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Tolerance and Cross-Tolerance among Psychotomimetic Drugs*

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Summary. A fixed-ratio schedule of milk reinforcement (FR 30) was used to study tolerance to the effects of D-LSD, L-LSD, BOL, psilocybin, mescaline, and D-amphetamine in the rat. A decrease in the amount of disruption of bar-pressing occurs with repeated daily administration of appropriate doses of all of the "psychotomimetic" compounds, and cross-tolerance was also demonstrated.

Key-Words: Hallucinogens — Drug Tolerance — Lysergic Acid Diethylamide — Mescaline — Psilocybin.

The effects of D-lysergic acid diethylamide (LSD) on the bar-pressing behavior of rats have recently been the subject of several investigations (e.g., APPEL and FREEDMAN, 1964; FREEDMAN *et al.*, 1964; GARDNER, 1965; JARRARD, 1964; APPEL and FREEDMAN, 1965) and tolerance to these effects on fixed-ratio (FR) and variable-interval (VI) schedules of positive reinforcement has been reported (FREEDMAN *et al.*, 1964). In the present experiment, an FR schedule is again used to determine whether or not tolerance occurs in the rat as well as in man (e.g., ABRAMSON and ROLO, 1965; ISBELL *et al.*, 1955, 1959, 1961, 1962) with compounds which are chemically or pharmacologically related to D-LSD, i.e., L-LSD, bromo-lysergic acid (BOL), psilocybin, and mescaline, and to demonstrate the phenomenon of cross-tolerance.

Methods and Materials

Behavioral Procedures and Apparatus. The general procedure has been described in detail elsewhere (FREEDMAN *et al.*, 1964). Ninety-day old male albino rats of Sprague-Dawley strain obtained from Charles River Laboratories, Wilmington, Massachusetts, were maintained on a 22 h food deprivation schedule. In addition, their food intake was restricted such that their weights remained at a relatively constant level throughout the investigation. About 10 g of Wayne Laboratory Chow was given each day in addition to whatever liquid food was obtained in the appa-

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ratus. Water was always available in individual home cages (but not in the experimental space) which were housed in a room kept on a 12 h day-night cycle and constant temperature and humidity.

The experiments were run in a box containing a single lever or bar which could be activated by a force of approximately 15 g. Sound and light attenuation were provided by enclosing the apparatus in an insulated and ventilated outer chamber. All experimental events were programmed and recorded automatically in an adjoining room by relay and timing circuits.

Each animal was run 1 h per day, seven days per week. Every bar-pressing response was initially rewarded by 3-sec access to a liquid diet of sweetened milk, eggs, and vitamins. The ratio of responses to reinforcements was subsequently increased gradually until all of the rats had to press the bar 30 times to obtain each reinforcement (FR 30).

Each animal was given at least 3 weaks of exposure to the FR 30 schedule before the first control injection was administered.

Pharmacological Procedures. Drugs or an injection of 0.5 ml of isotonic saline were administered intraperitoneally (i.p.) each day immediately before the animal was placed into the apparatus. At least 7 days always occurred between treatments. Tolerance was induced by 7—8 daily administrations of the same dose of the same compound. Doses were selected on the basis of results of pilot experiments in our laboratory. Cross-tolerance was studied by first giving the challenge drug, second, making the animal tolerant to the test drug one week later and third, giving a second treatment of the same dose of the challenge drug on the day after the final tolerance day. Alternatively, the "second" treatment was compared with the response to the challenge drug given one week *after* tolerance.

Three of the drugs (D-LSD, BOL, and psilocybin) were injected from ampules in dosages of 0.1 mg/ml, 0.5 mg/ml and 3 mg/ml respectively[1]. Solutions of the sulfate salts of mescaline (10 mg/ml) and D-amphetamine (1 mg/ml) and L-LSD, in the form of the free base dissolved in an acid solution (pH = 5.6) were prepared for use in these experiments in our laboratory.

Methods of Presenting Drug Effects. The effects of drugs are shown directly on cumulative-response curves of the performances of individual animals for sessions following injections of either control (saline) or active compounds or as the average percentage of control responses. The percentage for any animal is obtained by dividing the total number of responses on any drug day by the mean number of responses on the four days of saline preceding treatment.

[1] These ampules were provided by Sandoz Pharmaceuticals, Hanover, New Jersey.

In the study of tolerance, the percent of control responses (using the four saline days preceding the first drug day as a base line) is plotted as a function of days.

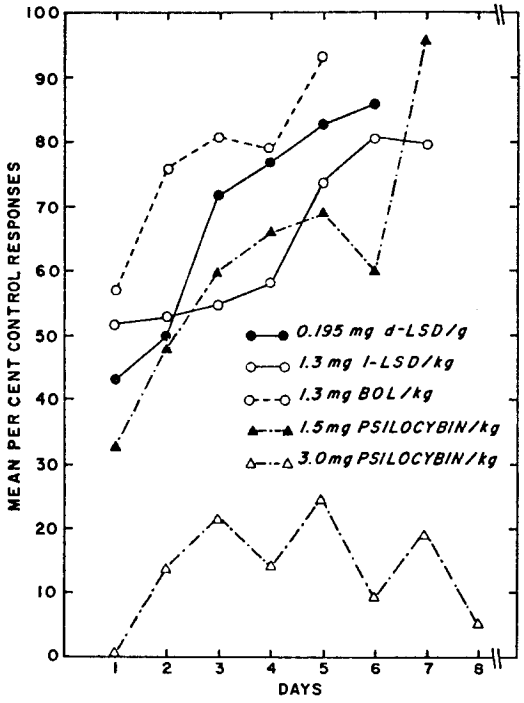


Fig. 1. Tolerance to psychotomimetic drugs on an FR 30 schedule of reinforcement in the rat. (See Method and Materials section)

Results

With appropriate dosage regimens, tolerance develops to all of the psychotomimetic drugs we have tested. That is, there is a decrement of effect contingent on repeated administrations of the same amount of the compound. This is shown for three different drugs in Fig. 1. Both D-LSD (0.195 mg/kg) and L-LSD (1.3 mg/kg) show at least partial tolerance, i.e., the rate of bar-pressing returns to 80% of normal in 5—6 days of daily treatment. With BOL (1.3 mg/kg), tolerance develops even more rapidly and, with 10 mg mescaline/kg (not shown), tolerance develops most rapidly of all, i.e., in 2—3 days. Tolerance can also be seen with 1.5 mg psilocybin/kg but not with 3.0 mg/kg of the same compound.

When tolerance occurs, the decrement of effect is primarily due to a shortening of the period(s) of no responding (FREEDMAN *et al.*, 1964).

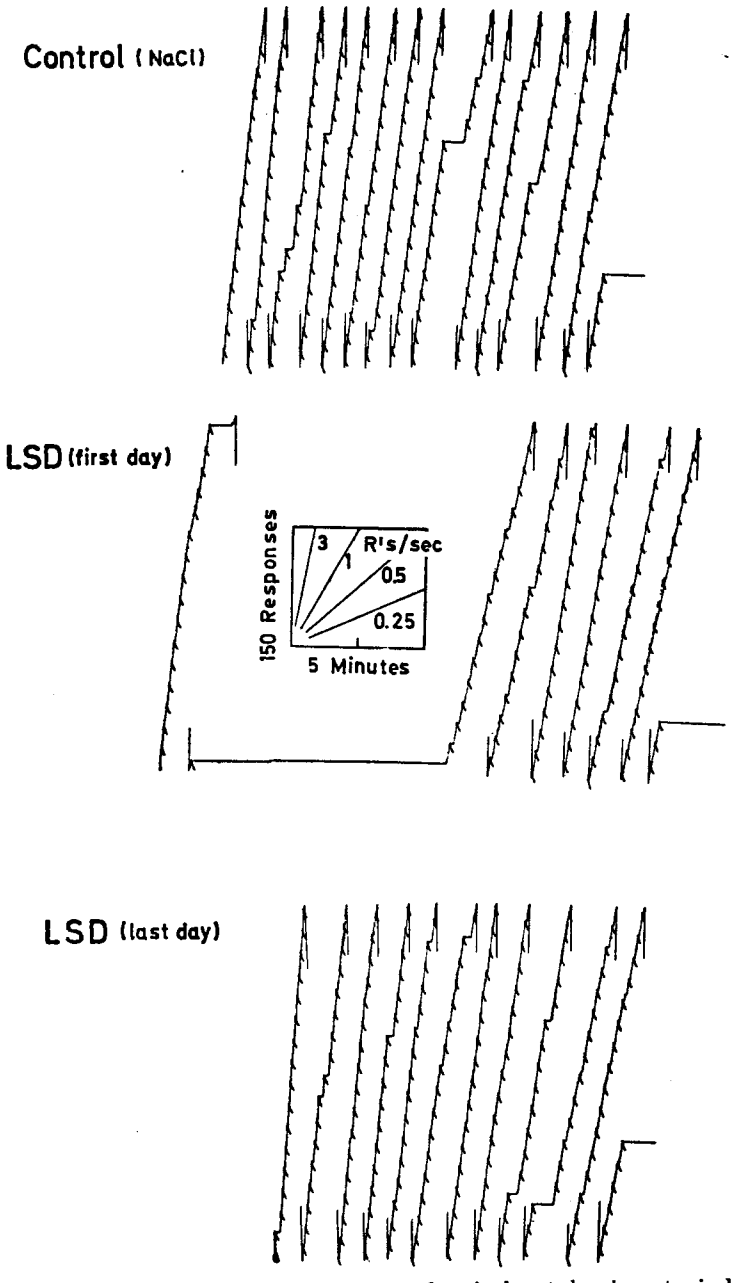
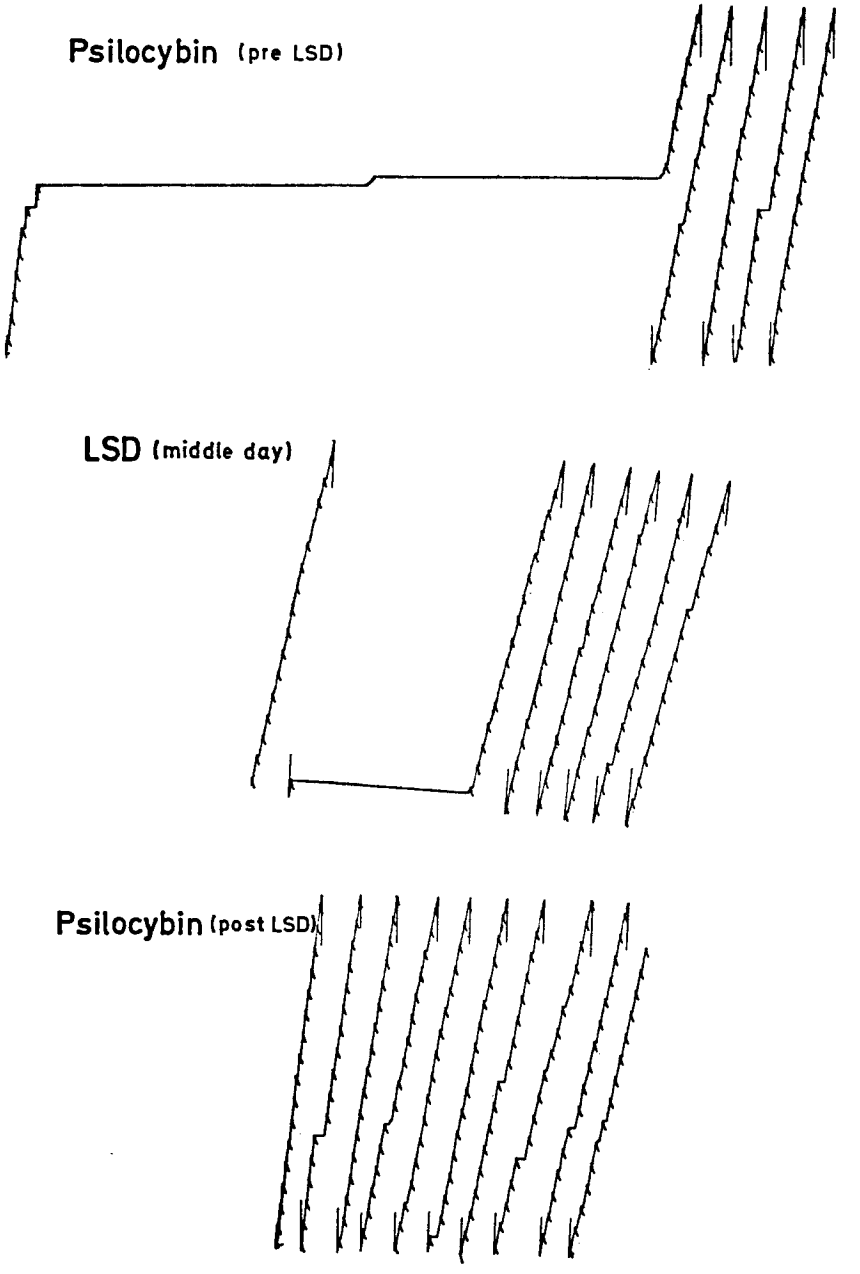


Fig. 2. Cumulative records of the performance of a single rat showing a typical con- between D-LSD and 1.5 mg psilocybin/kg. The slopes of these curves are pro-



trol session, the development of tolerance to 0.195 mg D-LSD/kg and cross-tolerance portional to rate of bar-pressing and reinforcements are indicated by vertical "pips"

This can be seen in the case of 0.195 mg LSD/kg in Fig. 2 where cumulative records of three sessions of treatment for a single animal are shown.

Perhaps the most interesting results of these experiments concern cross-tolerance. That is, if an animal is made tolerant to one psychotomimetic it will, at the same time, be tolerant to other related agents. This is shown in Fig. 2 where the effect of 1.5 mg psilocybin/kg given 10 days before treatment with D-LSD (pre LSD) is compared with administration of the same dose of the same compound on the day after tolerance to LSD (post LSD).

Cross-tolerance relationships for several animals are presented in the Table which shows the average effect of a number of compounds before ($\bar{X}$ % pre) and after ($\bar{X}$ % post) tolerance is induced and the results of statistical analyses. To demonstrate cross-tolerance, $\bar{X}$ % post must be $> \bar{X}$ % pre so that the percent change (Table, column 8) will be positive. While only two of the t_d tests which were run on these percent change or difference scores were significant at the 0.05 level of confidence (probably because of the relatively small number of subjects) all of the results with the exception of those with D-amphetamine were in the appropriate direction.

Table. *Cross-Tolerance*

Challenge Drug	Dose mg/kg	N	$\bar{X}$ % Pre	$\bar{X}$ % Post	% Change	t_d	p
A. Animals tolerant to D-LSD (0.195 mg/kg)							
BOL	1.30	4	54	68	+ 14	1.38	< 0.30
Psilocybin	1.50	5	36	68	+ 32	4.46	< 0.02
Mescaline	10.00	6	44	76	+ 32	2.78	< 0.05
	15.00	1	65	26	− 39		
Amphetamine	1.00	4	69	65	− 4		
	2.00	2	36	22	− 14		
B. Animals tolerant to BOL (1.3 mg/kg)							
D-LSD	0.13	5	56	81	+ 25	1.46	< 0.30
Psilocybin	1.50	4	22	30	+ 8	0.80	< 0.50
C. Animals tolerant to psilocybin (1.5 mg/kg)							
D-LSD	0.13	5	49	87	+ 38	1.74	< 0.20
D. Animals tolerant to mescaline (10 mg/kg)							
D-LSD	0.13	6	52	86	+ 34	2.23	< 0.10

The Table, Part A, shows that the effects of psilocybin, 10 mg mescaline /kg and perhaps BOL are attenuated by inducing tolerance to LSD. Since tolerance to BOL, psilocybin, and mescaline similarly alter the effects of LSD (Table, Parts B, C, and D) it can be assumed that cross-tolerance relationships among psychotomimetics probably go in both directions;

tolerance to appropriate doses of any one compound will induce cross-tolerance with any of the others.

Discussion

The results of these experiments confirm earlier findings that tolerance to the effects of D-LSD occurs in the rat (FREEDMAN *et al.*, 1964). It also can be observed with compounds which are chemically very closely related to D-LSD (e.g., L-LSD, BOL) or which have similar, but less potent psychotomimetic effects in man (e.g., psilocybin, mescaline). As was the case with D-LSD, the probability that tolerance will develop is directly related to dose, but it is no doubt also related to the type of behavior being measured and to a large variety of other pharmacological and physiological variables. The rate at which tolerance develops is also inversely related to the relative potencies of the test drugs (APPEL and FREEDMAN, 1965); i.e., tolerance develops most rapidly to the least potent compound, mescaline, and least rapidly to the most potent compound, D-LSD.

It is unlikely that tolerance can be explained by appealing to psychological processes such as "learning" since studies of acute tolerance show that it is not necessary to run an animal after each drug treatment to demonstrate tolerance (FREEDMAN *et al.*, 1964); tolerance and cross-tolerance are, in other words, pharmacological phenomena.

While these experiments suggest that cross-tolerance among chemically or pharmacologically similar compounds can occur, they do not demonstrate that it must occur. Similarly, cross-tolerance among pharmacologically dissimilar agents does not seem to develop, at least in the case of D-amphetamine (a CNS stimulant) and LSD (a psychotomimetic) but, of course, no experiments can show that it will never occur. It is not, however, unreasonable to assume that cross-tolerance is more likely to develop among chemically similar compounds than among completely unrelated compounds.

Finally, these results indicate that tolerance and cross-tolerance relationships among psychotomimetic compounds in the rat and man are remarkably similar. Thus, an FR schedule can be used to measure both the relative potencies of these compounds (APPEL and FREEDMAN, 1965) and the extent to which tolerance and cross-tolerance develops among them.

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