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Performance Influence on the Development of Tolerance to Amphetamine[1]

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CAMPBELL, J. C. AND L. S. SEIDEN. *Performance influence on the development of tolerance to amphetamine.* PHARMAC. BIOCHEM. BEHAV. 1(6) 703 708, 1973. -This experiment was performed to determine whether performance of a behavior in the drug state was necessary for behavioral tolerance to the effects of that drug to occur. Eight rats trained on a DRL 17.5-sec schedule received daily injections of 1.5 mg/kg *d*-amphetamine sulfate; four received amphetamine 30 min presession, and four received amphetamine 30 min postsession. Amphetamine given presession initially resulted in a disruption of timing behavior, an increase in response rate, an increase in short IRTs and a decrease in the number of reinforcements received. With continued administration of presession amphetamine the rats developed a partial tolerance to these disruptive effects. Postsession amphetamine had no effect on performance. When tolerance developed in rats receiving presession amphetamine, they were switched to postsession amphetamine; rats receiving postsession amphetamine were switched to presession amphetamine. Amphetamine produced the same disruption of performance in the rats switched to presession amphetamine as was observed in the initial pressession amphetamine group, indicating that tolerance did not develop to amphetamine given postsession. In addition changes in the pattern of responding were observed when amphetamine was initially administered presession.

D-amphetamine DRL schedule Amphetamine tolerance Chronic drug administration

CARLTON and Wolgin [1] found that the development of rats' tolerance to the anorexic effects of amphetamine was contingent on the relationship between the time of amphetamine administration and the time of food presentation. One of two groups of rats received daily prefeeding injections of *d*-amphetamine (2.0 or 3.0 mg/kg, intraperitoneally), and the other group received postfeeding injections of amphetamine. The group receiving the prefeeding injections of amphetamine showed an initial decrease in milk consumption followed by an increase that reached a level of intake equal to or greater than that obtained during a predrug control period. The rats that received postfeeding injections of amphetamine, on the other hand, showed no change in milk consumption. When the prefeeding drug group developed tolerance to the effects of amphetamine, the groups were switched so that this group now received postfeeding injections, and the group that initially received postfeeding injections of amphetamine now received prefeeding injections. The group of rats that was switched from postfeeding to prefeeding injections of amphetamine exhibited no initial tolerance although they had received the same amount of amphetamine as the original prefeeding amphetamine group that showed tolerance.

Sidman [7] and Segal [5] have shown that acute administration of amphetamine to rats responding on a differential-reinforcement-of-low-rate (DRL) schedule increases the rate of responding, and shifts longer interresponse times (IRTs) towards shorter IRTs and, therefore decreases the number of reinforcements received. Schuster and Zimmerman [4] found partial tolerance developed to the effects of chronic *dl*-amphetamine (1.0 mg/kg, intraperitoneally) administration in rats responding on a DRL 17.5–sec schedule. Upon initial administration, the effects of amphetamine were similar to those described by Sidman [7] and Segal [5], but with continued daily amphetamine administration the disruptive effects were diminished.

The present study was undertaken to determine if the results obtained by Carlton and Wolgin [1] in a food consumption test could be generalized to the operant situation in which animals emit a conditioned response. Specifically, this experiment was designed to answer the question: Would rats performing on a DRL schedule of reinforcement develop tolerance to the disruptive effects of amphetamine if the animals chronically received an amount of the drug following behavioral sessions or is presession adminstration of amphetamine necessary for the develop-

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ment of tolerance? It was found that the rats given presession injections of amphetamine initially showed a disruption in DRL performance and gradually developed a partial tolerance to the effects of the amphetamine. Rats receiving postsession injections showed no development of tolerance when amphetamine was given presession. That is, the changes in pattern of responding when amphetamine was administered presession were similar to the initial amphetamine induced changes.

METHOD

Animals

The experimental animals used were eight Sprague-Dawley rats, 60 days of age at the beginning of the experiment and weighed between 220 and 240 g. The rats were housed two to a cage, were water deprived for 23 hr before each session and had free access to Rockland Laboratory Chow. Following each training session water was available for a five-minute period. During chronic administration of *d*-amphetamine sweetened evaporated milk (evaporated milk:sugar:water; 1:1:2) was substituted for postession water in order to maintain the animals' body weights.

Apparatus

Four Lehigh Valley double-lever operant chambers (Model 1316) enclosed in soundproof chambers (Model 1316c) were used. For this experiment the right lever was removed from the chamber and the opening taped over from the outside. Each contained a houselight that illuminated the chamber. The programming of this experiment was accomplished with Massey Dickinson solid state

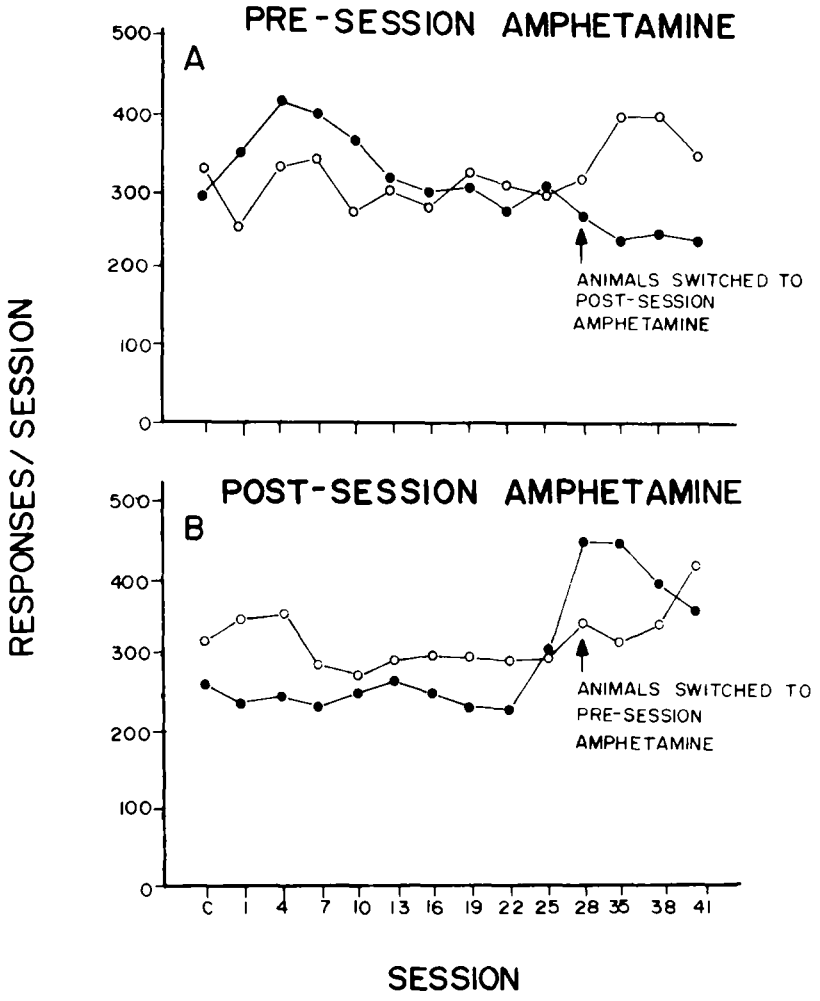

FIG. 1. Overall rates for four animals. A: Animals 1 ●–● and 7 ○–○ which initially received 1.5 mg/kg *d*-amphetamine sulfate presession; B: Animals 5 ●–● and 6 ○–○ which initially received 1.5 mg/kg *d*-amphetamine sulfate postsession. Each point is the average of the overall rate for three sessions, except for the last point which is the average rate for the last two sessions. C denotes the average rate for three predrug sessions immediately prior to drug administration, each number denotes the first session of the three averaged sessions. Animals 5 and 6 were switched to presession amphetamine on the 27th session, and Animals 1 and 7 were switched to postsession amphetamine on the 28th session.

modules. Reinforcement consisted of presentation of 0.01 cc of water to the animal. Data was collected and analyzed by a computational system described by Seiden *et al.* [6].

Schedule of Reinforcement

The DRL schedule is one in which a response is reinforced only if the time since the preceding response is equal to or greater than a specified value. Responses occurring before the end of the specified interval do not produce reinforcement but start the timing of the next interval [2]. Typically, interresponse time (IRT) distributions generated by the DRL schedule are bimodal, showing both very rapid responding and a narrow distribution of IRTs around the interval value specified by the schudule (reinforced IRTs). The overall rate of responding under this schedule is both low and stable [2].

Drugs and Administration

D-amphetamine sulfate was obtained from the Smith, Klein and French Co. It was dissolved in 0.9% saline 0.001 N HCl to a concentration of 0.75 mg/cc and injected intraperitoneally. All doses of amphetamine are expressed in terms of the salt.

PROCEDURE

Rats were shaped to lever press and were initially run on a fixed ratio 1 (FR 1) schedule of reinforcement for two days. Beginning with the second day, session lengths were one hour long throughout the experiment. A DRL 17.5-sec schedule was instituted on the third day and the animals were run until a stable baseline of responding was observed (about one month). Responding was considered stable when a rat's response rate deviated by less than 10% from the mean of the response rates of the three previous sessions. The rats were then divided into two groups, that had approximately equal response rates, at the end of the training period. The presession group received 1.5 mg/kg of *d*-amphetamine sulfate 20 min presession and 2 cc/kg of acidified saline 20 min postsession. The second, postsession group, received acidified saline injections 20 min presession and 1.5 mg/kg of *d*-amphetamine sulfate 20 min postsession. This drug regimen was continued daily until behavioral tolerance to amphetamine was exhibited by the animals receiving presession amphetamine injections. After tolerance was exhibited, the groups were switched. That is, presession amphetamine animals now received amphetamine postsession, and postsession animals now received amphetamine presession. The animals were run under this drug regimen for an additional 12 days.

Data Analysis

Initially, data were plotted in a histogram fashion. Each IRT was sorted into a bin depending on the length of that IRT. Each bin was three seconds long and there were ten bins. Further computer analysis was performed to obtain a more detailed analysis of the behaviroal changes. Each IRT was given an ordinal value depending on its position, in the series of IRTs relative to the preceding reinforcement. For example, the IRT directly following a reinforcement would be given an ordinal value of one. Ordinal values for the inter-response times in each bin were then plotted as a histogram. A mean ordinal value for the IRTs in each bin was also computed.

RESULTS

Animals responding on a DRL 17.5-sec shedule generally show a bimodal distribution of IRTs with one peak occurring at short IRTs (0.1–3.0 sec) and one peak including the long and/or reinforced IRTs (15.1–18.0 sec). Representation of the predrug control performances for four of the rats are shown in the upper panels of Figs. 2 and 3.

Rats injected with 1.5 mg/kg of amphetamine pressesion showed an increase in response rate (Fig. 1), an increase in the number of short IRTs and a shift in the mean IRT towards shorter values. For each rat the maximal disruptive effect appeared on the third or fourth day of drug administration. Over the course of presession drug administration, which lasted for 27 days, the IRT distribution gradually showed a partial return to the predrug control patterns (Fig. 2). The performances of the rats given postsession injections of amphetamine was similar in all respects to their predrug control performance (Fig. 3).

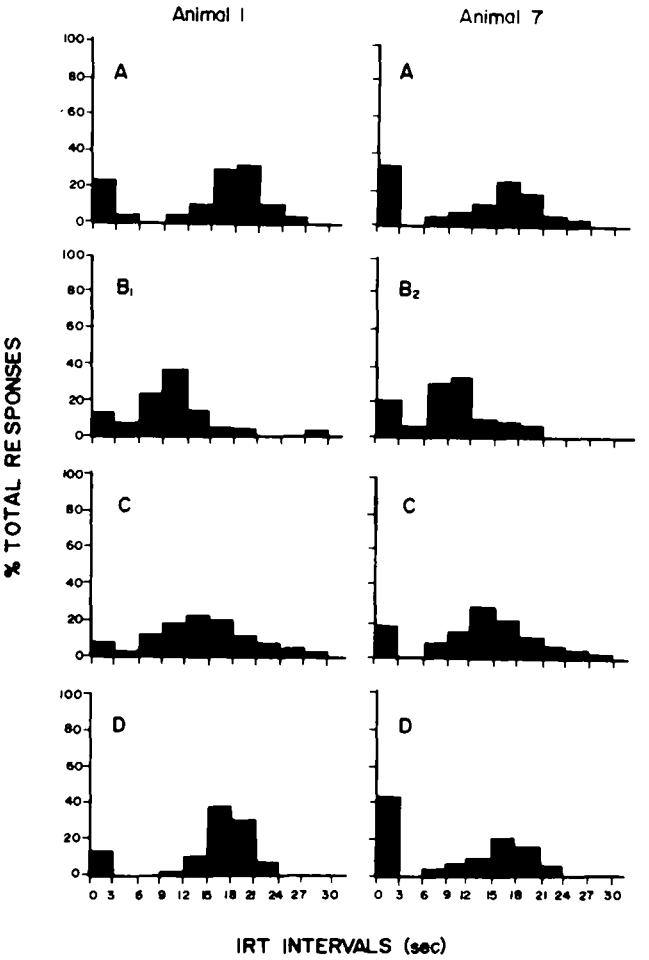

FIG. 2. IRT distribution for two rats that initially received injections of 1.5 mg/kg *d*-amphetamine sulfate 20 min presession; each IRT interval is 3 sec long. A–control day; B_1–5th day of presession drug administration; B_2–6th day of presession drug administration; C–tolerance day; D–postsession amphetamine administration.

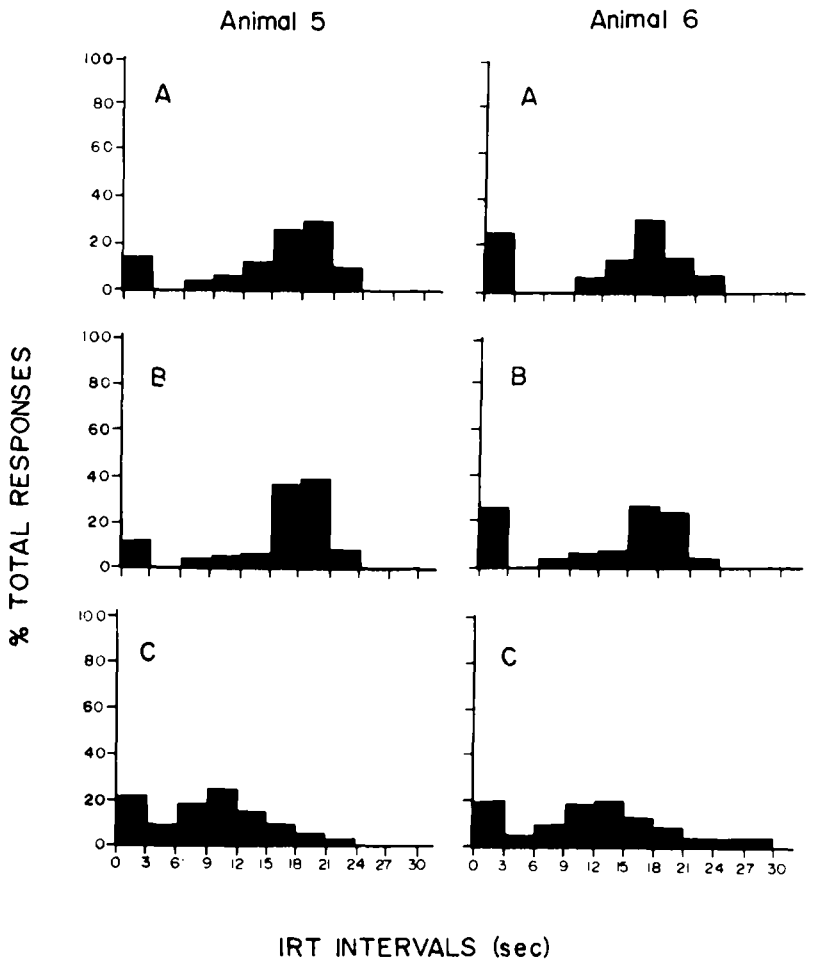

FIG. 3. IRT distribution for two rats that initially received injection of 1.5 mg/kg *d*-amphetamine sulfate 20 min postsession; each IRT interval is 3 sec long. A–control day; B--postsession amphetamine administration; C–3rd day of presession amphetamine administration.

When presession amphetamine rats were switched to postsession amphetamine administration, the IRT distributions assumed the essential characteristics of the predrug control days (Fig. 2). Postsession amphetamine rats switched to presession amphetamine injections exhibited the same initial drug effects as did presession amphetamine rats on the first few days of drug administration (Fig. 3).

Figure 4 shows the mean of ordinal values for one presession and one postsession animal on a representative control day and on several drug days. The upper panel of Fig. 4 shows the mean ordinal values of short IRTs for Animal 1, which initially received presession amphetamine. On the control day prior to drug administration, the mean ordinal value is 1.4 which indicates that most short IRTs directly follow a reinforcement. When amphetamine is administered presession the mean ordinal value of short IRTs is initially greatly increased, but as tolerance develops the mean ordinal value returns to control values as shown by the mean ordinal value on Day 27 (the last day of presession amphetamine administration). The mean ordinal value on this day is 5 as compared to 25 on Day 5 (the day of the largest drug effect for Animal 1). When the rat is switched from presession to postsession amphetamine the mean ordinal value returns to predrug control values, as seen on Day 35 of drug administration.

In the lower panel of Fig. 4, the mean ordinal value of short IRTs are plotted for one postsession amphetamine animal (Animal 5). The mean ordinal value for predrug control days is essentially the same as for Animal 1. The mean ordinal value of short IRTs when amphetamine is administered postsession (Days 2, 5, 11 and 24) are not significantly different from the predrug control day. On the first day of presession administration of amphetamine (Day 28) the mean ordinal value of short IRTs is increased, with the greatest value on the third day of presession amphetamine administration. With continued administration of presession amphetamine the mean ordinal value begins to return to predrug control values.

The increase in the mean ordinal value of short IRTs on days when amphetamine is administered indicates not only an increase in overall responding but also a change in the position of short IRTs in relation to the previous reinforcement. The increase in the standard error of the mean along with the increase in these mean ordinal values indicates an

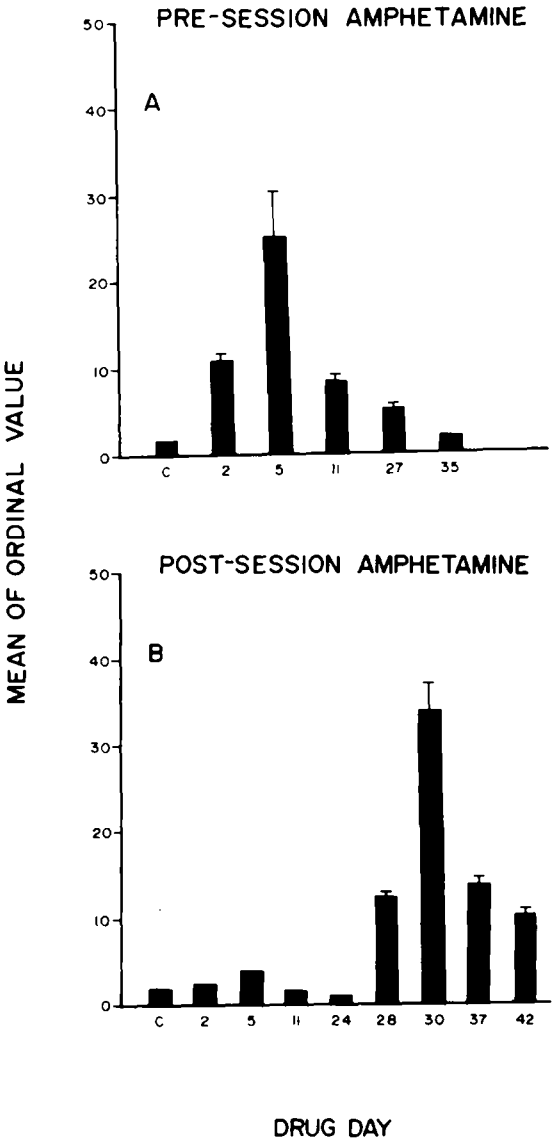

FIG. 4. Mean ordinal values of short IRTs (0.1–3.0 sec) for two animals. A: Animal 1 (initially received presession injections of 1.5 mg/kg *d*-amphetamine sulfate); C–control day, Day 5–day of maximum drug effect, Day 27–last day of presession drug injections, Day 35–representative day of postsession amphetamine injection. B: animal 5 (initially received postsession injections of 1.5 mg/kg *d*-amphetamine sulfate); C–control day; Days 2, 5, 11 and 24–postsession amphetamine administration; Day 28–first presession drug day; Day 30–day of maximum drug effect. The bars represent the standard error of the mean. Those histograms without bars had standard errors too small to be depicted on the graph.

increase in the variability of responding and a decrease in the predictability of the ordinal position of a short IRT.

Since the short IRTs are not normally distributed the mean ordinal value is not always an accurate representation of the modal value of the short IRTs. Therefore, the percentage of short IRTs directly following a reinforcement (ordinal value of one) was calculated for four rats (1, 5, 6 and 7) on predrug control days, presession drug days and postsession drug days. Presession drug days were divided into presession drug days and tolerance days for the two rats that were initially given amphetamine presession (Animals 1 and 7). The day on which these rats developed tolerance was determined by examining the shift in reinforced IRTs on their daily histograms as in Fig. 2. It was found for three of the four animals examined (1, 5 and 6) that on control days or when amphetamine was administered postsession, a reinforcement was followed by a short IRT greater than fifty percent of the time. For Animals 1, 5 and 6 the percentage of short IRTs directly following a reinforcement on postsession amphetamine days did not differ significantly from the percentage found on predrug control days. When amphetamine was given presession these three rats showed a decrease in percentage of short IRTs directly following a reinforcement which was significantly different from predrug control and postsession amphetamine values ($p<0.001$ for all cases) (Table 1). The percent change, of the percentage of short IRTs with an ordinal value of one, from predrug control days to presession amphetamine administration days for Animals 1, 5 and 6 were 63.2%, 90.6% and 78.6%, respectively. Differences in

TABLE 1

MEAN PERCENTAGE OF 0.1–3.0 SEC RESPONSES DIRECTLY FOLLOWING A REINFORCEMENT*

Time of Drug† Administration	ANIMAL NUMBER 1	7
Control‡	67.7 ± 3.3	39.0 ± 3.3
Presession	24.5 ± 2.7[a,b]	34.4 ± 3.9
Presession (Tolerance days)	40.5 ± 5.9[c,d,e]	38.1 ± 4.6
Postsession	72.5 ± 6.7	30.7 ± 3.3
	ANIMAL NUMBER 5	6
Control‡	52.7 ± 7.0	56.3 ± 5.0
Postsession	53.0 ± 7.9	54.6 ± 3.1
Presession	4.9 ± 1.0[a,b]	12.2 ± 1.6[a,b]

*All values are expressed as % ± standard error of the mean.
†Animals 1 and 7 initially received injections of 1.5 mg/kg *d*-amphetamine sulfate 20 min presession, and Animals 5 and 6 initially received amphetamine injections 20 min postsession.
‡No drug or vehicle injections
[a]Value significantly different from the control value ($p<0.001$)
[b]Value significantly different from the postsession value ($p<0.001$)
[c]Value significantly different from the control value ($p<0.02$)
[d]Value significantly different from the presession value ($p<0.05$)
[e]Value significantly different from the postsession value ($p<0.01$)

this change may be due to individual differences to the effects of this dose of amphetamine. In addition, the percentage of short IRTs directly following a reinforcement for Animal 1 on the days during which tolerance developed was between the control and presession values and significantly different from both ($p<0.02$ and $p<0.05$, respectively). Animal 7 (initially receiving presession amphetamine) showed no significant changes in the percentage of short IRTs directly following a reinforcement, throughout the study.

DISCUSSION

On a DRL scedule timing behavior is disrupted by administration of amphetamine as shown by the shift in IRTs. With rats responding on a concurrent variable-interval DRL schedule (con VI-DRL), Segal [5] found that amphetamine affected the response rates on the two components of the schedule about equally. This result was interpreted as showing that the main drug effect was motor excitatory and that amphetamine simply reduces the frequency of long IRTs. These findings support the interpretation that overt behavior mediates the temporal spacing of DRL responding and therefore amphetamine disrupts DRL responding by simply increasing the rate of emission of all overt behavior. However, closer analysis of the responses of each animal showed that there was also some change in the pattern of responding for some animals. Therefore, it is reasonable to conclude that the effects of amphetamine on DRL responding may also, in part, be due to these changes in patterns of responding as well as the increase in the rate of responding.

Sidman [8] has reported that animals emit a high frequency of short IRTs when reinforcement is made contingent on a specific time delay between responses. He found that these short IRTs are most frequent when the animal has waited almost long enough to produce a reinforcement, and therefore, the probability of a short IRT occurring is very low directly after a reinforcement. However, three of the four rats examined in this study show a high percentage of short IRTs directly following a long, but not reinforced IRT, was relatively low for these four animals. The percentage of short IRTs following a long, nonreinfoced IRT (15.0–17.4 sec) for animals 1, 5, 6 and 7 on predrug control days was 9.7%, 2.8%, 21.8% and 32% respectively.

The schedule in Sidman's experiment and this study were not exactly the same due to the presence of a second lever. Responses on the second lever in the presence of an auditory stimulus produced reinforcement and reset the interval on the first lever. Responses in the absence of the auditory stimulus had no effect, and as many as 65% of the responses on this lever occurred in the absence of the stimulus. These differences may account for the discrepancy between the data obtained in Sidman's study and this experiment.

It has already been reported that rats' performances on a DRL schedule develop tolerance to the effects of amphetamine when it is administered chronically before each session [4]. The results of this study corroborate these findings.

Schuster, Dockens and Woods [3] administered amphetamine to rats responding on a free-operant avoidance schedule or a multiple fixed-interval 30 sec DRL 30-sec schedule of positive reinforcement. Initially, rate increases occurred on both components of the multiple schedule and under the free-operant avoidance schedule. Tolerance to the behavioral effects of amphetamine developed only on the DRL component of the multiple schedule. It was therefore concluded that tolerance develops only when the action of the drug disrupts the animal's behavior such that it results in a decrease in the number of reinforcements received; when the actions of the drug enhance or do not affect the animal's behavior in meeting reinforcement requirements tolerance does not develop.

The finding that the animals in the present study that received postsession amphetamine exhibited no tolerance to the drug by the time that presession injection animals showed tolerance indicates that the development of tolerance was dependent on the relation between the time of injection and the time at which the operant task was performed. These results suggest that the tolerance that develops cannot be a physiological tolerance unless it is in some way related to performance under amphetamine, as previously suggested [1].

There are at least two variables important in the development of tolerance to amphetamine: (1) Behavioral variable, i.e., tolerance develops mainly to the disruptive effects of amphetamine. This implies that if the animal's performance is not disrupted tolerance will not develop [3]. (2) The relation between time of drug injection and performance of the required task is important.

REFERENCES

1. Carlton, P. L. and D. L. Wolgin. Contingent tolerance to the anorexigenic effects of amphetamine. *Physiol. Behav.* **7**: 221–223, 1971.
2. Ferster, C. B. and B. F. Skinner. *Schedules of Reinforcement*. New York: Appleton-Century-Crofts, Inc., 1957.
3. Schuster, C. R., W. S. Dockens and J. H. Woods. Behavioral variables affecting the development of amphetamine tolerance. *Psychopharmacologia* **9**: 170–182, 1966.
4. Schuster, C. R. and J. Zimmerman. Timing behavior during prolonged treatment with *dl*-amphetamine. *J. exp. Analysis Behav.* **4**: 327–330, 1961.
5. Segal, E. F. Effects of *dl*-amphetamine under concurrent VI DRL reinforcement. *J. exp. Analysis Behav.* **5**: 105–112, 1962.
6. Seiden, L. S., R. Schoenfeld and D. Domizi. A system for the recording and analysis of interresponse-time data using an AM tape recorder and digital computers. *J. exp. Analysis Behav.* **12**: 289–292, 1969.
7. Sidman, M. Technique for assessing the effects of drugs on timing behavior. *Science* **122**: 925, 1955.
8. Sidman, M. Time discrimination and behavioral interaction in a free operant situation. *J. comp. physiol. Psychol.* **49**: 469–473, 1956.