Effects of Phenobarbital in Combination with Phenytoin or Valproic Acid on the Delayed-Matching-to-Sample Performance of Pigeons

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EFFECTS OF PHENOBARBITAL IN COMBINATION WITH PHENYTOIN OR VALPROIC ACID ON THE DELAYED-MATCHING-TO-SAMPLE PERFORMANCE OF PIGEONS

by

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EFFECTS OF PHENOBARBITAL IN COMBINATION WITH PHENYTOIN OR VALPROIC ACID ON THE DELAYED-MATCHING-TO-SAMPLE PERFORMANCE OF PIGEONS

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Western Michigan University, 1991

The present study examined the effects of phenobarbital (5, 10, 20, and 40 mg/kg), phenytoin (2.5, 5, 7.5, and 15 mg/kg), and valproic acid (40, 60, 80, and 120 mg/kg), and those of phenobarbital (10 and 20 mg/kg) in combination with phenytoin (2.5, 5, and 7.5 mg/kg) or valproic acid (40, 60, and 80 mg/kg), on the delayed-matching-to-sample performance of pigeons. In general, high doses of each individual drug reduced accuracy. Drug combinations also reduced accuracy relative to control values. Reductions in accuracy produced by drug combinations were very similar in magnitude to those predicted by a response-addition model of drug interaction.
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INTRODUCTION

Epilepsy involves "sudden transitory episodes (seizures) of abnormal phenomena of motor (convulsions), sensory, autonomic, or psychic origin" (Rall & Schleifer, 1985, p. 446). These episodes have several subtypes which can be classified as described in the "International Classification of Epileptic Seizures" (Gastaut, 1970). Epilepsy is most effectively controlled by the use of drugs, although other treatments, for example behavior modification (Krafft & Poling, 1982), have shown some success as well.

Sir Charles Locock (1857) is credited with the introduction of pharmacotherapy for the management of seizure disorders. He accidentally discovered in 1857 that potassium bromide was capable of suppressing catamenial seizures.

In 1903, phenobarbital replaced bromides as the drug of choice in the treatment of seizures due in part to the severely deleterious side effects produced by the bromides (e.g., psychosis and painful skin eruptions). Phenobarbital was more effective in controlling seizures than were the bromides and also produced fewer deleterious side effects. In the late 1930s, phenobarbital was replaced by phenytoin as the most popular antiseizure medication. Unlike phenobarbital, phenytoin did not produce physical or psychological dependence. During the next 25 years, several other antiepileptic drugs were introduced into clinical practice in the United States (Swinyard, 1982). With respect to pharmacotherapy and its
clinical effectiveness, "It is generally held that complete control of seizures can be achieved in up to 50% of patients and possibly another 25% can be improved significantly. The degree of success is largely dependent on the type of seizure and the extent of associated neurological abnormalities" (Rall & Schleifer, 1985, p. 449).

In prescribing drugs for the treatment of epilepsy, one needs to be cognizant that some anticonvulsants "not only fail to control seizure activity in some patients, but frequently cause side effects that range in severity from minimal impairment of the CNS, to death from aplastic anemia or hepatic failure" (Rall & Schleifer, 1985, p. 449).

Although the adverse psychological side effects of antiepilepsy drugs are widely recognized (Rall & Schleifer, 1985; Woodbury, Penry, & Pippenger, 1982), their possible behavioral side effects have received little attention. Gibbs et al. (1982) address the potential importance of such side effects:

In 1942, shortly after the discovery of phenytoin, William Lennox expressed his concern that antiepilepsy medicines reduced the quality of a patient's life in the process of preventing seizures. However, for a variety of reasons, the medical community has given short shrift to the issue of subtle side effects. Nevertheless, it is becoming increasingly evident that patients experience subtle side effects even within and below SOTL (Suggested Optimal Therapeutic Level) ranges. These subtle side effects are most likely to involve short-term memory or problem solving. (p. 305)

All antiepilepsy drugs are relatively simple chemical compounds which have the potential to alter neuronal activity enough to prevent the buildup of seizures. There is no reason to expect these medications to act only upon nerve cells specifically involved in epileptic activity and leave the processes of problem
solving and short term memory unaffected. With the development of each new anticonvulsant there is nonetheless hope of fewer negative side effects. Carbamazepine and valproic acid are examples of drugs believed to have fewer deleterious side effects than previous anticonvulsants. Their increased popularity for this reason now appears premature (Bowdle, 1979; Boxer, Herzberg, & Scott, 1976).

As a whole, few strong conclusions regarding subtle side effects of anticonvulsants can be drawn from the literature; however, the more a study focuses on attention, concentration, and short term memory, the more conclusively the results support subtle side effects (Gibbs et al., 1982). The literature regarding the anticonvulsant phenytoin is a case in point concerning the inconsistency of findings with respect to subtle side effects (see Gadow & Poling, 1980; Poling & Picker, in press). The drug appears to be behaviorally active at clinically relevant doses, but the exact nature of its actions, and the factors that modulate these actions, cannot be precisely determined on the basis of the clinical literature. Phenytoin has been studied far more extensively than the other anticonvulsants, and the inconsistencies apparent in the findings with phenytoin are evident with other antiepileptic drugs as well. Reviews of the behavioral effects of antiepileptic medicines revealed via clinical studies are provided by Gibbs et al. (1982), Stores (1975), Poling and Picker (in press), and Gadow and Poling (1980). The consensus of the aforementioned reviews is in agreement with that of Stores (1975), who wrote "the psychopharmacology of anti-epileptic drugs is unsatisfactory at present because of the inadequate and unsophisticated
reporting of behavioral change and because of the common problem of polypharmacy" (p. 655).

Recent research by Poling and associates, reviewed by Poling and Picker (1987), has examined the effects of phenobarbital, phenytoin, clonazepam, valproic acid, ethosuximide, mephenytoin, methsuximide, and primidone on the behavior of nonhumans. Pigeons served as the subjects in most of the studies; rats were used in a few. The purpose of these studies was to profile the behavioral effects of commonly prescribed anticonvulsant drugs.

Several procedures were employed, including a "learning" assay (i.e., a repeated acquisition procedure) and a "short term-memory" assay (i.e., a delayed-matching-to-sample procedure). Results revealed qualitative as well as quantitative differences in the effects of the various anticonvulsants. Moreover, whether a particular drug disrupted behavior at a given dose depended in part on the method by which the behavior in question was maintained. For example, under a multiple fixed-ratio fixed-interval schedule of food delivery, a range of doses of valproic acid and ethosuximide affected responding similarly, but under delayed-matching-to-sample and repeated acquisition procedures their effects were very different (Picker & Poling, 1984; Picker, Thomas, Koch, & Poling, 1985).

Although several studies of the behavioral effects of anticonvulsants in nonhumans have appeared, the effects of drug combinations have been largely ignored. Polypharmacy is a common clinical practice (Stores, 1975), and is typically employed when either (a) two different seizure types occur in the same
individual, or (b) one drug is unable to control seizure activity. The overall efficiency of polypharmacy has, however, been questioned (Reynolds & Shorvon, 1981). Polypharmacy also appears to increase the likelihood of troublesome physiological reactions, and has been criticized on that basis.

The purpose of the present study was to examine the effects of phenobarbital, in combination with phenytoin or valproic acid, on the performance of pigeons under a delayed-matching-to-sample (DMTS) procedure. This procedure is of some interest to behavioral pharmacologists because it provides a sensitive assay of the effects of drugs on complex conditional discriminations and on what might be referred to as "short-term memory." The DMTS procedure, which requires subjects to match or "remember" stimuli separated by short intervals of time, has provided a wealth of information regarding the effects of drugs (Thompson, 1978). Acute and chronic effects of phenobarbital, phenytoin, and valproic acid under this procedure have been determined previously (Picker et al., 1985; Poling, Picker, Vande Polder, & Clark, 1986), but drug combinations have not been examined.

Phenobarbital and phenytoin, and phenobarbital and valproic acid, are two frequently used drug combinations (Kutt & Paris-Kutt, 1982). Some information is available concerning the physiological basis of their interactions but, with the exception of sedation, the behavioral side effects of these combinations in epileptic patients are unclear (Kutt & Paris-Kutt, 1982). A single investigation (Picker et al., 1985) has examined the effects of phenobarbital, in combination with phenytoin or valproic acid, on the operant behavior on nonhumans. In that
study, the effects of drug combinations on the responding of rats maintained under fixed-ratio and interresponse-time-greater-than \( t \) schedules of food delivery were very similar to those predicted by an effect-addition model of drug interaction, wherein the effects of individual drug doses are arithmetically summed to predict the effects of drug combinations (Woolverton, in press).
METHODS

Subjects

Three experimentally-native White Carneaux pigeons, food deprived to 80% of free-feeding body weights, served as subjects. Each bird was individually housed with unlimited access to water and grit in a constantly illuminated room.

Apparatus

Three identical Lehigh Valley Electronics (BRS/LVE, Lehigh Valley, PA) operant conditioning chambers, measuring 32 cm long, 36 cm high, and 35 cm wide, were used. On the front wall of each three response keys (2.5 cm diameter) were located 5.5 cm apart 23 cm from the chamber floor. Each key could be illuminated in red or blue-green. A minimum force of 0.2 g was required for key operation. A food hopper centered in the front wall 7.5 cm above the floor and illuminated by a 7-W white bulb allowed access to mixed grain. A 7-W white bulb (houselight) located on the front wall 33 cm from the floor provided ambient chamber illumination. Masking noise and ventilation was provided by an exhaust fan. A Digital Equipment Corp. (Maynard, MA) PDP8/A minicomputer using interfacing and software (SUPER-SKED) supplied by State Systems Inc. (Snapper & Inglis, 1969) was used to control experimental events and to collect data.
Behavioral Procedure

All subjects initially were exposed to an autoshaping procedure as described elsewhere (Picker et al., 1985). Once all birds reliably pecked all keys under the autoshaping procedure, they were exposed to the DMTS procedure. Under this procedure, discrete trials were programmed with a 10-second intertrial interval (ITI). Each trial was preceded by a 0.25-second darkening of the chamber, after which the center key was illuminated in either red or blue-green; illumination of the center key constituted presentation of the sample stimulus. A response to the center key extinguished the sample stimulus and initiated a delay interval of 0, 1, 2, 4, or 8 seconds. During the delay period, the houselight remained illuminated, responses had no programmed consequences, and the keys were dark. Delays were selected at random with each programmed to occur equally often. At the end of the delay period, the two side keys were illuminated in one of the two possible configurations of color and position (i.e., red on left key and blue-green on right key, or red on right key and blue-green on left key). Illumination of the side keys constituted presentation of the sample stimulus. A response to the comparison stimulus that matched the sample stimulus in color (i.e., a matching response) darkened both side keys and produced 3-second access to grain, then initiated the ITI. Nonmatching responses (errors) also darkened the keys and initiated a 10-second ITI. Such trials were repeated until the subject responded to the appropriate comparison stimulus. Repeating trials in which errors were made was intended to prevent the pigeon from developing position preferences.
When the percentage of correct responses \([\text{matching responses/matching responses + nonmatching responses}} \times 100]\) for individual birds showed no visually evident trend over 5 consecutive 140-trial sessions, the response requirement for extinguishing the sample stimulus was increased to 5 (i.e., a fixed-ratio 5 schedule was arranged) and only every second correct response was followed by food delivery. Correct responses not followed by food delivery were followed by a 1-second flash of the feeder light and then a 10-second ITI. Red and blue-green sample stimuli were presented randomly, with equal probability of occurrence, and each of the five delay values appeared twice during each block of 10 trials. Trials terminated if the response requirement for center-key pecks (i.e., those directed to the sample stimulus) was not met within 35 seconds of trial initiation, or if the subject failed to respond to one of the side keys within 35 seconds of the onset of presentation of comparison stimuli. Such aborted trials were repeated after a 10-second ITI, and were not recorded as incorrect responses. Total aborted trials under all experimental conditions were less than 5% of complete trials. During the experiment proper, sessions terminated after 140 trials or 60 minutes, whichever occurred first, and were usually conducted 6 days a week at about the same time each day.

Pharmacological Procedure

When each subject had completed 40 sessions under the DMTS procedure, the acute effects of phenobarbital (5, 10, 20, and 40 mg/kg), phenytoin (2.5, 5, 7.5, and 15 mg/kg), and valproic acid (40, 60, 80 and 120 mg/kg) were evaluated.
These doses were selected on the basis of prior findings from our laboratory (Picker et al., 1985). Phenobarbital (Sigma Chemical Co., St. Louis, MO) and valproic acid (Saber Laboratories, Morton Grove, IL) were dissolved in distilled water with sufficient sodium hydroxide added to neutralize the drug to the sodium salt. Phenytoin was injected as a commercially prepared solution (Parke-Davis, Morris Plains, NJ) diluted with isotonic saline solution. In all phases of the study:

1. Injections were given intramuscularly (IM) 30 minutes prior to the experimental session at an injection volume of one ml/kg. Prior findings (e.g., Poling & Picker, 1987) indicate that phenobarbital, phenytoin, and valproic acid are behaviorally active when given IM at this presession injection interval.

2. Drugs and doses were given in an irregular sequence, and the active drug was given no more often than twice a week.

3. Drug sessions always were preceded by vehicle control sessions, in which IM injections of one ml/kg isotonic saline were given 30 minutes prior to behavioral testing. Each subject received each dose of an individual drug once during initial dose-response testing.

Following dose-response testing for individual drugs, the effects of drug combinations were evaluated. Twelve combinations of drugs and doses were evaluated. Each subject received 10 and 20 mg/kg phenobarbital in combination with 2.5, 5, and 7.5 mg/kg phenytoin, and in combination with 40, 60, 80 mg/kg valproic acid. Initial dose-response determinations and prior data from our laboratory suggested that these combination doses would be behaviorally active, but would not suppress responding totally. When drug combinations were given,
each drug was injected separately, and one injection was administered on each side of the breast. Control injections for drug combinations were given in the same fashion. After the drug combinations were evaluated, a second dose-response determination for individual drugs was completed in the same manner as the initial dose-response evaluation.
RESULTS

Figure 1 shows the effects of individual drugs on accuracy (mean percent correct responses across the five delay values, expressed as percent change from baseline performance). In general, as in previous studies (Picker et al., 1985; Poling et al., 1986), acute administrations of the three lowest doses of phenobarbital, valproic acid, and phenytoin had little effect on accuracy, although reductions in accuracy were apparent at the highest dose of each drug. Initial and post-combination dose-response determinations yielded very similar data for phenobarbital and valproic acid. The two highest doses of phenytoin, however, produced greater reductions in mean accuracy during the second dose-response determination, largely because one subject responded very little, and made a very high percentage of errors, when exposed to these doses for the second time. Comparison of initial and post-combination dose-response curves for individual drugs indicates that tolerance did not develop during the course of the investigation.

Figure 2 shows the effects of drug combination. The most interesting aspect of the data involves the relatively small decrement in accuracy produced by the drug combinations. In general, phenobarbital in combination with phenytoin or valproic acid slightly increased errors; the magnitude of this effect was greater at higher combination doses. Reductions in accuracy, although small, were very similar in magnitude to those predicted by a response-addition model of drug
Figure 1. Effects of Acute Administrations of Individual Drugs on the DMTS Performance of Pigeons. Data are summed across five delay values for three birds, and are expressed as mean change (+ or -1 S.E.) from control performance. Control performance, calculated by determining the mean percent correct responses across all vehicle control sessions, was 92% correct, with a range across subjects and sessions of 85 to 96%. Circles represent the initial dose-response determination, triangles show the post-combination dose-response determination. For the post-combination phenytoin dose-response determination, values at 7.5 and 15 mg/kg (asterisks) are -33% (S.E. = 35) and -50 (S.E. =29), respectively.
Figure 2. Effects of Acute Administrations of Drug Combinations on the DMTS Performance of Pigeons. Data are summed across five delay values for three birds, and are expressed as mean change (+ or -1 S.E.) from control performance. Control performance, calculated as described in Figure 1, was 92% correct, with a range across subjects and sessions of 86 to 95%. Circles indicate actual performance, triangles represent performance predicted by an effect-addition model of drug interaction.
interaction (Woolverton, in press), in which the effects of individual drugs and
doses (initial dose-response determinations) were arithmetically summed to
predict the effects of drug combinations. A sign test (Kolstoe, 1969) indicated
that greater than predicted changes in accuracy did not occur significantly more
often (p>0.05) than smaller than predicted changes, that is, the effects were
simply additive.
The use of polypharmacy in the management of epilepsy has been questioned in two regards (e.g., Reynolds & Shorvon, 1981). One concerns whether drug combinations are more effective in reducing seizures than are single medications, an issue upon which the present data do not bear. Another concerns whether drug combinations produce more deleterious side effects than individual medications. The present data surely do not resolve this issue for the drug combinations on DMTS performance, as on schedule-controlled responding (Picker et al., 1985), are not synergistic. Insofar as the DMTS procedure is a sensitive assay of drug effects (Thompson, 1978), and is often offered as an assay of "short-term" memory, which is in epileptic patients sometimes impaired by anticonvulsant medications, this outcome may be of some interest.

It is clearly established that phenytoin elimination kinetics are dose-dependent, and that repeated exposure to phenobarbital predictably induces phenytoin induction (Kutt & Paris-Kutt, 1982). Such induction might be expected to result in infra-additive behavioral effects when the drugs are administered together. Phenobarbital also acts as a competitive inhibitor with phenytoin as substrate, since both drugs undergo parahydroxylation and glucuronidation. These effects often balance out, such that interaction of the drugs is neither infra-additive nor synergistic (Kutt & Paris-Kutt, 1982), as was the case in the present study. Chronic drug exposure might, however, lead to stronger induction and
infra-additive effects. When the drugs are given chronically, "the coadministration of valproic acid and phenobarbital inhibits the biotransformation and hydroxylation of phenobarbital to hydroxyphenobarbital, increases its elimination half-life, and ultimately causes elevation of serum phenobarbital levels" (Mattson, 1982, p. 581). Such an interaction might well produce synergistic behavioral effects, but would not be operative under the conditions of the present study, in which drugs were given acutely and their effects assessed relatively soon after administration.

Like chronic exposure, high combination doses might alter the nature of drug interactions due to dose-dependent kinetics. The DMTS procedure used in the present study appears, however, to be ill-suited for studying such combination, since strong (i.e., >40%) reductions in response rates were observed with the highest combination doses we examined.

The present study, like previous research by Poling and associates (e.g., Poling & Picker, in press), was concerned entirely with the behavioral effects of anticonvulsant drugs. Although much effort has been expended in exploring the neurophysiological actions of such drugs, results fail to reveal how these agents control seizures, or affect overt behavior.

As Rall and Schleifer (1985) indicate:

There are two general ways in which drugs might abolish or attenuate seizures: effects on pathologically altered neurons of seizure foci to prevent or reduce their excessive discharge, and effects that would reduce the spread of excitation from seizure foci and prevent detonation and disruption of function of normal aggregates of neurons. Most, if not all, antiepileptic agents that are presently available act at least in part by the second mechanism, since all modify the ability of the brain to respond to various
seizure-evoking stimuli. While a variety of neurophysiological effects of such drugs have been noted, especially effects on inhibitory systems that involve GABA, investigators frequently fail to define those effects that might be prominent at therapeutic concentrations of free drug in plasma or those that are not characteristic of local anesthetics or sedatives. Thus, it must be admitted that mechanisms of action of antiepileptic agents are only poorly understood. Useful antiepileptic agents may be capable of causing some mixture of mutually reinforcing actions that permits therapeutic responses without undue disruption of normal function. (p. 449)

The present findings, in accordance with those of a wide range of other preclinical (e.g., Kulig, 1980; Poling & Picker, 1987) and clinical (e.g., Gadow & Poling, 1980) studies, clearly indicate that anticonvulsants can disrupt normal function. That this is the case should not blind researchers and clinicians to the therapeutic value of these agents, which is great. It should, however, suggest appropriate caution in their use.
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