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Effects of Acute and Chronic Administration of Phenobarbital and *d*-Amphetamine on Schedule-Controlled Behavior

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HARRIS, R. A. AND D. SNELL. *Effects of acute and chronic administration of phenobarbital and d-amphetamine on schedule-controlled behavior.* PHARMAC. BIOCHEM. BEHAV. **12**(1) 47–52, 1980.—The effects of acute and chronic administration of phenobarbital and *d*-amphetamine were determined in rats responding under a multiple fixed-interval five minute fixed-ratio 30 (mult FI 5 FR 30) schedule of food presentation. After determining the acute effects of each drug, the drugs were injected daily with one group of rats receiving the drugs before each behavioral session while another group received the drugs immediately after each daily session. After four to seven consecutive injections, tolerance developed to the effects of phenobarbital on the average rates of responding under FI and FR schedule components only if the drug was administered before each session. Tolerance was more pronounced for responding during the terminal portions of the FI component than for responding during either the initial portions of the FI or the FR component. Evidence for a selective tolerance to the effects of the drug on responding during the final segments of the FI was also obtained in rats responding under an FI 5 schedule. In contrast, injection of *d*-amphetamine for seven to eight consecutive days failed to produce any tolerance to the effects of the drug on responding under mult FI 5 FR 30, FI 5, or FR 30 schedules. These results indicate that the development of tolerance to the effects of phenobarbital depended both upon the temporal relationship of the drug effects to the behavioral testing and upon the schedules controlling behavior. These findings are discussed in terms of theories of behavioral tolerance.

Phenobarbital *d*-Amphetamine Rat Schedule-controlled behavior Tolerance

THE development of tolerance to drug effects depends upon behavioral as well as pharmacological variables (for reviews see references [6] and [13]). In particular it has been shown, at least in the case of ethanol and morphine, that tolerance to the effects of the drug on a particular behavior is acquired more rapidly when the drug effect occurs in conjunction with the behavioral test than when the drug effect is temporally separated from behavioral testing. This has been termed "behaviorally augmented" tolerance [6]. In addition, based on experiments with *d*-amphetamine, Schuster *et al.* [10] have postulated that tolerance is most likely to develop to the effects of a drug which interfere with reinforcement. In view of these findings, the following study was undertaken to evaluate the role of behavioral factors in the development of tolerance to phenobarbital and *d*-amphetamine on responding maintained by FI and FR schedules of food presentation. To test for a "behavioral augmentation" of tolerance, the acute effect of each drug was first determined in all animals, then the animals were divided into two groups, one which received the drug shortly before each daily behavioral session and one group which received the drug immediately after each session. After four to eight consecutive daily injections, all animals were given one drug injection before the behavioral session. This design allowed us to compare the development of tolerance in animals exposed to the drug effect either during behavioral testing or in their home cage. In addition, the use of a mult FI FR schedule allowed for evaluation of tolerance development on behavior maintained by different contingencies.

METHOD

Animals

Seventeen male Long-Evans rats, obtained from Charles River Laboratories, Wilmington, MA, were maintained at 80% of their free-feeding weights during the experiments.

Apparatus

Three standard rat test cages (Grason-Stadler, West Concord, Massachusetts) 23-cm long, 29-cm wide, and 19-cm high were installed in ventilated, sound-attenuating chambers (Grason-Stadler, West Concord, MA). The manipulan-

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dum was a standard rat lever (G6312, Ralph Gerbrands Co., Arlington, MA) and depression of the lever (about 30 g force required at the tip) was recorded as a response. The lever protruded from the wall containing the food bin and was placed 9 cm above the cage floor and 9 cm from the adjacent side wall. Conventional relay programming and recording equipment located in an adjacent room controlled the delivery of food and recorded the patterns of responding.

Procedure

Nine rats were trained under a multiple fixed-interval 5 minute, fixed-ratio 30 response (mult FI 5 FR 30) schedule of food presentation, five rats were trained under a fixed-interval 5 minute (FI 5) schedule of food presentation and three rats were trained under a FR 30 schedule of food presentation [3].

For the experiments with the mult FI FR schedule, when the FR 30 component was in effect, a tone was present in the chambers and the 30th lever press produced one 45-mg food pellet (P.J. Noyes Co., Lancaster, NH). When the FI 5 component was in effect, no tone was present and the first response after 5 minutes produced two 45-mg food pellets. During each schedule component, a limited hold of 90-sec was programmed. This meant that during the FR 30 component the rat had a total of 90-sec to emit the 30 responses to obtain food, and during the FI 5 component the rat had 90-sec after the 5 min had elapsed to make a response to obtain food. Schedule components alternated after each food presentation or after the 90-sec limited hold had elapsed in either component. Sessions always started with the FR 30 component and ended after 26 schedule changes or 80 min, whichever occurred first. Under the FI 5 min schedule, the first lever press after five minutes had elapsed produced two 45-mg food pellets. During this schedule a limited hold of 90-sec was programmed. Sessions ended after 13 intervals or 80 min, whichever occurred first. Under the FR 30 schedule, the 30th lever press produced one 45-mg food pellet. During this schedule a limited hold of 90-sec was programmed. Sessions ended after 20 min. All groups of animals were tested seven days per week.

Drugs

The compounds, in the form in which dosages were expressed, were: phenobarbital and *d*-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO). Drugs were dissolved in 0.9% NaCl and injected subcutaneously (SC). The phenobarbital solution was adjusted to pH 7.5 with NaOH. A volume of 1 ml/kg was used for all injections.

Effects of Phenobarbital

To study the effects of acute and chronic administration of phenobarbital on responding under the mult FI 5 FR 30 schedule, nine rats were injected SC with 50 mg/kg phenobarbital 45 min before the beginning of the session. This treatment was repeated four days later. For the next five consecutive days following this treatment, four of the rats (no. 1, 8, 11 and 14) were injected with phenobarbital (50 mg/kg) 45 min before the session while the other five rats (no. 3, 5, 9, 12 and 20) were injected with the same dose immediately after the session. On the day following this series of daily injections, all nine rats were injected with phenobarbital (50 mg/kg) 45 min before testing. On the following day, all nine rats were injected with saline 45 min

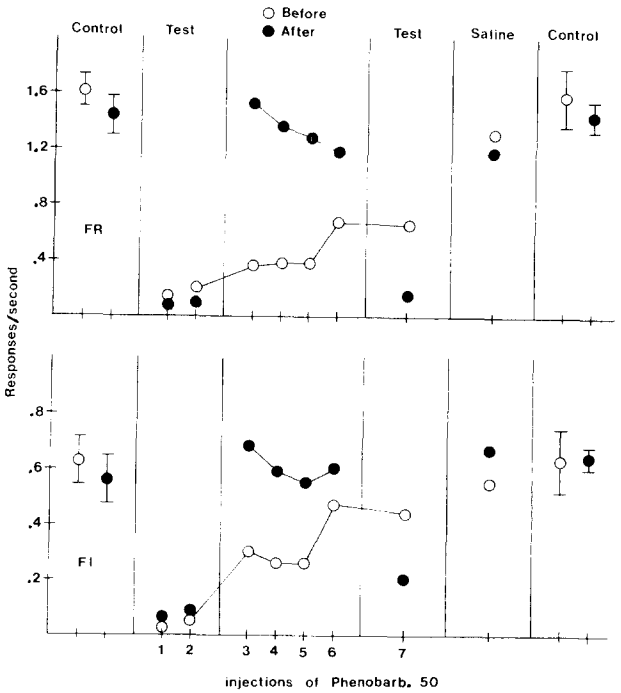

FIG. 1. Effects of acute and chronic administration of phenobarbital (50 mg/kg) on average rates of responding under the FR 30 (upper panel) and FI 5 (lower panel) components of a mult FI FR schedule. All rats received injections one, two, and seven before the sessions, while one group (open circles, n=4) received injections three thru six before each session and another group (filled circles, n=5) received injections three thru six after each session. The first and second injections were separated by four days while injections two thru seven were given on consecutive days. Saline was injected on the day following the last drug injection. Control rates were determined before and after the series of drug injections; Vertical bars represent ± SEM.

before the session. To determine the effects of acute and chronic administration of phenobarbital on responding under an FI 5 schedule, five rats (no. 2, 4, 7, 10 and 16) were injected with phenobarbital (50 mg/kg) 45 min before testing. This treatment was repeated three days later. Beginning four days after the second injection, a series of seven consecutive daily injections of phenobarbital (50 mg/kg, 45 min before testing) was begun. On the day following the last phenobarbital injection, saline was administered 45 min before the session.

Effects of d-*Amphetamine*

Beginning three weeks after completion of the phenobarbital study, the nine rats performing under the mult FI FR schedule were given 1 mg/kg *d*-amphetamine 20 min before the session. The next day four of the rats (no. 1, 3, 11 and 14) were again administered 1 mg/kg *d*-amphetamine 20 min before the session while the remaining five rats (no. 5, 8, 9, 12 and 20) were given the same dose of the drug after the session. These treatments were continued for six consecutive days. On the next day, all nine rats were injected with *d*-amphetamine 20 min before the session to evaluate the development of tolerance. On the following day, saline was injected SC 20 min before the session. Effects of *d*-am-

phetamine were also evaluated in rats performing under a FI 5 min schedule. Two weeks after completion of the phenobarbital study, five rats (no. 2, 4, 7, 10, 16) were injected SC with 1 mg/kg *d*-amphetamine 20 min before the session. Seven days later this treatment was repeated. Beginning four days after the second injection of *d*-amphetamine, the drug was administered 20 min before the session for eight consecutive days. On the following day, saline was injected SC 20 min before the session. The effects of *d*-amphetamine were also evaluated in three rats responding under a FR 30 schedule. The protocol for this study was identical to that used to study the effects of *d*-amphetamine on responding under the FI schedule.

Measurement of Drug Effects

The control response rates used for evaluating drug data were calculated using five to seven noninjection sessions occurring before and after each series of drug injections.

RESULTS

Effects of Acute and Chronic Administration of Phenobarbital on Average Rates of Responding Under Mult FI FR and FI Schedules

An initial injection of phenobarbital, as well as a second injection four days later, markedly reduced responding under both components of the multiple schedule (Fig. 1). When the same dose of phenobarbital was injected before the session for five consecutive days the average rate of responding increased progressively (open circles, Fig. 1), but when injections occurred after the session the rate of responding was not markedly affected (filled circles, Fig. 1). The seventh injection of the drug was given to both groups before the session and produced a marked suppression of responding in the group which had received daily injections after the sessions but did not markedly suppress responding in the group which had received the drug before the sessions. Thus, although both groups were exposed to similar amounts of the drug, tolerance development occurred only when the drug was administered before the session on a daily basis. In addition, tolerance development appeared somewhat more rapid and more complete under the FI component than under the FR component of the multiple schedule.

The effects of acute and chronic phenobarbital administration before the session were replicated in a separate group of rats responding under an FI 5 schedule. As can be seen from Fig. 2, little or no tolerance developed when injections were separated by three or four days (injections 1, 2 and 3), but tolerance developed rapidly when injections were given daily before each session. Complete tolerance did not develop to the effects of phenobarbital on overall rates of responding under either component of the multiple schedule or under the FI schedule as evidenced by the fact that after the last daily injection of phenobarbital response rates remained below control levels (Figs. 1 and 2).

Effects of Acute and Chronic Administration of Phenobarbital on Local Rates of Responding

The effects of acute and chronic administration of phenobarbital on local rates of responding during the FI component of the multiple schedule were evaluated by determining the rates of responding during successive fifths of the FI. In Table 1, the effects of acute and chronic treatment

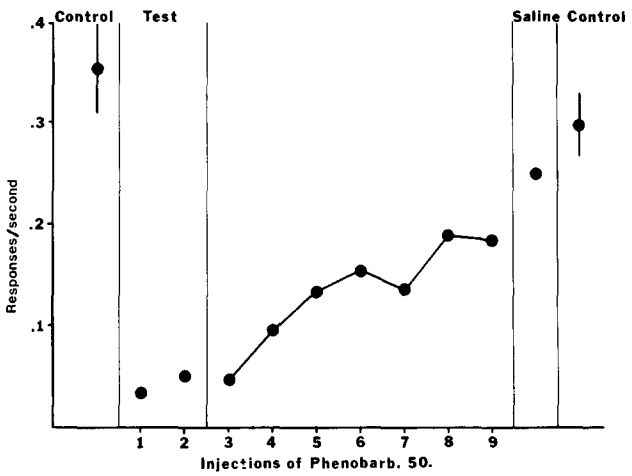

FIG. 2. Effects of acute and chronic administration of phenobarbital (50 mg/kg) on average rates of responding under an FI 5 schedule. Injections one and two were separated by three days, injections two and three were separated by four days, while injections three thru nine were given on consecutive days. The drug was always given before the session. Data represent the mean from five rats. Control responding was determined before and after the series of drug injections; vertical bars represent ±SEM.

with phenobarbital on responding during the initial and final fifths of the FI are presented for each of the nine animals, and the effects of drug administration on the overall rates of responding under the FR component are presented for the four rats performing under the multiple schedule. It can be seen that, in about half of the animals, acute treatment with phenobarbital increased rates of responded during the first fifth of the interval while in the other half of the animals the drug decreased responding during the initial fifth of the interval. In contrast, during the final fifth of the FI, acute administration of phenobarbital markedly reduced responding in all animals. After chronic treatment there was little evidence of a consistent development of tolerance to either the rate increasing or the rate decreasing effects of phenobarbital on responding occurring during the first fifth of the FI. However, in all animals there was evidence for the development of tolerance to the effects of the drug on responding occurring during the final fifth of the FI. Tolerance to the rate-decreasing effects of the drug during the final fifth of the FI was complete in three rats (no. 1, 2 and 10), although tolerance to the effects of the drug on responding during the initial fifth of the FI was either incomplete (no. 1 and 10) or undetectable (no. 2) for these same animals. Tolerance to the effects of the drug on the final segments of the FI did not occur in the animals which received the drug after each session (data not shown). In general, tolerance was less marked under the FR component of the multiple schedule than under the final fifth of the FI component (Table 1).

Effects of Acute and Chronic Administration of d*-Amphetamine on Average Rates of Responding under Mult FI FR, FI and FR Schedules*

Acute administration of 1 mg/kg of *d*-amphetamine re-

TABLE 1

EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF PHENOBARBITAL ON RATES OF RESPONDING DURING INITIAL AND FINAL SEGMENTS OF THE FI SCHEDULES

Rat number	Initial fifth of FI* Acute†	Initial fifth of FI* Chronic‡	Final fifth of FI Acute	Final fifth of FI Chronic	FR Acute	FR Chronic
1	440	241	2	80	10	50
8	261	112	4	57	6	54
11	0	737	0	33	1	7
14	53	33	9	66	33	58
2	72	69	17	98		
4	61	66	6	66		
7	268	450	7	35		
10	510	200	6	99		
16	200	900	2	16		

*All values represent percent of control. Control responding was determined before and after the series of injection (see Figs. 1 and 2). Rats 1, 8, 11 and 14 performed under a Mult FI5 FR30 schedule while rats 2, 4, 7, 10 and 16 performed under a FI5 schedule.

† Data for acute treatments are an average of values obtained from the first two injections of phenobarbital (see Figs. 1 and 2).

‡Data for chronic treatment are obtained from the last injection of phenobarbital (see Figs. 1 and 2).

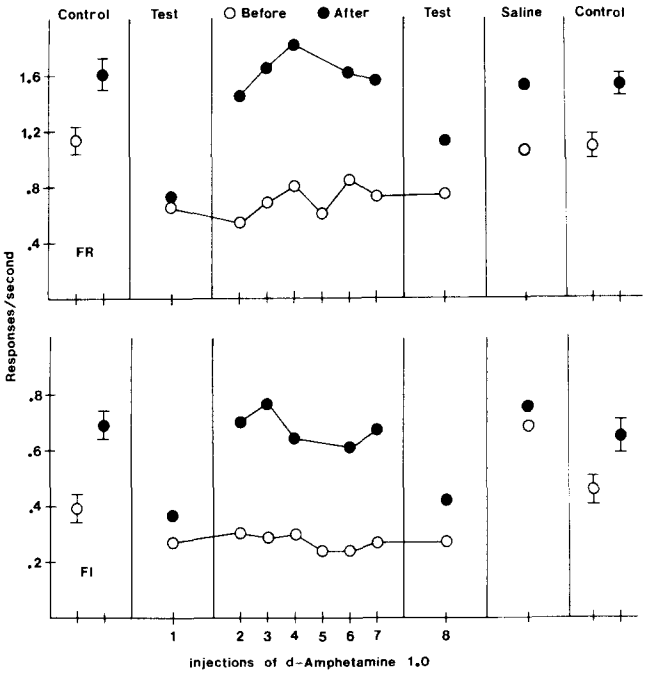

FIG. 3. Effects of acute and chronic administration of *d*-amphetamine (1 mg/kg) on average rates of responding under the FR 30 (upper panel) and FI 5 (lower panel) components of the multiple FI FR schedule. All rats received injections 1 and 8 before the sessions, while one group (open circles, n=4) received injections two thru seven before each session and another group (filled circles, n=5) received injections two thru seven after each session. Saline was injected on the day following the last drug injection. Control rates were determined before and after the series of drug injections; vertical bars represent ±SEM.

duced the average rates of responding under both components of the mult FI FR schedule (Fig. 3). When the drug was injected daily for eight days, there was no evidence of tolerance development (Fig. 3). Others have reported that daily injections of *d*-amphetamine produced tolerance to the effects of the drug on responding under FI or FR schedules [12,14]. In view of these findings, the effects of chronic *d*-amphetamine administration were studied in additional groups of rats performing under either an FI 5 schedule or an FR 30 schedule. As can be seen from Fig. 4, eight consecutive daily injections of *d*-amphetamine produced no evidence of tolerance in animals responding under the FR schedule and indications of only a very slight development of tolerance under the FI schedule.

Effects of Acute and Chronic Administration of d-*Amphetamine on Local Rates of Responding*

The effects of *d*-amphetamine on local rates of responding during the FI components of the schedules were also evaluated. Amphetamine produced an almost constant rate of responding throughout the interval, resulting in increased rates during the initial portions and decreased rates during the final portions of the FI, as compared to control sessions [4]. In contrast to the effects observed with phenobarbital, chronic treatment with amphetamine did not alter either the rate-increasing or the rate-decreasing effects of the drug (data not shown).

DISCUSSION

Repeated daily injections of phenobarbital or *d*-amphetamine produced distinct behavioral effects depending upon the drug, the parameter measured and whether the drug was administered before or after the behavioral session. In the case of phenobarbital, daily administration before the behav-

ioral sessions resulted in a clear tolerance to the rate-decreasing effects of the drug on average rates of responding maintained by a mult FI FR schedule or an FI schedule. This tolerance did not develop when the same dose of phenobarbital was administered after each behavioral session. Thus, tolerance cannot be attributed to altered disposition of the drug but is consistent with the concept of "behaviorally augmented" tolerance [6]. When drug injections were separated by three or four days tolerance also failed to develop, indicating the importance of a daily injection regimen. An analysis of the local rates of responding during the FI demonstrated that tolerance to the effects of phenobarbital on responding was more pronounced during the terminal portions of the FI, and in some animals tolerance was completely lacking during the initial portions of the FI. Thus, tolerance development was highly dependent upon behavioral variables. In addition to phenobarbital, the effects of daily *d*-amphetamine administration were evaluated. Tolerance did not develop to the effects of *d*-amphetamine on the average rates of responding under mult FI FR, FI or FR schedules when the drug was administered for eight consecutive days. The well-known "rate-dependent" effects of

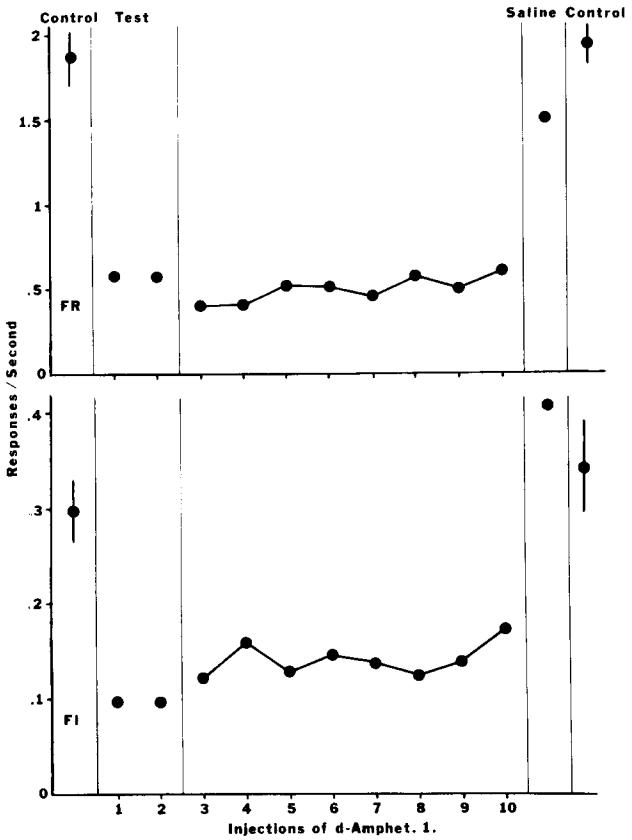

FIG. 4. Effects of acute and chronic administration of *d*-amphetamine (1 mg/kg) on average rates of responding under an FR 30 schedule (upper panel, n=3) and an FI 5 schedule (lower panel, n=5). All injections were given before the sessions. Injections one and two were separated by seven days, injections two and three were separated by 4 days, and injections three thru ten were given on consecutive days. Saline was administered on the day following the last drug injection. Control rats were determined before and after the series of drug injections; vertical bars represent ±SEM.

d-amphetamine (responding at a nearly constant rate throughout the interval, see reference [4]) were clearly demonstrated, and these effects were not altered by chronic administration of *d*-amphetamine. Thus, in contrast to phenobarbital, tolerance did not develop to the effects of *d*-amphetamine on the local rates of responding during the FI.

It is of interest to compare these results with other studies of drug tolerance. Tolerance is known to develop to many effects of the barbiturates [6], but the role of behavioral factors in the acquisition of barbiturate tolerance has received little attention. The present results demonstrate that under certain conditions the development of tolerance to daily injections of barbiturates is dependent upon the drug being administered before the behavioral sessions.

In a similar study, Tang and Falk [13] also demonstrated that injection of phenobarbital before testing resulted in greater tolerance than injection of the drug after testing. However, these investigators found a substantial degree of tolerance in the group exposed to phenobarbital after each behavioral session, which is in contrast to our results. It is likely that the differences between the studies are because Tang and Falk [13] used of a higher dose of phenobarbital for a longer period of time. These differences in drug exposure would be expected to increase pharmacological tolerance [6]. A behaviorally augmented tolerance has also been demonstrated for ethanol [7], Δ^9-tetrahydrocannabinol [1], mescaline [8] and LSD [8]. Several studies [12,14] have demonstrated that six to twelve daily injections of *d*-amphetamine produced a marked tolerance to the effects of the drug on responding under FI and FR schedules of food presentation. In addition, five to twenty daily injections of *d*-amphetamine produced partial tolerance to the effects on responding under a schedule of differential-reinforcement of low rates [9]. However, in the present study we were unable to demonstrate any tolerance after eight daily injections of *d*-amphetamine in rats performing under mult FI FR, FI or FR schedules. It is possible that a more prolonged injection regimen would result in tolerance. In a related study [5], we demonstrated that consumption of *d*-amphetamine in the drinking water for 32 days (at a dose of 5 to 15 mg/kg/day) resulted in a marked tolerance to the effect of the drug on FR responding.

One of the most interesting aspects of the present study is the observation that tolerance development was more pronounced for the effects of phenobarbital on responding during the final segments of the FI than during the initial portions of the FI or during the FR component of the mult FI FR schedule. This selective tolerance was not found after chronic *d*-amphetamine administration. These effects of phenobarbital on FI responding may be related to the postulate of Schuster *et al.* [10] that tolerance is most likely to develop to effects of drugs which decrease the frequency of reinforcement. Alterations in the rate of responding at the beginning of the FI will not affect the frequency of reinforcement, while a marked suppression of responding at the end of the FI will postpone reinforcement. Thus, tolerance might be expected to develop to the latter effect. However, decreased rates of responding during the FR component will also delay (or reduce) reinforcement, and tolerance would be expected to also develop to these effects. Surprisingly, tolerance developed more rapidly to the effects of phenobarbital on responding under the FI component than under the FR component. Thus, the present results are partially, but not entirely, consistent with the hypothesis of Schuster

et al. [10]. The present results also reflect on the hypothesis of Siegel [11] that tolerance is due to the conditioning of a compensatory response which offsets the drug effect. According to this theory, animals tolerant to phenobarbital would respond at a rate higher than their predrug control rate when given an injection of saline rather than phenobarbital. However, this effect was not observed, and the present results indicate that under the conditions of this study the development of tolerance is unlikely to be due to the acquisition of compensatory responses.

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