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Effects of Ethanol on Stimulus Properties of D-Amphetamine

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EFFECTS OF ETHANOL ON STIMULUS PROPERTIES OF D-AMPHETAMINE

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ABSTRACT

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The drug discrimination paradigm was used to investigate the effects of combinations of d-amphetamine sulfate (amph) and alcohol (etoh). Rats were trained to discriminate between the stimulus properties of 1.2 mg/kg amph and non-drug treatment in a two-lever food-motivated operant task. Once trained, rats were tested with .3 mg/kg amph, and showed an intermediate level of "amphetamine" responding. Combinations of this test dose with etoh 150 mg/kg or 300 mg/kg produced significantly increased amph-appropriate responding. This potentiation of the amph cue tended to be higher at etoh 150 mg/kg. The administration of etoh (150 or 300 mg/kg) alone did not result in amph-appropriate responding. These findings confirm earlier results from both drug discrimination and other behavioral paradigms, indicating that low doses of etoh in combination with low doses of amph can result in potentiation of amph effects.

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Introduction

The study of stimulus properties and interaction of drugs is gaining increasing significance in the field of drug research today. Knowledge of the mechanisms involved when certain drugs are combined is important at the therapeutic level, where such knowledge could prevent joint prescriptions, which could produce interactions detrimental to the person. Much careful research is necessary in order to document the interactive effects of drugs, which are extremely complex and often unpredictable from knowledge of the properties of the individual drugs (Hansten, 1973).

The present discussion will focus on behavioral effects and outcomes of the combined administration of stimulants and depressants. A brief review of the current knowledge of both depressant and stimulant effects follows.

Stimulant Effects

Drugs in this category have several effects on behavior. In particular, they activate the sympathetic nervous system. Further, thay are anorectic, i.e., they supress the appetite for food. In general, stimulants

cause an increase in arousal and activity levels. Increased dosages can also cause severe behavioral disruption, paranoid states in humans, and finally CNS seizures (some species) which can be fatal (cf. Iverson & Iverson, 1981; Ray, 1983).

Depressant Effects

Drugs such as alcohol and barbituates exert a depressant effect on the CNS. Generally, the activity level is lowered. Humans experience mood changes and reduced information- processing ability. In large doses depressants suppress ongoing behavior and induce sleep. Very high doses can also result in coma and eventually death.

Both stimulant and depressant effects seem to follow a roughly similar pattern which can be described somewhat simply in the following manner: First, increasing degrees of behavioral disruption, (intoxication) are seen, and finally death (preceded by sleep and coma for depressants, seizures and convulsions for stimulants) results.

This pattern is interesting from a scientific view point, because among other effects, both seem to "tap into" circuits controlling arousal level, a basic type of behavioral/brain function. As a result, numerous studies have been conducted investigating the effects of stimulants and depressants on behavior in many species, including humans. The knowledge emerging from such

studies also has a more practical, applied value because humans frequently use these drugs, both separately and in combination.

Common Uses of Stimulants

The different types of stimulants most commonly used are caffeine in coffee, nicotine in cigarettes and various amphetamine (amph) compounds such as "diet pills," and cocaine. Some of the more frequent reasons for the use of such stimulants are to relieve fatigue and boredom, to increase alertness and to maintain concentration, and to assist weight reduction.

Common Use of Depressants

Reasons for the common use of depressants include: to induce relaxation or sleep, to decrease inhibition and to be more "sociable."

Stimulants and Depressants in Combination

Joint usage of stimulants and depressants can be in the form of caffeine (in coffee and cola drinks) and alcohol; or various barbituates and amphetamines; or amphetamines, or cocaine and alcohol. Needless to say, especially the latter two combinations of stimulant and depressant are the most dangerous and widely abused today. The two most common reasons for joint usage are to counteract certain effects of depressant with a stimulant or vice versa. For instance, a person trying to reverse the intoxication effects of alcohol by drinking strong black coffee, or in reference to the latter reason, someone who is taking a depressant or a "tranquilizer" in

order to counteract some strong stimulant effects (e.g., taking valium to "come down from" cocaine).

Stimulant-Depressant Interactions

In general, stimulants are widely believed to have characteristics opposite to those of depressants. As mentioned, for both practical (clinical) and theoretical purposes, the effects of combinations of stimulants and depressants are of interest and have been studied extensively. Drug effects are quite complex in their own right. Some relevant factors are dose, behavior measured, species, tolerance and expectation (in humans). When measuring two drugs combined, the complications increase. The following is a summary and description of mechanisms of action and reactions in regard to behavior and physiology that may occur when stimulants and depressants are combined.

First, the two when combined could interact in an antagonistic manner (cf., Holloway & Holloway, 1978). The fact that amphetamine exerts an excitatory effect led to the common belief that the biological/behavioral reaction when the two are combined is an antagonistic one, meaning that their opposite effects would partially or totally cancel each other out. This belief lead early researchers (as well as many "lay persons" dealing with alcohol intoxication) to attempt to use stimulants to reverse alcohol intoxication. Early studies using human subjects were conducted by Reifenstein and Davidoff (1938, 1941)

who found that amphetamine was very effective in treating the acute phases of ethanol (etoh) intoxication in alcoholics.

In 1956, a more sophisticated study was conducted comparing the ability of subjects receiving a combination of amphetamines and etoh on such tests as balance, hand steadiness, visual fusion, and EEG patterns (Newman & Newman, 1956). The researchers concluded that amph did have a slight antagonistic effect on the alcohol produced depression.

Similar antagonistic effects were found by Jansen, Rutenfranz & Jansen, 1959; 1969) who studied the effects of etoh-amph combinations on driving-simulator performance. They found that the amph completely antagonized low doses of ethanol (BAL less than .06%), but incompletely antagonized higher effects of ETOH doses.

This result raises the question whether these antagonistic effects are limited to certain dose ranges. Various studies (Duncan & Cook, 1981; and Holloway & Holloway, 1978) have established that when etoh and stimulants are administered in combination, different types of interactive effects on behavior may be seen, depending on the dosage of each drug, the type of behavior measured, and the effects of each drug when administered separately (Duncan & Cook, 1981). Another study illustrating the importance of dosage in determining interaction effects of amph and etoh was conducted by Wallgren and Tirri (1963). In this experiment, the

investigators used a tilting-plane device to test for intoxication in rats. Their results revealed that the amphetamine antagonized the etoh when the dose of etoh was 2000mg/kg, but no antagonism was seen with 3000mg/kg etoh.

Similar results were obtained by Frommel and Seydoux (1964), who found antagonistic effects of amph and etoh when observing general motor activity. However, these authors attributed their results to the antagonistic influence of the depressant action of alcohol on the excitatory actions of amphetamine (Frommel & Seydoux, 1964).

Antagonism is certainly not the only possible result of combination treatment of amphetamine and etoh. The two agents combined can also interact synergistically (jointly act in the same direction), or can produce potentiation (whereby the effects of both drugs combined give a higher, more intense effect that either drug alone).

An early study which documented findings of both synergism and potentiation was by Weiss & Laties (1964). The authors measured operant response duration in dogs and found that when etoh and amph were administered separately, a shortened response duration occurred. However, when the two were administered in combination, the response duration was reduced even more.

Todzy et al. (1978) reported that etoh can potentiate the locomotor activity-increasing effect of amph in rats. Other studies, (Rech et al., 1978; Duncan & Cook, 1981)

have also reported potentiation of this amph-induced effect by administration of etoh in rats.

There are many possible mechanisms whereby the different types of drug interactions (antagonism, potentiation, synergism) can occur, and many variables which influence these mechanism. The drugs may mutually or non-mutually affect each others' absorption, metabolism, distribution and excretion (Hansten, 1973). Some of the physiological mechanisms involved and affected by the administration of drugs (especially etoh in combination with amph) are the metabolism, the rate of absorption of the drugs, and the release (or inhibition) of certain neurotransmitters within the brain, namely serotonin, GABA, glycine, acetylcholine and catecholamines.

Functions of Mechanisms of Action

Modification of absorption. When etoh and amph are administered in combination, a possible mechanism of interaction between the two drugs is the modification of absorption into the blood stream of one drug by another. Rinkel and Myerson (1941, 1942) were among the first investigators to study the effects of amph on the absorption of etoh. The subjects used in the study were pigeons. The animals received a treatment of etoh (orally) which was followed by IV treatment with amph. The researchers found that the amph did lower the "blood-alcohol curve." Rinkel & Myerson attributed this

inhibition to delayed absorption of the etoh from the gastronintestinal tract caused by the influence of the amph.

Modification of metabolism. Certain drugs share a common metabolic pathway (Metzey, 1976). Hence, another possible mechanism of interaction is the modification of the metabolism of such drugs. Metzey (1976) stated that etoh is metabolized mainly by the cytoplastic liver alcohol dehydrogenase, an enzyme which is not substrate inducible; thus it is assumed that etoh would not alter the metabolism of many drugs (Metzey, 1976). However, researchers (Iverson et al, 1975) have reported that although etoh had no significant effect on the toxicity of amph, it had a definite inhibitory effect on the metabolism of the amph. Such an effect would lengthen the duration of amph's presence in the body and thus prolong its action.

Neurotransmitter effects. Etoh has a direct effect on the membranes of cells within the brain (neurons). Research has shown (Kalant, 1971; cf. Ray, 1972) that a change in permeability of the neural membranes produces a reduction of movement of sodium ions and gives rise to the depressant effects of etoh. "This change in permeability results in the action potential rising more slowly and to a lowered maximum" (Kalant, 1971). Etoh has been found to increase the blood levels of Serotonin (Girard, 1962), as well as to increase the excretion of urinary tryptamine, a breakdown product of serotonim (Schenker, Kissin, Maynard & Schenker, 1966).

One of the numerous effects of amph and etoh is their influence on neurotransmitters, such as serotonim, gaba, glycine, acetylcholine, and catecholamines. Research on the direct effects of etoh on these transmitters is somewhat inconclusive, with the exception of cathecholamines. Two very important transmitters classified as catecholamines are dopamine (DA) and norepinephrine (NE). The pharmacological and biochemical properties of these two transmitters are very similar (Iversen & Iversen, 1975). Much research on the release, inhibition or concentration of DA and NE has been conducted with amph. Studies have shown (Holmes & Rutledge, 1976) that amph facilitates the release of these catecholamines, and also blocks their re-uptake. The effects of etoh on DA and NE are not as well understood. In 1975, Thadani conducted a study giving alcohol to rats and found what he called a "biphasic reaction." The alcohol initially caused an increase in the release of NE or a decrease in its re-uptake, followed by a decrease in release of NE from presynaptic terminals (Thadani, 1975). This biphasic reaction to etoh could explain findings by Matchett and Ericson (1977) of a biphasic behavioral effect of etoh. These latter authors found that adrenergic blocker phentolamine antagonized the initial stimulatory effect of etoh on behavior, as did the dopaminergic blocker spiroperidol; findings which implicate the involvement of both DA nd NE in etoh

actions. However, a study by Schechter (1974) found that propanolol, an adrenergic blocking agent, had no significant effect on the ability of rats to discriminate an etoh cue (Schechter, 1974).

Other studies (Alkana, Parker, Cohen, Birch and Noble, 1977) indicate that amph and etoh may have some similar effects on catecholamine systems, and to some extent, have the same mechanisms of action, which could produce a synergistic or potentiating interaction.

Stimulus Properties of Drugs

The foregoing review of possible amph-etoh interactions suggests that the knowledge resulting from the research has potential clinical as well as theoretical relevance. The study of drug discrimination has been a useful tool in the research of stimulus properties of drugs.

A great variety of responses are acquired through discrimination learning. Reinforcement is presented when a particular response is emitted in the presence of a particular stimulus. Likewise, an alternative response is rewarded only in the presence of a different stimulus. Discrimination requires the ability to choose between two alternative responses and to differentiate between the relevant stimuli.

Drugs can produce powerful, distinctive stimuli, due to their strong physiological effects. A difficulty in studying drugs as discriminative stimuli is the complex

and varied nature of drug effects. However, an advantage is that their effects are highly predictable, reliable and powerful (Barry, 1974).

There are several resons why discrimination learning has been used so extensively to study drug effects:

1. Drug discrimination (DD) is very sensitive to both type and dose of drugs used. When choosing the type of drug to be studied, the various mechanisms of action are relevant, and of course essentially all drug effects can be controlled by dosage.

2. When studying DD, the effects on the behavior of the animals used for the research are not very disruptive, mainly because relatively low doses can be used.

3. Finally, since much research has been done in this area, any results obtained can be compared to, and, integrated into a large amount of existing information. Additionally, experimental paradigms and techniques have been developed and extensively tested for this type of research.

In a few studies, highly discriminable drugs have acquired discriminative control somewhat more rapidly than any known exteroceptive stimulus conditions (Overton, 1964).

A review of the literature by Barry (1974) focuses on the research utilizing drug-discrimination learning. Generally, in this kind of research, laboratory rats are trained to make differential responses on the basis of

stimulus characteristics of drug treatments. Barry's review mainly concerns the distinctiveness, rather than the strength of drug stimulus effects (Barry, 1974).

As mentioned, in most studies reviewed by Barry, rats are trained to make differential responses under the drug and non-drug conditions. The differential response is reinforced by reward for the correct choice under the particular condition, and by non-reward or punishment for the alternative incorrect choice (Barry, 1974). A frequently used technique for studying stimulus properties of drugs involves the two-lever appetitive operant paradigm.

Cross-Drug Transfer

Etoh will generalize to other CNS depressants. The technique of drug-cue generalization, or cross-drug transfer, has been employed using etoh both as the training drug and as the novel test drug. The results of several such cross-drug transfer studies (Overton, 1964; Krimmer & Barry, 1973; Kubena & Barry, 1969) have shown that when etoh was used as the training drug, stimulus generalization to barbituates, minor tranquilizers and sedative hypnotics was observed (Krimmer and Barry, 1973; Overton, 1964). These results would indicate that the central cues produced by effective doses of etoh and other CNS depressant drugs are similar. In contrast, the

central stimulants d-amph and bemegride produced cues that were dissimilar to those produced by etoh (Kubena and Barry, 1969; Overton, 1964).

Stimulants such as amph also generalize to other stimulants. Generalization to amph responding in animals trained to discriminate amph from saline occurs with such other stimulants as cocaine, methamphetamine, methylphenidate and l-amphetamine (Schechter & Rosecrans, 1973; Winter, 1975). These drugs have similar mechanisms of action, and this common feature is apparently the basis for their similarity of stimulus properties. These findings also substantiate the reliability of drug discrimination for the use of drug classification.

In his review of the classification of drugs, Barry (1974) also describes the use of the ED_{50}, (effective dose 50) which is a basic technique used in pharmacology to qualify dose-response relations of drugs. There are several different methods by which an ED_{50} can be determined. Barry describes the use of the ED_{50} in drug discrimination studies as a standardized measure of drug potency. It implies that at this dose a specified response is elicited by a higher, but not by a lower dose of a test drug (Barry, 1974). For example, when a discriminative response is established at a certain training dose a much lower dose (usually less than half of the training dose) will result in an equal probability of a "drug" or "saline" response being emitted.

Basic Stimulus Properties of Amph

A common feature of stimulants such as amph is their agonistic effects on brain dopamine systems. Studies (Taylor and Snyder, 1973; Moore, 1973) have reported that amph exerts its locomotor stimulant effects via dopaminergic mechanisms. That this effect may be importantly involved in discriminative response control by these compounds is supported by the findings that generalization to amph is produced by direct dopamine receptor agonists (Schechter & Cook, 1975). Amph releases both dopamine (DA) and norepinephrine (NE) from neurons within the brain that contain these transmitters. The notion that amph acts by displacing catecholamines from storage sites in the brain is strongly supported by the findings that pretreatment with drugs that inhibit catecholamine biosynthesis completely prevents amph stimulation of behavior. Although amph appears to cause a release of both DA and NE in the brain, their stimulant effects result primarily from the release of DA (Iversen & Iversen, 1975).

As mentioned, amph is a potent CNS stimulant. Further, it also blocks the re-uptake and facilitates the release of 5-HT (serotonin) in nerve endings (Wong et al., 1973). Amph and related compounds have been studied extensively for their discriminative stimulus properties (Ho & Huang, 1975; Overton, 1971; Schechter & Rosecrans, 1973). Such discrimination studies have been useful for

the classification of drugs (Barry, 1974), and some reports have shown that stimulants do not generalize to other drug classes such as depressants and hallucinogens (Ho, Richards & Chute, 1978).

In 1975 Ando investigated the discriminiative control of operant behavior by IV injections of amph and etoh. Rats were trained to discriminate between the stimulus properties of amph and saline. The animals were then tested with an array of drugs such as cocaine, methamphetamine etoh and epinephrine. The study confirmed earlier results indicating that intravenously administered drugs can act as discriminative stimuli in controlling operant behavior (Ando, 1975). Specifically, rats trained to discriminate amph from saline responded to other stimulants as if they were amphetamine, but emitted "saline" responses after etoh treatment. Therefore, the amphetamine cue is not similar to the ethanol cue.

Another study by Garza & Johanson (1985) assessed the discriminative properties of cocaine in pigeons. Six pigeons were trained to discriminate IM injections of cocaine (2mg./kg.) from saline. Cocaine was found to be an effective discriminative stimulus. Furthermore, the authors concluded these discriminative effects of cocaine are pharmacologically specific in that other psychomotor stimulants like d-amph and l-cathinine consistently produced drug-appropriate responding while drugs with different pharmacological actions did not (Garza & Johanson, 1985).

Basic Stimulus Properties of Etoh

Etoh may be classified as a sedative-hypnotic agent since its principle action is depression of the CNS. Etoh was shown as early as 1951 (Conger) to acquire state-dependant control of discriminative responding (Ho, Richards & Chute, 1978). Etoh is a powerful CNS depressant and has been reported to generalize to other depressants also. A somewhat secondary mechanism of action of etoh is that it blocks the re-uptake as well as facilitates the release of catecholamines such as DA and NE. Of particular relevance to the main mechanisms of action of etoh is the relationship between levels of brain serotonin (5-HT) and certain behavioral effects of etoh. Several investigators (Feldstein, 1972; Barry & Krimmer, 1973) have reported that some of the effects of etoh can be altered by 5-HT depletion. The behavioral characteristic most frequently associated with 5-HT depletion in the brain is hyperactivity, hence the possibility of altering the depressant effects of etoh (cf. Ho, Richardson & Chute, 1978; Schechter, 1975).

Combination Effects of Amph and Etoh

Previous studies of combination effects of etoh and amph (Schechter, 1975; Krimmer et al., 1974) suggest possible antagonism between the two drug effects. Amph has been reported to antagonize etoh effects on performance in humans (Bernstein et al., 1965; Taylor et al., 1964).

As mentioned, amph has been reported to have different effects on etoh's action in human subjects. Some studies have reported an increase (Miller, 1944), some have shown a decrease (Hughes & Forney, 1964) and others have shown no effect (Brown et al., 1965). Results are more conclusive in studies using animal subjects. In rats, amph (4mg/kg) was shown to antagonize etoh's depressant effects on exploratory behavior in a T-maze (Leonard & Wiseman, 1970).

Caffeine has also been reported to antagonize the actions of etoh in humans (Graf, 1950; Nash, 1965). However, in rats, 100mg./kg. of caffeine and 1mg./kg. of etoh were shown to produce significantly greater depressant effects than 1mg./kg. of etoh alone (Alstatt & Forney, 1971).

In spite of the evidence discussed so far which points to the notion that amph may antagonize the effects of etoh, this is usually not the case. The following review of more recent research in this area will serve to clarify this statement.

The foregoing studies as well as the ones reviewed by Barry were largely based on drug vs. non-drug discriminations. However, several investigators (e.g. Duncan & Kao, 1979) have made use of the drug vs. drug paradigm. Generally, results have indicated that rats can disciminate between different types of drugs, and also between different doses of the same drug. An example of a study utilizing the drug vs drug paradigm is Duncan and

Kao (1979). The authors trained rats to discriminate amph (.8mg./kg.) treatment from pentobarbital treatment (10mg./kg.), in an appetitive-operant paradigm. When these trained rats were treated with etoh (200-1400mg./kg.), they responded by pressing the pentobarbital lever, with pentobarbital choice increasing as etoh dose increased. Hence, their conclusion, that in rats trained to discriminate sodium pentobarbital from amph at these dosages, etoh does not have a convincing degree of amph like stimulus properties at any dose tested, but does have depressant like stimulus properties at moderate dose levels (Duncan & Kao, 1979). However, when Duncan & Kao presented their animals with amph and etoh combined they still recognized the amph cue, even though etoh was also present.

In 1974, Barry stated in his review that rats trained with barbituate vs. saline (drug/non-drug design) did not emit "barbituate responses", even when tested with etoh. Comparison of these two results (Duncan & Kao, 1979; Barry, 1974) yields that etoh does not display any barbituate stimulus properties when barbituate is contrasted with a non-drug state such as saline. However, this is not the case when the barbituate is contrasted with another drug state such as amph. In such cases the etoh displays more barbituate-like stimulus properties than amph-like stimulus properties. Further, studies have shown that rats trained with saline vs. etoh respond to

the etoh stimulus in a test with barbituate, but not vice versa. Also, it seems that neither amph nor barbituates are antagonized by each other when animals are trained drug vs. saline.

Potentiation Effects of Amph-Etoh Combinations

Several investigators have reported potentiation of both etoh-induced and amph-induced effects on behavior at least in some tasks and for some drug dose combinations. Some of amph's enhancement of etoh induced effects include: decrease in shock avoidance by rats (cf. Holloway & Holloway, 1978; Allen & Ferguson, 1971), impairment of discriminated Y-maze performance in rats (Holloway, 1976) and decreases in human performance on simple addition tasks (cf. Holloway & Holloway, 1978; Hughes & Forney, 1964). Some instances of etoh's enhancement of an amph-induced effect were found also. Some of these include: hypermotility and sterotypy (cf. Holloway & Holloway, 1978; Todzy et al., 1978; Tritt & Walsh, 1971), hyperactivity and facilitation of Y-maze discriminated avoidance performance (Holloway, 1977).

In 1980 Duncan conducted a follow-up study to Duncan & Kao (1979). The main purpose of the experiment was to investigate the effects of potentiation and antagonism of amph stimulus properties in reference to conbinations of etoh and amph. Rats were trained with amph (.8mg./kg.) vs. saline, and tested with amph (.2mg./kg. and .4mg./kg.), and etoh (400 and 800mg./kg.). Tests were

also conducted with combinations of all of the above dosages. Duncan concluded that etoh did potentiate the amph cue, the best comination for this effect was amph .2mg./kg. and etoh 400mg./kg.

The result of the drug interaction seen in Duncan's (1980) study confirm previous findings (Todzy et al., 1978) that etoh can potentiate the amph-induced effect. In the Todzy (1978) experiment as mentioned briefly before, potentiation of the SMA-stimulant effect was seen at the lowest etoh dose administered (800 mg/kg in combination with 1 mg/kg of amph). Duncan and Cook (1981) conducted yet another study investigating possible potentiation as a result of combinations of etoh and amph. The investigators found that 800 mg/kg of etoh in combination with amph resulted in more SMA than did etoh alone (Duncan & Cook, 1981).

The present study was also an attempt to study possible potentiation and antagonism effects of combinations of etoh and amph on the stimulus properties of amph. A drug vs. non-drug paradigm was used. The basic procedures used by Duncan (1980) were replicated, however, a higher training dose of amph, and lower test doses of etoh were used.

Method

Subjects. Nine male rats of the Long-Evans strain were housed individually and given free access to water. The approximate initial body weight was about 350 grams. Food was restricted until 85% predeprivation weight was reached. Water was available at all times in the home cages, which were located in a vivarium with west-facing windows. Day-night cycles were thus determined by natural illumination. Training and testing was usually done between noon and six pm.

Apparatus. Basic operant training (establishing lever-pressing) was conducted in single-lever response chambers (Scientific Model Prototype #A101A). Drug discrimination training and testing was conducted in two-lever operant chambers (BRS/LVE RTC-022). Three such chambers were used, each was enclosed in a sound-attenuating shell. Reinforcement for lever-pressing was 45 mg. food pellets. Injection solutions were sterile distilled water, d-amphetamine sulfate (Sigma Chemical Company) dissolved in sterile water, and 190-proof grain alcohol (ethanol) diluted with sterile water to yield a 5% (volume/volume) ethanol-water solution. The concentration of d-amphetamine was .6 mg./ml. for training, and .15 mg./ml. for test sessions. Both concentrations and doses mentioned below) are expressed as weight of the salt.

Procedure

Training Phase: The rats were trained to lever-press for food pellets on a continuous reinforcement schedule in the single-lever boxes. Then daily training sessions commenced in the two-lever operant chambers. For five rats the left lever was designated as the amph lever, and for four rats, the right lever was the amph lever. During the first three sessions in the two-lever boxes, lever-pressing on the non-drugged (ND) lever was reinforced. The reinforcement schedule was gradually changed from CRF to VI-12 sec. over this three day period. For the next three days, responses on the amph lever were reinforced, again moving from CRF to VI-12 sec. schedule. Fifteen minutes prior to the first drug training session, amph at a dose of .6mg/kg was administered via intraperitoneal (IP) injection. On the second drug training day, the amph dose was increased to .9, and then finally, to 1.2 mg/kg for the third day. On the seventh training day in the two-lever boxes, a double alternation sequence (ND, ND, amph, amph) was initiated for the pre-training injection. Both solutions, distilled water or amph were injected 15 minutes prior to the start of each session, IP at a volume of 2 ml/kg. Daily sessions were approximately 15 minutes in duration. Each session started with a 30 second "test period", during which no reinforcement was available. This period was followed by a 14-1/2 minute training period, during which

reinforcement was contingent upon pressing the left or right lever, depending on the drug (amph or ND) condition. On the seventh day, the VI-12 sec reinforcement schedule was replaced by a tandem reinforcement schedule: a variable interval 12-second phase was followed by a FR 4 phase. The VI 12-second phase started again after each reinforcement. After training for about 20 days, the duration of daily sessions was reduced to ten minutes.

Training Condition Test Sessions: After the left/right lever response distribution during the 30-sec test period, and during the entire test-training session indicated that discrimination was established, the rats were presented with longer (120 sec) test periods on certain days. An approximately eight-minute training period immediately followed these test sessions. The criterion level for discrimination performance during the initial two-minute test period was 85% of total responses emitted on the drug-appropriate lever. These test sessions were given on the day after a training day with the opposite drug treatment. More test sessions with training drugs were given occasionally throughout the experiment to ensure that basic discrimination was still present.

Test Phase: Test periods similar to the two minute test periods described above were conducted for various amph, etoh, and combination treatments. However, during

these "novel treatment" test sessions, the rats were removed from the chamber at the end of each test period (120 sec), to prevent reinforcement for responses on either lever while in a novel drug state, since the animals were only to be reinforced after treatment with amph or ND. Each test phase was followed by two to three training days, some of which were training-condition (120 sec) test sessions. These training sessions were interspersed among the test sessions to maintain the basic operant response, as well as the amph/non-drug discrimination.

Experimental Design: The following novel drug treatments were given on test days in this sequence: (all dosages expressed in mg/kg) amph .3, amph.3 plus ethanol 300, ethanol 300, amph .3 plus ethanol 150, ethanol 150. As described above, at least two training days were interspersed between these test sessions. The sequence of novel drug treatments was then run through again, this time in reverse order as a counterbalancing feature. At least two test session results (data points) were collected for each drug treatment, one preceded by a amph training day, and the other by a non-drugged training day. If the percentage of amph responses for the two trials for a given novel drug treatment were quite dissimilar, a third trial for this treatment was conducted after the second sequence of treatments was completed. In some cases the variability among the trials was still

high, in such cases a fourth trial was conducted. When four data points for a given treatment were collected, the preceding training day treatments were again balanced.

Results

Training Phase

Results reported from the training phase refer to responses emitted during the initial thirty-second non-reinforcement period of each test session. Acquisition of amph-ND discrimination was reached by the 20th day of training. At this time, all rats had reached a performance level of at least 85% correct for both the amph and the ND condition. The means and standard error of the means (SEM) of percent of correct responses emitted during the 120-sec test periods (training condition test sessions) were as follows: after amph treatment (1.2 mg/kg) 92.1 (±8.9), after ND treatment 93.0 (±9.1).

Test Phase

Even though all animals reached the criterion level of 85 percent correct responses after 20 days of training with amph 1.2 mg/kg and ND, some needed a higher number of interspersed training days (once testing had started) in order to maintain the above-stated criterion.

Treatment with .3mg/kg Amph:

The group means of percent responses on the amph lever during test sessions are presented in Table 1. After treatment with amph .3mg/kg, the animals' responses were intermediate (39.9, ± 3.3) percent on the amph lever) and different from those seen after either ND (17.9, ±1.6) or amph 1.2 mg/kg treatment.

Treatment with Etoh:

Response distribution after etoh 150 mg/kg alone (mean and SEM: 13.8, ±3.2), as well as after treatment after etoh 300 mg/kg alone (mean and SEM: 21.3, ±4.6) did not differ significantly from that seen after ND treatment (refer to Table 1).

Amph-Etoh Contamination Treatment:

The combination of etoh (150 and 300 mg/kg) with amph .3 mg/kg produced a higher percentage of "amph" responses then did amph .3 treatment alone (mean and SEM amph .3 and etoh 150: 62.6, ±9.9; for amph .3 and etoh 300: 56.6, 4.6). These results were analyzed by means of a 2 (amph 0 and .3 mg/kg) by 3 (etoh 0, 150 and 300 mg/kg) ANOVA, repeated measures on both IV's, in order to determine the significance of the differences among the various treatment conditions (refer to Table 2). The results showed a significant main effect for amph ($f = 42.9$ $df = 1,8$ $p < .01$) and a significant interaction effect for amph-etoh combinations ($f = 4.2$ $df = 2,16$ $p < .05$). Further analyses was performed by means of the Newman-Keuls test which permitted comparisons between specific pairs of treatment conditions refer to (Table 3). These tests revealed a significant difference between amph .3mg/kg alone and amph .3 in combination with etoh 150 mg/kg (N-K D = 23.3), and between amph .3 mg/kg alone and amph .3 in combination with etoh 300 (N-K D = 17.3), which indicates potentiation of the "amphetamine" effect

at these dosages. Both of these Neuman-Keuls tests were significant at the $p < .05$ level. No significant difference was found between results of treatment with amph .3 mg/kg in combination with etoh 150 mg/kg and amph .3mg/kg in combination with etoh 300 mg/kg.

Effects in Individual Animals:

Since the present data were summarized and presented in the form of means, it is necessary to mention the response pattern of the individual animals. For five out of nine rats potentiation was most evident after combination treatment with amph .3 mg/kg and etoh 150 mg/kg (mean percent amph response: 81.7). Four out of nine rats showed greatest potentiation after treatment with amph .3 and etoh 300 (mean of percent amph response: 62.7). Also, four out of nine animals showed potentiation at both etoh dosages (two out of these four rats showed highest potentiation after treatment with etoh 150 mg/kg, and two after treatment with etoh 300 mg/kg).

Discussion

The basic strategy used in this experiment to examine possible potentiation of the amph-stimulus effect, required testing animals trained to discriminate amph 1.2mg/kg from ND with a lower dose of amph, intended to produce responses between and different from both training-drug conditions. Two recent reports (Glennon, Yong, Hack and McKenney, 1984; and Minnema, 1984) have determined the ED_{50} for rats trained to discriminate saline from amph doses slightly lower than that used here.

In the present experiment, the intermediate dose used for testing was .3mg/kg amph. At this dose the animals emitted less than half amph appropriate responses. These results are consistent with the findings by Minnema (1984) and Glennon et al. (1984). These investigators tested the discriminative effects of amph and LSD, as well as stimulus generalization to these agents. Results of these studies, using amph as a training drug, indicated that a dose less than half of the training dose will result in 50% amph appropriate responses at least 50% of the time. For example, in Glennon's experiment, rats were trained to discriminate 1.0 mg/kg amphetamine sulfate from saline, and the ED_{50} value obtained was .42 mg/kg.

In the present study, when animals trained to discriminate amph (1.2 mg/kg) from ND were tested with amph .3 mg/kg they emitted 39 percent amph-appropriate response.

When tested with etoh alone (150 and 300 mg/kg) the ND appropriate response was emitted, showing that etoh did not elicit the amph-trained drug response. This finding is supported by several other studies, (Barry & Krimmer, 1972; Overton, 1964) results of which have indicated that central cues produced by effective doses of etoh are dissimilar to those produced by central stimulants such as amph. In one study by Overton (1964), rats trained to discriminate pentobarbital from saline, chose the saline response in tests with d-amphetamine, bemegride, LSD and physostigmine. In another study of the discrimination between pentobarbital and saline (Barry and Krimmer, 1972), summarized by Barry (1974), the rats chose the saline response in tests with d-amphetamine, chlorpromazine and nicotine. In the study by Kubena & Barry (1969), rats trained to discriminate etoh from saline chose the saline response in tests with d-amphetamine and chlorpromazine. Likewise, tests with sedative drugs (pentobarbital, etoh of chlordiazeoxide), summarized by Barry (1974), showed that the saline response was chosen by rats trained to discriminate other categories of drugs (nicotine, tetrahydrocannabinol or chlorpromazine) from saline.

Similar results were also obtained by Ando (1975) who investigated discriminative control of operant behavior by administration of such drugs as amph or etoh. His results indicated that rats trained with amph vs. saline emitted the amph-appropriate response only when tested with other CNS stimulants such as metaamphetamine or cocaine, but not when tested with etoh.

In the present study, combination treatment with amph (.3 mg/kg) and etoh (150 and 300 mg/kg) resulted in a potentiation of the amph cue. Similar results were obtained by Duncan (1980 unpublished), who trained rats to discriminate amph (.8 mg/kg) from saline. In that study, Duncan tested the rats with amph .2 and .4 mg/kg, etoh 400 and 800 mg/kg and with combinations of these etoh and amph doses. Potentiation was highest after combination treatment of amph .2 mg/kg and etoh 400 mg/kg.

Another relevant study is that by Schechter (1985) who trained rats to discriminate 600 mg/kg etoh from saline. Once the response to etoh was established, apomorphine (.16 mg/kg) was administered, to test how pretreatment with this drug would effect the discriminative response. Results indicated that pretreatment with .16 mg/kg apomorphine produced an increased discrimination at all etoh doses. Thus, the co-administration of this direct dopaminergic agonist produced a significant increase in the rat's ability to differentially respond to the interoceptive cue produced by various doses of etoh.

Apomorphine-only treatment did not produce any etoh responses. A possible link between the behavioral effects of etoh and it's ability to affect brain dopamine levels was discussed by Schechter who further points out the parallelism between etoh and apomorphine, and suggests that the drugs are acting (at least partially) via a common site and/or mechanisms of action. Further, it seems that part of etoh's discriminative properties may involve central dopaminergic activity. Further studies are necessary to investigate the importance and involvement of dopamnine receptors in mediating or modulating etoh intoxication (Schechter, 1985).

Schechter's experiment and the present study complement each other nicely since he showed that a DA-agonist other than amph can potentiate the etoh cue, while this experiment demonstrates that etoh can potentiate the amph cue, which has been clearly shown to be mediated by DA receptor activation.

Although the present results indicate potentiation of the amph cue at both doses of etoh (150 and 300 mg/kg), it is necessary to point out certain dose differences among animals. As stated before, five out of nine animals showed highest potentiation after pretreatment with etoh 150 mg/kg (followed by administration of amph .3 mg/kg). This suggests that potentiation was "strongest" at these dosages. At best this can be explained by the fact that etoh is a DA and NE agonist, as is amph. However, this

agonistic effect is not one of the main mechanisms of action of etoh, and seems to be evident only after administration of small doses of etoh or shortly after administration.

The influence of etoh on amph effects seen in the present study are consistent with reports of many previous experiments in which other behavioral effects of amph were examined. As reported by Todzy et al. (1978), Duncan et al. (1979) and Holloway (1971), etoh can potentiate the locomotor activity - increasing effects of amph. In the Todzy experiment, potentiation of the SMA - stimulant effect was seen at the lowest etoh dose administered (800 mg/kg in combination with 1 mg/kg amph). Duncan & Cook (1981) reported that the combination of etoh 400 mg/kg and amph .8 mg/kg potentiated the amph-stimulant effect, but that higher doses of etoh (800, 1200 and 1600 mg/kg) counteracted the amph-produced increment in SMA. One of the advantages in utilizing the drug- discrimination paradigm is that it is sensitive to very low drug dosages. However, high doses of any drug tend to disrupt operant behavior, so that high doses usually cannot be tested. For instance, Duncan (unpublished, 1980) reported that many rats simply did not respond after administration of 800 mg/kg etoh. Hence, possible potentiation of amph effects by high doses of etoh cannot be investigated through this paradigm.

The above reported findings (Todzy et al., 1978; Duncan et al., 1981; Holloway, 1971) are generally consistent with those of the present study, since potentiation tended to be greater after treatment with amph .3 mg/kg and etoh 150 mg/kg, indicating potentiation of the amph appropriate response with a low dose of etoh.

Although, as briefly mentioned before, etoh may have some DA-agonistic effects, when administered alone, it does not produce any amph response. Likewise, a test with apomorphine will not result in any etoh response in animals trained to discriminate etoh from saline. However, the combined administration of such drugs will potentiate the training drug response. The mechanisms underlying this interaction are not clear at this point. Possible involvement of dopaminergic systems seems evident. Further studies are necessary to investigate the importance of dopaminergic mechanisms and their role in etoh-amph interactions, and the involvement of these mechanisms in mediating or modifying etoh-intoxication.

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Table 1

Mean Percent Responses on Amphetamine Lever After Non-Drug Amphetamine, Ethanol, and Combination Ethanol-Amphetamine Treatment

Amph Dose	Ethanol Dose		
	0	150 mg/kg	300 mg/kg
0	17.9 (±1.6)	13.9 (±3.3)	21.3 (± 4.6)
.3 mg/kg	39.3 (±3.3)	62.6 (±9.9)	56.6 (± 4.6)

Table 2

ANOVA SUMMARY

Source	SS	df	ms	F
Amph	16712.963	1	16712.963	42.96
Etoh	1211.3704	2	605.68	2.976
Amph x Etoh	1681..148	2	840.54	4.239

Table 3

Neuman-Keuls Comparisons

A. Amph .3 mg/kg and Etoh 0 Compared to Amph .3 mg/kg plus ETOH 150 mg/kg

	Amph .3 + Etoh 0 39.3	Amph .3 + Etoh 150 62.6	N-K*
39.3	--	23.3*	15.3
62.6		--	

B. Amph .3 mg/kg and Etoh 0 Compared to Amph .3 mg/kg plus Etho 300 mg/kg

	Amph .3 + Etoh 0 39.3	Amph .3 + Etoh 300 56.6	N-K*
39.3	--	17.3*	15.3
56.6		--	