FACTORS INFLUENCING THE ELEVATION OF VOLUNTARY ETHANOL INTAKE BY HYPOTHALAMIC STIMULATION IN RATS

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Abstract

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In an attempt to determine the locus of lateral hypothalamic (LHA) stimulation which maximizes voluntary ethanol intake, rats were stimulated at a variety of hypothalamic sites in a standard brain stimulation paradigm. The electrodes were aimed either .4 mm anterior, posterior, dorsal, or ventral to placements used by others. Only small, statistically unreliable, differences in ethanol intake levels were seen between the stimulated groups with different electrode placements. To further examine the small differences, a replication study was run with the addition of a group of non-implanted control animals. Again, there were only small, non-significant, differences in ethanol intake levels between the groups with varying electrode placements. The pattern of differences did not clearly replicate with the possible exception of the group with the more anterior placements which consumed the highest percentage of ethanol in both studies. In both the original study and the replication, the pooled stimulated animals drank significantly more ethanol than the implanted, unstimulated animals at day 30; however, this difference was only temporary. By the end of the experiment, day 72, there was no significant difference between the two groups. Histological confirmation of the electrode sites revealed that all the placements were dorsal

to the intended targets; thus the findings regarding locus of stimulation are not definitive and further work is needed in this area. The two control groups of non-stimulated rats differed in their level of ethanol consumption. The implanted control animals consumed significantly less ethanol than the non-implanted control animals by day 30; however, there was no significant difference at day 72. Thus, some aspect of surgery temporarily suppresses ethanol drinking. The stimulated animals, although implanted, did not show this suppression, indicating that stimulation counteracts in part the suppressive effects of surgery. This finding indicates an important and previously unappreciated influence on ethanol intake in brain stimulation studies.

Acknowledgements

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Many investigators have tried to develop an animal analogue for the human alcoholic. In doing so, two critical attributes of alcoholism have been considered to be linked: 1) the physical dependence of the organism on alcohol, and 2) a high level of voluntary consumption of alcohol.

It was generally assumed that if animals could be made physically dependent on alcohol, then they would also voluntarily consume large quantities of alcohol. This approach now seems unlikely to work. Several investigators have now successfully produced physical dependence, but none report abnormal voluntary consumption of concentrated ethanol solutions (Veale & Myers, 1969; Ratcliffe, 1972; Martin & Myers, 1972; Mello, 1973; Lester & Freed, 1973; Begleiter, (in press)).

Another approach to the problem has been to attempt to directly maximize voluntary ethanol intake without consideration of the development of dependence. This thesis deals with studies taking this approach.

A major problem has been in getting rats to voluntarily consume ethanol at high concentrations. Rats generally prefer water over a concentrated (e.g. 15% or higher) ethanol solution. If the ethanol concentration is as low

as 6%, rats may be willing to consume it in preference to water; but at concentrations above 6%, voluntary ethanol consumption decreases rapidly. At ethanol concentrations above 15% (20% for occasional rats, Mendelson & Mello, 1964), the ethanol solution is generally rejected in favour of water. (Richter & Campbell, 1940; Kahn & Stellar, 1960; Myers, 1966).

One method that has successfully reversed the normal preference for water over high ethanol concentrations was reported by Amit, Stern and Wise, 1970. Male Wistar rats which had stimulating electrodes implanted in the lateral hypothalamic area (LHA) and which drank in response to hypothalamic electrical stimulation, received 30 minute daily stimulation sessions for 30 days in the presence of ethanol solutions. The level of ethanol concentration was individually determined so that each rat was exposed to the ethanol concentration level at which he had previously refused to drink for 48 hours, (median concentration was 21% v/v). These animals drank the previously rejected ethanol solutions during the stimulation sessions and, more importantly, significantly increased their home cage consumption of ethanol over that of control animals. In the home cage, ethanol was presented every other day in a free choice situation with water. The stimulated animals' abnormal voluntary preference for ethanol over water developed throughout the 30 days

of stimulation and continued following termination of the stimulation sessions. Thus, this method of electrical LHA stimulation in the presence of ethanol succeeded in inducing a permanent reversal in preference for ethanol over water which persisted for the duration of the animal's life (some animals having been maintained for over a year; Amit, personal communication).

It was later reported (Amit & Stern, 1971) that the stimulation-induced drinking of ethanol during the electrical stimulation sessions was not necessary for the home-cage reversal in preference to occur. Wistar rats which received LHA stimulation in the presence of only tap water and also animals stimulated with no fluid present both developed a similar preference for previously aversive ethanol solutions presented in the home cage every other day in free choice with water. Since this permanent reversal of preference occurred both in animals that drank (drinkers) and also in animals that did not drink (non-drinkers) in response to the lateral hypothalamic stimulation, the important experimental manipulation was hypothalamic stimulation per se, and not the stimulation-induced drinking experience.

The general finding that LHA stimulation enhances home-cage ethanol preference was further substantiated by Wayner, Gawronski, Roubie & Greenberg (1970), who discussed

a single rat, a stimulation-induced drinker, which received electrical stimulation of the LHA in the presence of various ethanol solutions. This animal (and others which Wayner notes without details) appeared to confirm the general finding of Amit, Stern and Wise (1970) and Amit and Stern (1971) that daily lateral hypothalamic stimulation increases home cage voluntary ethanol consumption and reverses the normal preference for water over concentrated ethanol solutions.

Subsequently, failures to confirm the general findings of Amit et al., (1970) and Amit and Stern (1971) were reported. Wayner, Greenberg, Carey and Nolley (1971) reported data from four animals which received daily LHA stimulation in the presence of ethanol solutions for up to 25 days. These rats ingested intoxicating amounts of 20% ethanol during the test sessions but there was no significant change in the amount of their voluntary home cage ethanol consumption.

Martin and Myers (1972) trained rats to lick a drinkometer tube in order to receive stimulation of the LHA. They reported that the drinking response to obtain intracranial reinforcement decreased as the ethanol concentrations in the drinkometer increased. They also found that ethanol intake of the animals in their home cage did not increase either following the daily sessions during which ethanol was consumed in the test chamber or as a result of the repeated

LHA stimulation. This paradigm, however, differs very significantly from the others in terms of the total amount and pattern of brain stimulation given.

Wayner and Greenberg (1972) attempted to re-assess the contribution of LHA stimulation and ethanol presentation schedule to the increased consumption of previously aversive ethanol solutions. One of the groups of rats in this study was treated similar to those of Amit and Stern (1971). They received LHA electrical stimulation during daily 30 minute sessions in the presence of water only. The results from this group confirmed those of Amit and Stern (1971). The animals reversed their preference for water over previously aversive ethanol concentrations in their home cage and this effect persisted for 63 days at which time the animals were sacrificed.

Thus while dramatic elevation of home cage voluntary ethanol intake has been produced by lateral hypothalamic stimulation in the hands of some investigators, it has not been easily produced by others. Amit, Stern and Wise (1970) and Amit and Stern (1971) reported high levels of ethanol intake from their stimulated animals which differed significantly from the ethanol intake from their unstimulated controls. But, as mentioned above, with another paradigm, Martin and Myers (1972) did not find any change in home cage

ethanol consumption following LHA stimulation. Wayner and his colleagues followed the paradigm of Amit et al.(1970) more closely and were first successful (1970), then unsuccessful (1971), and finally successful (1972) in confirming the finding of Amit et al.(1970), although even in their recent successful study the observed elevation of ethanol intake is of lesser magnitude than that seen by Amit et al.(1970). Thus it would appear that unappreciated aspects of the paradigm of Amit et al.(1970) are important, and it seems necessary to determine which of many arbitrary procedures in their paradigm are critical for their findings. This thesis is a step toward such a determination.

Procedural variations among investigators are numerous. In order to assess the contribution of LHA stimulation to enhanced ethanol consumption, the effects of various other factors, i.e., strain differences, method of ethanol presentation, eleotrode placement, must be determined. As these factors were not kept identical by all investigators, they may account for some of the variability in results reported by independent investigators.

Strain Differences

One of the precedural differences noted involves rat strain. Amit and his colleagues, (1970, 1972) used

Wistar rats as subjects. Attempted confirmations have involved either Sprague-Dawley rats (Wayner et al., 1970; Wayner et al., 1971) or hooded rats, (Wayner et al., 1971; Wayner & Greenberg, 1972; Martin & Myers, 1972) as subjects.

Strain differences in alcohol intake have been widely documented in other paradigms (Rodgers & McClearn, 1962; McEwen, 1965; Eriksson, 1968); and Wise (1974) has shown that they can occur in rats which are presented with a 20% ethanol solution every other day in a free choice situation with water. Under these conditions, Wistar rats ingested twice as much ethanol relative to water as did Sprague-Dawley rats when both groups were obtained from one supplier. Wistar and Sprague-Dawley rats obtained from another breeder showed no significant differences in ethanol intake. Thus, these rats showed varying willingness to consume ethanol depending on both strain and supplier. It seems essential, therefore, to control for the strain of the animals within a particular study in order to reliably assess the amount of ethanol intake due to an experimental manipulation. This was not done in one attempted replication of Amit et al.'s paradigm which used both hooded and Sprague-Dawley rats in the same experiment (Wayner et al., 1971). It also seems necessary, when attemption to replicate findings involving ethanol consumption, that the same strain (and perhaps

breeder) be used in order to have comparable results.

Schedules of Ethanol Presentation

Another factor that contributes to the amount of ethanol consumed by experimental animals is the specific schedule of ethanol presentation. Since investigators have employed different schedules of ethanol presentation, this may account for some of the difficulty in attempting to compare results. Animals progressively exposed to increasing concentrations of ethanol solutions (acclimation) come to consume higher concentrations than would normally be ingested (Veale & Myers, 1969). Intermittent schedules of ethanol availability, in which the ethanol solutions are periodically presented and withdrawn also result in an increase in ethanol consumption (Sinclair & Senter, 1968; Wise, 1973; Wayner, 1971; Engel, 1972).

Wise (1973) investigated the effects of acclimation, forced versus free choice (ethanol presented alone or in choice with water) and schedule of ethanol presentation (every day versus every other day), on the amount of a 20% concentrated ethanol solution consumed in subsequent free choice situations. After 30 days on the every-day presentation schedule, the free choice group had a higher ethanol intake level than the forced choice group; but on the intermittent schedule, the forced choice group drank more

than the free choice group. Periodic withdrawal of the solution (the intermittent schedule), led to higher levels of intake than were seen with continuous availability of ethanol regardless of whether a free or forced choice was given. Engel (1972) studied the effects of brain stimulation in interaction with the above schedules of ethanol presentation and found similar results; the stimulated animals given ethanol every other day consumed at high levels, while those given ethanol every day drank minimal amounts. Thus, intermittent schedules of ethanol availability resulted in the most dramatic increases in ethanol intake.

A cycle of 2 days of ethanol presentation in free choice with water followed by water only for 2 days was compared to every-day exposure to ethanol and water by Wayner, Greenberg, Tartaglione, Nolley, Fraley and Cott, 1971. Their findings were consistent with those of Wise (1973) and Engel, (1972); intermittent ethanol exposure led to higher intake than continuous exposure.

The importance of the intermittent exposure schedule would appear to explain one of the failures to replicate the Amit et al. finding. In the Wayner et al. (1971) study in which LHA stimulation caused no change in home cage ethanol consumption, every day presentation of ethanol

solutions was employed. It would appear (as confirmed by Wayner & Greenberg, 1972) that the failure to produce high intake in this study was in great part due to this deviation from the intermittent presentation schedule used by Amit et al. (1970) and Amit & Stern (1971).

In addition to the intermittent schedule of ethanol availability, the animals in the Amit et al. (1970) and the Amit and Stern (1971) studies were given prior acclimation to increasing concentrations of ethanol. However, acclimation, itself, does not seem to contribute significantly, in the long run, to the increased levels of ethanol intake seen in animals which are given long exposure on an intermittent ethanol schedule (Engel, 1972; Wise, 1973). Wise (1973) has shown that long term intake of 20% ethanol solutions on intermittent schedules by animals that were given previous acclimation to 20% ethanol concentration is not significantly different from that of animals which received 20% solutions at the start of the intermittent schedule. Engel (1972) showed the same results in conjunction with the brain stimulation paradigm.

In summary, acclimation to increasing concentration of ethanol solutions does not seem to account for the higher, permanent ethanol intake levels seen in the Amit paradigm. However, periodic presentation and withdrawal

of the ethanol solution does appear to contribute significantly to the high drinking levels reached by animals in these studies.

Variations in Electrode Placements

It appears clear that in studies using an alternate day ethanol presentation schedule, animals which receive stimulation of the lateral hypothalamus increase their subsequent voluntary ethanol intake over that of animals which do not receive LHA stimulation. (Amit et al., 1970; Amit & Stern, 1971; Wayner & Greenberg, 1972). However, there are differences in the magnitude of increase reported in these different studies. The locus of stimulation sites mediating the strongest effects is not clearly established.

Animals stimulated in the LHA reversed their normal preference for water over ethanol, while those stimulated in the ventromedial hypothalamus (VMH) drank virtually no ethanol during the 30 day stimulation period (Amit & Stern, 1972). Thus the acquired preference for ethanol has been suggested (Amit & Stern, 1972) to be a specific function of electrical stimulation of the LHA and to be due to modifications in the hypothalamic substrate immediately adjacent to the tip of the stimulating electrode.

However, Wayner & Greenberg (1972) observed only a slight increase in ethanol consumption (1g/kg) due to LHA

stimulation when stimulated animals were compared to animals that received no stimulation. This differs from the findings of Amit et al., 1970 and Amit & Stern, 1971 in which a much larger increase in ethanol consumption (on the order of 3g/kg) was observed. Wayner & Greenberg (1972) have suggested on the basis of the results of their study that the electrical stimulation of the LHA contributes little by itself to the observed increased ethanol consumption and that periodic presentation of ethanol solution is the stronger effect.

However, an alternate explanation for these differences in efficacy of stimulation may lie in the differences in electrode placements within the lateral hypothalamus. Histological examination of electrode sites of the Wayner and Greenberg (1972) study show placements somewhat more dorsal than those of Amit et al. (1970). Amit et al. (1970) used sites supporting stimulation-induced drinking of fluids during the stimulation session, but none of the stimulated animals in the Wayner and Greenberg study showed these stimulation-induced behaviours. This suggests that the small differences between the Wayner & Greenberg and the Amit placements are functionally significant, at least in terms of drinking circuitry. The idea that they may be significant in terms of circuitry involved in ethanol consumption is supported by the finding that dorsal LHA lesions do not

abolish ethanol intake, where ventral LHA lesions do (Meade, Amit and Singer, 1974).

The purpose of the first of the present experiments was to assess the effects of electrical stimulation at differing sites within the LHA on subsequent voluntary ethanol intake. Perhaps variations of electrode placements among and within investigators may account in part for the differing amounts of increased ethanol intake seen in stimulated animals. Thus, the first experiment was concerned with whether the increased ethanol intake observed as a result of brain stimulation sessions varies as a function of the specific area of the LHA that is stimulated, and whether dorsal-ventral differences, in particular, can be critical in this regard.

Method

Experiment I

Subjects were 40 male albino rats of the Wistar strain obtained from Bio-Breeding Farms. They were approximately 100 days old and weighed approximately 350 grams at the beginning of the experiment. The animals were randomly divided into five groups of eight animals each. They were anesthetized with sodium pentobarbital (Nembutal) and supplemental injections of chloral hydrate as necessary. A

monopolar stainless steel stimulating electrode, insulated except at the tip, was stereotaxically aimed for the lateral hypothalamic area (LHA) and implanted in each animal. The stereotaxic coordinates were systematically varied from those used by Amit, Stern and Wise (1970). Amit et al.'s (1970) coordinates were 0.8 mm posterior to Bregma, 1.5 mm lateral of the sagital suture and 8.2 mm ventral to the skull surface. In this study the coordinates were changed by .4 mm to be either more anterior, (Group A); posterior, (Group P); dorsal, (Group D); or ventral, (Group V). A fifth group of animals was implanted in the LHA (some animals at each of the placements listed above) and served as unstimulated controls, (Group C). Each subject was injected with 60,000 units of Pennicilin G to reduce the chance of infection. A recovery period of at least 5 days followed surgery. Since there were 40 animals which were implanted, some experienced a longer recovery period (up to fourteen days) during which time the others were being implanted.

All animals were housed individually with free access to food and water and on alternate days were given free choice between water and a 20% (v/v) ethanol solution. A fixed arbitrary ethanol concentration was used for all animals following Engel's (1972) and Wise's (1973) modification of Amit's paradigm. As was reported in the

Introduction, Wise and Engel found that there were no significant long term differences in ethanol intake levels between animals on an intermittent ethanol presentation schedule which had received previous acclimation to 20% solutions and animals which did not receive acclimation. Thus, it was decided to use the more simplified technique of fixed concentrations, realizing that this is a modification of the original Amit et al. paradigm.

Water and 20% ethanol solutions were presented in graduated Richter-type drinking tubes and the position of the tubes was interchanged in a non-systematic order. Water was the only fluid available on the days between ethanol presentations. Ethanol and water intake were measured on ethanol presentation days; the animals were weighed once a week, and the ratio of ethanol solution to total fluid intake and the grams of ethanol consumed relative to body weight were calculated daily.

During Days 1-30, the animals in Groups A, P, D and V were administered electrical stimulation through the implanted electrode during daily 30 minute sessions. The general method of LHA electrical stimulation was similar to that reported by Amit and Stern (1971): stimulation consisted of a 20-seconds-on, 20-seconds-off cycle of 60 Hz sine wave current. However, a second deviation from the Amit et al.

paradigm involved stimulation intensity. In the Amit studies, stimulation intensity was adjusted for each animal to produce stimulation-induced ethanol drinking. However, in the present study, the electrode targets varied for each group. Thus, many electrode placements would not be expected to support stimulation-induced drinking. Moreover, in order to assess the effects of variations in electrode placement, it was necessary to attempt to equate the spread of current around each electrode by holding stimulation intensity constant. Thus, the stimulation intensity was fixed for all animals. Fifteen (15) μA stimulation intensity was chosen as it consistently produced forward searching movements in most animals in the present study and because it has been found to exceed the usual stimulation-induced drinking threshold under the present conditions for animals previously investigated in our laboratory. Three animals showed pronounced motor or aversive side-effects at 15 μA and they were stimulated at slightly lower current levels (10-13 μA).

Concerning ethanol availability in the test box, it has been shown (Amit & Stern, 1971) that stimulation without ethanol available is sufficient to elevate home-cage ethanol intake and that ethanol consumption in the test box is not essential. In the present study, since the varied electrode placements were not expected to support stimulation-induced -

drinking, this fact was exploited: animals in Groups A, P, D and V were stimulated in empty, individual test boxes. Animals in Group C were brought daily to the test room but did not receive electrical stimulation. On day 31, stimulation sessions were discontinued. All animals were maintained on the alternate-day free-choice fluid schedule, and ethanol and water intake continued to be monitered for an additional 42 days. After day 72, animals were sacrificed, perfused with saline followed by a 10% formalin solution, and the brains were removed and frozen. Forty micra sections of brain tissue were cut near the electrode track; they were stained with thionin and examined to determine the location of the electrode tips.

Experiment II

In order to assess the reliability of the differences in intake between groups in Experiment I, it was decided to replicate the study with an independent group of animals. At this time, it was realized that undergoing general surgical procedures might influence ethanol intake. Thus, an additional group of non-implanted animals was added to the replication study.

Subjects were 48 male albino rats of the Wistar strain obtained from Bio-Breeding Farms. They were approximately 90 days old and weighed approximately 275 grams at

the beginning of the experiment. All procedures were identical to those reported above with the addition of a sixth group of eight animals, which received no surgery and served as a non-implanted control group (Group UC).

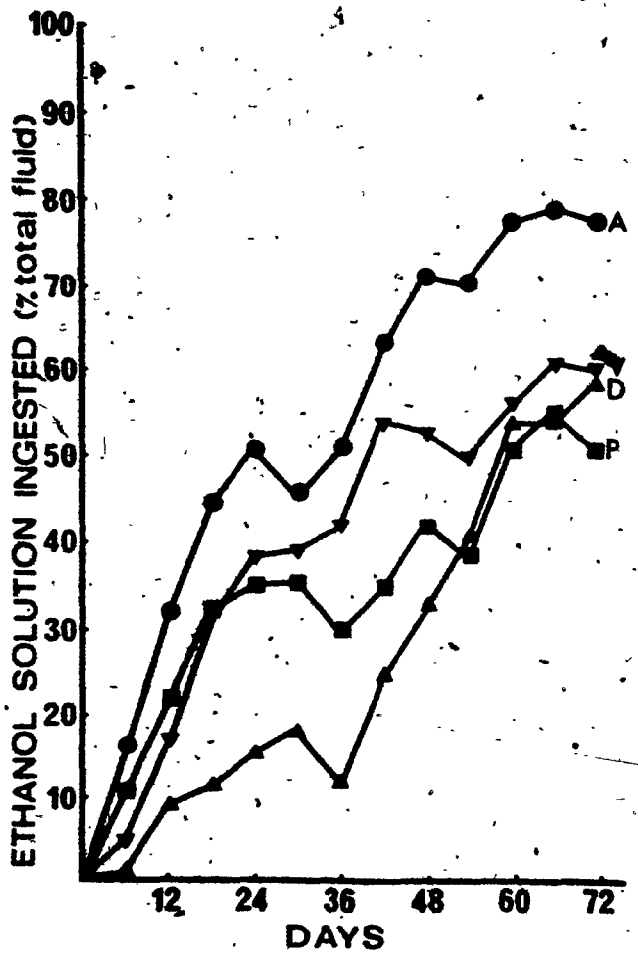
Results

The ethanol consumption data have been expressed in terms of the percentage of total fluid intake consumed as ethanol solution and the absolute grams of ethanol ingested per kilogram of body weight. These two aspects of the same data have been reported by other investigators in the past and are followed in the present study for purpose of comparison. As both reflect the same intake, only the preference data were subjected to statistical analysis.

Ethanol consumption as a function of electrode placement

The ethanol consumption levels of the four groups of stimulated animals with differing electrode placements in Experiment I are shown in Figure 1. There were small differences in the ethanol consumption levels between the four groups. However, there was a great amount of variability among animals in the amount of ethanol consumed. None of these differences proved to be statistically significant. The preference for ethanol between the four groups of

Figure 1. The relative and absolute ethanol intake of the four groups of stimulated animals in Experiment I. (● A = Anterior group; ▼ V = Ventral group; ▲ D = Dorsal group; ■ P = Posterior group).


ETHANOL SOLUTION INGESTED (% total fluid)
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90
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DAYS
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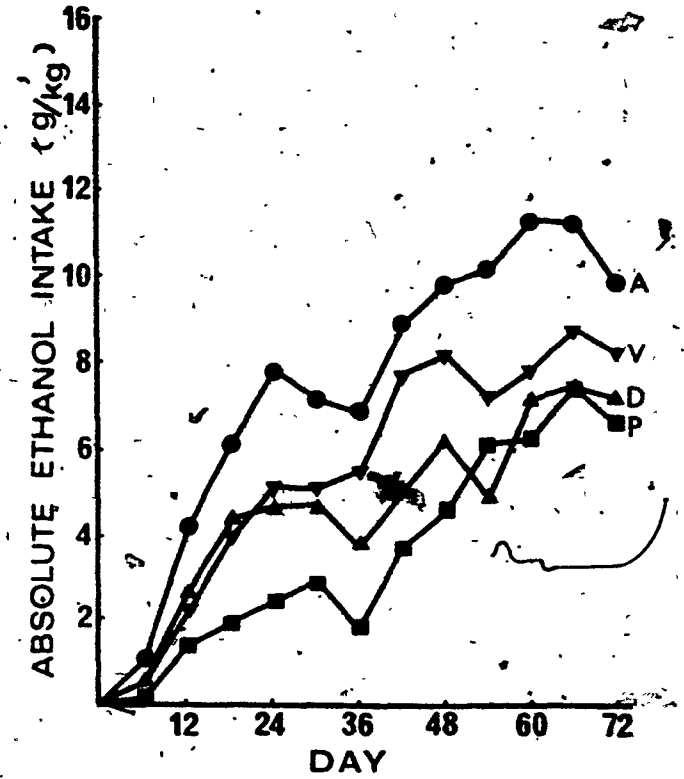
ABSOLUTE ETHANOL INTAKE (g/kg)
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stimulated animals in Experiment I was not significantly different by the end of the stimulation period (Day 30; Kruskal-Wallis; $H=4.19$; $d.f.=3$; $p > .05$) or at the end of the experiment (Day 72; Kruskal-Wallis; $H=2.15$; $d.f.=3$; $p > .05$).

The replication study with independent groups of animals, Experiment II, revealed a similar finding (Figure 2). Again, there was no significant difference in the ethanol preference levels between the four stimulated groups with different electrode placements at day 30 (Kruskal-Wallis; $H=3.20$; $d.f.=3$; $p > .05$) or at day 72 (Kruskal-Wallis; $H=0.73$; $d.f.=3$; $p > .05$).

Although none of the small differences between the groups with different electrode placements was significant, it was of interest to see if any of the trends seen in Experiment I would occur in Experiment II. Only one of these trends did stand up in replication. This was the more elevated drinking and preference levels seen in the group with the more anterior placements.

Stimulated animals compared with implanted controls

As the four groups of stimulated animals proved to drink ethanol at comparable levels regardless of the placement of the electrodes, they were pooled together and treated as one group, (stimulated animals), for comparison to

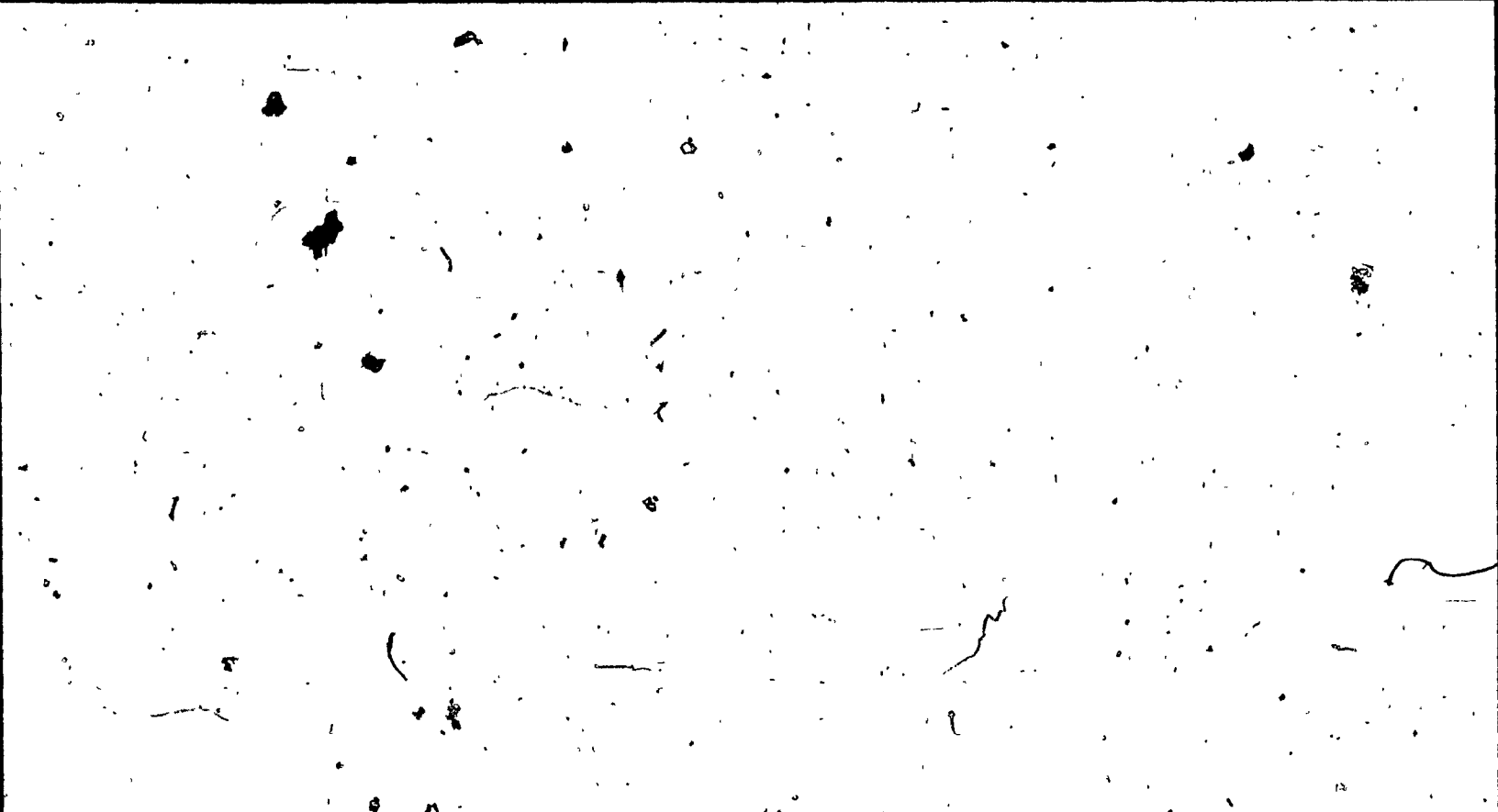
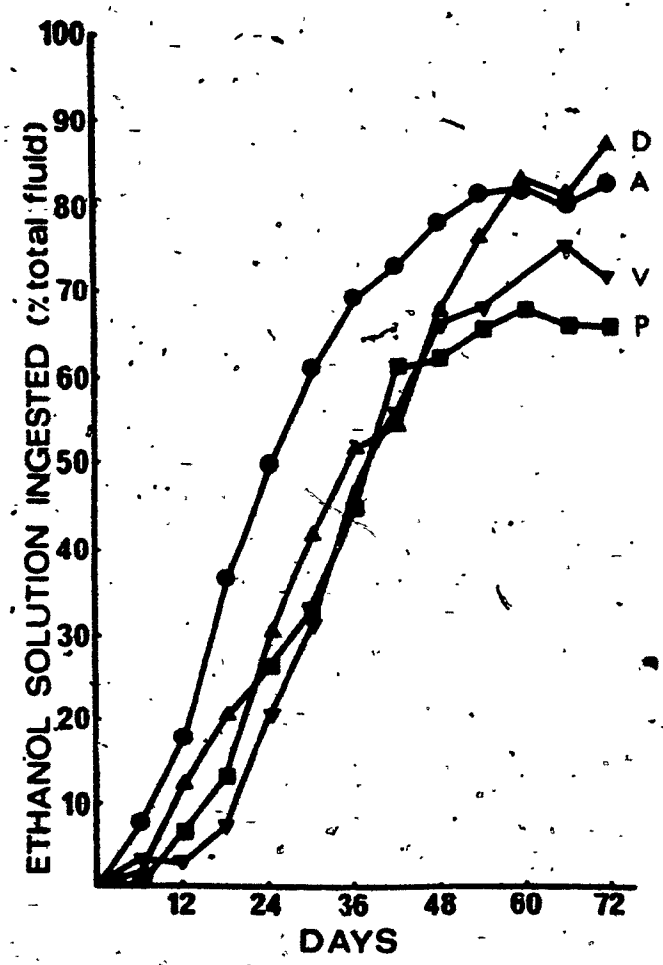


Figure 2. The relative and absolute ethanol intake of the four groups of stimulated animals in Experiment II. (● A = Anterior group; ▼ V =Ventral group; ▲ D =Dorsal group; ■ P =Posterior group).


ETHANOL SOLUTION INGESTED (% total fluid)
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DAYS
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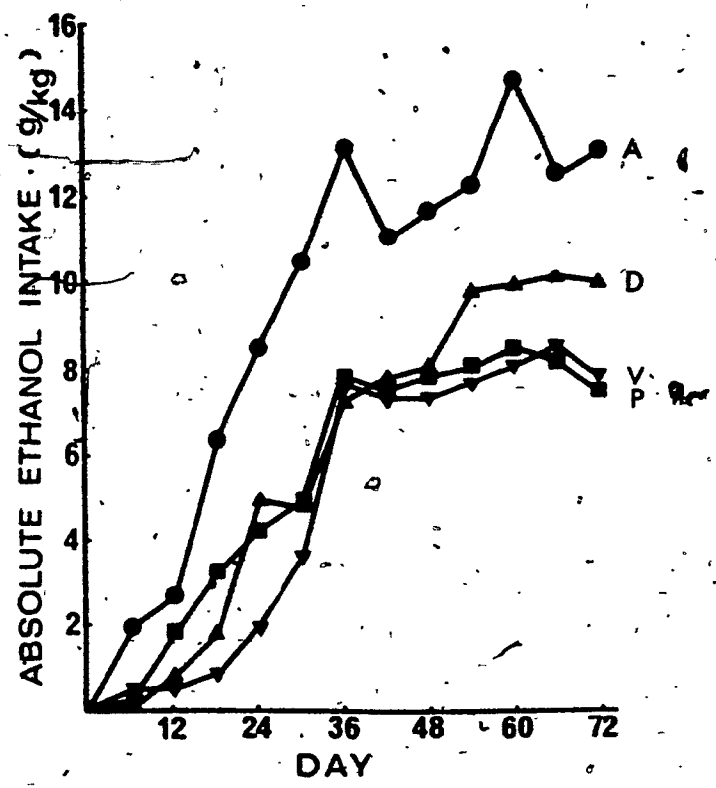
ABSOLUTE ETHANOL INTAKE (g/kg)
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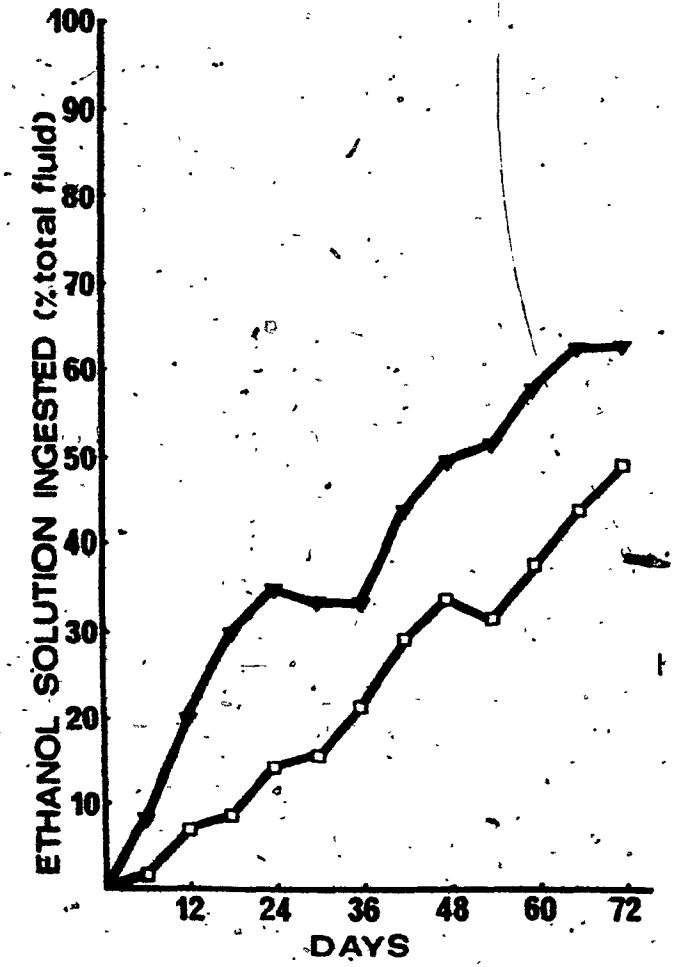
unstimulated control animals. The stimulated animals drank a significantly higher percentage of ethanol than the implanted, unstimulated controls by the end of the 30 day stimulation period in Experiment I (Figure 3; Mann-Whitney; $U=68$, $p < .05$). These higher drinking levels of the stimulated animals replicated in Experiment II, (Figure 4) where it was again found that at day 30 the stimulated animals consumed a significantly higher percentage of ethanol relative to water than the implanted, unstimulated control animals (Mann-Whitney; $U=56$, $p < .05$).

However, the difference between the ethanol intake levels in the stimulated animals and the implanted control animals was not permanent. By the end of the 42 day follow-up period, the stimulated animals in Experiment I were not significantly different from the implanted controls in terms of ethanol preference levels (Mann-Whitney; $U=167$, $p > .05$). This finding repeated itself in the replication study: the stimulated animals' preference to ethanol was not significantly different than that of the implanted controls by day 72 in Experiment II (Mann-Whitney; $U=108$, $p > .05$). Thus, stimulation significantly increased ethanol consumption in animals relative to implanted, unstimulated controls at day 30. However, this difference was only temporary; at the end of 72 days there was no significant difference in preference

Figure 3. The relative and absolute ethanol intake of the pooled stimulated animals compared to the implanted, non-stimulated animals in Experiment I. (▼ = Stimulated animals; □ = Implanted, non-stimulated animals)


ETHANOL SOLUTION INGESTED (% total fluid)
100
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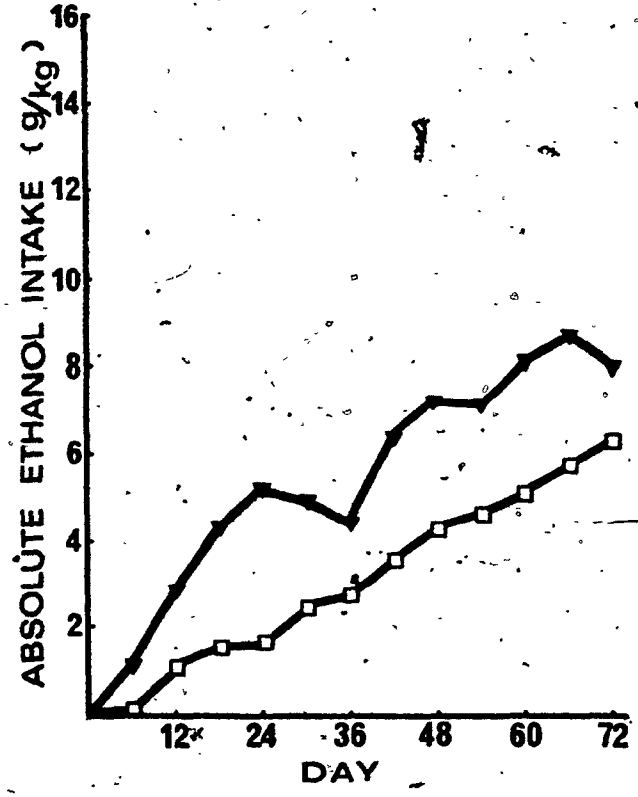
DAYS


ABSOLUTE ETHANOL INTAKE (g/kg)
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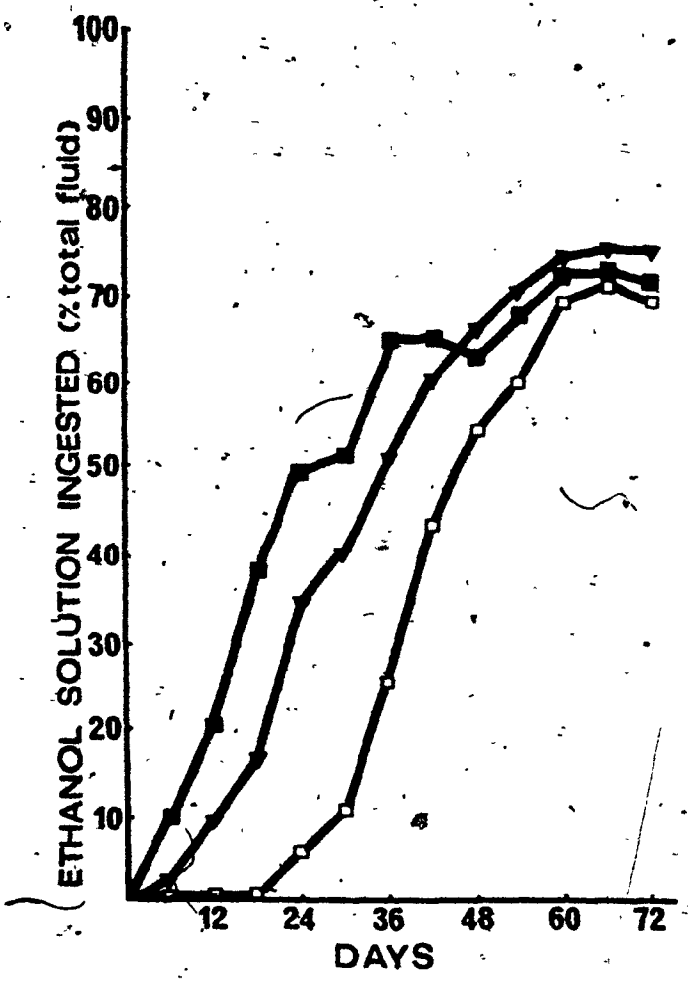
72
DAY

Figure 4. The relative and absolute ethanol intake of the pooled stimulated animals; the implanted, non-stimulated animals; and the non-implanted animals in Experiment II. (▼ = Stimulated animals; ■ = Implanted, non-stimulated animals; □ = Non-implanted animals)


ETHANOL SOLUTION INGESTED (% total fluid)
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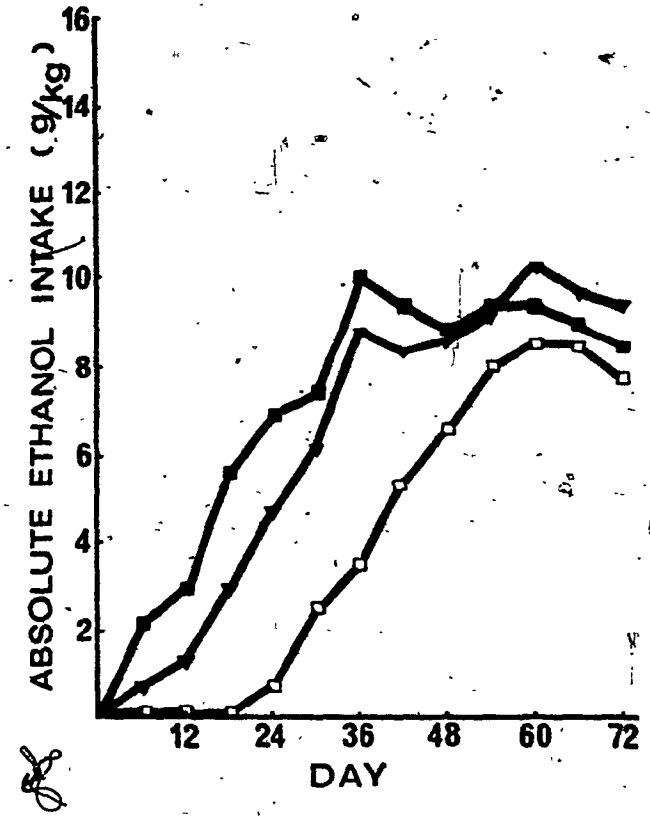
ABSOLUTE ETHANOL INTAKE (g/kg)
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72
DAY

to ethanol between the two groups.

Non-implanted controls compared to implanted controls

A comparison of the ethanol drinking levels in the two control groups of Experiment II, (Figure 4), revealed an interesting finding. The non-implanted control animals drank a significantly higher percentage of ethanol than the implanted controls at the end of 30 days (Mann-Whitney; $U=9$, $p < .05$). Whereas the implanted controls did not begin to drink the ethanol solutions in any measurable amount until day 18 and then only consumed less than 10% of their total fluid intake in ethanol by day 30, the non-implanted controls began drinking on day 1 and steadily increased their consumption of ethanol to approximately 52% of their total fluid intake by day 30. However, these differences were not permanent and by the end of the 72 day observation period, the two control groups were not significantly different in their preference to ethanol (Mann-Whitney; $U=29$, $p > .05$).

Non-implanted controls compared to stimulated animals

The temporary suppression of ethanol drinking seen in the implanted controls relative to the non-implanted controls was not seen in the other implanted group, the stimulated animals. The stimulated animals increased their amount of ethanol consumption at approximately the same rate

and to approximately the same levels as the non-implanted animals (Figure 4). The stimulated animals and the non-implanted animals did not differ significantly in percent of ethanol consumed at day 30 (Mann-Whitney: U=30, $p > .05$) or at day 72 (Mann-Whitney: U=86, $p > .05$).

Histology

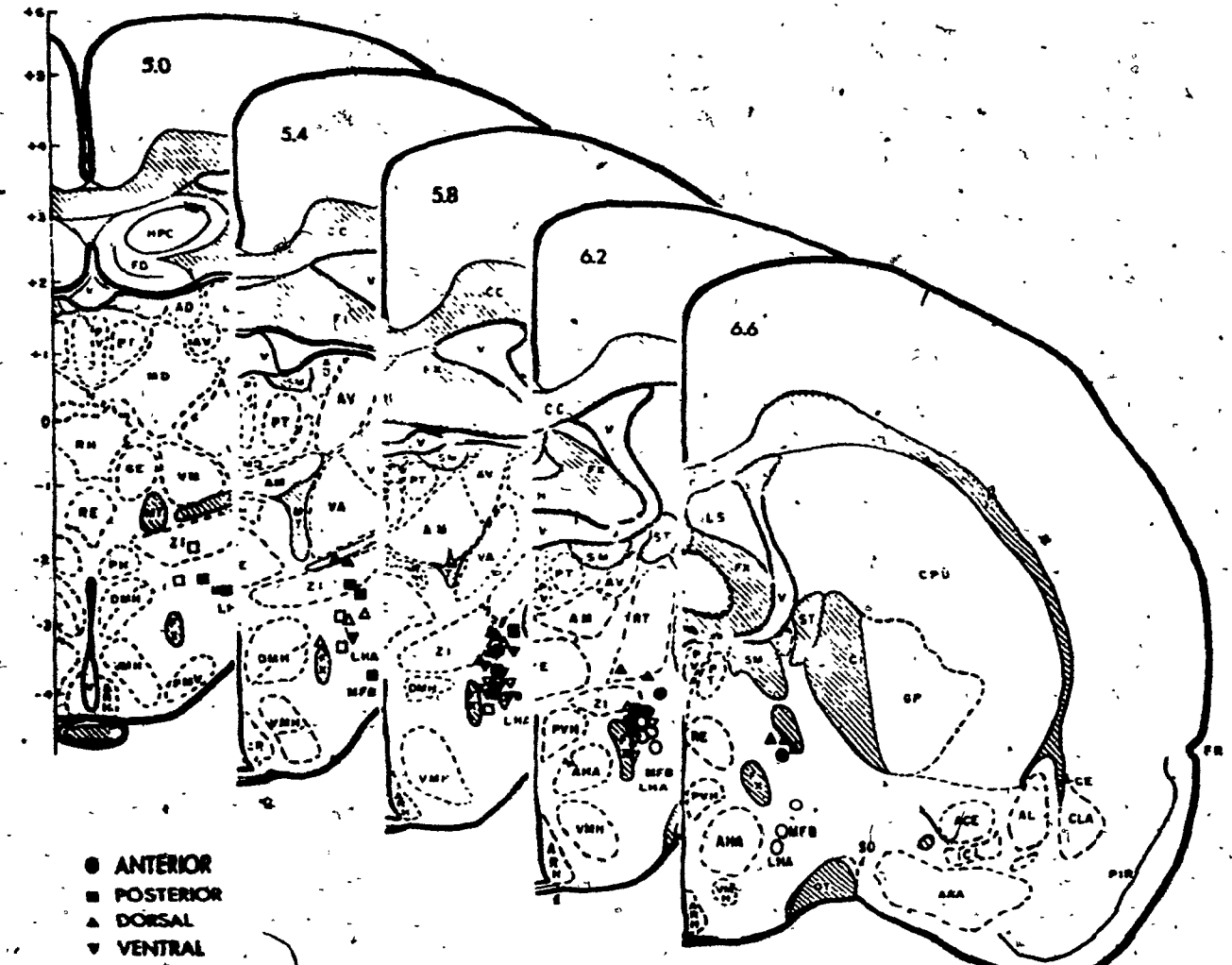
Reconstruction of the electrode placements based on histological verification is represented in Figure 5. A distinction between the anterior, posterior, ventral and dorsal groups was revealed, although a high percentage of the placements were consistently dorsal to the intended target. Even the more ventral placements were found to be dorsal to the Amit et al. coordinates.

Discussion

Effect of stimulation locus

Variations in electrode placements between the four stimulated groups resulted in no significant consistent differences in ethanol intake. The small differences in ethanol intake levels that were observed between the four stimulated groups in the first experiment were not consistent with the differences seen in the replication study with one exception: the anterior group drank the highest amount

Figure 5. Histological reconstruction of the electrode placements for Experiment I (solid symbols) and for Experiment II (open symbols).


5.0
5.4
5.8
6.2
6.6
ANTERIOR
POSTERIOR
DORSAL
VENTRAL

of absolute ethanol in both experiments and the most ethanol relative to water in Experiment I.

One possibility as to why the anterior group showed somewhat consistently higher intake levels is that the anterior placements seemed to be most closely correlated with the catecholamine trajectories of the medial forebrain bundle (MFB) as mapped by Ungerstedt (1971). It has previously been suggested (Amit and Stern, 1972) that it is stimulation of catecholamine fibers in the MFB that is critical for the elevation of ethanol intake seen in the stimulation paradigm. If this does turn out to be the critical region for stimulation to effectively increase ethanol intake, then perhaps the anterior group drank most because it was the group with the most favourable placements.

This apparent superiority of the anterior placements was unexpected, as the placements of greatest interest were the dorsal and ventral placements which proved not to be significantly different from one another. While the present study did not itself provide support for the view that dorsal-ventral differences are important, it was not a good test of this view because of the dorsal bias in the placements. Histological analysis revealed a high percentage of all placements in the present study to be dorsal to the intended target, the coordinates used by Amit et al. (1970),

and even the ventral placements which were aimed to be .4mm ventral to the target of Amit et al. (1970) were found to be dorsal to the original target.

As the placements generally were only in the dorsal region of the LHA, it is possible that more ventral placements would have resulted in higher intake and preference levels than were seen in the present study. Other new evidence suggests that the ventral portion of the LHA is the critical region for ethanol preference (Meade, Amit and Singer, 1974). In this study, lesions of the ventral portion of the LHA followed by stimulation of the more dorsal region decreased ethanol preference while lesions of the dorsal LHA followed by stimulation of the ventral hypothalamus rapidly increased ethanol preference to 82% of total fluid intake. The investigators concluded that stimulation and lesions influence intake only when the ventral portion of the MFB are involved.

Because of the above results, a post hoc analysis based on the histologically verified placements in the present study was carried out: ethanol intake of animals with placements which fell below the fornix were compared to those which fell above the fornix. However, this comparison also revealed no significant difference in ethanol preference levels (Mann-Whitney; $U=70$, $p > .05$). Thus those

animals with electrode placements which were closer to the intended target did not ingest significantly different amounts of ethanol than those animals whose placements were most off-target.

The present data thus suggest that variations in stimulation locus within the dorsal region of the LHA do not significantly alter the levels of ethanol intake. The efficacy of stimulation within the dorsal region of the LHA compared to the ventral region was not resolved by this study, due to the dorsal bias of the placements. Because of the fluctuations of ethanol intake levels seen between Experiment I and Experiment II, the apparent superiority of the anterior placements should be regarded with caution until further data are collected.

Level of intake in the stimulated animals

Although electrode placements were generally more dorsal than intended, the stimulated animals as a whole drank at levels which were consistent with levels seen in stimulated animals in most studies in the literature. In terms of percent of ethanol consumed relative to total fluid consumption, Amit, Stern and Wise (1970) reported mean levels of 90% by the end of the experiment; Amit and Stern (1971) reported intake levels of 85% (for animals exposed

to water during stimulation); and 60% (for animals stimulated in empty chambers); Wayner and Greenberg (1972) reported levels of 80%; and the present studies report levels of 62% (Experiment I) and 74% (Experiment II) of ethanol ingested relative to total fluid intake. The results are also comparable in terms of grams of ethanol intake per kilogram of body weight. In the present studies and Amit's studies (Amit et al. 1970; Amit & Stern, 1971), the final drinking levels were approximately 10 gm/kg. Although Wayner and Greenberg (1972) only found drinking at levels of 3.5 gm/kg, their animals were of a different strain, and were exposed to far weaker ethanol solutions than those used by Amit et al. or in the present studies.

Level of intake in the non-stimulated controls

The control animals of the present study on the other hand, exhibited much higher drinking levels than seen in the control animals of Amit et al. (1970) and Amit and Stern (1971). This was true of both the non-implanted control group and the implanted control groups by the end of the 72 day observation period. Whereas Amit et al.'s (1970) implanted controls leveled off their ethanol drinking at 25% of total fluid intake and Amit and Stern's (1971) non-implanted controls leveled off at 40%, the implanted controls

of the present study were consuming 50% of their fluid intake in ethanol in Experiment I and 70% in Experiment II and the non-implanted controls of Experiment II were drinking 72% of their total fluid intake in ethanol by the end of the experiment. This difference in the control animals was also seen in terms of absolute ethanol intake per kilogram of body weight. Amit et al. (1970) reported intake of approximately 3.2 gm/kg by the end of the 72 days and Amit and Stern (1971) reported 4 gm/kg (these amounts were estimated from Amit's data on absolute ethanol intake by assuming the animals to weigh approximately 500 grams). These levels are lower than were seen in the present study (Implanted controls of Experiment I - 6 gm/kg; Experiment II - 8 gm/kg; non-implanted controls of Experiment II - 8.5 gm/kg).

The high drinking levels of the non-stimulated controls are not, however, unique to the present study. Wise (1973) has seen similar high ethanol preference levels (80%) in unstimulated, non-implanted animals exposed only to every other day presentation of 20% ethanol solutions. Wayner and Greenberg (1972) also reported high (90%) preference levels in implanted, unstimulated animals which received ethanol every day for 30 days, then every other day for 30 days.

The difference between the control animals in Amit's studies and the control animals in Wise's and the present studies is not easily understood. Both the same strain and same schedule of ethanol presentation were used in these experiments. One difference in procedure which may prove to be important is that in Amit's studies, the ethanol concentrations presented to the animals were individually determined to be aversive; whereas in Wise's studies and the present study, a single, generally aversive concentration was used for all. While the same average concentrations were used in these studies (median concentration 21% in Amit et al., 1970; 20% in Wise's and the present study), the use of "initial rejection concentrations" (IRCs) by Amit and his colleagues starts all the animals off at concentrations they avoid equally, while the arbitrary use of 20% solutions for all animals permits more variation in drinking preference. This difference could have important long term consequences in terms of subsequent ethanol intake levels.

Wayner and Greenberg (1972) did use individually determined ethanol concentrations for their animals and did not report low drinking levels like those of Amit's control animals but rather high levels comparable to those seen in Wise's and the present study. The differing ethanol concentrations used by Wayner and Greenberg may not, however,

have been comparably aversive to the IRCs used in Amit's studies. They claimed it was difficult to find animals which would meet Amit's criterion of aversion, and appeared to use a less stringent criterion and considerably weaker concentrations in their study. In addition, ethanol was presented in ball-pointed drinking tubes rather than in the standard Richter-type graduated cylinders. It has been suggested by Amit, Amir & Corcoran (1973) that the use of ball-pointed drinking tubes results in an unreliable measure of the rats ethanol intake due to a marked leakage of ethanol solutions from tubes of this type. Thus the Wayner and Greenberg data do not help resolve whether the IRC paradigm of Amit and his colleagues leads to the lower levels of drinking seen in their animals as compared to those in the present study.

Another factor which might conceivably explain the different drinking levels, is the effect of the stab wound on subsequent ethanol drinking in the implanted controls. As the surgical procedure itself has been found to temporarily suppress subsequent ethanol drinking (to be discussed later), it is possible that the deeper stab wound (perhaps through more critical tissue) in Amit's studies (where more ventral electrode placements occurred) may have inhibited drinking more or for a longer period of time than the more dorsal

stab wound produced in the present and Wayner and Greenberg (1972) studies.

Only further investigation will reveal whether differences in drinking levels between the non-stimulated animals of Amit et al. (1970) and those of Wise (1974) and the present study are due to (i) the use of 20% solutions rather than IRCs, (ii) the effect of differences in depth of stab wounds, or (iii) other procedural differences. However, the difference appears reliable and in order to compare and analyse the results of the various stimulation studies, these differences in control baseline intake must be taken into account.

Relative intake of the stimu[redacted] and non-stimulated animals

The present study s[redacted] strong effect of stimulation on home cage intake. This [redacted]e apparent up to day 30 when clear cut differences in intake levels between the implanted controls and stimulated animals were observed. This finding supports the conclusion of Amit et al. (1970) and Amit and Stern (1971) that stimulation facilitates ethanol intake. However, the stimulated animals did not maintain their greater intake; the control animals caught up by Day 72. Thus stimulation, in the present study, produced only a slight, if any, permanent effect on ethanol intake.

Wayner and Greenberg (1972) also reported only a slight effect of stimulation. Although their stimulated animals were drinking at high levels, there was only a small difference in ethanol intake levels by the end of the study, between these animals and their comparison group that received no stimulation. They concluded that the increase in ethanol intake due to stimulation is only about 1 gm/kg and occurs almost immediately. Amit and his colleagues (1970, 1971) on the other hand reported a much stronger effect of stimulation on increased subsequent ethanol consumption. The effect of stimulation in their studies resulted in a permanent significant elevated intake over the control animals.

As previously mentioned, in Wayner and Greenberg's (1972) study and the present studies, higher baseline ethanol intake levels were observed in the control animals compared to the intake levels of controls in the studies of Amit et al. (1970) and Amit & Stern (1971). Thus, even though the stimulated animals in all these studies drank ethanol at comparable levels, the magnitude of difference in ethanol consumption between stimulated animals and unstimulated controls was different. In order to have shown comparable quantitative effects of stimulation on subsequent home cage intake, Wayner and Greenberg's and also the present stimulated animals would have had to drink ethanol at higher

preference levels and to higher amounts of absolute ethanol than Amit's stimulated animals since there was a major difference in baseline control ethanol intake levels. Thus the data from the present study compare with those of Wayner and Greenberg's (1972) study; only a slight effect of stimu[redacted] was observed on ethanol intake levels when the intake o[redacted] stimulated animals was evaluated relative to the baseline of implanted control animals at the end of the experiment. Amit et al. (1970) and Amit and Stern (1971) reported much higher intake levels in their stimulated animals relative to control animals, and the differences seen in their animals remained, unlike those seen in the present study, long after stimulation treatment was terminated.

An explanation for the different strength of the effect of stimulation on subsequent ethanol intake is suggested by the histological analysis of the loci of stimulation and has previously been mentioned: the electrode placements in the present study were dorsal to the intended target. Wayner and Greenberg's (1972) histology reveals a similar distribution: their placements were also dorsal to those of Amit et al. (1970). In the study by Wayner and Greenberg and in the present study only a slight increase in ethanol intake due to stimulation was observed while in the Amit studies (Amit et al. 1970; Amit and Stern, 1971),

with more ventral placements, a greater increase was reported.

In summary, comparable levels of ethanol intake in stimulated animals were observed in the present studies and in previous studies. There were, however, significant differences in the control animals' intake, which could be due to any of a number of seemingly trivial details of procedure. Considerable further investigation is needed before the differences in control intake levels seen in different laboratories can be fully understood. Two lines of investigation suggested by the present study are the possible differences in long-term ethanol intake due to the use of IRCs or 20% solutions, or the effect of varying amounts of damage to brain tissue due to different depths of electrode penetration in implanted control animals. The critical region in which the effect of stimulation will be maximal and can be reliably obtained is still not known. The present study indicates that stimulation in the dorsal region of the LHA results in only a slight permanent elevation of ethanol intake and other evidence suggests stronger effects may be found in the ventral portion of the LHA.

Effects of Surgery

In addition to the primary results discussed above, the study yielded interesting data on a secondary point of

interest. The non-implanted control animals of Experiment II drank significantly higher levels of ethanol than did the implanted control animals for approximately the first 30 days. Once the implanted controls began drinking ethanol, their pattern of intake was similar to that of the non-implanted controls but their initial drinking did not begin to appear until a month after the start of the experiment. Thus, the present data indicate that the procedure of electrode implantation can itself temporarily suppress voluntary ethanol intake.

Amit (personal communication) has also found a reliable difference between his non-implanted and implanted control animals, although the difference is of a lesser magnitude than reported here. This lesser difference seen by Amit might be due in part to the fact that his animals have a longer period of recovery from surgery (2-4 weeks more: this is the period required for the process of establishing IRCs) than the animals in the present study. Thus, it seems as though suppressive effects of surgery may be avoided if a long enough recovery period is allowed. In the present study the animals were given a five day to two week recovery period prior to the start of the experiment and then did not begin drinking until approximately four weeks later. This suggests that a total six week recovery

period may be necessary in order for implanted animals to ingest ethanol normally.

The stimulated animals, although implanted, did not show this suppression of ethanol consumption. The significant difference in ethanol intake levels between the two implanted groups (stimulated versus unstimulated) at day 30 indicates that the stimulation counteracts some of the suppressive effects of the surgical procedure and leads to drinking levels more comparable to those of animals which were not subjected to the implantation process. However, the stimulated animals did not elevate their ethanol consumption beyond the level of the unoperated control group by day 30, and, as previously discussed, by the end of the experiment all groups were drinking at approximately the same level.

It is not clear what aspect of surgery causes the suppression of ethanol drinking or what the stimulation does to overcome this effect. It has been shown (Wise & James, 1974) that neither anesthesia with sodium pentobarbital nor anesthesia with ether produces this suppression. Thus the critical factor must be some aspect of the surgical insult itself. The suppression of ethanol drinking may be due to general stress responses to the opening of the skin or skull, specific reactions to pathogens or foreign material

introduced into the brain or cranial tissue, neural damage caused by the electrode penetration, or to any of a number of other factors.

Due to the range of surgical factors that may be important, and due to the variability of surgical techniques practiced in different laboratories, it is difficult to assess the generality of the present finding. However, it indicates that studies cannot be adequately compared or interpreted without consideration of the duration of the recovery period allowed after surgery and it also underscores the need to use implanted animals to provide control baseline for stimulated animals, since the facilitory effect of stimulation overcomes in part the inhibitory effects of surgery.

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