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A Critical Evaluation of Tetrahydroisoquinoline Induced Ethanol Preference in Rats

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DUNCAN, C. AND R. A. DIETRICH. *A critical evaluation of tetrahydroisoquinoline induced ethanol preference in rats.* PHARMAC. BIOCHEM. BEHAV. 13(2) 265–281, 1980.—It has been reported [30] that certain tetrahydroisoquinoline compounds, especially salsolinol and tetrahydropaveroline (THP) when infused into the lateral ventricle of rats' brains results in increased preference for alcohol solutions. The effect is reported to be long-term, in that animals do not return to baseline drinking even months later. The current report provides a replication of the original experiments and also an extension of the work to complete dose-response curves for salsolinol and THP. Generally we have confirmed that rats of the Sprague-Dawley and Long-Evans strains do increase their alcohol intake in response to infused THP or salsolinol and that the effect is long lasting, up to 10 months. Such animals consume less alcohol at concentrations above 20% than below, in contrast to the previous reports where drinking was maintained at high concentrations of alcohol. While the animals will select alcohol in the face of a saccharin choice, they will not drink alcohol adulterated with quinine. We have failed to observe signs of dependence or withdrawal by these techniques and suggest that the original reports of these signs may have been a result of cellular damage caused by the long-term infusions. Additionally we have carried out extensive dose-response experiments with both salsolinol and THP. Doses of THP of 104 nmoles/day were inhibitory to alcohol drinking. We conclude that these compounds do shift these animals preference for alcohol relatively permanently, but not to the point of gross intoxication nor into the highly aversive range of alcohol concentration. We cannot confirm the reports that salsolinol or THP produce withdrawal symptoms when infused.

Ethanol Salsolinol Preference Tetrahydroisoquinolines Tetrahydropapaveroline

RECENT biochemical theories of alcohol dependence [8, 9, 12, 14, 34] have emphasized the role of certain products of the condensation of aldehydes with biogenic amines as possible causes of some of the aspects of alcohol dependence, withdrawal, and tolerance. The two most widely studied condensation products are the tetrahydroisoquinolines (TIQs), tetrahydropapaveroline (THP) and salsolinol. These are the dopamine -3,4,-dihydroxyphenyl acetaldehyde and dopamine-acetaldehyde condensation products, respectively [9,12].

In 1977, Melchior and Myers [26] reported that the chronic infusion of THP or salsolinol into the rat's cerebral ventricles increased the rat's preference for ethanol in a free choice situation, especially in the range of ethanol concentrations of 11–30%. In addition to the increased preference for ethanol, it was also reported by these investigators that THP or salsolinol chronically infused intraventricularly in the rat also resulted in certain withdrawal-like symptoms such as teeth chattering and "wet dog shakes". Some rats chronically infused with these condensation products were reported to develop tonic-clonic convulsions. Both THP and salsolinol were implicated in long-term if not permanent alteration of the rat's preference for ethanol.

The experiments reported here attempt to replicate specific aspects of the findings on THP and salsolinol as well as extending the original findings [3]. The primary rationale for the attempted replication of these experiments was based on the following considerations: (1) The findings of Myers and Melchior were of sufficient importance to warrant an attempt at replication; (2) Earlier published reports on attempted replications of the chronic infusion of dilute ethanol solutions in rats and other species [32] have been unsuccessful [15]; (3) Dose-response relationships for THP or salsolinol did not emerge from the earlier experiments on these compounds [25, 26, 30].

EXPERIMENT 1

THP's reported effects on alcohol preference, the long-term nature of the alteration and the role of certain taste properties of ethanol solutions were re-examined in this experiment. From the original publications, it would appear that the optimal dose for THP's effects on voluntary ethanol intake in the rat was 5.2 nmoles per day (given as 1 μl infusions of a solution containing 0.02 μg/μl every 15 min around the clock) and administered for a total of 14 days [25, 26, 30]. Earlier studies utilized a dose of THP of 520 nmoles per day (2 μg/μl/15 min/day) [30]. The experiments reported here on THPs long-term effects used what appeared to be the optimal dose for this compound for altering the rat's preference for ethanol.

GENERAL METHOD

Animals

Animals used in these experiments were male rats of the Sprague-Dawley strain (Charles River, Inc.) and a line of rats, Long-Evans (RR) [13], maintained at the University of Colorado Health Sciences Center. All rats used in these experiments were between 90–120 days old (350–450 g) at the beginning of chronic infusion experiments. These rats were housed in group cages (3–4 rats per cage) prior to surgery. Sprague-Dawley rats were maintained in isolation for 14 days before exposure to surgery or experimental procedures. All animals were maintained on food (Purina Lab Chow; Myers laboratory used Wayne Lab Block) and water ad lib throughout all experimental procedures.

Surgical Procedure

Rats were stereotaxically implanted with Teflon guide cannulae (C313G, Plastic Products, Co.) under sodium pentobarbital anesthesia (50 mg/kg) in the dorsal portion of the rat's left lateral cerebral ventricle with the head held in the König and Klippel orientation [22] and coordinates of −0.2 A.P., R.L. ± 1.5, H −3. A triangular array of stainless steel mounting screws (0-8×3/16 Plastic Products Co.) were fitted firmly into the rat's skull around the guide cannulae. The guide cannulae and mounting screws were fixed to the rat's calvarium with cranioplastic cement. The scalp incision was closed with surgical sutures (Ethicaon, 00-Chromic, G-123H). A 29 ga dummy cannulae was placed into the guide cannulae immediately after surgery and remained in place during those times when the rat was not being chronically infused. Rats were allowed a 48 hr recovery period from the surgical procedure before they were exposed to the chronic infusion experiments. Melchior and Myers [25] had used a stainless steel guide cannulae and placed the head in the DeGroot orientation.

Chronic Infusion Apparatus

During chronic infusion experiments the animals were placed in plywood infusion cages that were covered with flat grey paint [27]. The cages were constructed to be identical to the original description. The front of each infusion cage was fitted with 3 equally spaced clamps 6.5 cm apart which held graduated cylinders, fitted with rubber stoppers which held L-shaped glass spouts 23 cm in length. The glass spouts were prepared with openings 10–15 mm from the end. These drinking tubes were prepared to minimize spillage and evaporation of fluid. The graduated cylinders and glass spouts were placed above the floor of the infusion cage and required rats to rear in order to drink. The drinking tube arrangement caused no injuries to the rats or breakage of the glass spouts during the 18–20 months that these devices were in use.

Infusates were delivered by an infusion pump (Orion Research, Model 355), fitted with a Plexiglas syringe carriage modified to hold six 1 ml glass syringes that were matched for their calibrated volume. The plungers of these syringes were coated with vacuum grease (Lubrisol, Arthur Thomas Co.) once every 14 days. The barrels of these syringes were flushed with 70% ethanol, followed by distilled water every 7 days. Each syringe was fitted with a 23 ga needle which was press-fitted to PE 50 tubing (approximately 150 cm length). In line with the PE tubing was a water tight Teflon swivel 20 mm in length. The Teflon swivel was attached to the inner PE tubing of a spring-covered connector (Plastic Products Co.). The spring-covered connections were attached to the rear of each infusion cage with a 30 ga copper wire spring assembly located approximately 25 cm above the floor of the infusion cage. This arrangement allowed the rat complete freedom of movement in its infusion cage. The internal PE tubing of the connectors was fitted with 29 ga injection cannulae with 23 ga adaptors. This latter arrangement provided the basic connection between the infusion system and the rat. The 29 ga injector cannulae were cut to extend 0.5 mm—1 mm beyond the top of the guide cannulae after the spring-covered connector was in place over the guide cannulae.

The infusion pump was placed on the same level as the floor of the infusion cages. Recycling electromechanical timers controlled the operation of the infusion pump. The volume of each individual line (without injector cannulae in place) was calibrated by inserting the needle of a 50 μl Hamilton syringe directly into each infusion line in place of the cannulae in the rat's skull. The needle of the Hamilton syringe was filled with blue fluid to flow into the syringe. This technique allowed precise calibration of the volume and flow rate of each PE infusion line connected to its syringe which was positioned in the syringe carriage [27].

This infusion apparatus was pre-tested on several rats that had cannulae placed in the vicinity of the left lateral cerebral ventricle. These rats were infused with a solution of methylene blue (30 mg/250 ml) at a rate of 1 μl/15 min for 24 hr for a five day period. On the 6th day these rats were decapitated under sodium pentobarbital anesthesia and the ventricular system was exposed. This pre-testing procedure resulted in no observable misplaced cannulae and ensured the complete dispersal of the infusate throughout the ventricular system.

Preparation of THP and Salsolinol

THP-HBr was synthesized from papaverine by the method of Pyman [33]. Elemental analysis (Huffman Laboratories, 3830 High Court, Wheatridge, CO 80033) revealed: C-expected 52.32% found 53.29%; H 4.63% found 4.79%; 017.44% found 21.1%. Analysis by GC-MS performed by Drs. K. Clay and R. Murphy of this department, revealed single peak for the trimethylsialyl derivative and the expected mass spectra. More recent GC-MS analysis of the trifluoroacetyl derivatives of a number of THP samples has revealed small amounts of singly or doubly O-methylated THP in all of those samples tested. In 0.1 N HCl the THP-HBr had an absorption maximum of 283 nm and molar extinction coefficient of 6.30×10^3.

Salsolinol HBr was synthesized by mixing 3.8 gm of dopamine HCl, 200 ml H_2O and 2 gm of acetaldehyde at room temperature in the dark for 3 days. After evaporation, the residual oil was dissolved in a small volume of HBr seeded with a small amount of salsolinol HBr and then allowed to stand in the freezer for several days. The precipitate was collected and dried over P_2O_5 at 25°. It had an absorption maximum of 284 nm and an extinction coefficient of 2.63×10^3.

All THP HBr and salsolinol HBr solutions prepared for chronic infusion were dissolved in an artificial cerebrospinal fluid (CSF) [27] which contained 0.1 mg/ml ascrobic acid. The infusates (THP or salsolinol, or CSF for controls) were prepared in 200 ml batches, pH 3.8, and remained refrigerated except for those instances that required refilling syringes or flushing the PE infusion lines. THP which had

remained in the infusion line for 24 hr in the artificial CSF with ascorbic acid was analyzed by HPLC with electrochemical detection [21]. No instability was detected.

Blood Ethanol Determinations

Blood ethanol levels were determined enzymatically (Calbiochem-Behring) for selected groups for THP and salsolinol treated rats. In these instances blood was taken from the retro-orbital sinus by puncturing the sinus with a micropipette. The rats undergoing blood ethanol determinations were 500 gm or larger and required an ataxic dose of ethanol-free anhydrous-ether (J. T. Baker Chemical Co.) prior to the actual puncture of the sinus. Blood ethanol levels were assessed at 4:00 a.m. or 8:00 a.m.

Assessment of Withdrawal Symptoms

THP, salsolinol treated and CSF infused controls were routinely evaluated for behavioral characteristics and abnormalities associated with ethanol withdrawal in the following manner: (1) Each rat was observed for 10–15 min and handled briefly during the same time interval that observations took place. These times were routinely 8:00–9:00 a.m., 2:00–3:00 p.m. and 9:00–10:00 p.m. Behavioral and postural abnormalities were recorded; (2) Each rat was periodically evaluated for its susceptibility to audiogenic seizures by exposing it to a door buzzer for 10 sec (approximately 60 dB relative to 100 dynes/cm^2).

Histological Verification of Ventricular Placement

Animals from the THP experiments were anesthetized with sodium pentobarbital and injected at the site of cannulation with 10 μl of methylene blue dye as used before. Subsequently the animals were perfused intracardially with 100 ml phosphate-buffered formalin. Frozen coronal sections were cut on a cryostat (American Optical) at 40 μ with every 5th slice mounted on albumin-coated slides and stained with cresyl violet. This technique was necessary due to the long-term aspects of these experiments and the fact that most of the rats used in Experiment 1 had dislodged their cannulae within a few weeks after termination of the chronic infusion experiments and visual confirmation of cannulae placement was no longer possible. Ventricular staining and preparation of frozen sections allowed visual verification of the ventricular site of the infusion, the cannulae track and abnormalities of cerebral tissue that resulted from the chronic infusion procedure.

Experimental Procedure

The general procedure for these experiments followed the chronic infusion paradigm [25, 26, 30] used in the original studies of THP and salsolinol. In the present experiments on THP, each rat was placed in its infusion cage for a brief period of time. Then, the connector was placed over the guide cannulae and screwed into position without the injector cannulae in place. A few minutes later, the rat was removed from the infusion cage and the connector was fitted with an injection cannulae. The pump was activated several times until a drop of the infusate could be visualized at the tip of the injector cannulae. Following this procedure, the rat was again connected to the infusion line and placed in its infusion cage.

The infusion sequence, volume and infusion rate used those parameters [30] that appeared to be optimal for enhancing the rat's preference for ethanol throughout the 3–30% v/v ascending sequence of ethanol concentrations. Treated rats in this initial experiment were infused with a single concentration of THP (.02 mg/ml) at a rate of 1 μl/15 min over a 15 sec interval continuously for a 23 hr period. One hour was allowed for cleaning replenishment of the infusion fluids each day. Controls were infused with the artificial CSF containing 0.1 mg/ml ascrobic acid at the same volume and rate. The ascending series of ethanol concentrations began on the third day of the infusion sequence. The ethanol concentration was increased from 3% to 30% v/v over a 12 day period. The method of fluid presentation followed the 3-bottle, 2-choice random rotation method [25, 26, 30]. These experiments were replication of the Myers and Melchio experiment. In taste preference tests which involved random rotation of bottles filled with either saccharin (0.06% w/v), water, ethanol or quinine sulfate (0.05% w/v) were present in the alcohol. Measures of fluid intake consisted of the following determinations: (1) The proportion of ethanol consumed (total ethanol consumed divided by total fluid consumed); (2) Grams of ethanol consumed per kilogram body weight. These measures were assessed on a daily basis throughout all experimental procedures along with each rat's body weight. All test drinking fluids were prepared fresh daily with tap water. Fluids were usually given to these rats between the hours of 10:00 a.m. and 12:00 noon. The light dark cycle consisted of 12 hr light (on at 8:00 a.m.) and 12 hr dark (off at 8:00 p.m.).

The initial experiments on THP involved four groups of rats composed of 5 Sprague-Dawley males (SDT), 5 CSF infused Sprague-Dawley controls (SDC), 4 Long-Evans males infused (RRT) with THP and 5 CSF infused Long-Evans male rats (RRC). After the initial 14 day infusion sequence with either THP or the artificial CSF, the rats were placed in individual plastic cages fitted with wire tops with bottoms lined with wood shavings. These rats were given an additional 12 day sequence of the 3–30% (v/v) ascending series of ethanol concentrations at 30 and 60 days in these cages. At 90 days, following the termination of chronically infused THP or artificial CSF, these rats were exposed to a 3-bottle, 3-choice test between saccharin (0.06% w/v), ethanol 3–30% (v/v) and tap water. The concentration of the saccharin solution was selected from the equal preference curves between saccharin and sucrose [39].

One hundred twenty days after the chronic infusion was terminated, these same rats were exposed to a second 3-bottle, 3-choice test between saccharin, water and the ascending ethanol series where each ethanol concentration was in solution with a 0.05% (w/v) quinine sulfate.

In addition to these groups described above, 3 Sprague-Dawley male rats (SDP) were given an exposure to the 12 day ascending ethanol sequence prior to surgery. A second ethanol sequence was given to these rats, during the infusion of THP (0.02 mg/ml). This experiment was a replication of the earlier THP experiment [25, 26, 30] that resulted in the rat's enhanced preference for ethanol into range of aversive concentrations (>10%). These rats were maintained in individual cages for a period of 10 months after infusion with THP with free access to food and water. At the end of the 10 months these same rats were given an additional exposure to the ethanol sequence.

Another 3 rats were implanted with permanent indwelling cannulae in the vicinity of the 3rd ventricle (A.P. 4.0, L. ± 0, H - 3.8 from dura) with the head in the König and

Klippel orientation [22]. This group was included in order to insure access to THP to brain stem structures that have been implicated in the increase in the rat's ethanol preference caused by THP.

EXPERIMENT 2

Dose-response relationships did not emerge in the earlier experiments with chronically infused THP or salsolinol and this could have been due to several factors. (1) There may be a threshold effect for the TIQs; (2) There may be an upper limit to the effect and greater amounts become inhibitory. Inspection of the data from these experiments [25, 26, 30] does reveal a rudimentary optimal dose for THP (0.02 mg/ml, infused at 1 μl/15 min or 5.2 nmoles/day) and a concentration of THP increased by a factor of 100 (2.0 mg/ml, infused at 1 μl/15 min or 520 nmoles/day) caused a marked if not statistically significant decrease in the grams of ethanol consumed per kilogram body weight [30]. The present dose-response experiments were designed to examine a much wider range of concentrations for both THP and salsolinol than were used in earlier experiments on these compounds.

METHOD

Animals

Sprague-Dawley male rats (Charles River Co.) approximately 120–150 days old (380–450 g) were used in these experiments.

Surgical Procedure

The surgical procedure was the same as described in Experiment 1. For dose-response experiments special cannulae were prepared from Teflon or metal (10 mm×2.5 mm) and fitted with 23 ga stainless steel tubing (overall length of the cannulae assembly was 15 mm). These cannulae were fixed to the cranium with a cyanoacrylate cement which provided an assembly that remained intact long after the termination of chronic infusion. Cyanoacrylate cement did not cause any apparent tissue toxicity. Precautions were used during surgery to avoid placing this substance near the incision.

Chronic Infusion

For dose-response experiments PE-20 replaced PE-50 tubing used in earlier experiments and 27 ga injection cannulae were used and fitted directly into slightly flared PE-20 tubing. The spring-covered connectors were modified to hold 10×15 mm Lucite connectors which prevented breakage and provided a durable connection between the polyethylene tubing, injection cannulae and guide cannulae fixed to the rat's skull.

Experimental Procedure

Procedural variables were similar to earlier experiments on THP. THP was infused in concentrations ranging from 0.65 nmoles/day to 104.0 nmoles/day and salsolinol from 4.65 nmoles/day to 297.60 nmoles/day. Both compounds were infused in separate groups of rats at an infusion rate of 2 μl infused over a 30 sec interval every 12.5 min for a total of 14 days. The infusion interval was based on the half-life of salsolinol [24]. Animals were selected randomly and arbitrarily assigned to receive particular doses of CSF, salsolinol or THP. Each animal received only a single concentration of either THP or salsolinol. There was no pre-arranged schedule of ascending or descending dosages. The apparent optimal dose of THP [30] was used as the point of reference for which other concentrations of THP or salsolinol were prepared. For inclusion in the dose-response experiments, implanted rats were required to maintain ventricular patency as measured by the gravitation flow technique during chronic infusion as described by Myers and Oblinger [31]. Two independent dose-response curves were carried out for both salsolinol and THP. Data from only the second group is presented. The majority of the animals used for dose-response experiments were retained for histological verification of cannulae placement.

RESULTS EXPERIMENT 1

Table 1 presents results from Experiment 1 in comparison to the results of Myers and Melchior [30]. In our hands both Sprague-Dawley rats (the same strain used by Myers and Melchior) and Long-Evans rats show preference (i.e., 0.50 preference score) for alcohol during THP infusion only in the 3–9% range of ethanol concentrations. There is no major difference between our results and those of Myers and Melchior [30] in this preference range when their experiment is replicated by prior exposure of the animals to the increasing ethanol concentration in the choice situation (SDP in Table 1).

The other paradigm used for Table 1, that is infusing animals with THP that have never been exposed to ethanol, gives similar results in both the Long-Evans and Sprague-Dawley strains. Thus, we are able to replicate the essential finding of Myers and Melchior that chronic THP infusion into rats' lateral ventrical does result in increased ethanol consumption.

A more detailed presentation of these data is in Tables 2 and 3. As can be seen from the ANOVA, all comparisons have a p value less than 0.01. Additional comparisons across groups used Dunnet's procedure to compare the means of each THP group versus the combined means of g/kg ethanol consumed for the CSF controls were carried out. These results are available upon request.

Six simple effects analyses were performed for each group across blocks of ethanol concentrations. SD3, SDC and RRC groups did not reveal significant increases or decreases across blocks of ethanol concentrations.

The data from Table 3 (mean preference ratios) treated in the same way as the g/kg ethanol consumed data, indicated that groups were significantly different, $p<0.01$. There were also significant differences across blocks of ethanol concentrations, $p<0.01$. The groups×blocks interaction was not significant ($p>0.25$). Further statistical data is available on request.

Long-Term Effects of THP

SDT, RRT, SDC and RRC groups were re-tested for ethanol intake 30, 60, 90 and 120 days after cessation of the infusion of THP. The means of these groups' g/kg ethanol consumed and mean preference ratios were analyzed in the same manner as the chronic infusion data. An overall ANOVA was used on the means of three ethanol test periods (30, 60, 90 days). These results are reported (Table 4 and 5). It is important to note that the 90 day tests on ethanol intake was a 3-bottle, 3-choice test between water, ethanol and a dilute saccharin solution (0.06% w/v). The ANOVA is given

TABLE 1

COMPARISON OF THE PATTERN OF ETHANOL INTAKE FOR COMPARABLE GROUPS FROM THE PRESENT STUDY AND THE MYERS AND MELCHIOR STUDY [30]

Group	n	Proportion ETOH to total fluid intake ± SEM 3%–9%	11%–30%	Mean gm/kg/day ± SEM 3%–30%
THP 5.2 nmoles/day				
Sprague-Dawley* (SDT)	5	.75 ± .02‡	.40 ± .09¶	4.15 ± 0.71
Long-Evans (RR)* (RRT)	4	.67 ± .03‡	.21 ± .03¶	4.24 ± 0.93
Sprague-Dawley† (SDP)	3	.80 ± .09§	.42 ± .09¶	4.33 ± 0.84
Controls				
CSF-Sprague-Dawley (SDC)	5	.31 ± .08	.07 ± .08	1.32 ± 0.19
CSF-Long Evans (RR)	5	.20 ± .04	.11 ± .02	1.79 ± 0.15
UNOP. Controls	3	.16 ± .07	.08 ± .004	0.65 ± 0.16
Myers and Melchior [30]				
THP (5.2 nmoles/day)				
Sprague-Dawley†	4	.85	.55	6.55
CSF Controls	4	.15	.06	0.65
UNOP. Controls	20	.29	.08	0.78

*Rats not exposed to 12-day ethanol sequence prior to chronic infusion of THP (5.20 nmoles/day).

†Rats exposed to 12-day ethanol sequence prior to chronic infusion of THP (5.20 nmoles/day).

‡Significantly different from appropriate CSF controls $p<0.001$.

§Significantly different from appropriate CSF controls $p<0.01$.

¶Significantly different from appropriate CSF controls $p<0.05$.

below each table and shows significant ($p<0.01$) effects for each comparison except for the SDC group. Detailed analysis by Dunnet's procedure is available on request for these data as well.

From all these analyses it would appear that the most permanent change in the rat's ethanol intake pattern, resulting from THP administration, is ethanol preference in the range of concentrations from 3–9% v/v. Differences between preferences ratios during chronic infusion and subsequent re-testing were negligible. The absolute amount of ethanol consumed by THP treated rats also tended to remain constant. This fact has been somewhat obscured by the tendency of CSF treated controls to increase their ethanol intake during re-testing. This is especially clear for the RRC group.

After the termination of THP infusion, the SDP group was retained for a period of 10 months without further exposure to ethanol or THP. Dependent t-tests (two-tailed were used to compare this group's overall g/kg ethanol consumed during pre-exposure to ethanol and their ethanol intake during the chronic infusion of THP, $t(3)=5.94$, $p<0.05$. A similar comparison between g/kg ethanol consumed during THP infusion and ethanol intake 10 months after infusion was not significant, $p>0.05$. Comparison of this group's overall g/kg consumption during pre-exposure to ethanol and their ethanol intake during the chronic infusion THP was also significant ($p<0.05$). A similar pattern was clearly demonstrated for this group's ethanol preference. The SDP group body weight increased by 32% over 10 months (Table 6). Fluid intake also increased significantly.

At the end of the ethanol sequence, 312 days post THP infusion, the SDP group was given another ethanol sequence which terminated at each animal's most preferred ethanol concentration. These rats were exposed to this ethanol concentration for a period of 48 hr and blood ethanol concentrations (BAC) were tested at 8:00 a.m. Ethanol was undetectable in blood at 8:00 a.m., but at 4:00 a.m. the following day blood alcohol levels in two of these rats were 22 and 38 mg/dl.

The results of these experiments indicated clearly that the major effects of THP are on the rat's preference for ethanol. Additionally, this preference for ethanol tends to be maintained permanently and is strongest in the range of ethanol concentrations from 3–9% v/v.

Taste Preference Tests

At 90 days after THP infusion the SDT, RRT, SDC and RRC animals were exposed to a 0.06% w/v saccharin solution during the ascending ethanol sequence (Table 4). The ethanol preference pattern and g/kg ethanol consumed at this interval were similar to the overall analysis discussed above for the 30, 60 and 90 test sequences. The ANOVA results at 90 days for g/kg ethanol consumed revealed significant main effects of groups, $p<0.01$ and blocks of ethanol concentration, $p<0.01$.

The ANOVA for ethanol preference also revealed significant main effects of groups, $0.01<p<0.05$. The details of these analyses were similar in form to the overall analysis for 30, 60, 90 day means. Analysis of the mean ml saccharin over 4 blocks of 3 days each for the 12 consecutive days of the preference test was carried out. The ANOVA for saccharin intake revealed significant main effects of groups, $p<0.001$

TABLE 2

GRAMS OF ETHANOL CONSUMED PER kg BODY WEIGHT FOR 4 GROUPS OF RATS INFUSED WITH THP (5.20 nmoles/day) FOR 14 DAYS AND 2 GROUPS OF CSF INFUSED CONTROLS OVER 4 BLOCKS OF INCREASING ETHANOL CONCENTRATIONS (MEAN ± SEM)

		Ethanol Concentrations			
	n	3%–5%	6%–9%	11%–15%	20%–30%
THP (5.20 nmoles/day)					
Sprague-Dawley* (SDT)	5	2.44 ± 0.07	4.46 ± 0.42	5.83 ± 0.68	4.61 ± 0.15
Sprague-Dawley† (SDP)	3	2.18 ± 0.13	4.35 ± 0.39	6.23 ± 0.84	3.88 ± 0.36
Sprague-Dawley‡ (SD_1)	3	1.37 ± 0.28	1.62 ± 0.18	0.99 ± 0.68	0.89 ± 0.63
Long-Evans* (RRT)	4	3.22 ± 0.33	5.97 ± 0.50	2.12 ± 0.33	5.66 ± 0.55
Controls					
Sprague-Dawley (SDC)	5	1.02 ± 0.35	1.20 ± 0.22	0.95 ± 0.15	1.73 ± 0.19
Long-Evans (RRC)	5	1.02 ± 0.15	1.83 ± 0.32	1.92 ± 0.38	1.69 ± 0.45

ANOVA		F	p
Among groups	(5,19)	89.58	<0.01
Blocks of ethanol	(15,57)	4.27	<0.01
Simple effects across groups			
3–5%	(5,33)	4.28	<0.01
6–9%	(5,33)	13.32	<0.01
11–15%	(5,33)	17.28	<0.01
20–30%	(5,33)	15.56	<0.01
Simple effects across blocks of ethanol			
SDT	(3,57)	5.81	<0.01
SDP	(3,57)	10.48	<0.01
RRT	(3,57)	7.05	<0.01
SD_1, SDC, RRC	(3,57)	0.99–1.06	>0.25
Main effects of blocks	(3,27)	22.23	<0.01
Groups × blocks	(6,27)	15.65	<0.01

*Rats not exposed to 12-day ethanol sequence before chronic infusion of THP.
†Rats exposed to 12-day ethanol sequence prior to chronic infusion of THP.
‡Animals chronically infused with THP in 3rd ventricle not exposed to ethanol before chronic infusion.

TABLE 3

PROPORTION OF ETHANOL CONSUMED TO TOTAL FLUID FOR 4 GROUPS OF RATS INFUSED WITH THP (5.20 nmoles/day) AND 2 GROUPS OF CSF INFUSED CONTROLS OVER 4 BLOCKS OF INCREASING ETHANOL CONCENTRATIONS. MEAN ± SEM

	n	3%–5%	6%–9%	11%–15%	20%–30%
THP (5.20 nmoles/day)					
Sprague-Dawley†	5	.72 ± .03	.78 ± .06	.58 ± .09	.23 ± .04
Sprague-Dawley†	3	.75 ± .01	.88 ± .06	.60 ± .09	.26 ± .03
Sprague-Dawley‡	3	.36 ± .12	.31 ± .06	.12 ± .02	.05 ± .02
Long-Evans (RR)	4	.61 ± .04	.68 ± .05	.21 ± .04	.21 ± .02
Controls					
Sprague-Dawley	5	.40 ± .09	.19 ± .02	.09 ± .01	.07 ± .01
Long-Evans (RR)	5	.25 ± .04	.15 ± .01	.10 ± .02	.06 ± .02

ANOVA		F	p
Among groups	(5,19)	39.5	<0.01
Blocks of ethanol	(3,57)	70	<0.01
Groups × blocks	(15,57)	0.95	>0.25

*Rats not exposed to 12-day ethanol sequence prior to chronic infusion of THP.
†Rats exposed to 12-day ethanol sequence prior to chronic infusion of THP.
‡Animals chronically infused with THP in 3rd ventricle not exposed to ethanol before chronic infusion.

TABLE 4

G/kg (MEAN ± SEM) ETHANOL CONSUMED FOR SDT‡, RRT‡ and RRC‡ GROUPS AT 30, 60, 90 AND 120 DAYS POST THP INFUSION. (5.20 nmoles/day)

Group	n	3%–5%	6%–9%	11%–15%	20%–30%
30 Days					
SDT	5	3.39 ± 0.43	5.09 ± 0.39	3.99 ± 0.12	1.58 ± 0.32
RRT	4	3.15 ± 0.37	4.77 ± 0.34	2.66 ± 0.43	2.66 ± 0.43
SDC	4	0.37 ± 0.12	0.44 ± 0.15	0.53 ± 0.16	0.48 ± 0.10
RRC	3	0.93 ± 0.28	1.28 ± 0.84	2.41 ± 0.28	2.41 ± 0.28
60 Days					
SDT	5	2.61 ± 0.17	4.77 ± 0.50	2.48 ± 0.94	2.31 ± 0.41
RRT	4	3.56 ± 0.08	6.28 ± 0.41	3.69 ± 0.32	1.68 ± 0.33
SDC	4	0.53 ± 0.12	0.75 ± 0.06	0.75 ± 0.06	1.11 ± 0.10
RRC	3	1.38 ± 0.16	1.75 ± 0.38	2.71 ± 0.14	3.05 ± 0.19
90 Days*					
SDT	5	2.10 ± 0.54	5.17 ± 1.19	3.93 ± 0.57	2.83 ± 0.30
RRT	4	3.21 ± 0.16	4.13 ± 0.29	1.76 ± 0.13	2.92 ± 0.37
SDC	4	0.25 ± 0.07	0.43 ± 0.12	0.88 ± 0.13	1.03 ± 0.25
RRC	3	0.16 ± 0.05	0.32 ± 0.11	0.66 ± 0.10	0.67 ± 0.25
120 Days‡					
SDT	5	0.16 ± 0.02	0.09 ± 0.04	0.43 ± 0.06	0.63 ± 0.20
RRT	4	0.23 ± 0.02	0.25 ± 0.05	0.53 ± 0.09	0.67 ± 0.18
SDC	4	0.10 ± 0.01	0.20 ± 0.03	0.34 ± 0.05	0.58 ± 0.02
RRC	3	0.29 ± 0.06	0.26 ± 0.11	0.69 ± 0.15	0.56 ± 0.02

ANOVA		F	p
Groups	(3,12)	30.0	< 0.01
Blocks	(3,36)	23.6	< 0.01
Groups × blocks	(9,36)	15.0	< 0.01
Simple effects within blocks			
3–5%	(3,18)	9.8	< 0.01
20–30%	(3,18)	3.56	< 0.025
Simple effects across blocks			
SDT	(3,36)	33.2	< 0.01
RRT	(3,36)	29.2	< 0.01
SDC	(3,36)	1.0	> 0.25
RRC	(3,36)	5.2	< 0.01

*At 90 days the choice between was H_2O, ethanol and 0.06% saccharine.

†At 120 days the choice was between H_2O, 0.06% saccharine solution and ethanol adulterated with quinine (0.05%).

‡SDT: Sprague-Dawley rats infused with THP; RRT: RR-Long-Evans rats infused with THP; SDC: Sprague-Dawley control animals; RRC: RR-Long-Evans control animals.

and blocks of ethanol concentration, $p<0.025$. The groups×blocks of days interaction was not significant. A comparison of SDT versus SDC on mean ml saccharin over 12 days was significant, $p<0.05$ (SDC Mean ml ± SD=31 ± 7 versus SDT Mean ml ± SD=16 ± 10). The RRT versus RRC comparison was significant, $p<0.005$. The main effect of blocks which represented saccharin intake over 12 consecutive days for all groups was significant in a comparison of the first block (Days 1–3, Mean ml ± SD=20 ± 13) versus the last block (Days 10–12, Mean ml ± SD=26 ± 11), $p<0.001$. The significant effect of blocks was due to the increase in saccharin intake of the SDT and RRT groups which corresponded to the decrease in these groups' ethanol preference (a reduction in ml ethanol consumed).

The saccharin intake for CSF controls was considerably lower than expected. A group of naive Sprague-Dawley males (n=4), approximately the same age and weight of THP and CSF infused groups at the time of their exposure to saccharin were given a single exposure to the same concentration of saccharin in the presence of the ascending ethanol concentrations. A comparison of these five groups (SDT, SDC, RRT, RRC and naive controls) revealed significant main effects for groups ($p<0.01$) and blocks ($p<0.01$). The groups×blocks interaction was not significant. A comparison between the mean ml saccharin for naive controls versus mean ml for SDC's controls was significant ($p<0.025$). The naive controls consumed substantially more of the 0.06% w/v saccharin solution (mean ml ± SD=62 ± 19) than the CSF control groups (mean ml ± SD=33 ± 7). The lower saccha-

TABLE 5

MEAN ± SEM OF MEAN PREFERENCE RATIOS FOR SDT‡, RRT‡, SDC‡, and RRC‡ GROUPS AT 30, 60, 90 AND 120 DAYS AFTER CHRONIC INFUSION OF THP (5.20 nmoles/day)

		Blocks of ethanol concentration			
Group	n	3%–5%	6%–9%	11%–15%	20%–30%
30 days					
SDT	5	.91 ± .01	.90 ± .03	.61 ± .03	.12 ± .04
RRT	4	.85 ± .02	.81 ± .06	.37 ± .13	.17 ± .03
SDC	4	.20 ± .06	.08 ± .02	.07 ± .03	.03 ± .01
RRC	3	.22 ± .06	.28 ± .06	.07 ± .02	.08 ± .01
60 days					
SDT	5	.87 ± .03	.77 ± .06	.21 ± .04	.18 ± .03
RRT	4	.92 ± .01	.93 ± .02	.39 ± .02	.10 ± .02
SDC	4	.24 ± .04	.15 ± .03	.12 ± .03	.09 ± .01
RRC	3	.31 ± .04	.22 ± .02	.18 ± .01	.09 ± .01
90 days*					
SDT	5	.66 ± .11	.78 ± .08	.41 ± .08	.21 ± .08
RRT	4	.74 ± .04	.66 ± .08	.16 ± .01	.13 ± .01
SDC	4	.14 ± .04	.12 ± .01	.10 ± .02	.07 ± .02
RRC	3	.25 ± .02	.18 ± .03	.07 ± .02	.05 ± .01
120 days†					
SDT	5	.07 ± .01	.02 ± .01	.06 ± .01	.06 ± .01
RRT	4	.07 ± .01	.04 ± .01	.04 ± .01	.05 ± .01
SDC	4	.05 ± .01	.04 ± .01	.05 ± .01	.03 ± .01
RRC	3	.06 ± .02	.04 ± .03	.04 ± .03	.06 ± .03

ANOVA		F	p
Groups	(3,12)	158.0	<0.01
Blocks	(3,36)	270.0	<0.01
Groups × blocks			
3–5%	(3,22)	103.0	<0.01
6–9%	(3,22)	127.0	<0.01
11–15%	(3,22)	71.2	<0.01
20–30%	(3,22)	5.9	<0.01
Simple effects across blocks			
SDT	(3,36)	182.7	<0.01
RRT	(3,36)	127.0	<0.01

*At 90 days the choice was between H_2O, ethanol and 0.06% saccharine.

†At 120 days the choice was between H_2O, 0.06% saccharine solution and ethanol adulterated with quinine (0.05%).

‡SDT: Sprague-Dawley rats infused with THP; RRT: RR-Long-Evans rats infused with THP; SDC: Sprague-Dawley control animals; RRC: RR-Long-Evans control animals.

rin intake for CSF controls could be due to some permanent effect of the chronic infusion procedure or to other extrinsic factors not controlled for in these experiments.

At 120 days after the infusion with THP and 30 days after exposure to saccharin H_2O and increasing ethanol concentrations, a second 3-bottle, 3-choice test was given to these groups among 0.06% saccharin, H_2O, and the ethanol concentrations prepared in a 0.05% quinine–HCl solution. During this preference test the ethanol solutions were avoided by all groups and the g/kg ethanol consumed were below 1 g/kg/day over the 12 day preference test. These results are presented in Table 4 and 5. A comparison of the mean g/kg/day and the mean preference ratios across blocks of ethanol concentrations for individual animals in these four groups did not reveal important differences among these groups.

Body weight and total fluid were examined over the 4 blocks of the 12 consecutive days at 30, 60, 90 and 120 days tested by Friedman's test (no ties permitted). Friedman's test for body weight was significant $\chi^2(3)=8.78$, $p<0.05$. The rank order of body weights were SDC SDT RRT RRC and there was very little change in the ranks of these groups from the beginning of these experiments. Friedman's test on total fluid showed no important differences among groups $\chi^2(3)=6.3$, $p>0.05$.

RESULTS EXPERIMENT 2

THP Dose-Response Results

The results of the second THP dose-response experiment have been placed in Table 7 (g/kg/ethanol consumed) and 8 (mean preference ratios). Results of experiments with animals chronically infused with salsolinol have been placed in Table 9A and 9B, neither the THP nor the salsolinol dose-response curve differs significantly from the first dose-response experiments performed. An ANOVA for THP dose-response curves (2.6, 10.4, 41.6 nmoles/day) when the data is calculated as g/kg ethanol consumed is presented in Tables 7 and 8. As can be seen there are significant effects in doses of THP and blocks of ethanol as well as group×block interaction and simple effects across groups.

Sheffé comparisons ($F'=6.84$, $p<0.05$) of the means of CSF infused rats and rats treated with THP (2.6, 10.4 and 41.6 nmoles/day) within the four blocks of ethanol concentrations indicated that THP infused groups differed from the CSF controls only at the 6–9% and 11–15% blocks of ethanol concentrations ($p<0.05$). There were not important differences between controls and THP treated groups at the 3–5% block of ethanol concentrations or at the 20–30% block ($p>0.05$).

Analysis of the mean preference ratios for CSF controls and THP treated rats revealed a pattern similar to the g/kg data, as can be seen in the ANOVA (Table 8). Sheffé comparisons ($F=6.84$, $p<0.05$) for CSF control versus THP infused groups again indicated marked differences between controls versus THP treated groups. Each block of ethanol concentrations showed significant differences between the CSF controls and THP treated rats at the 3–5%, 6–9% and 11–15% blocks of ethanol concentrations ($p<0.05$). There were not important differences between the CSF controls and THP treated groups at the 20–30% block ($p>0.05$). Further Sheffé comparisons between the 2.60 nmoles/day group and the 10.4 and 41.6 nmoles/day groups showed that the latter two groups have substantially higher preference ratios at the 6–9% and 11–15% blocks ($p<0.05$). A final comparison between the rats infused at 10.6 nmoles/day and rats infused at 41.6 nmoles/day over the 11–15% revealed that the latter maintained substantially higher preference ratios (.77 versus .47 for the 10.6 nmoles/day group), $p<0.05$. Blood ethanol levels were assessed at 8:00 a.m. for the 10.4 and 41.6 nmoles/day groups for the 11%, 15% and 25% alcohol solutions. These values, expressed as percent along with g/kg ethanol consumed and preference ratios, have been placed in Table 9. Blood ethanol levels for these rats were either very low or undetectable. Yet,a total of 4 rats in these groups did maintain the unusually high preference pattern described by Myers and Melchior [28, 29, 33]. One rat (THP

TABLE 6

MEAN g/kg ± SEM AND MEAN PREFERENCE RATIOS ± SEM OVER 4 BLOCKS OF ETHANOL CONCENTRATION DURING PRE-EXPOSURE, THP INFUSION, AND 10 MONTHS AFTER FOR THE SDP GROUP

	3%–5%	6%–9%	11%–15%	20%–30%	Weight gm ± SD	Total fluid ml
Pre-exposure						
g/kg	0.28 ± 0.06	0.39 ± 0.08	0.47 ± 0.12	1.17 ± 0.14		
Pref. ratio	.22 ± .04	.15 ± .05	.08 ± .01	.08 ± .02		
THP infusion (5.2 nmoles/day)					529 ± 4	54 ± 6
g/kg	2.18 ± 0.13	4.35 ± 0.39	6.23 ± 0.84	3.88 ± 0.36		
Pref. ratio	.75 ± .01	.88 ± .06	.60 ± .09	.26 ± .03		
10 months post-THP infusion					697 ± 8	64 ± 9
g/kg	2.78 ± 0.42	3.91 ± 0.23	6.30 ± 1.31	3.16 ± 0.63		
Pref. ratio	.85 ± .03	.81 ± .06	.71 ± .08	.26 ± .07		

No. 70) infused at 10.4 nmoles/day demonstrated preference for ethanol through the 20–30% block of ethanol concentrations. At this rat's peak of ethanol consumption at 25%, it consumed 12.0 g/kg. However, the blood ethanol level taken between 8:00 and 9:00 a.m. was only 87 mg/dl.

Salsolinol Dose-Response Results

The results and the ANOVA on g/kg/day data for groups infused with different concentrations of salsolinol and CSF controls is presented in Table 10A. Significant differences were found for the effects listed. Sheffé comparisons ($F'=11.72$, $p<0.05$) of the means of CSF controls and salsolinol infused groups indicated that these groups differed significantly in ethanol intake ($p<0.05$). An additional comparison between the lowest ethanol consuming group and highest ethanol consuming salsolinol group (272.64 nmoles/day) was significant ($p<0.05$). Additional comparisons between these groups was not statistically meaningful.

An ANOVA for the mean preference ratios is presented in Table 7B for CSF controls and salsolinol infused groups. Significant effects were found for main effect of groups and blocks, but not for the interaction. Sheffé comparisons ($F'=11.72$, $p<0.05$) for the overall mean preference ratios between CSF controls and salsolinol groups was significant ($p<0.05$). The conclusions for the salsolinol dose-response relationships are very similar to the THP dose-response data.

The dose-response for THP and salsolinol for all blocks of ethanol concentrations is graphically represented in Figs. 1 and 2. It is seen that THP exhibits a maximal effect at 41.6 nmoles/day and that a dose of about 100 nmoles/day completely blocked drinking above control levels. Similar results were obtained in an earlier experiment (results not reported).

TABLE 7

DOSE-RESPONSE EFFECT WITH THP

		Ethanol concentration (v/v) g/kg consumed ± SEM			
Dose/day	n	3%–5%	6%–9%	11%–15%	20%–30%
2.6 nmoles	6	1.27 ± .32	1.34 ± .44	1.38 ± .54	.60 ± .28
10.4 nmoles	6	2.53 ± .24	4.38 ± .34	4.38 ± .93	3.17 ± 1.40
41.6 nmoles	6	2.63 ± .23	4.69 ± .39	6.31 ± .53	3.84 ± .84
104.0 nmoles	6	1.32 ± .23	.55 ± .16	.16 ± .09	.14 ± .06
CSF vehicle	6	.81 ± .44	.64 ± .11	.37 ± .19	.53 ± .19

ANOVA		F	p
Main effect group of doses THP	(3,20)	21.0	<0.01
Main effect blocks ethanol	(3,60)	2.9	0.01
Groups × blocks	(9,60)	2.9	<0.01
Simple effect across groups			
3–5%	(3,34)	2.9	<0.05
6–9%	(3,34)	14.9	<0.01
11–15%	(3,34)	25.8	<0.01
20–30%	(3,34)	10.2	<0.01

TABLE 8

DOSE-RESPONSE EFFECT WITH THP

Dose/day	n	Ethanol concentration (v/v) mean preference ratios ± SEM 3%–5%	6%–9%	11%–15%	20–30%
2.6 nmoles	6	.45 ± .13	.25 ± .11	.20 ± .13	.03 ± .01
10.4 nmoles	6	.79 ± .05	.76 ± .06	.47 ± .08	.23 ± .12
41.6 nmoles	6	.86 ± .01	.85 ± .03	.77 ± .05	.28 ± .06
104.0 nmoles	6	.45 ± .10	.14 ± .05	.02 ± .01	.01 ± .005
CSF Vehicle	6	.26 ± .13	.13 ± .04	.03 ± .01	.03 ± .01

ANOVA		F	p
Main effect groups of doses THP	(3,20)	23.5	<0.01
Main effect blocks ethanol	(3,60)	36.8	<0.01
Groups × blocks	(9,60)	3.1	<0.01
Simple effect across groups			
3–5%	(3,95)	36.2	<0.01
6–9%	(3,95)	58.4	<0.01
11–15%	(3,95)	46.4	<0.01
20–30%	(3,95)	7.6	<0.01

Salsolinol appears to be both less potent and less powerful than THP when the data is plotted on preference ratios (Fig. 2).

Rats infused with salsolinol at the largest dose used in the experiments (272.6 nmoles/day) were re-tested 90 days after the chronic infusion sequence. Interestingly, these rats demonstrated somewhat higher mean preference ratios at the 90 days preference test over the 3–5% and 6–9% blocks of ethanol concentrations. These results must be viewed cautiously due to the fact that only these salsolinol infused rats were re-tested and the replications of salsolinol groups have been less extensive THP dose-response groups. Nevertheless, the long-term preference pattern for ventricularly infused salsolinol at its maximal dose may not be substantially different from the maximal effects of THP.

Blood ethanol levels were determined for rats (n=3) infused with salsolinol at 272.6 nmoles/day after the 90 day re-test by placing these rats on their individual concentrations of highest ethanol intake in the standard preference situation. Two of these rats maintained a rather substantial

TABLE 9

BLOOD ETHANOL LEVELS (BAL IN PERCENT AT 8:00 a.m.) g/kg AND PREFERENCE RATIOS OF INDIVIDUAL RATS INFUSED WITH THP AT 10.40 nmoles/day OR 41.60 nmoles/day AT ETHANOL CONCENTRATIONS OF 11%, 15% AND 25%

A. THP (10.40 nmoles/day)

	11%			15%			25%		
Rat #	BAL	g/kg	E/T	BAL	g/kg	E/T	BAL	g/kg	E/T
61	.011	6.33	.77	.043	5.62	.91	—	3.57	.15
73	.010	4.99	.88	—	.34	.05	—	—	—
70	—	2.03	.17	.057	7.45	.68	.087	11.86	.70
60	—	1.71	.30	—	1.45	.16	—	.82	.04
76	—	2.40	.38	—	2.50	.17	—	3.38	.14
58	.020	7.80	.79	—	2.56	.15	—	—	—

B. THP (41.60 nmoles/day)

Rat #	BAL	g/kg	E/T	BAL	g/kg	E/T	BAL	g/kg	E/T
59	.025	7.74	.71	—	4.73	1.00	—	1.75	.08
74	.037	7.82	1.00	.010	5.09	1.00	.001	4.99	.58
80	.020	8.01	.82	.001	5.50	.56	—	3.26	.31
75	.003	7.39	.73	.020	6.04	.67	.012	5.54	.31
62	—	2.35	.50	.030	7.58	.71	.010	5.20	.21
77	.010	4.79	2.00	—	2.37	.18	—	—	—

TABLE 10

DOSE-RESPONSE EFFECT WITH SALSOLINOL

Salsolinol dose/day	n	A. Ethanol concentration (v/v) g/kg consumed ± SEM 3%–5%	6%–9%	11%–15%	20%–30%
4.62 nmoles	4	1.29 ± 0.18	1.20 ± 0.42	1.04 ± 0.02	1.75 ± 0.15
17.04 nmoles	5	1.84 ± 0.31	3.62 ± 0.56	2.59 ± 0.32	2.02 ± 0.37
68.16 nmoles	4	2.20 ± 0.56	2.80 ± 0.51	3.81 ± 1.76	3.80 ± 0.96
272.64 nmoles	4	3.09 ± 0.20	3.97 ± 0.97	5.31 ± 1.80	4.24 ± 0.90
CSF Vehicle	6	0.81 ± 0.44	0.64 ± 0.11	0.37 ± 0.19	0.53 ± 0.19

ANOVA	F		p
Main effect groups (doses Salsolinol)	(4,10)	8.18	<0.01
Main effect blocks (ethanol)	(3,54)	2.03	>0.05
Groups × blocks	(12,54)	1.82	>0.05

dose/day	n	B. Mean preference ratios ± SEM 3%–5%	6%–9%	11%–15%	20%–30%
4.62 nmoles	4	.45 ± .07	.30 ± .07	.10 ± .01	.09 ± .01
17.04 nmoles	5	.66 ± .08	.62 ± .08	.25 ± .02	.14 ± .02
68.16 nmoles	4	.57 ± .16	.58 ± .10	.40 ± .14	.18 ± .04
272.64 nmoles	4	.65 ± .03	.55 ± .15	.44 ± .15	.25 ± .05
CSF Vehicle	6	.26 ± .13	.13 ± .04	.03 ± .01	.03 ± .02

ANOVA	F		p
Main effect groups (doses Salsolinol)	(4,18)	8.57	<0.01
Main effect blocks (ethanol)	(3,54)	24.01	<0.01
Groups × blocks interaction	(12,54)	1.26	>0.25

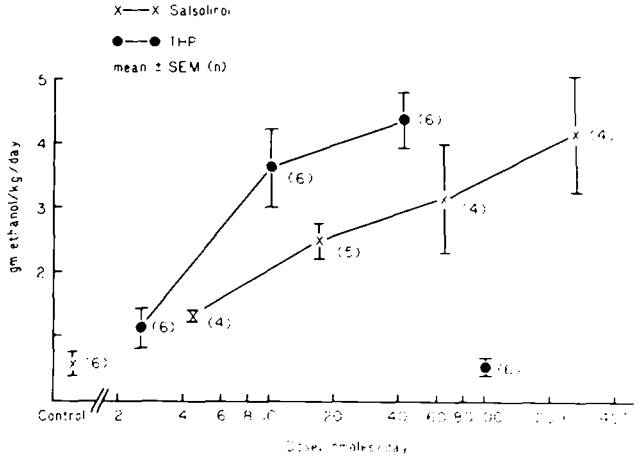

FIG. 1. Dose-response curve for salsolinol and THP expressed as mean gm/kg/day combined over ethanol concentrations of 3–30%.

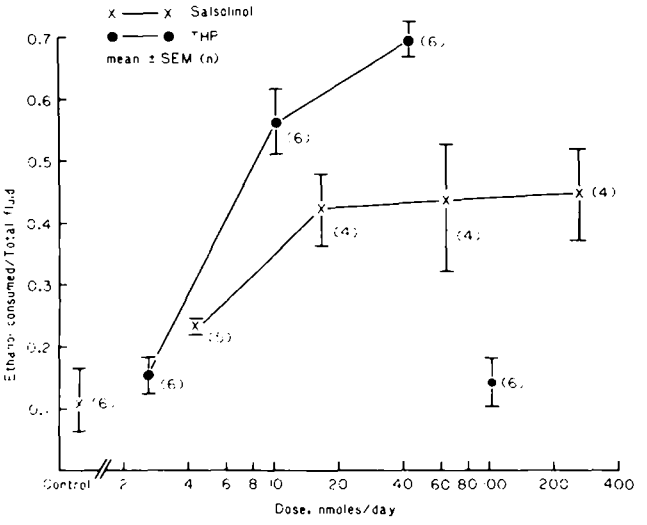

FIG. 2. Dose-response curve for salsolinol and THP expressed as mean preference ratio (ethanol consumed/total fluid) over ethanol concentrations of 3–30%.

TABLE 11

INFUSION OF DOPAMINE AND THP PLUS SALSOLINOL

A. Dopamine nmoles/day	n	Ethanol Concentration 3%–5%	6%–9%	11%–15%	20%–30%
			g/kg consumed ± SEM		
23.90	6	1.45 ± 0.29	1.40 ± 0.58	0.86 ± 0.13	0.71 ± 0.18
CSF Control (Table 7)	6	0.81 ± 0.44	0.64 ± 0.11	0.37 ± 0.19	0.53 ± 0.19
			Mean Preference ± SEM		
23.90	6	.55 ± .13	.26 ± .11	.10 ± .03	.03 ± .01
CSF Control (Table 8)	6	.26 ± .13	.13 ± .04	.03 ± .01	.03 ± .01
			g/kg consumed ± SEM		
239.00	6	1.49 ± 0.32	0.89 ± 0.33	0.70 ± 0.30	1.10 ± 0.43
			Mean Preference ± SEM		
239.00	6	.57 ± .12	.21 ± .08	.09 ± .03	.08 ± .02
B. THP & Salsolinol nmoles/day	n	3%–5%	6%–9%	11%–15%	20%–30%
			g/kg consumed ± SEM		
5.20 THP + 272.64 Salsolinol	5	2.47 ± 0.23	2.59 ± 0.45	2.27 ± 0.49	1.04 ± 0.37
			Mean Preference ± SEM		
5.20 THP + 272.64	5	.81 ± .43	.61 ± .09	.30 ± .09	.05 ± .04

ANOVA g/kg/day		F	p
Main effect groups (doses)	(1,9)	37.1	<0.01
Main effect blocks (ethanol)	(3,27)	3.6	<0.05
Groups × blocks interaction	(3,27)	3.2	<0.05
Preference			
Main effect groups (doses)	(1,9)	38.7	<0.01
Main effect blocks	(3,27)	20.5	<0.01
Groups × blocks interaction	(3,27)	7.0	<0.01

ethanol intake at 9% (mean g/kg 24 hr=6.67) averaged over 48 hr. The third rat in this group maintained a preference for ethanol at 11% concentration (mean g/kg 24 hr=5.90) averaged over 48 hr. The BAC levels determined at 4:00 a.m. were 43, 52, 27 mg/dl, rsepctively.

The Chronic Infusion of Dopamine, or THP and Salsolinol

As can be seen in Table 11A, infusion of dopamine itself caused no significant increase in alcohol intake at doses of 23.9 or 239 nmoles/day. An ANOVA comparison of the g/kg daga is presented in Table 11B for rats treated with both THP (5.2 nmoles/day) and salsolinol (277.7 nmoles/day) revealed significant main effects for groups, $p<0.01$ and significant differences for the main effect of blocks of ethanol concentrations, $p<0.05$. The groups×blocks of ethanol concentrations interaction was significant, $p<0.05$. There were significant differences between these groups at the 3–5%, 6–9% and 11–15% block of concentrations ($p<0.01$). However, these differences in intake were not present in the 20–30% block ($p>0.01$).

The ANOVA for the mean preference ratios for these groups also revealed significant main effects for groups, $p<0.01$. The main effect of blocks of ethanol concentrations was significant, $p<0.01$. The groups×blocks of ethanol concentrations was significant, $p<0.01$. Four simple effects

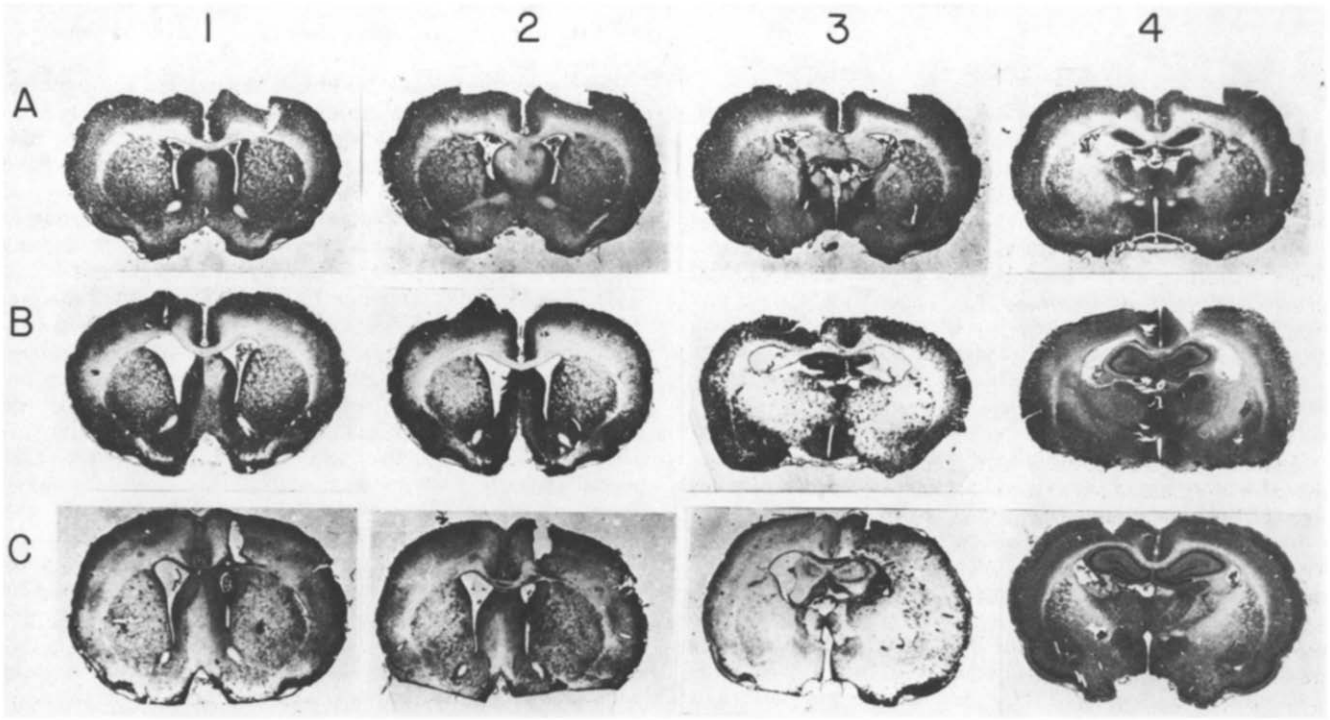

FIG. 3. Histological sections from brains of rats infused with THP or CSF for 14 days compared to an uninfused animal. Cresyl violet stain. Sections are from site of cannulation through brainstem. (A) non-infused, naive rat; (B) CSF infused control from preliminary THP experiments; (C) Rat infused with THP (10.6 nmoles/day) during a dose-response experiments.

analyses within blocks of ethanol concentrations revealed significant differences between groups at the 3–5%, 6–9% and 11–15% blocks of ethanol concentrations ($p<0.01$). The preference data did not show important differences among groups at the 20–30% block of concentrations ($p>0.25$).

Histological Aspects of the Chronic Infusion Process

The ventricular staining and histological procedures described earlier were completed on four of the five SDT rats from Experiment 1, all three of the SDP group, four SDC (CSF infused controls) and three rats infused in the third ventricle. Long-Evans rats (RRT and RRC) underwent ventricular staining and parasagital exposure of the ventricular system.

Most of these rats had dislodged their cannulae prior to histological procedures and ventricular staining could be accomplished only by pushing a 23 ga needle through the burrhole in the calvarium where the cannulae had been placed. SDT and SDC groups revealed a uniform histological trend in that all of these rats had symmetrical, bilateral enlargement of the ventricular lumen, both anterior and posterior, of the cannulae track. While direct observation of the original cannulae track was not possible, this enlargement is taken as evidence of accurate placement of the cannulae originally. There were also symmetrical, bilateral lesions of the fimbria, hippocampi and aspects of the dorsal hippocampus. There were not discerniable differences of this histological pattern for THP treated and CSF controls. The SDP rats demonstrated somewhat greater distortion of the lateral ventricle in the left hemisphere (site of cannulae placement) with moderate amounts of cellular debris visible in the ventricles of both hemispheres under a light microscope. Rats infused in the third ventricle showed a slight degree of non-specific tissue damage to the third ventricle without observable tissue damage to circumventricular structures posterior to the site of cannulation.

Histological aspects of the brains of rats used in the dose-response experiments with THP and salsolinol, paralleled the findings described for the SDP group; distortion of the ventricular lumen was restricted to the immediate vicinity of the cannula without apparent tissue damage to circumventricular structures, but with some distention of the ventricular system. In most cases the cannulae remained in place until histological sections were taken for these rats. Rats infused with THP at 10.6 or 41.6 nmoles/day from an earlier replication (n=4 for both groups) underwent complete histological examination as described earlier (data not presented). For rats included in the final replication of the dose-response experiments all rats infused at 2.6 nmoles/day and 104 nmoles/day/THP were examined by ventricular staining and histological sections. Three rats infused at 10.4 nmoles/day and three infused at 41.6 mmoles/day were treated similarly with the remainder of the rats in these groups retained for additional preference testing and drug treatment. Two rats were randomly selected from salsolinol dose-response curve for complete histological examination. There was not evidence of misplaced cannulae with the rats. Representative brain sections (40 μ) from the forebrain to brain stem for animals from preliminary experiments and dose-response experiments have been placed in Fig. 3. Similar sections for a naive, non-infused rat have been included in Fig. 3 for purposes of comparison.

DISCUSSION

We have been able to confirm the major findings of Melchior and Myers [25, 26, 30]. Animals infused with either THP or salsolinol consumed more alcohol than did CSF infused controls or naive controls. The greatest intake occurred at alcohol concentrations of 11–15% and declined at 20–30% for Sprague-Dawley rats which were of the same strain as those used by Melchior and Myers. Some rats did closely approximate the intake levels seen by Melchior and Myers (Table 9). In addition, we have observed long-term effects of these compounds on alcohol preference.

On the other hand, we did not observe intoxication, ataxia or behavioral symptoms characteristic of alcohol withdrawal.

Generally, blood ethanol levels were low and this was also characteristic of the original TIQ experiments [25], even though these earlier experiments revealed a number of rats consuming ethanol over the 11–30% range. Consequently, there has not been a demonstration that the TIQs shift the ethanol intake level to a point that could be considered intoxicating. It is clear from the several ANOVAs in the present experiments that the magnitude of the effects of the TIQs is greatest for the ethanol preference data with the g/kg/day data indicating that most TIQ infused rats regulate their ethanol intake so as to avoid blood levels that remain high during the day.

We have extended the work to develop dose-response curves. These data suggest that dosages from 5.2 to 41.6 nmoles/day of THP are effective in shifting the rat's preference for ethanol. The higher dose of THP used in these experiments, 104 nmoles/day, did not induce any shift in preference and resulted in animals drinking only control levels of alcohol. The dual action of THP on the rat's intake of ethanol is in agreement with some indications in the original experiments.

The ascending part of the THP dose-response curve also describes the salsolinol dose-response curve. Higher doses of salsolinol underwent rapid decomposition at ambient temperatures so that the question cannot be answered whether or not salsolinol's effect on ethanol intake is also antagonistic at higher concentrations. The data from the present experiment suggest that salsolinol is about three times less effective than THP (17.24 nmoles/day for salsolinol versus 5.20 nmoles/day for THP). The differences between the dose which produces maximal effects of these compounds is much greater (41.6 nmoles/day for THP and 272.6 nmoles for salsolinol). There are not meaningful differences among THP and salsolinol treated groups in terms of g/kg/day ethanol consumed at these doses. The important differences among THP and salsolinol infused rats appeared on the assessment of these groups mean preference ratios across blocks of ethanol concentrations. Again, the magnitude of the effects of THP or salsolinol on ethanol preference clearly points to THP's greater efficacy on this aspect of ethanol intake. If, as discussed below, the actions of these compounds are irreversible, one would not expect classic dose-response curves. The greatest difference between the present results and those of Myers and Melchior [25, 26, 30] is marked decrease in drinking brought about by doses of THP of 104 nmoles per day, whereas Melchior and Myers gave as much as 669 nmoles/day THP [26] without observing marked decreased drinking. They also report giving as much as 1073 nmoles salsolinol per day, a dose that we were not able to achieve, and again they observed no decrease in drinking.

The possibility that TIQ effects are related to opiate receptors in this area is an intriguing one. However, there is no substantial evidence to support this hypothesis at the moment. Studies of the binding of THP to opiate receptors have yielded IC 50 values in the range of 9×10^{-5} M [40] and 2×10^{-5} M [17,37], concentrations much higher than either we or Melchior and Myers have found effective in altering alcohol preferences. It can be calculated from the half-life of THP in the brain of 17.3 min [24] and a dose of 5.2 nmoles/day given in divided doses every 15 min, that the steady state concentration of THP in the brain would be about 6×10^{-8} M. An additional consideration arguing against reversible occupation of the opiate receptor by TIQs is that the effects are essentially irreversible. Myers and Melchior [25, 26, 30] demonstrated this, and we have confirmed it. This would indicate that some structural alteration has taken place as a consequence of the procedures used here.

The analgesic effects and epileptiform seizure patterns (EEG signs and behavioral manifestations) produced by morphine and the opiate-like peptides [4,16] are mediated by different regions in brain and certainly suggest different classes of opiate receptors in the mediation of analgesia and seizures. However, there has not been a demonstration of addictive-liability associated with the exogenous administration of the TIQs, nor their withdrawal. Although we have not observed TIQ induced epileptiform seizure patterns in the rat, it appears that these disturbances would have to be anatomically dissociated from brain sites that mediate the ethanol preference phenomenon. The application of TIQ alkaloids at specific brain loci should be capable of producing extreme motor disturbances in a dose-dependent manner, but not necessarily at the same sites as those that increase drinking behavior. Myers and Hoch [28] have reported that single injections of THP in some brain areas increasing drinking. Other areas are inactive, but no mention is made of any withdrawal-like effects at any site.

The actual extent and pattern of underlying tissue damage resulting from our own use of the chronic infusion technique has been presented, but in spite of these changes we observed no epileptiform seizures. It is possible that the behavioral effects reported in the earlier TIQ experiments [25, 26, 30, 31] were related to non-specific tissue damage resulting from the method of administration of the TIQ (see [35] also).

There is evidence of high density opiate receptor populations in the striatum [1] and additional experimental evidence suggesting that the TIQ alkaloids and morphine inhibit basal levels of dopamine sensitive adenylate cyclase [10,36]. At high concentrations neither substance inhibits dopamine sensitive adenylate cyclase in the striatum. Consequently, the evidence suggests that the influence of opiates on dopaminergic functions is not on the adenylate cyclase-linked dopamine receptor. There is evidence for two types of dopamine receptors mediating behavior [38]. The proximity of opiate and dopaminergic receptors certainly suggest some modulatory activity is possible between major putative transmitter substances and those mechanisms that possibly mediate the effects of opiate-like peptides under pathophysiological conditions. One possible outcome of this arrangement could be shared receptor mechanism or cooperativity between an opiate-modulated dopaminergic mechanism under pathophysiological conditions. The latter assumption is important in that there is no apparent physiological role for aberrant dopamine metabolites under basal

metabolic conditions. The unique pathophysiological condition associated with alcoholism possibly involve metabolic alterations of a major dopamine metabolite in the brain that interacts indirectly with opiate-receptor mechanisms. It is a possibility that such a system in brain could mediate the euphoric or rewarding properties of both ethanol and the opiates. Such a system would involve complex anatomical and neurochemical relationships between the telencephalon and brain stem [11].

Additional considerations of the TIQ's activity in the brain and their role in ethanol preference must take into account the following: (1) The complex pharmacological profiles of the TIQ alkaloids [2,8] clearly implicate TIQ activity restricted to catecholaminergic mechanisms with some recent evidence that these metabolites may influence serotonergic mechanisms in brain [19]; (2) The TIQ compounds would be most likely to act in those areas where they can be most easily formed. For example, the endogenous levels of catecholamines in the dorsal tegumentum are very low and it is unlikely that there would be substantial formation of the TIQ alkaloids in this region of the brain; (3) The conformational aspects of the TIQ alkaloids or their more complex metabolites would suggest efficacious binding at a dopaminergic receptor [7].

Additional experiments on dopaminergic systems that could be implicated in the TIQs influence on ethanol intake have used 6-OHDA injected into the ventral striatum (1 μg/4 μl) or into the vicinity of the lateral hypothalamus and ventromedial forebrain bundle (4 μg/2 μl) of THP treated rats and controls. Both of these treatments abolished the preference for ethanol in THP treated rats that demonstrated a stable preference for ethanol in the dilute range of concentrations (unpublished data). The non-specificity of 6-OHDA is well known and its action at major dopaminergic sites eliminates the rats ability to express a number of behaviors [6], especially food and water intake. Since ethanol preference, by definition, is a consummatory behavior, 6-OHDA's long-term influence on ethanol intake could suggest that intact dopaminergic systems are necessary for rats to experience the rewarding properties of ethanol. Others have observed the inhibiting effect of 6-OHDA on alcohol drinking not induced by TIQs [29].

In preliminary experiments, haloperidol (0.5 mg/kg or 1 mg/kg, IP) administered to THP treated rats with stable ethanol intake levels in the dilute range of concentrations and ethanol non-preferring controls resulted in different findings than the 6-OHDA experiments. The low dose of haloperidol (0.5 mg/kg) resulted in the typical immobilization syndrome and lowered fluid intake in control animals, when administered at 4:00 p.m. daily for a 10 day period. However, in THP treated, ethanol-preferring rats, the CNS depressant effects of haloperidol were not manifest nor were fluid intake and preference for ethanol drastically reduced when these rats were compared to controls. The high dose of haloperidol (1 mg/kg) resulted in immobilization and reduced fluid intake in both THP treated, ethanol-preferring rats and controls. However, haloperidol did not have permanent effects on ethanol preference as did 6-OHDA. The interesting aspect of this data is that the low dose of haloperidol did not alter the preference pattern of THP treated rats. Haloperidol's specificity for the adenylate cyclase sensitive dopaminergic receptor at low doses could mean that the TIQ alkaloids alter dopaminergic transmission or affect a class of dopaminergic receptors that are not linked to an adenylate cyclase. Higher doses of haloperidol could be non-specific and influence a wider range of receptors, one of which could mediate the TIQs effect on ethanol preference. This latter question could be answered by additional experiments with haloperidol at specific dopaminergic sites in the brain.

The major difficulties in assessing the TIQ's role in ethanol preference continues to be the absence of detailed knowledge about the distribution pattern of these compounds or their metabolites in brain. There is recent evidence from mass fragmentography that 3',4'-deoxynorlaudonsoline carboxylic acid (DNCLA), a TIQ alkaloid derived from dopamine and phenylpyruvic acid, is present in the brains of rats with experimentally induced hyperphenylalaninemia (PKU) at concentrations that are physiologically significant [23]. The distribution of DNCLA appears to be restricted to the medulla-pons, midbrain, cortex and cerebellum. PKU rats have reliably shown substantial increases in their brain levels of DNCLA primarily on cortex and cerebellum. There also is speculation that THIQ derivatives are involved in the abnormal movements of Parkinsonism [20]. In both of these instances TIQ alkaloids are present in diseased brains or experimentally induced conditions that are indicative of drastic alterations of the brain's metabolic activity. Alcoholism is postulated to have its own unique effect on brain metabolism. Although there is now some evidence that ethanol treated rats have detectable amounts of O-methylated salsolinol in their brain [18], it is not clear whether or not continuously high acetaldehyde levels or blood ethanol levels do indeed enhance the formation of these alkaloids or their more complex multi-ringed metabolites in the alcohol dependent human brain. Administration of exogenous TIQs in rat brain has not resulted in ethanol dependence in the rat probably because there has not been a tendency for THP treated, ethanol preferring rats to consume more ethanol than can be metabolized, or to maintain high blood ethanol levels. These objections to the exogenous administration of the TIQs as a model for alcoholism do not argue against a role for these substances in alcoholism, although drastic alterations in brain metabolism may be required for the enhanced formation of these metabolites. Most importantly, however, the availability of an assay which is capable of detection of THP or its metabolites at the levels of at least 20 ng/g (6×10^{-8} M) or lower is imperative.

Brown *et al.* [5] have recently reported that they are unable to induce drinking in Wistar rats by intraventricular infusion of THP. Any one of several differences between the present work and that of Myers and Melchior on the one hand and that of Brown *et al.* could explain this discrepancy. However, it would seem most likely that the explanation may be found in the strain of animal used. Sprague-Dawley and Long-Evans rats used in this study have a low preference for alcohol normally, while Wistar rats used by Brown *et al.* have a much higher preference. In fact, the control levels of drinking reported by Brown *et al.* are nearly as high as we are able to achieve by TIQ infusions. Since our data and a very large literature indicates that rodents will not imbibe alcohol to any significant extent above their metabolic capacity, this may serve as an upper limit on the amount of alcohol that an animal will select, given a choice in the matter.

Another major difference is ours and Myers' use of ascorbic acid to retard oxidation of the TIQs, but this is not done by Brown *et al.*. If there is an interaction between ascorbate, alcohol and TIQs, the present experiments would not detect it.

We have confirmed the major findings of Melchior and

Myers. The important differences between these experiments are summarized: (1) The present data has demonstrated a low frequency of animals with mean preference ratios above 0.5 at the aversive range of concentrations, 20–30%; (2) These experiments have not demonstrated that the intraventricular infusion of THP or salsolinol induce behavioral symptoms indicative of ethanol withdrawal; (3) We have observed decreased preference at high doses of THP. Although the effects of both THP and salsolinol on ethanol preference suggest a permanent alteration of brain biochemistry, there is no evidence that the permanent change in the rat's preference for ethanol can offer substantive model of alcoholism.

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