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Multiple Exposures to Ethanol Facilitate Intravenous Self-Administration of Ethanol by Rats[1]

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NUMAN, R. *Multiple exposures to ethanol facilitate intravenous self-administration of ethanol by rats.* PHARMAC. BIOCHEM. BEHAV. 15(1) 101–108, 1981.—In Experiment 1, male hooded rats (N=11) were implanted with jugular cannulas, and housed in sound attenuated operant chambers 24 hr/day. The rats were exposed to periodic cycles of forced ethanol infusions (30% v/v, 9–16 g/kg/day over 4–6 days for each cycle). Following each cycle, forced infusions were discontinued, but the rats were allowed access to a lever for self-administration of ethanol on a fixed ratio 1 schedule (FR1). Each lever press infused 0.2 ml of ethanol (20% v/v). The rats were maintained on self-administration for at least 24 hr. If a rat did not develop self-administration behavior (SAB) within 24 hr, the next forced cycle of ethanol exposure was initiated. Eight of the 11 rats developed SAB after a mean of 5.25 cycles of exposure to ethanol, and were then tested for a mean of 15 days on self-administration under FR1, FR2, and FR3 schedules of reinforcement. All rats were tested on FR1 and self-administered a mean of 10.43 g ethanol/kg/day over a mean of 10.75 days. Four rats were subsequently tested on FR2 and FR3 and increased lever presses in order to maintain daily ethanol intake comparable to FR1. Following self-administration testing, the rats were placed on withdrawal and exhibited mild to severe withdrawal symptoms, suggesting that SAB maintained physical dependence. In Experiment 2, rats (N=6/group) were allowed to self-infuse either saline or ethanol (20% v/v). These rats had no prior exposure to either saline or ethanol, and forced infusions were never administered. The rats remained in their operant chambers for 21 days under FR1 contingencies. Each lever press led to a 0.2 ml infusion. None of the rats developed SAB, but the saline controls made more lever presses than the ethanol rats ($p<0.01$). These results suggest that the ethanol parameters yielding SAB in Experiment 1 are aversive to ethanol naive rats.

Self-administration Ethanol Intravenous infusion Rats Ethanol physical dependence Withdrawal hypothesis

A NUMBER of investigators have recently attempted to develop an animal model of alcoholism [7, 11, 23, 24, 25, 30]. Ideally, such a model should include at least two components: (1) physical dependence upon ethanol, and (2) self-administration of substantial quantities of ethanol in a free-choice situation. The first criterion has been achieved in a number of species, using a variety of methods [9, 11, 13, 20, 23, 24, 27, 28]. The criterion of self-administration has met with some success, especially when schedule induced polydipsia, and/or low concentrations of ethanol have been employed [2, 10, 21, 22, 24, 31]. Other procedures, the most striking being the intravenous work with monkeys [4,34], have also produced positive data. However, many other attempts to achieve substantial self-administration of ethanol have been plagued with difficulties, especially when high, nonpreferred concentrations of ethanol were employed [2, 6, 7, 8, 23, 24]. These difficulties seem to be related to an aversion to the gustatory-olfactory, and postingestional effects of ethanol [6, 8, 18, 21]. Further, this aversion may be conditionable, limiting the effectiveness of flavor masking procedures [3, 6, 8]. Oronasal sensory aversions may be bypassed by the use of intravenous or intragastric methodology [4, 7, 31, 34]. However, the aversive postingestional effects of ethanol are probably not reduced when these methods are employed. Another approach has attempted to overcome ethanol aversions by exposing animals to multiple withdrawal episodes. This procedure has been used with the expectation that the animals would learn the association between alcohol intake and relief of withdrawal distress. Further, cyclical exposure to dependence induction and withdrawal may also facilitate tolerance to ethanol and increase withdrawal discomfort, which in turn might promote self-administration. Walker and Zornetzer [33], using a liquid diet procedure in mice, found that alcohol withdrawal symptoms were more severe following a second withdrawal period. The results suggested to the authors that physical

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dependence develops more rapidly and is more severe in previously dependent animals. Further, Kalant *et al.* [16] suggest that tolerance to alcohol is more readily induced in previously tolerant rats than in alcohol naive animals. These findings have been extended by Baker and Cannon [1]. Hunter *et al.* [14] used a liquid diet procedure to study the effects of multiple withdrawal episodes in rats on volitional ethanol consumption. They found that rats would not consume ethanol in a free choice test, despite severe withdrawal symptoms, following one or two periods of forced alcohol intoxication. However, following a third exposure to forced alcohol intoxication there was a substantial increase in voluntary consumption. Hunter *et al.* [14] conclude that laboratory animals may be able to learn the association between alcohol and relief of withdrawal symptoms if a number of withdrawal episodes are experienced. Deutsch and Walton [7] have recently confirmed these findings in rats using a gastric intubation procedure. While these findings are encouraging, only a few rats were used, self-administration was not maintained, and it is not clear whether or not the ethanol self-administration maintained physical dependence.

While much work is still necessary, the data just reviewed suggest that initial consumption of high (>10%) concentrations of ethanol is aversive. However, multiple exposures to alcohol intoxication and withdrawal may cause a decrease in this aversion, while increasing the severity of abstinence distress. This dual effect might, therefore, increase 'drug hunger' and lead to an enhanced volitional consumption of alcohol.

The present research presents data that lend further support to this hypothesis. The methods employed not only exposed rats to cycles of intoxication and withdrawal, but also bypassed oronasal sensory receptors. We used an intravenous (IV) procedure to expose rats to multiple withdrawal episodes during which ethanol could be self-infused. The IV procedure has not been a popular candidate for use in animal models of alcoholism. While a few investigators have used the IV procedure in monkeys with some success [4, 5, 17, 34], other less expensive species have not been tested. However, recent work has successfully applied the IV method in rats to assess the reinforcing effects of low doses of ethanol [31], and our laboratory has used the IV method to induce physical dependence upon ethanol in rats [27]. Therefore, it seemed to us that the IV method, which bypasses the oronasal sensory pathways, and directly enters the bloodstream in quantifiable amounts, might lead to superior self-administration data in rats.

EXPERIMENT 1

METHOD

Animals and Apparatus

Eleven male hooded rats of the Long-Evans strain which weighed between 280 and 366 g (mean weight: 318 g) were used. Each rat was implanted with an indwelling jugular cannula while under Nembutal anesthesia (50 mg/kg). The cannula was passed from the jugular vein, subcutaneously, to exit at the dorsal region of the animal's neck. The rat was then placed in a harness which had a spring (30 cm in length) attached to it, and the cannula was passed through this protective spring. Each rat was then individually housed in an operant chamber that was enclosed in a sound attenuated cubicle. The spring and cannula tubing were attached to a cannular feed-through swivel (BRS/LVE) positioned above the center of the operant chamber. The swivel, in turn, was connected by way of polyethylene tubing to an injection system (Harvard Apparatus Compact Syringe Pump, Model 975) located outside of the sound attenuating cubicle. More detailed descriptions of the surgical procedure, and directions for cannula and harness construction can be found in a recent publication [32].

The animals remained in the chambers 24 hours a day throughout the entire experiment. Food (granulated) and water (in calibrated drinking tubes) were freely available at all times. Food deprivation or weight reduction procedures were never employed. The chambers were well ventilated, temperature controlled (23±1°C) and internal lighting alternated on a 12 hr day-night (0800–2000 hr) cycle. The scheduling of forced saline or ethanol infusions was automatically programmed with electromechanical circuitry. Self-infusion could also be initiated, when programmed, by the depression of a centrally located lever available in each operant chamber.

Procedure

During the first 3 postoperative days the rats received 1 IV infusion of sterile saline (3 ml at a rate of 0.3 ml/min) every 4 hr around the clock. These 3 saline days served as an habituation and post-operative recovery period, and allowed food and water intake to stabilize.

Following this habituation period, the rats were exposed to periodic cycles of forced ethanol infusions. Each of these cycles was administered as previously described, and reliably induces physical dependence on ethanol [27]. Briefly, for each cycle, ethanol (30% v/v prepared from 95% ethanol and sterile saline) was administered (IV) over a 4–6 day period. A daily dose of 9–16 g ethanol/kg was infused in fractional quantities at 5 hr intervals around the clock. The infusion rate was 0.3 ml/min, and infusion time ranged between 8 and 15 minutes/infusion depending on dose. To prevent death from ethanol overdose, the dosing guidelines outlined by Majchrowicz [20] were followed. In general, the dose administered per infusion was controlled so as to maintain deep intoxication as determined by daily observations. However, periods in a comatose state were avoided by dosage reductions when necessary [20]. More specifically, the individual dose per infusion was controlled so as to produce a loss of righting reflex during the period immediately after an infusion, which was followed by moderate ataxia at 2–3 hr after a given infusion, and general sedation but without motor incoordination just prior to the next scheduled infusion. Majchrowicz [20] finds that these intoxication signs correlate well with blood ethanol levels (loss of righting reflex: about 500 mg/dl, moderate ataxia: about 350 mg/dl, and general sedation: about 250 mg/dl; see Majchrowicz [20] for details). Lever presses during these forced cycles of ethanol exposure did not lead to ethanol infusions (the lever circuitry was disconnected from the infusion pump).

Following each cycle, forced ethanol infusions were discontinued, but now the rats could depress the lever for self-administration of ethanol under a schedule of continuous reinforcement (CRF). Each lever press infused 0.2 ml of ethanol IV (20% v/v prepared from 95% ethanol and sterile saline) over a 1 sec period. Lever presses that occurred during a given infusion were counted, but did not lead to additional infusions; thus, only interresponse times of 1 sec were effective in producing infusions. The rats were maintained on self-administration for at least 24 hr, or longer if substan-

tial self-administration occurred (>5 g/kg/day). If a rat did not develop self-administration behavior (SAB), the next forced induction cycle was initiated. If a rat did develop SAB (daily intake maintained above 5 g/kg/day) it was maintained on self-administration, progressing, when possible, from CRF to fixed ratio (FR) schedules of reinforcement with infusion parameters the same as under CRF. FR schedules were employed to determine if lever press output would increase in order to maintain a constant g/kg/day intake of ethanol. It should be noted that since a constant volume of ethanol was infused for lever presses during self-administration, there was some variability in the unit dose (g/kg) of ethanol received/self-infusion across rats (since the rats varied in weight). The mean unit dose received/self-infusion was 0.093 g/kg (S.E.M. 0.0005, range 0.08–0.11 g/kg). There was no relationship between the unit dose received by a given rat and SAB.

Throughout testing, food intake, water intake, and body weights were recorded daily, along with the number of infusions, lever presses, and milliliters of ethanol infused. For 4 rats, hourly records of SAB were also sampled. In addition, all rats were observed, a few times each day, for gross signs of intoxication or withdrawal (but we did not attempt to induce withdrawal) and for behavior directed at lever pressing. Unfortunately, blood ethanol levels were not determined.

Self-administration testing was terminated, and withdrawal severity determined when: (1) it was felt that sufficient data were available for a given rat, (2) the cannula became inoperative, or (3) a rat would not transfer from CRF to FR. Withdrawal data were collected at 1 hr intervals between 6 and 15 hr of withdrawal. As reported previously [27], this is the time period during which peak withdrawal severity can be observed using the present methods. For these withdrawal observations, the doors to the sound attenuating cubicle and operant chamber were opened, but the rat was not removed. Each rat was observed for 5 min. The occurrence of withdrawal signs was recorded, and each sign was rated on a scale of 0 (absent) to 3 (severe) according to its intensity. At the end of each 5 min observation, a bunch of keys were jangled (duration: until seizure onset, or 20 sec) in front of the rat in an attempt to induce audiogenic seizure activity. The signs rated included spontaneous seizure, audiogenic seizure, tremor, tail stiffening, and body rigidity (see [15, 20, 27] for a characterization of these symptoms). Based on the constellation of symptoms observed, each rat was assigned an overall severity score which was derived by summating the maximum intensity ratings observed for each withdrawal sign. Using this method, the maximum score possible is 15 (5 signs×3 rating). See Numan and Gilroy [27] for further details. Severity scores between 1 and 5 were classified as mild withdrawal, between 6 and 10 as moderate withdrawal, and above 10 as severe withdrawal.

It should be pointed out that the data reported here were derived from rats that allowed sufficient testing to accumulate reliable data. Nine additional rats were also attempted, but 5 developed cannula damage (leaks, tears) and 4 died (thus, overall mortality was 20%) prior to the collection of reliable data. It should also be noted that available equipment only allowed the testing of 4 animals at any one time; however, as soon as a chamber became available, a new rat was initiated. The data reported here were collected over a 1 year period.

RESULTS AND DISCUSSION

Of the 11 rats tested, 8 showed evidence of SAB, while 3 rats failed to develop such behavior. The rats that did not develop SAB were exposed to 4, 5 and 12 cycles of forced dependence induction respectively. During the withdrawal episodes, these rats never reached a stable SAB infusion criterion of >5 g ethanol/kg/day even though withdrawal discomfort was clearly evident. While many variables could be responsible for these failures [21], it is also possible that some rats [14,31], like monkeys [4,34], will not develop alcohol preferences, even in the face of withdrawal discomfort.

TABLE 1

SUMMARY DATA FOR RATS THAT SELF-ADMINISTERED ETHANOL

Rat	Cycles*	Extent of Testing	Days Tested			
			CRF	FR2	FR3	Total
1	3	FR3	8	4	4	16
2	6	FR3	6	6	8	20
3	4	FR2	10	8	—	18
4	6	FR2	8	4	—	12
5	5	CRF	6	—	—	6
6	8	CRF	16	—	—	16
7	7	CRF	26	—	—	26
8	3	CRF	6	—	—	6

*Number of cycles of forced infusions and withdrawal prior to the acquisition of self-administration behavior.

Table 1 summarizes the extent of testing for the 8 rats that did show evidence of SAB. A mean of 5.25 (range 3–8) cycles of forced dependence induction were necessary before reliable SAB developed. Since all 8 rats were exposed to at least 3 cycles of dependence induction, a few statistical comparisons were carried out to determine if tolerance to ethanol developed during the first 3 cycles of forced ethanol infusions. All comparisons were carried out with 2-tailed dependent t-tests and compared data from the first and third forced cycles. During the first cycle, a mean of 10.48 g ethanol/kg/day (SEM: 0.42) could be safely (see [20,27] and above discussion) infused. In contrast, the rats were able to tolerate a mean of 13.36 g ethanol/kg/day (SEM: 0.23) during the third cycle. This increased ethanol tolerance is significant, $t=10.841$, $p<0.001$. Tolerance was also reflected in food and water consumption. During the first cycle, the rats averaged 5.50 g food/day (SEM: 0.69). In contrast, food consumption during the third cycle increased to a mean of 11.80 g/day, SEM: 1.40, $t=5.026$, $p<0.01$. Water intake also increased from a mean of 13.89 ml/day (SEM: 0.99) during the first cycle to a mean of 20.96 ml/day (SEM: 1.62) during cycle 3, $t=3.367$, $p<0.02$. Individual data for these parameters are shown in Table 2. In contrast to these findings, mean body weight remained stable, $t=0.788$, $p>0.20$, between the first (285 ± 10.44 g) and third (290 ± 10.03 g) cycles. However, these body weights following cycle 3 represent a significant, $t=3.630$, $p<0.01$, weight reduction (25 ± 7.10 g) compared to the mean initial body weight on the day of surgery (315 ± 9.92 g, and see Table 3).

During self-administration, Table 1 shows that all rats were tested on CRF, 4 were tested through FR2, and 2 were tested through FR3. Overall, the rats self-administered ethanol for a mean of 15 days (range 6–26; see Table 1 for a breakdown of the number of days tested on each schedule of reinforcement).

TABLE 2

CHANGES IN ETHANOL INFUSED, FOOD INTAKE AND WATER INTAKE DURING CYCLES 1 AND 3 OF FORCED DEPENDENCE

	Mean/Day					
	Ethanol Infused (g/kg)		Food Consumed (g)		Water Consumed (ml)	
Rat	Cycle 1	Cycle 3	Cycle 1	Cycle 3	Cycle 1	Cycle 3
1	11.48	13.64	6.00	9.70	11.00	23.30
2	9.50	12.32	2.00	7.25	17.00	21.25
3	9.93	13.53	6.50	20.00	11.25	28.00
4	10.54	13.68	4.14	10.40	12.41	13.00
5	9.45	13.25	4.70	10.20	15.30	20.60
6	12.90	14.49	8.50	12.25	17.50	22.50
7	10.31	12.86	5.33	14.80	10.83	22.80
8	9.76	13.10	6.83	9.80	15.80	16.20

Table 3 shows food and water consumption, and body weight changes for each rat during its period of self-administration. The rats averaged 17.53 (SEM: 0.76) g food/day and 27.62 (SEM: 3.65) ml water/day. Compared to their initial weights, the rats also averaged a 31 g (SEM: 16.19) increase in body weight. Further, calories supplied from ethanol during SAB averaged 30% (SEM: 1.78) of the total daily caloric intake.

Tables 4 and 5 present the self-administration and withdrawal data respectively. Overall results will be described first, followed by a description for each animal. During CRF, the rats self-administered an average of 10.43 (SEM: 0.85) g ethanol/kg/day and emitted a mean of 141 (SEM: 7.0) lever presses/day. The four rats tested on FR2 averaged 9.41 (SEM: 1.10) g ethanol/kg/day and 240 (SEM: 16.0) lever presses/day, and the two rats tested on FR3 averaged 11.63 (SEM: 1.40) g ethanol/kg/day with 409 (SEM: 3.0) lever presses/day. Observations of lever pressing behavior suggested that these responses were 'purposeful' rather than accidental. The rats generally approached the lever and held it with forepaws or a forepaw and snout. Sometimes the lever was depressed, and held in the depressed state until the infusion was complete, after which another lever press was emitted. Bursts of lever presses were also observed, especially during the ratio schedules.

During withdrawal observations, 4 rats exhibited mild withdrawal, 2 showed moderate withdrawal, and 1 rat was classified as severe. As will be explained below, we were unable to obtain withdrawal data from one rat.

Rat 1 showed the most impressive performance. This rat self-administered a daily amount of ethanol that ranged between 12.02 and 14.23 g/kg during its 16 days on CRF, FR2 and FR3, with lever presses increasing in a consistent manner with schedule contingencies. During withdrawal, this rat showed all the major abstinence signs, and was the only rat to show evidence of spontaneous seizure. This rat received a severe withdrawal classification with a score of 11. Rat 2 maintained a daily intake of ethanol between 7.18 and 13.42 g/kg throughout its 20 day period of self-administration. This rat averaged above 10 g ethanol/kg/day during CRF and FR3 (range: 9.29–13.42), but showed a slightly reduced intake (range: 7.18–9.51) during FR2. During withdrawal observations this rat received a mild withdrawal classification with a score of 4. Rat 3 averaged 7.02 g ethanol/kg/day (range: 5.78–8.48) during 10 days on CRF and 8.03 g ethanol/kg/day (range: 6.91–9.42) during 8 days on FR2, and received a mild withdrawal classification with a score of 4. Rat 4 maintained an average intake of 9.42 g ethanol/kg/day during 8 days on CRF (range: 7.56–10.84) and 8.68 g ethanol/kg/day (range: 8.32–9.04) during 4 days on FR2. This rat received a moderate withdrawal classification with a score of 6. Of course, in order to maintain ethanol doses on the ratio schedules, lever presses increased accordingly (see Table 4) for all rats.

Rats 5–8 were only tested on CRF. Rat 5 was tested for only 6 days and then terminated because of a leak that developed in its cannula during the night. This leak also made the acquisition of a reliable withdrawal score impossible. During the 6 days of self-administration, this rat maintained a mean intake of 12.93 g ethanol/kg/day (range: 10.66–14.60). Rat 6 was tested for 16 days on CRF and maintained an average intake of 9.21 g ethanol/kg/day (range: 8.09–11.44). FR2 was attempted with this rat, but daily intake of ethanol dropped to below 6 g/kg/day by day 6 of FR2, so this rat was terminated and withdrawal observations recorded. This rat re-

TABLE 3

FOOD AND WATER CONSUMPTION AND BODY WEIGHT CHANGES DURING SELF-ADMINISTRATION

		Mean for Entire Period of Self-Administration			
Rat	Initial Weight* (g)	Food (g/day)	Water (ml/day)	Body Weight (g)	Weight† Change (g)
1	292	15.06	27.68	284	−8
2	365	18.70	43.75	386	+21
3	340	21.83	25.33	389	+49
4	325	18.17	18.67	322	−3
5	315	16.00	14.50	332	+17
6	292	17.13	36.88	375	+83
7	315	17.69	35.46	424	+109
8	280	15.67	18.67	260	−20

*Body weight on day of surgery.

†Difference between mean weight during self-administration and initial weight.

TABLE 4

AMOUNT OF ETHANOL SELF-INFUSED AND LEVER PRESSES DURING SELF-ADMINISTRATION

	Mean/Day ± S.E.M.					
	CRF		FR2		FR3	
Rat	Ethanol Infused*	Lever Presses	Ethanol Infused*	Lever Presses	Ethanol Infused*	Lever Presses
1	13.38 ±0.33	140 ±8	12.68 ±0.55	274 ±13	13.02 ±0.99	412 ±27
2	11.04 ±1.23	183 ±18	8.23 ±0.68	224 ±16	10.23 ±0.30	406 ±17
3	7.02 ±0.58	139 ±11	8.03 ±0.53	256 ±26	—	—
4	9.42 ±0.69	128 ±9	8.68 ±0.36	204 ±12	—	—
5	12.93 ±1.17	157 ±16	—	—	—	—
6	9.21 ±0.35	128 ±7	—	—	—	—
7	7.88 ±0.37	132 ±6	—	—	—	—
8	12.53 ±0.14	124 ±3	—	—	—	—

*g ethanol/kg.

TABLE 5

MAXIMUM INTENSITY OF ETHANOL WITHDRAWAL SIGNS

	Maximum Intensity Rating for Each Withdrawal Sign*					Overall Severity	Withdrawal
Rat	SS	AS	T	TS	R	Score†	Classification†
1	2	2	2	2	3	11	Severe
2	0	1	1	1	1	4	Mild
3	0	1	1	1	1	4	Mild
4	0	1	1	2	2	6	Moderate
5	—	—	—	—	—	—	—
6	0	2	1	1	1	5	Mild
7	0	1	1	1	1	4	Mild
8	0	2	2	2	1	7	Moderate

*SS—spontaneous seizure, AS—audiogenic seizure, T—tremor, TS—tail stiffening, R—rigidity.
†See text for explanation.

ceived a mild withdrawal classification with a score of 5. Rat 7 was tested for 26 days on CRF and maintained an average ethanol intake of 7.88 g/kg/day (range: 5.60–10.69). This rat also refused to increase lever presses during 2 days on FR2 and was terminated. This rat received a mild withdrawal classification with a score of 4. Finally, rat 8 was tested for only 6 days on CRF. Like rat 5, a leak developed in the cannula which precluded further testing, but with this rat a reliable withdrawal rating was obtained since the leak was detected early. During the 6 days on CRF, this rat self-administered a daily amount of ethanol that averaged 12.53 g/kg/day (range: 12.27–12.75) and received a moderate withdrawal classification with a score of 7.

For the four rats (1, 2, 4, and 6) for which hourly infusion rates were sampled, it was noted that more ethanol was self-administered during the night cycle (62% of the infusions) compared to the day cycle (38%). The largest number of infusions (20%) occurred during the 3 hr period following light offset (2000–2300 hr) and the smallest number of infusions (7%) occurred between 1400 and 1700 hr.

During periods of self-administration, the rats were frequently observed for gross signs of intoxication or withdrawal. While careful quantification was not attempted, it was clear that self-intoxication only rarely occurred; the rats seemed to infuse just enough ethanol to block withdrawal distress. More specifically, while self-intoxication, as as-

TABLE 6
SELF-ADMINISTRATION OF SALINE OR ETHANOL BY ETHANOL NAIVE RATS

		Mean ± S.E.M.				
Condition	N	ml Infused/ Day*	Lever Presses/ Day*	Food g/day*	Water ml/day*	Weight Increase (g)*
Saline	6	3.42 ±0.76	24.45 ±5.60	24.69 ±1.68	45.08 ±5.06	52.17 ±11.43
Ethanol	6	1.17 ±0.15	6.01 ±0.38	23.02 ±1.48	39.28 ±2.85	45.00 ±7.01
$p^{\dagger}$	—	<0.02	<0.01	>0.20	>0.20	>0.20

*Data shown in table are means calculated over the 21 day test period.
†Two-tailed t-test.

sessed by moderate to severe ataxia, was occasionally observed in all rats, the most frequently observed behavioral state during self-administration consisted of neutrality (see Majchrowicz [20]; consisting of normal body and muscle tone, and normal reflexes like grooming) or mild hyperarousal and mild body rigidity suggestive of the initial stages of the ethanol withdrawal syndrome [20,27]. Otherwise, all rats appeared healthy during periods of self-administration.

A few additional observations, during self-administration, should be mentioned as they strengthen our view that the rats were motivated to self-infuse ethanol. For rat 1, lever press data were collected during the first 24 hr of withdrawal. Prior to withdrawal, this rat averaged 412 (±27) lever presses/day on FR 3. During withdrawal, lever presses were counted, but they did not activate the infusion pump (extinction). During the first 12 hr of withdrawal this rat emitted 1,010 lever presses, while during the second 12 hr period this rat only emitted 40 lever presses—a typical extinction pattern. While some of the lever presses emitted during the first 12 hr of withdrawal were related to hyperactivity, most were 'purposeful' as described above. Similar data were serendipitously collected in rat 2 during CRF testing. On the fifth day of CRF, this rat emitted an average of 19.7 (SEM: 1.3) lever presses/hr between 5 a.m. and 9 a.m. At 9 a.m. the syringe containing the ethanol was emptied, and we decided to collect lever press data for an additional hour under extinction conditions prior to refilling the syringe. Between 9 a.m. and 10 a.m. (extinction) this rat emitted 183 lever presses. We then refilled the syringe with 20% v/v ethanol. Lever presses recorded for the next 5 hr (reinforced) averaged 15.4 (SEM: 0.8) lever presses/hr.

EXPERIMENT 2

In Experiment 1 it was found that rats would self-administer ethanol IV in substantial quantities; however, SAB only developed after a minimum of 3 cycles of exposure to forced ethanol infusions. This result suggests that, upon initial exposure, the ethanol parameters used here may have aversive effects and that experiential and/or biochemical changes induced through cycles of ethanol exposure are necessary to overcome this aversion. In the present experiment we attempted to obtain further support for the view that the ethanol parameters employed in Experiment 1 were not reinforcing, and perhaps are aversive to the ethanol naive rat.

METHOD

Subjects and Apparatus

Twelve male hooded rats of the Long-Evans strain which weighed between 250–400 g (mean: 312 g) were used. The animals were implanted with chronic jugular cannulas and housed in sound attenuated behavioral chambers as described for Experiment 1. The animals remained in the behavioral chambers 24 hr/day throughout the entire experiment with food and water freely available. Housing conditions and equipment were identical to those described for Experiment 1.

Procedure

Forced infusions were not employed in this experiment. Rather, the rats remained in the chambers for 21 days. During this time, the rats always had access to a lever for self-infusions of solutions under a schedule of continuous reinforcement. The rats were randomly assigned to either a saline condition (N=6) or an ethanol condition (N=6). Depending on their respective conditions, each lever press led to a 0.2 ml infusion of either sterile saline or 20% v/v ethanol infused over a 1 sec period. Ethanol solutions were prepared as described in Experiment 1. Each day, lever presses, milliliters of solution infused, food and water intake, and body weights were recorded. Lever presses that occurred during the 1 sec infusion period were counted, but did not lead to additional infusions. The animals were tested in 4 batches of 3 rats each. Each batch consisted of at least 1 saline and 1 ethanol animal, with the condition of the third rat alternating between batches. Each of the 3 operant chambers employed in this experiment were used to test 2 saline and 2 ethanol animals.

RESULTS AND DISCUSSION

Table 6 presents the results. Statistical comparisons were carried out with 2-tailed t-test. The data suggest that the ethanol parameters that yielded significant self-administration in Experiment 1 are aversive to ethanol naive rats. During the 21 day test period, the saline animals infused an average of 3.42 ml of saline each day, while the ethanol rats averaged only 1.17 ml/day. Thus, while none of the rats developed SAB, the saline animals infused almost three times more solution ($p<0.02$) and made four times as many

lever presses ($p<0.01$) as the ethanol rats. In contrast, average daily food and water intakes were comparable ($p>0.20$), as was weight gain ($p>0.20$) over the 21 day period. While it can be argued that these results could be accounted for by a nonspecific depression of both lever pressing and other behavior by the infused ethanol, this possibility seems unlikely. The ethanol rats never infused enough ethanol to produce even mild intoxication. Further, if a non-specific depression of behavior was produced by the ethanol, one might also expect a depression of food and water intake. This did not occur. In addition, a number of studies have shown that low doses of ethanol (<1 g/kg) stimulate motor activity, including lever presses, while only higher doses (>1 g/kg) lead to a depression of responses (see Pohorecky [29]). In our experiment, the average daily intake of ethanol for the experimental rats was only 0.57 g/kg, and during the last 3 days of the 21 day test period the ethanol rats self-administered a mean of only 0.42 g/kg/day (SEM: 0.10, range 0.14–0.76). However, since the possibility of non-specific depressive effects cannot be completely ruled out, future studies might control for these effects by having a second lever in the operant chamber to measure "activity" lever presses.

Two additional control rats were also tested, but received somewhat different treatments. Both rats were cannulated, and housed as described above. One rat was exposed to 3 cycles of forced ethanol infusions and withdrawal as described for Experiment 1. However, this rat was allowed access to saline for self-infusion during the withdrawal periods (0.2 ml/lever press administered over 1 sec), rather than ethanol. Following these 3 cycles, this rat had access to saline for self-infusion over a 5-day period under a CRF schedule. Self-infusions were low, and averaged 32 infusions/day (SEM: 10.9). The second rat received 3 cycles of forced saline infusions with access to ethanol (20% v/v, 0.2 ml/lever press administered over 1 sec) for self-infusion during the 24 hr saline withdrawal periods. Following these 3 saline cycles, this rat was allowed to self-administer ethanol for 5 days on CRF. The mean infusion rate during these 5 days was 16.8 infusions/day (SEM: 2.4). The mean daily dose of ethanol received at this infusion rate was 1.59 (SEM: 0.23) g/kg/day. The findings from these two rats compliment the results discussed above, and further support the view that rats will not self-administer (IV) significant quantities of ethanol (at the given parameters) without prior exposure to ethanol.

GENERAL DISCUSSION

The results of Experiments 1 and 2, taken together, suggest that for the infusion parameters used here, ethanol is not reinforcing for alcohol naive rats. However, after at least 3 cycles of forced dependence induction separated by 24 hr periods of withdrawal with access to ethanol, SAB is acquired. Prior to this time, the rats never self-administered more than 5 g ethanol/kg/day. Further, the results also show that once SAB is acquired, it maintains the previously induced physical dependence. During SAB, the rats always self-administered more than 5 g ethanol/kg/day. The factors responsible for this reversal to SAB are not, of course, clear from the current investigation. Biochemical, physiological, and experiential factors could all be responsible for the observed effects. However, the suggestion that the rats tended to self-administer ethanol only in quantities sufficient to block withdrawal distress, rather than to induce intoxication lends some support to the conclusions of previous studies [7,14] suggesting that rats may be able to associate alcohol with relief of withdrawal discomfort if a number of withdrawal periods, with access to ethanol, are experienced. This possibility is also supported by a recent study [19] demonstrating that an ethanol associated conditioned taste aversion can be replaced by an ethanol associated conditioned taste preference if rats are first made physically dependent upon ethanol prior to conditioning. These authors [19] also suggest that such induced preferences are related to a reduction in withdrawal distress, since the degree of preference for the taste (saccharin) paired with ethanol was directly related to the duration of prior ethanol exposure. However, since neither the LeMagnen *et al.* study [19], nor the present study are definitive with regard to the role of withdrawal, it may be more parsimonious, at present, to simply suggest that prior exposure to ethanol facilitates a preference for ethanol, without specifying a critical role for withdrawal distress reduction. For example, it is possible that programmed infusions of ethanol that do no lead to physical dependence might, nonetheless, still facilitate ethanol self-administration.

The data from the FR schedules clearly show that the rats were motivated to self-administer ethanol. As the response requirement for an ethanol infusion increased, most rats increased lever press output accordingly in order to maintain a relatively constant intake of ethanol. While all rats did not transfer to the FR schedules, 4 of the 6 for which transfer could be tested (2 rats developed cannula damage which precluded further testing) were successful. The failure of the two rats to transfer to FR is not clear. It is possible that these rats were going through a period of spontaneous rate decrement similar to that reported for monkeys [4,34]. While response rates for these rats may have increased again if further testing was attempted [34], we did not want to risk the loss of withdrawal data.

While SAB did maintain physical dependence, the withdrawal scores tended to be low. A number of factors were probably related to this generally mild withdrawal. First, most rats self-administered less ethanol than they received during their dependence induction cycles. Two exceptions were rats 1 and 8. These rats self-administered ethanol in daily amounts comparable to the amounts received during the dependence induction cycles. Importantly, these rats also received the highest withdrawal ratings. Secondly, since blood ethanol levels were not obtained for this study, we do not know what levels of blood ethanol were maintained during SAB, nor the blood ethanol levels during the withdrawal observations. Further, stable blood ethanol levels are necessary to induce and maintain physical dependence upon ethanol in rodents [12,30]. The fact that there was some variability in the amount of ethanol self-administered during SAB may have resulted in fluctuating blood ethanol levels, which in turn maintained only mild to moderate dependence. Importantly, however, it seems unlikley that the withdrawal signs that we did observe were due to the forced dependence cycles since ethanol withdrawal symptoms are known to be complete by 24–72 hr following withdrawal in rodents [27], even when they are exposed to multiple cycles of intoxication [12]; and our rats were tested on self-administration for an average of over 2 weeks prior to withdrawal.

Our findings are also interesting because, unlike some data collected from monkeys [4], they suggest that rats will maintain body weight and good health during periods of SAB. It is doubtful [5] that ethanol was self-administered solely for its caloric value. If so, we would expect higher

rates of self-administration following the initial cycles of forced dependence induction when food intake and body weights were reduced, rather than following the later cycles when food intake and body weights increased. However, this possibility cannot be entirely ruled out without the use of a control group allowed to self-infuse a control solution isocaloric to ethanol during withdrawal cycles.

In conclusion, the self-administration data reported here support the view that multiple exposures to dependence induction and withdrawal can overcome an initial ethanol aversion, and also lend some support, at least for rats, for a withdrawal hypothesis of SAB. Future studies should add additional controls such as an isocaloric non-ethanol group, collect blood ethanol levels, and test modifications of the various infusion parameters and other aspects of the methodology employed here in order to further facilitate SAB.

REFERENCES

1. Baker, T. B. and D. S. Cannon. Potentiation of ethanol withdrawal by prior dependence. *Psychopharmacology* **60:** 105–110, 1979.
2. Cicero, T. J. Animal models of alcoholism? In: *Animal Models in Alcohol Research,* edited by J. D. Sinclair and K. Kiianmaa. New York: Academic Press, 1980, pp. 99–117.
3. Cunningham, C. L. Flavor and location aversions produced by ethanol. *Behav. Neural Biol.* **27:** 362–367, 1979.
4. Deneau, G., T. Yanagita and M. H. Seevers. Self-administration of psychoactive substances by the monkey. *Psychopharmacologia* **16:** 30–48, 1969.
5. Denoble, V. J. and H. Begleiter. Alcohol self-administration in monkeys (*Macaca Radiata*): The effects of prior alcohol exposure. *Pharmac. Biochem. Behav.* **8:** 391–397, 1978.
6. Deutsch, J. A. and A. Eisner. Ethanol self-administration in the rat induced by forced drinking of ethanol. *Behav. Biol.* **20:** 81–90, 1977.
7. Deutsch, J. A. and N. Y. Walton. A rat alcoholism model in a free choice situation. *Behav. Biol.* **19:** 349–360, 1977.
8. Deutsch, J. A., N. Y. Walton and T. R. Thiel. The importance of postingestional factors in limiting alcohol consumption in the rat. *Behav. Biol.* **22:** 128–131, 1978.
9. Essig, C. F. and R. C. Lam. Convulsions and hallucinatory behavior following alcohol withdrawal in the dog. *Archs Neurol.* **18:** 626–632, 1968.
10. Falk, J. L. and M. Tang. Schedule induction and overindulgence. *Alcoholism: Clin. exp. Res.* **4:** 266–270, 1980.
11. Freund, G. Animal models of ethanol withdrawal syndromes and their relevance to pharmacology. In: *Biological and Behavioral Approaches to Drug Dependence,* edited by H. D. Cappell and A. E. LeBlanc. Toronto: Addiction Research Foundation of Ontario, 1975, pp. 13–25.
12. Goldstein, D. B. Rates of onset and decay of alcohol physical dependence in mice. *J. Pharmac. exp. Ther.* **190:** 377–383, 1974.
13. Goldstein, D. B. and N. Pal. Alcohol dependence produced in mice by inhalation of ethanol: Grading the withdrawal reaction. *Science* **172:** 288–290, 1971.
14. Hunter, B. E., D. W. Walker and J. N. Riley. Dissociation between physical dependence and volitional ethanol consumption: Role of multiple withdrawal episodes. *Pharmac. Biochem. Behav.* **2:** 523–529, 1974.
15. Irwin, S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* **13:** 222–257, 1968.
16. Kalant, H., A. E. LeBlanc, R. J. Gibbons and A. Wilson. Accelerated development of tolerance during repeated cycles of ethanol exposure. *Psychopharmacology* **60:** 59–65, 1978.
17. Karoly, A. J., G. Winger, F. Ikomi and J. H. Woods. The reinforcing property of ethanol in the rhesus monkey. II. Some variables related to the maintenance of intravenous ethanol-reinforced responding. *Psychopharmacology* **58:** 19–25, 1978.
18. Kulkosky, P. J. Effect of addition of ethanol and NaCl on saccharin + glucose polydipsia. *Pharmac. Biochem. Behav.* **10:** 277–283, 1979.
19. LeMagnen, J., P. Marfaing-Jallat and D. Miceli. A bioassay of ethanol-dependence in rats. *Pharmac. Biochem. Behav.* **12:** 707–709, 1980.
20. Majchrowicz, E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacologia* **43:** 245–254, 1975.
21. Meisch, R. A. Ethanol self-administration: Infrahuman studies. *Adv. Behav. Pharmac.* **1:** 35–84, 1977.
22. Meisch, R. A. and T. Thompson. Rapid establishment of ethanol as a reinforcer for rats. *Psychopharmacologia* **37:** 311–321, 1974.
23. Mello, N. K. A review of methods to induce alcohol addiction in animals. *Pharmac. Biochem. Behav.* **1:** 89–101, 1973.
24. Mello, N. K. Animal models for the study of alcohol addiction. *Psychoneuroendocrinology* **1:** 347–357, 1976.
25. Moscatelli, E. A., M. K. Switzer and W. D. Blaker. A model for chronic ethanol consumption in rats. *Res. Communs Psychol. Psychiat. Behav.* **3:** 267–283, 1978.
26. Myers, R. D. and W. L. Veale. The determinants of alcohol preference in animals. In: *The Biology of Alcoholism, Vol. II,* edited by B. Kissin and H. Begleiter. New York: Plenum Press, 1972, pp. 131–168.
27. Numan, R. and A. M. Gilroy. Induction of physical dependence upon ethanol in rats using intravenous infusion. *Pharmac. Biochem. Behav.* **9:** 279–282, 1978.
28. Pieper, W. and M. J. Skeen. Induction of physical dependence upon ethanol in rhesus monkeys using oral acceptance techniques. *Life Sci.* **11:** 989–997, 1972.
29. Pohorecky, L. A. Biphasic action of ethanol. *Biobehav. Rev.* **1:** 231–240, 1977.
30. Rogers, J., S. G. Wiener and F. E. Bloom. Long-term ethanol administration methods for rats: Advantages of inhalation over intubation or liquid diets. *Behav. Neural Biol.* **27:** 466–486, 1979.
31. Smith, S. G. and W. M. Davis. Intravenous alcohol self-administration in the rat. *Pharmac. Res. Communs.* **6:** 397–402, 1974.
32. Smith, S. G. and W. M. Davis. A method for chronic intravenous drug administration in the rat. In: *Methods in Narcotics Research,* edited by S. Ehrenpreis and A. Neidle. New York: Marcel Dekker, 1975, pp. 3–32.
33. Walker, D. W. and S. F. Zornetzer. Alcohol withdrawal in mice: Electroencephalographic and behavioral correlates. *Electroenceph. clin. Neurophysiol.* **36:** 233–243, 1974.
34. Winger, G. D. and J. H. Woods. The reinforcing property of ethanol in the rhesus monkey: I. Initiation and termination of intravenous ethanol-reinforced responding. *Ann. N. Y. Acad. Sci.* **215:** 162–175, 1973.