rats (Bio-Lab, St. Paul, MN) were used. The rats varied in weight between 360 and 380 g at the beginning of the experiment.

Apparatus

Throughout the experiment, the rats were housed in individual wire mesh rat cages (Hoeltge) in a continuously lighted room with the temperature constant at 24°C. They were removed from the cages for about 20 sec each day to record body weights. The rats were habituated to the handling and weighing procedure two days before the experiment began. Drinking solutions were presented to the rats in 250 ml bottles attached to the front of the cages. Food (45 mg standard Noyes pellets) was also presented inside the front of the cage in stainless steel containers (10.2×5.1×2.5 cm).

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Procedure

Throughout the experiment, each day between 7:30 and 9:00 a.m., bottles and food containers were removed from the cages and the amounts consumed were measured and recorded. Corrections were made for spillage. The body weight of each rat was then recorded, and food and liquid were returned to the cages. During this procedure, each rat was without food and liquid for 20 min.

The 34 rats were divided into four groups. The experimental treatments were arranged such that each group received conditions of unlimited access to a 5 μg/ml etonitazene solution (E) *or* water (W) *and* food deprivation (FD) *or* food satiation (FS). The four groups were labeled according to their respective treatment: E-FD (N=9); E-FS (N=8); W-FD (N=9); and W-FS (N=8).

The experiment was divided into four phases. during Phase 1 (5 days), mean water intake was determined for all groups. Each group received ad lib access to tap water and 40 g of food pellets each day. Phase 2 (5 days) consisted of a five-day period in which Groups E-FD and E-FS were allowed continuous access to the etonitazene solution, and Groups W-FD and W-FS continued to receive ad lib water. In Phase 3 (23 days), the food-deprived groups (E-FD and W-FD) were reduced to 75% of their mean body weights as determined during Phase 2. This was accomplished by feeding each rat 8 g of pellets per day until the 75% weight was reached, then an amount necessary (usually 12-14 g) to maintain that weight for at least 5 days. Groups E-FS and W-FS continued to receive 40 g of food per day. Phase 4 (30 days) was identical to Phase 2. Each rat in all groups received 40 g of food per day and ad lib access to either etonitazene (E-FD and E-FS) or water (W-FD and W-FS). At the end of Phase 4, the food-deprivation and food-satiation conditions were repeated once for Group E-FD for a within-group comparison of experimental treatments. During Phases 1, 2 and 4, the rats never ate all 40 g of food.

Drug Solution

The etonitazene HCl (NIDA: Research Triangle Institute) solution (5.0 μg/ml) was mixed daily from a stock solution containing 12.5 μg/ml which was mixed weekly in tap water. Concentrations are expressed in terms of the salt.

RESULTS

Figure 1 shows liquid intake for each group as a function of the drinking solution presented and feeding conditions. There were no differences among the four groups during the five-day water period (Phase 1). During Phase 2, there were also no differences in intake of the etonitazene solution and water among the four groups. Even on the first day of etonitazene access, liquid intake did not fall below the water levels of Phase 1.

At the onset of food deprivation (Phase 3), changes in the volumes of liquid consumed were immediately apparent. Group E-FD's mean etonitazene intake dropped slightly below water levels for the first two days, then it steadily increased. By the 17th day, Group E-FD's intake had stabilized at a level substantially above all other groups. As expected, food deprivation produced an immediate decrement in water intake for Group W-FD. This group consumed one-half of their usual amount of water under these conditions of food deprivation. The two food-deprived groups

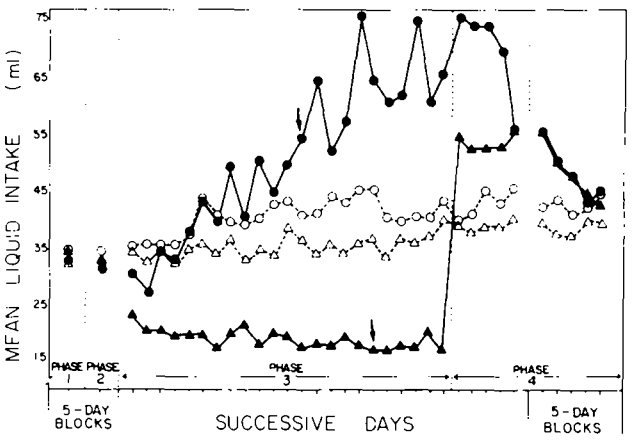

FIG. 1. Mean liquid intakes for the four groups: E-FD (●——●); E-FS (○– –○); W-FD (▲——▲); W-FS (△– –△) are presented for the four experimental phases (Panels 1–4). Data for Phases 1, 2 and part of Phase 4 (Panels 1, 2 and 4) are presented in blocks of five-day means. Daily group means are presented for Phase 3 (Panel 3) and the first five days of Phase 4 (Panel 4). Arrows indicate the day when all animals in the food-deprived groups had reached their 75% body weight.

reached their 75% weight in 13–18 days following the onset of Phase 3. No systematic changes in drinking were observed as these rats were fed slightly more to maintain their 75% weight. It was noted, however, that Group E-FD lost weight at a slightly more rapid rate than group W-FD. Group E-FS showed a small, steady increase in etonitazene intake over the first half of Phase 3, and the mean intake stabilized at a level that was consistently above that of the water control group (W-FS). The water control group, W-FS, showed no systematic increases or decreases in liquid intake throughout the experiment.

When the two food-deprived groups were abruptly food-satiated during Phase 4, both etonitazene (E-FD) and water (W-FD) intake increased on the first day. Etonitazene intake in Group E-FD remained at its previous food-deprived level for 4 days, then began to steadily decline. Water intake in Group W-FD increased by 40 ml on the first day of food satiation; and it remained at that level for 10 days, then slowly declined. A separate comparison of the variability around each of the daily group means showed large differences among groups and treatment conditions. Since there were large differences among group means, a comparison of the magnitudes of standard errors or standard deviations was not appropriate [25]. Thus, the coefficient of variation, which expresses the standard deviation as a percentage of the mean (SD/mean×100), was calculated for each group daily and used as an estimate of variability. The coefficients of variability for Groups E-FS, W-FD and W-FS ranged from 9.5 to 35.9 during the four phases, and there was considerable overlap among the groups. However, the etonitazene food-deprived group (E-FD) showed substantially more intersubject variability than the other three groups during Phases 3 and 4. The coefficient of variability for this group ranged 36.9 to 71.2 during Phase 3, and increased steadily. During the first 15 days of Phase 4, the coefficient decreased from 59.3 to 26.8 (within the range of the other groups).

The intersubject variability of Group E-FD was a result of an erratic drinking pattern found in all rats of this group. They typically drank large (100–250 ml) and small (30–60 ml) volumes of the etonitazene solution on alternate days. This oscillating pattern is only partially revealed in Fig. 1, since the nine rats in this group were not synchronized in their pattern of alternation. Figure 2 presents data for individual rats and illustrates the alternating pattern more clearly. Liquid intake for a single rat in each group is presented for Phases 3 and 4. The individual data shown were selected as the pattern closest to the group mean. The highly variable pattern of etonitazene drinking for Group E-FD began approximately a week after food deprivation (Phase 3) and, throughout this phase, increased directly with increases in the mean volume consumed. The other three groups showed no differences in individual drinking patterns.

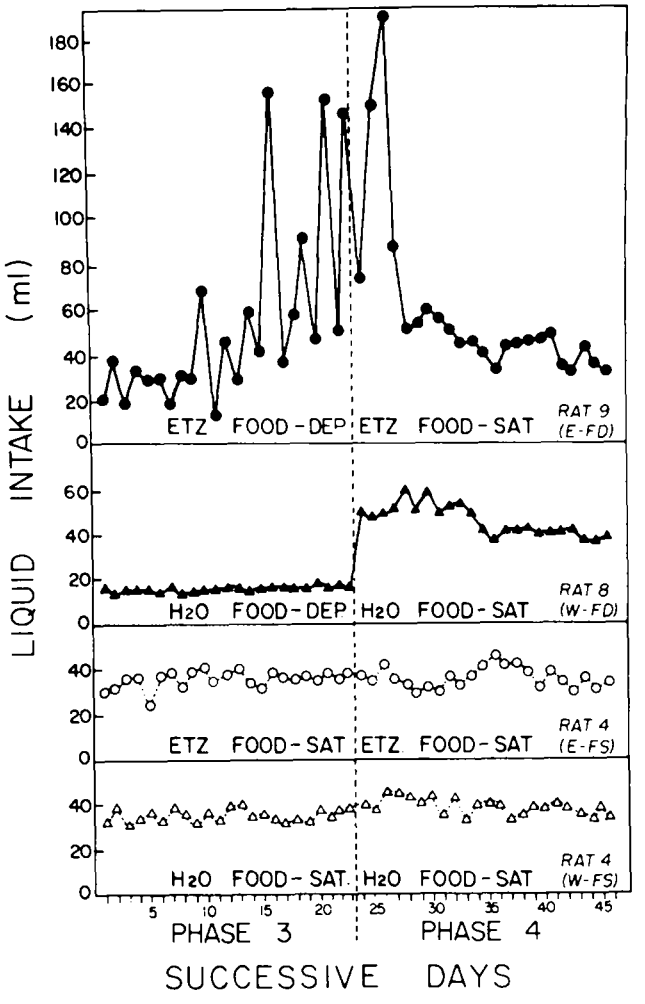

FIG. 2. The amount of liquid consumed per 24 hr is presented for a series of successive days during Phases 3 and 4. Data for individual rats from each group (in parentheses) are presented as most representative of the mean of the group.

The weights of each group of rats were changing at different rates throughout the experiment as a function of the food-deprivation/-satiation conditions and liquid consumed; thus, estimates of actual drug intake (μg/kg) cannot be accurately made based on Fig. 1. Table 1, therefore, summarizes the data on volume consumed per unit of body weight (ml/kg) for all four experimental phases. The most significant change was apparent in Group E-FD during Phase 3. The volume of drug intake and quantity of drug consumed per kg of body weight were more than two times higher than in Phases 1, 2 or 4. A much smaller increase in drug intake over time was found in the etonitazene food-satiated group (E-FS). Group W-FD showed a decline in ml per kg of water consumed during the food deprivation phase. There were no systematic increases or decreases in ml/kg in Group W-FS.

TABLE 1

MEAN 24 HR LIQUID INTAKE IN ML/KG AND ETONITAZENE (5.0 μg/ml) INTAKE (MG/KG) DURING THE LAST 5 DAYS OF EACH EXPERIMENTAL PHASE*

Group		Experimental Phase 1	2	3	4
E-FD	ml/kg	84.8	80.0	224.2	108.9
	mg/kg		0.40	1.12	0.54
E-FS	ml/kg	89.2	89.5	97.8	99.9
	mg/kg		0.45	0.49	0.50
W-FD	ml/kg	87.0	81.5	73.1	89.1
W-FS	ml/kg	88.5	81.1	89.3	81.6

*Each value is the mean of 40 or 45 values (8 or 9 rats × 5 observations each).

At the end of Phase 4, the food-deprived and food-satiated conditions were replicated in the experimental group, E-FD, in order to provide further evidence that the dramatic changes in drug intake found in the group were specifically related to feeding conditions. The increases that occurred during Phase 3 were slow in onset and did not stabilize for 17 days. This suggested tolerance and/or a learning mechanism may be involved. A within-group replication would also provide information in support of these hypotheses. For instance, an immediate increase in drug intake would support a learning interpretation; while a gradual increase might suggest the re-establishment of tolerance. Figure 3 shows the results of repeated food-deprivation/food-satiation phases in Group E-FD. Increases in etonitazene drinking occurred immediately during the second food-deprivation phase (FD-2). However, decreases in etonitazene drinking occurred at a slower rate during the second food-satiation phase (FS-2). The first food-satiation phase was characterized by a steady decline in mean liquid intake, while the second phase was characterized by a general decline in etonitazene intake interspersed with periods (3–5 days) of high intake. The alternating pattern of drinking was present in all rats of this group during both food-deprivation phases. This pattern disappeared during the food-satiation phases.

In addition to the large quantities of etonitazene consumed and the erratic drinking patterns of Group E-FD, other changes in behavior were seen during the food-deprivation phases. Stereotypy occurred in all rats in the group from the onset of the food deprivation. It was predominantly characterized by self-mutilation, specifically bit-

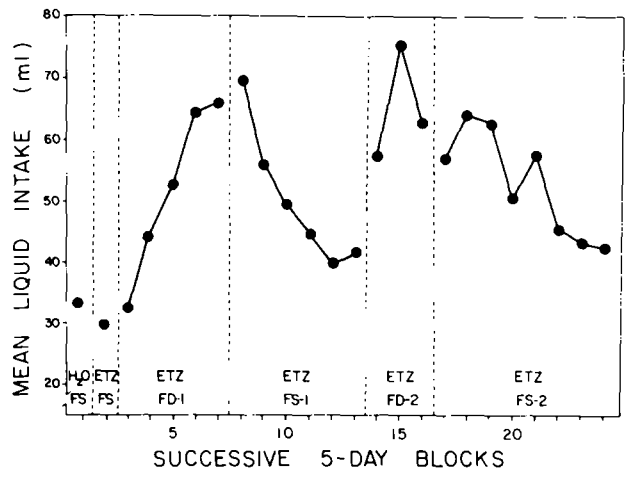

FIG. 3. Mean liquid intake is presented over a series of successive five-day blocks for Group E-FD. The first four panels represent the Experimental Phases 1–4. The last two panels of data points represent a replication of the food-deprivation (FD-2) and food-satiation (FS-2) conditions. Note: Block 7 consists of a 3-day mean.

ing of the front paws. By the end of Phase 3, all rats in Group E-FD had chewed all digits off the front paws. The rats also engaged in rocking behavior and biting of the grid floor. Several of the rats consumed over 200 ml of the etonitazene solution on alternating days. Self-mutilation became progressively severe as these high rates of drinking emerged. However, self-mutilation and other stereotyped behavior ceased abruptly when the rats were food-satiated, and this behavior did not occur in any of the other groups.

DISCUSSION

Food deprivation increased the mean amount of etonitazene consumed by rats by more than 100% (Group E-FD). The increase in etonitazene drinking began approximately three days after the onset of food deprivation and increased over a two-week period. Gross self-mutilation, stereotypy, and highly variable drinking patterns were also noted in the food-deprived group drinking etonitazene. Similar forms of self-mutilation have been reported [4,22], and variable drinking patterns [4] have been noted previously. Tolerance alone is not sufficient to explain the increase in etonitazene consumed, as a food-satiated control group (E-FS) increased their etonitazene consumption by only 25% over the same length of time. Food deprivation alone did not account for increases in etonitazene intake, since food deprivation resulted in a decrease in liquid intake for a group drinking water. Group W-FD decreased their mean water consumption by about 50% when food-deprived. Futhermore, the increase in etonitazene intake in both food-deprived and food-satiated groups was not a result of increased hydrational needs in young adult rats. The food-satiated control group's (W-FS) water intake did not show any systematic changes throughout the entire period.

The results of this experiment suggest there is an interaction between food deprivation and the effects of etonitazene consumption, which produces gross changes in behavior. The increased etonitazene drinking found during food deprivation in the present study is in agreement with earlier findings from this laboratory concerning increases in ethanol intake in food-deprived rats [15,16]. The increases in ethanol intake could be interpreted as caloric replacement; however, this would not apply to the present etonitazene experiments. Others have briefly noted that nicotine intake of rats maintained at reduced body weights was considerably higher than that of a group of free-feeding rats [8]. Thus, it appears that this food deprivation effect occurs with three of the four major classes of drugs abused by humans. The increasing generality of this finding argues against interpretations involving specific pharmacological actions of a class of drugs; such as the CNS depressant action of the general depressants, anorectic effects produced by opioids, or a specific role of the endorphins. Instead, these results suggest a more general mechanism whereby the interoceptive stimuli related to food deprivation become associated with the reinforcing properties of the drugs.

Food deprivation is routinely used in several major areas of research concerning drugs, including many animal behavioral tests used in the initial screening of drugs for classification and abuse liability. An implication of the present research is that food deprivation may be an important variable for consideration in future studies involving drug self-administration.

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