Pharmacology Biochemistry & Behavior, Vol. 22, pp. 389–393, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Effects of Phenytoin, Phenobarbital, and Valproic Acid, Alone and in Selected Combinations, on Schedule-Controlled Behavior of Rats

MITCHELL PICKER, JASPER THOMAS, CAROLYN KOCH AND ALAN POLING[1]

Department of Psychology, Western Michigan University, Kalamazoo, MI 49008

Received 26 March 1984

PICKER, M., J. THOMAS, C. KOCH AND A. POLING. *Effects of phenytoin, phenobarbital, and valproic acid, alone and in selected combinations, on the schedule-controlled behavior of rats.* PHARMACOL BIOCHEM BEHAV 22(3) 389–393, 1985.—The present study examined the effects of phenytoin (20, 30, 40, and 50 mg/kg), phenobarbital (10, 20, 30, and 40 mg/kg), and valproic acid (80, 120, 160, and 240 mg/kg), and those of phenobarbital (10 and 30 mg/kg) combined with phenytoin (20, 30, and 40 mg/kg) or valproic acid (80, 120, and 160 mg/kg), on the lever pressing of rats maintained under fixed-ratio and interresponse-time-greater-than-*t* schedules of food delivery. High doses of each individual drug significantly decreased mean group response (and reinforcement) rate under the fixed-ratio schedule. No dose of an individual agent significantly affected mean group response rate under the interresponse-time-greater-than-*t* schedule, although high doses of phenobarbital and valproic acid significantly reduced the mean group reinforcement rate under this schedule. When given in combination, phenobarbital and phenytoin and phenobarbital and valproic acid significantly reduced response (and reinforcement) rate under the fixed-ratio schedule and reinforcement rate under the interresponse-time-greater-than-*t* schedule. These reductions did not significantly differ in magnitude from those predicted by an additive model of drug interaction.

Phenytoin Phenobarbital Valproic acid Fixed-ratio schedule
Interresponse-time-greater-than-*t* schedule Drug combinations Anticonvulsant drugs Rats

PHENOBARBITAL and phenytoin are the most commonly prescribed anticonvulsant medications. Recently, their popularity for treating seizure disorders has been threatened by the addition of valproic acid to physicians' armamentarium of anticonvulsant drugs. Chemically unrelated to other anticonvulsant compounds, valproic acid has been used in France since 1964, in the United Kingdom since 1973, and in the United States since 1978. Beyond being effective as an anticonvulsant, valproic acid has been reported to be virtually free of serious side effects [5].

In order to achieve the desired level of seizure control, anticonvulsant medications are frequently administered in combination, although accumulating evidence indicates that anticonvulsant drug combinations are commonly associated with adverse physiological side effects [3,17]. Phenobarbital and phenytoin, and phenobarbital and valproic acid, are two of the most used anticonvulsant combinations [11]. With the exception of sedation, the behavioral side effects of these combinations are unclear [10, 11, 12].

Despite the large number of studies examining the anticonvulsant properties of phenobarbital, phenytoin, and valproic acid, little is known concerning their effects, alone or in combination, on operant behavior. Recent investigations [8,9] found that phenytoin produced dose-dependent decreases in response rates of rats and pigeons performing under fixed-ratio (FR) and fixed-interval (FI) schedules of food delivery, but had little reliable effect on performance under an interresponse-time-greater-than-*t* (IRT>*t*) schedule. Phenobarbital produces quite different effects; low to moderate doses typically increase FR and FI responding with higher doses nonselectively suppressing behavior [1, 13, 14]. Nothing has been reported concerning the effects of valproic acid on schedule-controlled performance, although the drug has been evaluated in pigeons performing under repeated acquisition and delayed-matching-to-sample procedures [15,16]. Valproic acid increased errors and decreased response rates under both procedures. Phenobarbital also increased errors, but typically enhanced response rates. Phenytoin increased errors under the repeated acquisition procedure but not under the delayed-matching-to-sample procedure, although it decreased response rates under each. To date, no published studies have examined the effects of combinations of phenobarbital and phenytoin, or phenobarbital and valproic acid, on operant behavior.

The present study examined the effects of phenobarbital, phenytoin, and valproic acid, given alone and in selected

[1]Requests for reprints should be addressed to A. Poling.

combinations, on the responding of rats maintained under FR and IRT>t schedules of food delivery. These schedules characteristically engender different rates and temporal patterns of responding. Since drug effects often differ as a function of rate and pattern of responding in the absence of drug [2, 6, 18], it is of some interest to evaluate the effects of anticonvulsants under these schedules.

METHOD

Subjects

Six experimentally naive adult male Sprague-Dawley rats were used. They were food deprived to approximately 80% of free-feeding weights (range across subjects 267–354 g) and were housed individually with unlimited access to water.

Apparatus

Three plastic and aluminum operant conditioning chambers were used. Each contained a work panel equipped with two response levers and a pellet trough into which 45 mg Noyes rat pellets could be delivered. Constant ambient illumination was provided by a 7-W white light located on the left wall of the chamber. Scheduling of experimental events and data collection were accomplished through the use of a Digital Equipment Corporation PDP/8A minicomputer using interfacing and software (SUPERSKED) supplied by State Systems Incorporated (Kalamazoo, MI).

Behavioral Procedure

The rats were first trained to lever press under an FR 1 schedule of reinforcement, where delivery of a food pellet followed each response. When all subjects responded consistently under this schedule, they were randomly assigned into two groups of three. For one group, the FR value was gradually increased to 30. The second group was exposed to an IRT>15-sec schedule. Under this schedule, a food pellet followed the first response emitted at least 15 sec after the preceding response; each response emitted before that time reset the interval. These schedules were in effect throughout the balance of the study. Sessions were 30 min in duration and were conducted 6 days per week at about the same time each day.

Pharmacological Procedure

After each rat responded consistently under its terminal schedule, an injection regimen was begun in which each animal received four doses of phenobarbital (10, 20, 30, and 40 mg/kg), phenytoin (20, 30, 40, and 50 mg/kg), and valproic acid (80, 120, 160, and 240 mg/kg). Drugs were given only when an individual rat's performance was stable across three consecutive sessions, one of which was a vehicle control session in which a 1 ml/kg injection of isotonic saline solution was given intraperitoneally (IP) 30 min prior to the experimental session. Responding was assumed to be stable when the mean rate of responding varied by less than 10% across the three sessions. Each subject received each dose of every drug on one occasion; drugs and doses were administered in an irregular sequence that varied across subjects. A minimum of six sessions separated the completion of a dose-response determination for one drug and the start of another.

At the completion of the initial dose-response determination, each rat was exposed to 10 and 30 mg/kg phenobarbital combined with the three lowest doses of valproic acid and phenytoin. These 12 combinations were administered in an irregular order that varied across subjects. Active drug was given only when responding during control sessions was stable according to the criterion described above.

After each subject had received all of the combination doses, a second dose-response determination was made for each of the three drugs given alone; procedures were identical to those described for the pre-combination dose-response determination. One animal in each group died of unknown causes before the completion of the post-combination dose-response determination.

All doses of individual drugs and drug combinations were injected IP (1 ml/kg body weight) 30 min prior to the session. Phenytoin was prepared as a commercially available injection (Parke-Davis, Morris Plains, NJ) diluted with 0.9% sodium chloride solution. Valproic acid (Saber Laboratories, Morton Grove, IL) and phenobarbital (Sigma, St. Louis, MO) were dissolved in distilled water with sufficient sodium hydroxide added to neutralize the drug to the sodium salt. Drug doses, expressed as the sodium salt, were selected on the basis of prior studies conducted in our laboratory which indicated the drugs to be behaviorally active across these dose ranges [8, 9, 15, 16].

RESULTS

The effects of individual drugs and drug combinations in animals performing under the FR 30 schedule are shown in Fig. 1. For simplicity, all data are group means expressed as percent of baseline (the three sessions immediately prior to drug administration) response rate. Across all control sessions, the mean group control rate was 155.7 responses per min, with a range across animals of 131.3 to 176.4 responses per min. In general, each of the individual drugs was associated with dose-dependent decreases in responding under the FR schedule. The pre- and post-combination dose-response determinations yielded similar data; this indicates that tolerance did not occur. The 10 mg/kg dose of phenobarbital in combination with the various doses of phenytoin reduced the group response rate relative to control values in 6 of 6 instances; the 30 mg/kg dose of phenobarbital in combination with phenytoin did likewise. Valproic acid in combination with 10 mg/kg phenobarbital had little effect on mean group response rate. However, 30 mg/kg phenobarbital in combination with each of the three doses of valproic acid reduced responding relative to control levels.

A repeated measures analysis of variance [4] performed on all data save the second dose-response determination for individual drugs indicated a significant treatment effect, $F=3.23$, $p<0.05$, under the FR schedule. Planned comparisons tests (Fisher's protected least significant difference (T_{LSD}) tests, see [4]) were used to compare response rates at each drug dose to control values. Results of these tests indicated that response (and reinforcement, since number of food deliveries per min is prefectly correlated with number of responses per min under the FR schedule) rates under the FR schedule were significantly ($p<0.05$) lowered at the 40 mg/kg dose of phenobarbital alone, at the 40 and 50 mg/kg doses of phenytoin alone, and at the 240 mg/kg dose of valproic acid alone. No drug combination involving 10 mg/kg phenobarbital significantly reduced response (and reinforcement) rate under the FR schedule; all drug combinations involving 30 mg/kg phenobarbital did so. In 7 of 12 instances, drug combinations produced greater mean rate

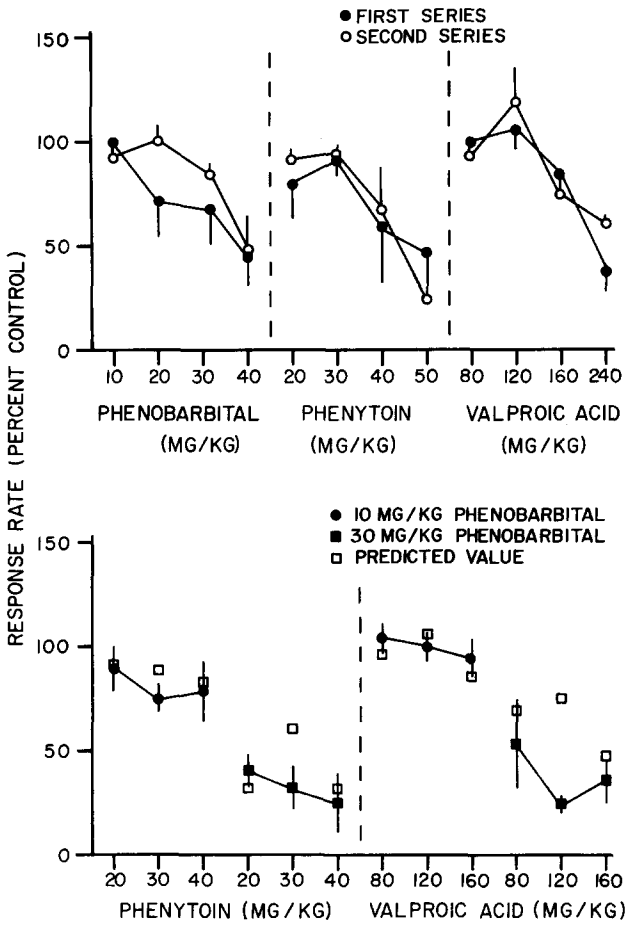

FIG. 1. Effects of phenytoin, valproic acid, and phenobarbital, alone and in selected combinations on the mean group response rate of rats responding under an FR 30 schedule of food delivery. Response rates during sessions in which drugs were given are expressed as a percentage (+1 or −1 standard error) of the rates obtained across the three sessions immediately prior to drug administration. In the upper frame, filled circles represent pre-combination dose-response determinations and open circles represent post-combination dose-response determinations. In the lower frame, effects of drug combinations predicted by a simple additive model are indicated by open squares and obtained values are indicated by filled circles (10 mg/kg phenobarbital plus phenytoin or valproic acid) or filled squares (30 mg/kg phenobarbital plus phenytoin or valproic acid).

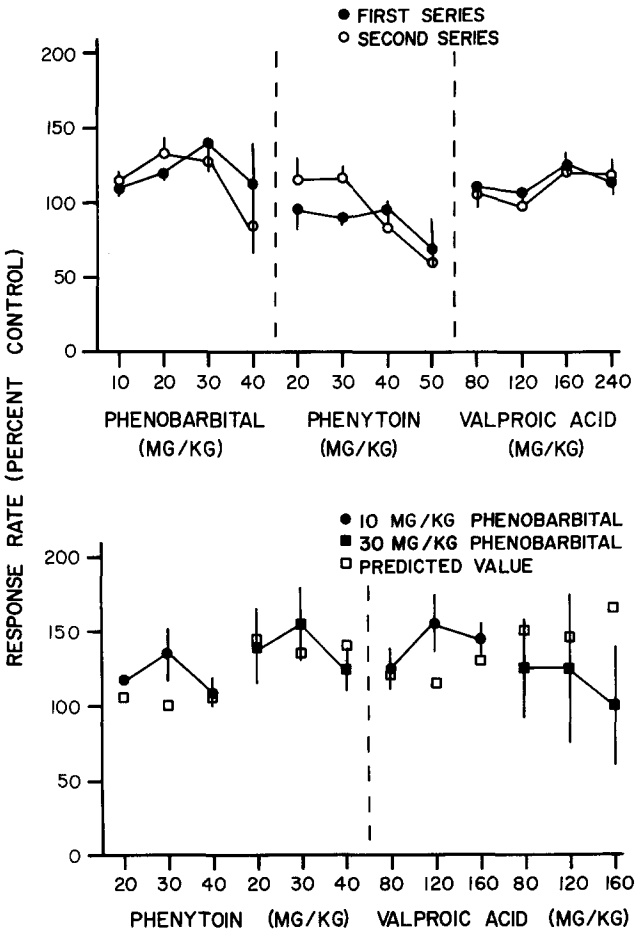

FIG. 2. Effects of phenytoin, valproic acid, and phenobarbital, alone and in selected combinations, on the mean group response rate of rats responding under an IRT>15-sec schedule of food delivery. Details are as in Fig. 1.

reductions under the FR schedule than predicted on the basis of an arithmetic summation of the effects of the individual agents. A sign test [7] indicated that greater than predicted rate reductions did not occur significantly more often ($p>0.05$) than smaller than predicted rate reductions. This may be taken to indicate that, overall, drug combinations did not produce supra-additive effects.

Mean response and reinforcement rates of animals responding under the IRT>15-sec schedule when exposed to individual drugs and drug combinations are shown in Figs. 2 and 3, respectively. Across all control sessions, the mean group response rate was 4.1 responses per min (range across animals of 4.0 to 4.4) and the mean group reinforcement rate was 66.5 food deliveries per min (range across animals of 57.1 to 75.2). A repeated measures analysis of variance performed on all data save the second dose-response determination for individual drugs indicated that, for response rate under the IRT>15-sec, there was not a significant treatment effect, $F=1.2$, $p>0.05$. However, there was a significant treatment effect for mean reinforcement rate, $F=3.3$, $p<0.05$.

Planned comparisons tests (t_{LSD}) indicated that the mean reinforcement rate was significantly ($p<0.05$) below control values at the 30 and 40 mg/kg doses of phenobarbital alone, and at the 160 mg/kg dose of valproic acid alone. The 10 mg/kg dose of phenobarbital in combination with 30 mg/kg phenytoin, and with 120 and 160 mg/kg valproic acid also significantly reduced reinforcement rate, as did 30 mg/kg phenobarbital combined with 20 and 30 mg/kg doses of phenytoin and with all doses of valproic acid. In 7 of 12 instances, drug combinations produced greater changes in mean response rate under the IRT>t schedule than predicted on the basis of an arithmetic summation of the effects of individual agents; in 6 of 12 instances, reinforcement rate was similarly affected. Sign tests indicated that greater than predicted changes in response and reinforcement rates did not occur significantly more often ($p>0.05$) than smaller than predicted rates. This suggests that, overall, drug effects under the IRT>t schedule were not supra-additive.

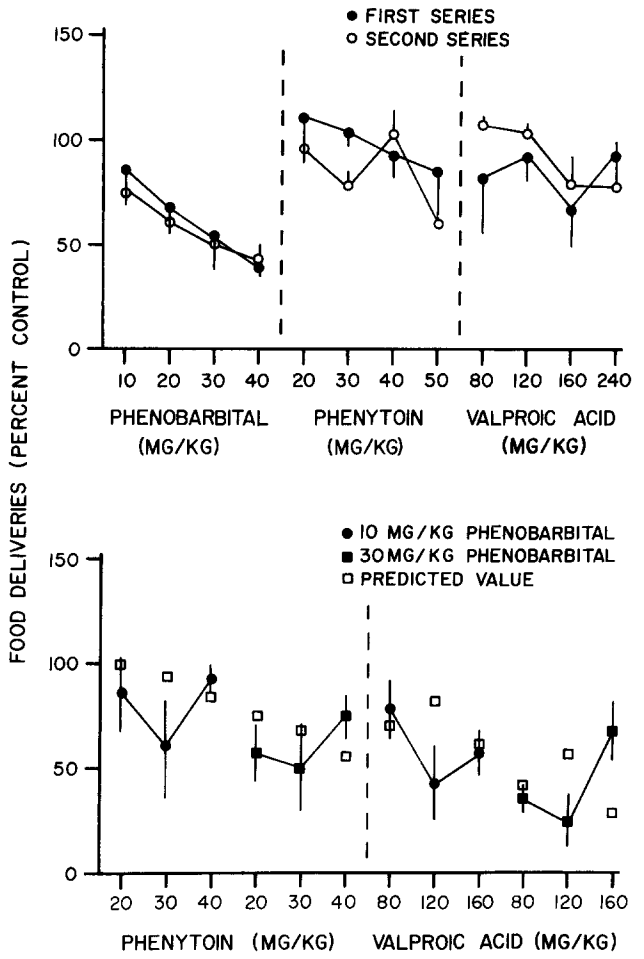

FIG. 3. Effects of phenytoin, valproic acid, and phenobarbital, alone and in selected combinations, on the mean group reinforcement rate (food deliveries/min) of rats responding under an IRT>15-sec schedule of food delivery. Details are as in Fig. 1.

DISCUSSION

The finding that phenytoin decreased FR 30 responding at doses that had no reliable effect on response rate under the IRT>15-sec schedule is consistent with the results of earlier investigations [8,9]. The effects of phenobarbital observed in the present study also are similar to those previously described for phenobarbital and other barbiturates (e.g., [1, 13, 14]), although in some cases these drugs have been shown to increase FR responding. Such an action was not evident with phenobarbital at the doses tested here. Valproic acid, a drug allegedly free of clinically important behavioral side effects [5], significantly reduced responding under the FR schedule at the highest dose tested. Like phenobarbital and phenytoin, valproic acid did not significantly affect responding under the IRT>t schedule. This suggests that the behavioral effects of these drugs may be rate-dependent, since the FR schedule engendered far higher control (nondrug) response rates than the IRT>t schedule. Although none of the drugs significantly affected response rate under the IRT>t schedule, certain doses of phenobarbital and valproic acid significantly reduced mean reinforcement rate under that schedule.

Earlier studies have made it clear that the effects of anticonvulsants differ qualitatively as well as quantitatively when evaluated in animals performing under repeated acquisition [15] and delayed-matching-to-sample [16] procedures. In those studies, phenytoin, phenobarbital, and valproic acid produced similar error-increasing effects under the repeated acquisition procedure, although most doses of phenytoin and valproic acid decreased response rates, whereas most doses of phenobarbital increased responding. Phenobarbital and valproic acid, but not phenytoin, also increased errors under the delayed-matching-to-sample procedure. Another anticonvulsant, ethosuximide, typically decreased response rates under both procedures, but had little effect on errors. The actions of combinations of anticonvulsants were not assessed in these studies, nor in other prior evaluations of the behavioral effects of such drugs.

Unfortunately, as others have pointed out (e.g., [19]), evaluating the behavioral effects of drug combinations is a difficult task. Among the difficulties are (a) possible shifts in baseline response rates over the necessarily protracted course of the study, (b) possible tolerance or cross-tolerance resulting from repeated dosing, and (c) the absence of a generally accepted standard for assessing when obtained effects are actually infra-additive or supra-additive. Despite these potential confounds the importance of evaluating the behavioral effects of drug combinations commonly used in a therapeutic context cannot be denied. The present investigation is of significance primarily because it represents an initial attempt to evaluate the behavioral actions of two drug combinations frequently prescribed for epileptic patients.

Overall effects of these combinations on the schedule-controlled performance of rats did not differ significantly from those predicted on the basis of a simple additive model, wherein the effects of individual agents are arithmetically summated to predict their combined action. While this finding is of interest, it should not be taken to indicate that supra-additive effects would not be observed under other conditions, nor that clinical concern for the untoward side effects of anticonvulsant medications (e.g., [17]) is misguided. Though studies of drug effects on schedule-controlled responding are capable of yielding a wealth of information concerning drug effects (see [13]), fully profiling the behavioral actions of anticonvulsant drugs and drug combinations will require the use of schedules and procedures (e.g., delayed-matching-to-sample, repeated acquisition) not examined in the present study. In the absence of further investigations, strong statements concerning the behavioral effects of combinations of anticonvulsants are unwise as well as unwarranted.

ACKNOWLEDGEMENTS

The reported research was supported by National Institutes of Health Grant No. 1 RO1 NS 20216-01. The authors wish to thank Mr. Scott Wallace for the excellent technical assistance he provided.

REFERENCES

1. Commissaris, R. L., W. H. Lyness, K. E. Moore and R. H. Rech. Central 5-hydroxytryptamine and the effects of hallucinogens and phenobarbital on operant responding of rats. *Pharmacol Biochem Behav* **14**: 595–601, 1981.

2. Dews, P. B. and G. R. Wenger. Rate-dependency of the behavioral effects of amphetamine. In: *Advances in Behavioral Pharmacology*, vol 1, edited by T. Thompson and P. B. Dews. New York: Academic, 1977, pp. 167–229.

3. Goth, A. *Medical Pharmacology*. St. Louis: Mosby, 1984.
4. Huitema, B. *The Analysis of Covariance and Alternatives*. New York: Wiley, 1980.
5. Jeavons, P. M. Valproate toxicity. In: *Antiepileptic Drugs*, edited by D. M. Woodbury, J. K. Penry and C. E. Pippenger. New York: Raven, 1982, pp. 601–610.
6. Kelleher, R. T. and W. H. Morse. Determinants of the specificity of the behavioral effects of drugs. *Ergeb Physiol* **60:** 1–56, 1968.
7. Kolstoe, R. H. *Introduction to Statistics for the Behavioral Sciences*. Homewood, IL: Dorsey, 1969.
8. Krafft, K., D. O. Lyon and A. Poling. Effects of phenytoin on schedule-controlled responding of rats. *Psychopharmacology (Berlin)* **78:** 93–95, 1982.
9. Krafft, K. and A. Poling. Acute and chronic effects of phenytoin on fixed-ratio performance of pigeons. *Pharmacol Biochem Behav* **16:** 843–846, 1982.
10. Kutt, H. Phenytoin interactions with other drugs. In: *Antiepileptic Drugs*, edited by D. M. Woodbury, J. K. Penry and C. E. Pippenger. New York: Raven, 1982, pp. 329–340.
11. Kutt, H. and H. Paris-Kutt. Phenobarbital interactions with other drugs. In: *Antiepileptic Drugs*, edited by D. M. Woodbury, J. K. Penry and C. E. Pippenger. New York: Raven, 1982, pp. 329–340.
12. Mattson, R. H. Valproate interactions with other drugs. In: *Antiepileptic Drugs*, edited by D. M. Woodbury, J. K. Penry and C. E. Pippenger. New York: Raven, 1982, pp. 579–590.
13. McKearney, J. W. and J. E. Barrett. Schedule-controlled behavior and the effects of drugs. In: *Contemporary Research in Behavioral Pharmacology*, edited by D. E. Blackman and D. J. Sanger. New York: Plenum, 1978, pp. 1–64.
14. Middaugh, L. D., L. A. Blackwell, W. O. Boggan and J. W. Zemp. Brain concentrations of phenobarbital and behavioral activation or depression. *Pharmacol Biochem Behav* **15:** 723–728, 1981.
15. Picker, M. and A. Poling. Effects of anticonvulsants on learning: Performance of pigeons under a repeated acquisition procedure when exposed to phenobarbital, clonazepam, valproic acid, ethosuximide, and phenytoin. *J Pharmacol Exp Ther* **230:** 307–316, 1984.
16. Picker, M., W. White and A. Poling. Effects of phenobarbital, clonazepam, valproic acid, ethosuximide, and phenytoin on the delayed-matching-to-sample performance of pigeons. *Psychopharmacology*, in press.
17. Richens, A. Interactions with antiepileptic drugs. *Drugs* **13:** 266–275, 1977.
18. Sanger, D. and D. E. Blackman. The effects of tranquilizing drugs on timing behavior in rats. *Psychopharmacologia* **44:** 153–156, 1975.
19. Thompson, D. M. and J. M. Moerschbaecher. Phencyclidine in combination with *d*-amphetamine: Differential effects on acquisition and performance of response chains in monkeys. *Pharmacol Biochem Behav* **20:** 619–627, 1984.