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A Silicone Delivery System for Producing Binge and Continuous Ethanol Intoxication in Rats

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ELLISON, G., S. STAUGAITIS AND P. CRANE. *A silicone delivery system for producing binge and continuous ethanol intoxication in rats*. PHARMAC. BIOCHEM. BEHAV. **14**(2) 207–211, 1981.—A silicone delivery system for administering large quantities of ethanol (ETOH) to rats is described. When implanted subcutaneously and filled with 95% ETOH (v/v), lethal quantities can be administered within 5 days. Two different models are described: a rapid release thin-walled silicone pillow which can be filled with 95% ETOH for 3 hours daily so as to induce binge-like, transient high blood ETOH levels and motor impairment, and a thicker pillow which can be filled daily with 84% ETOH (v/v) so as to produce continuous ETOH administration. When different groups of rats administered similar amounts of ETOH using these two different regimens were compared, it was found that caloric intake (food+ETOH) and body weight were reduced in the Binge group whereas the Continuous group gained weight although their total caloric intake was similar to Controls. This delivery system can deliver appreciable amounts of ETOH to rats in the absence of gustatory stimulation and in two intake patterns which have been reported to occur in alcohlics.

Ethanol Silicone delivery system Binge vs continuous drug regimen Withdrawal and intoxication

THERE are a variety of methods available for administering ethanol to animals, including the liquid diet [3,8], intravenous [4] or intragastric [5] infusion, ethanol vapors [9], gastric intubation [10] schedule induced polydipsia [11], and silicone release systems [7]. Each technique has certain advantages and disadvantages (for review, see [2]). In this paper we present a silicone release system called the ethanol pillow which is capable of administering large quantities of ethanol to rats.

In designing this system we attempted to achieve two goals. The first was to induce appreciable levels of blood ethanol without the necessity of combining the silicone delivery system with intraperitoneal injection as in the case of the SERT described by Erickson *et al.* [7]. Secondly, we attempted to design a system which could deliver ethanol in Continuous versus Binge patterns. Jellinek [10] described different types of alcoholics in terms of Binge or Continuous drinking patterns. In the case of amphetamine administration, continuous levels were more toxic to the brain than binge administration of the same amounts [6].

METHOD

Subjects

Male albino rats (387–505 g from Simonson Laboratories, Gilroy, CA) were housed in individual cages under continuous illumination and provided with Purina Rat Pellets and water ad lib. For the main experiment, rats were assigned to one of 3 groups (N=10 each) with equal mean body weight (418 g) called "Binge", "Continuous", and "controls".

Pillow Construction

The ethanol pillow (Fig. 1) was constructed from Silastic Medical Grade Sheeting (Dow Corning) of 0.02 inch (50 mm) thickness (for the Continuous pillow) and 0.005 inch (0.127 mm) thick sheeting (for the Binge pillow). In each case, a 64×76 mm piece of sheeting was folded in half lengthwise, a short segment of Silastic tubing (ID=0.062 inch, 1.58 mm; OD=0.125 inch, 3.18 mm) was inserted into one end, and the edges were clamped between cardboard pieces with spring type paper clips. Silicone Type A Adhesive was applied to the edges and allowed to dry. Silastic elastomer was used to coat rough edges. Each pillow had final dimensions of 78×32 mm and a releasable surface area of 3800 mm^2. Finally, a piece of Marlex Mesh (Davol) was attached to the tubing to secure the filling tube of the pillow in place and prevent the exit hole from enlarging or becoming infected after implantation. The end of the filling tube was plugged with a short length of steel wire.

Surgery

The pillows were sterilized in 70% (v/v) ethanol for 24 hours prior to surgery and then dusted with Neosporin powder. Rats were anesthetized with Nembutal (50 mg/kg, IP)

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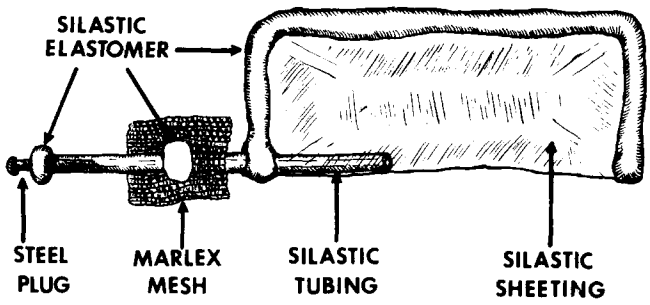

FIG. 1. Drawing of the alcohol pillow.

and given 0.04 mg Atropine (IP), shaved on the back above the front and rear legs and scrubbed with an iodine solution.

A 3 cm transverse incision (3 cm) was made in the back and a subcutaneous cavity made with blunt scissors. The pillow was held with a curved hemostat, inserted into the subdermal cavity, and then pushed rostrally so that the tubing exited through a small incision in the neck region. The pillow was positioned so that the mesh was against the skin beneath the opening. The caudal incision was sutured, a topical antibiotic (Mycitracin) was applied and a Bicillin injection (0.1 cc, IM) was given. The pillow was filled with water and plugged. The rats were given one week to recover from surgery.

Ethanol Administration

In early tests of the pillow, it was found that only ethanol and not water diffused out of the pillows. This conclusion was based on an analysis of the contents remaining in the pillow 24 hr after filling using gas chromatography. This meant that the amount of ethanol released could be calculated from the difference in volumes of the pillow's contents at filling and emptying times. The amount of ethanol released by the pillows *in vitro* was considerably greater than when the same amount of solution was tested *in vivo* and the rate of ethanol release *in vivo* decreased with time. Further experiments were conducted only with pillows implanted in rats, and different volumes and concentrations of ethanol were tested in order to choose Binge and Continuous regimens.

The Binge group was filled at the same time each day with 10.0 ml of 95% (v/v) ethanol; three hours later the remaining fluid was removed, measured, and the pillow was refilled with 10.0 ml of water. The pillows of the Continuous group were filled at the same time each day with 7.0 ml of 84% ethanol (v/v); 24 hours later the remaining fluid was removed and measured, and the pillow was refilled with new solution. The pillows of the Control group were filled daily with water; half of the Controls were implanted with pillows like that of the Binge animals and handled similarly, and half treated like Continuous animals. All rats were handled at three hours after filling and at this time the area around the tubing was cleansed using Povodone.

Open Field Activity

Activity in open field was observed on Days 1, 4, and 12 at 3, 6, and 24 hours after filling the pillows with ethanol. The open field was a flat surface (115×70 cm) painted with a square grid (3×5, 23 cm squares). The rat was placed in the center square and during the following minute the number of squares entered was counted. Motor coordination in 5 rats from each group was rated by a trained observer who was using a rating scale modified from Majchrowicz and Hunt [12]: (1) absence of righting reflex, (2) crawling, rights slowly, (3) staggering or gait impairment, (4) sedation or reduced muscle tone, (5) neutral. In the same rats, food and water were weighed for three days prior to ("Baseline") and on each day of ethanol administration.

Plasma Ethanol Determination

Following each observation, 0.2–0.3 ml of blood was collected by nicking the tail vein with a sharp scalpel. The samples were centrifuged (10,000 g, 5 min) and the plasma removed. Plasma specimens were stored at −20°C, and thawed immediately before injection. Ninety microliters of plasma were mixed with ten microliters of internal standard solution (20 mg n-propanol per ml distilled water). A 0.1 microliter aliquot of the mixture was injected into a Packard 419 Gas Chromatograph. The GC column was a 6 ft×2 mm (ID) glass column packed with 0.2% Carbowax 1500 on Carbopack C (80–100 mesh). Duplicate aliquots were injected and the peak areas of ethanol and n-propanol were integrated with a Varian Model 85 Integrator. This system was linear above ethanol concentrations of 0.10 mg/ml.

RESULTS

In pilot experiments, rats (mean wt=450 g) implanted with continuous type pillows were filled daily with 10 ml of 95% (v/v) ethanol. The amount released was 4.4 ml of ethanol in 24 hours (i.e., 7–9 g/kg/day). The mean plasma ethanol levels (PEL) were 2.5 mg/ml after 24 hours and 5.6 mg/ml after 48 hours. The animals became comatose and mortality occurred after 3.5 days. Other animals (mean wt.=320 g) were given doses of 8–10 g/kg/day by filling the pillow with smaller volumes of 95% (v/v) ethanol. After 3 days of intoxication, the ethanol dose was reduced by filling the pillows with solutions of low ethanol concentration. When the dose was 3 g/kg or less per day, withdrawal symptoms such as audiogenic seizures, excitability, broadbased gait, arched tail and piloerection were observed during the next 24 hours.

In the experiment proper, when 95% and 84% ethanol solutions were left in the Binge and Continuous pillows for 3 and 24 hours, respectively, the amount of ethanol administered (mean ± SEM) was 2.0 ± 0.2 ml daily for the Binge group and 3.4 ± 0.1 ml for the Continuous group. These values did not vary more than 0.2 ml from day to day and did not change in pillows that were reused. The average daily doses given to the Binge and Continuous groups were 4.1 and 6.1 g/kg, respectively.

The mean activity in open field (squares entered) for each group is shown in Fig. 2. Variability on this test was high with the mean ± SEM for the 3 hr test on the first day being 7.9 ± 1.9 for the Controls, 0.5 ± 0.3 for the Binge group, and 8.2 ± 2.3 for the Continuous animals. Compared to Controls, activity in the Binge group was decreased significantly at nearly all observations (see caption to Fig. 2). The Continuous group generally showed an activity pattern that was similar to Controls although on Days 1 and 4 at 6 and 24 hours after administration, their activity was slightly decreased.

TABLE 1

MEAN PLASMA ETHANOL LEVELS ON DAYS 1, 4, AND 12 OF ETHANOL ADMINISTRATION AT 3, 6, AND 24 HOURS AFTER FILLING THE PILLOWS WITH ETHANOL SOLUTIONS (OR WATER FOR CONTROLS)

		Mean Plasma Ethanol (mg/ml ± SEM)		
Group	Hours*	Day 1	Day 4	Day 12
Binge				
	3	3.05 ± .61 (4)†	3.58 ± .40 (7)	3.26 ± .20 (7)
	6	2.84 ± .31 (4)	3.44 ± .65 (6)	3.41 ± .57 (5)
	24	<.1 (5)	<.1 (5)	<.1 (5)
Continuous				
	3	0.17 ± .04 (4)	0.13 ± .03 (10)	0.19 ± .05 (9)
	6	<.1 (4)	0.22 ± .05 (10)	0.20 ± .05 (10)
	24	<.1 (4)	<.1 (5)	<.1 (5)
Control		<.1	(10 samples randomly chosen)	

*Hours after filling the pillows with ethanol.
†Number of samples.

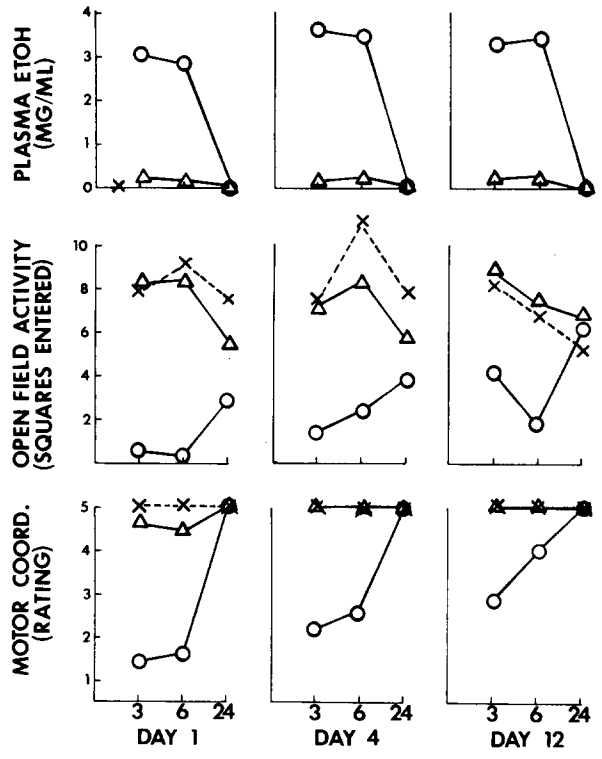

FIG. 2. Blood ethanol levels, activity in open field, and locomotion rating (described in *Method*) for the Control group (×---×), the Continuous group (△——△) and the Binge group (○——○). Observations were made on Days 1, 4, and 12 of ethanol administration at 3, 6, and 12 of ethanol administration at 3, 6, and 24 hr after filling the pillows. Compared to controls open field activity was significantly reduced in the binge animals at all points on day 1 and at the 3 and 6 hr points on Days 4 and 12 and motor coordination was decreased at the 3 hr test on all days ($p<0.01$, t-tests).

When motor coordination was rated the Binge group showed a decreased impairment across successive observation days (Fig. 2), implying the development of tolerance. On Day 1 at 3 hours the Binge group generally displayed a slowed righting reflex and locomoted by crawling, while at the same time on Day 12, they were able to walk with their abdomens raised although they staggered. At 24 hours after filling the pillow (and 21 hours after emptying the pillow) on all observation days there were no longer signs of ethanol intoxication in the Binge group. The Continuous group did not show motor impairment during any observation.

The analysis of the blood samples taken from each rat immediately after each observation period revealed that the mean plasma ethanol level (PEL) of the Binge group was very high 3 hours after filling their pillows with ethanol (Table 1). The mean PEL was still elevated at the 6 hour observation (i.e., 3 hours after ethanol was removed from the pillows), but ethanol was not detectable in the 24 hour samples. During the first six hours, the Continuous group had detectable but negligible PELs.

The average body weight and food and water consumption of 5 rats from each group during 3 days prior to ethanol administration ("Baseline") and during the first and second weeks of the 14 days of ethanol administration are given in Table 2. The Continuous group showed a 7% weight gain compared to pre-administration body weight whereas the mean body weight of the Binge group decreased by about 10%. Compared to "Baseline", mean food consumption decreased significantly in all groups during the first week (t-test, $p<0.05$). During the second week both ethanol-treated groups decreased their food consumption significantly (t-test, $p<0.05$). The total caloric intake from food (3.6 kcal/g) and ethanol (7.1 kcal/g) are also given for all groups in Table 2. The total number of calories received by the Binge group was less than Controls but the calories obtained by the Continuous group was similar to Controls. Water consumption increased significantly after two weeks of ethanol administration in the Binge group (t-test, $p<0.05$). The Continuous

TABLE 2

MEAN BODY WEIGHT AND FOOD AND WATER CONSUMPTION (±SEM) IN THE THREE GROUPS

	Baseline	First Week	Second Week
Body weight (g)			
Control	423 ± 4	419 ± 6	425 ± 11
Continuous	410 ± 11	431 ± 8*	440 ± 10
Binge	411 ± 15	391 ± 11	380 ± 13
Food consumption (g)			
Control	23.6 ± 1.3	20.5 ± .9*	22.4 ± 1.0
Continuous	24.2 ± 1.5	14.5 ± .4*	16.6 ± .9*
Binge	22.3 ± 2.1	10.1 ± 1.3*	14.7 ± 1.6*
Total calories			
Control	85.0 ± 4.7	73.8 ± 3.2*	80.6 ± 3.6
Continuous	87.1 ± 5.4	71.3 ± 1.4*	78.8 ± 3.2*
Binge	80.3 ± 7.6	47.7 ± 4.7*	64.1 ± 5.8*
Water consumption (cc)			
Control	31.1 ± 1.6	30.9 ± 1.3	32.6 ± 1.8
Continuous	33.7 ± 2.9	24.5 ± 1.1	24.6 ± 1.5
Binge	25.7 ± 1.0	30.1 ± .8	39.2 ± 1.7*

*Means significantly different from Baseline, $p<0.05$, t-test using paired comparisons.

group tended to decrease water consumption while the Controls did not change.

After three weeks the pillows were removed and the surrounding tissue inspected. A collagenous sac, often filled with fluid, had formed around the pillow. Patches of infected tissue were observed in members of all groups, including Controls.

DISCUSSION

The technique for ethanol administration described here has several distinctive features. Using silicone pillows, large quantities of ethanol can be administered to rats and high plasma ethanol levels can be produced when either type of pillow is filled with concentrated ethanol. Different daily doses and regimens can be achieved by changing the volume and concentration of the ethanol solution or the thickness of the silicone sheeting. The amount of ethanol released is easily measured and reliable across days, and the materials for construction of these silicone pillows are readily obtained and, when cleaned and dried thoroughly, the pillows are reusable.

One objective in developing the ethanol pillow was to directly compare the effects of Binge (3 hour) and Continuous (24 hour) administration regimens, and to facilitate this it was initially intended that the same dose of ethanol be given to both Binge and Continuous groups. However, pilot experiments showed that an appreciable daily dose could be given during Binge administration without mortality, but that the same daily dose given in a Continuous fashion produced plasma ethanol levels which were not detectable. As a result the Continuous group in the present experiments were administered slightly more ethanol per day than the Binge animals (6 vs 4 g/kg). A 6 g/kg/day ethanol dose administered continuously is within a rat's metabolic capacity (0.3 g/kg/hr) [14] so that the mean blood ethanol levels obtained from the continuous group were low. However, the ethanol administered was sufficient to significantly affect feeding behavior.

Both ethanol administration regimens produced alterations in food and water consumption and body weight. After 14 days of daily ethanol administration, the Binge group was emaciated as evidenced by significant reductions in food consumption and body weight. The total caloric intake of the Continuous group was the same as Controls, but like the binge group they consumed less calories as Rat Chow. Reduced food consumption may have been a response to the caloric content of ethanol, since this also occurs when nutrients such as glucose or casein are administered subcutaneously to rats [1]. Although the caloric intake of both Continuous and Control groups were similar, the Continuous group gained weight. It may be hypothesized that ethanol administration decreased basal metabolic rate or activity levels which were not detected during open field observations. Finally, the Binge group increased their daily fluid intake, a phenomenon also observed in dogs given acute doses of ethanol [13], but a continuous dosage caused rats to reduce water consumption. To summarize, while both Binge and Continuous regimens had the same effect on food consumption, they had opposite effects on fluid intake and body weight. When similar amounts of ethanol are delivered in these two different regimens, there are a variety of differences in the effects of ethanol on body weight, fluid intake, and plasma ethanol levels.

There are several advantages to using this device for ethanol administration. Precise but large amounts of ethanol can be administered and the duration and schedule of ethanol administration are easily manipulated. The surgical procedures are much simpler than those required for intravenous [4] or intragastric [5] infusion techniques and the animal is not restrained by the infusion apparatus. Both the liquid diet [3,8] and schedule induced polydipsia techniques [11] are highly regarded as ethanol administration methods because animals can be maintained in such condition for a number of months. However for short term chronic treatment, the ethanol pillows may be preferred since no weight reduction is necessary, the need for food or water does not determine whether ethanol is administered, and pair fed controls may be incorporated into the experimental design. Finally, using the pillow method, ethanol is administered over a period of time and high blood ethanol levels can be maintained for several hours. This experimental control means that human ethanol intake patterns can be modeled more closely than by acute injection or intubation, where blood ethanol levels peak and decline rapidly.

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