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Intraventricular Ethanol and Ethanol Intake: A Behavioral and Radiographic Study[1]

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FRIEDMAN, H. J. AND D. LESTER. *Intraventricular ethanol and ethanol intake: A behavioral and radiographic study.* PHARMAC. BIOCHEM. BEHAV. 3(3) 393–401, 1975. – Ethanol (10% w/v) was infused intraventricularly at a rate of 11 μl/hr, delivered over 50 sec every 10 min for 10 days into 5 Sprague-Dawley and 5 Wistar rats. Thereafter, preference testing with ascending concentrations of alcohol solutions vs. water vs. food gave no significant differences between treated and sham-operated controls, in contradiction to previously reported increases in alcohol consumption. Ethanol's rate of elimination from the ventricle and its pattern of diffusion into the brain were determined using radioactive ethanol: elimination from the brain is rapid with a half-life of 24 to 35 sec, and the amount diffused throughout the brain small, with a maximal concentration in any one section of 0.004% (such a concentration is at least 20 times less than would result from a moderately intoxicating parenteral dose of ethanol). The character of the elimination and the lack of effect on alcohol intake found here indicate that intraventricularly administered ethanol is a technique with no usefulness in elucidating the processes affecting alcohol addiction.

Ethanol drinking Ethanol administration Intraventricular infusion Ventricular ethanol elimination Brain ethanol diffusion

INGESTION of alcohol is an obvious and principle requirement for an animal model of alcohol addiction [30]. Various manipulations have been used to induce animals to increase their consumption of alcohol in order to understand the operative physiological and behavioral factors which control its choice. Among the factors investigated have been: stress [5], genetic differences [12,32], polydipsia [13.28], and alcohol deprivation [46,47].

A few studies have attempted to change alcohol intake by indirect manipulation of the central nervous system, e.g., electrical stimulation of the lateral hypothalamus [3], while other studies have sought an association between the levels of putative neurotransmitters, e.g., serotonin [1] and the level of alcohol intake. Notable in this category is the finding that the injection of minute quantities of ethanol into the lateral ventricles of rats results in an increase in the animals' oral intake of alcohol [33]; these workers observed that volumes of up to 3 μl of 10% (v/v) ethanol infused over 86 sec every 15 min for 10 days caused the animals to consume ethanol in preference to water [33,36]. This technique, resulting in a long-term increase in alcohol intake (and a seemingly permanent alteration in the CNS [33,34]), seemed to have great promise in the study of alcohol choice and addiction [29]. The only question raised about these findings was the possibility that the tonicity of the infusion was related to the effect [29].

Intraventricular infusions of acetaldehyde, methanol, and paraldehyde in rats are also reported to result in increases in alcohol intake [39]. The mechanism of action of the various infused solutions was unclear to the authors: they speculated (1) that perhaps the infusions interfered with serotonin (5-HT) metabolism and (2) that intraventricular alcohols and aldehydes block the sensation of noxious olfactory and gustatory qualities of ethanol. The first speculation is based on questioned [7,45] findings that inhibition of 5-HT metabolism by p-chlorophenylalanine (p-CPA) can affect alcohol intake [38]. The second speculation may be valid because oro-sensory cues are important factors in alcohol intake [20, 27, 41], but it should be noted that even anosmic rats [20] do not consume the concentrations or quantities of ethanol reportedly consumed after intraventricular infusions. In addition, the results of the study reported here indicate the unlikelihood that intraventricular infusions reach areas involved in olfactory or gustatory sensibility and thereby reduce the sensitivity of these senses.

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Intraventricular 5-hydroxytryptophol (5-HTOL) was next found to cause an increase in alcohol intake in rats [36]. What relationship this might have to the hypothesis of serotonin involvement in ethanol intake is not readily apparent since 5-HTOL, a liver metabolite of 5-HT, is not present in appreciable amounts in the brain [11, 49, 50]. The results of 5-hydroxytryptophan (5-HTP) administration, both systemically and intraventricularly, also cast doubt on the serotonin hypothesis; 5-HTP and p-CPA have opposite biochemical effects [23], yet Myers and colleagues [36,38] found that both cause decreases in alcohol intake. The question regarding tonicity [29] was answered by Myers *et al.* [36]: isotonic solutions of ethanol, acetaldehyde, paraldehyde, and 5-HTOL, infused intraventricularly, all produced increases in alcohol selection.

Two other groups have attempted to extend these findings to other species: Jones *et al.* [19] infused comparable amounts of ethanol into dogs, and Koz and Mendelson [25] infused 1 monkey for 1 hr/day. Both were unsuccessful in increasing alcohol intake and attributed their negative findings to species differences; unfortunately, neither attempted to replicate the reported results in the species used by Myers.

On the other hand, Myers *et al.* [40] have since partially extended their findings to monkeys. Ethanol, paraldehyde, and acetaldehyde were infused intraventricularly; ethanol produced intermittent increases in EtOH intake, but the other substances produced no reliable effect. The authors believe the intraventricular effect not to be species specific; Koz and Mendelson's [25] negative results are attributed to a different infusion pattern while those of Jones *et al.* [19] are attributed to too high an ethanol concentration in the preference test.

Cicero and Smithloff [8] have infused rats intraventricularly with ethanol in an attempt to increase their EtOH intake; no increases were found. Although the authors could not explain the discrepancy between their results and Myers' [33], they stated that the intraventricular route is not useful for studying alcohol selection. However, it is difficult to conclude that Cicero and Smithloff's results disprove Myers' original findings [33] because of several differences in procedure. The animals were given a preinfusion exposure to alcohol, had alcohol available as a sole fluid during infusion, had a one day hiatus in both oral and intraventricular administration and then had water-ethanol preference testing during which no infusion was given; the infusion pattern was not experimenter controlled, but was determined by the animals' drinking, resulting in a nonuniform rate of administration. Their 5 μl infusions were delivered over 15 sec, a rate 9 times higher than that used in Myers' [36] experiments or in that reported in this paper and which might unduly raise intracranial pressure, an issue mentioned by Cicero and Smithloff [8].

The relationship between the substance infused and the substance consumed is not known. The intraventricular route is non-specific as to stimulus since ethanol, methanol, acetaldehyde, paraldehyde, and 5-HTOL all reportedly produce similar effects although their chemical relatedness vis-a-vis possible receptors is unclear. The specificity of the response is as yet untested: will infusion of specific substances produce consumption of specific solutions, or will infusion of any substance produce consumption of any sapid fluid?

The initial purpose of our experiments was to resolve the question of generality and then to investigate the mechanisms by which the infusion produced alcohol drinking – and thereafter to study the relationship of such mechanisms to alcohol addiction. However, it was first necessary to attempt to unravel the conflict between the results of Koz and Mendelson, Jones *et al.*, and Cicero and Smithloff and Myers' numerous findings.

The first experiment attempted to replicate Myers' findings on rats of the same strain [16]. Since an effect of the infusion could not be found, we were unable to proceed with experiments about generality and the mechanisms involved. The second and third experiments were initiated as additional indicants of whether or not intraventricular ethanol might have a central effect; C^{14}-ethanol was infused and its pattern of diffusion and rate of elimination determined: the rate of ethanol elimination from the ventricular space is extremely rapid and brain ethanol levels remain far below those generally considered minimally effective [21], a result indicating that the infusion is unlikely to have an effect on drinking.

From the data it can be concluded that the differences in findings previously reported can only be attributed to unknown differences in methodology enabling one researcher to obtain an effect otherwise not reproducible. These experiments, supported by previous findings [8, 19, 25], indicate that the intraventricular infusion of ethanol is not an effective technique for research in alcohol addiction.

EXPERIMENT 1

Method

Animals. Male Sprague-Dawley and Wistar rats were obtained from Charles River Breeding Laboratories. They were experimentally naive and weighed 200–300 g at the start of the experiment. They were maintained under a 12 hour light – 12 hour dark cycle.

Apparatus. The cannula system (Fig. 1) consists of an outer guide cannula of 22 ga stainless steel tubing and an inner cannula of 28 ga tubing. The guide cannula is silver soldered into a stainless steel Luer-Lok syringe hub, the base of which has been trimmed flush. The 22 ga tubing extends about 5 to 6 mm below the base of the hub and about 2 mm above it.

The inner cannula is held inside a 1.5 cm long 13 ga needle with hub and extends about 3 mm above it. A 5 mm length of 22 ga tubing is slipped over the 28 ga tubing, and both are silver soldered inside the 13 ga needle; the joint is tested for water tightness. The inner cannula can then be lowered into the outer cannula, and the hub of the 13 needle screwed into the Luer-Lok hub, locking the inner cannula in place, about 1 to 2 mm below the guide cannula.

When the inner cannula is not in place, loss of cerebrospinal fluid (CSF) is prevented by inserting a stilette in the outer cannula. The stilette is a 28 ga rod soldered into another 13 ga needle and hub.

The infusion solution is contained in 1 ml B-D disposable plastic syringes mounted on a Harvard Apparatus Continuous Infusion Pump (Model 945); the pump was programmed to operate for 50 sec every 10 min. Each syringe was connected by polyethylene tubing (Clay Adams, PE 50) to a swivel device (Lehigh Valley Electronics, Model 192–03) suspended over each cage. The swivel in turn is connected to the inner cannula by another length of PE 50 tubing which is forced over the 22 ga tubing sheathing the 28 ga inner cannula. A spring (Newark

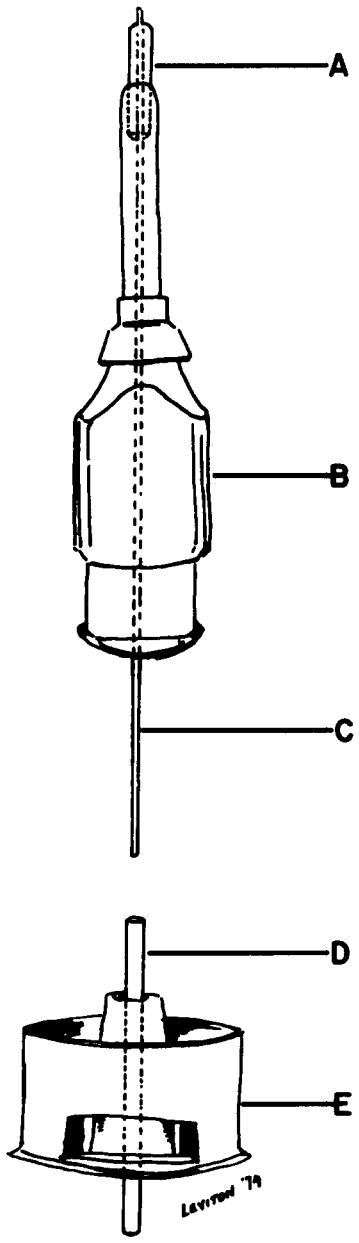

FIG. 1. A piece of 22 ga tubing (A) sheathes the top of the 18 ga inner cannula (C), and both are silver soldered inside the barrel of a 13 ga needle (B). The inner cannula (C) fits into the outer cannula (D), a piece of 22 ga tubing soldered inside a Luer-Lok hub (E).

Spring Co., 526) covers the PE 50 tubing, protecting it from puncture by the animal; the spring is attached at one end to the swivel and to the 13 ga needle at its other end.

The animal living chambers were galvanized steel chambers, 30 cm high and 26 cm in diameter; three 2.5 cm^2 opening, 5 cm apart, were provided for drinking bottles, and two screened openings (8 X 10 cm) were on either side of the bottle openings, 13 cm above the base. The smooth inside surfaces of the the chambers prevented damage to the cannula system.

Procedure. Five Wistar and 5 Sprague-Dawley rats were implanted with the cannula while under Nembutal-chloral hydrate anesthesia, so that the tip of the inner cannula was in the lateral ventricle: coordinates, AP, 6.1 mm; horiz., 2.0 mm; lateral, 1.5 mm [24]. After the operation the inner cannula was replaced by the stilette, and the animals were given a 5 day recovery period with food (Purina rat chow) and tap water available ad lib. Drinking solutions were presented in Richter drinking tubes.

All ethanol solutions were prepared from 95% ethanol and distilled water. The infusion solution was 10% (w/v) EtOH and was infused at 11 μl/hr, delivered over 50 sec every 10 min – a mean of 1.83 μl/injection. The infusion began after the recovery period and lasted for 10 days with food and water ad lib.

Five other Sprague-Dawley rats underwent sham operations and were used as controls. A cannula was lowered into the ventricle and then removed. Thereafter the control animals were treated identically to the infused animals, except for the infusion.

During the subsequent preference testing period all animals were 23 hr food and fluid deprived and had a food-water-alcohol choice in a one hour preference test. The fluids were presented in a 3-bottle, 2-fluid manner [37], and the bottles were rotated randomly each day. On the first day of testing (Day 11) the ethanol concentration was 3% (w/v) and was increased in 1% steps daily. The infusions in the 10 treated animals continued during testing.

At the end of testing methylene blue was injected into the cannula to verify patency, and histological examinations verified cannula placement.

Results

No increases in alcohol intake were found in the animals as a result of the alcohol infusion. The alcohol intake, expressed as ml 10% ethanol/ml total fluid intake, is shown in Fig. 2. The two groups of treated animals, Wistar and Sprague-Dawley, are represented together since analysis of variance showed no significant difference between them ($p>0.50$). More important, there was no significant difference between the treated animals and the control animals ($p>0.50$).

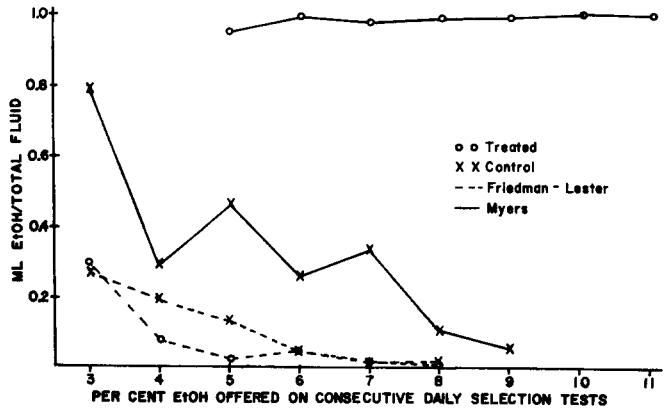

FIG. 2. Ethanol as proportion of total fluid intake. Myers' data from [33].

Calculated as mean grams ethanol/kg body weight, the controls show a somewhat higher (not significant, $p>0.10$) intake than the treated animals (Fig. 3). Once again the results of the two treated groups are combined since there

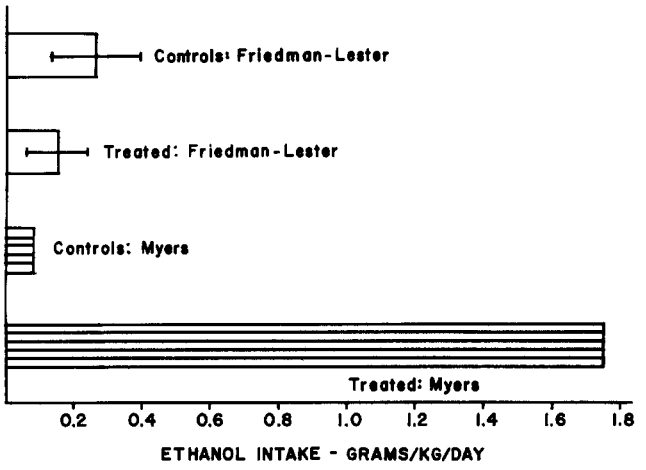

FIG. 3. Mean daily ethanol intake, grams/kg. Myers' data from [35].

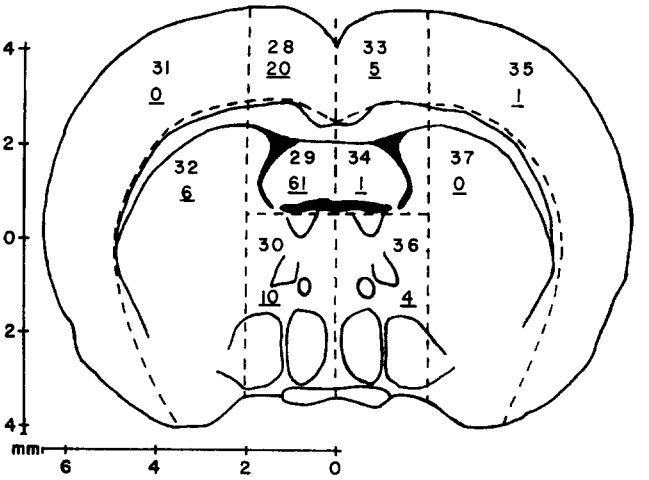

FIG. 4. Posterior surface (AP, 6.3 mm) of a coronal section of the acutely treated animal (rat 2). Thickness of section was 1.2 mm. Dotted lines indicate division of the section into samples. Non-underlined numbers are sample numbers, and underlined numbers are dpm/10 mg found in that sample.

was no significant difference between them, nor were significant differences ($p>0.50$) found in total fluid intake (ml fluid/kg body weight).

EXPERIMENT 2

C^{14}-EtOH was infused and its pattern of diffusion and rate of elimination determined, providing evidence as to whether the intraventricularly administered alcohol might be affecting areas involved in drinking, olfaction, or taste.

Method

Animals. Three female Sprague-Dawley rats, 200–300 g, were used. Food and water were available ad lib throughout the experiment.

Procedure. The operative procedure was the same as in Experiment 1 except that the verification of cannula placement was made by obtaining a back-flow of fluid out of the cannula. The infusion solution was again 10% ethanol, to which an appropriate amount of EtOH-C^{14} (New England Nuclear, 2 mCi/mM) was added. The amount of radioactivity of the solution was determined as described later. One of the animals was infused for 5 days at the same rate as the animals in Experiment 1. The animal was sacrificed immediately after the end of the last infusion by placement in a CO_2 gas filled chamber; death occurred within 2 min. The animal was then packed in dry ice and frozen to prevent volatilization and any further diffusion of the ethanol.

After freezing, the brain was excised and mounted on a chilled metal plate (held over dry ice) between two pairs of blocks which provided a cutting guide for a Stadie-Riggs knife. The brain was cut into coronal sections of varying thickness (1–3 mm), and each section was further cut, along anatomical boundaries if possible. The size of the sections and pieces depended positively on the proximity to the cannula site. An example of the sectioning is shown in Fig. 4. Each tissue section was weighed immediately after being added to preweighed scintillation vials containing 1 ml of distilled-deionized water; then 3 ml of 95% EtOH and 11 ml of scintillation fluid (340 ml Triton X-100, 718 ml toluene, 42 ml Liquifluor) were added to each vial. Radioactivity was counted in an Intertechnique SL-30 scintillation counter; disintegrations per minute (dpm) were obtained from the counts (cpm) after correcting for tissue quenching by use of an external standards ratio method and a series of variably quenched standards.

To determine the radioactivity of the infusion solution and to verify the volume given per 50 sec pulse, several 5 μl samples of the infusion solution and several 50 sec infusions delivered by the pump through the cannula were injected into counting vials for scintillation analysis.

To determine the elimination rate constant of ethanol from the ventricle, a second animal was given a single 1.8 μl dose of the labelled 10% EtOH, infused over 50 sec. Two minutes after the infusion the animal was immersed in liquid nitrogen, resulting in an almost immediate cessation of all diffusion. The brain was removed and analyzed as described.

To test the accuracy of the techniques used the third animal was infused with 5 μl of the solution after being killed by being placed in a CO_2 chamber. After infusion the animal was frozen with dry ice and the described procedure followed. Any ethanol not recovered would be due to the technique and not diffusion.

Results

The results with the control animal (Rat 3) verify that essentially no alcohol was lost as a result of the procedure: a 5 μl dose directly into scintillation vials averaged 91,426 dpm: recovery from the brain was 91,189 dpm, essentially complete. Radioactivity not recoverable in the other animals can be assumed to have been removed before the animal was killed and the analytical process begun.

In the acutely treated animal (Rat 2) 400 dpm were recovered, an amount consistent with the idea that the infused ethanol rapidly diffuses throughout the body; three 1.83 μl standards had a mean of 25,464 dpm. Since the drug was administered at a constant rate, administration was considered a zero-order process, while the rate of elimination of the ethanol was considered first order and proceeding according to the equation $C=C_o e^{-kt}$.

An iterative computer calculation was performed every 0.01 seconds assuming 0.01 sec pulses of EtOH being administered and subjected to elimination processes together with the reminder from previous administrations; solving, k=0.029 sec^{-1}. The half-time of the elimination process equals 0.693/k; solving, $t_{1/2}$ = 24 sec. The distribution of the infused alcohol in one coronal section is shown in Fig. 4. Since the sections were not of uniform size (the sizes are shown in the figure), the absolute values (dpm) were converted to dpm/10 mg frozen tissue to denote relative alcohol concentration. The coronal section shown is the one in which the cannula was located and has the highest values of dpm/10 mg found. The large value of Section 28 is probably an artifact caused by ethanol being wiped off the cannula as it was removed.

In the animal which received the chronic administration (Rat 1), a total of 16,067 dpm were recovered from the brain, an average of 30,599 dpm being administered per 50 sec pulse (about 22,000,000 dpm/5 day period). The distribution of the radioactivity in the coronal section with the highest levels, the section in which the cannula was located, is shown in Fig. 5. If it is assumed that the radiocarbon arises from ethanol alone in this animal, then the elimination constant would be 0.004 sec^{-1} and $t_{1/2}$ = 174 sec when death (and cessation of life processes) is taken as 2 min from the end of injection. The lengthy half-time appeared compatible with the half-time found in the acute animal only if most of the radioactivity arises from incorporated C^{14} rather than ethanol.

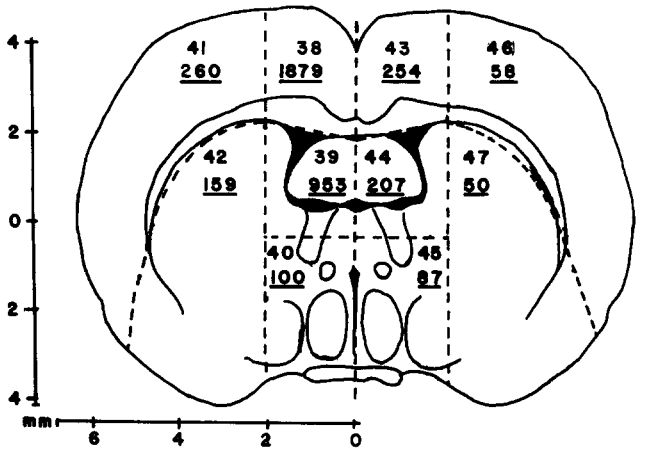

FIG. 5. Posterior surface (AP, 6.4 mm) of a coronal section of the chronically treated animal (Rat 1). The thickness of the section was 1.0 mm. Dotted lines, non-underlined numbers and underlined numbers have the same designation as in Fig. 4.

EXPERIMENT 3

At least 6% of a dose of ethanol is incorporated into tissue constituents before being converted to CO_2 [6,53]. In the chronically treated animal much of the C^{14} found thus represents C^{14} incorporated into other compounds. In Experiment 2, most of the radioactivity in Rat 1 most probably arises from nonethanol C^{14}. To determine the extent of this incorporation and to assess the amount of ethanol in the brain, samples from infused animals were heated to volatilize the ethanol and were compared to samples from which the ethanol had not been removed; the difference between such samples was taken as the maximum amount of ethanol present in the tissue.

Method

Animals. Four female Sprague-Dawley rats, 200–300 g, were used.

Procedure. Two of the animals were implanted with intraventricular cannulae. The operative procedure and ethanol administration were the same as with the chronic animal in Experiment 2. At the end of 5 days of infusion the animals were immersed in liquid nitrogen until frozen, the first animal at 90 sec and the second at 60 sec after the end of the last infusion. The whole brains and 1–2 g samples of liver were removed and weighed, and each was homogenized at 0°C in an equal weight of water using a motor driven teflon pestle (Arthur H. Thomas Co., Philadelphia, Pa., 3431–F50). Aliquots, 100–200 mg, of the homogenates were transferred to pre-weighed scintillation vials and the actual weight of the sample determined by reweighing the vial. The various fluids (water, EtOH, and scintillation fluid) were added to 2 samples of each homogenate, and the radioactivity was counted. Two other samples of each homogenate were placed, in uncapped vials, in a 100°C oven for 1 hr to volatilize the ethanol. Preliminary studies had shown that heat volatilization and freeze-drying were equally reliable and reproducible in removing ethanol but heat volatilization was less time consuming. After volatilization the various fluids were added and the radioactivity counted.

The other two animals received 1.5 g C^{14}-EtOH/kg, 10% (w/v) EtOH, intraperitoneally. They were killed in a CO_2 chamber, one 4 hr and the second 24 hr after injection. The brains were removed, frozen with dry ice, and analyzed as described above.

Results

The radioactivity in the volatilized and non-volatilized brain samples from the first intraventricular animal was, respectively, 39.1 dpm/5 mg tissue and 43.7 dpm/5 mg, as shown in Table 1. The difference between the volatilized and non-volatilized samples, presumably C^{14}-ethanol, is 4.6 dpm/5 mg or 1611 dpm/whole brain and 5.6 μg/g brain. The amount of ethanol injected per nominal 1.8 μl pulse had a mean of 29,670 dpm. Using the procedure described in Experiment 2, k = 0.026 sec^{-1} and $t_{1/2}$ = 27 sec. The calculated concentration of liver ethanol was 33.4 μg/g.

In the second intraventricular animal, the radioactivity due to C^{14}-ethanol was 18.6 dpm/5 mg or 6026 dpm/whole brain, equivalent to 21.79 μg EtOH/g brain. Since the amount of ethanol injected per pulse had a mean of 30,728 dpm, K = 0.0196 sec^{-1} and $t_{1/2}$ = 35 sec. The ethanol concentration was 32.7 μg/g.

Four hours after an IP dose of C^{14}-ethanol containing 90,961 dpm/5 μl the ethanol concentration was 750 μg/g brain.

Brain samples 24 hours after an IP dose of C^{14}-ethanol containing 39,704 dpm/5 μl gave a brain ethanol concentration of 6 μg/g.

DISCUSSION

Although we had hoped to find an increase in alcohol intake resulting from the infusion and thus obtain some insight into the processes which affect alcohol selection, no

TABLE 1

C^{14} RECOVERED FROM VOLATILIZED AND NON-VOLATILIZED BRAIN HOMOGENATES AND THE CORRESPONDING ETHANOL CONCENTRATIONS FROM EXPERIMENT 3

Rat	1	2	3	4
Time of death (post-injection)	90 sec	60 sec	4 hr	24 hr
Brain (grams)	1.77	1.62	1.80	1.93
Total C^{14} (dpm/5 mg brain)	43.7	67.8	846	44.4
Non-volatilized C^{14} (dpm/5 mg brain)	39.1	49.2	165	41.9
Total C^{14} (dpm/5 mg liver)	145.8	133.9		
Non-volatilized C^{14} (dpm/5 mg liver)	118.3	105.7		
EtOH-C^{14} (dpm/brain)	1628	6026		
EtOH (μg/g brain)	5.6	21.8	749*	6.3
EtOH (μg/g liver)	33.4	33.0		
$t_{1/2}$	27	35		

*The amount (749 μg EtOH/g brain) reflects (assuming a water content of the brain of 78% and of 65% for the body water generally) a metabolism in this rat of about 220 mg EtOH/kg, 23% less than usually reported after cold ethanol, a decrease not surprising in view of the great lethargy and depression produced by the large amount of radiocarbon injected (124 μCi/kg).

such effect was found; it was, therefore, impossible to proceed with experiments directed to the question of specificity or the mechanisms involved.

This study, with its negative results, is in direct contrast to the overwhelming effect reported by Myers. However, the results here are in agreement with those of Koz and Mendelson [25], Jones *et al.* [19], and Cicero and Smithloff [8]. Koz and Mendelson [25] and Jones *et al.* [19] attributed their negative findings to species differences, while Myers [40] speaks of a different infusion pattern and alcohol concentrations. Cicero and Smithloff [8] did indeed use a different infusion pattern and procedure, but the species was the same. The negative findings of this study, however, cannot be attributed to any of these factors: the infusion pattern was similar, the alcohol concentrations were similar, and the species and one of the strains identical.

Non-ventricular administration has had various effects on alcohol intake. McClearn and Nichols [31] in C57B1 mice and Thiessen and Rodgers [48] in C57B1 and R111 mice found IP injections of ethanol to decrease subsequent ethanol selection. Under some conditions, prior exposure to alcohol results in a greater alcohol intake. Rats exposed to alcohol in a free-choice situation gradually increased their alcohol intake over several months [52,54]; forced administration of ethanol (as the sole fluid available) increased subsequent free-choice of ethanol when gradual acclimation to increasing concentrations was used during the sole fluid period [51] or when a long duration sole fluid period (330–350 days) was used [51,54], while a short, single EtOH concentration exposure resulted in a decrease in alcohol selection [52]. Barry and Perhach [4] found that long-term IP injections of alcohol at 4 g/kg increased choice of alcohol in rats; a dose of 2 g/kg had no effect. Deutsch and Koopmans [10] have reported that six days of intragastric administration of EtOH, at a level which maintained the animals in an ataxic state and on the verge of consciousness, produced a large and sustained increase in subsequent alcohol selection.

One must conclude that to increase alcohol selection requires doses large enough to produce either behavioral signs of intoxication [4,10] and/or long-term free-choice or gradually increasing concentration acclimation [51, 52, 54]. The intraventricular method fulfills neither condition. The alcohol administration lasts only 10 days whereas the shortest non-ventricular exposure producing an increase is 18 days [52]. This study, along with that of Myers [34] and that of Cicero and Smithloff [8], finds that the infusion produces no discernible signs of intoxication; inasmuch as the amounts of alcohol infused were small, the lack of

intoxication is not surprising. On the other hand, this study finds no increase in aggressiveness due to the infusion, in contradiction to previous reports (cited by [29,34]). The whole brain ethanol concentration 1 min after the end of the infusion (the second intraventricular animal of Experiment 3) was 0.0022%. This level is substantially lower than body fluid concentrations generally considered as minimal levels (0.025 to 0.04%) necessary for behavioral and physiological effects [21]. The alcohol levels found successful for increasing alcohol intake have been about 0.2% [4, 10, 21]. The brain ethanol level 24 hours after the IP dose of 1.5 g ethanol/kg was of a magnitude equal to the levels found shortly after the intraventicular infusion: 100-fold higher levels were found in brain 4 hours after an IP dose of 1.5 g/kg. For the brain as a whole then, the alcohol levels achieved are higher and more sustained after IP injection than after intraventricular infusion.

With a calculated half-life of 24 to 35 sec, virtually all the ethanol of one pulse is eliminated before the next pulse is delivered. The distribution of ethanol which would be reasonably expected after each pulse in a chronically infused animal can, therefore, be considered to be essentially the same and can also be considered identical to the distribution in an animal given a single infusion, as in Experiment 2. In the acutely infused animal all of the C^{14} can be considered to be ethanol since not enough time has elapsed to allow any appreciable amount of metabolism and incorporation. The highest ethanol concentration found in any section was only 0.004% (Fig. 4, section 29).

It is unlikely that intraventricular ethanol could increase alcohol intake by affecting the gustatory or olfactory qualities of ethanol, as theorized by Myers and Veale [39], or by affecting drinking behavior. None of the areas generally considered to be involved in drinking [15], taste [26, 42, 55], or olfaction [2] have appreciable levels of ethanol.

Cicero and Smithloff [8] believe that the infusion is ineffective because their 5 μl dose is diluted 300 times by the 1500 μl of brain water before reaching any target sites, including areas immediately surrounding the ventricle. This assumes instantaneous, uniform distribution throughout the brain, quite different than would be expected from a diffusion process in which areas proximal to the ventricle would have the highest levels initially. Our experiments clearly show that the infusion is ineffective because ethanol is rapidly eliminated rather than merely diluted; in Experiment 3 only 6026 dpm were recovered out of over 26 X 10^6 dpm ethanol administered. So little alcohol remains in the ventricles that pharmacologically important amounts do not appear in adjacent areas.

The choroid plexus, the tissue which projects into the ventricles and secretes the cerebrospinal fluid, is the most likely exit route for the infused EtOH. The choroid plexus contains an extensive venous network only one cell layer removed from the surface, comprising a large surface area of villi and microvillous projections [9]. Active transport mechanisms within the plexus are involved in the removal of various solutes from the CSF in a number of species [22,44] while a large proportion of solutes are removed from the CSF by passive diffusion into the blood. Pappenheimer *et al.* [44] found that the passive diffusion component is quite rapid: inulin, a large molecule relative even to glucose, diffused out of the CSF into the blood at the rate of 3 moles/sec/gm of tissue, 6 times the rate in muscle. Tritiated water diffuses into the blood from the CSF in the goat at 10 times the rate of inulin [43]. It seems not unlikely, therefore, that the bulk of the infused ethanol would also be eliminated from the CSF as is tritiated water or inulin. Davson [9] believes that diffusion into the blood is probably ethanol's exclusive route of exiting the ventricle, and Pappenheimer and Heisey [43] report that ethanol, a small and essentially non-ionized molecule like water, diffuses into the blood at a rate comparable to water. As Experiment 3 shows, after intraventricular ethanol administration higher ethanol levels are found in the liver than in the brain. Myers' [34] speculation that intraventricular ethanol does not pass into the general circulation is thus untenable.

Intraventricular administration has been used with other drugs to separate systemic and central effects, e.g., histamine [14], and to determine central sites of drug action, e.g., morphine and antagonists [17,18]. From the initial reports of Myers and his colleagues [33, 34, 35] the intraventricular route seemed suitable for doing similar determinations with ethanol [29]. However, this study has found that ethanol's rate of elimination is so rapid and the amount of ethanol diffused into the brain tissue so small that central effects are neither likely nor detectable.

We believe a final comment on the quality of the research in this area is appropriate; five groups have tried intraventricular infusions of ethanol [8, 16, 19, 25, 33, this paper] and only one has obtained a series of positive results [33, 34, 35, 36, 39, 40]. Koz and Mendelson [25] and Jones *et al.* [19] avoided the issue by their seeming acceptance of Myers' results, attributing their own findings to species differences: not finding an effect with dogs or with one monkey hardly verifies an effect with rats. The use of a different procedure [8, 19, 25] and the use of a single subject [25] in an experiment of this nature make for an inadequate and inappropriate replication and inconclusive results. We conclude that there is no effect, that neither species nor procedure nor ethanol concentration determine the negative result, but that there is an artifactuality of some kind which has yielded the positive series of results of Myers.

The weight of evidence indicates that intraventricular infusion of ethanol produces no increase in its oral intake and that this technique has no discernible usefulness in elucidating the processes affecting alcohol addiction.

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