Discriminative Stimulus Properties of Amphetamine and Structurally Related Phenalkylamines

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I. INTRODUCTION

Amphetamine serves as an effective discriminative stimulus in animals and is one of the oldest and most popular of training drugs. In tests of discriminative control of behavior, animals are normally trained to distinguish (i.e.

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discriminate) between the effects produced by one agent versus those produced by another. The second agent may be a different drug, a different dose of the first drug, or vehicle. Once the animals have been trained to discriminate a particular agent (i.e. the training drug) from, for example, saline vehicle, various studies can be conducted. Such studies can be directed towards the characterization and further understanding of the stimulus properties of the training drug. Or, once the training drug has been well studied and its stimulus properties are relatively well understood, studies can be conducted in order to investigate novel agents that (a) might produce stimulus effects similar to those of the training drug, in tests of stimulus generalization, and/or (b) might antagonize the stimulus effects of the training drug, in tests of stimulus antagonism. Such studies can have direct application to explorations of structure-activity relationships, and investigations of metabolism and mechanism of action. Because of amphetamine's extensive use as a training drug, it was felt that a thorough review of this topic might serve as a classical example of the application of drug discrimination to CNS pharmacology/medicinal chemistry. There have been no detailed reviews on amphetamine as a discriminative stimulus, although two reviews on the stimulus properties of psychomotor stimulants (covering the literature up to about 1978) have appeared.[1,2] Amphetamine, being a prototypic member of this class of agents, was accorded its share of attention in these reviews. As a consequence, this present review will place somewhat less emphasis on the earlier discrimination studies involving amphetamine and will attempt to highlight those areas that either are novel or that were covered in less detail in the two earlier reviews.

II. METHODOLOGICAL CONSIDERATIONS

A number of different procedures have been developed to study the discriminative stimulus properties of amphetamine (Table I). Each of them arranges an environment in which behavioral responses have particular consequences under drug and vehicle treatment conditions. The types of apparatus, for example, differ in the behavioral response required, the kinds of consequences, and the relative ease with which the responses may be measured. The operant chamber and T-maze are particularly well-suited for drug discrimination tasks, that is, for those in which a subject must choose between two (or more) alternatives (e.g. levers or alleyways). In these studies the

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Table I
Animal Species, Apparatus and Tasks Employed in Discrimination Studies Using Amphetamine as the Training Drug.

Species/Sex	Apparatus[a]	Task[b]	Reference
Rat (F)	2-Lever	Multiple FR-50 DRL-20 sec	Harris and Balster (1971)[3]
Rat (M)	2-Lever	Multiple DRL-15 sec DRL-15 sec	Waters et al (1972)[4]
Rat (F)	3-Chambers	Shock-escape	Schechter and Rosecrans (1973)[5]
Rat (M)	2-Lever	DRL-15 sec	Huang and Ho (1974)[6]
Rat (M)	2-Lever	DRL-15 sec	Huang and Ho (1974)[7]
Rat (M)	2-Lever	DRL-15 sec	Huang and Ho (1974)[8]
Rat (M)	2-Lever	DRL-15 sec	Jones et al (1974)[9]
Rat (M)	2-Lever	Tandem (VI-1', RF-10)	Kuhn et al (1974)[10]
Rat (M)	2-Lever	DRL-15 sec	Ho and Huang (1975)[11]
Rat (F)	3-Chambers	Shock-escape	Schechter and Cook (1975)[12]
Rat (M)	2-Lever	RI-15 sec	Tilson et al (1975)[13]
Rat (M)	T-maze	(+)-Reward (food)	Bueno et al (1976)[14]
Rat (M)	2-Lever	FR-10	Colpaert et al (1976)[15]
Rat (M)	2-Lever	DRL-15 sec	Ho et al (1976)[16]
Rat (M)	2-Lever	DRL-15 sec	Jones et al (1976)[17]
Rat (M)	2-Lever	VI-15 sec	Rosecrans et al (1976)[18]
Rat (F)	2-Lever	Tandem (VI-1', FR-10)	D'Mello and Stolerman (1977)[19]
Rat (M)	2-Lever	VI-15 sec	Schechter (1977)[20]
Rat (M)	2-Lever	FR-10	Colpaert et al (1978)[21]
Rat (M)	2-Lever	VI-15 sec	Schechter (1978)[22]
Rat (M)	2-Lever	FR-10	Wilson and Buelke (1978)[23]
Cat (F)	2-Lever	FR-10	Kilbey and Ellinwood (1979)[24]
Gerbils (M)	T-maze	Shock-escape	Jarbe and Kroon (1980)[25]
Rat (M)	2-Lever	VI-15 sec	Schechter (1980)[26]
Rat (M)	2-Lever	Shock-avoidance	Shannon (1980)[27]
Rat (M)	2-Lever	Tandem (VI-30 sec, FR-5)	Silverman and Ho (1980)[28]
Rat (M)	2-Lever	Tandem (VI-30 sec, FR-5)	Silverman and Ho (1980)[29]
Rat (M)	2-Lever	VI-20 sec	Barrett and Leith (1981)[30]
Rat (M)	2-Lever	Tandem (VI-1', FR-10)	D'Mello (1981)[31]
Rat (M)	2-Lever	Tandem (VI-1', FR-10)	Stolerman and D'Mello (1981)[32]
Rat (M)	2-Lever	VI-20 sec	White and Barrett (1981)[33]
Rat (M)	2-Lever	FR-10	D'Mello (1982)[34]
Pigeon (M)	2-Key	FR-15	Jarbe (1982)[35]
Rat (M)	2-Lever	VI-20 sec	Barrett and Stearn (1983)[36]
Rat (M)	T-maze	Shock-escape	Jarbe and Kroon (1983)[37]
Mouse (M)	2-Photo-beam cells	FR-20	Snoddy and Tessel (1983)[38]
Human (M/F)	Verbal response	Reward[c]	Chait et al (1984)[39]
Rat (M)	2-Lever	VI-15 sec	Glennon and Young (1984)[40]

(*continued*)

Table I
(Continued)

Species/Sex	Apparatus[a]	Task[b]	Reference
Rat (M)	2-Lever	VI-15 sec	Glennon and Young (1984)[41]
Rat (M)	2-Lever	VI-15 sec	Glennon et al (1984)[42]
Rat (M)	2-Lever	VI-15 sec	Glennon et al (1984)[43]
Rat (M)	2-Lever	FR-10	Minnema and Rosecrans (1984)[44]
Rat (M)	2-Lever	FR-10	Porsolt et al (1984)[45]
Rat (M)	2-Lever	VI-15 sec	Glennon et al (1984)[55]

[a]A 2-lever procedure refers to a typical operant chamber.
[b]See text for explanation of tasks.
[c]Monetary reward was used.

investigator records a change in the probability of occurrence of a specified response, when the delivery of a particular reward is made contingent upon the completion of that response. The responses most commonly studied are lever pressing and T-maze running, using rats, mice, gerbils, or cats as subjects, or key pecking, using pigeons.

A. Operant Methodology

Operant learning involves responses controlled by an organism. The responses of a subject operate on the environment to produce reinforcing consequences. Responses that lead to satisfying effects are strengthened. Operant learning requires that the correct response usually be followed by a reinforcing event. Responses may be strengthened by either positive or negative reinforcement. Positive reinforcement is provided by the presentation of food, water, etc.; these reinforcers are frequently referred to as rewards. Negative reinforcement is provided by the opportunity to escape from shock, or some other aversive event. Thus, positive reinforcers make the actions they follow more probable by their occurrence, negative reinforcers make the actions they follow more probable by their termination or nonoccurence.

A piece of laboratory equipment called an operant chamber is the setting for experiments in operant learning. On the chamber's inside wall is a device the subject can operate, for example, a lever. Near the lever is an opening in which the reinforcer appears. A typical rat chamber has a tray on which small pellets of food can be delivered or an opening permitting the animal access to a water dipper. Finally, the floor of the operant chamber may be comprised of metal rods through which electric shocks can be administered for aversive events.

A typical operant experiment might involve a rat that is food deprived (the animal's weight is reduced to ca. 75–80% of its expected free-feeding body weight by partial food deprivation) and placed in an operant chamber. However, the rat does not rush over to the lever and press it. The initial training period is referred to as "shaping."

Shaping is a process in which a series of responses by the rat are developed, by the investigator, in the direction of a final, desired response. The exper-

imenter shapes the lever-pressing response. The rat is initially rewarded for approaching the lever. Next, the animal is rewarded for touching the lever, and finally for pressing the lever. The desired final response in this case, pressing the lever, has been achieved by a series of successive approximations in which only the responses that approximate the desired response are reinforced. T-maze studies also sometimes permit some pretraining habituation to the apparatus in the form of a period of unrewarded exploration of the alley and/or the rats may be placed in the goal box and are allowed access to food.

Reinforcement is the pivotal concept in operant learning; it enables an organism to learn the correct response. The subject learns the correct response because its response is reinforced. If every response is reinforced, continuous reinforcement (CRF) or fixed ratio one (FR1) responding is said to be controlling behavior. More often, reinforcement is programmed on various schedules to control different rates and patterns of responding. A distinction can be made between simple schedules of reinforcement in which a single schedule is continuously in effect, and higher-order schedules, in which the subject is exposed to more than one simple schedule. The two most popular simple schedules are ratio and interval schedules, each of which can be either fixed or variable. In a fixed ratio (FR) schedule an animal must complete a fixed number of responses in order to obtain each reinforcer. In a fixed-interval (FI) schedule, reinforcement is delivered for the first response after a given interval of time has elapsed. On variable-ratio (VR) and variable-interval (VI) schedules, the number of responses required, or seconds elapsing, before reinforcement is delivered varies around the mean value specified by the schedule. The differential reinforcement of low rate (DRL) schedule is also used quite often. Under this schedule an organisms' responses are reinforced only when emitted at a sufficiently low rate. For example, on the DRL 15 sec schedule only responses that follow one another by more than 15 seconds are eligible for reinforcement. If responses do occur during the interval, the timing of the interval is restarted. Thus, reinforcement is contingent upon the occurrence of a response separated from the preceding response by some specified interval. Higher-order schedules involve more than one simple schedule. A multiple schedule, for example, involves two or more schedules, with each component (i.e. schedule) signaled by a different external cue (e.g. a light or tone).

Reinforcement follows the completion of each component. Another higher-order schedule is a tandem schedule, which also involves two or more simple schedules. However, the subject must satisfy the requirements of the first schedule in order to enter into the second schedule. Reinforcement occurs after both schedule components have been completed.

B. Two-lever Drug Discrimination

General Concepts

If the drug discrimination paradigm is to be successful, then the correct response must occur at the proper moment. In other words, the response (e.g. correct lever pressing, under a particular schedule of reinforcement, in

the operant chamber) must be performed when a particular drug/vehicle stimulus is presented. Thus, the rat must press, for example, the left lever when given drug and press the right lever when given vehicle. In drug discrimination experiments, the subject is taught to distinguish between the presence and absence of a particular dose (i.e. the training dose) of a particular compound (i.e. the training drug). This is achieved by reinforcing only the correct responses that are made in the presence of either the drug or vehicle stimulus, and by not reinforcing (or, alternatively, by punishing with shock) any incorrect responses made in the presence of either drug or vehicle stimulus.

Extinction

In operant learning, extinction occurs when the reinforcer is withheld. This causes the subject to respond to the drug/vehicle stimulus with decreasing frequency, until the animal ceases to respond altogether. In the operant box the lever-pressing activity will ultimately be extinguished if the reinforcing event(s) cease to be presented. The time required for extinction of a response pattern varies according to the method of reinforcement. Generally, the stronger the conditioning, the more difficult it is to extinguish. In drug discrimination tasks, stimulus control often is evaluated during occasional short periods (at the beginning of the behavioral session) of extinction. The rationale for using extinction sessions is that an animal's performance (i.e. discrimination learning) should be evaluated independently of the reinforcer.

Subjects

An examination of Table I reveals that the use of the drug discrimination paradigm to study the effects of amphetamine is not confined to a single species. Organisms from a number of species have served as subjects in amphetamine discrimination studies: for example, rat, mouse,[38] gerbil,[25] pigeon,[35] cat,[24] and human.[39] The most popular species has been the rat. Generally the results of drug discrimination studies involving amphetamine have shown good agreement between species, and within a species (e.g. in studies using male and female rats). Specifically, the amphetamine stimulus does not generalize to LSD (rats[5,10,27] and pigeons[35]), fenfluramine (rats[5] and gerbils[25]), and p-hydroxyamphetamine (rats,[7,9] gerbils,[25] and pigeons[35]). Complete generalization has been reported for cocaine (rats[7,16,19,21] and cats[24]). Similarly, the dopamine antagonist haloperidol antagonizes the amphetamine stimulus in rats,[12,15,21,28] gerbils,[25] and pigeons.[35] Inconsistent results, in tests of stimulus generalization, have been reported for morphine (pigeon: no substitution;[35] cat: partial substitution[24]), apomorphine (pigeon[35] and gerbil:[25] partial substitution; rat: partial[11,14] or complete substitution[12,20,33]), and Δ^9THC (gerbil[25] and pigeon:[35] no substitution; rat: no substitution[10] or complete substitution[14]).

Within species, consistent results have been obtained between sexes in the rat with cocaine (generalization[7,16,19,21]) and LSD (no generalization).[5,10,27] Complete or partial generalization of the amphetamine stimulus to apomorphine has been reported in four studies using male rats,[11,14,20,33] while one study which used female rats reported complete generalization.[12]

C. Comparisons with Different Behavioral Tasks

A comparison of (+)-amphetamine discrimination studies using three different behavioral tasks, i.e. 3-chambered shock-escape, T-maze, and 2-lever operant choice, indicates fairly consistent agreement in generalization and antagonism results. Specifically, in the 3-chamber shock-escape procedure and in the 2-lever operant choice task (+)-amphetamine stimulus generalization did not occur with mescaline [5,7,10,28] or LSD,[5,10,35] whereas generalization did occur upon administration of (−)-amphetamine.[5,7,9,22,41] In all three tasks haloperidol was found to attenuate the (+)-amphetamine-stimulus,[12,21,25,28] while propranolol did not block the (+)-amphetamine cue in the 3-chamber shock-escape task[12] or the 2-lever operant choice task.[35] Partial or complete (+)-amphetamine stimulus generalization to apomorphine has been reported in all three tasks.[11,12,14,20,25,32,33,35] Inconsistent (+)-amphetamine generalization results have been obtained with Δ^9THC. One study, in which a T-maze apparatus was used, reported complete generalization,[14] whereas in another T-maze study[25] and in two 2-lever operant choice task studies,[10,35] a lack of stimulus generalization was reported.

D. Comparisons with Different Operant Schedules

A review of the existing literature reveals that good agreement is generally found amongst the results of stimulus generalization and stimulus antagonism studies, using (+)-amphetamine as the training drug, between different laboratories, and using different operant schedules of reinforcement. For example, using animals trained to discriminate (+)-amphetamine from vehicle under DRL or tandem schedules of reinforcement, stimulus generalization was reported to occur with methamphetamine[7,10] and methylphenidate.[16,28] Stimulus generalization did not occur with DOET[7,28] or mescaline.[7,10,28] A discrepancy of results is noted with 4-methylamphetamine, where both partial generalization,[7] and no generalization[28] have been reported. In stimulus antagonism studies, the (+)-amphetamine-stimulus was not attenuated by cinanserin[11,28] or methysergide.[11,28]

Under DRL and VI schedules of reinforcement (+)-amphetamine-stimulus generalization has been reported with (−) amphetamine.[9,22,41] Partial or complete generalization has been reported with 4-methoxyamphetamine[7,55] and amantadine.[11,20] Consistent generalization results have been obtained under tandem and FR schedules, with no (+)-amphetamine-stimulus generalization to Δ^9THC[10,35] or LSD,[10,35] and under tandem and VI schedules, with no (+)-amphetamine stimulus generalization to S(+)-DOM,[28,41] and partial generalization to R(−)-DOM.[13,28]

In animals trained to discriminate (+)-amphetamine from vehicle under DRL, tandem, and FR schedules of reinforcement, stimulus generalization has been reported to occur to cocaine,[7,16,19,21,24,32] but not to *p*-hydroxyamphetamine.[7,32,35] Under DRL, tandem, and VI schedules, however, no generalization, or partial generalization, has been observed with 2,5-DMA[7,28,55] and DOM.[7,28,40] In stimulus antagonism studies, the (+)-amphetamine-stimulus has been blocked by haloperidol under tandem,[28] VI,[26] and FR[21,35] sched-

ules of reinforcement. Finally, the (+)-amphetamine-stimulus generalized partially or completely to apomorphine under DRL,[11] tandem,[32] VI[20,33] and FR[35] schedules of reinforcement.

III. PROPERTIES OF THE AMPHETAMINE STIMULUS

A. Dose and Temporal Parameters

(+)-Amphetamine has been used as a training drug in drug discrimination studies involving a variety of animal species (Table I), but the rat seems to be the most common subject; although training doses of (+)-amphetamine have ranged from 0.5 to 8.0 mg/kg (in a single study involving human subjects, an oral dose of 10 mg/kg was employed[39]), the most commonly employed dose (in studies involving rats) is either 0.8 or 1.0 mg/kg (Table II). (−)-Amphetamine has not seen extensive application as a training drug, but here also, doses of 0.8 to 1.0 mg/kg appear to be most common.[6,23] Racemic amphetamine has been used as a training drug at doses ranging from 0.16 and 0.3 to 2.5 mg/kg.[4,15] Typically, amphetamine is administered via intraperitoneal injection, although subcutaneous,[21,27,33] intramuscular,[24,35] oral,[39] and intraventricular[2] routes have also been employed. When amphetamine is administered via the intraperitoneal route to rats, the optimal pre-session injection interval (i.e. injection-to-test interval) seems to be 15 (10–30) min. Similar pre-session injection intervals have been used with other species of animals, including gerbils,[25] pigeons[35] and cats.[24]

Acquisition of the amphetamine stimulus can be measured, in discrimination studies involving operant responding, by plotting percent amphetamine-appropriate responding as a function of time. The rate of acquisition appears to be somewhat dependent upon the dose, particular procedure and schedule of reinforcement being employed. Typical learning curves for acquisition of the (+)-amphetamine stimulus are shown in references 7 and 28.

In a study involving intraperitoneal administration of (+)-amphetamine (pre-session injection interval = 15 min) to rats, Silverman and Ho[29] found that the onset of effect was quite rapid, but not instantaneous. Tested 1 to 2 min post-injection, the animals responded on the saline-appropriate lever, whereas 3 to 4 min post-injection, 55–65% of responses were made on the amphetamine-appropriate lever. Thereafter, responding was primarily on the amphetamine-appropriate lever. The authors commented that these results were consistent with the report that, in single unit recording studies, the central effects of amphetamine were evident within 5 min of intraperitoneal administration of amphetamine.

Drug discrimination studies are rather unique in that, unlike with most other pharmacological techniques, a given agent can have more than a single ED_{50} value. The ED_{50} value of an agent is a function both of the training drug and its training dose. As the training dose of the training drug increases, the ED_{50} value of the training drug increases,[46] and the ED_{50} value of agents to which the training drug generalizes can also increase. For example, Waters et al[4] obtained an ED_{50} for (±)-amphetamine of 0.15 mg/kg in a group animals trained to discriminate 0.3 mg/kg of racemic amphetamine from saline; in another group of animals trained to discriminate 2.5 mg/kg, the ED_{50} was approximately 1 mg/kg. Kuhn et al[10] reported an (estimated) ED_{50} for (+)-

Table II
Doses, Route of Administration and Temporal Parameters Employed in Studies of the Stimulus Properties of (+)-Amphetamine in Rats.

Dose (mg/kg)	Route of Administration[a]	Pre-session Injection Interval (min)[b]	Extinction Period (min)[c]	Ref.
4.0	i.p.	10	—	(5)
0.8	i.p.	15	5/10	(6)
0.8	i.p.	15	5/10	(7)
0.8	i.p.	15	5/10/15	(8)
0.8	i.p.	15	10	(9)
1.0	i.p.	30	5	(10)
0.8	i.p.	15	5/10	(11)
2.0	i.p.	15	—	(12)
1.0	—	15	2.5	(13)
1.0	i.p.	15	—	(14)
0.8	i.p.	15	15	(16)
0.8	i.p.	15	10	(17)
1.6	i.p.	15	10	(17)
2.4	i.p.	15	10	(17)
0.9	i.p.	15	2.5	(18)
1.0	i.p.	30	5	(19)
0.8	i.p.	15	2.5	(20)
1.25	s.c.	30	—	(21)
0.8	i.p.	15	2.5	(22)
0.6	i.p.	15	2.5	(26)
1.0	s.c.	30	—	(27)
1.0	i.p.	15	10	(28)
1.0	i.p.	15	10	(29)
0.5	i.p.	15	2.5/5	(30)
1.0	i.p.	15	2.5/5	(30)
1.5	i.p.	15	2.5/5	(30)
1.0	i.p.	10	5	(31)
1.0	s.c.	15	5	(33)
1.0	i.p.	30	—	(34)
0.5	i.p.	15	2.5	(36)
1.5	i.p.	15	2.5	(36)
8.0	i.p.	20	—	(37)
1.0	i.p.	10	—	(38)
3.2	i.p.	10	—	(38)
1.0	i.p.	15	2.5	(40)
1.0	i.p.	15	2.5	(41)
1.0	i.p.	15	2.5	(42)
1.0	i.p.	15	2.5	(43)
0.9	i.p.	15	—	(44)
0.6	i.p.	30	—	(45)

[a]I.p. = intraperitoneal; s.c. = subcutaneous.
[b]Time between administration of (+)-amphetamine and testing.
[c]Period of non-reinforced responding.

amphetamine of 0.23 mg/kg in animals trained to discriminate 1.0 mg/kg of (+)-amphetamine from saline, whereas Schechter[22] obtained an ED_{50} value of 0.122 mg/kg for this same agent in animals trained to discriminate 0.8 mg/kg of (+)-amphetamine from saline.

Several investigators have studied the duration of action of the amphetamine stimulus. In addition to their standard 30-min pre-session injection

interval, Kuhn et al[10] conducted tests of stimulus generalization in rats with (+)-amphetamine using pre-session injection intervals of 60, 90, 120 and 150 min. With the 60-min interval, percent (+)-amphetamine-appropriate responding decreased from 80% to 74%; when the animals were tested 90 min post-injection, responding was about equally distributed on the amphetamine and saline levers. With pre-session injection intervals of 120 and 150 min, amphetamine-appropriate responding was decreased to 33% and 14%, respectively. Huang and Ho[7] obtained comparable results in a similar study. In this latter study, rats were trained to discriminate 0.8 mg/kg of (+)-amphetamine from saline using a 15-min pre-session injection interval. The percent amphetamine-appropriate responding dropped sharply after 45 min, and was less than 50% after 120 min. One of the most comprehensive temporal studies is that of Jones et al,[17] wherein three groups of rats were trained to discriminate (+)-amphetamine (0.8, 1.6 and 2.4 mg/kg) from saline using a 15-min pre-session injection interval. Following acquisition of the stimulus, the pre-session interval was varied from 0-240 min. The onset of stimulus properties was fairly rapid and occurred within 10 min; maximal effects were attained by 15–30 min, and stimulus effects were minimal or absent 120–240 min post-injection. The time-course of effects for each of the doses of (+)-amphetamine was identical (when each group was administered its respective training dose). However, when the animals were challenged with doses other than those under which they were trained, the time-course of effects varied as a function of training dose, test dose, and pre-session injection interval. The authors offer, as a possible explanation, that stimulus generalization curves depend not only upon the dose employed during training, but also upon the absolute dose administered during testing.[17]

The possibility exists that the time-course of effects may be different in different animals species; however, this has not been systematically examined. For example, variation of the pre-session injection interval from 15–120 min, in pigeons trained to discriminate (+)-amphetamine from saline, had little effect on amphetamine-appropriate responding.[35] Although the duration of action of amphetamine appears to be longer in pigeons than in rats, Jarbe has suggested, however, that this might be a reflection of the route of administration (intramuscular) rather than a species difference.[35]

B. Nature of the Stimulus

Stimulus Stereoselectivity

Optical isomers of biologically active agents commonly display differences in potency, and, on occasion, can also display differences in effect. Racemic amphetamine can be resolved to afford S(+)-amphetamine (i.e. dextroamphetamine or *d*-amphetamine) and R(−)-amphetamine (*l*-amphetamine) (Figure 1). With respect to their peripheral actions, S(+)-amphetamine and R(−)-amphetamine are essentially equivalent in potency; however, S(+)-amphetamine is several-fold more active than its enantiomer in producing central effects.

Although thorough dose-response studies were not always conducted, early work with (+)-amphetamine-trained animals demonstrated that (+)-am-

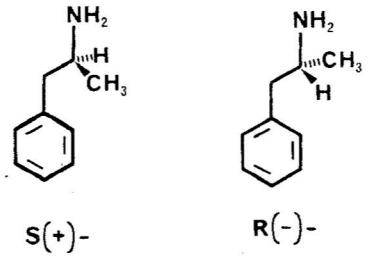


Figure 1. Structures of S(+)- and R(−)-amphetamine.

phetamine was two- to three-times more potent than its enantiomer.[5,7,9] Comparable results were obtained with animals trained to discriminate (−)-amphetamine from saline.[9,23] The first enantiomeric potency comparison based on ED_{50} doses was that reported by Schechter[22] in 1978. Using rats trained to discriminate 0.8 mg/kg of (+)-amphetamine on a variable interval 15 sec schedule of reinforcement, ED_{50} values of 0.122 and 0.603 mg/kg were obtained for (+)- and (−)-amphetamine, respectively; this constitutes an enantiomeric potency ratio of approximately five. More recently, we obtained, using animals trained to discriminate 1.0 mg/kg of (+)-amphetamine from saline under a similar schedule of reinforcement, ED_{50} values for (+)-, (±)- and (−)-amphetamine of 0.42, 0.62 and 1.23 mg/kg, respectively (Figure 2).[41] This results in an enantiomeric potency ratio of approximately three. Thus, it is probably safe to conclude from these and other studies that (+)-amphetamine is two- to five-times more potent than its enantiomer in tests of discriminative control of behavior in rats. However, species differences might exist; for example, Jarbe[35] has reported that (+)-amphetamine is less than twice as potent as (−)-amphetamine in pigeons.

It has been stated that amphetamine produces a discriminative stimulus that is stereospecific.[8] The term "stereospecific stimulus" should probably be reserved for those cases where optical isomers produce dissimilar effects. It would be more appropriate to refer to the amphetamine stimulus as being

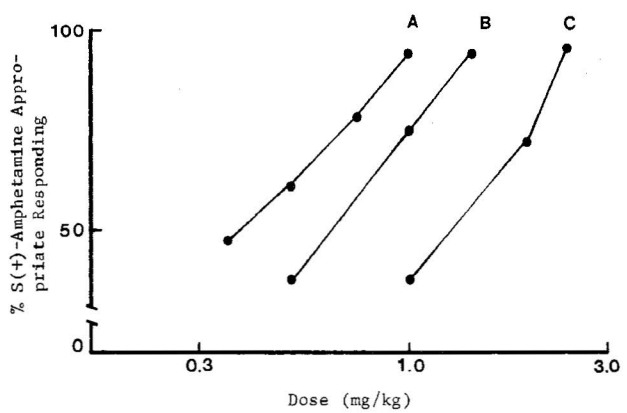


Figure 2. Dose-response relationships for S(+)-amphetamine (A), (±)-amphetamine (B), and R(−)-amphetamine (C) in rats trained to discriminate 1.0 mg/kg of (+)-amphetamine from saline.

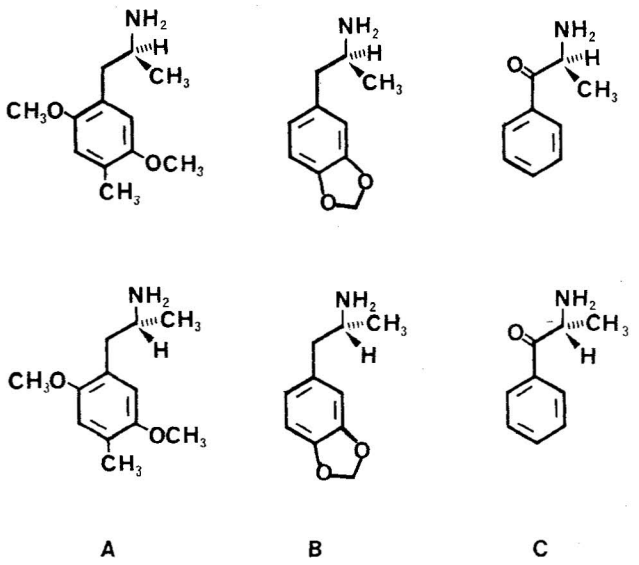


Figure 3. Structures of the S- (upper panel) and R-isomers (lower panel) of DOM (A), 3,4-MDA (B), and cathinone (C).

stereoselective, inferring that both optical isomers produce similar stimuli but that one isomer is more potent than the other.

Very few pairs of optical isomers have been studied in tests of stimulus generalization in amphetamine-trained animals. For example, Silverman and Ho[28] examined the effects of R(−)-DOM and S(+)-DOM; unfortunately, the (+)-amphetamine-stimulus did not generalize to either isomer. (+)-Amphetamine-trained rats recognized the effects produced by (±)- and S(+)-MDA, but not those produced by R(−)-MDA[41] (stereospecific generalization); as a consequence, an enantiomeric potency comparison was not possible. (+)-Amphetamine-stimulus generalization did occur, however, with the β-keto analog of amphetamine (i.e. cathinone); the ED_{50} values for S(−)-, (±)- and R(+)-cathinone (in rats trained to discriminate 1.0 mg/kg of (+)-amphetamine from saline) are 0.34, 0.72 and 4.41 mg/kg respectively.[42] Thus, the R/S potency ratio is about 10. (See Figure 3 for the structures of these agents.)

In rats, then, amphetamine produces a stimulus that is stereoselective with the S(+)-isomer being several-fold more potent than the R(−)-isomer. Amphetamine-stimulus generalization has also been demonstrated to occur to various agents and this generalization can be either stereoselective or stereospecific (with regard to the challenge drug).

Stimulus Specificity

The focus of many of the initial studies using amphetamine-trained animals was to establish the specificity of the amphetamine stimulus. Indeed, administration of various centrally acting agents, including analgesics, hallucinogens, monoamine oxidase inhibitors, CNS depressants, anorexics, etc. (Table III) did not, as a rule, result in stimulus generalization. The reverse situation

Table III
Results of Stimulus Generalization Studies Involving (+)-Amphetamine-trained Animals[a]

A. Agents to which (+)-amphetamine-stimulus generalization has been demonstrated to occur.

Amantadine[b](20)	Methamphetamine (7,10)
Amfonelic Acid (49)	Methylphenidate (7,16,31)
Apomorphine[b](12,20)	Piribedil (20)
Cathinone (42)	PMA (7)
Cocaine (7,16,19,21,24,35)	Propyl noraporphine (20)
Deprenyl (45)	THC[b](14)
Ephedrine (7)	Tranylcypromine (45)

B. Non-phenalkylamines to which the (+)-amphetamine-stimulus did not generalize.

Amantadine[c](11)	Moclobemide (45)
Apomorphine[c](11,14,25,44)	Morphine (24,35)
Atropine (11)	Nialamide (45)
Caffeine (10)	Nicotine (5,11)
Chlorpromazine (11)	Nikethamide (6)
Cimoxatone (45)	Oxotremorine (11)
Cinanserin (11)	Pargyline (45)
Clonidine[d](37)	Phentolamine (11)
Clorgyline (45)	Picrotoxin (6)
Ethanol (14)	Pimozide (11)
Iproniazid (8)	Propranolol[d](11)
LSD (5,10)	Psilocybin (10,28)
LY-51641[e](45)	Quipazine (44)
MD-240,928[f](45)	Strychnine (6)
α-Methyltyrosine (11)	THC[c](10,25,35)
Methysergide (11)	Toloxotone (45)

C. Phenalkylamine derivatives to which the (+)-amphetamine-stimulus did not generalize

2,5-DMA (7,28)	MDA[g](27)
DOET (7,8)	Mescaline (5,7,10,28)
(±)-DOM (7,27)	4-Me PIA[h](7,28)
(−)-DOM (13,28)	MMA[g] (7)
(+)-DOM (28)	Tyramine (7)
Fenfluramine (5,25)	PEA[i] (8)
4-OH PIA[h] (5,25,35)	

[a]Each entry is followed by the appropriate literature citation in parenthesis. See Tables I and II for dose of training drug, animal species, route of administration, etc. See also Table VI for results of those agents used in the SAR studies. [b]See Part B of this table. [c]See Part A of this table. [d]Produced approximately 50% amphetamine-appropriate responding. [e]N(2-Chlorophenoxyethyl) cyclopropanamine. [f]R(+)-3-[4-[(3-chlorophenyl)methoxy]phenyl-5-methylaminomethyl]-2-oxazolidinone. [g]See also Table VI. [h]kPIA = phenylisopropylamine. [i]PEA = phenethylamine.

also appears to be true; that is, animals trained to discriminate either ethanol, LSD, pentobarbital, mescaline or nicotine from saline did not recognize amphetamine as producing similar stimulus effect (see ref. 2 for a review). There are, however, a few exceptions that deserve further discussion. For example, Bueno et al[14] have reported that the stimulus effects of 5 mg/kg of Δ^9-THC are similar to those of 1.0 mg/kg of (+)-amphetamine (training drug). These findings are not supported by the results of at least three other studies using amphetamine as a training drug.[10,25,35] Furthermore, generalization did not

occur when (+)-amphetamine (0.5, 1.0 and 2.0 mg/kg) was administered to animals trained to discriminate 5.0 mg/kg of Δ^9-THC from saline.[14] The authors attempted to explain this one-way (i.e. asymmetric) generalization on the basis that chronic amphetamine administration might result in the formation of a false transmitter that could be involved in the stimulus properties of the drug.[14] While this is an interesting possibility, it has not received any further attention. A second exception is seen with the hallucinogen R(−)-DOM. Administration of 0.5 and 0.75 mg/kg of R(−)-DOM to animals trained to discriminate 1.0 mg/kg (+)-amphetamine from saline provided results (68% and 69% (+)-amphetamine-appropriate responding, respectively) that are claimed not to differ significantly from those obtained by administration of the training dose of the training drug (i.e. 76% (+)-amphtamine-appropriate responding).[13] In addition, animals trained to discriminate 0.75 mg/kg of R(−)-DOM (i.e. 74% R(−)-DOM-appropriate responding) from saline responded on the drug-appropriate lever when administered 0.75 mg/kg of (+)-amphetamine (i.e. 80%), but not when administered higher doses of (+)-amphetamine.[13] Unfortunately, as in the studies with THC, others have been unable to reproduce these results. For example, using animals trained to discriminate 1.0 mg/kg of (+)-amphetamine from saline, stimulus generalization did not occur with (±)-DOM,[27,28,40] S(+)-DOM,[28,41] or R(−)-DOM.[28] Furthermore, animals trained to discriminate (±)-DOM from saline did not recognize the effects of amphetamine.[28,47]

Amphetamine is classified as a central stimulant. Huang and Ho[6] addressed the question of whether amphetamine produced a specific stimulus or whether the stimulus was solely related to the general excitatory effects of the drug, by evaluating other agents known to possess central stimulant properties. The (+)-amphetamine-stimulus did not generalize to nikethamide, picrotoxin or strychnine.[6] In other studies, the (+)-amphetamine stimulus failed to generalize with nicotine,[5,11] caffeine,[10] and oxotremorine.[11] Whereas generalization with methylphenidate was observed when (+)-amphetamine[7,16,28] or racemic amphetamine[3] was employed as the training drug, generalization failed to occur in animals trained to discriminate (−)-amphetamine from saline.[23] Other agents with central stimulant properties, such as ephedrine,[7] cocaine,[7,16,19,21,24,25] and cathinone,[42] have also been demonstrated to produce amphetamine-like stimulus effects. Early on in their work, Silverman and Ho[1] suggested that the stimulus produced by amphetamine could not be accounted for on the basis of non-specific central arousal, but that it was probably a more specific phenomenon, of central origin, and mediated via a dopaminergic mechanism.

Table III is a compilation of many of the stimulus generalization studies that have been conducted with (+)-amphetamine as the training drug. However, where stimulus generalization has been reported, it should be kept in mind that generalization from one treatment to another does not necessarily indicate that the treatments produced identical stimuli; stimulus generalization merely implies that the treatments produced similar stimulus effects.[2] Furthermore, in certain instances, the published results may have represented "borderline" situations; the reader is cautioned concerning the interpretation of this table and is urged to consult the original literature for greater detail.

C. Locus and Mechanism of Action

The stimulus produced by (+)-amphetamine appears to be of central origin; there are several lines of evidence to support this idea. The optical isomers of amphetamine are essentially equiactive with respect to their peripheral actions whereas (+)-amphetamine is several-fold more active than (−)-amphetamine in producing central effects. The finding that (+)-amphetamine is more potent than (−)-amphetamine in tests of stimulus generalization suggests the observed effects are centrally mediated. In addition, (+)-amphetamine is 5-times more potent as a discriminative stimulus when administered in an intraventricular manner than when administered systemically.[2] Finally, stimulus generalization does not occur between amphetamine and a metabolite of amphetamine (i.e. para-hydroxyamphetamine) which does not readily penetrate the blood-brain barrier.[7,9,25,35] Interestingly, Colpaert et al[15] have found that the discriminative stimulus properties of low racemic amphetamine doses differ qualitatively from those of higher doses. Using a training dose of 0.16 mg/kg of (±)-amphetamine, stimulus generalization was obtained with para-hydroxyamphetamine, but not with high (although non-disruptive) dose (5 mg/kg) of (±)-amphetamine.[15] The authors suggested that the stimulus produced by 0.16 mg/kg of (±)-amphetamine, unlike that produced by higher training doses, is probably of peripheral origin. There is also some evidence to suggest that the (−)-amphetamine stimulus itself may possess a substantial peripheral component.[23]

Pre-treatment of animals trained to discriminate amphetamine from saline with α-methyl para-tyrosine, an inhibitor of catecholamine synthesis, resulted in a dose-dependent decrease in amphetamine-appropriate responding.[10–12] Depletion of serotonin by pre-treatment of the animals with para-chlorophenylalanine (oral or intraperitoneal administration) had no significant effect on amphetamine-appropriate responding.[12] Likewise, pre-treatment with disulfiram,[12] phenoxybenzamine,[12] phentolamine,[44] atropine[44] and propranolol[11,12,35] were without effect (although in one study involving gerbils, propranolol attenuated the stimulus effects of amphetamine[25]). Based, in part, on the results obtained with α-methyl para-tyrosine, Ho and Huang[11] suggested that dopamine might play a major role in the discriminative stimulus produced by amphetamine; they further suggested that the stimulus might be more dependent on newly formed dopamine as opposed to direct interaction of amphetamine with dopamine receptors. Subsequent studies investigated the ability of the amphetamine-stimulus to generalize to various dopaminergic agents. For example, it was found that amphetamine-stimulus generalization occurred with the dopamine precursor L-DOPA (in combination with the decarboxylase inhibitor RO 4-4602).[11] N-Propylnoraporphine and piribedil also produced amphetamine-like effects.[20] Less clear are the results obtained with amantadine and apomorphine. Amantadine has been reported to act on dopaminergic neurons, and Schechter found that (+)-amphetamine-stimulus (0.8 mg/kg) generalization occurred with high doses (50 mg/kg) of amantadine.[20] Wilson and Buelke[23] obtained similar results using animals trained to discriminate (−)-amphetamine from saline. On the other hand, in a study by Ho and Huang,[11] administration of amantadine (50 mg/kg)

to (+)-amphetamine-trained (0.8 mg/kg) animals produced only partial (51%) generalization. However, in this same study, co-administration of L-DOPA (in combination with RO 4-4602) and amantadine (50 mg/kg) did result in amphetamine-appropriate (100%) responding. Perhaps more confusing are the results obtained with apomorphine. In two separate studies, Schechters group has found that apomorphine produces amphetamine-appropriate responding[12,20] (Table IV). Results obtained by Ho and Huang,[11] Bueno et al,[14] and Jarbe and Kroon,[25] clearly indicate partial generalization. The only study to report a lack of generalization is that by Minnema and Rosecrans;[44] however, because no data were presented in this latter study, it is not possible to determine whether partial generalization occurred or whether there was a complete lack of generalization at all doses tested. It is clear, from the majority of the studies, that apomorphine can produce an effect that is, at least, somewhat similar in nature to that produced by amphetamine. This supports an earlier suggestion that a direct dopamine receptor interaction may not be as important to producing the amphetamine stimulus as is the release of newly synthesized dopamine.[11] However, as suggested by Jarbe and Kroon,[25] if the discriminable effects of amphetamine were entirely restricted to direct or indirect stimulation of dopaminergic pathways, depletion or destruction of these neurochemical sites ought to prevent discriminative response control by amphetamine. However, rats depleted of dopamine learned and maintained amphetamine discrimination as efficiently as did controls. In a more recent study, Minnema and Rosecrans[44] trained two groups of adult rats, one group being neonatally depleted of dopamine, to successfully discriminate 0.9 mg/kg of (+)-amphetamine from saline; however, while exhibiting similar

Table IV
Results of Stimulus Generalization Studies with Apomorphine in Amphetamine-trained Animals.

Training Dose of (+)-Amphetamine (mg/kg)	Animal Species/ Route of Administration	Dose of Apomorphine (mg/kg)	Result (AMPH-appropriate Responding)	Ref.
0.8	Rat/i.p.	0.25	30%	(11)
		0.50	56%	
		1.0	58%	
		2.0	41%	
2.0	Rat/i.p.	0.5	50%	
		1.25	84%	
		2.5	79%	
		5.0	81%	
1.0	Rat/i.p.	0.5	65%	(14)
		1.0	67%	
0.8	Rat/i.p.	1.0	51%	(20)
		2.0	73%	
		2.5	82%	
8.0	Gerbil/i.p.	4.0	*ca* 40%	(25)
		8.0	*ca* 50%	
		16.0	*ca* 40%	
0.9	Rat/i.p.	—[a]		(44)

[a]No experimental results presented. Statement made that generalization did not occur.

performance under saline and amphetamine training-dose conditions, the individual groups exhibited significantly different dose-response relationships. In a study employing rats trained to discriminate 0.16 mg/kg of apomorphine from saline, (+)-amphetamine and amfonelic acid, an agent that supposedly promotes mobilization of dopamine from pools different than those mobilized by amphetamine, were studied in tests of stimulus generalization; neither agent produced apomorphine-like effects.[48] However, amfonelic acid did produce amphetamine-appropriate responding in amphetamine-trained (1.0 mg/kg) animals.[49] Schechter has suggested that these agents probably produce their stimulus effects via different types of dopaminergic mechanisms.[48] That is, whereas amphetamine produces it stimulus effects via release of dopamine (and, perhaps, by some additional action upon norepinephrine neurons), apomorphine may act solely as a postsynaptic dopamine agonist.[48] Further, on the basis of electrical brain-stimulation experiments, D'Mello has suggested that mesolimbic dopamine systems may play a role in the discriminative stimulus produced by amphetamine.[31] However, Snoddy and Tessel[38] have recently demonstrated that, in mice, (+)-amphetamine-stimulus generalization occurs to the selective norepinephrine uptake inhibitor nisoxetine; similar results were obtained in nisoxetine-trained animals. Because *in vivo* administration of nisoxetine has little effect on dopamine uptake, spontaneous dopamine efflux or amphetamine-induced dopamine release in brain tissue at concentrations that affect similar parameters of noradrenergic function,[38] the above results suggest that in mice norepinephrine may play a role in the discriminative stimulus properties of amphetamine, or at least in those properties that it shares with nisoxetine.

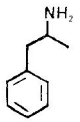
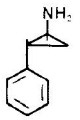
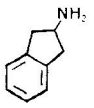
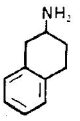
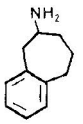
McMillen has suggested that behavioral stimulants can be sub-divided into two classes of drugs.[50] Amphetamine-like agents are direct releasers, as well as re-uptake inhibitors, of dopamine and norepinephrine both *in vivo* and *in vitro*. Certain non-amphetaminergic stimulants (e.g. amfonelic acid, cocaine, methylphenidate) block dopamine and/or norepinephrine re-uptake, but enhance dopamine release only *in vivo*, and differentiation between monoamines is due to differences in storage and releasable pools of dopamine and norepinephrine. Thus, the results of various stimulus generalization studies, such as those described above, might reflect these mechanistic differences and/or may involve various dose-related phenomena.

Another mechanistic approach to studying discriminative stimuli involves stimulus antagonism experiments. That is, pre-treatment of animals with a particular neurotransmitter antagonist should attenuate the effects of a given agent if that particular neurotransmitter system is involved in its mechanism of action. Some of these antagonism studies were already described; additional studies are described below. For example, administration of (+)-amphetamine to animals trained to discriminate the purported serotonin agonist quipazine from saline resulted in partial generalization.[51] One possible explanation is that amphetamine and quipazine might share common mechanistic components involving serotonin (although it now appears more likely that quipazine might actually possess some dopaminergic activity). All attempts to abolish or attenuate the stimulus effects of amphetamine (in amphetamine-trained animals) by pre-treatment of the animals with serotonin antagonists have, however, been unsuccessful; antagonists examined have included: cin-

there are several additional potential problems. For example, in some of the more early studies of stimulus generalization, complete dose-response relationships were not investigated; conclusions may have been based on the results of only one or two doses of test compound (that resulted in saline-appropriate responding), and the effects of higher doses of these agents remain unknown. In other studies, it may have been concluded that an agent was amphetamine-like (even though it may have produced only, for example, 65% amphetamine-appropriate responding) on the basis that the results were statistically different from those produced by saline, or statistically similar to those produced by amphetamine, when, in fact, >80% amphetamine-appropriate responding was never experimentally demonstrated. Results such as these obfuscate the formulation of reliable SAR. As a consequence, somewhat less emphasis has been placed on the results of certain early studies.

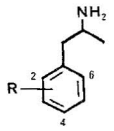
The first detailed study aimed expressly at elucidating the SAR of amphetamine using drug discrimination methodology was that by Huang and Ho;[7] the results of this study are included in Table III. We have more recently undertaken a comprehensive and systematic investigation of these SAR and have examined approximately fifty molecular modifications of the amphetamine structure. Some of our results are summarized in Tables V and VI. What follows is an SAR discussion drawn primarily from our work and from that of Huang and Ho; in both sets of studies (+)-amphetamine was used as the

Table V
Results of Stimulus Generalization Studies Using the Conformationally-Retricted Analogs.

Agent	Result
	Racemic Amphetamine: ED_{50} = 0.62 mg/kg (41)[a] (2.6 μmoles/kg)
	Cyclopropyl derivative: 75% amphetamine-appropriate responding; less potent than amphetamine (45)
	Aminoindane derivative (2-AI): ED_{50} = 2.12 mg/kg (42)[a] (12.5 μmoles/kg)
	Aminotetralin derivative (2-AT): ED_{50} = 1.20 mg/kg (42)[a] (6.6 μmoles/kg)
	Aminobenzocycloheptane derivative: saline-like effects at doses of up to 20 mg/kg (42)[a]

[a]Animals trained to discriminate 1.0 mg/kg of (+)-amphetamine from saline. All agents were tested as racemic mixtures.

Table VI
Results of Stimulus Generalization Studies with Methoxy-substituted Phenylisopropylamine Derivatives in (+)-Amphetamine-trained Rats[a].



Agent	R_2	R_3	R_4	R_5	R_6	Result[b]
(±)-AMPH	H	H	H	H	H	0.62 mg/kg
(+)-AMPH	H	H	H	H	H	0.42 mg/kg
(−)-AMPH	H	H	H	H	H	1.23 mg/kg
(±)-OMA	OMe	H	H	H	H	7.82 mg/kg
(±)-MMA	H	OMe	H	H	H	3.44 mg/kg
(±)-PMA[c]	H	H	OMe	H	H	1.91 mg/kg
(−)-PMA	H	H	OMe	H	H	S(12% at 2.0)
(±)-2,3-DMA	OMe	OMe	H	H	H	D(14% at 5.5)
(±)-2,4-DMA	OMe	H	OMe	H	H	D(50% at 6.75)
(±)-2,5-DMA	OMe	H	H	OMe	H	D(43% at 13.5)
(±)-2,6-DMA	OMe	H	H	H	OMe	S(12% at 15.0)
(±)-3,4-DMA	H	OMe	OMe	H	H	D(7% at 6.5)
(±)-3,5-DMA	H	OMe	H	OMe	H	S(9% at 7.0)
(±)-2,3-MDA	O—CH_2—O		H	H	H	D(12% at 2.75)
(±)-3,4-MDA	H	O—CH_2—O		H	H	2.29 mg/kg
(+)-3,4-MDA	H	O—CH_2—O		H	H	0.90 mg/kg
(−)-3,4-MDA	H	O—CH_2—O		H	H	D(25% at 2.0)
(±)-2,3,4-TMA	OMe	OMe	OMe	H	H	D(2% at 10.0)
(±)-2,3,5-TMA	OMe	OMe	H	OMe	H	D(3% at 10.0)
(±)-2,4,5-TMA	OMe	H	OMe	OMe	H	D(46% at 3.0)
(±)-2,4,6-TMA	OMe	H	OMe	H	OMe	D(51% at 8.0)
(±)-3,4,5-TMA	H	OMe	OMe	OMe	H	D(45% at 4.75)
(±)-DOM	OMe	H	Me	OMe	H	D(32% at 1.75)
(+)-DOM	OMe	H	Me	OMe	H	D(46% at 6.0)

[a]Data are from Glennon et al[55]

[b]Where stimulus generalization occurred, an ED_{50} value is given. D designates that disruption of behavior was observed; the number in parenthesis is the % amphetamine-appropriate responding at that dose (in mg/kg) just prior to the dose that produced disruption. S indicates that at the highest dose tested, saline-appropriate responding was observed; the number in parenthesis in the % amphetamine-appropriate responding at the highest dose (in mg/kg) evaluated.

[c]A 5-min pre-session injection interval was used for this particular agent; see ref 55.

training drug, and a 15-min pre-session injection interval was employed. Relevant results from other studies (employing the same training drug) will be incorporated into the discussion where appropriate.

A. Side Chain and Terminal Amine Modifications

These two categories were combined primarily because relatively little has been done with the side chain, and even less has been done with the terminal amine.

The simplest N-alkyl derivative of amphetamine is its N-monomethyl derivative, i.e. methamphetamine. Kuhn et al[10] found that 1.0 mg/kg of methamphetamine exerted the same effect as 1.0 mg/kg of (+)-amphetamine; how-

ever, the stereochemistry of the methamphetamine was not specified. Huang and Ho published similar results.[7] More recently, we found that S(+)-methamphetamine is equipotent with S(+)-amphetamine.[41] No other simple alkyl analogs of amphetamine have been investigated. Compounds such as ephedrine, methylphenidate and fenfluramine might be considered as N-substituted amphetamine analogs. Whereas the (+)-amphetamine-stimulus generalized to methylphenidate and ephedrine, generalization did not occur to fenfluramine (Table III). However, because each of these agents bears structural modifications in addition to their N-alkyl substituent, it is impossible, at this time, to comment on the effect of their N-alkyl groups.

Amphetamine possesses a methyl group α to the terminal amine, and, as such, is optically active. As already discussed in Section II.B., the S(+)-isomer of amphetamine is 2–5 times more potent than its enantiomer in tests of discriminative control of behavior. Removal of the α-methyl group of amphetamine results in β-phenethylamine (PEA). Administration of PEA to amphetamine-trained animals did not result in stimulus generalization;[8,42] however, this may be the result of rapid metabolism of PEA by monoamine oxidase. Pre-treatment of the amphetamine-trained animals with the monoamine oxidase inhibitor iproniazid did result in amphetamine-stimulus generalization to subsequent doses of PEA.[8] α-Alkyl homologs of amphetamine have not been investigated.

The results of nuclear magnetic resonance studies suggest that, in solution, the preferred side chain conformation of various phenylisopropylamines is an extended trans-phenylamino arrangement (see ref. 42 for further discussion). This conformation can be mimicked to some extent by the conformationally-restricted analog 2-aminotetralin (2-AT), and to a lesser extent by 2-aminoindane (2-AI). Indeed, 2-AI, and in particular 2-AT, are capable of producing a number of amphetamine-like effects, including anorexia and locomotor stimulation in animals. Administration of several conformationally-restricted amphetamine analogs to amphetamine-trained animals revealed that 2-AT was active, but approximately half as potent as racemic amphetamine[42] (Table V).

Perhaps the simplest conformationally-restricted analog of amphetamine is the monoamine oxidase inhibitor trans-2-phenylcyclopropylamine (tranylcypromine). In a recent study, Porsolt and co-workers found that administration of 1.5 mg/kg of racemic tranylcypromine to animals trained to discriminate 0.6 mg/kg of (+)-amphetamine from saline resulted in approximately 75% amphetamine-appropriate responding (higher doses were not examined).[45] Although monoamine oxidase inhibitors do not generally produce amphetamine-appropriate responding (see Table III), it does appear that tranylcypromine is amphetamine-like. Interestingly, however, none of the conformationally-restricted analogs was as potent as amphetamine itself.

Very little work has been done on amphetamine analogs bearing substituents β to the amine (i.e. on the benzylic carbon atom). As mentioned above, the amphetamine-stimulus generalized to ephedrine,[7] an agent that possess a β hydroxyl group. Administration of norephedrine produced 70–75% amphetamine-appropriate responding at 8 mg/kg (in animals trained to discriminate 0.8 mg/kg of (+)-amphetamine from saline);[7] however, higher doses were not evaluated. Methylphenidate, with a β-methyl ester, also produced

amphetamine-appropriate responding.[7] The β-keto analog of amphetamine, i.e. cathinone, is a naturally occuring central stimulant. Introduction of this carbonyl group appears to have little effect on potency. Cathinone produced amphetamine-like responding, and, as with amphetamine, the S-isomer of cathinone is more potent than its enantiomer.[42,54] S(−)-Cathinone is equipotent with S(+)-amphetamine; however, whereas the enantiomeric potency ratio for amphetamine is 2–5, the ratio for cathinone is about 10. Again, as with amphetamine, removal of the α-methyl group, to afford α-demethylcathinone, results in a compound that produced saline-appropriate responding at a dose 10-times greater than that of the ED_{50} dose of S(−)-cathinone.[42]

B. Modification of the Aromatic Nucleus

Simple fusion of the b(e)-face or the c(d)-face of racemic amphetamine with a benzene ring is detrimental to activity. The b-fused compound (i.e. the 1-naphthyl analog of amphetamine; 1-NAP) produced saline-like effects at low doses and disruption of behavior at 10-times the ED_{50} dose of (+)-amphetamine.[42] The c-fused analog (i.e. the corresponding 2-naphthyl analog; 2-NAP) produced saline-like effects at just under 10-times the ED_{50} dose of (+)-amphetamine (higher doses were not evaluated).[42]

Aromatic substitution results in amphetamine analogs that are, in general, not amphetamine-like. Several groups of investigators have demonstrated that the 4-hydroxy analog of racemic amphetamine (i.e. 4-OH PIA) does not produce amphetamine-appropriate responding.[5,25,35] This is consistent with the finding that 4-OH PIA does not readily penetrate the blood-brain barrier. On the other hand, amphetamine-stimulus generalization was observed when 4-OH PIA was administered to animals trained to discriminate a low dose (0.16 mg/kg) of (±)-amphetamine from saline;[15] however, it might be noted that low amphetamine doses have been speculated to produce a stimulus that is peripheral in nature.[23] Decreasing the polarity of this hydroxyl group (and consequently enhancing the ability of the resultant derivative to penetrate the blood-brain barrier) by O-methylation affords the 4-methoxy analog of amphetamine, i.e. PMA. It has been demonstrated, in two separate studies, that the amphetamine-stimulus does generalize to PMA, although PMA appears to be somewhat less potent than amphetamine.[7,55] There are two other mono-methoxy isomers of amphetamine: the 3-methoxy (MMA) and 2-methoxy (OMA) analogs. Huang and Ho showed that at 4 mg/kg MMA produced partial generalization in amphetamine-trained animals.[7] Glennon et al[55] obtained similar results at this dose but found that doses higher than 4 mg/kg resulted in amphetamine-stimulus generalization. Likewise, the 2-methoxy derivative, OMA, also produced amphetamine-like effects. In terms of relative potency, MMA and OMA are, respectively, 5-and 10-times less potent than racemic amphetamine. The 4-methyl derivative of amphetamine produced approximately 50% amphetamine-appropriate responding at the highest dose at which it was tested.[7,28]

There are six possible dimethoxy analogs of amphetamine (i.e. 2,3-DMA, 2,4-DMA, 2,5-DMA, 2,6-DMA, 3,4-DMA and 3,5-DMA) and all six have been evaluated in amphetamine-trained animals (Table VI). The amphetamine-stimulus did not completely generalize to any of these dimethoxy analogs.[55]

2,6-DMA and 3,5-DMA produced saline-like effects at the highest doses tested (15 mg/kg and 7 mg/kg respectively), whereas 2,3-DMA and 3,4-DMA both produced saline-like effects, followed, at higher doses, by disruption of responding. Likewise, the amphetamine-stimulus did not generalize to either 2,4-DMA or 2,5-DMA; however, these agents differed from the other dimethoxy analogs in that both produced 50–55% amphetamine-appropriate responding at doses just below those that resulted in disruption of behavior. It may be that 2,4-DMA and 2,5-DMA possess some amphetamine character, but that as their doses are increased, some other effect predominates. Interestingly, in tests of stimulus generalization using the hallucinogenic agent DOM as the training drug, 2,4-DMA and 2,5-DMA were the only simple dimethoxy derivatives that resulted in DOM-stimulus generalization.

Similar studies have been conducted with five different trimethoxy analogs (TMA's) of amphetamine (i.e. 2,3,4-TMA, 2,3,5-TMA, 2,4,5-TMA, 2,4,6-TMA and 3,4,5-TMA).[55] 2,3,4-TMA and 2,3,5-TMA produced saline-like effects, followed, at higher doses, by disruption of behavior. The remaining three trimethoxy analogs also resulted in disruption of behavior, but in all cases, these agents produced between approximately 45–55% amphetamine-appropriate responding prior to those doses that disrupted behavior. Again, it might be speculated that some of these agents possess an amphetamine-like component of action. Also, DOM-stimulus generalization has been demonstrated to occur with all five of these agents.

It seems that of those nuclear-substituted amphetamine analogs evaluated, none is as potent as amphetamine itself, and, in most cases, the actions of these derivatives is not clearly amphetamine-like. The monomethoxy derivatives, while less potent than amphetamine, do produce amphetamine-like effects. As the number of methoxy substituents is increased to two or three, the action appears to shift from amphetamine-like to DOM-like; however, activity and potency are dependent upon the exact location of these methoxy substituents. These results tend to parallel the results of studies in human subjects, where such data are available. For example, certain methoxy-substituted derivatives such as 2,4-DMA and 3,4,5-TMA are hallucinogenic in man, but also appear to produce a considerable amount of amphetamine-like stimulation.[56]

Additional studies have been conducted on a few other 2,5-dimethoxy derivatives. The 4-methyl and 4-ethyl derivatives of 2,5-DMA (i.e. DOM and DOET, respectively) are reportedly hallucinogenic in humans. Amphetamine-stimulus generalization did not occur to either of these agents, nor to either R(−)-DOM or S(+)-DOM.[7,28,40,41] Conversely, in animals trained to discriminate DOM from saline, DOM-stimulus generalization occurred to R(−)-DOM, S(+)-DOM and DOET, but not to amphetamine.[28,47]

One remaining group of agents worthy of mention are the methylenedioxy analogs of amphetamine. There are two simple methylenedioxy analogs possible: 1-(2,3-methylenedioxyphenyl)-2-aminopropane (2,3-MDA) and 1-(3,4-methylenedioxyphenyl)-2-aminopropane (3,4-MDA), 2,3-MDA has not been evaluated in humans, whereas 3,4-MDA is a hallucinogenic agent with a strong stimulant component of activity. In animals trained to discriminate 1.0 mg/kg of amphetamine from saline, 2,3-MDA did not produce amphetamine-like effects.[43] Likewise, Shannon reported that at doses of up to 3.0 mg/kg,

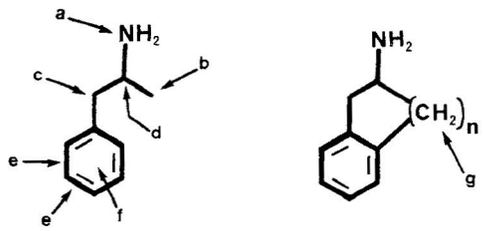
3,4-MDA failed to cause the animals to make greater the 10% of their responses on the amphetamine-appropriate lever, and that at higher doses the animals' behavior was disrupted.[27] In a more recent study, we found that amphetamine-stimulus generalization does, indeed, occur to 3,4-MDA.[40] It might be noted, however, that the two studies differed with respect to methodology, pre-session injection interval and route of administration. This finding of stimulus generalization was rather surprising in view of our earlier demonstration of DOM-stimulus generalization to 3,4-MDA.[57] Interestingly, while the DOM-stimulus generalized to (±)-MDA[57] and to R(−)-MDA, generalization did not occur to S(+)-MDA. These results, coupled with the considerable depression in response rates at that dose of 3,4-MDA to which amphetamine-stimulus generalization occurred, prompted us to examine the individual optical isomers of 3,4-MDA in amphetamine-trained animals. It was found that amphetamine-stimulus generalization occurred to S(+)-MDA, but not to R(−)-MDA.[41] In other words, racemic 3,4-MDA is capable of producing amphetamine-like and DOM-like effects. Apparently, the S(+)-isomer is responsible for one type of activity while the R(−)-isomer appears to be responsible for the other. These results are consistent with the general finding that for those agents that produce DOM-like effects, it is the R isomer that is more active than the racemic mixture and/or S isomer, while the reverse is true for those agents that produce amphetamine-like activity. Furthermore, whereas N-methylation decreases the potency, or abolishes the activity, of DOM-like agents, it seems to have little effect on amphetamine-like stimulus potency (e.g. compare the potencies of amphetamine and methamphetamine). Consequently, we examined the N-monomethyl derivative of (±)-3,4-MDA (i.e. MDMA). Consistent with the above generalizations, MDMA did not produce DOM-like effects in DOM-trained animals,[57] whereas it was slightly more potent than (±)-3,4-MDA in amphetamine-trained animals.[41]

Considerable work remains to be done with respect to amphetamine SAR; what little is known has been summarized in Figure 4. Nevertheless, the studies with MDA serve as an example of how SAR results can be applied to the investigation of subsequently studied agents.

V. OTHER PHENALKYLAMINES AS TRAINING DRUGS

A. Phenethylamine

β-Phenethylamine, i.e. 1-phenyl-2-aminoethane, is the α-demethyl analog of amphetamine. Certain pharmacological aspects of phenethylamine (PEA) are similar to those of amphetamine, and Sandler and Reynolds[58] have suggested that phenethylamine might constitute an "endogenous amphetamine." As described above, phenethylamine did not result in amphetamine-appropriate responding when administered to amphetamine-trained animals. It was hypothesized that this might be a consequence of its rapid metabolism and, indeed, amphetamine-stimulus generalization was observed when the animals were pre-treated with a monoamine oxidase inhibitor prior to administration of phenethylamine. Goudie has raised the issue that pre-treatment of animals with a monoamine oxidase inhibitor not only interferes with the metabolism of phenethylamine, but it also elevates the levels of other endogenous amines in brain tissue;[59] in other words, an artificial "state" may



SAR SUMMARY

a. N-Monomethylation has no effect on activity/potency.
b. α-Demethylation abolishes amphetamine-like activity.
c. Introduction of carbonyl has little effect on potency.
d. Optical isomers: S(+)- more potent than R(-)-isomer.
e. Benz-fusion abolishes activity.
f. Methoxy substitution: mono-methoxy derivatives are amphetamine-like, di- and tri-methoxy are not (see text).
g. Conformationally-restricted derivatives: active but less potent than amphetamine when n = 1 or 2; inactive when n = 3.

Figure 4. An SAR summary for amphetamine-like discriminative stimulus properties.

be produced. In order to better understand the stimulus properties of phenethylamine, Goudie attempted to train twelve rats to discriminate 40 mg/kg (i.p.) of this agent from saline. After an extensive period of training, only half of the animals achieved criteria; the remaining animals were excluded from the remainder of the study. The stimulus effects of PEA were dose-dependent and of short duration. In tests of stimulus generalization, three of the six animals recognized (+)-amphetamine; these same three animals also recognized cocaine and deprenyl. Goudie has suggested that the stimulus produced by phenethylamine is a complex, multidimensional one, and, because of the rapid metabolism of phenethylamine, that the animals were probably required to discriminate a rapidly changing brain/plasma level of this agent.[59]

In a separate study, Goudie et al[74] trained twelve hooded rats to discriminate PEA (30 mg/kg; i.p.) from saline using a 2-lever operant procedure (FR-10; pre-session injection interval = 15 min.) Naloxone (10 mg/kg) had no effect on PEA discriminability, and the PEA-stimulus failed to generalize to fenfluramine. The PEA-stimulus did generalize, however, to several metabolites of PEA (ED_{50} = 24.7 mg/kg), including phenethanolamine (ED_{50} = 19.7 mg/kg) and N-methyl PEA (ED_{50} = 36.1 mg/kg). Stimulus generalization was also demonstrated with deuterated (α,α-d_2) PEA (ED_{50} = 11 mg/kg).[74]

B. Cathinone

S(−)-Cathinone, i.e. 1-phenyl-2-aminopropanone, is one of the active central-stimulant components of khat (*Catha edulis*). The wide spread abuse of khat in certain areas of the world, coupled with the recent isolation and identification of cathinone, has spurred interest in the pharmacological properties of this agent. Recent reviews of this topic suggest that cathinone and amphetamine share a number of similar pharmacological effects.[60,61] In tests of stimulus generalization using amphetamine-trained animals, cathinone produced amphetamine-appropriate responding;[42,54] furthermore, as with amphetamine, the S-isomer was more active than its enantiomer.[42] Racemic

cathinone, itself, has been employed as a training drug; Schechter et al[62] have demonstrated that 0.6 mg/kg of cathinone serves as an effective discriminative stimulus in rats when paired with saline. Subsequent studies have shown that S(-)-cathinone is approximately 3-times more potent than R(+)-cathinone and about 8-times more potent than (+)-cathine, i.e. (+)-nor-pseudoephedrine, a reduced analog of cathinone.[63] Several other cathinone analogs, including α-demethylcathinone, failed to produce cathinone-stimulus generalization at doses of up to about 6-times the ED_{50} dose of racemic cathinone.[63] The cathinone stimulus, however, did generalize to (±)-methamphetamine and cocaine.[64] The results obtained thus far do support the concept that amphetamine and cathinone are pharmacologically similar.

More recently, Goudie et al[65] have trained animals to discriminate racemic cathinone from saline; they also found that cathinone stimulus generalization occurs with amphetamine and cocaine. Generalization did not occur with 4-OH PIA, haloperidol, fenfluramine, chlordiazepoxide or fentanyl.

C. PMA

PMA, or 1-(4-methoxyphenyl)-2-aminopropane, is the para-methoxy analog of amphetamine. This agent has been reported to produce a psychotomimetic effect in humans,[56] but while its discriminative stimulus effects are different from those of (±)-DOM,[47] administration of PMA to (±)-amphetamine trained animals does result in stimulus generalization.[7,55] Winter has trained rats to discriminate 3.0 mg/kg of PMA from saline using a fixed ratio 10 schedule of reinforcement.[66] Investigations of stimulus generalization using LSD yielded intermediate results, i.e. the distribution of responses on the PMA-designated lever and saline-designated lever was significantly different from that observed following administration of either PMA or saline. In a second experiment, using animals trained to discriminate 0.1 mg/kg of LSD from saline, intermediate results were again obtained. While 1.0 mg/kg of PMA produced 48% LSD-appropriate responding, 3.0 mg/kg resulted in 75% LSD-like responding; however, at the higher dose, only five of the ten animals responded. In tests of stimulus antagonism, it was found that pizotyline, a serotonin antagonist that effectively attenuates the stimulus effects of DOM, was without significant effect in the PMA-trained animals, at doses of 3 and 10 mg/kg. In contrast the intermediate results produced by PMA and the LSD-trained animals were significantly antagonized by 3 mg/kg of pizotyline. Winter has suggested that PMA produces a compound stimulus and that in establishing stimulus control of behavior, the relative importance of the component stimuli may vary with the circumstances of training and testing.[66] That is, PMA and LSD may share an effect that is serotonergic in nature, but an effect that is non-essential for the induction of stimulus control by PMA.

D. 3,4-MDA

(±)-3,4-MDA, i.e. 2-(3,4-methylenedioxyphenyl)-2-aminopropane, the agent to which both an amphetamine-stimulus and a DOM-stimulus generalized, has been used as a training drug in drug discrimination studies. Glennon and Young have trained rats to discriminate 1.5 mg/kg of racemic 3,4-MDA from saline in a two-lever operant procedure using a variable interval schedule

of reinforcement.[40,41] The results obtained in their studies compliment the results of the stimulus generalization studies with 3,4-MDA in animals trained to discriminate either (+)-amphetamine or (±)-DOM from saline. In other words, the 3,4-MDA-stimulus generalized both to (+)-amphetamine and (±)-DOM. Furthermore, the 3,4-MDA-stimulus also generalized to S(+)-methamphetamine and cocaine (agents to which (+)-amphetamine but not DOM generalizes) and to (+)-lysergic acid diethylamide (LSD) (an agent to which DOM but not amphetamine generalizes).[67] Both R(−)- and S(+)-MDA produced (±)-3,4-MDA-like effects. These results support the suggestion that R(−)-3,4-MDA and S(+)-3,4-MDA produce different effects and that the effect of the racemate may reflect the overall actions of both optical isomers.

E. DOM

DOM, or 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane, is a hallucinogenic phenalkylamine analog.[56] As previously discussed, stimulus generalization did not occur between DOM and (+)-amphetamine regardless of which was employed as the training drug. The discriminative stimulus properties of DOM have been recently reviewed in detail.[47]

F. Mescaline

In humans, mescaline is a weak hallucinogenic agent.[56] Several groups of investigators have trained animals to discriminate mescaline from saline; this has been recently reviewed.[47] While stimulus generalization occurs between mescaline, LSD and DOM, regardless of which of the three hallucinogenic agents is used as the training drug, stimulus generalization does not occur between mescaline and amphetamine, either when mescaline or (+)-amphetamine (Table III) is used as the training drug. All available evidence suggests that mescaline and amphetamine produce dissimilar discriminative stimuli.

G. Fenfluramine

The anorectic agent fenfluramine (N-ethyl-α-methyl-m-(trifluoromethyl)phenethylamine hydrochloride has been used as a training drug in three drug discrimination studies.[68–70] These studies used the two-lever operant task, with rats responding on fixed ratio schedules of reinforcement, for a water or milk reward. Training doses employed were 1.0 and 3.0 mg/kg (i.p.), with pre-session intervals ranging from between 20–60 min. In one study,[70] the time course effect of a 3.0 mg/kg (−)-fenfluramine HCl stimulus was studied. In addition to their standard 60-min pre-session injection interval, McElroy and Feldman conducted tests of stimulus generalization with fenfluramine using pre-session injection intervals of one minute to 960 minutes. The fenfluramine stimulus had a relatively rapid onset; with a 10 min pre-session injection interval, fenfluramine resulted in approximately 70% drug-appropriate responding. Optimal fenfluramine responding (95–100%) occurred with 60–240 minute pre-session injection intervals. With injection intervals of 480, 720, and 960 minutes, fenfluramine-appropriate responding was decreased to approximately 70%, 55%, and 20% respectively.

Racemic fenfluramine can be resolved to afford (+)- and (−)-fenfluramine. As with amphetamine, it has been stated that fenfluramine produces a discriminative stimulus effect that is stereospecific.[70] Again, the term stereoselective would be more appropriate. That is, in rats trained to discriminate (±)-fenfluramine from saline, both individual isomers were recognized (i.e. stimulus generalization occurred) (+)-fenfluramine being slightly more potent than (−)-fenfluramine.[70]

In several species, including the rat, fenfluramine is de-alkylated to norfenfluramine. This suggests that the stimulus effects of fenfluramine could be due to its metabolite norfenfluramine. In two studies of (+)-fenfluramine discrimination,[68,70] fenfluramine generalization occurred to (±)-norfenfluramine, with the latter being 1.5 to 2.0 times more potent than the former. This indicates that norfenfluramine probably exerts a major role in the discriminative stimulus profile of fenfluramine.

In tests of generalization the (±)-fenfluramine stimulus generalized completely to quipazine,[69] lisuride,[69] MK-212,[69] p-chloroamphetamine[69,70] and *p*-fluoramphetamine.[70] Partial substitution has been observed with LSD[69] and cocaine.[69] (−)-Fenfluramine did not generalize to (+)-amphetamine.[69]

In stimulus antagonism tests, the (±)-fenfluramine cue was blocked by the serotonin antagonists cyproheptadine,[69] methysergide,[70] cinanserin,[70] and methiothepin.[69] The dopamine antagonist haloperidol did not attentuate the fenfluramine-stimulus.[69]

H. Misc. Phenalkylamines

Other than for those agents described above, very little is known about the discriminative stimulus properties of substituted phenalkylamines (as training drugs). For the sake of completeness, results with several miscellaneous phenalkylamines are described below.

3,4-Dimethoxyphenethylamine (DMPEA)

DMPEA is the agent that produces the so-called "pink spot" in the chromatographic analysis of urine from schizophrenic patients. Structurally, it differs from mescaline only in the absence of one of the meta methoxy groups. Winter[71] trained a small group of rats to discriminate between mescaline and DMPEA. The results of this study suggested that the stimulus effects of the two agents are dissimilar. Bueno was unable to train animals to discriminate between DMPEA and saline.[72] Because the animals responded appropriately (*ca.* 80% correct responding) when administered DMPEA but divided their responses evenly between the two levers when administered saline, the author commented that an extended learning training period might prove beneficial. As in the study by Winter, however, Bueno was successful in training animals to discriminate DMPEA from mescaline.[72]

2,3,4-Trimethoxyphenethylamine (2,3,4-TMPEA)

This agent is a positional isomer of mescaline in which the 5-methoxy group of the latter has been moved to the nuclear 2-position. This compound may also be visualized as the phenethylamine counterpart of 2,3,4-TMA (i.e. α-

demethyl 2,3,4-TMA). Whereas mescaline is hallucinogenic in humans, the hallucinogenic potential of 2,3,4-TMPEA has not been firmly established. Winter determined that 2,3,4-TMPEA could serve as a discriminative stimulus in rats when paired with saline, but not when paired with mescaline.[73]

4-Hydroxyamphetamine (4-OH PIA)

Amphetamine and 4-OH PIA are equipotent in the periphery, but the latter lacks significant central activity, when administered systemically, presumably due to its relative inability to penetrate the blood-brain barrier. In an attempt to better characterize the stimulus properties of amphetamine and to provide evidence as to the central or peripheral nature of its cue, Jones et al[9] attempted to train animals to discriminate 4-OH PIA from saline for the purpose of conducting stimulus generalization studies. However, using a two-lever operant procedure, they were unable to train a group of rats (n = 6) to discriminate 0.8 mg/kg of 4-OH PIA (i.p. administration; 15 min pre-session injection interval) from saline.[9]

VI. SUMMARY

The drug discrimination paradigm can be a powerful tool for the investigation of centrally-active agents. Hopefully, this review has served to demonstrate the application of this technique as it relates to amphetamine. (+)-Amphetamine can act as a discriminative stimulus in animals. The stimulus produced by (+)-amphetamine is both time- and dose-dependent and is, most likely, of central origin. The stimulus is also steroselective, and evidence suggests that it may involve a catecholaminergic (DA and/or NE) mechanism. Results from various laboratories reveal a general consistency, even though a variety of different training and testing procedures, behavioral tasks, training doses, and animal species have been employed. More often than not, the observed differences are more of a quantitative nature than of a qualitative one; that these differences may also be related to the training dose of amphetamine is nicely demonstrated by the low-dose study conducted by Colpaert et al.[15] Nevertheless, caution should still be used when comparing results from different laboratories because of the above-mentioned differences in conducting discrimination studies. Stimulus generalization studies reveal that the (+)-amphetamine stimulus generalizes to agents that tend to produce amphetamine-like effects in other types of pharmacological tests, particularly if those agents produce their effects by a relatively similar mechanism. That is, the (+)-amphetamine-stimulus does not necessarily generalize to general CNS stimulants (such as strychnine and caffeine, which are thought to possess a different mechanism of action) or to other anorectic agents (e.g. fenfluramine). On the other hand, stimulus generalization does occur with agents thought to act (directly or indirectly) as dopamine agonists, whereas known dopamine antagonists can attenuate the discriminative stimulus effects of amphetamine. There are relatively few inconsistencies amongst the results of generalization studies, and here too, where inconsistencies do exist, procedural differences may have played an important role. For example, in some of the early studies, only one or two doses of a compound may have been examined in tests of stimulus generalization; thus, a low dose of a particular

challenge drug might have been too low (and afforded saline-appropriate responding), whereas a higher dose may have been too high (and resulted in disruption of behavior). The importance of thorough dose response studies can not be overemphasized. Another problem that is occasionally encountered relates to the generalization criteria employed, and to the arbitrary definition (and a recognized lack of understanding) of "partial generalization." For example, there are instances in the literature where 60–65% drug-appropriate responding has been suggested to constitute generalization whereas the majority of investigators would probably refer to such an effect as partial generalization. At this time, there are varying opinions as to what partial generalization means with respect to similarity of effect, and this issue will not be addressed here. However, it should be recognized that certain agents have been demonstrated to produce 60–65% drug-appropriate responding, and then, at a slightly higher dose, produce disruption of behavior. Thus, it is difficult to draw conclusions, in the absence of data on the effect of intermediate doses, as to the similarity of effect between such an agent and that of the training drug.

The results of various structure-activity studies, which are far from being complete, reveal that aromatic substitution of the amphetamine nucleus by methoxy groups generally reduces potency (or abolishes activity) of the resultant agent with respect to amphetamine-like stimulus effects. Modifications of the side chain and terminal amine have not been sufficiently studied to warrant any generalization with regard to SAR; however, N-monomethylation (i.e. methamphetamine) and incorporation of a benzylic keto group (i.e. cathinone) have little effect on amphetamine-like effects. Interesting, though, is that relatively simple structural modifications, such as introduction of two methoxy groups (e.g. 2,4-DMA and 2.5-DMA), or introduction of a trifluoromethyl and N-ethyl substituents (e.g. fenfluramine), dramatically alter activity.

Hopefully, the results reviewed above have highlighted some of the strengths and weaknesses of the drug discrimination procedure; perhaps future studies in this area will attempt to take advantage of its strengths, while, at the same time, make efforts to better understand and improve upon those features that might be considered its weaknesses.

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