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Behavioral Variables Affecting the Development of Amphetamine Tolerance*

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Amphetamine administration produces a disruption of timing behavior in subjects who are reinforced for responding at a low rate (SIDMAN, 1956; DEWS and MORSE, 1958). With continued daily administration of amphetamines, performance changes progressively toward that observed under saline control conditions (SCHUSTER and ZIMMERMANN, 1961; ZIMMERMAN and SCHUSTER, 1962). General activity measures taken from the same subjects are consistently elevated over the course of the chronic-drug period. The evidence suggests a certain specificity in what behaviors will show the development of tolerance to chronically administrated amphetamines. The present report deals with a series of experiments designed to analyze the role of reinforcement contingencies as one class of variables that influence the development of behavioral tolerance to amphetamines.

Experiment I

Materials and Methods

Subjects. Three Sprague-Dawley male rats were used that ranged in weight from 300—320 g. The subjects were gradually reduced to 70% of their original body weight and maintained at this level by adjusted feedings after each experimental session.

Apparatus. The experimental chamber was a standard Gerbrands rat box containing a lever operandum, a Gerbrands pellet dispenser that delivered 45 mg Noyes rat pellets and two 5-Watt bulbs to provide visual stimuli. The experimental chamber was enclosed in a modified

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picnic chest which was enclosed in a large sound-attenuating chamber. Programming of stimulus events and response recording was accomplished by switching and timing circuits, a cumulative recorder, and electrical impulse counters.

Procedure. The animals were initially conditioned to press a response lever for a pellet of food. After one session in which every response was reinforced with food, the subjects were placed under the contingencies of a 2-ply multiple schedule of reinforcement. The multiple schedule consisted of fixed-interval (FI) and differential reinforcement of low rate (DRL) components. In the FI component the subject was reinforced with a presentation of food for the first lever response occurring after 30″ had elapsed from the previously reinforced response. Responses occurring before the 30″ had elapsed were recorded but had no other programmed consequences. The house lights were illuminated continuously during the FI. After 10 minutes on the FI schedule a 30″ black-out period occurred during which all lights in the experimental chamber were turned off. Lever responses during the 30″ black-out period had no experimentally specified consequences and were not recorded. Following the black-out period, the DRL schedule was presented for 10 minutes. During this period the subject was reinforced with food for those responses which occurred a minimum of 30″ after the preceding response. Responses occurring prior to the 30″ minimum time interval reset the 30″ timer and therefore postponed reinforcement opportunity by 30″. During the DRL component the house lights flashed in an irregular pattern with an average of 2 per second. The total session length of 62.5 min was comprised of three 10 minute FI periods alternated with three 10 minute DRL periods. The 30″ black-out period occurred after each schedule change. The subject's performance stabilized in both schedules after 75 consecutive daily sessions.

The total number of lever-pressing responses and food reinforcements were recorded separately for the FI and DRL components. In addition, the subject's lever-pressing responses in the FI component were recorded separately in each of the 6 consecutive 5″ periods covering the 30″ FI length. The sixth counter cumulated all responses occurring from 25″ on. This method of recording allows the analysis of the temporal distribution of FI responses. A convenient way of summarizing these data is to determine the average length of time expired before 25 and 75% of the total number of responses had occurred.

The subject's DRL lever-pressing responses were recorded in an 11—5″ compartment inter-response time (IRT) distribution (SIDMAN, 1956). For example, a response occurring between 30—35″ from the previous response was recorded in counter 7. Counter 11, the final counter, recorded all responses occurring 50″ or more from the previous

response. To present the large amount of data accrued in the present report the IRT data was simplified using a method described by HODOS (1963). In this method the mode of the IRT distribution is selected by visual inspection exclusive of the first compartment. The variability of responses around the mode is quantified by computing the interquartile range disregarding the first compartment. The first IRT compartment is not used in this computation or in the selection of the mode since the large number of responses occurring here reflect a response "burst" rather than temporally spaced responses.

Drug Administration. d-Amphetamine SO_4 was dissolved in physiological saline in a concentration of 1.0 mg/cc. This solution was diluted appropriately so that a constant volume (.1 cc/100 g of body weight) was given for all dosages. Drug solutions were freshly prepared every 5 days. The drug was administered subcutaneously along the flank of the animal thirty minutes prior to the experimental session. Control injections of physiological saline were administered in the same manner.

Pre-Chronic Drug. A dose-response curve for d-amphetamine was obtained spacing the drug administration so that 4 non-drug sessions intervened between each drug session. Dosages of .125, .25, .50 and 1.0 mg/kg of d-amphetamine SO_4 were tested in a random order in addition to six saline control sessions.

Chronic Drug. The subjects were placed on a chronic-drug regimen in which 1.0 mg/kg of d-amphetamine was administered 30 minutes prior to each experimental session, for 30 consecutive days. Following this chronic-drug regimen the subjects performed daily for approximately one month (26—32 days) under saline control conditions.

Post-Chronic Drug. A post-chronic drug dose-response curve for d-amphetamine was obtained using the same procedure and dosages as above (pre-chronic drug).

For the saline control periods and the chronic-drug regimen the FI-DRL data were analyzed in 6 session averages. For the averages the standard error of the mean was computed as a measure of variability.

Results. Under saline control conditions the subjects' performance in the FI components showed the typical "scallop" shaped temporal distribution of lever-pressing responses. The low rates of lever responding and the frequency of reinforcement in the DRL component approximate that observed in previous experiments using DRL schedules alone (ZIMMERMAN and SCHUSTER, 1962).

Fig. 1 (Pre-chronic drug) shows the effects of various dosages of d-amphetamine on the total number of lever-pressing responses in the FI and DRL components. As can be seen for subject R-2 the total responses in the FI and DRL show a marked increment as a function of d-amphetamine dosage. Subject R-5 shows a slight increase in the total number of

responses in the FI and a marked increase in the total number of responses in the DRL. Subject R-4, on the other hand, shows a marked decrement in total FI responses and negligible change in total DRL responses at the dosages tested.

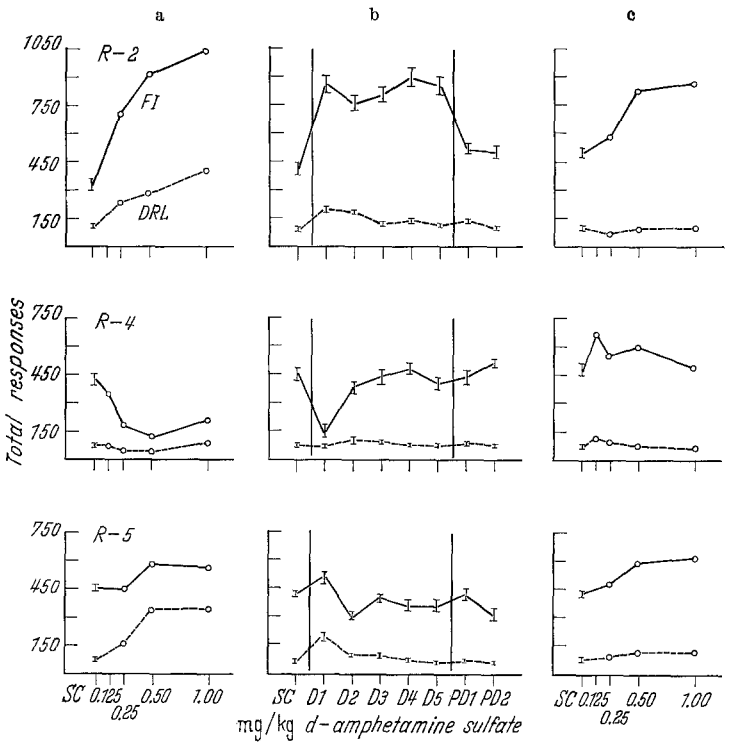

Fig. 1 a—c. The effects of various dosages of d-amphetamine on total responses in the FI and DRL schedules of reinforcement prior to the chronic drug regimen (a). Average number of responses in the FI and DRL under saline control conditions (SC, PD, and PD_2) and during the chronic drug regimen (D_1—D_5). Each point represents an average of 6 sessions (b). Repetition of dose-response function after the chronic-drug regimen (c). FI ———, DRL — — —

Fig. 1 (Chronic drug) shows the average total responses for the FI and DRL components under saline control conditions and over the course of the chronic-drug regimen. For subject R-2 the total number of FI responses shows a marked and sustained increase throughout the 30-day drug period. The total number of responses in the DRL component, however, shows, after an initial increment, a gradual decline over the course of the drug period. The total number of responses in the FI for subject R-4 shows a marked decrement in the initial period of the chronic-drug regimen followed by a return to the normal number of responses throughout the last 24 days of the chronic-drug period. The total number of

responses in the DRL component for this subject was unaffected by this dosage of d-amphetamine. Subject R-5 shows no consistent change in the total responses in the FI over the chronic-drug period. The total number of responses for subject R-5 in the DRL component, however, shows a marked increment in the first 6 days of the chronic-drug regimen followed by a gradual return to the rate observed under saline control conditions.

Table 1. *The mode and interquartile range (Q_1—Q_3) for DRL inter-response times under saline control and chronic d-amphetamine administration. Values are six session averages*

Drug Treatment condition	Subjects					
	R 2		R 4		R 5	
	Mode	Q_1—Q_3	Mode	Q_1—Q_3	Mode	Q_1—Q_3
Saline Control	33.0	20.5—36.0	27.5	24.0—37.0	35.0	26.0—40.0
Drug Periods D1	7.5	9.0—22.0	12.5	19.0—41.0	12.0	13.0—31.5
D2	7.5	8.0—28.0	17.5	16.5—29.0	17.5	17.5—30.0
D3	17.5	13.0—34.0	22.5	19.0—31.5	22.5	17.5—29.0
D4	18.5	12.5—32.0	22.5	22.0—33.0	27.5	21.0—40.0
D5	23.0	16.5—36.0	27.5	22.5—40.0	32.5	27.5—45.0
Post Drug Saline Control PDSC1	15.5	12.5—31.0	27.0	21.0—47.0	30.5	23.0—38.5
PDSC2	26.0	16.5—31.0	32.0	23.0—42.0	32.5	26.0—41.5

A more refined analysis of the subjects' DRL performance under saline control and chronic-drug conditions is given in Table 1. The mode, Q_1, and Q_3 values of the IRT distributions are shown in this table. For all three subjects the mode of the IRT distribution under the pre-drug saline control condition closely approximates the 30 second minimum interval by which responses were required to be separated for reinforcement. In the initial portion of the chronic-drug regimen (D1) the mode shows a marked decrement indicative of more frequent short inter-response times. With continued administration of the drug, however, (D2—D5) the mode and the Q_1 and Q_3 values show a progressive increment ultimately reaching a value closely approximating that observed under the pre-drug saline control condition.

Table 2 presents Q_1 and Q_3 values for the FI response time distributions under saline control and chronic-drug conditions. These measures show no change as a function of the chronic drug regimen for any of the subjects. This is particularly impressive in the case of subjects R-2 and R-4, who showed marked and opposite changes in the total number of responses.

Table 3 shows the average number of reinforcements received in the FI and DRL components under saline control and chronic-drug condi-

tions. Total number of FI reinforcements for subjects R-2 and R-5 was unaffected by the chronic-drug regimen. In contrast, the total number of DRL reinforcements is lower at the beginning of the chronic-drug regimen (D1) than at the end (D5). Subject R-5 has an average number of DRL reinforcements as great at the end of the chronic-drug regimen (D5) as ever observed under saline control conditions. Subject R-4 shows an

Table 2. *Time elapsed before 25% (Q_1) and 75% (Q_3) of the total FI responses are emitted under saline control and chronic d-amphetamine administration. Values are six session averages*

Drug Treatment condition	Subjects		
	R 2	R 4	R 5
	Q_1—Q_3	Q_1—Q_3	Q_1—Q_3
Saline Control	22.5—28.5	22.0—28.0	24.0—28.5
Drug Periods D1	20.5—29.0	23.5—27.0	23.5—28.5
D2	20.5—28.0	22.0—27.5	22.5—28.0
D3	21.0—28.0	23.5—28.5	24.5—28.5
D4	21.0—28.0	24.0—29.0	23.5—28.5
D5	21.0—28.5	24.5—28.0	24.0—29.0
Post-Drug Saline Control			
PDSC1	24.0—28.5	22.5—28.5	22.0—27.5
PDSC2	23.0—28.5	23.0—27.5	23.5—28.0

initial decrement in average FI and DRL reinforcements in the first 6 sessions of the chronic-drug regimen (D1) followed by a gradual trend towards saline control values.

Fig. 1 (Post-Chronic drug) shows the dose response function obtained one month after the chronic-drug regimen. The total number of FI responses of subjects R-2 and R-5 show an increment as a function of dosage comparable to that observed in the prechronic drug dose-response curve. The total responses in the DRL for these subjects, however, do not show an increment at any dosage comparable to that seen in the prechronic drug dose-response curve. In the post-chronic drug dose-response curve for R-4 the total number of responses in the FI shows an increment at the lower dosages. This is in contrast to the marked decrement observed at these dosages in the pre-chronic drug dose-response function. The total number of DRL responses for R-4 shows an increase only at the lowest dosage tested in the post-chronic drug-response curve.

Discussion. The disrupting effects of amphetamines on the accuracy of timing behavior generated by a DRL schedule of reinforcement are by now well confirmed (SIDMAN, 1956; DEWS and MORSE, 1958; SCHUSTER and ZIMMERMAN, 1961; ZIMMERMAN and SCHUSTER, 1962). The gradual diminution in the drug's effect observed in the present experiment with

Table 3. *Average number of total reinforcements for the FI and DRL components under saline control conditions and chronic d-amphetamine administration. Values are six session averages and variability is expressed as the standard error of the mean*

Drug Treatment condition		Subjects					
		R 2		R 4		R 5	
		FI	DRL	FI	DRL	FI	DRL
Saline Control		55.0 ± 1.0	29.0 ± 2.1	55.0 ± 1.1	26.0 ± 1.5	55.0 ± 1.0	31.0 ± 1.1
Drug Periods	D 1	54.0 ± 1.0	9.0 ± 2.4	41.0 ± 2.4	19.0 ± 1.1	55.0 ± 1.0	16.0 ± 3.9
	D 2	52.5 ± 1.3	16.0 ± 2.3	49.0 ± 1.8	15.0 ± 1.6	52.0 ± 1.1	16.5 ± 2.1
	D 3	54.5 ± 1.1	14.5 ± 1.8	51.0 ± 2.0	17.5 ± 1.3	55.0 ± 1.0	14.5 ± 1.3
	D 4	53.0 ± 1.0	13.0 ± 2.1	52.5 ± 1.1	21.0 ± 2.1	50.0 ± 2.3	24.0 ± 1.8
	D 5	54.0 ± 1.5	19.5 ± 1.7	54.0 ± 1.3	23.0 ± 2.6	53.5 ± 1.0	30.0 ± 2.0
Post-Drug Saline Control							
	PDSC 1	54.5 ± 1.3	12.5 ± 1.8	54.0 ± 2.1	27.0 ± 1.6	53.0 ± 1.0	28.0 ± 1.5
	PDSC 2	55.0 ± 1.1	15.5 ± 2.4	55.0 ± 1.3	29.5 ± 1.8	52.5 ± 1.0	32.0 ± 2.0

continued daily administration of d-amphetamine, corroborates the previously noted development of behavioral tolerance using simple or multiple DRL schedules (SCHUSTER and ZIMMERMAN, 1961; ZIMMERMAN and SCHUSTER, 1962). We do not imply a mechanism by suggesting that the term behavioral tolerance be applied to this phenomenon, rather that the term can be used with operational clarity when we observe a gradual decrease in a behavioral effect of amphetamines with repeated administration.

The dose-response curves for amphetamine reveal marked individual differences in our subjects. It has been our experience that this occurs frequently with amphetamines particularly with complex schedules of reinforcement. We have attempted to utilize this variability in the present experiment. In this regard subjects R-2 and R-4 are of particular interest. Subject R-2 showed a marked increment in the total number of FI responses which was sustained throughout the entire chronic-drug regimen. The stimulating effects of amphetamine in the FI performance showed no diminution with repeated administration. Therefore, by definition, this subject's FI performance did not show the development of tolerance. The post-chronic drug dose-response function of this subject showed comparable stimulation in the total FI responses to that observed in the pre-chronic drug dose-response function. Subject R-4, in contrast, showed an initial depression in total FI responses followed by a progressive decrement in the effect of the drug with repeated administration. This gradual diminution with repeated administration of the depressant effect of the drug on the subject's FI performance fits our conception of behavioral tolerance.

For both the DRL and FI performance tolerance was observed in the post-chronic drug dose-effect curves. That is, those subjects who developed tolerance during the chronic-drug regimen remained resistant to the actions of amphetamine thirty days after the cessation of chronic administration of the drug. Further parametric experimentation is needed to determine the variables controlling the permanence of amphetamine tolerance.

We are now faced with the question of what common variables may account for the observed behavioral tolerance to repeated administration of d-amphetamine in DRL performance while tolerance is observed in FI performance only when the rate of response is decreased by the action of the drug. Clearly the common physiological mechanisms responsible for drug tolerance cannot be appealed to as an explanation. If the tolerance observed was attributable to changes in absorption or metabolism, there would be no explanation for the differential development of tolerance in the different behaviors. Table 3 which shows the average number of FI and DRL reinforcements under saline control and chronic-

drug conditions may hold the key to this problem. Where the initial effect of the drug on either DRL or FI performance was such that the reinforcement frequency fell, we have observed the development of behavioral tolerance. We shall delay a more explicit statement of our hypothesis regarding this relationship between reinforcement frequency and amphetamine tolerance until our discussion following the next experiment.

Experiment II

Shock-Avoidance

The second experiment in this series was undertaken to determine whether or not behavioral tolerance would develop to chronically administered d-amphetamine where the drug enhances conditions of reinforcement through changes in behavioral output. A second question which this experiment was designed to answer was whether or not the facilitating effects of amphetamines would transfer after long term chronic administration to the non-drug condition. Previous reports have shown that amphetamines have a facilitating effect upon avoidance behavior generated by a Sidman avoidance schedule (VERHAVE, 1958). This effect is particularly pronounced in subjects whose avoidance is below optimum (HEARST and WHALEN, 1963). We selected ,therefore, for our investigation "poor avoidance" animals trained in a modified Sidman-avoidance procedure (SIDMAN, 1953).

Method and Apparatus

Subjects. The subjects were four Sprague-Dawley rats approximately 350—375 g in body weight. Subjects were given ad-lib food and water in their home cages.

Apparatus. The experimental chamber was a standard Gerbrands rat box containing a lever operandum for the rat to depress, with a grid floor wired for the delivery of electric shock. A single 5 watt bulb provided illumination during the experimental session. The experimental chamber was enclosed in a modified picnic chest which in turn was located in a sound-attenuating chamber. Programming of the onset, duration, and intensity of the electric shock was controlled by timers and a Grason-Stadler shock source and grid-scramber. The shock intensity was set at 2.0 ma. throughout the experiment. The subjects' lever-pressing performance was recorded on electrical impulse counters, running time meters, and a Gerbrands Model C cumulative recorder.

Procedure. The subjects were exposed daily to a 90 minute session of shock avoidance. In this schedule failure to make a lever pressing response for a period of 30 seconds resulted in the onset of shock which continued until the lever was depressed or for a maximum of 10 seconds. It should

be noted that in this procedure there is no exteroceptive warning stimulus prior to the shock. The total number of lever responses, shocks, and escape latencies were recorded.

After 125 hours of training four subjects were selected from a larger group of animals because of their "poor" but stable avoidance performance. Following 7 saline control sessions they were started on a thirty-five day chronic-drug regimen in which 1.0 mg/kg of d-amphetamine was administered subcutaneously 30 minutes prior to each daily session. This condition will be referred to as chronic-drug I.

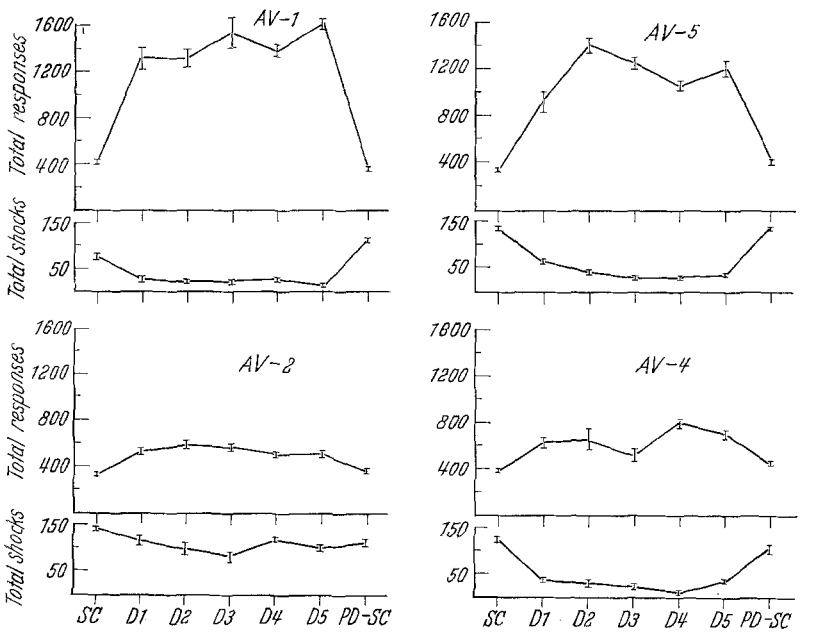

Fig. 2. Average number of total responses and shocks under saline control conditions (SC, PD—SC) and during the chronic-drug regimen (D_1—D_5). Each point represents an average of 7 sessions and the brackets indicate the standard error of the mean

After 20 saline control sessions two of the subjects were again placed on a chronic-drug regimen for 20 days. In the 2nd chronic-drug period the dosage of the drug was begun at 1.0 mg/kg and reduced daily by .05 mg/kg. An additional seven saline control sessions were run at the end of this 2nd chronic-drug regimen.

Results. Fig. 2 shows the total number of lever responses and shocks for the 4 subjects under saline control (SC and PDSC) and chronic drug (D_1—D_5) conditions. The total number of responses was increased for all subjects throughout the entire course of the chronic-drug regimen. Subjects AV-1, AV-4, AV-5, showed a large increment in number of

responses at this dosage of d-amphetamine and the number of shocks received was markedly decreased over the course of the chronic-drug regimen. AV-2 showed a smaller increment in response rate and did not show a clear decrease in number of shocks received. The drug regimen did not affect the approximate .6 second escape latencies for any subject.

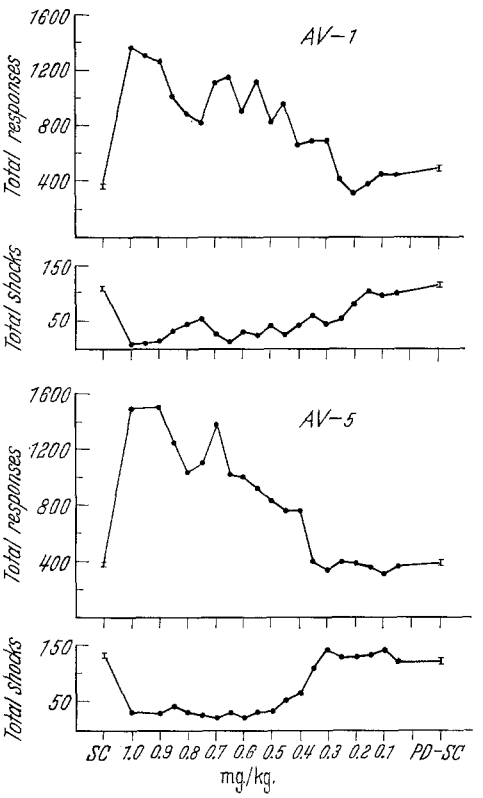

Fig. 3. Total responses and shocks under saline control conditions (SC and PD—SC) and as a function of daily decreasing dosages of d-amphetamine

When the subjects were returned to the saline control condition their performance immediately returned to that observed prior to the chronic drug regimen.

Fig. 3 illustrates the effects of the gradual withdrawal of amphetamines on the avoidance performance of AV-1 and AV-5. The subject's total number of responses shows an orderly decline and total number of shocks increases as a function of diminishing dosage of the drug. The drug regimen did not affect the subjects' escape latencies. Again the animals' saline control performance following this second chronic-drug

regimen shows no change from the pre-chronic drug avoidance performance.

Discussion. The administration of 1.0 mg/kg d-amphetamine to "poor" Sidman avoidance subjects resulted in a clear-cut facilitation in 3 of the 4 subjects. This dosage was fixed in order to make relevant comparisons to the first experiment in this series. Facilitation of the avoidance performance might have been obtained with a higher dosage in the case of subject AV-2 (HEARST and WHALEN, 1963). The important consideration here is the fact that the increased total number of responses and the decrement in shock frequency showed no tendency to diminish with prolonged daily administration of this dosage of d-amphetamine. Clearly these subjects' avoidance performance did not reflect the development of behavioral tolerance to this dosage of d-amphetamine.

It is also of some importance to note that despite the subjects' prolonged experience with higher response rates leading to diminished shock frequency there was no permanent improvement following the chronic drug regimen. This was true whether the drug was abruptly withdrawn (Chronic drug I) or gradually diminished in dosage (Chronic drug II). Despite the more favorable reinforcement conditions under the drug, the subjects did not transfer any improved performance from the drugged to the non-drugged states.

General Discussion – Experiment I und II

On the basis of this preliminary evidence we have evolved the following working hypothesis concerning the role of reinforcement contingencies in determining what aspect of an organism's behavioral repertoire will show the development of tolerance to amphetamines.

Behavioral tolerance will develop in those aspects of the organism's behavioral repertoire where the action of the drug is such that it disrupts the organism's behavior in meeting the environmental requirement for reinforcements. Conversely, where the actions of the drug enhance, or do not affect the organism's behavior in meeting reinforcement requirements we do not expect the development of behavioral tolerance.

This hypothesis is not intended as a replacement for the classical physiological theories of drug tolerance (EDDY, 1941; SOLLMAN, 1948). Rather this hypothesis is put forth as an additional variable which may be operative in those behavioral situations where tolerance develops in a manner not predictable from the classical conceptions.

Summary

The behavioral effects of chronic administration of d-amphetamine in rats at a dosage of 1 mg/kg were studied with baselines involving either food or shock reinforcement. Food reinforcement was assigned according

to a fixed interval or on the basis of differential reinforcement of low rate in a multiple schedule of reinforcement. Behavioral tolerance was observed in response to chronic administration of d-amphetamine when the action of drug led to a decrease in frequency of food reinforcement regardless of the schedule of reinforcement. In the second experiment, a shock avoidance situation was employed in which each avoidance response postponed the onset of grid shock. An escape contingency was provided for occasions on which an avoidance response did not occur. The chronic administration of d-amphetamine led to a uniform increase in response rate throughout the drug regimen with the consequence of decreasing rate of shock reinforcement. An hypothesis was put forward on the basis of these results which considers the development of behavioral tolerance to amphetamine administration to be a function of the drug's action in relation to its effects on the organism's behaviour in meeting reinforcement requirements.

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