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Ethanol Intakes and Preferences in the Desalivate Rat[1]

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ROEHRS, T. A. AND H. H. SAMSON. *Ethanol intakes and preferences in the desalivate rat.* PHARMAC. BIOCHEM. BEHAV. 12(2) 223–227, 1980.—Desalivate and control rats were tested for ethanol versus water preference (5%, 10% and 20% (v/v) ethanol solutions). Each concentration of ethanol was presented for six days in an ascending, descending, or mixed presentation schedule. Following preference tests, intakes of first 10% (15 days), then 5% (15 days), and finally 10% (5 days) ethanol as the only available fluid were determined. Blood ethanol concentrations were measured (22:00 hr, 24:00 hr, 02:00 hr) during the final 10% ethanol intake test. Desalivate and control rats showed similar aversions to ethanol at all concentrations with relative ethanol intake being a negative function of concentration. During ethanol and water intake tests, desalivates drank significantly greater amounts (ml/100 g body weight) of all drinking fluids than controls. However, for both groups intake of 10% ethanol was reduced significantly from water baseline levels. Although desalivates consumed as much ethanol as controls, their blood ethanol concentrations at all times tested were slightly lower than controls. During the ethanol intake test desalivate rats lost body weight, while control rats gained body weight.

Desalivation Ethanol intake Ethanol preference Blood ethanol Rat

DESALIVATION produces a chronic dry mouth, and as a result, to facilitate dry food ingestion, the desalivate rat develops a prandial pattern of drinking in which a small draught of water is drunk immediately after a morsel of food is taken [6, 11, 12]. Following recovery from surgery and the acquisition of a prandial pattern of drinking, the desalivate rat maintains a markedly increased total daily water intake when the diet is dry food [3,12]. This increased water intake, two or three times control levels, is a product of the prandial pattern of drinking. Desalivation also produces alterations in gustatory preferences, presumably due to the role of saliva in stimulating taste receptors [9]. The presence or absence of saliva is important in regulating the amount of NaCl a rat will ingest [12]. Desalivate rats show a greater preference for isotonic saline and a reduced preference for hypertonic saline compared to controls [15,16]. Further, the quinine rejection threshold of desalivates is raised relative to that of controls [2,12].

The characteristics of the ingestion pattern suggest that the desalivate rat may be a useful experimental preparation for studying chronic and excessive ethanol intake. The purpose of the present study was to investigate ethanol preference and intake in the desalivate rat. Since desalivation results in altered gustatory preference, the desalivate rat may accept and prefer higher concentrations of ethanol. Furthermore, since the daily water intake of the desalivate is twice that of a normal rat, the desalivate may ingest sufficient amounts of ethanol so as to maintain consistently high blood ethanol levels when ethanol is the only available drinking fluid.

METHOD

Animals and Experimental Environment

Twenty-three male Long Evans rats, 120 days old at the start of the experiment, were used. They were housed individually in stainless steel rodent cages. Drinking fluids were available from ball-point drinking tubes and standard water bottles attached to the cages. Standard laboratory chow in unlimited quantities was available continuously on the cage floor. There was artificial illumination from 7:30 to 19:30 hours.

Procedure

Table 1 outlines the experimental procedure.

Desalivation. Daily water intake and body weights were recorded for seven days prior to surgery. All animals were anesthetized with sodium pentobarbital (42 mg/kg, IP). Thirteen rats, randomly chosen, were desalivated and the re-

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TABLE 1
EXPERIMENTAL PROCEDURE

Phases		Fluid	Number of bottles	Number of days tested
Pre Op	baseline	water	1	7
Post Op				
1. desalivation recovery		water	1	15
2. drink latency test & recovery		water	1	3
3. ethanol preference tests				
a. baseline		water/water	2	6
b. 5, 10, or 20% ethanol		water/ethanol	2	6
c. 5, 10, or 20% ethanol		water/ethanol	2	6
d. 5, 10, or 20% ethanol		water/ethanol	2	6
4. ethanol intake tests				
a. baseline		water	1	7
b. 10%		ethanol	1	15
c. 5%		ethanol	1	15
d. 10%		ethanol	1	5

maining animals served as controls. Desalivation was accomplished by the ligation of the parotid ducts and extirpation of the submaxillary-sublingual gland complex [6]. The ducts were ligated as they passed along the lateral surface of the masseter muscle and care was taken not to include the nearby ramus mandibularis nerve. The procedure for control rats involved an incision and exposure of the salivary glands. Following surgery daily water intake and body weights were recorded for 15 recovery days (Post Op Phase 1). All rats were food deprived for 24 hr and then tested for drink latency (Post Op Phase 2). Time (sec) to the first drinking tube contact after placing 25 g laboratory chow in the cage was defined as drink latency. In order to insure that desalivation was complete and that a prandial drinking pattern had been acquired, only desalivate rats showing a drink latency of less than 30 sec and weighing at least the presurgery values were included in subsequent testing.

Ethanol preference tests. Following two days of ad lib food and water after the drink latency test all rats were given access to two drinking tubes and bottles filled with water for six days (Post Op Phase 3a). Each day tubes were switched from left to right and intake from each fluid container was recorded in order to determine a tube or location preference. Thereafter, an ethanol solution of 5%, 10%, or 20% (v/v) concentration was placed in the preferred tube or position according to three different sequences (Post Op Phases 3b, c, and d). One-third of both desalivate and control rats received an ascending series of concentrations, one-third a descending series, and the final third received the series of 10%, 5%, and 20% ethanol. Each concentration was presented for six days. Intake from the water and the ethanol containers was recorded daily and body weights were recorded every third day. Preference for ethanol was assessed by determining the proportion of ethanol to total fluid intake.

Ethanol intake tests. Following the ethanol preference tests all rats were given a single drinking tube and fluid (Post Op Phase 4). For the first seven days water was the only available fluid. Then a 10%, followed by a 5% ethanol solution, each for 15 days, replaced the water. Finally, a 10% ethanol solution was returned for a second five-days determination. Fluid intake was recorded daily and body weights were recorded every third day. On the last day of the second 10% ethanol intake test, blood samples were collected from all rats. Samples were taken from one-third of both desalivate and control rats at 22:00 hr, from the next third at 24:00 hr, and from the last third at 02:00 hr. Blood ethanol levels were determined by the enzymatic method [1].

RESULTS

After the 18 day postoperative recovery (Post Op Phase 1 and 2), nine of the thirteen rats which underwent the desalivation procedure had regained at least their presurgery body weights and had drink latencies of 30 sec or less upon access to 25 g laboratory chow following 24 hr food deprivation. Control rats all had drink latencies of greater than 180 sec. The results presented here are for these nine desalivate rats and the ten control rats.

The daily water intake of desalivate and control rats before and after surgery is presented in Table 2 as means over five day blocks. All fluid intake data have been expressed as ml/100 g body weight in order to equate for the disparity in body weights [4,5]. Statistical tests were performed on the transformed data. The daily water intake of desalivate rats increased significantly during the postoperative recovery to a level twice that of control rats, $F(1,17)=132.6, p<0.001$. The intake of the desalivate rats remained elevated during the baseline period of the ethanol preference test when two bottles, both with water, were available. Table 3 presents the mean body weight of desalivate and control rats during the preoperative and postoperative phases of the experiment. By Days 10–15 after surgery the mean body weight of the desalivate rats was greater than their presurgery level, although significantly lower than the mean body weight of the control rats ($t=2.26$, $p<0.05$). After the two-week recovery period and during phases of the experiment when at least one bottle with water was available, desalivates maintained and even gained body weight. Across Phase 1c–3a desalivates lost 1 g and controls gained 32.5 g, while over the ethanol preference test (Phase 3) desalivate and control rats gained 9 and 8 g, respectively.

TABLE 2
DAILY WATER INTAKE (ml/100 g BODY WT)

Phases	Control	Desalivates
Pre Op	10.8 ± 0.73*	11.1 ± 0.52
Post Op		
1a†	8.9 ± 0.36	13.6 ± 1.76‡
1b	9.7 ± 0.30	23.4 ± 0.86‡
1c	10.1 ± 0.43	22.1 ± 1.50‡
3a	11.7 ± 0.80	19.9 ± 1.26‡

*Mean ± standard error.
†Phase 1a, 1b, and 1c are five day blocks of desalivation recovery.
‡$p<0.01$.

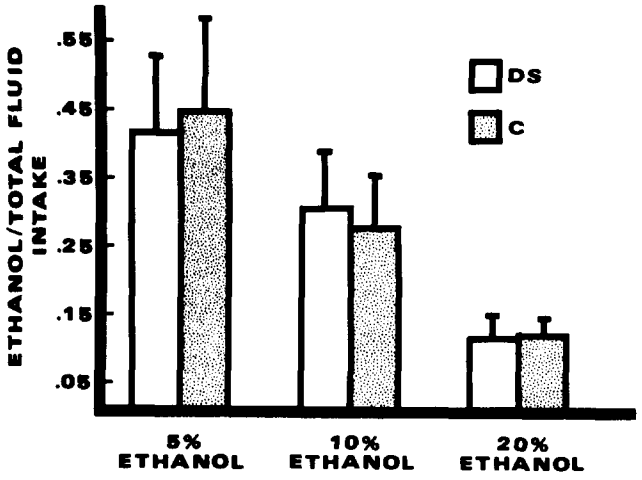

FIG. 1. Proportion of ethanol to total fluid intake in desalivate and control rats as a function of ethanol concentration (DS=desalivate rats, C=sham operated control rats). Data presented are means and standard error of the means.

TABLE 3
BODY WEIGHT

Phases	Control	Desalivates
Pre Op	447.6 ± 19.46*	405.2 ± 15.96
Post Op		
1†	447.8 ± 20.83	419.3 ± 15.40
3a	480.3 ± 21.40	418.4 ± 15.56
3b	483.4 ± 23.25	422.0 ± 16.62
3c	485.2 ± 23.01	421.3 ± 16.26
3d	488.4 ± 23.17	427.8 ± 17.20
4b	494.3 ± 23.09	420.3 ± 16.31
4c	512.2 ± 23.43	420.4 ± 16.40
4d	501.8 ± 18.48	416.4 ± 16.19

*Mean ± standard error.
†days 11–15.

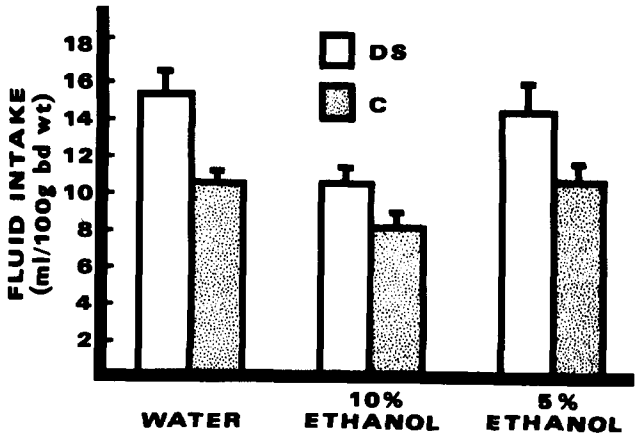

FIG. 2. Daily fluid intake of desalivate and control rats when water or ethanol solution is the only available drinking fluid (DS=desalivate rats, C=sham operated control rats). Data presented are means and standard error of the means, taken over the last five days of each exposure.

The results of the ethanol preference test are presented in Fig. 1. Desalivate and control rats did not differ in response to the ethanol preference test. Both groups had a greater preference for water than ethanol at all concentrations presented. Relative ethanol intake was a negative function of the ethanol concentration with intake of the 20% concentration being approximately 12% of the total intake and intake of the 5% concentration about 45% of the total. The testing sequence had a slight, but nonsignificant, effect on relative ethanol intake. Rats in both groups receiving the 20% concentration first showed a greater overall aversion to ethanol than those receiving the 5% concentration first (27.3 ± 17.40% vs 33.8 ± 17.73%).

The fluid intake of desalivate and control rats when water or ethanol was the only available drinking fluid is presented in Fig. 2. Means were calculated across the last five days of each exposure. The values for 10% ethanol represent Phase 4b. For both groups, intake of 10% ethanol was reduced significantly from the water baseline levels, $F(2,32)=40.02$, $p<0.001$, while intake of 5% ethanol was more similar to the water baseline. The desalivate group took significantly greater amounts of all drinking fluids, $F(1,17)=7.21$, $p<0.025$. The body weights of desalivate and control rats during the ethanol intake test (Phase 4) are found in Table 2. Across Phases 3d–4d control rats showed an overall gain in body weight while desalivate rats lost body weight. This difference in body weight change between the groups was significant ($t=5.15$, $p<0.001$).

Ethanol intakes and blood ethanol concentrations (mg/100 ml) for desalivate and control rats are presented in Table 4. Desalivate rats consumed a slightly, but not significantly, greater amount of ethanol than control rats. This small difference was a product of a slightly, but not significantly, larger cumulative intake of the 10% ethanol solution and the significantly smaller body weight of the desalivate rats ($t=3.48$, $p<0.01$). However, the blood ethanol concentration of desalivate rats, although not significantly different than that of control rats, was somewhat lower.

DISCUSSION

Desalivation did not alter the response to ethanol preference testing in that both groups showed a similar aversion to

TABLE 4
ETHANOL INTAKE (g/kg) AND BLOOD ETHANOL (mg/100 ml)

	Control	Desalivates
Intake (ml)*	18.22 ± 1.36†	20.56 ± 2.77
Weight (g)	501.80 ± 18.48	416.40 ± 16.19
Ethanol g/kg bd. wt.	2.93 ± 0.29	4.01 ± 0.61
Blood ethanol (mg/100 ml)	38.20 ± 11.06	30.43 ± 11.49

*Cumulative intake of 10% ethanol from 12 noon to time of blood sample.
†Mean ± standard error.

ethanol at all concentrations presented. For both groups intake of 5% ethanol, which was the lowest concentration presented, was approximately 45% of total fluid intake. This finding is fairly consistent with previous studies where the ethanol preference threshold, defined as that concentration in an ascending or descending series of which ethanol constitutes 50% of the rat's daily fluid intake, usually is found to be 4–6% [7,8].

As previously demonstrated [3,12], the daily water intake of the desalivate rats increased to twice that of the control group. This phenomenon can be attributed to a prandial pattern of drinking acquired during the post-operative recovery. This increased intake was sustained throughout those phases of the experiment in which at least one bottle of water and food was available. During these phases of the experiment (Phase 1 and 3) the desalivate rats maintained and even gained body weight. However, when an ethanol solution was the only available drinking fluid, the fluid intake and body weight of the desalivate rats declined. Apparently the ethanol solutions were sufficiently aversive so as to alter the drinking pattern of the desalivates, thereby decreasing the total daily fluid intake. It is interesting that this reduction in fluid intake, at the expense of normal body weight increase, occurred after the 18 day ethanol preference test, during which desalivates should have had an opportunity to become acclimated to ethanol under choice conditions. It has been shown that repeated exposure to ethanol solutions with water present as an alternative drinking fluid enhances ethanol intake over successive free-choice tests [13,14]. Thus, despite the acclimation period, the desalivate rats did not drink sufficient quantities of the ethanol solution so as to facilitate food ingestion and maintain normal body weight increases.

The blood ethanol concentrations of the desalivate rats were slightly lower than those of the control rats. The lower blood ethanol level of the desalivate, despite ethanol intakes comparable to controls, could be due to the fact that the desalivates may have distributed their fluid intake over longer time intervals. Although the drinking pattern of desalivates when ethanol was the sole drinking fluid was not assessed directly, desalivates may have maintained a drinking pattern similar to the typical prandial pattern, but reduced their total food and fluid consumption. A prandial pattern of drinking is characterized by frequent small bouts of drinking, typically 0.1 ml in size, which alternate with short episodes of eating [6]. Consequently, although the total intake of the desalivate was comparable to controls, the rate may have been slower. Thus, in the present experiment the rate of ethanol intake for desalivates did not exceed the rate of ethanol metabolism by as large a margin as in controls. This finding corresponds with the results of a previous study which indicates that not just amount, but the distribution of drinking within a given time period, is important in determining blood ethanol concentrations [10].

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