

**Reports from the Research Laboratories
of the
Department of Psychiatry
University of Minnesota**

**Changes in Activity and Drug, Food, and
Water Intake in 24Hr/Day
d- and l- Amphetamine and Methylamphetamine
Self-Administration by Rats**

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ROBERT A. YOKEL and ROY PICKENS

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There have been many reports of intravenous self-administration of the dextro isomers of amphetamine and methylamphetamine by humans (Griffith, et al., 1966; Hawks, et al., 1969). Intravenous self-administration of d-amphetamine and d-methylamphetamine has also been found with rats (Pickens, and Harris, 1968), rhesus monkeys (Schuster, et al., 1969) and squirrel monkeys (Stretch et al., 1971). In rhesus monkeys and rats, 24 hr/day access to these compounds produces alternating periods of drug responding and no responding (abstinence), long but relatively constant inter-injection intervals during drug responding periods, and an almost constant rate of drug intake across a wide range of injection dose (Pickens, et al., 1967; Deneau, et al., 1969). Apparently, similar effects are also seen in intravenous stimulant self-administration by humans (Kramer, et al., 1967).

Recently, rats have been found to self-administer the levo as well as the dextro isomers of amphetamine and methylamphetamine (Yokel and Pickens, 1973). During 6 hr/day drug access, uniform spacing of drug responses and an almost constant regulation of drug intake across a range of injection doses were seen with l-amphetamine and l-methylamphetamine, as with d-amphetamine and d-methylamphetamine. However, for both amphetamine and

methylamphetamine, the levo isomers were less potent than the dextro isomers in maintaining self-administration.

These results suggest that both optical isomers of amphetamine and methylamphetamine may be subject to human abuse. To date, however, there have been no reports of abuse of l-amphetamine or l-methylamphetamine by humans. The failure to observe human abuse of the levo isomers may be related to the drugs' relative unavailability, rather than to an absence of reinforcing effects.

No chronic studies of l-amphetamine or l-methylamphetamine self-administration in animals have been reported. Because of its relationship to problems of human drug abuse, the present study was conducted to compare the effects of dextro and levo isomer self-administration on eating, drinking, and sleep-activity cycles in rats.

METHOD

Subjects

Rats, 105 to 230 days old, were randomly divided into four groups (N = 8), with each group receiving only one of the four drugs tested.

Apparatus

The subjects were individually housed for the duration of the experiment in sound-attenuated operant conditioning chambers, 10 x 10 x 9 inches high, in constant illumination and at 24°C. Each chamber was equipped with a rat response lever (Gerbrands G6312), programmed to deliver drug injections, a similar response lever to deliver food pellets, a drinkometer, an ultrasonic activity recorder (Alton Electronics) which was sensitive to disturbances caused by the animal moving through an ultrahigh frequency sound field, and a small (1.2 w) stimulus light located

above the drug lever.

Procedure

Each subject was surgically equipped with a chronic jugular catheter through which drug solution could be intravenously administered from an infusion pump (Milton Roy Co., Instrument Minipump, Model 196-31), mounted outside of the experimental chamber (Pickens and Thompson, 1968). Responses on the drug lever were programmed to deliver intravenous drug injections to the rat of a constant volume (0.5 ml) and duration (30 seconds) on a continuous reinforcement schedule. The drug delivery system allowed the animal almost unrestricted movement in the experimental chamber. Responding on the food lever delivered a 45 mg Noyes pellet on a continuous reinforcement schedule. The stimulus light was illuminated for the duration of each drug injection.

After catheterization, the subjects were placed in the self-administration chambers with the drug lever covered to prevent responding. The subjects received hourly saline injections to prevent blood clotting in the catheter tubings until drug was made available. After food responding stabilized with little variation in daily response rate, a 48-hr baseline level of food and water intake, activity, and body weight was obtained. Since behavior was relatively stable within this period, the short baseline used was due to the limited lifetime of the catheter system. Following baseline determinations, drug solution was made available with the drug lever uncovered, and the subjects were allowed to respond on a continuous reinforcement schedule ad lib for drug injections until death or catheter malfunction.

All experimental events were controlled by electromechanical equipment located in an adjoining room. Food, water, and drug intake and activity counts were recorded in 12-hr blocks. Gross behavioral and physical observations were also made at this time, and the subject weighed unless asleep.

Drug doses were selected to produce about equal drug response rates (inter-injection intervals) and therefore uniform daily injection volumes. Drugs and doses used were 0.25 mg/kg d-methamphetamine hydrochloride (Sigma Chemical Company, St. Louis, Mo.), 0.5 mg/kg d-amphetamine sulfate and 1.0 mg/kg l-amphetamine sulfate (K and K Laboratories, Plainview, N.Y.), and 2.0 mg/kg l-methamphetamine hydrochloride (converted from base supplied by Abbott Laboratories, North Chicago, Ill. by precipitation from ether by hydrochloride gas). All dosages expressed as salt. The optical purity of all samples was confirmed by optical rotation and melting point determinations. All drug solutions were prepared in saline.

RESULTS

Pre-drug baseline behavior consisted of short periods of activity about every 3-4 hours around the clock, during which time eating and drinking occurred (see top half of Figure 1). Since the animals were maintained in constant illumination, no day-night cycles of eating and drinking were observed. Removal of the block over the drug lever resulted in exploratory behavior around the drug lever and subsequent lever presses. Figure 1, Day 1, shows the initiation of self-administration responding for a representative animal. As a result, almost all rats began self-administration on the first day of drug availability, and the remainder began within several days thereafter. With the initiation of self-administration

Figure 1

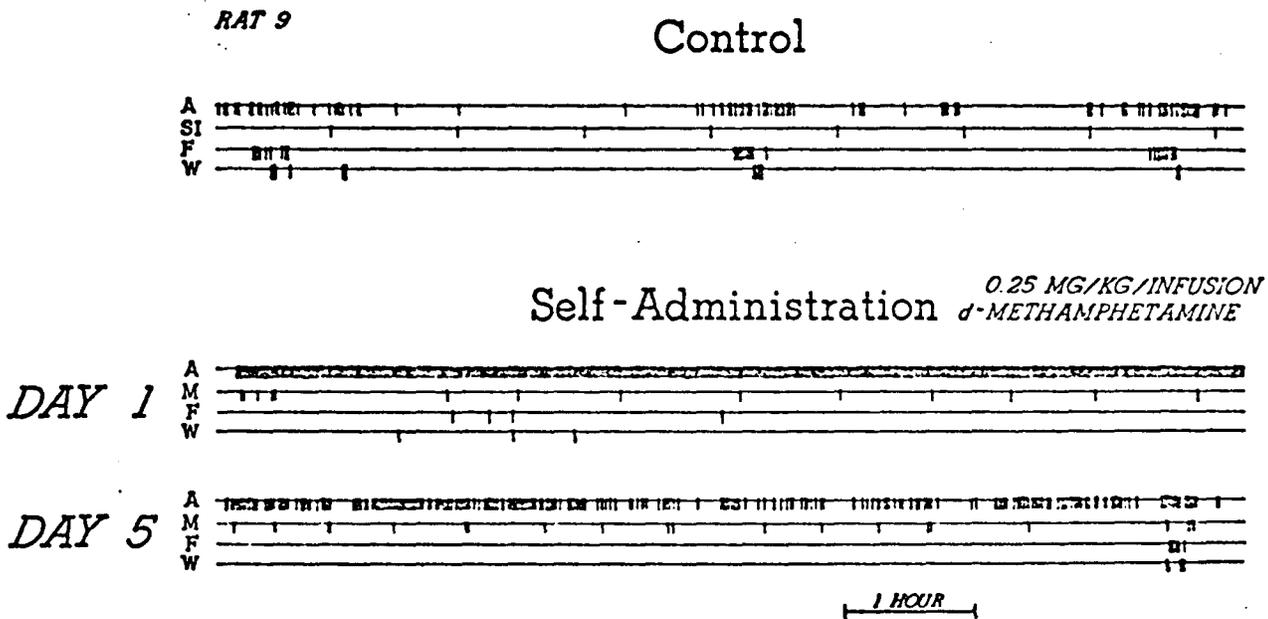


Figure 1. Changes in activity and in eating and drinking response during self-administration of d-methamphetamine. A = activity, as measured by the Alton Ultrasonic Motion Detector; SI = automatic hourly infusion of saline, necessary to keep the catheter open; F = responses for 45 mg food pellets; W = licks for water; M = lever presses for injections of d-methamphetamine. Each record segment is eight hours long.

drug lever responding for all four compounds quickly stabilized with alternating periods of drug intake and abstinence (a period of at least two hours without a drug response).

With the onset of self-administration, a high level of undifferentiated activity and no eating, drinking or sleeping was seen. Drug responses occurred about every thirty minutes. On observation, the activity was found to consist primarily of stereotypic back-and-forth head movements near the floor of the cage with little locomotion, similar to that described by Randrup and Munkvad (1967). There appeared to be no qualitative difference in activity produced by the four compounds. During drug abstinence periods, the animals slept, and eating and drinking resumed. Over the course of chronic self-administration, however, this pattern changed. Figure 1, Day 5 shows data for a representative animal during the fifth day of self-administration. As can be seen, the rate of responding for drugs has increased somewhat, and there is a partial attenuation of the initial suppression of eating and drinking (Day 1).

Table 1 shows mean daily drug intake, food and water intake, and activity level for each drug group over the course of the study. Data in 3-day blocks are shown to represent short and longer term effects of the drugs. Also shown are the significant changes from Days 1-3 to Days 12-14 of self-administration, to indicate possible tolerance to the drug effects. With the onset of drug self-administration (Days 1-3), mean injections/day were 39.8 for 0.25 mg/kg/injection d-methamphetamine (10.0 mg/kg/day), 54.8 for 2.0 mg/kg/injection l-methamphetamine (109.5 mg/kg/day), 36.4 for 0.5 mg/kg/injection d-amphetamine (18.2 mg/kg/day), and 49.0 for 1.0 mg/kg/injection l-amphetamine (49.0 mg/kg/day), indicating about similar daily response rates and injection volumes for each drug group. Between

Table 1: Changes in daily drug intake (mg/kg of salt), food intake (45 mg pellets), water intake (licks on water tube) and activity (counts on motion detector) over chronic self-administration for each drug group. Number in parentheses indicates animals surviving at the end of each period of time. Data are shown as mean \pm S.E.

	d-Amphetamine 0.50 mg/kg/injection	l-Amphetamine 1.00 mg/kg/injection	d-Methamphetamine 0.25 mg/kg/injection	l-Methamphetamine 2.00 mg/kg/injection
Drug Intake				
Days 1-3	18.2 \pm 1.1 (8)	49.0 \pm 6.0 (8)	10.0 \pm 0.6 (8)	109.5 \pm 5.9 (8)
Days 7-9	28.0 \pm 5.3 (4)	32.3 \pm 9.7 (2)	18.2 \pm 1.7 (4)	149.0 \pm 21.7 (7)
Days 12-14	23.5 \pm 7.9 (2)	41.8 \pm 9.6 (2)	28.2 \pm 4.8 (4) ^d	137.7 \pm 20.4 (4)
Food Intake				
Baseline	398.6 \pm 39.8	395.2 \pm 34.5	393.9 \pm 48.0	419.9 \pm 36.7
Days 1-3	19.5 \pm 11.7 (8) ^a	53.1 \pm 19.9 (8) ^a	15.7 \pm 6.7 (8) ^a	4.5 \pm 2.5 (8) ^a
Days 7-9	252.1 \pm 50.1 (4)	303.2 \pm 81.6 (2)	367.0 \pm 35.5 (4)	263.4 \pm 40.6 (7)
Days 12-14	407.0 \pm 90.3 (2) ^d	292.0 \pm 84.5 (2)	336.0 \pm 71.1 (4) ^c	332.8 \pm 34.9 (4) ^c
Water Intake				
Baseline	3205.4 \pm 346.2	3455.2 \pm 687.9	4398.5 \pm 1068.1	5185.8 \pm 1110.4
Days 1-3	1439.8 \pm 494.9 (8) ^b	2907.1 \pm 1262.8 (8)	2907.3 \pm 760.9 (8)	3722.9 \pm 1575.2 (8)
Days 7-9	1043.4 \pm 304.3 (4)	7935.3 \pm 5212.6 (2)	2800.6 \pm 675.7 (4)	4659.8 \pm 1444.2 (7)
Days 12-14	2042.0 \pm 835.7 (2)	2988.5 \pm 1019.5 (2)	3488.3 \pm 106.4 (4) ^d	3712.7 \pm 1210.0 (4) ^d
Activity				
Baseline	462.6 \pm 123.0	892.0 \pm 304.3	2408.3 \pm 1793.8	957.9 \pm 256.4
Days 1-3	1142.0 \pm 317.6 (8) ^b	1359.3 \pm 396.2 (8)	12428.8 \pm 4345.7 (8)	2580.2 \pm 526.0 (8) ^b
Days 7-9	693.0 \pm 234.4 (4)	792.5 \pm 295.2 (2)	6867.1 \pm 2175.1 (4)	3693.6 \pm 1028.5 (7)
Days 12-14	1814.8 \pm 740.9 (2)	1369.7 \pm 396.9 (2)	7600.7 \pm 2301.9 (4)	3713.2 \pm 1794.7 (4)

a Significantly different from baseline P < .001

b Significantly different from baseline P < .05

c Significantly different from Days 1-3 P < .001

d Significantly different from Days 1-3 P < .05

Days 1-3 and Days 12-14, drug intake increased significantly for the d-methamphetamine animals only.

No significant differences were found among the four groups in baseline food or water intake. Since the activity recording devices could not be uniformly set for all animals, activity comparisons across groups were not possible, and therefore within animal comparisons only were made.

During Days 1-3 of self-administration, food intake decreased significantly from baseline levels in all groups. Initially, eating was completely suppressed for all animals (range 4-148 hours, mean 56 hours). Between Days 1-3 and Days 12-14, food intake increased significantly for the d-amphetamine, d-methamphetamine, and l-methamphetamine groups, but in no case recovered to baseline levels. Food intake for the l-amphetamine group increased, but not significantly ($.1 > P > .05$).

During Days 1-3, drinking decreased significantly from baseline for the d-methamphetamine and d-amphetamine groups, but failed to reach significance for the l-amphetamine and l-methamphetamine groups. Drinking was initially suppressed for most animals (range 0-120 hours, mean 26 hours). No significant change in drinking occurred over the self-administration period (Days 1-3 to Days 12-14).

Activity increased significantly from baseline levels during Days 1-3 for the d-amphetamine, d-methamphetamine, and l-methamphetamine groups, but not for the l-amphetamine group. Between Days 1-3 and Days 12-14, activity decreased for the d-amphetamine group and increased for the other three groups, but in no case was the change significant.

Figure 2 shows mean daily drug intake for each group (solid lines) and individual records of drug intake for the animal surviving longest in each group (dashed lines). The number of animals surviving is shown in parenthesis

Figure 2

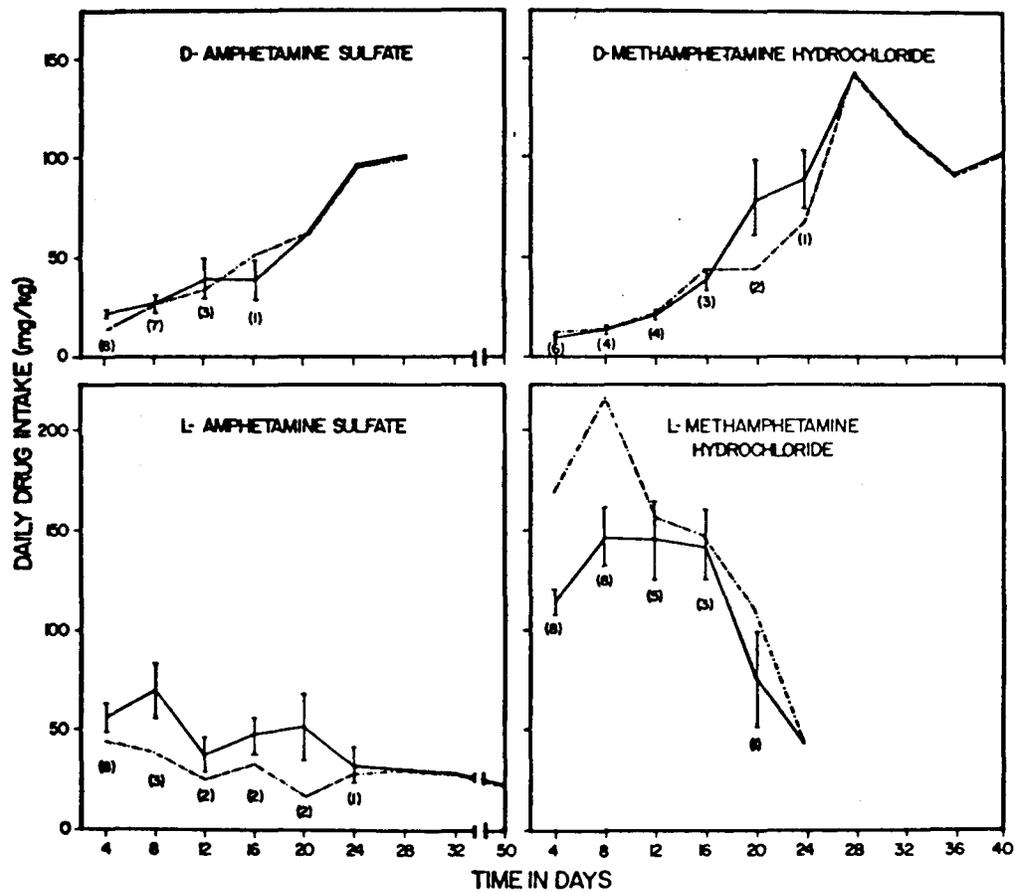


Figure 2. Drug intake for each drug group. Solid lines: Mean for all animals. Dashed lines: Longest surviving animal.

below each data point. Since group scores reflect the mean daily score for all animals surviving any part of each four-day block, the group score may differ from the individual score initially when only one animal is shown remaining in each group. As can be seen, the longest surviving animal was representative of the entire group in terms of daily drug intake.

Figure 3 shows patterns of drug intake and abstinence for two animals in each group surviving longer than 12 days. In some cases animals survived longer than the 700 hours shown, but since no further change was seen beyond this point, the curves have been truncated to conserve space. While four animals in each methamphetamine group survived longer than 12 days, the curves for only two animals are presented. The curves for the remaining animals in each group were intermediate to those shown, with drug intake times of 73.1% and 79.5% for the d-methamphetamine and 68.3% and 69.7% for the l-methamphetamine animals. As these data show, an almost constant amount of time was spent in drug intake periods for each animal over the entire self-administration period. In all except one animal, drug intake time ranged from 63.9% to 80.4% of the total self-administration time. The lengths of a given drug intake period and the following abstinence period were not significantly correlated, and the lengths of a given abstinence period and the following drug intake period were significantly correlated ($P < .01$) in only one case out of 12 animals tested. Significantly more intake and abstinence periods occurred during the latter half than the first half of the self-administration period (sign test, $P < .01$).

The stereotypic behavior observed in all animals often lead to body licking of paws, legs, and ventral body surfaces and resulted in denuding

Figure 3

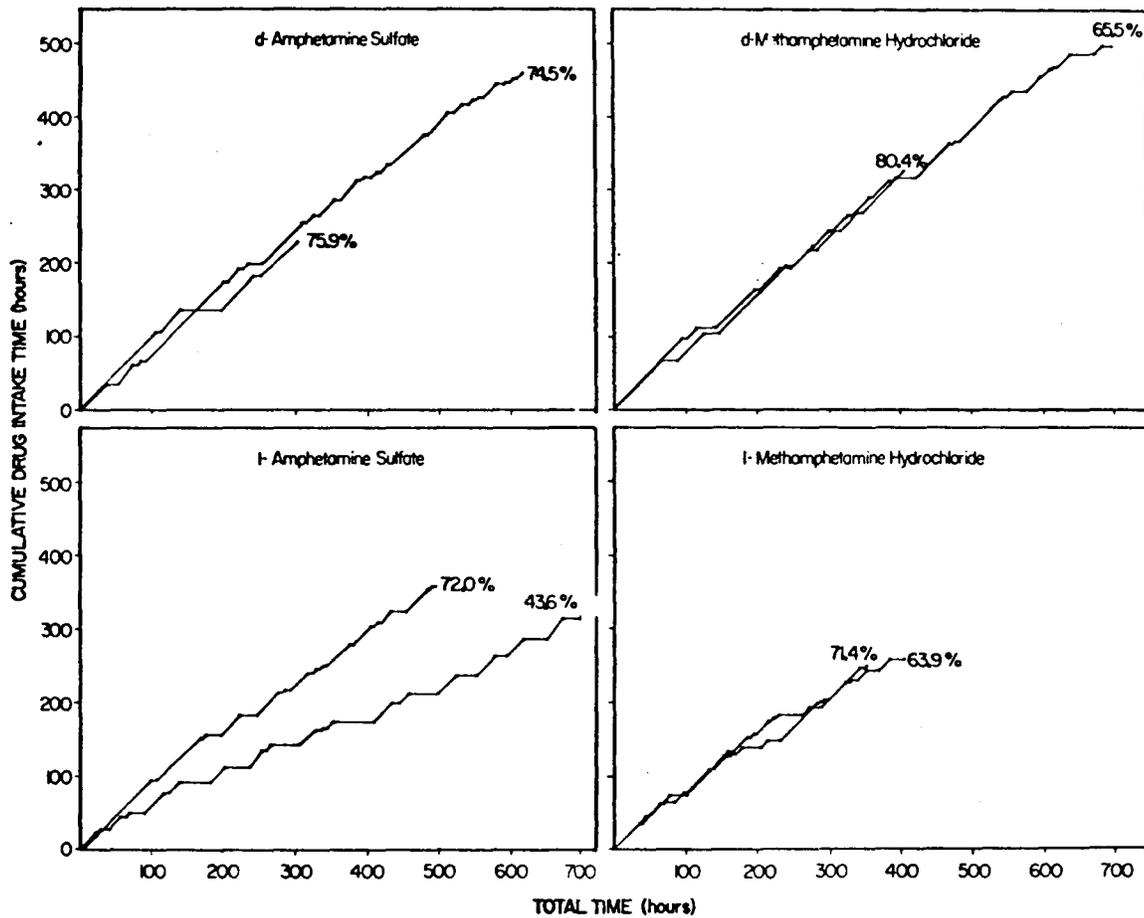


Figure 3. Patterns of drug intake (cumulative vertical lines) and abstinence (horizontal lines) for two animals in each drug group.

of these areas and, in some animals, irritation to the point of bleeding and digit loss. Table 2 shows the physical history of each group of animals. An analysis of time to onset of body self-irritation (as evidenced by a denuded area, most often on a forelimb, when the subject was weighed) revealed significant differences among the drug groups (F test, $p < .05$). Onset of self-irritation occurred earlier in the d-methamphetamine group than in either the l-methamphetamine (t-test, $p < .01$) or l-amphetamine ($p < .05$) group. Differences between the d-methamphetamine and d-amphetamine groups and all other group comparisons, however, were not significant. No significant group differences were found for time of death or body weight loss over the drug self-administration period. No significant correlations were found between the time of death (duration of self-administration) and the subjects' age or weight at the onset of self-administration, the duration of the initial complete suppression of eating, drug intake of the first day of self-administration, or the observed onset of self-irritation.

DISCUSSION

Over the course of the study (Figure 2), d-methamphetamine and d-amphetamine intake increased, l-methamphetamine intake initially increased and then decreased, and l-amphetamine intake decreased. The percent time spent in drug intake and abstinence remained relatively constant, however, with only an increase in the number of such periods. Since rats maintain an almost constant mg/kg/hr level of drug intake, (Yokel and Pickens, 1973), an increase in drug intake at a constant injection dose indicates an effective lowering of the injection dose of the drug. Since the intake of d-methamphetamine increased and d-amphetamine appeared to increase in the present study, this suggests the possibility of tolerance developing in the self-administration of the dextro, but not the levo isomers. This supports the view that

Table 2: Incidence and time to self-irritation and death and weight loss for each drug group (Mean \pm S.E.)

Physical history of drug groups

	Self-Irritation		Death		Weight	
	Number	Onset (hours)	Number	Time (hours)	Pre-Surgery (grams)	Percent Weight Loss at Death
<u>d</u> -Methamphetamine	7/8	46 \pm 4 ^{a,b}	6/8	213 \pm 77	435 \pm 19	28 \pm 3
<u>l</u> -Methamphetamine	5/8	125 \pm 23	4/8	244 \pm 49	416 \pm 23	42 \pm 5
<u>d</u> -Amphetamine	6/8	72 \pm 24	8/8	251 \pm 60	408 \pm 23	34 \pm 1
<u>l</u> -Amphetamine	6/8	69 \pm 6	6/8	191 \pm 64	429 \pm 17	33 \pm 4

^a Significantly different from l-methamphetamine (p<.01)

^b Significantly different from l-amphetamine (p<.05)

amphetamine tolerance in rats results from the formation of p-hydroxynorephedrine which is formed by the dextro, not the levo isomer of the drugs (Costa and Groppetti, 1970; Brodie et al., 1970; Goldstein and Anagnoste, 1965). Following the onset of self-administration, the return to eating indicates tolerance developing to the anorexigenic effects of d-methamphetamine, l-methamphetamine, and d-amphetamine groups. While eating also returned somewhat in the l-amphetamine group, the change only approached significance ($.1 > p > .05$). The development of tolerance to the decreased food intake but not to the increased activity effect agrees with data obtained with dl-amphetamine in the rat (Tormey and Lasagna, 1960), and further supports multiple mechanisms in amphetamine tolerance (Schuster, et al., 1966; Schuster and Zimmerman, 1961).

Deneau et al., (1969) have also reported increasing intake of d-amphetamine in rhesus monkeys, but with the intake leveling off at 2-3 weeks, about the time an appreciable increase was seen in the present study. Kramer et al. (1967) observed humans to increase their injection dose within a period of drug intake, whereas the present study found that the higher level of drug intake from one drug period was carried into the next drug period. The shorter abstinence periods in the rat may not allow for the loss of tolerance seen with the longer abstinence periods in man.

To the extent that drug self-administration techniques can be used to predict human drug abuse liability, the present findings suggest that the levo isomers of amphetamine and methamphetamine may possess sufficient reinforcing properties to pose an abuse problem in humans if such compounds were more readily available.

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