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**LA THÈSE A ÉTÉ  
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MEDIATION OF THE POSITIVE REINFORCING PROPERTIES  
OF ETHANOL BY CENTRAL NORADRENERGIC MECHANISMS:  
IMPLICATIONS FOR TREATMENT PROCEDURES FOR  
ALCOHOLICS

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A Thesis  
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ABSTRACT

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MEDIATION OF THE POSITIVE REINFORCING PROPERTIES OF ETHANOL BY CENTRAL NORADRENERGIC MECHANISMS: IMPLICATIONS FOR TREATMENT PROCEDURES FOR ALCOHOLICS

The putative role of central norepinephrine in the mediation of the positive reinforcing properties of ethanol in laboratory rats was investigated. In the first experiment, it was demonstrated that selective destruction of catecholamine-containing neurons by treatment with 6-hydroxydopamine produced differential effects on the maintenance of ethanol self-administration. The observed attenuation on ethanol consumption was attributed to the relative depletions of norepinephrine rather than dopamine. Furthermore, in the second experiment it was shown that when norepinephrine levels were reduced by treatment with FLA-57, a dopamine-beta-hydroxylase inhibitor, ethanol intake was markedly suppressed. This effect was specific to ethanol since similar treatment had no significant effect on the consumption of either morphine or quinine solutions.

In the final experiment, extinction procedures were em-

ployed in order to determine the effectiveness of FLA-57 in permanently suppressing ethanol intake in laboratory rats. Ethanol-experienced animals, forced to drink ethanol while being treated with FLA-57, subsequently reduced their preference for ethanol when presented in a free-choice with water. Implications for treatment procedures for human alcoholics based on this extinction paradigm were discussed.

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For centuries man has been familiar with the effects of drinking alcohol. Because of its availability and its social acceptability in most Western cultures, in addition to being a popular beverage, alcohol has become a serious liability to society. It is generally considered that alcohol abuse is a significant contributing factor to automobile accidents, crime, health disorders, family breakdown and lost work productivity (see Le Dain, 1973).

It seems apparent that in most cases man consumes alcohol for its euphoric properties suggesting that alcohol must have psychologically reinforcing effects. In recent years, a number of experimental studies have shown that laboratory animals will also self-administer ethanol. Despite its aversive taste at higher concentrations (Kahn & Stellar, 1960; Richter & Campbell, 1940; Wilson, 1972) rats can be induced to voluntarily ingest ethanol solutions by a variety of techniques. Temporary withdrawal or intermittent presentation of ethanol generally results in an increased preference (Amit, Stern & Wise, 1970; Sinclair & Senter, 1968; Wayner, Greenberg, Tartaglione, Nolley, Fraley & Cott, 1972; Wise, 1973). Schedule-induced polydipsia of ethanol has been demonstrated by a number of investi-

gators (e.g. Falk, Samson & Winger, 1972; Meisch & Thompson, 1972; Senter & Sinclair, 1967) and in some cases the elevated intake of ethanol persisted even after termination of food reinforcement (Meisch & Thompson, 1974). Sinclair (1974) has reported that non-deprived rats will learn to press a lever for solutions of ethanol with greater frequency than for water. Furthermore, ethanol has been shown to be self-administered by laboratory animals both intragastrically (Amit & Stern, 1969; Marfaing-Jallat, 1975; Smith, Werner & Davis, 1976; Yanagita, Ando, Takahashi & Ishida, 1969) and intravenously (Deneau, Yanagita & Seevers, 1969; Winger & Woods, 1973; Woods, Ikomi & Winger, 1971). The fact that animals will drink ethanol despite its aversive taste or will work for ethanol by performing an operant suggests that ethanol has reinforcing properties.

Although the ability of alcohol to act as a reinforcer may be evident, the nature of its reinforcing properties is somewhat less certain. A negative reinforcement model for explaining the self-administration of alcohol suggests that a physically dependent organism must continue to ingest alcohol in order to avoid the aversiveness of withdrawal symptoms. Al-



ternatively, a positive reinforcement model proposes that the self-administration behavior is perpetuated because of the hedonic or reward value of alcohol itself. Lester and Freed (1973) claimed that physical dependence is a necessary criterion for an animal model of human alcoholism. The variety of methods used to induce physical dependence in laboratory animals is described in a review by Mello (1973). Presenting animals with ethanol as the only source of liquid (Ratcliffe, 1972) or as a partial source of calories in a liquid diet (Freund, 1973) readily produced physical dependence as demonstrated by an abstinence syndrome when the ethanol was withdrawn. Physical dependence was also induced by intragastric intubation (Begleiter, 1975; Myers, Stoltman & Martin, 1972) or by alcohol vapor inhalation (Goldstein, 1974). Although physical dependence on ethanol could be induced by these methods, in no case did animals exhibit a subsequent tendency to voluntarily drink alcohol, suggesting that the withdrawal syndrome as a negative reinforcer does not play a role in the initiation of maintenance of ethanol self-administration. Furthermore it has been demonstrated that monkeys trained to lever press for intravenous infusions of ethanol, would

self-impose periods of abstinence during which they would manifest severe withdrawal symptoms (Deneau et al, 1969; Woods et al, 1971). Walker, Hunter and Riley (1975) also showed that rats made physically dependent on ethanol through a liquid diet subsequently drank ethanol solutions but in an inconsistent pattern broken by periods of self-withdrawal. Similarly, in a study with human alcoholics, Mello and Mendelson (1972) reported that patients would work for tokens with which they could "buy" a drink of alcohol. Instead of using the tokens to maintain a regular intake of alcohol, they tended to accumulate tokens and then embark on a drinking episode followed again by a period of abstinence and saving tokens during which time they manifested mild to severe withdrawal symptoms. It therefore appears that avoidance of the abstinence syndrome is not a primary motivating factor in the self-administration of alcohol. The alternative to the negative reinforcement model of alcoholism is the suggestion that alcohol is self-administered for its positive reinforcing properties with physical dependence possibly having some potentiating effect.

Another method by which rats can be induced to reverse their preference for water over alcohol is by

electrical stimulation of the lateral hypothalamus (Amit & Stern, 1971; Amit et al, 1970; Wayner & Greenberg, 1972; Amit, Note 1). Following determination of concentrations of alcohol that were rejected by individual animals, lateral hypothalamic electrical stimulation was administered for 30 minutes daily over a 30 day period. Home cage intake of the previously rejected ethanol solutions presented in a free choice with water increased substantially. This preference for alcohol persisted beyond the electrical stimulation phase and was also resistant to attenuation when the ethanol solutions were adulterated with quinine. Furthermore, when the presentation of ethanol was terminated, the animals did not exhibit any withdrawal symptoms.

A number of investigators have shown that laboratory animals will learn to self-stimulate through electrodes implanted in the area of the lateral hypothalamus (e.g. Olds & Milner, 1954; Olds, Travis & Schwing, 1960; Stein, 1968). Furthermore, recent neuroanatomical studies indicated that the region of the lateral hypothalamus is traversed by some of the major catecholamine pathways (Lindvall & Björklund, 1974; Ungerstedt, 1971). It has therefore been hypo-

thesized that catecholaminergic systems support self-stimulation and possibly subserve reward in general (see German & Bowden, 1974). Since electrical stimulation of the lateral hypothalamus appears to potentiate the self-administration of ethanol in rats (e.g. Amit & Stern, 1971), it is conceivable that the same catecholamine mechanisms which subserve intra-cranial self-stimulation also mediate the positive reinforcing properties of ethanol.

In an experiment designed to investigate the involvement of catecholamines in the behavioral effects induced by alcohol, human subjects were treated with alpha-methyltyrosine prior to programmed drinking of alcohol (Ahlenius, Carlsson, Engel, Svensson & Sodersten, 1973). Alpha-methyltyrosine is a potent inhibitor of tyrosine hydroxylase, the rate limiting enzyme in the biosynthesis of catecholamines (Engelman, Jequier, Udenfriend & Sjoerdsma, 1968). It was reported that pre-treatment with alpha-methyltyrosine resulted in a suppression of alcohol-induced stimulation and euphoria suggesting that catecholamines play a functional role in the mediation of alcohol's reinforcing effects.

Possible interactions between alcohol and the

catecholamines have also been investigated in a number of studies which examined the effects of ethanol treatment on the activity of central catecholamine-containing neurons. Wayner, Ono and Nolley (1975) reported that neurons in the lateral hypothalamus were highly sensitive to ethanol applied electrophoretically. It has been shown further that injections of ethanol in rats increased catecholamine turnover as determined by elevated concentrations of catecholamine metabolites (Karoum, Wyatt & Majchrowicz, 1976). Similar studies indicated that acute ethanol treatment in laboratory rats resulted in increased release and synthesis of norepinephrine while dopamine was only minimally affected (Corrodi, Fuxe & Hökfelt, 1966; Pohorecky, 1974; Pohorecky & Jaffe, 1975).

The arguments thus far presented propose that catecholamines are involved in the mediation of alcohol self-administration. There are however some suggestions that norepinephrine plays the dominant role in subserving the pharmacological effects of alcohol. The anti-alcoholic properties of disulfiram (Antabuse) are generally attributed to its capability of inhibiting aldehyde dehydrogenase (Mardones, 1963). Aldehyde dehydrogenase is the enzyme necessary for the break-

down of acetaldehyde which is the primary metabolite of alcohol. It is presumed that ingestion of alcohol following pre-treatment with disulfiram produces an emetic effect due to the accumulation of toxic acetaldehyde, consequently deterring further alcohol intake. Moreover, disulfiram also inhibits dopamine-beta-hydroxylase, the enzyme necessary for the synthesis of norepinephrine from dopamine (Goldstein, Anagnoste, Lauber & McKereghan, 1964). Because of the putative role of catecholamines in the mediation of the positive reinforcing properties of alcohol, it was suggested by Collier (1972) that the effectiveness of disulfiram as an anti-alcoholic agent may be due to its ability to act as a dopamine-beta-hydroxylase inhibitor rather than as an aldehyde dehydrogenase inhibitor. To investigate this possibility further, Amit, Meade and Corcoran (1975) compared the effects of disulfiram, Temposil and FLA-63 on ethanol consumption in laboratory rats. As previously mentioned, disulfiram inhibits both aldehyde dehydrogenase and dopamine-beta-hydroxylase. Temposil acts exclusively as an aldehyde dehydrogenase inhibitor whereas FLA-63 inhibits dopamine-beta-hydroxylase with only minimal effects on aldehyde dehydrogenase (Amit, Levitan & Lindros, 1976). The results of this experiment indicated that while

Temposil had virtually no effect on ethanol intake and disulfiram had only a moderate effect, FLA-63 markedly attenuated ethanol consumption. This suppression of drinking in the FLA-63-treated animals was presumed to be due to concomitant reductions in central norepinephrine levels. However, due to the high toxicity of FLA-63, no definitive conclusions could be drawn from this study.

The first two experiments in the present study were undertaken to test the hypothesis that norepinephrine plays a role in the mediation of the primary reinforcing properties of alcohol. Experiment I examined the effect of selective destruction of central noradrenergic and dopaminergic neurons by the neurotoxin 6-hydroxydopamine on the intake of ethanol. In Experiment II, norepinephrine levels were reduced by FLA-57 a non-toxic dopamine-beta-hydroxylase inhibitor and the consequent effects on the maintenance of ethanol self-administration were observed.

A variety of treatment procedures for alcoholism have been developed but only a few have met with limited success. In a recent evaluation of the various methods used for the treatment of alcoholics, Amit & Sutherland (1975/76) subdivided the popular approaches

into three main categories. One method is to induce abrupt cessation of drinking whereby alcoholic patients are deprived of alcohol with the consequent experience of withdrawal symptoms. Since relapse of alcohol drinking following such treatment is extremely high, the value of these procedures is minimal. Moreover, animal studies suggest that temporary deprivation invariably leads to an increased consumption of ethanol (Sinclair & Senter, 1968). A second class of treatment procedures involves substituting an alternate and incompatible response to alcohol-drinking behavior. Unless the performance of the alternate response is continually possible, it is likely that alcohol consumption would be resumed. In a recent study, it was shown that ethanol-preferring rats would switch to drinking a sodium saccharine solution when it was offered as a third-fluid choice (Amit & Amir, Note 2). However, when the saccharine solution was no longer available, ethanol drinking immediately recovered. The third category of treatments includes a number of variations of application of punishment. The initiation of alcohol drinking may be paired with an aversive stimulus such as shock, or the cessation of drinking may be negatively reinforced by the termination of



an aversive stimulus. Although these techniques may discourage drinking during the conditioning sessions, alcohol consumption is readily resumed thereafter.

In studies with infra-human subjects it has been shown that these procedures induce an elevation rather than a suppression of ethanol intake (e.g. Casey, 1960).

Punishment of alcohol drinking may also be invoked by emetic agents such as apomorphine or emetine or by drugs like disulfiram or Temposil which induce nausea following alcohol consumption. The basis for these treatment procedures is the conditioned taste aversion paradigm (e.g. Garcia & Koelling, 1966; Kalat & Rozin, 1970; Revusky & Garcia, 1970). Since conditioned taste aversions can only be induced with novel tasting fluids, it cannot be expected that alcohol intake would be effectively reduced in experienced drinkers. At best, treatment of alcoholics with emetic agents may provide periods of temporary alcohol abstinence during which time other forms of therapy may be introduced. In addition, pharmacological treatment with a variety of tranquilizing, anti-depressant or mood-altering drugs produces only limited and temporary effects on problem drinking.

Underlying the relative ineffectiveness of the

various treatment procedures described, are a number of general theoretical considerations. As previously argued, a negative reinforcement model of alcoholism is tenuous since physical dependence and relief from withdrawal are neither necessary nor sufficient conditions for the self-administration of alcohol. Therefore, alcoholics that are forced to abstain from drinking and pass through the alcohol withdrawal phase, generally tend to relapse despite the absence of the negative reinforcer (the threat of withdrawal symptoms). Furthermore, alcohol self-administration can be viewed as an acquired behavior (Conger, 1956; Kepner, 1964; Kingham, 1958) and therefore may be subject to the general laws of learning. In a treatment model for alcoholism based on conditioning principles, the most effective method of suppressing or attenuating alcohol-oriented behaviors would be to permit the performance of the drinking response in the absence of reinforcement. This procedure would be analogous to extinction paradigms used to abolish an operant response for food in laboratory animals. Simply depriving an animal of the opportunity to bar press will generally result in a reinstatement of the original response when the bar is again made available. Similarly,

treatment procedures for alcoholics which involve merely depriving the patient of alcohol, either by forced abstinence or punishment, cannot be expected to have lasting deterrent effects. Alternatively, if an animal is permitted to perform a learned operant response but with food reward no longer being delivered, the response will ultimately be extinguished. Therefore, in treating human alcoholics, the drinking response may be extinguished only if the subsequent reinforcement is eliminated. However, since alcohol-drinking behavior is reinforced by the inherent pharmacological effects of the ingested alcohol, it becomes a difficult task to separate the response from the reinforcement.

Since catecholamines and possibly norepinephrine in particular, may be mediating the positive reinforcing pharmacological properties of alcohol then conceivably if central norepinephrine levels are reduced, the reinforcing effects of alcohol may be inhibited. Experiment III was therefore designed to examine the effectiveness of FLA-57 induced depletions of norepinephrine on the extinction of ethanol self-administration in laboratory animals.

### Experiment I

It has been demonstrated that electrical stimulation of the lateral hypothalamus, an area traversed by catecholamine pathways, resulted in a reversal of preference for water over alcohol in laboratory rats (Amit & Stern, 1971; Amit et al, 1970; Wayner & Greenberg, 1972; Amit, Note 1). Furthermore, rats that were lesioned electrolytically in the ventral portions of the lateral hypothalamus exhibited a reduced preference for ethanol (Amit, Meade, Levitan & Singer, 1976).

A useful procedure for investigating the functions of different catecholamine systems is to selectively destroy catecholamine neurons by intra-cranial infusions of the neurotoxin 6-hydroxydopamine (Breese & Traylor, 1971; Uretsky & Iverson, 1969, 1970). By pre-treating animals with pargyline, a monoamine oxidase inhibitor or with desmethylimipramine, a norepinephrine re-uptake blocker, the cytotoxic effects of 6-hydroxydopamine on norepinephrine and dopamine-containing neurons can be systematically varied (Breese & Traylor, 1971; Sachs & Jonsson, 1975; Stricker & Zigmond, 1974).

A number of recent studies have examined the effects of 6-hydroxydopamine-induced depletions of cate-

cholamines on ethanol self-administration in laboratory animals. Destruction of fibers in the dorsal noradrenergic bundle by 6-hydroxydopamine in ethanol-preferring rats resulted in a significant increase in ethanol intake over a prolonged period (Kiianmaa, Fuxe, Jonsson & Ahtee, 1975). The elevation in ethanol intake was explained as a compensatory response necessary to maintain the reinforcing properties of ethanol in a partially damaged system. Ho, Tsai, and Kissin (Note 3) also reported an increase in ethanol preference following intracerebral infusions of 6-hydroxydopamine. On the other hand, Myers and Melchior (1975) found a decrease in the self-selection of increasing concentrations of ethanol in animals treated with 6-hydroxydopamine.

In the present experiment, norepinephrine and dopamine systems were selectively destroyed by treatments with 6-hydroxydopamine and the subsequent effects on the maintenance of ethanol-self-administration in laboratory rats were examined.

#### Method

##### Subjects

Male Wistar rats weighing approximately 225-275 grams were obtained from Canadian Breeding Farm Laboratories

Ltd. Animals were individually housed in stainless steel cages in a room regulated for constant temperature and humidity and a 12-hour light cycle. Water and ethanol solutions were presented in calibrated glass Richter tubes (Kimax) mounted in front of the cage and Purina rat chow was available ad libitum at all times.

#### Procedure

Surgery. Animals were anaesthetized with intraperitoneal injections of sodium pentobarbitol (60 mg/kg; Abbott Laboratories) and supplements of chloral hydrate (300 mg/kg) when necessary. In each of the animals a 22 gauge stainless steel cannula (Plastic Products Inc.) was stereotaxically aimed at the left ventricle of the brain. With the incisor bar set at zero the coordinates were 1.0 mm posterior to bregma, 1.5 mm lateral to the sagittal suture and 3.6 mm ventral to the dura. The cannula was secured in position by cranioplast cement anchored to the skull by 4 stainless steel screws.

Ethanol screening. Following a 5 day period of recovery from surgery each rat was screened to drink ethanol by a modification of a procedure described by Amit et al (1970). On alternate days animals were

offered a free choice between water and increasing concentrations of ethanol presented in Richter tubes mounted in front of the home cages. On the intervening days only water was made available in both tubes. Ethanol solutions were prepared by mixing 95% ethanol with tap water. On the first day of screening a 3% (v/v) ethanol solution was presented in a free-choice with water. If an animal drank more than half of its total daily fluid intake in the form of the ethanol solution then on the subsequent presentation the concentration was increased by 2%. This procedure was continued until the animal reached a concentration at which ethanol consumption stabilized at approximately 50% of its total daily fluid intake. The concentration so established was used as the test solution for the respective animal for the remainder of the experiment. Animals that refused to drink a minimum concentration of 8% (v/v) ethanol were eliminated from the experiment. In order to control for possible position bias the ethanol and water tubes were alternated with each presentation. Body weight and intake of fluids were recorded daily.

Treatments. Following induction of ethanol drinking and determination of individual test concentrations,

animals were randomly assigned to one of the 3 treatment groups. The first group (6-HDA) received 2 infusions of 6-hydroxydopamine (200  $\mu$ g) spaced by 24 hours. 6-hydroxydopamine hydrobromide (Regis Chemical Co.) was dissolved in a vehicle composed of a 0.1% (w/v) ascorbic acid solution prepared with 0.9% saline. All infusions were administered with a Harvard Apparatus infusion pump connected to a 26 gauge internal cannula (Plastic Products Inc.) which was inserted into the chronically implanted external cannula. 200  $\mu$ g of 6-hydroxydopamine in a volume of 10  $\mu$ l was delivered over a period of 30 seconds. The second group (6-HDA/pargyline) was treated in exactly the same way as the 6-HDA group except that 45 minutes prior to each 6-hydroxydopamine infusion animals were injected intraperitoneally with 50 mg/kg of pargyline hydrochloride (Sigma Chemical Co.). Pretreatment with pargyline, a monoamine oxidase inhibitor has been shown to potentiate the neurotoxic effects of 6-hydroxydopamine (Breese & Traylor, 1971). Animals in the third group (6-HDA/DMI/pargyline) received only a single infusion of 200  $\mu$ g of 6-hydroxydopamine 45 minutes after injection with pargyline (50 mg/kg i.p.) and desmethylinipramine (25 mg/kg i.p.; CIBA Company Ltd.).



Desmethylinipramine is a norepinephrine re-uptake blocker and selectively protects noradrenergic neurons from destruction by 6-hydroxydopamine (Cooper, Breese, Grant & Howard, 1973). Following these treatments, animals were returned to their home cages where food, water and ethanol were available as usual.

Post-treatment. For 3 to 5 days following treatment animals suffered from weight loss and consumption of food and fluids were depressed. However, by the fifth day the animals seemed to stabilize and recording of intake of alcohol was resumed for the next 18 alternate days at which point the experiment was terminated.

Biochemical assays. At the termination of the experiment the animals were killed by cervical dislocation and were decapitated. The brains were rapidly removed and were immediately frozen on dry ice. Whole brain norepinephrine and dopamine levels were analyzed by flourometric assay procedures (Chang, 1964; Shellenberger & Gordon, 1971). Additional animals of the same age as the experimental animals served as intact assay controls.

## Results

### Behavioral

Following the treatment phase of the experiment, animals in all groups displayed temporary aphagia and adipsia to varying degrees. Four to 5 days after treatment, body weight stabilized and food and fluid consumption returned to normal. Ethanol intake for the 3 groups during the baseline and post-treatment periods was recorded in terms of volume consumed (Figure 1) and preference ratios (Figure 2). Preference ratios were calculated as the volume of ethanol ingested compared to the total daily fluid intake. An analysis of variance and subsequent tests for simple main effects revealed that only the 6-HDA/pargyline group significantly attenuated its mean ethanol intake following treatment in terms of both volume consumed ( $F(1, 15) = 20.90, p < .001$ ) and preference ratio ( $F(1, 15) = 26.10, p < .001$ ).

### Biochemical Assays

A complete biochemical analysis was precluded due to a technical malfunction in which 4 of the 6 brains in the 6-HDA group were destroyed. Nevertheless, the relative depletions of norepinephrine and dopamine obtained appear to be consistent with other reports (e.g. Breese & Traylor, 1971; Cooper et al, 1973).

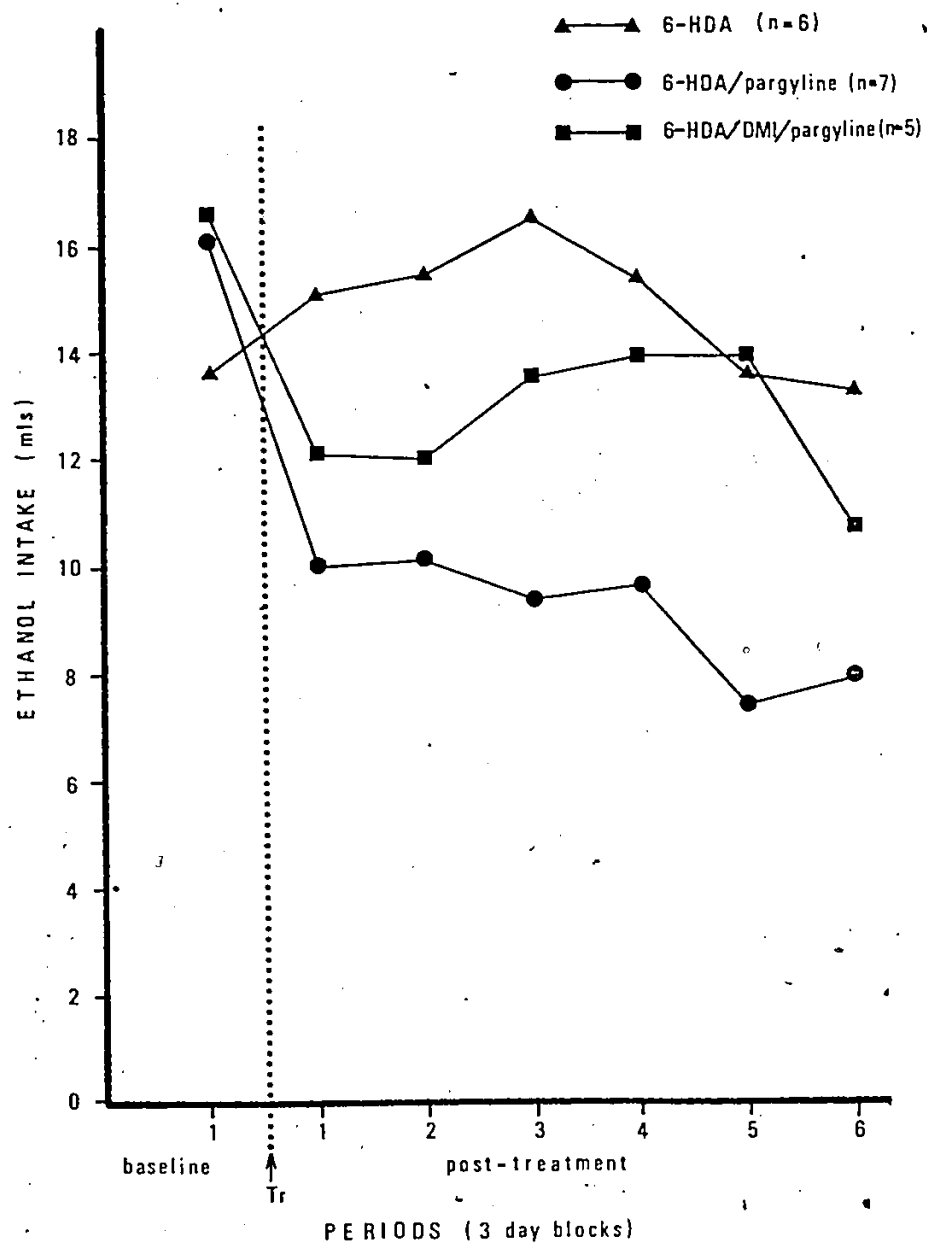


Figure 1. Ethanol intake in terms of mean volume consumed (mls) in rats treated differentially with 6-hydroxydopamine. (Tr = treatment and recovery period).

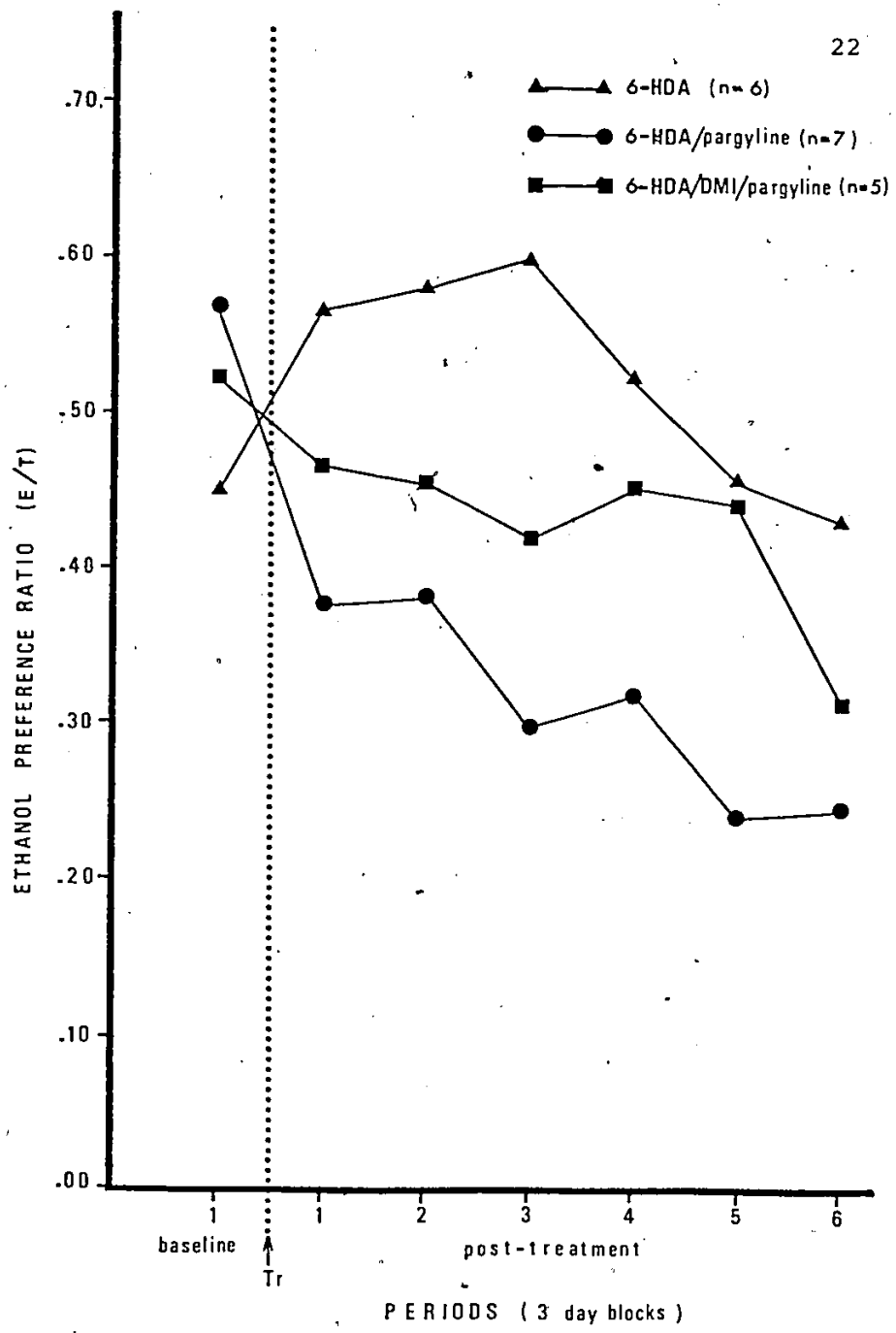


Figure 2. Ethanol consumption in terms of mean preference ratios (E/T = volume of ethanol/volume of total daily fluid intake) in rats treated differentially with 6-hydroxydopamine.

Table 1  
 Effect of 6-hydroxydopamine Treatments on the Concentration  
 of Catecholamines in Whole Brain

Treatment	n	NOREPINEPHRINE		DOPAMINE	
		Levels (ng/g)	% control	Levels (ng/g)	% control
Controls	5	231.6	-	623.9	-
6-HDA 2 x (200 ug 6-HDA)	2 a	32.0*	13.8	427.3*	68.4
6-HDA/pargyline 2 x (50 mg/kg par- gyline + 200 ug 6-HDA)	7	29.6**	12.8	141.1**	22.6
6-HDA/DMI/pargyline 1 x (30 mg/kg DMI + 50 mg/kg pargyline + 200 ug 6-HDA)	5	198.9	85.9	121.2**	19.5

a 4 of the 6 brains from the 6-HDA animals were lost. \* p < .01 \*\* p < .001

Table 1 summarizes the whole brain content of norepinephrine and dopamine for the 3 different groups as compared to intact control animals. Dopamine levels were significantly lower than control in all cases with the 6-HDA group showing smaller depletions than the other two groups (6-HDA:  $t(5) = 4.15$ ,  $p < .01$ ; 6-HDA/pargyline:  $t(10) = 15.16$ ,  $p < .001$ ; 6-HDA/DMI/pargyline:  $t(6) = 13.75$ ,  $p < .001$ ). Whole brain norepinephrine levels were significantly reduced in both the 6-HDA group ( $t(5) = 5.8$ ,  $p < .01$ ) and in the 6-HDA/pargyline group ( $t(10) = 11.73$ ,  $p < .001$ ), while the 6-HDA/DMI/pargyline group did not have a significant norepinephrine depletion ( $t(8) = 1.37$ ,  $p > .05$ ).

#### Discussion

The results of this experiment indicate that selective catecholamine depletions have differential effects on the maintenance of ethanol self-administration. With the most profound depletions of norepinephrine and dopamine produced by treatment with pargyline plus 6-hydroxydopamine, ethanol intake was markedly attenuated. These data therefore support the hypothesis that catecholamines may be involved in the mediation of the positive reinforcing proper-

ties of alcohol. When norepinephrine neurons were protected from the cytotoxic effects of 6-hydroxydopamine by pre-treatment with desmethylinipramine, the decrease in ethanol intake was not significant. Since the dopamine levels in both the 6-HDA/pargyline and the 6-HDA/DMI/pargyline groups were reduced to the same extent, the differential effects on ethanol self-administration would appear to be attributable to norepinephrine.

The ethanol intake results obtained for the 6-HDA group are consistent with the reports of Kiianmaa et al, (1975) and Ho et al (Note 4). However, based on a hypothesis of noradrenergic mediation of ethanol self-administration, according to the relative depletions produced it would be expected that the attenuation in ethanol drinking for the 6-HDA group would have been somewhat less than for the 6-HDA/pargyline group and greater than for the 6-HDA/DMI/pargyline group. According to Stricker and Zigmond (1976), the extent of recovery of function following destruction of catecholaminergic neurons does not necessarily correlate directly with the degree of damage. They proposed that following 6-hydroxydopamine treatments that produce only moderate neural

destruction, the main compensatory reaction is at the pre-synaptic level (e.g. increased tyrosine hydroxylase activity, increased re-uptake, monoamine oxidase inhibition, etc.) resulting in a partial recovery of function. With more extensive depletions, in addition to increased pre-synaptic activity, post-synaptic receptors become supersensitive with a consequent restoration of function to near normal levels. Finally, when neural destruction is maximized by treatment with pargyline plus 6-hydroxydopamine, the compensatory mechanisms break down almost completely generally resulting in severe and perhaps permanent behavioral deficits. The results of this experiment are consistent with this explanation in that only the 6-HDA/pargyline group showed a significant attenuation in ethanol self-administration. Although not quite evident from the limited assay results of this experiment, animals treated with 6-hydroxydopamine alone tend to have lesser depletions of norepinephrine than animals treated with pargyline plus 6-hydroxydopamine (Cooper et al, 1975). Therefore, according to Stricker and Zigmond (1976) the 6-HDA group would be expected to show the greatest recovery of function due to elicitation of both pre-synaptic and post-synaptic compen-



satory mechanisms. The 6-HDA/DMI/pargyline group, having the least depletion of norepinephrine, recovered only partially as evidenced by the moderate but non-significant attenuation in ethanol intake.

These data therefore strengthen the hypothesis that catecholamines are involved in the mediation of alcohol self-administration. Furthermore, norepinephrine rather than dopamine appears to play the dominant role in subserving the positive reinforcing properties of alcohol.

#### Experiment II

There is some evidence which leads to the possible implication of norepinephrine in the mediation of alcohol self-administration. It has been shown that acute injections of ethanol in laboratory rats altered the synthesis and release of norepinephrine but did not affect dopamine (Corrodi et al, 1966; Pohorecky, 1974; Pohorecky & Jaffe, 1975). Furthermore, Collier (1972) suggested that the anti-alcoholic properties of disulfiram may be due to its effect on norepinephrine via dopamine-beta-hydroxylase inhibition. Amit et al (1975) have demonstrated that the dopamine-beta-hydroxylase inhibitor, FLA-63, markedly

attenuated ethanol intake in laboratory rats. Unfortunately, this result was confounded by the fact that FLA-63 has toxic side-effects. It has also been reported that propranolol, a beta-adrenergic blocker, temporarily reduced the effects of alcohol in alcoholic patients (Tyrrer, 1972; Mendelson, Rossi & Bernstein, Note 4). Furthermore, in Experiment I of this study where catecholamines were selectively depleted by treatment with 6-hydroxydopamine, the results implicated norepinephrine rather than dopamine in the mediation of ethanol self-administration.

In order to verify the extent to which norepinephrine may subserve alcohol's positive reinforcing properties, we undertook to examine the effects of chronic injections of a non-toxic dopamine-beta-hydroxylase inhibitor, FLA-57, on the maintenance of ethanol self-administration in laboratory rats.

### Method

#### Subjects

Subjects used in this experiment were male Wistar rats (Canadian Breeding Farm Laboratories Ltd.) weighing 225-275 grams at the beginning of the experiment. Animals were individually housed in stainless steel

cages in a room regulated for constant temperature and humidity and a 12-hour light cycle. Drinking fluids were presented in calibrated glass Richter tubes (Kimax) mounted in front of the cage. Purina rat chow was available ad libitum throughout the experiment.

#### Procedure

Experiment II(a). Animals were screened to drink ethanol on an alternate-day free-choice paradigm as previously described in the procedure for Experiment I. The alternate-day schedule was maintained throughout the remaining phases of the experiment. Following alcohol screening and stabilization of baseline intake, animals were randomly assigned to one of the three treatment groups. Animals in the first group (FLA/25) were injected with the dopamine-beta-hydroxylase inhibitor FLA-57 (25 mg/kg i.p.; Astra Chemical Co.) approximately 3 to 4 hours prior to each alcohol presentation for 5 consecutive alternate days. FLA-57 was first dissolved in 1 N sodium hydroxide and then the pH was adjusted to 8.0-8.2 with 1 N acetic acid. Using Ringer's solution, the volume of the solution was increased to yield a concentration of 25 mg/ml of vehicle. At

this concentration, the drug precipitated and was injected as a suspension. The vehicle was also buffered to a pH of 8.0-8.2. Following the 5 injections of FLA-57, ethanol intake was recorded for 5 additional post-injection presentations. The second group (FLA/40) was treated in the identical manner as the FLA/25 group except that FLA-57 was injected in a dose of 40 mg/kg. The control group (Vehicle) was subjected to the same procedure as the two experimental groups except that during the injection period the animals received equivalent volumes of the vehicle. Body weight and fluid intake were recorded daily throughout the experiment.

Experiment II(b). Except for a few modifications and extensions, the procedure followed in this experiment was similar to that of Experiment II(a). Using the alternate-day free-choice paradigm, animals were screened to drink increasing concentrations of ethanol to a maximum of 15% (v/v). Animals failing to drink this concentration of ethanol were eliminated from the experiment. At this point, the schedule was switched from an alternate-day to an every-day free-choice between ethanol (15% v/v) and water. The every-day free-choice paradigm was maintained for the

balance of the experiment. After recording 5 days of baseline intake, animals were randomly assigned to either the experimental or control group. The experimental group (FLA/45) received 5 consecutive daily injections of FLA-57 (45 mg/kg i.p., 3 to 4 hours prior to the daily refilling of drinking tubes) while the control group (Vehicle) was injected with equivalent volumes of the vehicle. In order to avoid precipitation of the FLA-57 and to ensure more uniform doses upon injection, FLA-57 was prepared to yield a concentration of only 15 mg/ml. This solution was injected in a volume of 3 ml/kg of body weight resulting in a dose of 45 mg/kg. Alcohol and water intake as well as body weight were recorded during the injection period and for an additional 10 days thereafter.

In order to determine the specificity of the effects of FLA-57, 2 more groups of animals were added. One group (morphine/FLA) was initially screened to drink increasing concentrations of morphine sulphate (May & Baker Canada Ltd.) dissolved in a .05% (w/v) sodium saccharine solution. Morphine solutions were presented daily in a free-choice with water. Using a final concentration of .2 mg/ml, morphine intake

was allowed to stabilize for a 5-day baseline period. Animals were then injected intraperitoneally with FLA-57 at a dose of 45 mg/kg for 5 consecutive days and were then observed in the post-injection period for an additional 10 days. The second group (quinine/FLA) was treated in exactly the same way as the morphine/FLA group except that a .02 mg/ml concentration of quinine hydrochloride dissolved in a .05% (w/v) sodium saccharine solution was used instead of morphine-saccharine. Body weight and fluid intake were recorded as usual.

Biochemical assays. Assays were done to determine the effect of treatment with FLA-57 on the whole brain content of norepinephrine and dopamine. Additional animals of equivalent weight to those used in Experiment II(b) were maintained for assay purposes. One group of animals was injected with FLA-57 (45 mg/kg i.p.) prepared in the same way as described in the procedure for Experiment II(b). The remaining animals received an injection of the vehicle solution. Four hours after injection, all animals were decapitated, the brains were removed and were immediately frozed on dry ice. Using the procedures described by Chang (1964) and Shellenberger and Gordon (1971),

whole brain norepinephrine and dopamine levels were determined.

## Results

### General observations

A pilot study done prior to the present experiments revealed that treatment with FLA-57 did not produce any observable toxic side-effects. Since body weight and fluid intake were not affected during the injection and post-injection periods in the present experiments, the lack of toxic effects of FLA-57 was confirmed.

### Experiment II(a)

As can be seen from Figure 3 which represents the daily ethanol consumption expressed as grams of ethanol per kilogram of body weight, intake during the injection period was suppressed for both FLA-57-injected groups. An analysis of variance and subsequent tests for simple main effects did not yield a significant difference between the three groups ( $F(2,24) = 1.17, p > .05$ ). However, an analysis of the data for each individual group over the baseline, injection and post-injection periods produced the following results. The Vehicle group showed no change

in alcohol intake over time ( $F(2,48) = 2.76, p > .05$ ). Post hoc Tukey tests reveal that for the FLA/25 group, the mean ethanol consumption during the injection period was significantly lower than baseline ( $p < .01$ ) and that despite the partial recovery seen in the post-injection period, intake was still significantly lower than the original baseline ( $p < .01$ ). The FLA/40 group showed a significant attenuation in ethanol consumption during the injection period ( $p < .01$ ) but in the post-injection phase, intake was no longer different from baseline ( $p > .05$ ).

Figure 4 represents data from the same animals expressed as an ethanol preference ratio (volume of ethanol/total daily fluid intake). Post hoc Tukey tests performed subsequent to an analysis of simple main effects indicated that both FLA-57-injected groups differed significantly from the Vehicle group ( $p < .01$  in both cases). Additional post hoc analyses (Tukey tests) showed that while the Vehicle group developed an increased preference for ethanol over time ( $p < .01$ ), both FLA-57 groups reduced their mean ethanol preference during the injection period ( $p < .01$  in both cases). In the post-treatment period the FLA/40 group recovered to levels not different from



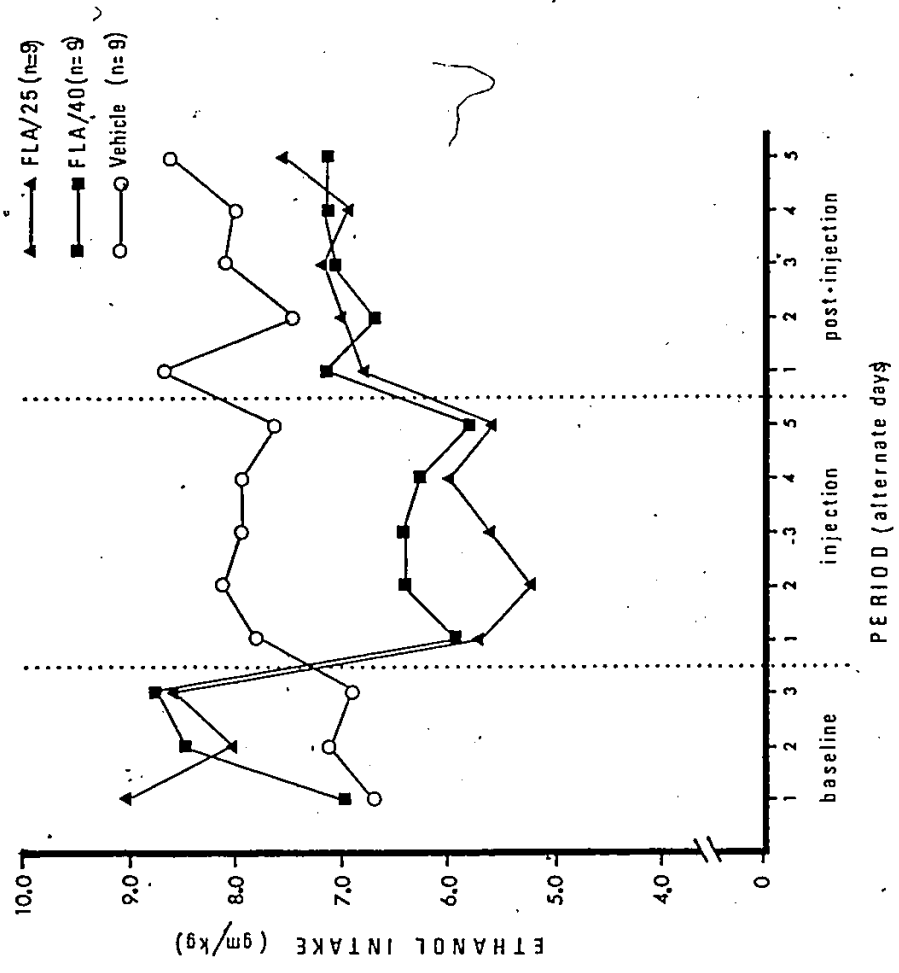


Figure 3. Ethanol intake in terms of mean absolute ethanol consumption (gm/kg) in rats treated with FLA-57.

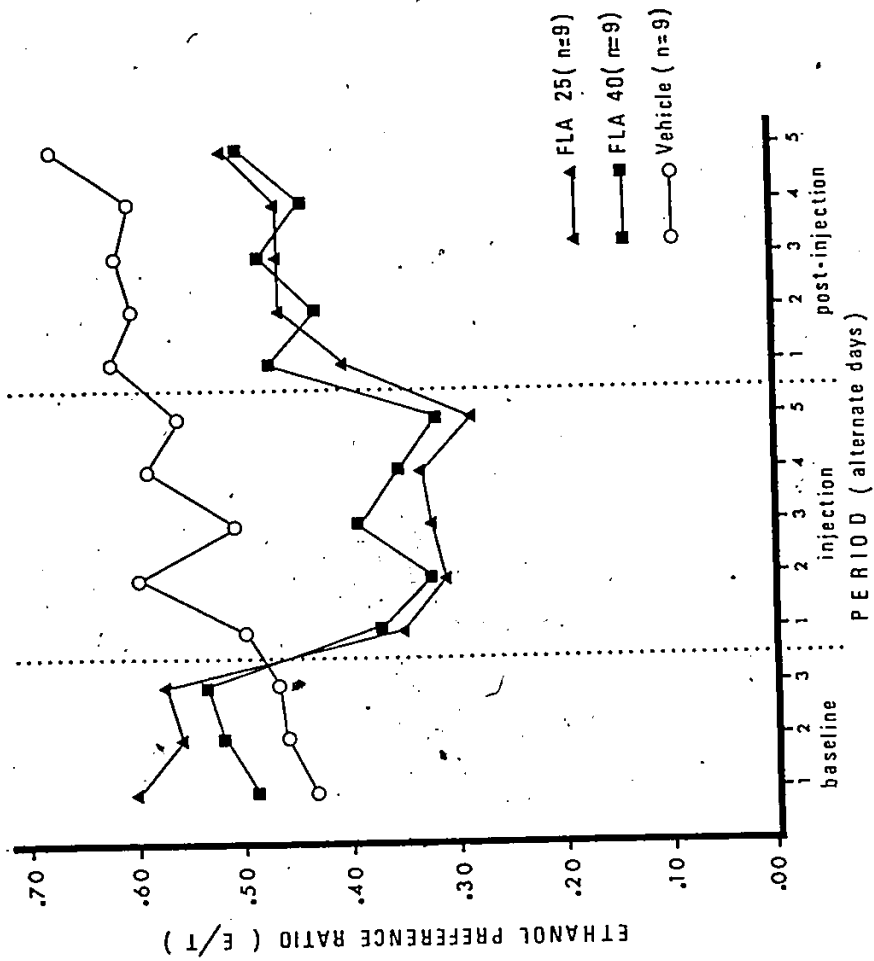


Figure 4. Ethanol consumption in terms of mean preference ratios (E/T = volume of ethanol/volume of total daily fluid intake) in rats treated with FLA-57.

baseline ( $p > .05$ ) while the preference ratio of the FLA/25 was still slightly but significantly depressed ( $p < .05$ ).

#### Experiment II(b)

Despite the procedural modifications, the results of Experiment II(b) are similar to those obtained in Experiment II(a). Figure 5 shows the mean ethanol intake (gm/kg) over the different phases of the experiment. A posteriori tests of simple main effects indicated that the mean ethanol intake during the injection period was significantly lower for the FLA/45 group than for the Vehicle group ( $F(1,8) = 5.45, p < .05$ ). Furthermore, the Vehicle group did not show any change in ethanol intake over time ( $F(3,24) = 1.08, p > .05$ ) while the FLA/45 group although showing a significant attenuation in absolute ethanol intake during the injection period (post hoc Tukey test,  $p < .01$ ) recovered to baseline levels in the post-injection period ( $p > .05$  for both the first and last 5 days of the post-injection period).

The same data expressed as a preference ratio are shown in Figure 6. During the injection period, the preference level for the experimental group differed significantly from that of the control group ( $F(1,8) =$

7.66,  $p < .05$ ). Again, in terms of preference levels, only the experimental group manifested a significant reduction during the injection period (post hoc Tukey test,  $p < .01$ ) with a subsequent increase back to baseline levels in the post-injection periods ( $p > .05$ ).

The effects of injections of FLA-57 on the consumption of either morphine or quinine solutions are shown in Figures 7 and 8 respectively. Whether analyzed in terms of volume consumed or preference ratios, the results indicated that there were no significant changes over time (Morphine/FLA: volume  $F(3,12) = 3.23$ ,  $p > .05$ ; preference  $F(3,12) = 1.78$ ,  $p > .05$ . Quinine/FLA: volume  $F(3,12) = 0.38$ ,  $p > .05$ ; preference  $F(3,12) = 1.14$ ,  $p > .05$ ).

#### Biochemical assays

Table 2 summarizes the results of the assay done to determine the effects of FLA-57 (45 mg/kg i.p.) on whole brain norepinephrine and dopamine levels. Dopamine content was slightly but not significantly increased by the treatment ( $t(11) = 1.81$ ,  $p > .05$ ) while norepinephrine was significantly reduced to 56.7% of control levels ( $t(11) = 5.45$ ,  $p < .001$ ).

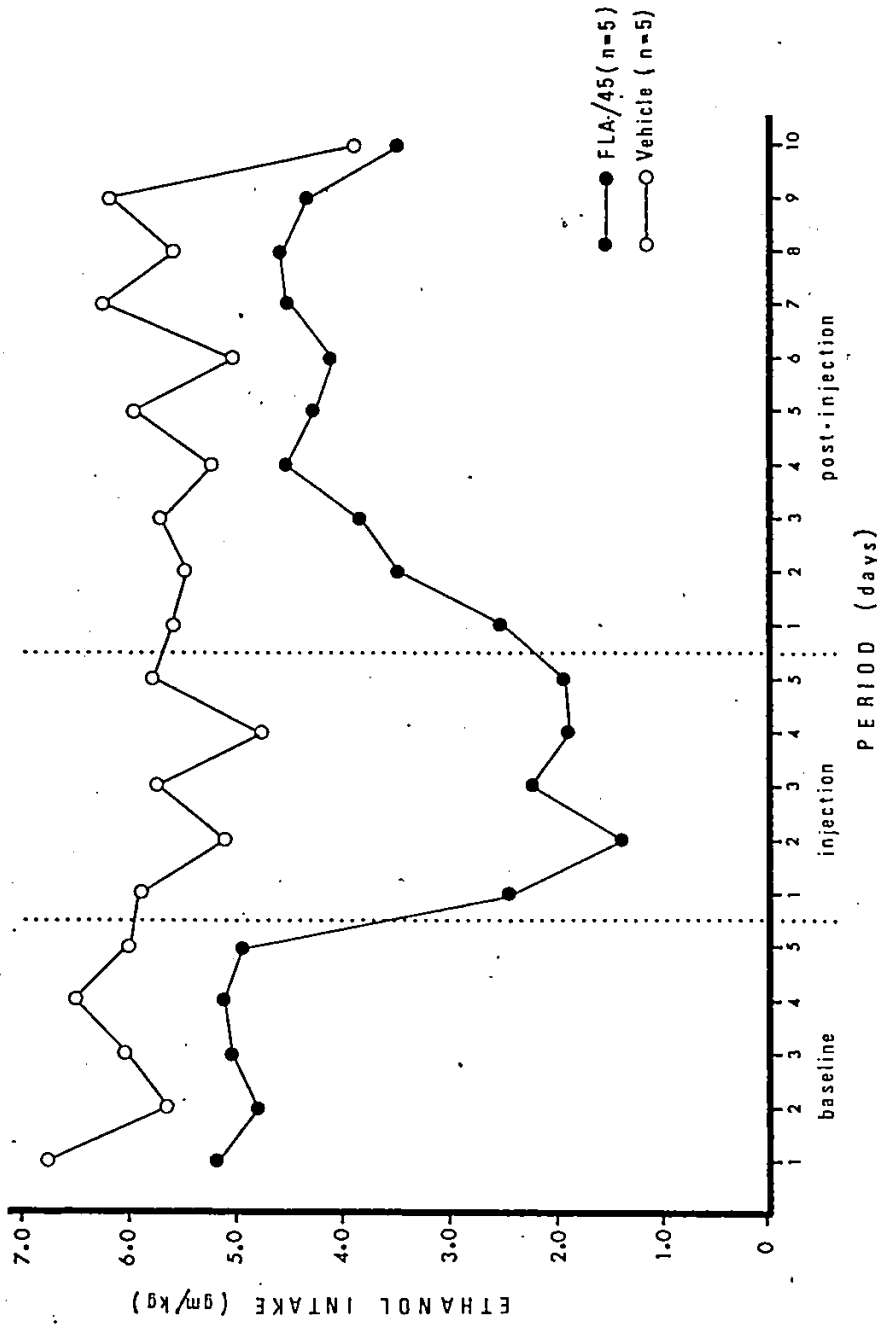


Figure 5. Ethanol intake in terms of mean absolute ethanol consumption (gm/kg) in rats treated with FLA-57.

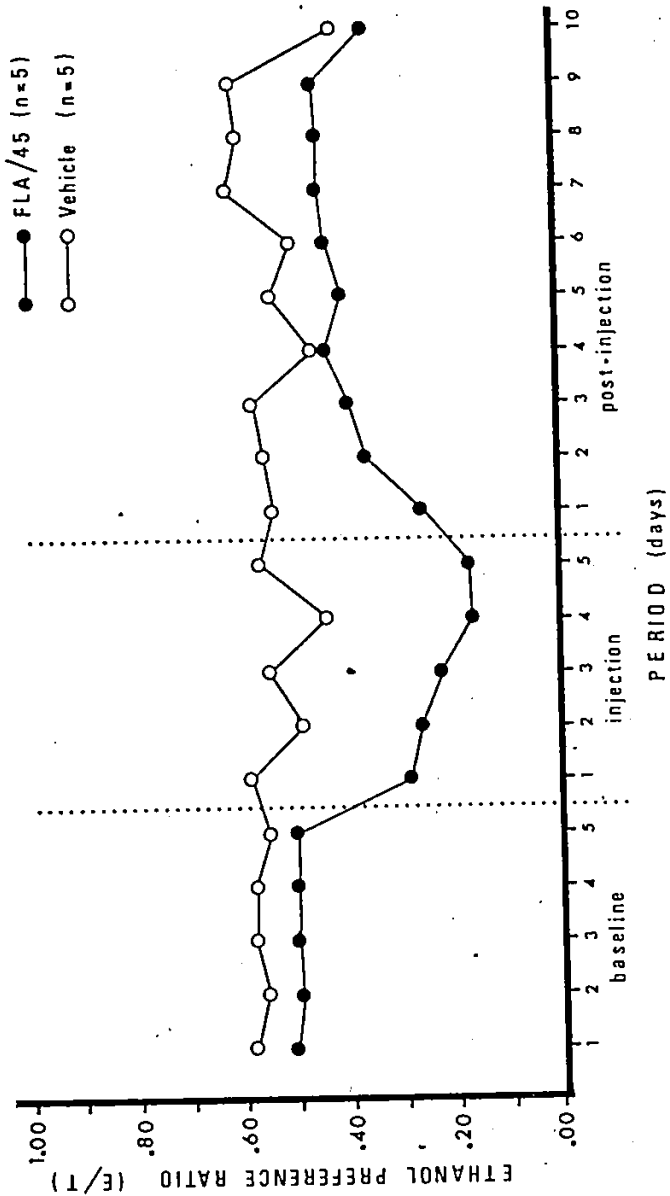


Figure 6. Ethanol consumption in terms of mean preference ratios (E/T) = volume of ethanol/volume of total daily fluid intake) in rats treated with FLA-57

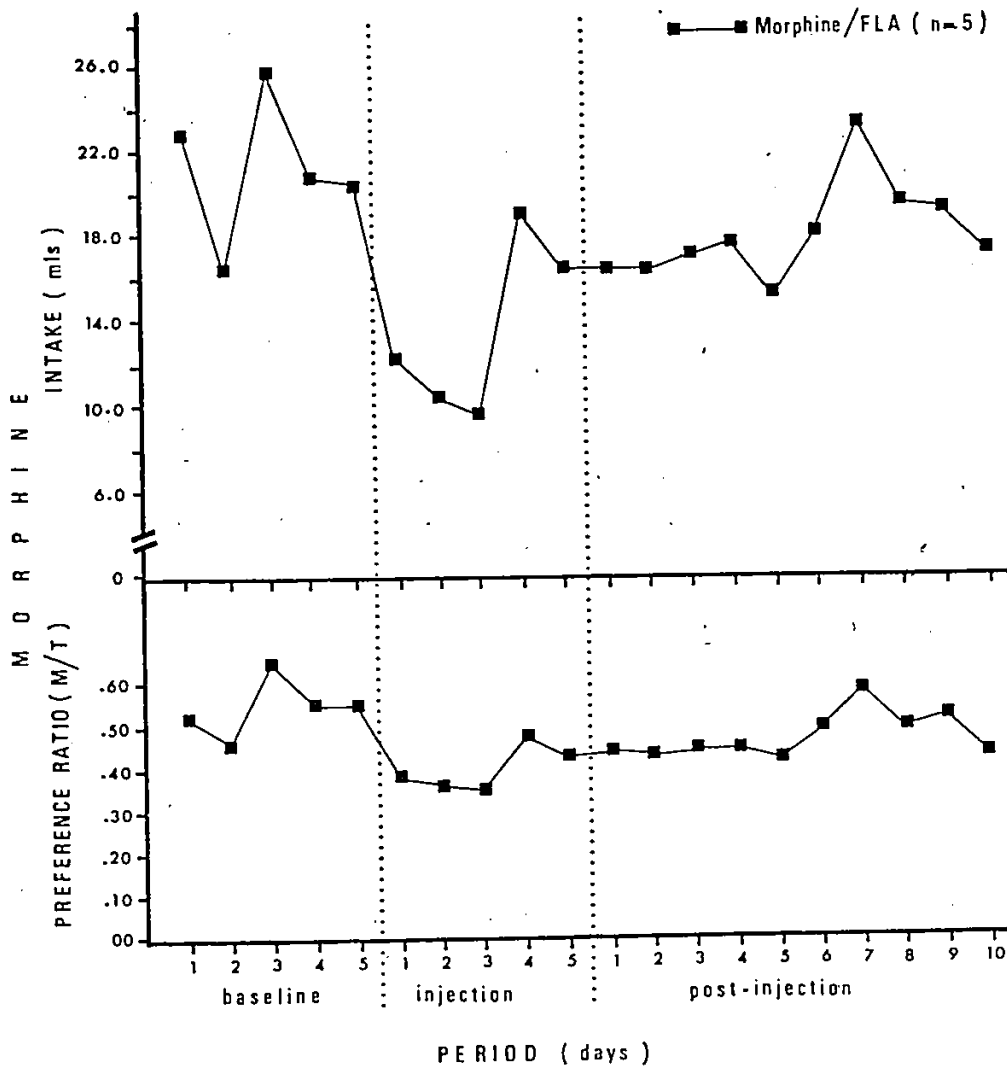


Figure 7. Morphine intake in terms of either volume consumed (mls) or preference ratios ( $M/T$  = volume of morphine solution/volume of total daily fluid intake) in rats treated with FLA-57.

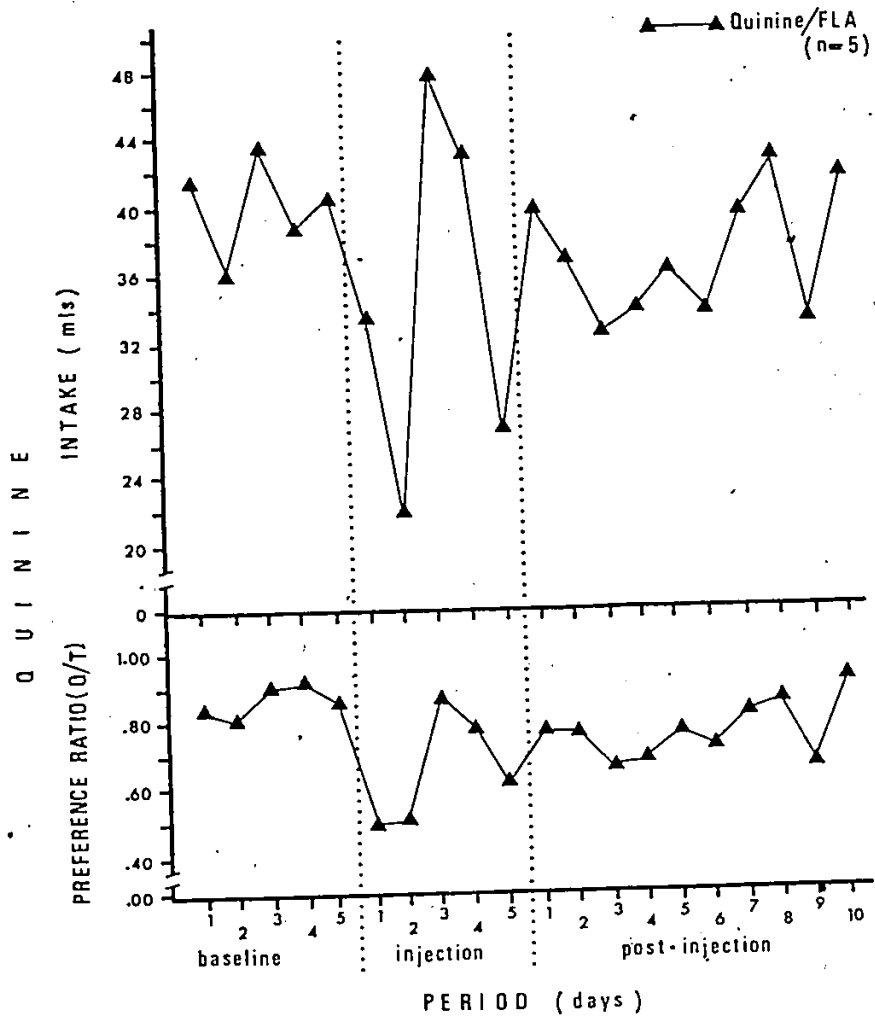


Figure 8. Quinine intake in terms of either volume consumed (mls) or preference ratios (Q/T = volume of quinine solution/volume of total daily fluid intake) in rats treated with FLA-57.



Table 2  
 Effect of Treatment with FLA-57 (45 mg/kg i.p.) on the Concentration  
 of Catecholamines in Whole Brain

Treatment	n	NOREPINEPHRINE		DOPAMINE	
		Levels (ng/g)	% control	Levels (ng/g)	% control
Controls	8	305.1	-	629.2	-
FLA-57 (45 mg/kg)	5	173.1*	56.7	727.4	115.6

\* $p < .001$

### Discussion

Although the catecholamines have previously been implicated in the mediation of the positive reinforcing properties of alcohol (e.g. Amit & Stern, 1971), the present findings suggest that norepinephrine alone plays an important role in the maintenance of alcohol self-administration. The dopamine-beta-hydroxylase inhibitor, FLA-57 has been shown to significantly reduce central norepinephrine levels without any measureable toxic side-effects. Furthermore, Lindros (Note 5) has determined that FLA-57, in the range of doses used in these experiments, produces no elevation of blood acetaldehyde levels following treatment with ethanol. Therefore, unlike the effect of disulfiram which is both a dopamine-beta-hydroxylase inhibitor and an aldehyde dehydrogenase inhibitor (Collier, 1972), the attenuation in alcohol self-administration demonstrated during treatment with FLA-57 would appear to be attributable to the concomitant depletion of norepinephrine. Since FLA injections had no consistent effect on the ingestion of a quinine-saccharine solution, the results obtained with the ethanol-drinking rats would not appear to be related to taste factors. However, this possibility cannot be entirely discounted based on the present data.

As a further test of the specificity of the ef-

fects of FLA-57 on ethanol consumption, a group of animals drinking a morphine-saccharine solution was subjected to treatment. It has been recently shown that U14,624 and diethyldithiocarbamate which are also inhibitors of dopamine-beta-hydroxylase, prevented the reacquisition of a conditioned response for intravenous injections of morphine (Davis, Smith & Khalsa, 1975). Furthermore, there are suggestions in the literature that some commonality between alcohol and morphine may exist (see Amit & Levitan, 1975). However, in the present experiment, FLA-57 produced only a small and non-significant attenuation in morphine consumption.

FLA-57 has been shown in the present experiments to be an effective agent in suppressing the reinforcing properties of ethanol via central norepinephrine depletions. Because of the apparent lack of toxicity, FLA-57 may be of considerable use in the development of effective treatment procedures for human alcoholics.

### Experiment III

Attempts have been made to suppress alcohol self-administration in both humans and laboratory animals by forced deprivation, substitution of alternate re-

sponses, aversive conditioning or pharmacological treatment (see Amit & Sutherland, 1975/76). However, the effectiveness of these treatment procedures proves to be minimal as evidenced by the high rate of relapse.

Since alcohol drinking may be considered a learned behavior (Conger, 1956; Kepner, 1964; Kingham, 1958), the most effective way of extinguishing alcohol-oriented behavior would be to allow the response to occur in the absence of reinforcement. However, because alcohol reinforces its own self-administration, it would be difficult to dissociate the response from the reinforcement.

The results of the first two experiments of the present study supported the hypothesis that noradrenergic mechanisms are involved in the mediation of the reinforcing properties of ethanol. By reducing norepinephrine levels, FLA-57 may have in effect blocked the reinforcing properties of ethanol resulting in the observed attenuation in ethanol self-administration. Since ethanol intake recovered soon after the FLA-57 injections were terminated, it suggests that this form of treatment only temporarily suppresses but does not extinguish ethanol-drinking behavior.

Conventional extinction procedures dictate that the original acquired response must be performed in the absence of reinforcement. In Experiment II, although alcohol reinforcement may have been effectively blocked by FLA-57 injections, the other component of the extinction procedure, namely performance of the learned response, was missing. In the present experiment an attempt was made to extinguish ethanol self-administration by forcing animals to drink ethanol during periods when its reinforcing properties were reduced by treatment with FLA-57.

#### Method

##### Subjects

Male Wistar rats (Canadian Breeding Farm Laboratories Ltd.) initially weighing 200-225 grams were housed individually in stainless steel cages in a room regulated for constant temperature and humidity and a 12-hour light cycle. Water and ethanol solutions were presented in calibrated Richter tubes mounted in front of the cages. Purina rat chow was always available ad libitum.

##### Procedure

Animals were screened to drink increasing concen-

trations of ethanol on an alternate-day free-choice paradigm similar to the procedure described in Experiment I. The final ethanol concentration used was 15% (v/v) and animals that failed to drink this solution were eliminated from the experiment. The alternate-day schedule was maintained for the duration of the experiment. After recording three days of stabilized baseline ethanol intake, animals were randomly assigned to either the experimental or control group.

During the first treatment period all animals were switched to a forced-choice alternate-day schedule where both Richter tubes were filled with the 15% (v/v) ethanol solution. On the intervening days only water was made available. Approximately 3 to 4 hours prior to each of the forced-choice presentations over 10 consecutive alternate days, the animals in the experimental group (FLA/30-E) were injected with FLA-57 (30 mg/kg i.p.). The FLA-57 solution was prepared as previously described in Experiment II(b) and was injected in a volume of 2 ml/kg. The control group (Vehicle-E) was subjected to the same procedure as the experimental group except that instead of FLA-57 injections they received equivalent volumes of the

vehicle. Following the first treatment phase, all animals were again given a free-choice between alcohol (15% v/v) and water for 10 consecutive alternate days. The second treatment period was identical to the first except that only 5 injections were administered. Free-choice ethanol intake was again recorded for 10 additional alternate days following the second treatment phase. Body weight and fluid intake were monitored daily throughout the experiment.

#### Biochemical assays

Additional animals were available for assay determination of whole brain catecholamine levels following injection of FLA-57 (30 mg/kg i.p.). Animals were decapitated 4 hours after injection and the extracted brains were immediately frozen on dry ice. Norepinephrine and dopamine levels were determined by fluorometric procedures (Chang, 1974; Shellenberger & Gordon, 1971).

#### Results

During the treatment phases, forced-choice ethanol consumption for both the experimental and control animals was equivalent to baseline levels. As can be seen from Figure 9 the absolute ethanol consumption (gm/kg) for the FLA/30-E group was markedly attenuated in the post-treatment periods while the Vehicle-E group maintained a relatively stable intake.

An analysis of variance and subsequent tests of simple main effects indicated that the FLA/30-E group differed significantly from the Vehicle-E group for the initial 5 days of the first post-treatment period ( $F(1,14) = 21.4, p < .001$ ) as well as for the last 5 days of the first post-treatment period ( $F(1,14) = 10.03, p < .01$ ). Although the difference in mean ethanol consumption between the groups was still significant during the first 5 days following the second treatment phase ( $F(1,14) = 5.89, p < .05$ ), in the final 5 days, ethanol intake did not differ ( $F(1,14) = 1.34, p > .05$ ). Additional tests of simple main effects of groups revealed that the Vehicle-E group did not alter its ethanol intake over time ( $F(4,56) = 0.34, p > .05$ ). On the other hand, the FLA/30-E group showed a significant attenuation in ethanol intake for each of the post-treatment periods when compared to the original baseline (post hoc Tukey tests,  $p < .01$  in all cases). Further post hoc analysis (Tukey tests) revealed that although the mean ethanol intake during the first 5 days immediately following the second treatment phase did not differ significantly from the last 5 days after the first treatment period ( $p > .05$ ) the increase in the final 5 days of the experiment was significant



( $p < .01$ ). It therefore appears that the second treatment phase did not have any effect in further attenuating ethanol self-administration.

Figure 10 represents the data expressed in terms of ethanol preference ratios (volume of ethanol/total daily fluid intake). Tests of simple main effects done following an analysis of variance indicated that the experimental group differed significantly from the control group in all phases of the post-treatment periods except for the final 5 day segment (Post-treatment I-Days 1-5:  $F(1,14) = 29.9$ ,  $p < .001$ ; Post-treatment I-Days 6-10:  $F(1,14) = 14.17$ ,  $p < .01$ ; Post-treatment II-Days 1-5:  $F(1,14) = 9.55$ ,  $p < .01$ ; Post-treatment II-Days 6-10:  $F(1,14) = 2.05$ ,  $p > .05$ ). Post hoc Tukey tests showed that the Vehicle-E group increased its preference for ethanol from the baseline to the final 5 days of testing ( $p < .05$ ). For the experimental group the first extinction treatment significantly lowered the mean preference level ( $p < .01$ ). However, the second treatment phase failed to produce any further attenuation ( $p > .05$ ) and in the final 5 days of the experiment ethanol preference had returned to baseline levels ( $p > .05$ ).

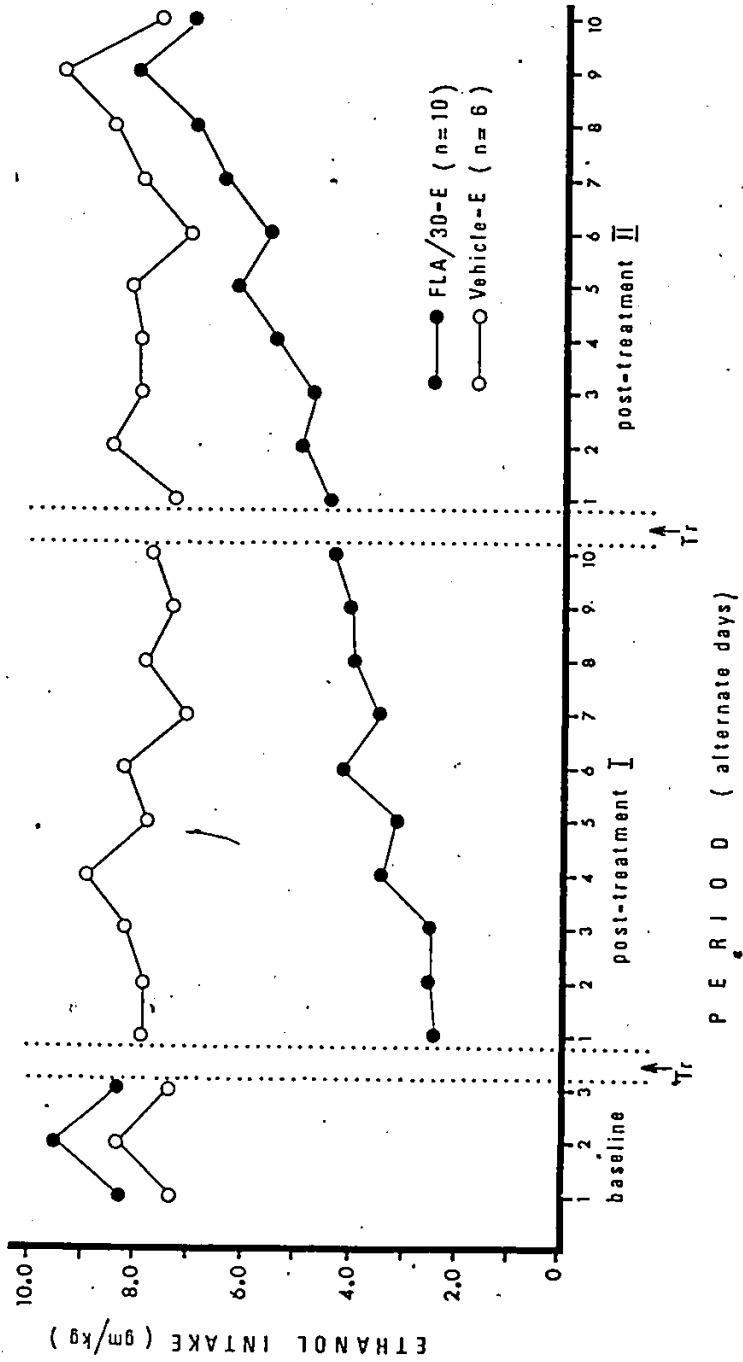


Figure 9. Ethanol intake in terms of mean absolute ethanol consumption in rats subjected to extinction treatments (Tr) with FLA-57.

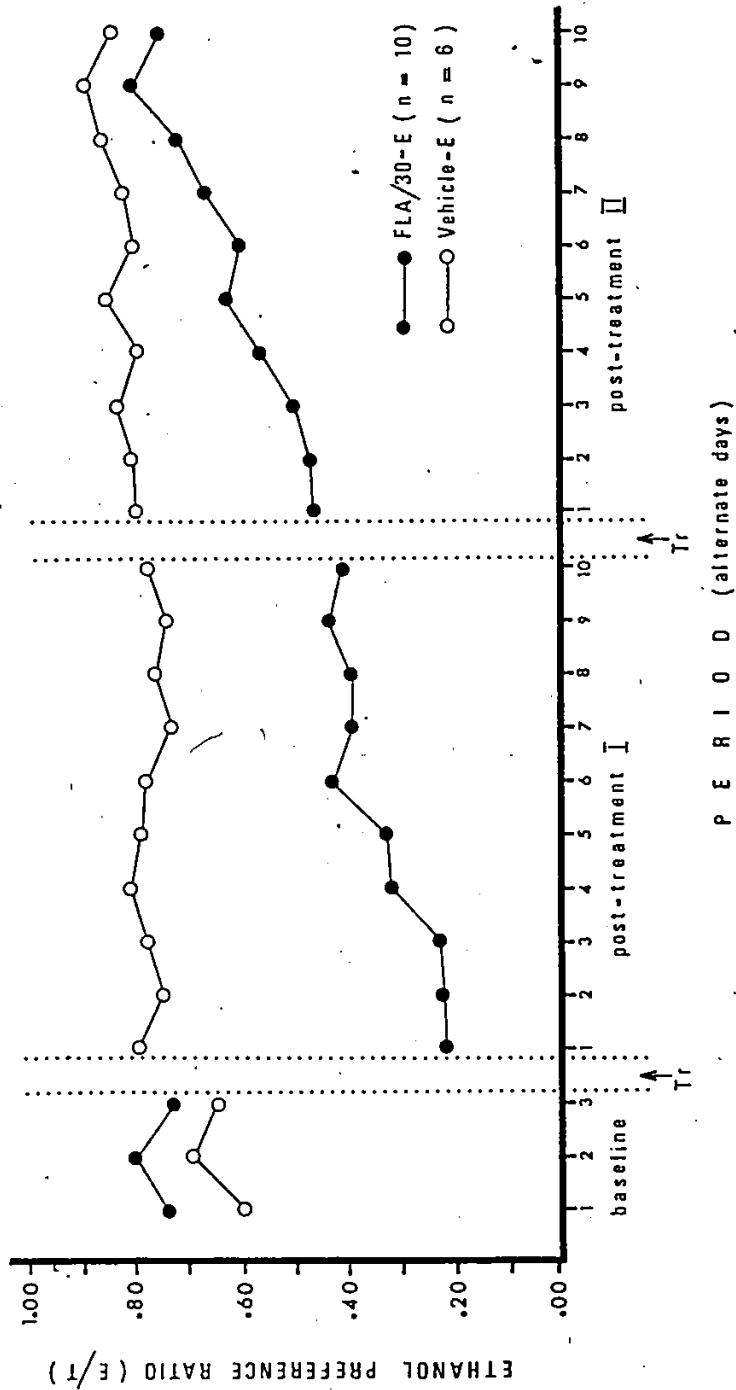


Figure 10. Ethanol consumption in terms of mean preference ratios (E/T = volume of ethanol/volume of total daily fluid intake) in rats subjected to extinction treatments (Tr) with FLA-57.

Table 3

Effect of Treatment with FLA-57 (30 mg/kg i.p.) on the Concentration of Catecholamines in Whole Brain

Treatment	n	NOREPINEPHRINE		DOPAMINE	
		Levels (ng/g)	% control	Levels (ng/g)	% control
Controls	13	335.1	-	756.2	-
FLA-57 (30 mg/kg)	7	201.9*	60.3	785.9	103.9

\*p < .001

### Biochemical assays.

Whole brain levels of the catecholamines following treatment with FLA-57 (30 mg/kg i.p.) are shown in Table 3. Dopamine levels were not affected ( $t(18) = .47, p > .05$ ) while norepinephrine levels were significantly reduced ( $t(18) = 7.93, p < .001$ ).

### Discussion

The results of this experiment suggest that it is possible to extinguish ethanol self-administration in laboratory rats. In Experiment II it was shown that by lowering central norepinephrine levels, FLA-57 was capable of reducing the reinforcing effects of ethanol. However; since intake was depressed following the initial injection of FLA-57, the subsequent injections could no longer be effective in producing extinction of the ethanol-drinking behavior. The procedure of the present experiment was more conducive to extinguishing ethanol self-administration since both criteria necessary for extinction were met. With the positive reinforcing properties of ethanol being inhibited by treatment with FLA-57, the experimental animals were forced to continue responding for ethanol. Consequently, it was demonstrated that in the

post-treatment periods ethanol intake and preference were significantly lower than baseline levels. The partial recovery of drinking that developed with the passage of time may be partly due to the relatively short duration of the treatment phases. Possibly by modifying the extinction treatment parameters, a more profound and permanent suppression of ethanol self-administration may be exhibited.

In addition to the primary reinforcing effects of drugs, conditioned reinforcers in the environment may often develop. Wikler (1973) proposed that relapse long after detoxification may be partly due to exteroceptive conditioned stimuli which the addict is confronted with when returned to his original drug-taking environment. In the development of therapeutic procedures for human alcoholics, FLA-57 may prove to be an effective agent since not only could it block the primary reinforcing properties of alcohol but it may also extinguish the effects of environmental conditioned reinforcers.

#### General Discussion

A number of studies have implicated catecholamines in the mediation of the positive reinforcing

properties of alcohol (Ahlenius et al, 1973; Amit & Stern, 1971; Amit et al, 1970; Wayner & Greenberg, 1972). The results of the present experiments suggest that central noradrenergic mechanisms play the dominant role in subserving the self-administration of ethanol in laboratory rats. In Experiment I it was shown that selective destruction of norepinephrine-containing neurons by 6-hydroxydopamine resulted in differential effects on the maintenance of alcohol consumption. Based on the speculation that the anti-alcoholic properties of disulfiram may be due to its effect on norepinephrine rather than on blood acetaldehyde levels (Collier, 1972), it was demonstrated in Experiment II that FLA-57, a non-toxic dopamine-beta-hydroxylase inhibitor, is capable of attenuating ethanol intake. Although ethanol self-administration was depressed during the injection period, the behavior was resumed relatively quickly when the FLA-57 injections were no longer administered. Therefore, although FLA-57 was shown to have the capacity to block the reinforcing effects of ethanol, the procedures used in Experiment II did not extinguish alcohol-oriented behavior. This failure to produce extinction of alcohol self-administration was attributed to the fact

that following the initial injection of FLA-57, alcohol drinking was significantly diminished thereby precluding the effect of subsequent injections on the extinction of the behavior. Therefore, in the final experiment, performance of the alcohol-drinking response was forced during treatment with FLA-57. True extinction of ethanol self-administration was produced as evidenced by the suppressed alcohol intake during the injection-free periods following treatment.

In the development of treatment procedures for human alcoholics the extinction paradigm described may be of critical importance. In one regard, with voluntary consumption of alcohol being depressed following FLA-57-induced extinction, it would be possible to introduce alternative incompatible behaviors necessary for the rehabilitation of the alcoholic. Furthermore, since FLA-57 can be administered to the alcoholic in his natural environment, not only will the response to the primary pharmacological effects of alcohol be extinguished but the effects of secondary reinforcing stimuli may also be eliminated. What remains to be determined is the extent to which cognitive factors may influence the effectiveness of these procedures with human alcoholics.



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