AN EXAMINATION OF THE ROLE OF CATECHOLAMINES IN THE SELF-ADMINISTRATION OF MORPHINE AND ETHANOL IN THE RAT

Robert George Meade

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Abstract

Robert George Meade

An Examination Of The Role Of Catecholamines In The Self-Administration Of Morphine And Ethanol In The Rat

Three experiments are presented which examined the effects of functional catecholamine depletions on self-administration of morphine and ethanol in rats. In Experiment 1, the effects of chronic catecholamine depletion on oral self-administration of morphine were examined. The method of depletion employed was the intracerebral administration of 6-hydroxydopamine. Working with more transient depletions, Experiment 2 attempted to delineate which of the two catecholamines might be more critically involved in morphine self-administration. Experiment 3 was similar to Experiment 2, however, here the effects of transient catecholamine depletions on oral self-administration of ethanol were examined. Both of these experiments utilized enzyme inhibition and receptor blocking as means of manipulating catecholamines. Experiment 3 was also concerned with the relative efficiency of dopamine-beta-hydroxylase inhibition and aldehyde dehydrogenase inhibition as anti-alcoholic-manipulations. The results support the notion that reducing the levels of catecholamines reduces the tendency to self-administer morphine and ethanol. No evidence was obtained to support the idea that one of the catecholamines

might be more important than the other in mediating drug self-administration behavior. The results of Experiment 3 suggest that inhibition of dopamine-beta-hydroxylase is a more important factor in reducing ethanol intake than is inhibition of aldehyde dehydrogenase.

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Traditionally, drug self-administration behaviour has been viewed as a concomitant of drug addiction. Operationally, this viewpoint sees drug self-administration as the response made by an addicted organism in order to avoid the drug abstinence syndrome (Seevers & Deneau, 1963). As the scientific study of both behavioral and physiological mechanisms of drug action has grown, evidence has come to light for which this theoretical formulation does not provide answers. In 1957, the World Health Organization attempted to clarify the issue by omitting the term addiction and substituting the concepts of physical dependence and psychic dependence. A secondary goal of this redefinition was a reduction in the moralistic and mentalistic loading which had come to be associated with the term addiction. While this clarification was somewhat useful, the WHO definitions of physical and psychic dependence (WHO Expert Committee, 1957) were still negative definitions in that they were built around the concept of the abstinence syndrome. The result of this was that an organism could be shown to be dependent on a drug only by observation of the behaviour of the organism upon withdrawal of the drug. More recently, an alternative concept which has been put forward is that of behavioral dependence. Behavioral dependence is defined as the tendency of the organism to self-administer a drug and is measured by direct observation of self-administration behavior (Thompson & Ostlund, 1965). The usefulness of the concept of behavioral dependence may be seen more clearly after reviewing some of the animal drug self-administration

data of the last thirty years.

Traditionally, three classes of drugs have been thought to produce physical dependence. They are opiates, barbiturates, and alcohol. The present thesis is concerned with self-administration of morphine (opiate) and alcohol and the review of the literature will be appropriately restricted. It is important to note that the current investigation is concerned with the biological factors involved in drug self-administration. Therefore, it is important to minimize the effect of non-biological variables such as social learning. Since it is assumed that the degree and complexity of social learning is greatest in the human species, discussion of drug self-administration behavior will be restricted to that of infrahuman species.

One of the earliest reports of drug-seeking behavior in animals was that of Spragg in 1940. Spragg presented detailed descriptions of chimpanzees leading the experimentor to the injection room, struggling to get into the room, and eagerly mounting the apparatus where the injections were given. In an attempt to measure the strength of this behavior, Spragg devised a test situation in which the animals were required to choose either a black or a white box, one containing palatable food, the other a morphine injection. The subjects were run through a series of 48 trials, one per day, under four experimental conditions - 1) hungry and morphine-deprived, 2) hungry and morphine-satiated, 3) food-satiated and morphine-deprived, 4) food-satiated and morphine-satiated. It was hypothesized that

the subjects would choose the morphine-containing box under conditions 1 and 3, and the food-containing box under conditions 2 and 4. The results were highly significant and in accord with the experimental hypothesis. Spragg concluded that a clear-cut desire for morphine injections had been demonstrated in morphine-dependent chimpanzees.

In an effort to demonstrate purposeful, drug-seeking behavior in a species other than primates, Beach (1957) devised a test using rats in a Y-maze in which the goal boxes were differentiated by color. In these experiments, naive animals were tested for goal-box preference in the maze prior to receiving morphine. Once preference had been determined, the animals received daily saline injections in the preferred box and morphine injections in the non-preferred box. The subjects were then re-tested for goal-box preference and the results showed that animals switched preference in response to the drug injections. These results held true both for animals whose last injection had been several hours prior to testing and for those who had been injected immediately prior to testing. Further, animals who were withdrawn from the drug and re-tested three weeks later continued to demonstrate a learned preference for the morphine goal-box.

Although the above studies may be interpreted as demonstrating drug-seeking behavior and therefore, a tendency to self-administer drugs, much more conclusive evidence was offered by Weeks and Collins in 1968. These investigators maintained rats for as long as three months

with chronic indwelling venous catheters. After establishing physical dependence by administering an ascending series of passive injections of morphine, the animals were given the opportunity to self-inject morphine by performing a lever-press response. Subjects maintained a relatively constant intake of the drug on fixed-ratio schedules as high as FR-75. At higher ratios, intake gradually declined until insignificant amounts of morphine were ingested at ratios around FR-180. The animals demonstrated a somewhat imprecise regulation to changes in dose level and non-contingent presentation of other opioids, orally or intravenously. Infusion of nalorphine, a morphine antagonist, caused an increase in responding for morphine. Rats who were withdrawn from the drug for a period of four weeks showed a rapid return to pre-withdrawal levels when returned to the self-administration situation.

In 1968, Nichols reported a procedure whereby rats could be induced to voluntarily consume a solution of morphine in tap water. Physical dependence, induced by passive morphine injections combined with twenty-four hour periods of fluid deprivation, produced substantial morphine drinking. However, in subsequent choice tests of morphine and tap water, mean morphine intake declined sharply. The results of this study may be taken to mean that although the animals were physically dependent, the aversive taste properties of the drug solution were sufficient to cause the animals to discontinue self-administration of the drug.

While the above studies clearly demonstrate the pur-

poseful intake of drugs by infrahuman species, they contain an important common factor - the existence of physical dependence. Physical dependence is herein defined as a set of symptoms which include piloerection, tremors, abdominal cramps, lacrimosis, etc. and which occurs after drug administration has been terminated. In addition, strong cravings for the drug occur when the drug is no longer available to the organism (Eddy, Halbach, Isbell, & Seevers, 1965). These studies then, do not provide evidence that drug self-administration behavior is maintained in infrahuman species without the existence of physical dependence.

Several studies have separated self-administration from physical dependence by demonstrating the existence of one of these phenomena without the other, in the same organism. Woods and Schuster (1968) reported the maintenance of stable lever-pressing rates for fifteen days by monkeys self-administering morphine in non-dependence producing doses. Their subjects were prepared with chronic jugular catheters and were trained to lever-press for food and water. Subsequently, they were given access to the drug for four one-hour periods daily, signalled by a light. It is interesting to note that during the extinction phase of the experiment, when saline was substituted for the morphine solution, responding by those animals who had been receiving non-dependence producing doses quickly tapered off while those animals who had been self-administering much

larger doses showed a marked increase in response rate, lasting about three days. Deneau, Yanagita, and Seevers (1969) reported that naive monkeys will initiate and maintain intravenous self-administration of morphine, cocaine, and amphetamine while this behavioral pattern is not seen with saline or nalorphine, a morphine antagonist.

Stolerman and Kumar (1970) attempted to induce in rats a preference for oral solutions of morphine over water. Preference for a solution is said to occur when more than 50% of total fluid intake is of that solution. Their subjects were given access to fluids for seven hours per day. Every third day, the animals had a free choice between morphine solution and water, while on the two intervening days, only morphine solution was available. While preference for the morphine solution developed after 2-3 choice trials, they found no differences in latencies to develop preference nor in proportion of morphine to total fluid intake between naive animals and those who had received a series of twenty morphine injections prior to testing.

Ratcliffe (1972) reported that rats who had been exposed to ethanol (ETOH) solutions as their only source of fluid for seven weeks showed no subsequent preference for ETOH solutions over water in a free choice situation. The rats had been exposed to a series of ascending concentrations of ETOH and cessation of the forced-choice intake resulted in hyper-irritability and increased susceptibility to audiogenic seizures, both of which are considered to be

integral components of the alcohol abstinence syndrome. Whereas the animals had the opportunity of alleviating this syndrome by increasing ETOH intake, they maintained consumption at about 20% of total fluid intake. Both Freund (1969) and Goldstein & Pal (1971), were able to induce physical dependence on ETOH in mice, as evidenced by the existence of the abstinence syndrome when the drug was withdrawn. Freund did so by utilizing a diet which contained ethanol, while Goldstein & Pal forced mice to inhale the drug in vapor form. Both studies report that the existence of physical dependence, although manifested by withdrawal symptoms, was not accompanied by increased voluntary oral intake of the drug.

Approaching the question of separation of self-administration and physical dependence from the opposite angle, Amit & Stern (1971) induced a preference for oral solutions of ETOH over tap water, using electrical stimulation of the lateral hypothalamus (LH). After rats had been preferring ETOH for up to 50 days, the drug was withdrawn. With the exception of a small degree of weight loss, possibly accounted for by the caloric factors in ETOH, no signs of stress or abstinence syndrome were observed. In this study, the authors were unable to differentiate the drug-abstinent animals from controls. It is worth noting that in a previous study, Amit, Stern, & Wise (1970) reported a difference between ETOH preferrers and controls during the withdrawal phase.

In a recent report, Begleiter (1974) details the effects

of physical dependence on ETOH on subsequent oral self-administration of the drug. Begleiter intubated one group of rats, and in this manner, was able to force them to ingest ETOH. A second group was maintained on a liquid diet to which ETOH was added in increasing amounts. Although cessation of the drug administration resulted in withdrawal symptoms, there was no change in the ETOH preference ratio, when measured against pre-dependence baselines. These results held true over a range of ETOH concentrations from 6% to 20%.

The above evidence suggests, I believe, at least a partial separation of the phenomena of physical dependence and drug self-administration. Since it has been shown that drug self-administration is initiated and maintained without the existence of physical dependence, it must be assumed that this behavioral response is being positively reinforced. Further, since no signs of the abstinence syndrome are evident, it can be concluded that such reinforcement does not come from relief of withdrawal symptoms. Clearly, relief from physical illness may be construed as being positively reinforcing. It is somewhat more difficult to explain the reinforcing effect of drugs in cases where physical illness is not a factor i.e. where the organisms being tested manifest no signs of physical dependence.

It is not the purpose of this thesis to investigate the physiological substrates of reward or drive, therefore for purposes of discussion, reward will be herein defined as a neural event which an organism will work to reproduce.

One example of such an event is that of electrical stimulation of brain tissue (Olds & Milner, 1954). The phenomenon of rewarding electrical stimulation of the brain (ESB) is demonstrable by the implantation of a chronic electrode into specific sites of the brain. Subsequently, the subject is placed in an operant learning paradigm with the operant response being reinforced by electrical stimulation through the electrode. The typically high rates of responding reported suggest that such stimulation is positively reinforcing. While the neurochemical basis of this behavior has not been conclusively established, the existing evidence suggests the involvement of the putative neurotransmitter substances - the catecholamines (CA). The known brain catecholamines are norepinephrine (NE) and its precursor, dopamine (DA). Evidence exists which suggests that both support self-stimulation (SS).

Much of the evidence supporting NE as the transmitter substance involved in SS has come from Stein and various collaborators. Wise & Stein (1969) implanted electrodes in the LH of rats and after recovery, trained them to lever-press for ESB. They then injected the animals with DBH inhibitors, blocking the final step in the biosynthesis of NE. Both of the inhibitors that they used (Disulfiram & DEDTC) reduced responding to less than 20% of control level. Subsequent intraventricular infusions of NE reversed this behavioral suppression to a large extent, within several minutes. Stein & Wise (1969) implanted electrodes in both amygdaloid and lateral hypothalamic sites and push-pull

cannulae downstream from the electrodes. After training rats to self-stimulate, they injected radioactive NE tracer into the lateral ventricle, then measured the level of radioactivity in the perfusate obtained via the push-pull cannula. They found an increase in the amount of radioactivity during rewarding ESB, while non-rewarding ESB produced no such increment. They concluded that the release of NE from LH sites is at least partially responsible for the rewarding aspect of ESB in these loci. Further support for this theory later came from Ritter & Stein (1974) who obtained reliable rates of SS from sites in the locus coeruleus of rat brains. The cell bodies in the locus coeruleus were shown by Ungerstedt (1971) to be almost exclusively noradrenergic and are thought to be the source of the dorsal noradrenergic bundle which runs through the LH. Attempts to implicate another monoamine, serotonin (5-HT) in self-stimulation have produced results which are less clear than those obtained with the catecholamine manipulations.

Poschel & Ninteman (1963) have argued that NE is the neurotransmitter involved in SS of the lateral hypothalamus. After implanting electrodes in this area of rat brains, they allowed the animals time to recover, then trained them to lever-press for ESB. When the animals achieved reliable rates of this behavior, they were injected with various monoamine oxydase inhibitors (MAOI). Monoamine oxydase is one of the enzymes essential to the biodegradation of CA. Inhibition of this enzyme therefore functionally inhibits this biodegradation. [illegible] so interfering with the

synthesis-degradation balance of CA, the levels of catecholamines are increased. Poschel & Ninteman demonstrated greatly enhanced SS rates and concluded that, since NE had been shown to be associated with excitatory functioning, and since the LH is an area rich in NE, the increased rate of responding was due to increased levels of NE.

Lippa, Antelman, Fisher & Canfield (1973) attempted to delineate the neurochemistry of SS at lateral hypothalamic sites. They reported that rats who were chronically depleted of 90% of brain NE fully recovered baseline SS rates within seven days. These depletions were accomplished by means of intracranial administration of 6-hydroxydopamine (6-OHDA). Since it could be argued that only 10% of brain NE is necessary to support SS behavior, they subsequently administered phentolamine, an adrenergic receptor blocking agent. This procedure further reduced functional utilization of NE. This treatment had no effect on animals who had recovered their pre-6-OHDA rates of responding. To investigate this question further, they ran a second experiment which examined the effects on SS rates of 1) phentolamine, 2) haloperidol - a dopamine receptor blocker, and 3) FLA-63 - an inhibitor of dopamine-beta-hydroxylase (DBH), the rate-limiting enzyme in the conversion of dopamine to norepinephrine. While neither of the NE manipulations had a significant effect on SS, response rates in the dopamine-blockaded group were reduced to 46% of mean SS rate for the three days preceding administration of the drugs.

Cooper, Cott, & Breese (1974) also produced compelling evidence for the involvement of DA in SS elicited from sites in the LH. Using 6-OHDA, in conjunction with various other pharmacological agents, they reduced brain NE content in rats by 90% with no significant changes in SS responding. Response decrements of 46% were seen however in animals whose brain DA was reduced by 70%. It should be noted that, even in these animals, responding returned to normal within one week of treatment. Finally, German and Bowden (1974), in an extensive review, concluded that, "1) intracranial self-stimulation exists in numerous species from fish to men; 2) the brain areas supporting intracranial self-stimulation are similar across species and would appear to correspond to CA systems as mapped in the rat and human fetus; and 3) in man, intracranial self-stimulation of such areas as the septal area and the caudate nucleus is accompanied by pleasurable responses, and those areas contain portions of CA systems."

Reasoning that drug self-administration and SS responding are both subserved by the same neurochemical system, the next logical area of investigation should be the effect of catecholamine manipulations on drug self-administration. Several investigators have examined this relationship, with interesting and encouraging results. Pozuelo & Kerr (1972) trained monkeys to self-administer morphine intravenously by lever-pressing. Once stable responding had been established, they tested the effects of alpha-Methyl-para-Tyrosine

(aMpT) on this behavior. aMpT inhibits tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of CA, thereby reducing CA levels. This treatment completely suppressed self-administration behavior, however when administration of aMpT was discontinued, self-administration responding returned to its pre-treatment level. While this study does not clearly demonstrate which of the catecholamines was critical in producing the results, Neff & Costa (1966) have demonstrated that aMpT reduces brain dopamine earlier and more dramatically than brain NE. Coupled with the report that lesions aimed at mainly dopaminergic pathways reproduce the effects of aMpT on morphine self-administration (Kerr & Pozuelo, 1971), these authors favor a hypothesis involving dopamine.

Glick, Zimmerberg, & Charap (1973) investigated the effect of aMpT administration on initial liability to consume oral morphine solution by rats. Beginning three days before morphine was made available as the only source of fluid, a regimen of two daily aMpT injections was begun. A control group received saline injections. While the groups showed no difference in water consumption, morphine intake was significantly reduced for the aMpT treatment group throughout the nine days that it was available. To eliminate the possibility that the results were due to gustatory factors, a similar experiment was run with an equiaversive solution of quinine being substituted for morphine. They found no difference in consumption of the

quinine by the two groups.

Schwartz & Marchok (1974) investigated the neurochemical substrate of morphine reward using the Y-maze procedure described by Beach (1957). Like Beach, they found that morphine injections associated with the initially non-preferred goal-box reversed this preference. However, they also found that groups treated with aMpT, 6-OHDA, or haloperidol did not show the reversal of preference. Depleting only NE, by using a DBH inhibitor (DEDTC) had no appreciable effect. They concluded that interference with dopamine activity reduced the behaviorally reinforcing effects of morphine.

Amit, Corcoran, Amir, & Urca (1973) investigated the effect of two-stage, bilateral hypothalamic lesions on oral intake of morphine solution by rats. One week after induction of the second stage of the lesions, the animals received an ascending series of morphine injections which lasted for sixteen days. Beginning on the day of the last injection, the animals were presented with a morphine solution as their only source of fluid for five days. While those animals who had lesions in the ventral portion of the LH demonstrated almost total reluctance to drink the solution, both the sham-operated and the dorsally lesioned groups drank. Since the animals showed no reluctance to drink a quinine solution, it was concluded that taste was not the determining factor. Further, since the ventral lesions were shown, a posteriori, to be located in the area

through which catecholaminergic fibers pass, the authors concluded that interruption of these fibers blocked the reinforcing efficacy of morphine.

Davis & Smith (1973) studied the effect of aMpT on the reinforcing properties of morphine in rats. After teaching rats to lever-press for intravenous infusions of morphine at a low dose, the response was extinguished. The animals were then divided into two groups, one of which received injections of aMpT, the other received injections of saline. The rats were then reintroduced into the operant chamber and tested for re-acquisition of the response. During this testing session, the total responses of the aMpT group were significantly less than those of the saline group. In order to ensure that the results of this experiment were not due to motor debilitation, the same authors ran a second experiment in which rats were trained to perform a lever-press response. Following this, one group received injections of aMpT, while the other group was injected with saline. Both groups were then subjected to 100 non-contingent pairings of a buzzer with intravenous infusions of morphine. To ensure that the drugs had been eliminated from the organism, the animals were tested several days later for re-acquisition of the lever-press response, using both saline infusions and the buzzer as reinforcers. It was reasoned that if the aMpT had blocked the reinforcing properties of morphine, the buzzer would have acquired the properties of a conditioned reinforcer for the saline

group, but not for the aMpT group. In fact, the saline group did perform significantly better than the aMpT group.

It is worth mentioning that Friedler, Bhargava, Quock, and Way (1972) have reported that 6-hydroxydopamine pretreatment enhanced the morphine abstinence syndrome in rats, as measured by weight loss and withdrawal jumping. This evidence argues against the hypothesis that CA-depleted subjects voluntarily reduce drug intake because the severity of the abstinence syndrome is ameliorated.

That catecholamines and ethanol, or its primary metabolite, acetaldehyde, may interact in the CNS has been studied by several investigators, on the biochemical level. Davis & Walsh (1970) have demonstrated in vitro that the presence of ETOH or acetaldehyde interferes with the metabolism of the CA, dopamine. This interference is characterized by the production of pharmacologically active metabolites called alkaloids. The authors argue that this metabolic aberration may be, in fact, the biochemical basis of physical dependence on alcohol.

Cohen & Collins (1970) also offered biochemical evidence of a relationship between ETOH and catecholamines. They demonstrated, in adrenal tissue, the formation of alkaloids from norepinephrine and acetaldehyde. Since these alkaloids are structurally similar to CA, the authors suggest the possibility that they may function either as false transmitters or as CA receptor blockers.

Amit & Stern (1972) have hypothesized that the

development of ETOH preference by rats during 30 days of electrical stimulation of the LH may be due to increased turnover of NE resulting from stimulation of the medial forebrain bundle. More recently, Sinclair (1974) has shown that, in both acute and chronic conditions, oral intake of ETOH by rats is reduced by injections of morphine. Since water consumption is increased, the effect is apparently not due to disruption of the fluid regulatory system. Since the acute animals were clearly not dependent, this eliminates the possibility that the analgesic action of morphine blocked the production of abstinence symptoms, so that the animals could withdraw themselves from ETOH. While the explanation of these results remains unclear, a relationship between the two drugs is clearly demonstrated.

The accumulation of the above evidence suggests a possible role for catecholamines in the neurochemical mediation of drug self-administration behavior. The following set of experiments was designed to further investigate this role. The first experiment examines the effect of permanent catecholamine depletions on oral intake of morphine by rats. The second and third experiments examine the effects of transitory functional depletion of CA on oral intake of both morphine and ethanol.

Method

Experiment 1

While several techniques for functionally depleting CA are available (electrolytic lesions, radio-frequency lesions, enzyme inhibition, receptor blocking, administration of 6-OHDA), the administration of 6-OHDA offers some advantages which the others do not. Pharmacologic agents, injected systemically, produce changes in peripheral adrenergic functioning as well as the intended CNS alterations. Due to the proliferation of adrenergic nerve terminals in the brain, it is virtually impossible to eliminate adrenergic functioning in the CNS using electrolytic or radio-frequency lesions. 6-OHDA, however, appears to have a selective neurotoxic effect on CA-containing nerve terminals in the brain (Ungerstedt, 1968). Although the mechanism whereby 6-OHDA produces destruction of nerve endings and resulting axonal degeneration of CA-containing neurons is unknown, it has been shown to have no effect on brain levels of other monoamines (Uretsky & Iverson, 1970). It has also been shown that dopamine-containing neurons are more resistant to the effects than are norepinephrine-containing neurons (Uretsky & Iverson, 1970; Breese & Traylor, 1970). Finally, the effects of 6-OHDA are long-lasting; the duration of the depletions having been determined to be at least 78 days (Breese & Traylor, 1970).

It is worth emphasizing that recently, several investigators have criticized this technique and reported damage to non-CA containing tissue by this neurotoxin

(H.C. Fibiger, personal communication).

Twenty-five male Hooded rats were obtained from Canadian Breeding Farms Limited. Upon arrival, the weights of the animals were recorded as ranging from 250 g. to 300 g. The animals were housed individually in stainless steel cages, with Purina dry lab chow available ad libitum. The subjects were allowed four days during which they were handled briefly by the experimentor each day. On the fifth day, a chronic indwelling stainless steel cannula was stereotactically implanted in the lateral ventricle of each animal. The cannulae were constructed of 22-gauge stainless steel tubing, the upper portion of which was embedded in plastic. The plastic was threaded to allow the attachment of either dummy inner cannulae or inner infusion cannulae. The stereotaxic coordinates were 1.5 mm. lateral to the mid-saggital suture, 1.0 mm. posterior to bregma, and 3.0 mm. ventral to the inferior skull surface. Surgery was performed under sodium pentobarbital (50 mg. $kg.^{-1}$) and chloral hydrate (300 mg. $kg.^{-1}$) anaesthesia. As determined by the surgery schedule, animals were allowed a minimum of seven days to recover. During the recovery period, they were handled briefly each day. On the seventh and eighth post-surgery days, the animals received intraventricular infusions according to the following program:

GROUP	DOSE OF 6-OHDA	VOLUME & VEHICLE
I (n=6)	350 µg.	10 µl. of 0.1% ascorbic acid
II (n=4)	350 µg.	10 µl. of 0.1% ascorbic acid
III (n=5)	350 µg.	10 µl of 0.1% ascorbic acid
IV (n=5)	- -	10 µl. of 0.1% ascorbic acid
V (n=5)	250 µg.	10 µl. of 0.1% ascorbic acid

The 6-OHDA was given in the form of hydrochloride salt and all doses are calculated as this salt of the drug. Doses in the above table refer to each of two infusions. Infusions were delivered in 30 seconds through a 28-gauge inner cannula. The 6-OHDA solution was mixed just prior to the infusion and was delivered from a B-D numbered glass syringe via an infusion pump. Following the first infusion, the following fluids were made available to the subjects in a forced choice condition. Groups I, IV, and V received a solution of morphine hydrochloride in tap water (0.5 mg. $ml.^{-1}$), while Group II received tap water and Group III was presented with a solution of quinine sulphate in tap water (0.25 mg. $ml.^{-1}$). All fluids were presented in standard type glass Richter tubes. Fluid intake was measured daily for five consecutive days prior to the first infusion and for five consecutive days thereafter.

Experiment 2

Thirty-two male Hooded rats were obtained from Canadian Breeding Farms Limited. The animals were housed individually in stainless steel cages with Purina dry lab chow available ad libitum. The subjects were allowed one week during which they were handled briefly by the experimentor each day. At the end of this period, the range of the subjects' weight was between 350 g. and 400 g. To establish a baseline of fluid consumption, water intake was then measured for five days. All subjects had continual access to water which was presented in Richter tubes. Following this period, a 0.05% morphine solution was substituted for tap water and morphine intake was recorded for fourteen days. At the end of this period, subjects who were not consuming an amount equal to or in excess of their daily H_2O baseline intake were omitted from the experiment. The remaining animals were randomly distributed into four groups.

Group I (n=4) received intraperitoneal (IP) injections of FLA-63 (20 mg. $kg.^{-1}$). The FLA-63 was dissolved in acetic acid, then bufferred to pH 7.00 with tricine. FLA-63 is an inhibitor of dopamine-beta-hydroxylase, which is the rate-limiting enzyme in the conversion of dopamine to norepinephrine. Administration of low to moderate doses of this compound results in temporarily decreased levels of NE, while DA remains unaffected. Group II (n=7) received injections of haloperidol (5 mg. $kg.^{-1}$). The haloperidol was obtained in injectable form and measurement showed the pH to be 7.00. This compound is known to temporarily block

DA receptors and thereby functionally deplete dopamine. Group III (n=6) received injections of RO4-4602 (400 mg. $kg.^{-1}$). Due to the rapid metabolism and elimination of this compound in the organism, subjects in this group were injected twice daily at twelve hour intervals. The drug was dissolved in Ringer's solution and the pH was verified at 7.00. RO4-4602 is an inhibitor of dopa decarboxylase, the rate-limiting enzyme in the conversion of dopa to dopamine. Administration of this compound results in temporarily decreased levels of brain DA, with no known effect on other CA. Group IV (n=5) received daily IP injections of acetic acid which was bufferred to pH 7.00 with tricine. A sufficient amount of Ringer's solution was added to equate the injection volume of this group to that of the other three groups.

Experiment 3

As well as examining the effects of catecholamine manipulations on ETOH self-administration this experiment was designed to probe another question. Disulfiram is used as an anti-alcoholic agent in humans. It is known to inhibit both dopamine-beta-hydroxylase and aldehyde dehydrogenase. The relative contribution of the inhibition of each of these substances to the depression in alcohol intake is unknown. Experiment 3 was designed to explore this question by inhibiting a) dopamine-beta-hydroxylase, b) aldehyde dehydrogenase, c) both of these enzymes in rats who were already consuming substantial amounts of ETOH.

Forty male Wistar rats were obtained from Canadian Breeding Farms Limited. The animals were approximately 90 days old. Upon arrival, the animals were housed individually in stainless steel cages with Purina dry lab chow available ad libitum. The subjects were allowed four days during which they were handled briefly by the experimentor each day. Beginning on the fifth day, the subjects were screened for individual concentrations of ETOH by giving them a free choice between ascending concentrations of ETOH and tap water. The free choice situation was presented on alternate days. On non-alcohol days, subjects were presented with two tubes containing tap water. All fluids were presented in Richter tubes. All subjects were initially presented with a choice between tap water and a 3% (v/v) ETOH solution. Subsequent increments were of 2%. If an animal consumed more than 3 mls. of the ETOH solution, the concentration of the ETOH solution

was increased by 2% for presentation on the next alcohol day. When an animal consumed less than 3 mls. of ETOH solution for three consecutive alcohol days, this concentration was then maintained for that subject in the free choice situation.

The highest ETOH concentration used was 30% (v/v). When individual ETOH concentrations had been determined for all animals, alternate day presentation was continued for two weeks prior to treatment.

The animals were then randomly divided into four groups (n=10) and each group received a series of five IP injections. All injections took place on alcohol days. Group I received injections of Disulfiram (25 mg. $kg.^{-1}$). This compound was dissolved in Ringer's solution. Disulfiram is a commonly used anti-alcoholic agent. It is thought to produce no noticeable behavioral effects except when administered in conjunction with ETOH. In such a situation, the inhibitory action of Disulfiram on the enzyme, aldehyde dehydrogenase, causes an accumulation of acetaldehyde. In humans, this accumulation results in an acute period (30 min.) of extreme physical illness. Disulfiram is also known to inhibit DBH. Group II received injections of FLA-63 (17.5 mg. $kg.^{-1}$). FLA-63 is a DBH inhibitor which does not produce the dramatic elevations in blood acetaldehyde which are seen after Disulfiram administration (K. Lindros, personal communication, 1975). FLA-63 was prepared for injection as in Experiment 2. Group III received injections of haloperidol (1 mg. $kg.^{-1}$). This compound was obtained in injectable form. Group IV received injections of Temposil (25 mg. $kg.^{-1}$). Temposil

is an anti-alcoholic agent which is known to inhibit aldehyde dehydrogenase. It has no known effect on DBH. The Temposil was dissolved in Ringer's solution. the pH of all solutions was verified at 7.00 prior to injection. The volume of all injections was 1 cc. per kg. of the Ss body weight.

Results

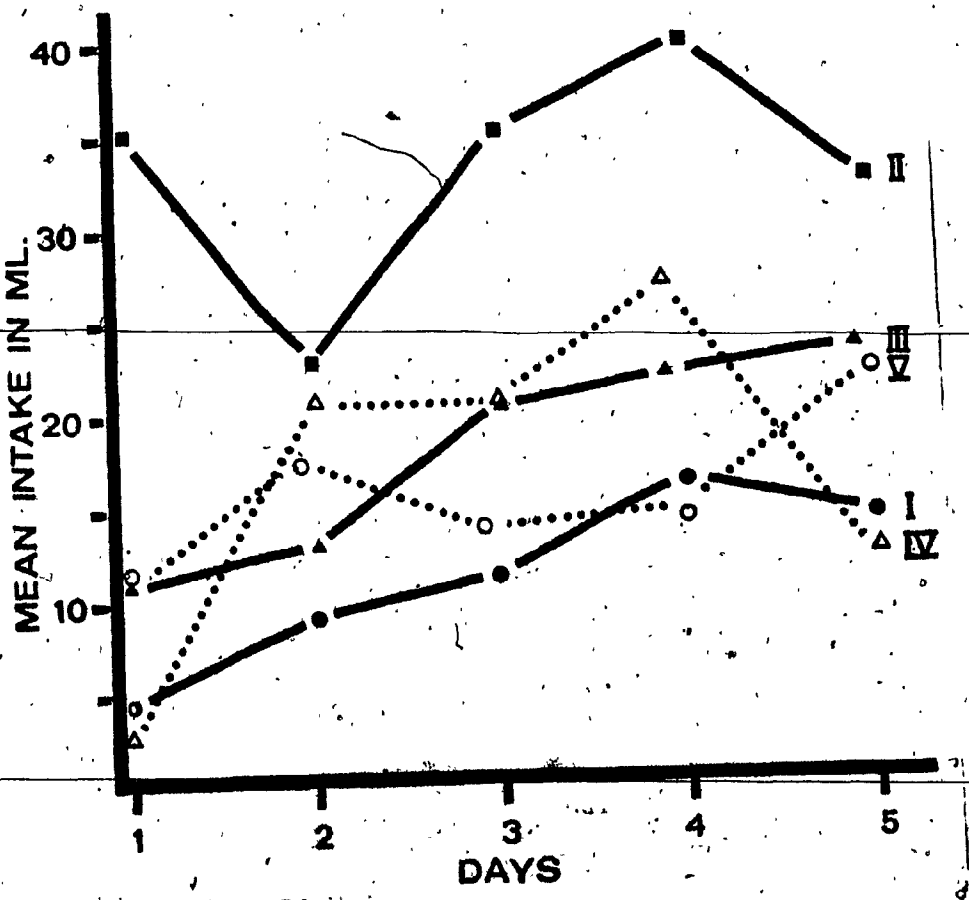
Experiment 1

Figure I shows the mean intake of the various solutions of each of the groups over the five-day post-infusion period. The intake patterns of the various groups appear to be substantially different, however, closer inspection reveals that the crucial treatment comparison, Group I, which received 6-OHDA vs Group IV, which received ascorbic acid, yields no significant difference (Kruskal-Wallis $H=1.32$, $p > .05$). In other words, the fact that less morphine was consumed than either quinine or water cannot be attributed to catecholamine depletion caused by infusion of 6-OHDA. The fact that in Group II, which received 6-OHDA, no significant difference (Wilcoxon $T=1.5$, $p > .05$) exists between H_2O intake for the five days prior to infusion and five days post-infusion indicates that the 6-OHDA treatment did not have a generalized depressant effect on fluid intake.

LEGEND

GR. I 350 µg. 6-OHDA - Mor.
GR. II 350 µg. 6-OHDA - H_2O
GR.III 350 µg. 6-OHDA - Quin.
GR. IV asc. acid - Mor.
GR. V 250 µg. 6-OHDA - Mor.

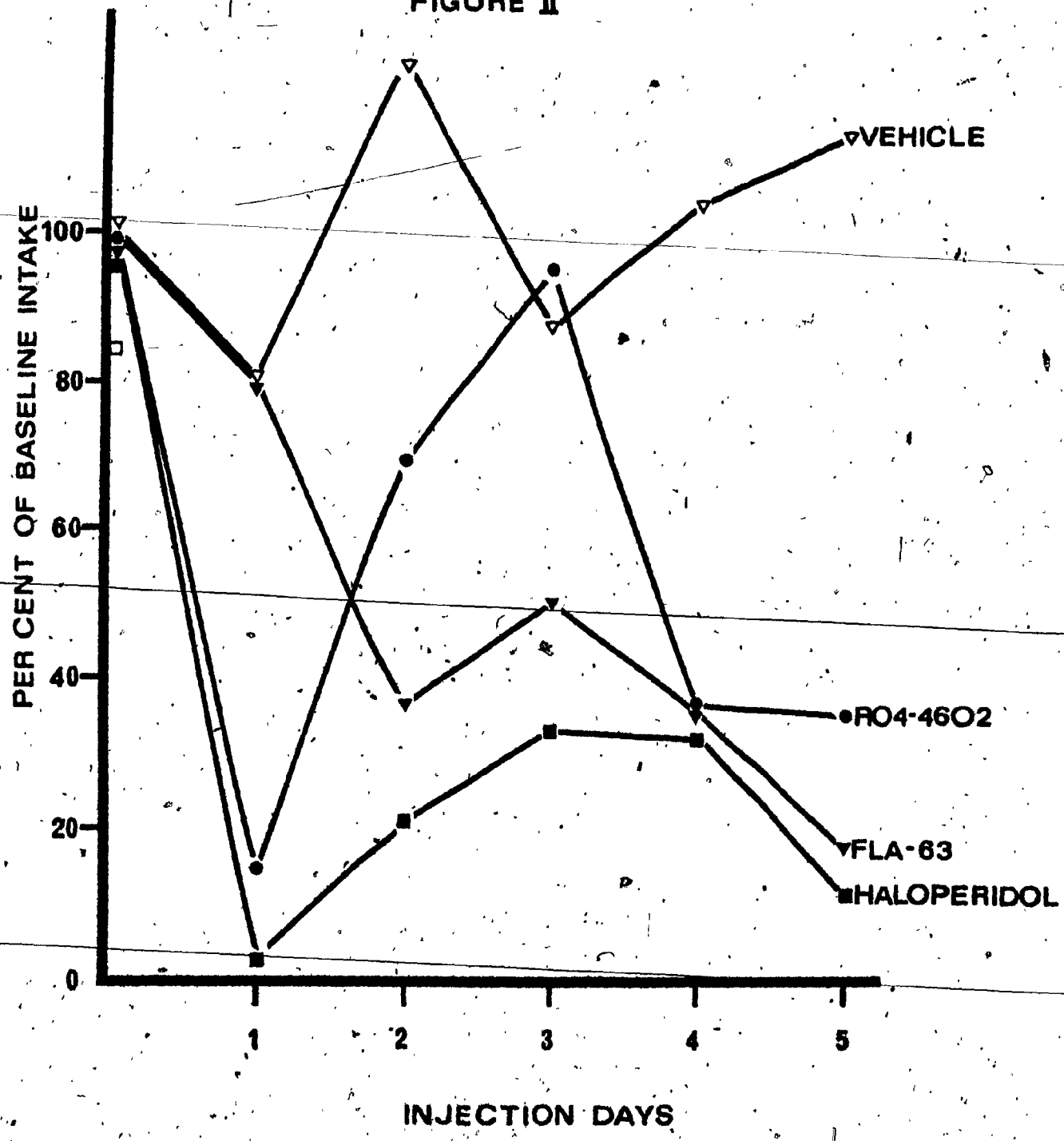
FIGURE 1



Experiment 2

Figure II shows the mean intake of morphine solution of each of the groups over the five-day injection period, calculated as a per cent of mean intake for the five-day pre-injection baseline period. A Kruskal-Wallis one-way analysis of variance by ranks indicates a significant difference between groups ($H=8.69$, $p < .05$). Looking at the differences between individual groups, a significant difference was found between Group I (FLA-63) and Group IV (saline) ($H=6.8$, $p < .05$), and between Group II (RO4-4602) and Group IV (saline) ($H=4.81$, $p < .05$). No significant difference was seen between Group II (RO4-4602) and Group I (FLA-63) ($H=.01$, $p > .05$). There was a significant difference between the two dopamine treatment groups, Group II (RO4-4602) vs. Group III (Haloperidol) ($H=3.93$, $p < .05$).

FIGURE II


PER CENT OF BASELINE INTAKE
100
80
60
40
20
0
1
2
3
4
5
INJECTION DAYS
VEHICLE
RO4-4602
FLA-63
HALOPERIDOL

Experiment 3

Figure III shows the mean fluid intake of each of the groups over the five injection days, calculated as a per cent of mean fluid intake for the five-day pre-injection baseline period. All scores reflect alcohol as a per cent of total fluid intake for the twenty-four hour period. A Kruskal-Wallis one-way analysis of variance by ranks indicated a significant difference between groups ($H=14.9$, $p < .05$). Comparisons between pairs of groups shows a significant difference between Group I (Disulfiram) and Group IV (Temposil) ($H=3.1$, $p < .05$) as well as between Group I (Disulfiram) and Group II (FLA-63) ($H=5.8$, $p < .05$). No significant difference was found between Group III (Haloperidol) and Group II (FLA-63) ($H=2.5$, $p > .05$).

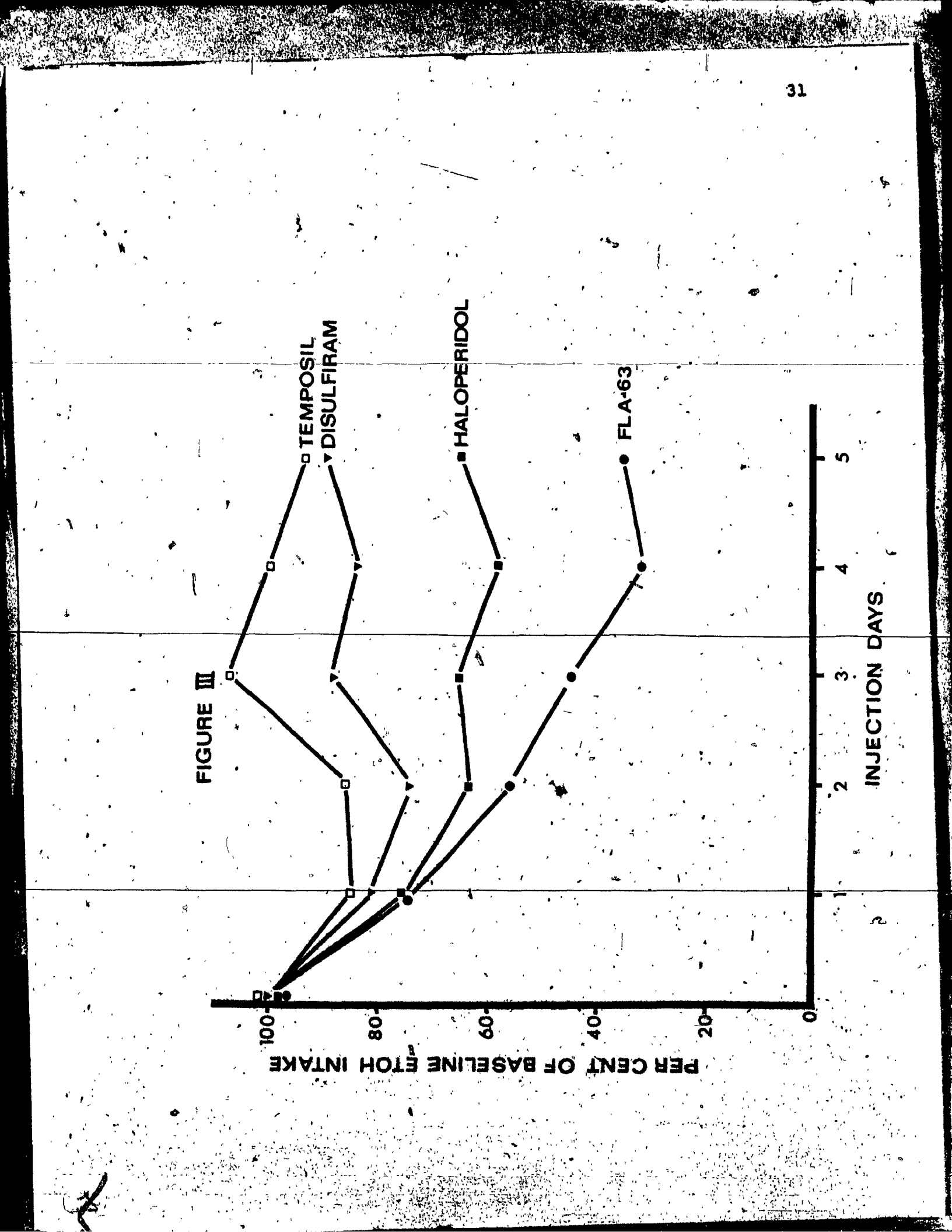

TEMPOSIL
DISULFIRAM
HALOPERIDOL
FLA-63
PER CENT OF BASELINE ETOH INTAKE
100
80
60
40
20
0
1
2
3
4
5
INJECTION DAYS

FIGURE III

Discussion

Experiment 1

The results of experiment 1 are inconclusive in that the small amounts of morphine ingested cannot be definitively attributed to catecholamine depletion. This is so since there was no significant difference between the morphine consumption of the 6-OHDA treated Group I and that of Group IV, which was treated with the vehicle. Given such previous results as those of Pozuelo & Kerr (1972) and Glick, Zimmerberg, & Charap (1973), the lack of effect in the present experiment is difficult to understand. These results might be interpreted to mean either 1) that central administration of 6-OHDA has no effect on morphine intake, or 2) that central administration of a .01% ascorbic acid solution affects morphine intake in a similar manner to that of 6-OHDA. One of the problems associated with the use of 6-OHDA is that it is rapidly oxydized upon contact with air and therefore must be administered in an ascorbic acid solution in order to retard oxydation. This requires the administration of a solution with a pH value of approximately 1.8. Whereas the compound has been extensively used and its specific effects on CA are well-established, effects of ascorbic acid which are unrelated to CA are not known. Due to the fact that Group II, who received the 6-OHDA in the ascorbic acid vehicle, did not alter its water consumption during the treatment period, it can be assumed that neither substance creates a depressant effect on fluid intake, at least for the dose levels employed in

the present study.

An alternate way of testing the catecholamine-morphine hypothesis would have been to present the morphine groups with the drug for several days before the 6-OHDA treatment and thus obtain a pre-treatment baseline level of morphine intake. In this way, post-treatment consumption of the drug could have been measured against baseline intake. This paradigm was considered and rejected due to the results of Amit, Corcoran, Amir, & Urca (1973) which suggests that neural manipulations affect drug self-administration differentially in drug-naive subjects and subjects who have had prior drug experience.

Therefore, one is left to consider the possibility that CA depletion induced by central administration of 6-OHDA has no effect on oral consumption of morphine by rats, or that non-catecholamine related damage produced by central administration of a highly acid solution depresses morphine intake.

Experiment 2

While there is ample evidence suggesting that catecholamines are involved in drug self-administration, evidence concerning specificity of function between dopamine and norepinephrine has been much more difficult to produce. The source of this difficulty is unclear since existing technology lacks the refinement necessary to ensure discrete manipulations. Permanent lesioning techniques (electrolytic, radio-frequency, 6-OHDA) are known to produce damage which is non-specific to catecholamines, and methods of pharmacological intervention have not yet demonstrated the capability to wholly and discretely affect only one of the catecholamines. As in other relatively unexplored fields, technological advances create sometimes the impetus for new research, and at other times, negate previous findings. Such is the case with 6-OHDA, which was initially thought to be CA-specific. Further investigation and technological refinement has shown that this is not the case (e.g. Hokfelt & Ungerstedt, 1973). The above difficulties are compounded by the phenomenon of functional redundancy in the CNS. The proportion of any discrete physical area or neurochemical system which is necessary to support specific behaviors remains unknown. A technique of CA manipulation which has recently become widely used is that employed in the present study - enzyme inhibition. The results of the present study support the thesis that CA are involved in drug self-administration, in that Groups I (FLA-63) and II (RO4-4602)

both consumed significantly less morphine that Group IV (saline). However, investigation of CA specificity was unsuccessful in that there was no significant difference in the amounts of morphine consumed by Groups I and II. Inhibition of both dopamine and norepinephrine synthesis reduced morphine intake. However, since there is no difference in the amount of reduction seen in the two groups, this experiment does not illuminate which, if either of these two catecholamines, is primarily involved in the mediation of morphine self-administration. Group III, which was treated with Haloperidol, consumed significantly less of the drug that either of the groups treated with enzyme inhibitors. It had been assumed that, since both Haloperidol and RO4-4602 act as functional depletors of dopamine, the results of Groups II and III would not differ significantly. The difference which was found could be interpreted to mean that, at the dosages used, Haloperidol was a more effective functional depletor than RO4-4602.

Experiment 3

Once again, the results of Experiment 3 support the notion that CA are involved in drug self-administration. One of the purposes of this experiment was to investigate the relative efficacy of DBH inhibition and aldehyde dehydrogenase inhibition as anti-alcoholic manipulations. The results of the present study support the hypothesis that inhibition of DBH is a more potent method of reducing ETOH intake than is aldehyde dehydrogenase inhibition. Group I, which was treated with Disulfiram, reduced its ETOH intake significantly more than did Group IV, which was treated with Temposil. Both of these substances are commonly used in the treatment of human alcoholics. Disulfiram is known to inhibit both of the above enzymes, whereas current literature indicates that Temposil inhibits only aldehyde dehydrogenase. Group II, which was treated with FLA-63, reduced its ETOH consumption significantly more than did Group I, the Disulfiram group. FLA-63 is an experimental compound which was developed as a DBH inhibitor. It seems reasonable to assume that the results obtained from this group are due to inhibition of DBH. There was no significant difference between the group functionally depleted of dopamine and the group functionally depleted of norepinephrine. Somewhat parallel to the results observed with morphine in Experiment 2, the results of the present study with ETOH do not support the idea of catecholamine specificty as regards drug self-administration.

General Discussion

Although the results of Experiment 1 are inconclusive, the results of Experiments 2 and 3 serve to confirm the hypothesis that catecholamines are involved in the self-administration of morphine and ethanol. In both of these experiments, the reductions in drug intake evidenced by all of the CA-treated groups were significantly greater than the reductions seen in the control groups. This is taken as a suggestion that reducing the availability of catecholamines reduces the reinforcing properties of both of these drugs. These results are substantially in agreement with those of Davis & Smith (1973), who found that administration of aMpT blocked the efficacy of morphine as a reinforcer of an operant response. Glick et. al. (1973) found that aMpT reduced oral intake of morphine in rats and Pozuelo & Kerr (1972) reported that this same compound suppressed intravenous self-administration of morphine in monkeys. Further, these results may be interpreted as concurring with those of Amit et. al. (1973), who found that lesions of the LH substantially blocked oral consumption of morphine in rats.

An important feature which is lacking in the present studies is the verification of CA depletions by biochemical assays. All catecholamine manipulations were derived from other studies in which the results of these techniques were verified. At the time that the present studies were initiated, such verification was planned, however, technological difficulties did not permit this to be carried out. Therefore, it should be emphasized that CA depletions in the present

studies are assumed and have not been verified as such.

There were two major purposes to this investigation. The first was to further illuminate the role of catecholamines in drug self-administration in non-dependent subjects. The second was to attempt to delineate some specificity of catecholaminergic functioning underlying drug self-administration. It was speculated that the results of this investigation might support the notion that one of these neurotransmitters is more critically involved in drug self-administration than the other or that self-administration of certain drugs might be subserved by one of the catecholamines while self-administration of other compounds is subserved by the other CA. The results of these experiments do not, in fact, provide any evidence to support these speculations. Indeed, the data from the present study indicate that tampering with either dopamine or norepinephrine had similar effects on self-administration of morphine or ethanol. This is not offered as conclusive evidence of lack of catecholamine specificity. As pointed out earlier, some of the compounds used are in the experimental stages of development, and even as concerns those which are widely marketed, precise pharmacological effects are unknown. FLA-63 has been shown to not only functionally deplete NE, but also to increase brain levels of DA, although at higher doses than those used here (Svensson, 1973). The precise effects of Temposil on CNS enzymes are unknown as are those of FLA-63 on liver enzyme action. Pharmacologic agents for neurochemical manipulation and techniques for measuring the effects of

these agents are currently being developed. A major problem seems to be that of synchronization of the various sciences. Some of the questions that behavioral scientists are currently interested in investigating, such as drug self-administration, are not necessarily the priorities of other disciplines such as physiology and pharmacology.

The most important contribution of the present studies is to add support to the notion that drugs are not self-administered as an escape from punishment due to inadvertent physical dependence. Such social factors as peer pressure or relief from stress are clearly not operating here. These factors are often used to explain the initiation of drug use among humans. The subjects in Experiment 2 had no previous drug experience until the presentation of morphine; therefore, they were clearly not drinking morphine due to physical dependence. Previous data (Amit & Stern, 1971) strongly suggest that the animals in Experiment 3 were not physically dependent, yet they consumed ETOH even with water available. Thus, in concurrence with Deneau et. al. (1969), one sees that non-dependent animals will initiate and maintain self-administration of certain drugs. The present results, in concordance with others previously mentioned, indicate that reducing the availability of CA reduces the propensity to self-administer drugs. This suggestion is particularly interesting in that other investigators have shown that reducing CA levels reduces the liability to engage in positively reinforcing behaviors such as self-stimulation (e.g. Cooper, Cott, & Breese, 1974) and sex (Caggiula,

A.R., Antelman, S.M., & Zigmond, M.J., 1973). The evidence from the present study and others suggests that it is possible that organisms self-administer drugs primarily because this behavior is positively reinforcing. Should the accumulation of evidence become sufficient to warrant general acceptance of this idea, a complete restructuring of the problem and treatment of human drug use will be necessitated. This view of drug self-administration concurs with results of studies of human drug users such as that of Himmelsbach (1943), which indicates that human heroin users often voluntarily undergo withdrawal to reduce heroin tolerance for monetary reasons.

References

Amit, Z., Corcoran, M.E., Amir, S., & Urca, G. Ventral hypothalamic lesions block the consumption of morphine in rats. Life Sciences, 13, 805, 1973.

Amit, Z., & Stern, M.H. A further investigation of alcohol preference in the laboratory rat.induced by hypothalamic stimulation. Psychopharmacologia, 21, 317, 1971.

Amit, Z., & Stern, M.H. Electrochemical interaction in the medial forebrain bundle and enhanced preference in rats. In O. Forsander & E.K. Eriksson (Eds.), Biological Aspects of Alcohol Consumption, Finnish Foundation, 225, 1972.

Beach, H.D. Morphine addiction in rats. Canad. J. Psychol., 11, 104, 1957.

Begleiter, H. Ethanol consumption subsequent to physical dependence. In M.M. Gross (Ed.), Alcohol intoxication and withdrawal: experimental studies II, Plenum Press, New York, in press.

Breese, G.R., & Traylor, T.D. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. J. Pharm. Exptl. Ther., 174, 413, 1970.

Cagguila, A.R., Antelman, S.M., & Zigmond, M.J. Disruption of copulation in male rats after hypothalamic lesions: a behavioral, anatomical, and neurochemical analysis. Brain Research, 59, 273, 1973.

Cohen, G., & Collins, M. Alkaloids from catecholamines in adrenal tissue: possible role in alcoholism. Science, 167, 1749, 1970.

Cooper, B.R., Cott, J.M., & Breese, G.R. Effects of catecholamine-depleting drugs and amphetamine on self-stimulation of brain following various 6-hydroxydopamine treatments. Psychopharmacologia, 37, 235, 1974.

Davis, V.E., & Walsh, M.J. Morphine and ethanol physical dependence: a critique of a hypothesis. Science, 170, 1113, 1970.

Davis, W.M., & Smith, S.G. Blocking of morphine based reinforcement by alpha-methyl-tyrosine. Life Sciences, 12, 185, 1973.

Deneau, G.A., Yanagita, R., & Seevers, M.H. Self-administration of psychoactive substances by the monkey. A measure of psychological dependence. Psychopharmacologia, 16, 30, 1969.

Eddy, N.B., Halbach, H., Isbell, H., & Seevers, M.H. Drug dependence: its significance and characteristics. Bulletin of the World Health Organization, 32, 721, 1965.

Freund, G. Alcohol withdrawal syndrome in mice. Arch. Neurol., 21, 315, 1969.

Friedler, G., Bhargava, H.N., Quock, R., & Way, E.L. The effect of 6-hydroxydopamine on morphine tolerance and physical dependence. J. Pharmacol. Exptl. Ther., 183, 49, 1972.

Glick, S.D., Zimmerberg, B., & Charap, A.D. Effects of alpha-methyl-para-tyrosine on morphine dependence. Psychopharmacologia, 32, 365, 1973.

Goldstein, D.B., & Pal, N. Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. Science, 172, 288, 1971.

Himmelsbach, C.K. With reference to physical dependence. Fed. Proc., 2, 201, 1943.

Hokfelt, T., & Ungerstedt, U. Specificity of 6-hydroxydopamine induced degeneration of central monoamine neurons: an electron and fluorescence microscopic study with special reference to intracerebral injection in the nigro-striatal dopamine system. Brain Research, 60, 269, 1973.

Kerr, F.W.L., & Pozuelo, J. Suppression of physical dependence and induction of hypersensitivity to morphine by stereotaxic hypothalamic lesions in addicted rats: a new theory of addiction. Mayo Clin. Proc., 46, 653, 1971,

Lippa, A.S., Antelman, S.M., Fisher, A.E., & Canfield, D.R. Neurochemical mediation of reward: a significant role for dopamine? Pharmacol. Biochem. Behav., 1, 23, 1973.

Neff, N.H., & Costa, E. The influence of monoamine oxydase inhibition on (rat brain and heart) catecholamine synthesis. Life Sciences, 5, 951, 1966.

Nichols, J.R. How opiates change behavior. Sci. Amer., 212, 80, 1965.

Nichols, J.R. Morphine as a reinforcing agent: laboratory studies of its capacity to change behavior. In A. Wikler (Ed.), The addictive states. Baltimore: Williams and Wilkins, 1968.

Olds, J., & Milner, P. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. Comp. Physiol. Psychol., 47, 419, 1954.

Poschel, B.P.H., & Ninteman, F.W. Norepinephrine: a possible excitatory neurohormone of the reward system. Life Sciences, 10, 782, 1963.

Pozuelo, J., & Kerr, F.W.L. Suppression of craving and other signs of dependence in morphine-addicted monkeys by administration of alpha-methyl-para-tyrosine. Mayo Clin. Proc., 47, 621, 1972.

Ratcliffe, F. Ethanol dependence in the rat: its production and characteristics. Arch. Int. Pharm. Ther., 196, 146, 1972.

Ritter, S., & Stein, L. Self-stimulation in the mesencephalic trajectory of the ventral noradrenergic bundle. Brain Research, 81, 145, 1974.

Schwartz, A.S., & Marchok, P.L. Depression of morphine-seeking behavior by dopamine inhibition. Nature, 248, 257, 1974.

Seevers, M.H., & Deneau, G.A. Physiological aspects of tolerance and physical dependence. In W.S. Root & F.G. Hofmann (Eds.), Physiological pharmacology. Vol. 1, New York: Academic, 1963.

Sinclair, J.D. Morphine-induced suppression of voluntary alcohol drinking in rats. Nature, 246, 425, 1973.

Spragg, S.D.S. Morphine addiction in chimpanzees. Comp. Psychol. Monogr., 15, 1, 1940.

Stein, L., & Wise, C.D. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. J. Comp. Physiol., Psychol., 67, 189, 1969.

Stolerman, I.P., & Kumar, R. Preference for morphine in rats: validation of an experimental model of dependence. Psychopharmacologia, 17, 137, 1970.

Svensson, T.H. Increased dopamine concentration in the striatum in the mouse by FLA-63, a dopamine-beta-hydroxylase inhibitor. Letters to the editor. J. Pharm. Pharmacol., 25, 73, 1973.

Thompson, T., & Ostlund, W., Jr. Susceptibility to re-addiction as a function of the addiction and withdrawal environments. J. Comp. Physiol. Psychol., 60, 388, 1965.

Ungerstedt, U. 6-Hydroxydopamine induced degeneration of central monoamine neurons. Eur. J. Pharmacol., 5, 107, 1968.

Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand., 82, 1, 1971.

Uretsky, N.J., & Iverson, L.L. Effects of 6-hydroxydopamine on catecholamine containing neurons in the rat brain. J. Neurochem., 17, 269, 1970.

Weeks, J.R., & Collins, R.J. Patterns of intravenous self-injection by morphine-addicted rats. In A. Wikler (Ed.), The addictive states. Baltimore: Williams and Wilkins, 1968.

WHO Expert Committee on Addiction-Producing Drugs. Seventh Report: WHO Techn. Rep. Ser., 116, 1957.

Wise, C.D., & Stein, L. Facilitation of brain self-stimulation by central administration of norepinephrine. Science, 163, 299, 1969.

Woods, J.H., & Schuster, C.R. Reinforcement properties of morphine, cocaine, and spa as a function of unit dose. Int. J. Addict., 3, 231, 1968.