NIGROSTRIATAL DOPAMINE AND ALCOHOL INTAKE

THE EFFECTS OF LESIONS IN THE NIGROSTRIATAL DOPAMINE SYSTEM ON THE DEVELOPMENT OF ALCOHOL PREFERENCE BY HYPOTHALAMIC STIMULATION

by

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M.A. Psychology

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Abstract

The present study examined the effects of nigrostriatal lesions on the development of alcohol preference by hypothalamic stimulation.

The 24 animals were assigned to 1 of 4 conditions: A nigrostriatal lesion followed by 30 days of stimulation in the lateral hypothalamus; a nigrostriatal lesion alone; hypothalamic stimulation alone; or neither hypothalamic stimulation nor a lesion. All animals had free access to both water and alcohol for 60 days.

Electrical stimulation of the lateral hypothalamus failed to induce a preference for alcohol. The lesion in the nigrostriatal dopamine system significantly inhibited the normal elevation of alcohol intake which occurrs over time ($p < .01$).

These results indicate that the nigrostriatal system contributes to the regulation of alcohol consumption. Hypotheses were put forward to explain the failure of the stimulation to elicit alcohol preference.

M.A. Psychologie

L'ACTION DES LESIONS DE LA SYSTEME NIGROSTRIATAL SUR LE DEVELOPPMENT DE LA PREFERENCE POUR L'ALCOOL PAR LA STIMULATION ELECTRIQUE DU HYPOTHALAMUS

Penny S. Arnold

Résumé

Cette expérience a examiné l'action d'une lesion nigrostriatal sur le développment de la préférence pour l'alcool par la stimulation electrique du hypothalamus.

Les 24 animaux étaient assignés à un sur quatre conditions: une lesion nigrostriatal suivée par 30 jours de la stimulation électrique du hypothalamus latéral; seulement une lesion nigrostriatal; seulement la stimulation hypothalamique; ni la lesion ni la stimulation hypothalamique. Tous les animaux avaient le choix de l'eau et de l'alcool pendent les 60 jours.

La stimulation électrique du hypothalamus latéral n'est pas produit une préférence pour l'alcool. La lesion nigrostriatal a empêché l'élévation de l'ingestion de l'alcool qui se recontre au cours des 60 jours ($p < .01$).

Ces résultats suggerent que la système nigrostriatal aide à la régulation de la consumption d'alcool. Les hypothèses étaient presentées pour expliquer l'insuccès de la stimulation hypothalamique d'obtenir l'ingestion de l'alcool.

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Introduction

The present study was designed to examine the effects of lesions in the nigrostriatal dopamine pathway on the development of alcohol preference. Much evidence has suggested that the formation of tetrahydropapaveroline (THP) from dopamine may be an important factor in the self-administration of alcohol. However, none of this evidence was supported by studies conducted *in vivo* in animals that had exhibited prolonged voluntary ingestion of alcohol in high concentrations. Such investigations have been impeded by the rats' consistent rejection of alcohol in high concentrations. The technique developed by Amit, Stern and Wise (1970) for inducing alcohol preference in the laboratory rat has circumvented this problem and now provides the opportunity to examine the role of dopamine in long-term alcohol ingestion.

As background for the present study is the belief held by many behavioral scientists that the biological mechanisms underlying human alcoholism can be effectively studied in animals. For this reason, investigators have concentrated on a search for an animal paradigm, particularly in the laboratory rat, which approximates to human alcoholism. Such at-attempts have been difficult since rats typically drink water in preference to alcohol if the concentration exceeds 6% (Kahn and Stellar, 1960; Myers, 1966; Richter and Campbell, 1940). This behavior has stimulated many investigators to design techniques to increase the ingestion of aversive alcohol solutions. Such techniques have included schedule-induced polydipsia (Lester, 1961; Falk, 1961, 1972), forced alcohol consumption by restriction of other fluids (Myers, 1966), or addition of ethanol to

a liquid diet (Freund, 1969), intragastric (Amit and Stern, 1969) or intravenous self-administration (Deneau, Yanagita and Seevers, 1969), and injection into the cerebral ventricles (Myers, 1972). While many of these studies demonstrated increased ethanol consumption during the experimental treatment, few have succeeded in developing a permanent preference for alcohol over water.

One technique which has proven effective in producing a persistent alcohol preference is electrical stimulation of the lateral hypothalamus (Amit, Stern and Wise, 1970). The rationale for this study was based on four major ideas: First, alcohol preference was considered to be an acquired homeostatic phenomenon which is controlled by neural tissue (Malmo, 1967). Secondly, the neural tissue involved was thought to be the lateral hypothalamus since this nucleus plays an important role in the regulation of consummatory behavior (Roberts, Steinberg and Means, 1967; Morgane, 1964) in general and drinking behavior in particular (Grossman, 1960; Morgane, 1964; Peck and Novin, 1971). Thirdly, electrical stimulation of the lateral hypothalamus appeared to be a viable technique for eliciting consummatory behavior (Coons, 1964; Roberts, Steinberg and Means, 1967), and also for changing the neural substrate underlying the behavior (Valenstein, Cox and Kakalewski, 1968, 1969). Finally, some evidence indicated that stimulation of the hypothalamic nuclei could alter normal alcohol intake (Segal, Nerobkova and Rybalkina, 1969).

These findings led to the notion that voluntary alcohol consumption might be increased as a function of prolonged electrical stimulation of the lateral hypothalamus. Consequently, rats were subjected to daily thirty-minute sessions of hypothalamic stimulation for a period of thirty days. Alcohol solutions were presented in the home cage on alter-

nate days while food and water were continuously available. Alcohol and water intake were monitored during the thirty-day period and for 135 days after the cessation of stimulation. A large proportion of the stimulated animals reversed their preference for water over alcohol and, more importantly, this preference became a permanent feature of their behavior. The non-stimulated rats did not exhibit a reversal in preference. This effect was not abolished by prolonged withdrawal of the alcohol nor by adulteration of the alcohol solution with quinine (Amit, Stern and Wise, 1970). Further research determined that this effect was restricted to stimulation of the lateral hypothalamus and not other structures, and that it involved some modification of hypothalamic tissue (Stern and Amit, 1972).

If one accepts this paradigm as a viable animal model, the next step is to examine the neural mechanisms that may be involved. The crucial question to be answered is how the electrical stimulation acts on the brain to modify alcohol intake. Recent evidence has suggested that this effect may be mediated by the catecholamines, dopamine and noradrenaline.

There are a number of reasons that the catecholamines might be implicated in the development of alcohol preference by electrical stimulation. First, the catecholamines have recently been shown to act as neurotransmitters (Arbuthnott, Crow, Fuxe, Olson, and Ungerstedt, 1970;Fuxe and Gunne, 1964), and their fiber systems pass through the area of the hypothalamus stimulated by Amit, Stern and Wise (1970) (Ungerstedt, 1971a). Moreover, ethanol affects the catecholamines, both by depleting them from their storage sites (Duritz and Truitt, 1966; Gursey and Olson, 1960) and by altering their metabolic disposition (Davis, Brown, Huff and Cashaw, 1967; Davis, Cashaw, Huff, Brown and Nicholas, 1967). Since both ethanol ingestion and electrical stimulation influence the catecholamines,

it is feasible that dopamine or noradrenaline or both contribute to the development of alcohol preference. In the present study dopamine was selected for investigation. Although noradrenaline might be equally important, practical considerations limited the scope of the investigation to dopamine. Evidence supporting its importance in alcohol preference will be cited following a description of the location of dopamine systems in the brain.

Using histochemical florescence, Ungerstedt (1971a) demonstrated the existence of three dopaminergic systems in the brain. The nigrostriatal pathway originates from the A9 cell group in the zona compacta of the substantia nigra. These ascending fibers turn ventromedially and then rostrally to pass through the lateral hypothalamus at the dorsolateral level of the medial forebrain bundle. Rostral to this they enter the internal capsule, spread out in the globus pallidus, and innervate the caudate-putamen. Terminals of this system are also found in the nucleus centralis of the amygdala. The cell bodies of the mesolimbic system (A10) lie dorsal and lateral to the interpeduncular nucleus, and the axons ascend medial to those of the nigrostriatal pathway. These fibers innervate the olfactory tubercle, the nucleus accumbens, and the interstitial nucleus of the stria terminalis. The tubero-infundibular pathway arises from cells situated mainly in the arcuate nucleus (A12). These cell bodies extend to the dorsomedial nucleus of the hypothalamus and gather in a dense group just ventral to the mammilothalamic tract (A13). The A12 fibers terminate in the median eminence while the location of the A13 terminals is unknown.

In the present investigation, the nigrostriatal system alone was

selected for study for several reasons. First, since the cell bodies of this system lie quite lateral to the noradrenergic fibers, they can be lesioned electrolytically without inflicting damage on the noradrenaline system. Elimination of the meso-limbic or the tubero-infundibular pathways however would undoubtedly produce some noradrenergic destruction. Moreover, the nigrostriatal system contributes to the regulation of consummatory behavior since its bilateral removal produces the adipsia and aphagia characteristic of the lateral hypothalamic syndrome (Ungerstedt, 1970, 1971c). If alcohol ingestion depends on the integrity of the lateral hypothalamic motivational systems, then the nigrostriatal pathway is strongly implicated.

Much evidence has accrued to indicate that the presence of dopamine may be an important factor in alcoholism. The relevant studies can be discussed in three major sections: alcohol and the TIQ alkaloids; possible CNS functions of these alkaloids; and the role of electrical stimulation and acclimation.

It has frequently been speculated that alcohol evokes a change in the metabolism of the catecholamines such that morphine-like compuunds are formed (Malmo, 1967). These compounds are known as the tetrahydroisoquinolines or the TIQ's and one of them, tetrahydropapaveroline (THP) is formed from dopamine in the presence of alcohol. The formation of THP could theoretically occur as follows: Although dopamine is the precursor in the biosynthesis of noradrenaline (Blaschko, 1959), it can also be metabolized along other pathways. Its major pathway is the direct amine-aldehyde-acid route in which dopamine is first converted to 3,4,dihydroxyphenylacetaldehyde by monoamine oxidase. This aldehyde is then converted by the action of aldehyde dehydrogenase to

3,4,dihydroxyphenylacetic acid (Davis, 1971). When ethanol is introduced, its principal metabolite acetaldehyde inhibits the oxidative metabolism of the intermediate aldehyde (Davis, Brown, Huff and Cashaw, 1967) by competitively inhibiting aldehyde dehydrogenase (Lahti and Majchrowicz, 1969). This results in elevated concentrations of the highly reactive aldehydes which can then condense with the parent amine to form complex alkaloids. In the case of dopamine, 3,4,dihydroxyphenylacetaldehyde can condense with dopamine to form THP. The probability of this reaction's occurring would likely be enhanced in tissues such as the brain which have a low aldehyde-oxidizing capacity (Davis and Walsh, 1971). Unfortunately, this idea has only been substantiated in vitro on rat brain and liver homogenates (Davis and Walsh, 1971).

If THP is actually formed in brain tissue during ethanol metabolism, then what are its possible effects on CNS function? First, the TIQ's in general retain a catecholamine-like structure and they are taken up and stored in sympathetic nerve endings like regular neurotransmitters (Cohen, Mytilineon, and Barrett, 1972). This finding suggests the following possible actions of the TIQ's and hence THP: 1) They may be more effective as neurotransmitters than the parent catecholamines; 2) They may be completely ineffective and thus act as blocking agents or 3) They may bind to the enzymes which metabolize the catecholamines and in this way potentiate responses to normally-secreted transmitter. In all three cases THP is capable of altering physical and psychological states normally controlled by the catecholamines. In alcoholism, behavior such as hallucinosis, seizures, and tremulousness typically persist after the blood alcohol level has

either declined or disappeared. Since these changes cannot be explained by the presence of alcohol or acetaldehyde, they might be attributed to to the actions of TIQ alkaloids formed during ethanol metabolism (Cohen, 1971). In addition to mediating the pharmacological actions of ethanol, TIQ's may also function to induce physical dependence. Since the TIQ's and hence THP are intermediates in the formation of morphine, alcohol may derive its addictive potential from the presence of morphine-like compounds (Cohen, 1971; Davis, 1971). Another possibility not yet investigated is that THP itself possesses addictive properties.

In conclusion, THP, a compound formed from dopamine in the presence of alcohol, may be of substantial importance in the development of alcohol preference. It may mediate some of the behavioral and pharmacological actions of alcohol and it may also be significant in the establishment of physical dependence.

The ability of lateral hypothalamic stimulation to increase alcohol intake may be related to the formation of THP from dopamine. Unlike the other unsuccessful techniques, this paradigm may somehow provide the optimal conditions for the synthesis of THP and hence for the development of alcohol preference. Possibly the most crucial factor in the synthesis of THP is the presence of sufficient amounts of acetaldehyde and unbound dopamine in the system. The paradigm of Amit, Stern and Wise (1970) ensures that the levels of both these substances are elevated in the central nervous system. The release of dopamine is facilitated by electrical stimulation of the lateral hypothalamus. The nigrostriatal pathway passes through the hypothalamus on its way to the caudate nucleus and dopamine is undoubtedly released when these fibers are

electrically activated (Von Voigtlander and Moore, 1971; Arbuthnott, Fuxe and Ungerstedt, 1971). The acclimation procedure in which rats are gradually exposed to increasing concentrations of ethanol, ensures that animals will ingest sufficient amounts of alcohol in high concentrations to produce elevated levels of acetaldehyde.

In summary, the formation of THP from dopamine may be an important factor in the development of alcohol preference by hypothalamic stimulation. If the release of dopamine is necessary for the acquisition of preference, then the stimulation of animals lesioned in the nigrostriatal pathway should have little effect on alcohol intake; that is, the lesioned animals should drink alcohol at or below the levels of the non-stimulated control subjects.

Method

Subjects

The subjects were 40 male adult Wistar rats obtained from Canadian Breeding Farms. They were housed individually in steel cages with ad libitum access to food and water. Each cage was supplied with a food hopper for lab chow and two 100 cc Richter tubes, one always containing water and the other containing either alcohol or water.

Apparatus

Brain stimulation was delivered by a 60 Hz sine wave stimulator. The on-off pattern of stimulation was controlled by a tape timer which opened and closed the relay at twenty-second intervals. Subjects received stimulation in a wooden box, 12" x 12" x 18", with a one-way mirror on the front wall. The interior of the box was illuminated by a 60 Watt light bulb surrounded by a metal reflector.

Procedure

Under sodium pentobarbital anesthesia (35 mg/kg), animals were implanted with both a monopolar stimulating electrode aimed at the lateral hypothalamus (Kreig coordinates,-.8; 1.5; 8.5) and a lesion electrode aimed at the zona compacta of the substantia nigra (-4.6; 2.1; 8.1).

After seven days of post-operative recovery, the animals were screened for their initial rejection concentrations of alcohol (IRC). Each subject was provided with two Richter tubes, one containing tap water and the other containing a 3% (v/v) alcohol solution made from

95% ethanol and water. Both tubes were present in the home cage for 24 hours. If an animal drank more than two milliliters of the alcohol solution, the concentration was raised by 1% for the next 24 hour period. This procedure was continued until the alcohol solution was rejected by an individual animal for seven consecutive days. Once this occurred, the concentration of alcohol was further raised by 3% and this concentration, the initial rejection concentration, became the test solution for the animal. In this way each subject was provided with an individually-determined aversive alcohol solution. The Richter tube positions were reversed daily to counteract any position preference.

Once the IRC's had been established, all animals were tested for stimulation-induced behavior. Animals were connected to the stimulator and placed in a stimulation box containing lab chow pellets and two Richter tubes filled with water. Stimulation was delivered on a 20-second-on /20-second-off schedule. The current intensity was initially set for two microamperes and was raised by two microampere steps after each stimulation until some stimulation-bound behavior was exhibited or until aversive responses were observed.

On the basis of these results the animals were divided into four groups : the stimulation group (S/NL) which received 30 days of hypothalamic stimulation; the stimulation/lesion group (S/L) which received a nigrostriatal lesion followed by hypothalamic stimulation; a control group (C/NL) which received no stimulation and no lesion; and a control/lesion group (C/L) which received a lesion but no stimulation. Animals which exhibited no stimulation-bound behavior were randomly assigned

to either the C/NL or the C/L groups. Those subjects which ate, drank, or gnawed under stimulation were assigned to the S/NL or the S/L groups. This was done to ensure that the animals receiving daily hypothalamic stimulation (S/NL and S/L) had properly placed electrodes.

Following this, the animals in the lesion groups (S/L and C/L) sustained lesions through the electrode in the zona compacta of the substantia nigra. The animals were lightly anesthetized with ether and the anode of a DC lesion maker was connected to the electrode while the cathode was attached to a rectal probe. A total of 1.5 milliamperes was passed for 15 seconds.

For thirty days after the lesion, animals in the S/NL and S/L groups received daily 30-minute sessions of intermittent electrical stimulation at the previously-determined current levels. Stimulation was administered in empty wooden boxes. During this time subjects in all groups had a free choice between alcohol and water in the home cage. Each subject was provided with two Richter tubes filled with water; on alternate days one of these tubes was filled with the appropriate alcohol solution. Every 24 hours fluid intake was measured and the position of the tubes was reversed.

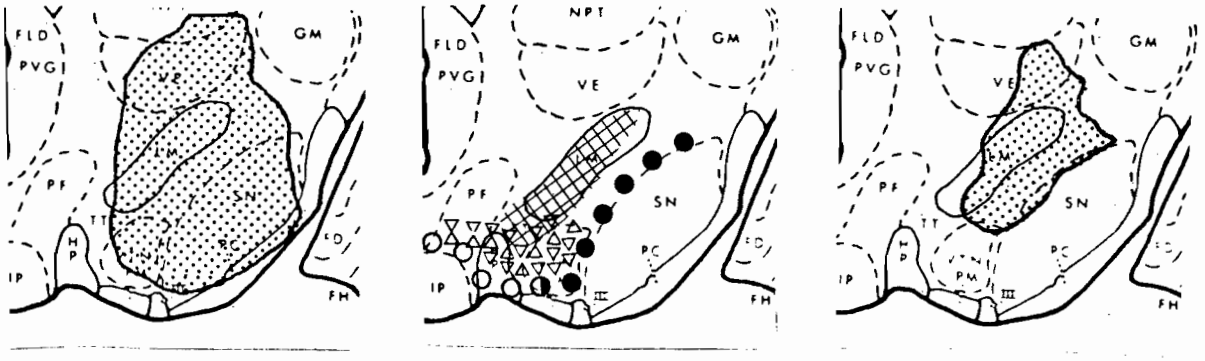
At the end of the experiment animals were killed with an overdose of sodium pentobarbital and perfused with saline followed by a 10% formol saline solution. The brains were frozen, sliced in 40 micron sections, and stained with thionin.

Results

Out of the original 40 animals, 16 were discarded due to illness or to misplaced electrodes. This left 5 animals in the S/L group, 6 in the S/NL group, 4 in the C/L group, and 9 in the C/NL groups. All animals in the lesion groups (S/L and C/L) sustained damage to the dopamine cell bodies in the zona compacta of the substantia nigra (Figure 1). The lesion extended from the level of the most rostral tip of the pons to the rostral tip of the substantia nigra. The destruction typically included the entire substantia nigra and zona compacta, the ventral tegmental nucleus of Tsai, and part of the medial leminiscus. In many cases, the ventral noradrenergic bundle and the meso-limbic dopamine system appeared to partially eliminated. In the S/L group, none of the lesions was closer than one millimeter to the stimulating electrodes.

The stimulating electrodes in the S/NL and the S/L groups were all located in the lateral hypothalamic area adjacent to the fornix (Figure 2). The majority of these placements however were at least one-half millimeter more anterior than were the stimulation sites of Amit, Stern and Wise (1970). The anterior placements in the present study were possibly in a position to stimulate only noradrenaline fibers while the posterior placements were likely capable of activating both catecholamines. Interestingly, in the S/NL group, there was a slight tendency for the animals with the posterior stimulation sites to ingest more alcohol than those with the anterior electrodes.

In order to evaluate the alcohol intake of the four groups, two



A B C

Figure 1. A) Example of one of the larger substantia nigra lesions
B) Approximate location of catecholamine neurons in this area. Open circles refer to A10, closed circles to A9, triangles to dopamine fibers, and cross-hatch to noradrenaline fibers. (Ungerstedt, 1971a)
C) Example of one of the smaller substantia nigra lesions.

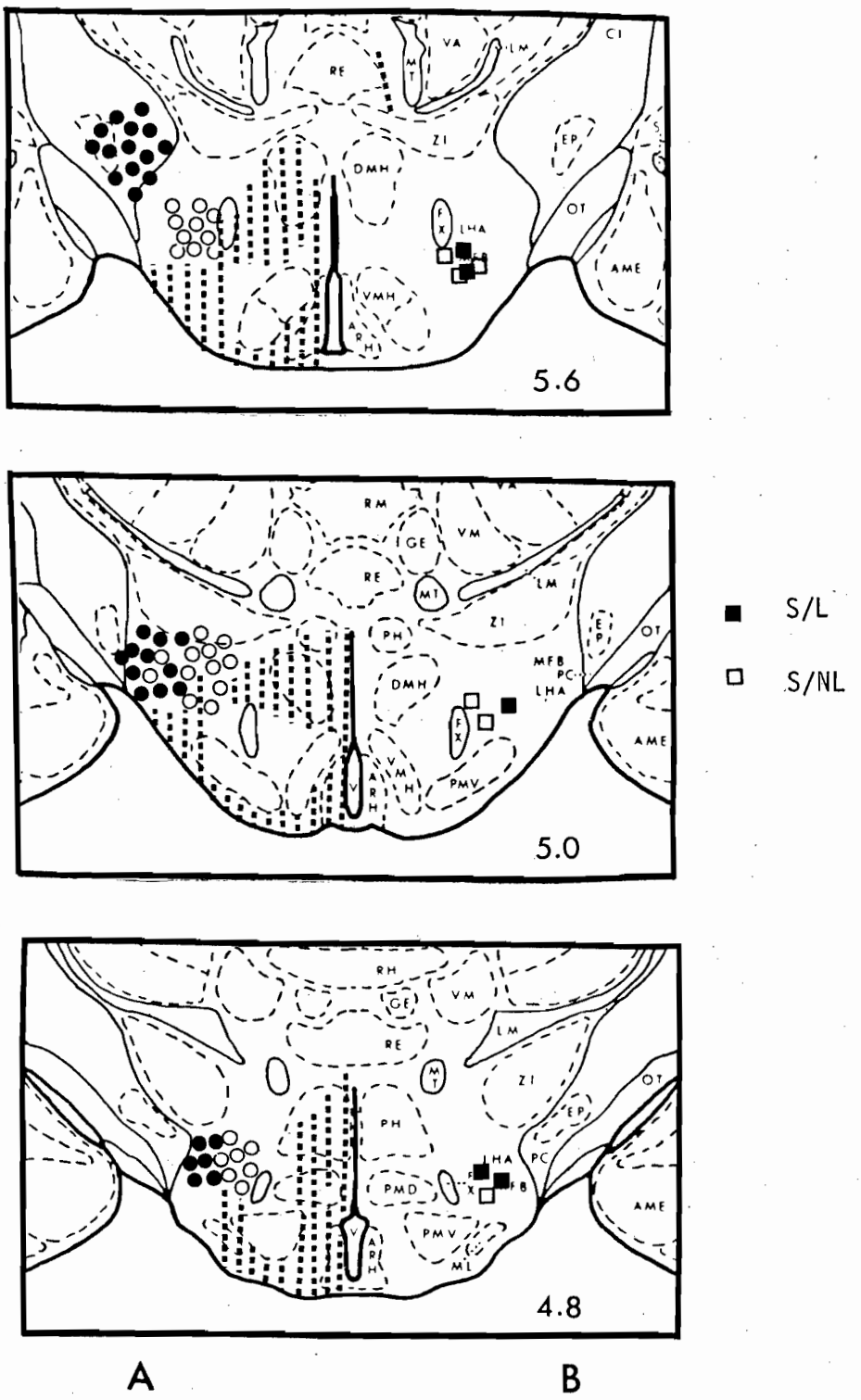


Figure 2. A) Approximate distribution of catecholamine fibers and terminals. Closed circles indicate dopamine fibers, open circles noradrenaline fibers, and dashed lines noradrenaline terminals (from Ungerstedt, 1971a)

B) Location of stimulating electrodes in the S/L and S/NL groups.

dependent variables were examined - percent of total fluid intake and absolute alcohol consumption. Figure 3 shows the percent fluid intake of the four groups over the 60 day testing period. One can see from this graph that the intake of animals receiving hypothalamic stimulation (S/NL) was not much greater than that of the control subjects (C/NL). By the end of the 30 day stimulation period, the mean alcohol intake of the S/NL animals was 37.13% of total fluid intake whereas the mean intake of the C/NL animals was 27.61%. The average alcohol consumption of both groups increased over the subsequent 30 days until by Day 60, the S/NL group was drinking 48.40% of their total fluid in alcohol while the C/NL group was drinking 46.58%. Moreover, the stimulated animals exhibited no reversal of preference since they always ingested less than 60% of their fluid intake in alcohol.

The lesion however had a striking effect on alcohol consumption. Both the S/L group and the C/L group exhibited a much smaller increase in alcohol intake over time than did the non-lesioned animals. The lesioned groups began at a slightly higher level than the non-lesioned animals and their intake did not increase substantially over the 60 days. This effect is clearly demonstrated in Figures 3 and 4.

These data were evaluated statistically by a three-way analysis of variance. The three factors were Lesion, Stimulation, and Period (Day 2, Day 30, and Day 60). The results of this analysis are shown in Table 1 and they support the data presented above. The most important finding is the significant Lesion x Period interaction which indicates that the lesioned and the non-lesioned animals differed in their alcohol intake over time. Newman-Keuls tests performed on these data demonstrated that the lesioned animals maintained a stable level of alcohol consumption

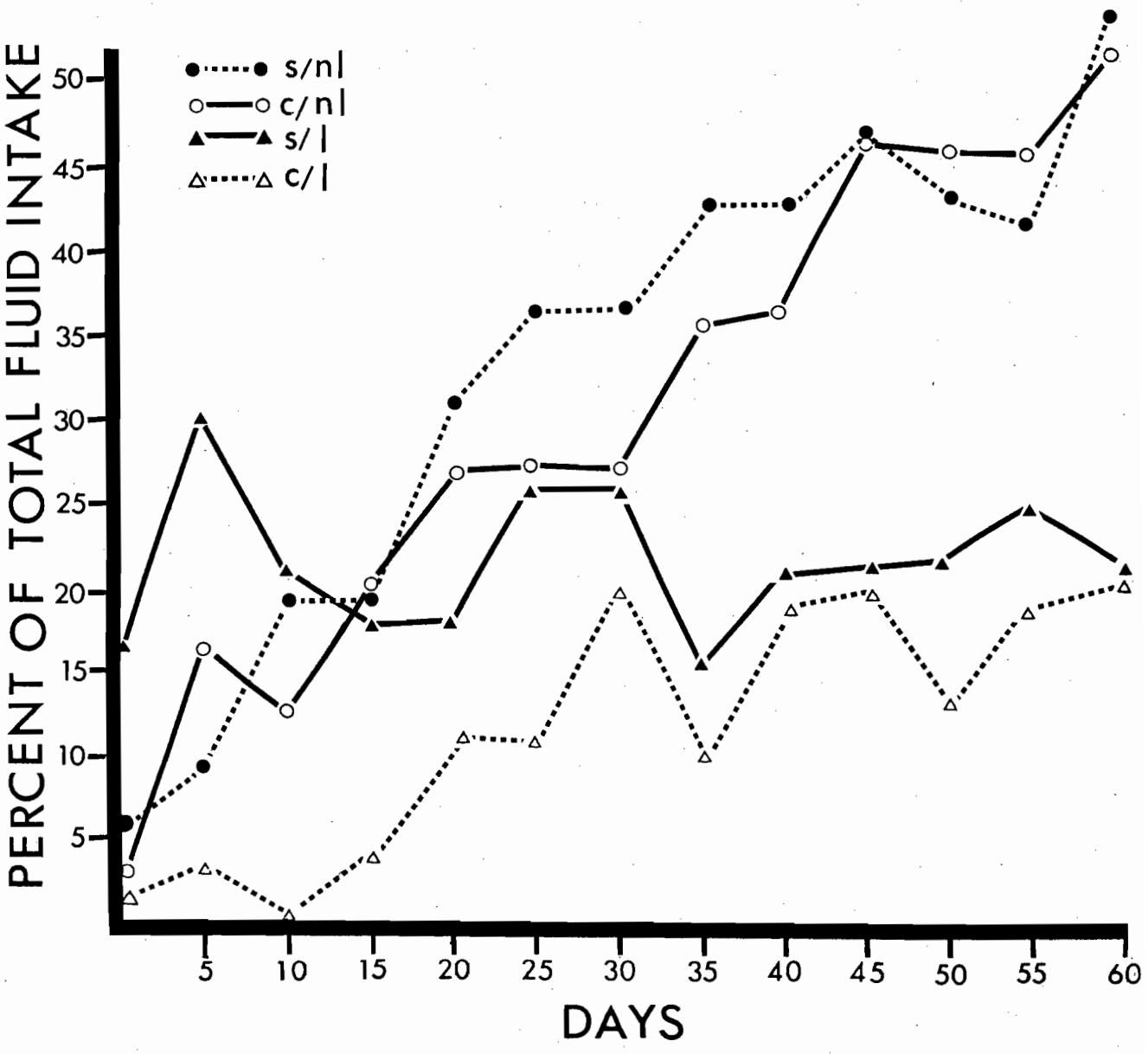


Figure 3. Percent of total fluid intake ingested in alcohol solution for all four groups over the 60 day testing period. The S/L and S/NL groups received hypothalamic stimulation from Day 1 through Day 30.

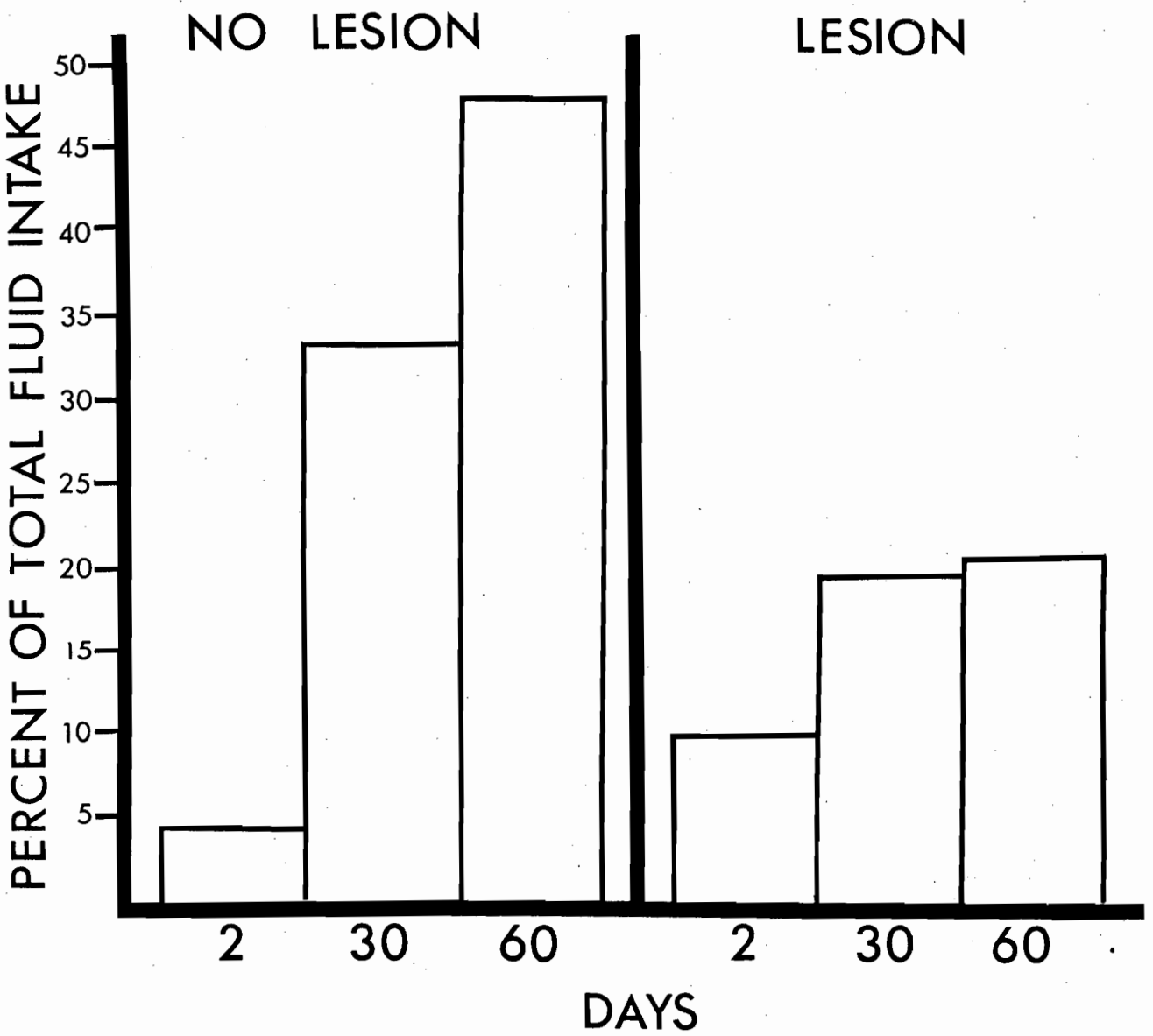


Figure 4. Percent of total fluid intake ingested in alcohol solution for the lesioned (S/L + C/L) and non-lesioned (S/NL + C/NL) subjects for the three periods.

Table 1

Analysis of Variance for Percent of Total Fluid Intake

Source	df	MS	F
Lesion (L)	1	2,672.72	2.93
Stimulation (St)	1	799.56	.88
L x St	1	46.26	.05
Ss	20	912.87	
Period (P)	2	3,858.57	13.48***
L x P	2	1,889.02	6.60*
St x P	2	30.59	.11
L x St x P	2	156.41	.55
Ss x P	40	286.27	

* $p < .01$
*** $p < .0001$

over the three time periods while the non-lesioned animals consistently increased their alcohol intake. The latter group drank significantly more alcohol on Day 30 than on Day 2 ($p < .01$), and they also drank more on Day 60 than on Day 30 ($p < .05$). Hence the lesion prevented the normal elevation in alcohol ingestion which occurs over time (Figure 4).

The data derived from the second dependent variable, absolute alcohol consumption, paralleled the results presented above. The lesion suppressed the normal rise in alcohol ingestion over the 60 days and the stimulation failed to influence alcohol intake (Figure 5). A three-way analysis of variance (Table 2) demonstrated a significant Lesion x Period interaction as above (Figure 6). Subsequent Newman-Keuls tests showed that the non-lesioned subjects increased their alcohol consumption up to Day 30 (Day 2 vs Day 30, $p < .01$), after which their intake stabilized (Day 30 vs Day 60, n.s.). The intake of the lesioned animals however remained relatively constant from Day 2 to Day 60.

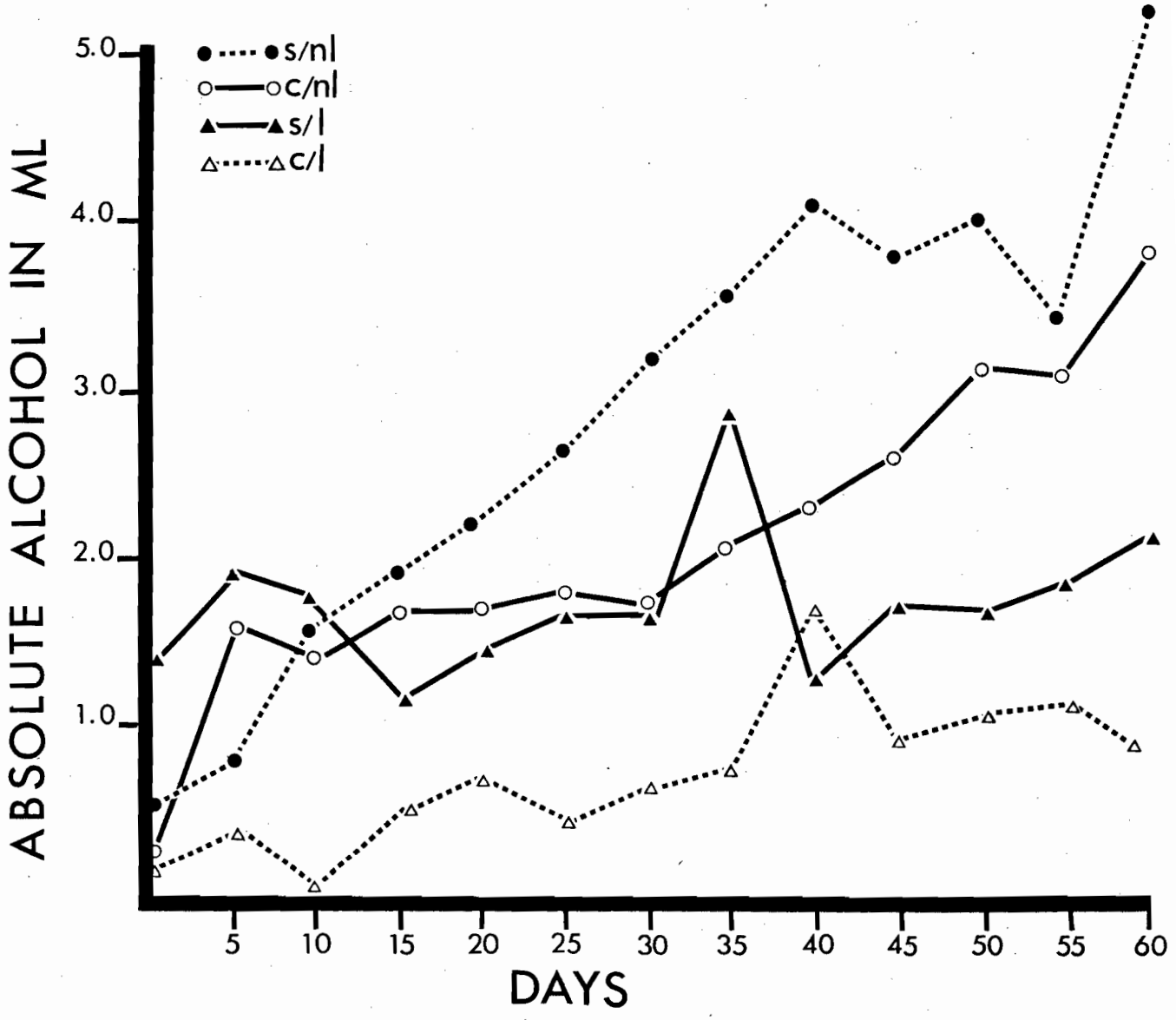


Figure 5. The absolute alcohol consumption in milliliters for all four groups over the 60 day testing period. The S/L and S/NL groups received hypothalamic stimulation from Day 1 through Day 30.

Table 2

Analysis of Variance for Absolute Alcohol Intake

Source	df	MS	F
Lesion (L)	1	23.37	4.14
Stimulation (St)	1	23.99	4.25
L x St	1	.26	.05
Ss	20	5.65	
Period (P)	2	20.79	8.65**
L x P	2	12.17	5.07*
St x P	2	1.56	.65
L x St x P	2	1.77	.74
Ss x P	40	2.40	

* $p < .01$

** $p < .001$

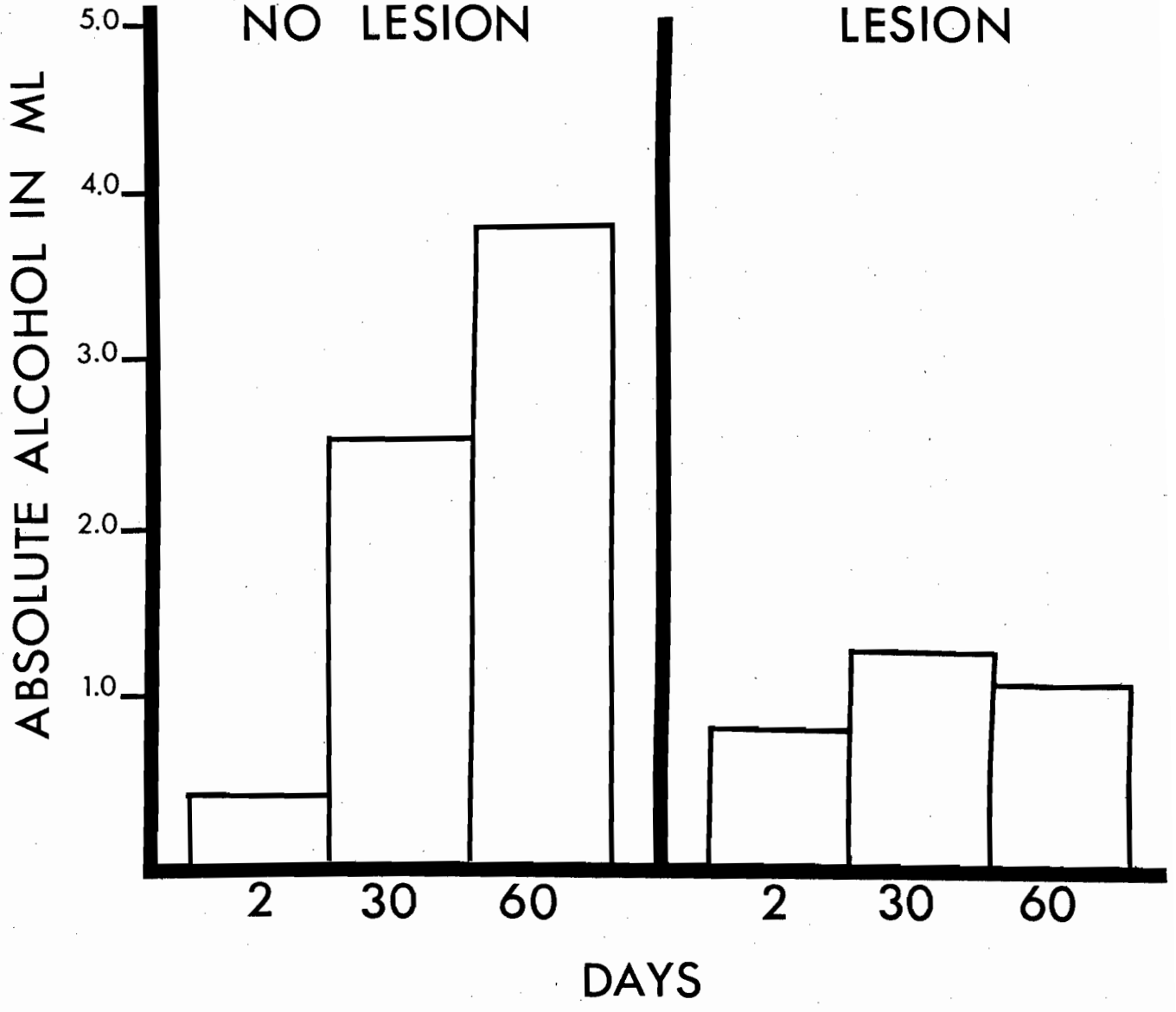


Figure 6. The absolute alcohol consumption in milliliters for the lesioned (S/L + C/L) and non-lesioned (S/NL + C/NL) subjects for the three periods.

Discussion

The purpose of the present study was to investigate the effects of nigrostriatal lesions on the development of alcohol preference by hypothalamic stimulation. Contrary to the findings of Amit, Stern and Wise (1970), in the present investigation electrical stimulation of the lateral hypothalamus failed to induce a preference for alcohol over water. The stimulation and control groups (S/NL and C/NL) ingested similar quantities of alcohol and both groups drank less than 60% of their fluid intake in alcohol solution. The nigrostriatal lesion however significantly inhibited alcohol intake in both stimulated and control subjects. This finding suggests that the nigrostriatal dopamine system contributes to the regulation of alcohol consumption.

Although this study failed to replicate the work of Amit et al. (1970), it should not be concluded that hypothalamic stimulation is ineffective in the establishment of alcohol preference. Several hypotheses can be forwarded to explain this discrepancy.

First, alcohol ingestion in the laboratory rat is a highly variable behavior, both before and after experimental intervention. Although alcohol intake remains relatively constant within a given animal, there is considerable variability between animals of the same sex and strain, both in the concentrations of alcohol they will reject and in their susceptibility to the effects of hypothalamic stimulation. For example, Amit et al. (1970) reported initial rejection concentrations which ranged from 9% to 40%, and in their stimulated groups, 9 of the 33 subjects failed to develop a preference for alcohol. This

varibility was attributed to underlying constitutional factors which may have determined the animals' resistance to the effects of alcohol or hypothalamic stimulation or both. In the present sample, the inclusion of several such animals could conceivably have lowered the group mean.

An alternative explanation of the failure to replicate Amit, et al. (1970) can be found in the fact that the stimulation group in this study included no stimulation-bound drinkers. Although Amit et al. (1970) did not demonstrate a direct correlation between stimulation-bound drinking and alcohol preference, they did obtain more consistent results when only stimulation-bound drinkers were employed as experimental subjects. Moreover the differences between their stimulated, experimental and control, non-stimulated animals was further enhanced when the animals were permitted to consume alcohol during the stimulation session. This suggests that stimulation-bound drinking may act as a selective factor in determining alcohol intake and, also that the close contiguity of alcohol ingestion and stimulation further facilitates the acquisition of preference. In the present study, no stimulation-bound drinkers were included. Hence the animals would not have ingested alcohol during the stimulation period even if it had been provided. These factors may have functioned to suppress the alcohol intake of the stimulated group.

Probably the most important factor that affected the present results was the placement of the stimulating electrodes. Although all the electrodes were contained within the lateral hypothalamus, histological examination showed them to be consistently more anterior than the sites of Amit et al. (1970). This importance of this factor is

emphasized by the finding that animals in the S/NL group with posterior placements tended to consume more alcohol than those with the anterior electrodes. If one makes approximations to the maps of Ungerstedt (1971a), it appears that the more anterior electrodes were in a position to stimulate only noradrenergic fibers and terminals. The dopamine fibers at this level have turned laterally into the internal capsule. The posterior placements however seemed to be capable of activating both catecholamines. If the release of dopamine is critical for the development of alcohol preference, then it follows logically that the posterior electrodes should be more effective in elevating alcohol intake. However the lack of histofluorescent examination makes this suggestion highly speculative.

In the absence of histfluorescence it is difficult to conclude that the lesions in this study actually destroyed the nigrostriatal system. In light of other evidence, however, this speculation appears more valid.

The first piece of evidence derives from direct behavioral observation. When the nigrostriatal system is destroyed unilaterally, animals typically exhibit a pronounced rotation towards the lesioned side (Ungerstedt, 1968; Anden, Dahlstrom, Fuxe, and Larsson, 1966). This effect has been attributed to an imbalance in the concentrations of dopamine in the two nigrostriatal systems such that the animals turn towards the side containing the least amount of dopamine (Ungerstedt, Butcher, Butcher, Anden and Fuxe, 1969). Although rotation was not quantified in the present experiment, it was observed that the lesioned animals consistently turned towards the lesioned side dur-

ing the test period. This behavior was not a function of the stimulation since it was exhibited both before and after current was applied. This evidence suggests that the lesion succeeded in eliminating at least some of the dopamine fibers on that side of the brain. Unfortunately no observations were made on the C/L animals to determine their response to the lesion.

Secondly, other studies have demonstrated that electrolytic lesions in the antero-ventral part of the substantia nigra cause a complete disappearance of dopamine from the ipsilateral caudate-putamen (Hokfelt and Ungerstedt, 1969; Ungerstedt, 1971a). Although immediately following the lesion there is an increase in striatal dopamine, within 56 hours an almost complete loss of transmitter is reported (Hokfelt and Ungerstedt, 1969; Ungerstedt, 1971b). Hence it seems likely that the lesions in the present study disrupted the nigrostriatal pathway and that, moreover, dopamine was depleted from the nerve terminals at an early point in the experiment. Unfortunately it is difficult to specify if the destruction was restricted to this system. Some fibers and cell bodies of the meso-limbic system were undoubtedly affected and in many cases, the ventral noradrenergic bundle appeared to sustain damage. However, the nigrostriatal system probably received the most widespread destruction.

If one accepts the fact that the lesion removed the nigrostriatal dopamine system, then dopamine appears to be of great importance in the regulation of alcohol intake. All animals sustaining lesions drank considerably less alcohol than the non-lesioned subjects. Interestingly, the stimulated animals (S/L) were slight-

ly less affected by the lesion than the control subjects (C/L). This might be attributed to the action of the stimulation in releasing dopamine from any remaining intact terminals and thus slightly compensating for the effects of the lesion. Moreover, on Day 1, the lesioned stimulated animals ingested more alcohol than any other group (17.9% or total fluid intake, as compared to 1.75%, 2.65%, and 5.67%). They reached a peak of 30% of total fluid intake on the third day of alcohol presentation after which their intake declined and remained stable at a lower level. This unusual finding may be explained by the fact that dopamine levels in the striatal terminals increase following electrocoagulation (Hokfelt and Ungerstedt, 1969; Ungerstedt, 1971b). Release can be effected by drugs such as amphetamine (Ungerstedt, 1971b)and also by electrical stimulation (McLennan, 1965; Portig and Vogt, 1968, 1969). Hence the stimulation of lesioned animals might at first elicit greater dopamine release than the stimulation of intact animals. This might also lead to greater alcohol consumption which, like the levels of dopamine, would decline after several days.

The demonstration that nigrostriatal dopamine exercises a critical role in alcohol ingestion raises a number of important questions. First, it is unusual that a unilateral lesion should exert such a striking and long-lasting influence on any behavior. Such findings have previously been reported only with unilateral damage in humans or with bilateral lesions in animals. The only analogous result for the rat brain has been observed in self-stimulation studies. Various investigations in this field have indicated that structures from both sides

of the brain participate in an integrated system of reward which subserves self-stimulation (German and Holloway, 1973; Ungerleider and Coons, 1970). Hence unilateral electrical stimulation probably activates the entire bilateral substrate. This idea seems tenable in view of the number of decussating fibers in the medial forebrain bundle (Umemoto, 1968; Wolf and Sutin, 1966), the major fiber system supporting self-stimulation (Olds and Olds, 1963). Further support for this hypothesis comes from the demonstration that procaine injections (which produce a functional lesion) into medial forebrain bundle sites markedly reduced self-stimulation rates elicited from contralateral structures (Madryga and Albert, 1971; Nakajima, 1972). This suggests that a disruption of any part of the bilateral system would influence the function of the entire system, and would result in an overall reduction of the rewarding effect. Since both self-stimulation (German and Bowden, 1974) and alcohol ingestion are mediated to some degree by the catecholamines, it is feasible that their substrates might function in an analogous manner. If so, then one might consider the present results in the following light. If the substrate which supports alcohol consumption is also a bilaterally integrated system, then damage to one part of the system would probably be reflected in the behavior as a whole. Thus if the nigrostriatal pathways are, or contribute to, the crucial fibers, then their unilateral destruction would be expected to modify alcohol intake.

Secondly, it is tempting to attribute the suppression of alcohol intake by nigrostriatal lesions to a reduction in THP formation. As mentioned previously, THP is an alkaloid formed from dopamine in the

presence of alcohol and has been thought to subserve some of the pharmacological and behavioral properties of the drug. Presumably, a reduction in dopamine concentrations could decrease THP formation and would consequently attenuate alcohol intake. Although a depletion of dopamine did depress alcohol ingestion in this study, there is no basis to ascribe this effect to a deficit in THP. Such a conclusion is contingent upon the assumption that the intake of the nonlesioned animals was a function of THP formation. This seems improbable since heavy consumption of alcohol is required for the production of THP (Davis and Walsh, 1971). The animals in this study can hardly be characterized as "heavy drinkers" and thus it is unlikely that THP played a critical role in their alcohol intake.

Moreover, before any THP hypothesis can be forwarded further research is required to determine if this effect is specific to the nigrostriatal pathway. Other catecholamine and non-catecholamine systems were destroyed by the lesion and could have feasibly contributed to the suppression of alcohol intake. Before any definite conclusions can be drawn about dopaminergic transmission, the effects of other manipulations on this system and on other systems must be examined.

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