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BRIEF COMMUNICATION

Effects of Chronic Phenobarbital Administration on Forgetting Functions in Pigeons

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WHITE, K. G., D. N. HARPER AND J. E. WATSON. *Effects of chronic phenobarbital administration on forgetting functions in pigeons.* PHARMACOL BIOCHEM BEHAV 49(2) 427–431, 1994.—The present study examined the effect of chronic phenobarbital administration on forgetting functions based on delayed matching-to-sample performance in pigeons. The effects of IP injections of 10 and 20 mg/kg administered over five consecutive training sessions were compared to each other and a baseline (no drug) condition. Percent correct was reduced as a function of both increasing delay and dose of phenobarbital. A quantitative analysis using a negative-exponential function fitted to bias-free measures of discriminability demonstrated that impaired performance following administration of phenobarbital was reflected in both an increase in the rate of forgetting as well as a decrease in initial discriminability. Furthermore, the influence of proactive interference arising from stimuli on previous trials was attenuated at the highest dose level (20 mg/kg). Thus, chronic phenobarbital administration impairs memorial function and limits the influence of information gained from previous trials on subsequent performance. The current effects of chronic phenobarbital administration are consistent with the effects of acute administration on forgetting functions reported in prior studies.

Chronic phenobarbital Forgetting functions Proactive interference Initial discriminability
Rate of forgetting Delayed matching to sample

SEVERAL lines of evidence support the conclusion that phenobarbital, a commonly used anticonvulsant, has a number of undesirable side effects. Administration of phenobarbital to children with epilepsy may result in hyperactivity, sleep disturbances, and impaired functioning on spatial-motor and a variety of intellectual performance tasks (13). In particular, phenobarbital can impair the shorter-term aspects of memory function in both humans (4,11) and nonhumans (2,5,9).

Studies using the delayed-matching-to-sample (DMTS) procedure with pigeons as subjects have been very informative about the nature and locus of the memory impairment following acute phenobarbital administration. The DMTS procedure has proven to be particularly valuable in separating memorial and perceptual or attentional aspects of memory performance (14). In the DMTS procedure a sample stimulus is presented and then removed. After a short delay or retention interval, comparison stimuli are presented, and the subject has to correctly identify which stimulus matches that originally seen at the beginning of the trial. Picker, White, and Poling (6) examined the effects of acute phenobarbital administration (5 mg/kg to 40 mg/kg) on the performance of pigeons in a DMTS task with delays of 0.5 to 8 s. They found that at even very low doses, overall accuracy was impaired and accuracy at the longest (8 s) delay decreased to chance level.

In a recent study, Watson and White (12) demonstrated that phenobarbital decreased DMTS accuracy in terms of an increase in the rate of forgetting (i.e., performance was more impaired by the drug at longer delays relative to shorter ones) and also in terms of a general decrease in initial discriminability with increasing doses (i.e., performance was lower at a 0 s delay). Watson and White (12) also investigated the effect of phenobarbital on the influence of proactive interference from

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previous trials. Consistent with previous evidence (1), they found that proactive interference was evident in terms of a greater rate of forgetting for trials in which the sample stimulus differed from the sample in the immediately preceding trial, compared to consecutive trials where the sample stimuli were the same. However, increasing dose levels of phenobarbital resulted not only in an increase in the rate of forgetting but also removed the differential effect on the rate of forgetting according to whether the sample stimulus on the previous trial was the same or different as on the current trial. Thus, the acute administration of phenobarbital also attenuated the influence of events from the previous trial on memory performance.

The aim of the present study was to examine the effects of chronic phenobarbital administration on DMTS performance in pigeons. The effects of chronic phenobarbital administration have not received much attention despite their significance, given that epileptic patients often receive anticonvulsant medication chronically. Furthermore, there is some evidence that the effect of anticonvulsant drugs on memory may differ according to whether they are administered acutely or chronically. For example, Poling, Picker, Polder, and Clark (7) showed that two anticonvulsant drugs—clonazepam and valproic acid—impaired DMTS accuracy, but that tolerance may develop with continued administration and, thus, attenuate the impairment. In the present study, we used the same basic task as used by Watson and White (12) to examine the effects of phenobarbital on DMTS accuracy and proactive interference, but instead of being administered acutely, phenobarbital was administered on a chronic basis. An important feature of the present study was the use of bias-free measures of accuracy and a quantitative model of memory performance (14) to separate changes in initial discriminability from changes in rate of forgetting. This analysis has been shown to be sensitive to changes in the rate of forgetting, which can be hidden when accuracy is measured using percent correct (14,16).

METHOD

Subjects

Five adult homing pigeons with extensive experience in DMTS procedures and previously administered acute doses of phenobarbital (12) (the same for all birds) were maintained using postsession feed at 80 ± 15% g of their ad lib body weights. Experimental sessions were conducted daily unless a bird's weight was outside the prescribed range. Water and grit were continuously available in the home cages in a holding room maintained on a 12 L : 12 D cycle.

Apparatus

Pigeons were trained and tested in a sound-attenuating experimental chamber, 31 cm wide, 34 cm deep, and 33 cm high. Three response keys were mounted on one wall, with a hopper opening below the center key. The translucent keys were 2.5 cm in diameter, 10 cm apart, and situated 23 cm above the grid floor. A force of at least 0.1 N was required to operate the microswitch located behind each key. Keys were illuminated either red or green. Experimental events were controlled and recorded by a PDP computer using SKED software.

Behavioral Procedure

Daily experimental sessions consisted of 129 trials. Each trial began with a sample phase in which the center key was illuminated either red or green. The fifth peck darkened the key and initiated a delay that lasted for 0.2, 1.0, 4.0, or 12 s. During the delay, the chamber was darkened and responses were ineffective. Following the delay, the comparison side keys were illuminated (one lit red and the other lit green). Subjects were required to respond on the key lit the same color as originally presented on the center key in the sample phase. A single correct response darkened both keys, produced 2-s access to grain (accompanied by illumination of the food hopper), and initiated a 10-s intertrial interval (ITI). Incorrect responses produced 2-s blackout followed by the ITI. Upon completion of the ITI, the next trial began. Each session consisted of 128 trials plus an initial 'dummy' trial at the beginning of the session which did not contribute to the analysis. The order of red and green sample stimuli were arranged in a pseudorandom fashion. The sequence of trials within each session was constrained so that the sample on the current trial was the same (red-red or green-green) or different (red-green or green-red) from the sample on the preceding trial, equally often for each delay and each sample color. The left-right position of the correct comparison stimuli occurred equally often within each session and across delays. Equal numbers of trials at each delay were arranged within a session.

Pharmacological Procedure

Phenobarbital, obtained from commercial suppliers, was diluted to either 10 or 20 mg/kg with distilled water (vehicle). Each dose of phenobarbital was administered to subjects at a volume of 1 ml/kg, intraperitoneally, 15 min prior to the start of the experimental session. There were three conditions compared in the present study—a baseline condition during which no drugs were administered, a 10 mg/kg condition, and a 20 mg/kg condition. [In previous studies we have found that the effects of vehicle control are consistent with no-drug baseline performance (12).] Stable baseline performance was established over 18 sessions before drug administration. The sessions contributing to the analysis for the baseline condition were the five sessions of DMTS training immediately prior to phenobarbital administration and sessions 4 to 8, inclusive, of further baseline training that followed completion of the 20 mg/kg condition. Of the two drug conditions, the 10 mg/kg condition was given first and drugs were given prior to each and every session during drug administration. Thus, data from five consecutive sessions of DMTS performance under 10 mg/kg and 20 mg/kg of phenobarbital were compared to each other and data from the 10 sessions of baseline performance. Drug administration was discontinued after five sessions in the 20 mg/kg condition because it was found that after five sessions several of the pigeons became unable to complete all the trials within a given session.

Performance Measures

In addition to the use of percent correct, memorial and perceptual aspects of performance were examined here using an analysis (14,15) in which accuracy is measured using the bias-free measure of discriminability, Log d. Log d is analogous to d′ in signal detection theory and is calculated using the following equation (14):

$$\text{Log d} = 0.5 \cdot \text{Log}\,[(\text{Cr}/\text{Er}) \cdot (\text{Cg}/\text{Eg})]$$

where Cr and Cg are the total number of correct responses to the red and green stimuli, respectively, and Er and Eg are the total number of error responses to the red and green stimuli,

respectively. There are several advantages of using measures derived from a signal detection type analysis (such as Log d) when assessing accuracy in a memory task [see (3,8,10) for further discussion on measures of accuracy]. For example, Log d is on a nonbounded scale and, thus, can reveal small differences at high overall levels of performance. Also, Log d is bias-free in that it takes account of the tendency of the subject to prefer one stimulus over the other irrespective of memory for that stimulus. However, a particular advantage of using Log d is that there is considerable evidence that this measure decreases in a negative exponential manner with increasing delay duration. Thus, the reduction in Log d across delays is well described by the following equation (14,15):

$$\text{Log d} = \text{Log d}_0 \cdot \exp(-bt)$$

In the exponential function, t is the delay, Log d_0 is the Y-axis intercept, and, thus, provides a measure of initial discriminability or performance at the zero second delay, and b is the rate of change in the value of Log d across the delays. Thus, fitting the exponential function to Log d measures obtained in the present experiment allows a quantitative analysis of the perceptual or attentional (Log d_0) and the memorial (b) aspects of performance. The parameters Log d_0 and b are independent, and have been interpreted as providing measures of initial discriminability and rate of forgetting, respectively (14,15).

RESULTS

Figure 1 shows that the chronic administration of phenobarbital caused both a decrease in initial discriminability as well as an increase in the rate of forgetting as dose levels were increased. The top panel of Fig. 1 shows mean percent correct for the group as a function of delay and dose. As delay increases, there is a monotonic reduction in accuracy. Likewise, increasing the concentration of phenobarbital caused a greater disruption to accuracy (i.e., a reduction in accuracy at all delays). Figure 2 shows the mean accuracy (averaged across subjects and delays) for each of the five sessions in the first

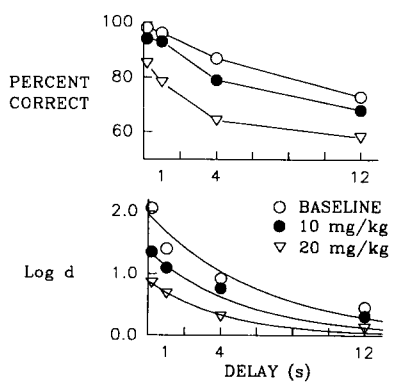

FIG. 1. DMTS performance under chronic administration of phenobarbital. The top panel shows mean percent correct as a function of delay interval for baseline (no drug), 10 mg/kg, and 20 mg/kg doses of phenobarbital. The bottom panel shows mean discriminability (Log d) as a function of delay interval for baseline (no drug), 10 mg/kg, and 20 mg/kg doses of phenobarbital. The solid lines in the bottom panel are negative exponential functions fitted to discriminability values by a nonlinear least squares method. (The variance accounted for by the fitted functions ranged from 91 to 98%.)

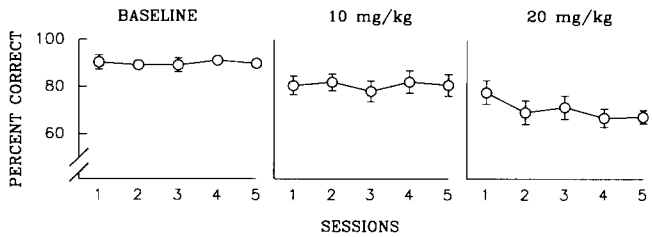

FIG. 2. Mean percent correct averaged over delays in each session in the initial baseline phase, and 10 mg/kg and 20 mg/kg conditions. Error bars show the standard error at each data point.

baseline and two drug conditions. This panel shows that accuracy was overall lower at 10 mg/kg compared to baseline and at 20 mg/kg compared to the other two conditions. However, there were no systematic changes in accuracy across session within a given condition. This conclusion was supported by a repeated measures analysis of variance which revealed a significant effect of condition, $F(2, 8) = 33.4$, $p < 0.01$, but not of session or of the interaction between session and condition ($p > 0.05$). Thus, a tolerance to drug effects did not appear with continued administration.

A major question in the present study was the effect of phenobarbital on the perceptual (initial discriminability) and memorial (rate of forgetting) aspects of DMTS performance. The bottom panel in Fig. 1 shows the mean Log d values according to delay and dose for the group. Solid lines represent the forgetting functions obtained by fitting the negative exponential function to the Log d measures using a nonlinear least-squares regression method. As with percent correct, increasing delays and doses reduced the obtained value of Log d. Fitting the negative exponential function also revealed that there was a systematic decrease in Log d_0 as the dose of phenobarbital increased (1.96 to 1.36 to 0.87, respectively, for baseline, 10 mg/kg, and 20 mg/kg conditions). Furthermore, there was a systematic increase in b as dose increased (0.161 to 0.198 to 0.245, respectively, for baseline, 10 mg/kg, and 20 mg/kg conditions). The trends in Log d shown in Fig. 1 were confirmed by a repeated-measures analysis of variance on the factors of delay and dose, which revealed that there was a main effect of both delay, $F(3, 12) = 136.2$, $p < 0.001$, and dose, $F(2, 8) = 24.8$, $p < 0.001$, as well as an interaction between dose and delay $F(6, 24) = 6.7$, $p < 0.001$. Figure 3 shows that the changes in the log d_0 and b measures for the group data in Fig. 1 were consistent with the data for each bird. With the exception of the 20 mg/kg condition of Bird D1, every bird showed a systematic increase in b and a decrease in log d_0 with increasing dose level of the drug. Thus, consistent with the effects of acute phenobarbital administration (12), chronic administration of phenobarbital in the present study impaired DMTS performance both in terms of a decrease in initial discriminability as well as in terms of an increase in the rate of forgetting.

The current study also examined the effects of phenobarbital on the influence of proactive interference arising from the stimulus presented on the immediately previous trial. To examine this issue, trials were divided according to whether the previous sample stimulus (on trial n-1) was the same or different from the sample stimulus on the current trial (trial n). The top row of Fig. 4 shows mean group performance separated according to whether the previous trial was the same (unfilled circles) or different (filled circles) at each dose level. Solid

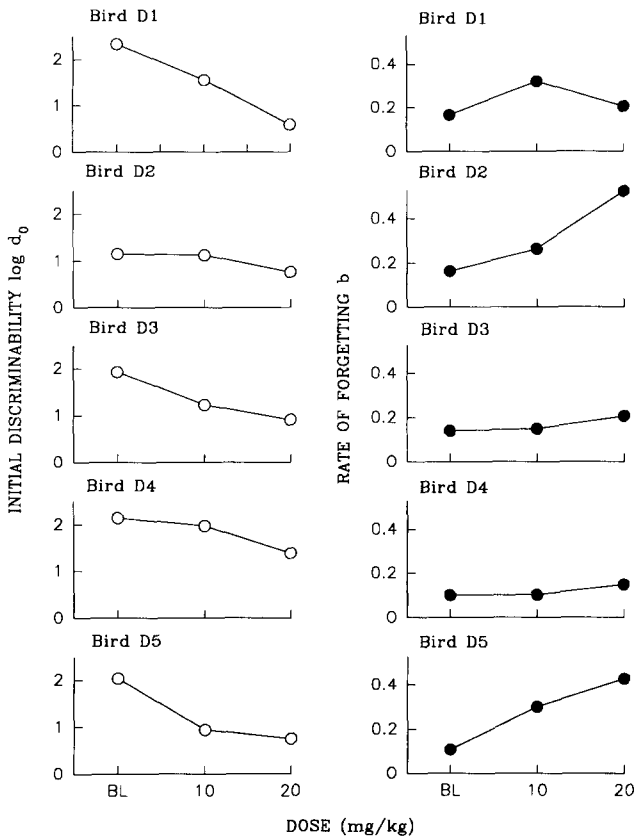

FIG. 3. Initial discriminability Log d_0 (left column) and rate of forgetting b (right column) parameter values in each condition for individual subjects. For the purposes of calculating individual performances, 0.5 was added to Cr, Cg, Er, and Eg (see Eq. 2) to avoid undefined parameter values that may arise at very high levels of accuracy. BL is the baseline condition.

lines are exponential functions fitted to the discriminability measures, Log d, by a nonlinear least-squares regression method. The two lower panels show the changes in the parameter values Log d_0 (left panel) and b (right panel) for the exponential functions displayed in the top panels. Consistent with previous evidence (1,12), Log d_0 did not depend on whether the previous stimulus was the same or different, and Log d_0 decreased to the same extent as the dose of phenobarbital increased for both types of trial. A repeated-measures analysis of variance comparing changes in Log d_0 for individual birds as a factor of trial type (i.e., previous stimulus same vs. different) and dose level showed there was a main effect of dose, $F(2, 8) = 21.7, p < 0.001$, but no significant effects of trial type or an interaction between trial type and dose $p > 0.05$. Also consistent with previous evidence, the rate of forgetting (b) generally increased as dose increased (12). Furthermore, whether the stimulus was the same or different from the current stimulus was highly influential on b, but only during baseline and 10 mg/kg conditions. That is, the rate of forgetting was greater on trials where the sample stimulus differed from that on the preceding trial for the baseline and 10 mg/kg conditions. At 20 mg/kg the differential effect of sample stimulus on the previous trial disappeared. A repeated measures analysis of variance comparing changes in b for individual birds as a factor of trial type (i.e., previous stimulus same vs. different) and dose level showed that the main effect of dose approached significance, $F(2, 8) = 3.7, p = 0.07$, and that there was a significant main effect of trial type, $F(1, 4) = 8.3, p < 0.05$. The conclusion that increasing phenobarbital dose diminishes the effect on rate of forgetting of whether sample stimuli on consecutive trials were the same of different, was supported by a significant interaction between dose and trial type, $F(2, 8) = 5.0, p < 0.05$.

DISCUSSION

The present results showed that the chronic administration of phenobarbital impaired DMTS performance both in terms of a decrease in initial discriminability as well as in terms of an increase in the rate of forgetting. Furthermore, at high doses, subjects become increasingly less able to use information from previous trials in guiding performance on the current trial. Thus, these present results were consistent with those observed under an acute regime of phenobarbital administration (12). However, the effects of phenobarbital on the rate of forgetting and susceptibility to influence from events on previous trials are findings that are dissimilar from those observed following administration of another barbiturate, sodium amylobarbitone (3). Hulme, Sahgal, and Iversen (3) used a similar DMTS task with pigeons and showed that although accuracy was reduced by sodium amylobarbitone, the disruption was solely in terms of an overall decrease in accuracy, and no interaction with proactive interference effects was found. Thus, as indicated by the study of Hulme et al. (3) and others (6,7), the various drugs that are often classed together, as anticonvulsants or barbiturates, may not always disrupt performance via the same behavioral mechanisms.

The similar effects of chronic phenobarbital administration

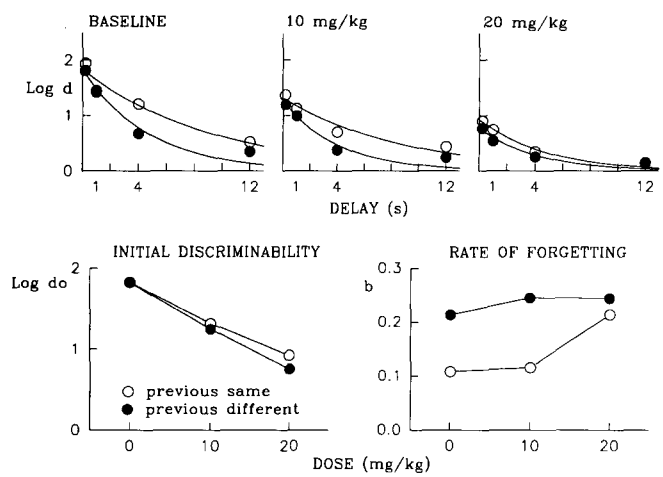

FIG. 4. DMTS performance under chronic administration of phenobarbital separated for trials on which consecutive sample stimuli were the same ○ or different ●. The top panels show mean discriminability (Log d) as a function of delay interval for baseline (no drug), 10 mg/kg, and 20 mg/kg doses of phenobarbital. Solid lines are negative exponential functions fitted to discriminability values by a nonlinear least squares method. (The variance accounted for by the functions ranged from 92 to 98%.) The lower two panels show changes in the two parameters, Log d_0 and b, respectively, according to dose. Parameter values were obtained from the exponential functions fitted to the mean group discriminability data displayed in the top panels.

in the present study and acute phenobarbital administration in the prior study (12) suggest that continued chronic administration of phenobarbital is likely to be just as disruptive as acute administration of phenobarbital. Additionally, there was no evidence for tolerance over the 5-day administration period. Therefore, any possible habituation to the memory-impairing aspects of phenobarbital is unlikely to occur, at least in the short term. The implication of the present findings is that the continued administration of phenobarbital as an anticonvulsant in humans may also produce severe memory impairments that may remain unameliorated despite continued drug delivery.

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