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Brain Concentrations of Phenobarbital and Behavioral Activation or Depression

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MIDDAUGH, L. D., L. A. BLACKWELL, W. O. BOGGAN AND J. W. ZEMP. *Brain concentrations of phenobarbital and behavioral activation or depression.* PHARMAC. BIOCHEM. BEHAV. 15(5) 723–728, 1981.—Brain concentrations of phenobarbital and its effects on locomotor activity and lever responding for food reinforcement were determined at several intervals following injections into C57BL/6J mice. Phenobarbital either elevated or depressed both types of behavior depending on dose and time after injection. Excitation was noted at times and doses when brain concentration was 9 μg–11.5 μg/g tissue. Depression was initially noted at approximately 20 μg/g tissue. Lever responding was altered when brain concentrations of the drug were lower than those associated with corresponding effects on locomotor activity. Excitatory and depressive effects were most extensive when basal response rates were moderate or high respectively. Hence, whether phenobarbital is excitatory or depressive depends on a complex interaction of brain concentration, rate of ongoing behavior and the stimulus conditions maintaining the behavior.

Barbiturates Locomotor activity Operant behavior C57BL/6J mice Rate dependency

BARBITURATES are recognized primarily for their sedative-hypnotic properties [6] however, an excitatory action on both humans [1,12] and laboratory animals [5, 11, 13, 14] has been reported. Read *et al.* [13], and Millichamp and Millichamp [11] reported that certain doses of phenobarbital elevated locomotor activity of mice prior to depression; and Waters and Walczak [14] reported activation following barbiturate depression. The phenobarbital induced biphasic action noted in these two studies is presumably related to the amount of phenobarbital at critical sites in the brain. Although data relating brain concentrations of the drug to behavior is lacking, activation is presumed to occur with low brain concentration initially following injection and then after the depressive effects when concentration is reduced through clearance from the brain. Consistent with this interpretation is Waters and Walczak's [14] report that low doses of phenobarbital produced only an elevation of locomotor activity, a finding which they interpret to indicate that different neural mechanisms mediate the excitatory and depressive effects of phenobarbital. Dews [5] on the other hand emphasizes that the particular effect of barbiturates, as well as other drugs, on behavior is heavily influenced by the environmental conditions maintaining the behavior. Thus, barbiturates can either elevate or reduce response output depending on the schedule according to which reinforcement is given; and, in general, have more of an excitatory effect when the basal response rates produced by the reinforcement schedules are low.

Although barbiturate induced excitation is documented, the conditions which lead to excitation or depression have not been thoroughly investigated and data on the relationship of brain concentrations of the drug to these states is absent. The primary purpose of the present study was to determine brain concentrations of phenobarbital at doses and injection times which elevate or reduce behavioral output. Since the effects of the drug seem to depend on the conditions under which the behavior is examined, three separate behavioral experiments were conducted. In the first experiment, doses of phenobarbital which elevate or reduce locomotor activity (a reflexive behavior) were established. To determine the generality of the effect noted in the first experiment, the effect of the same three doses on lever responding for food delivered according to a random interval schedule of reinforcement (a learned behavior) was examined. In the third behavioral experiment, the effect of phenobarbital on lever responding under a fixed interval schedule of reinforcement was determined. This particular schedule was used because it generates periods of both low and high response rates thus provides a mechanism for determining whether or not the behavioral effects of the drug are dependent on basal response rates. This experiment was also used to establish more closely the minimum dose necessary to depress behavior. In the final experiment, mice were injected with the phenobarbital doses used in the behavioral experiments and animals were killed at times when behavior was either elevated or reduced to allow a comparison of brain concentrations with behavioral effects.

METHOD

Male C57BL/6J mice 120–140 days of age were subjects for all four experiments. They were maintained six per cage

on a 12 hr Light:Dark cycle, with lights on at 0700 hr, and had continuous access to Purina Lab Chow and water except as noted below. Sodium phenobarbital was dissolved in 0.9% saline to provide doses of 20 mg (P_{20}), 40 mg (P_{40}), 60 mg (P_{60}) or 80 mg (P_{80})/g body weight. The particular doses used will be specified below for each separate experiment. Injections of phenobarbital or saline vehicle (S) were given subcutaneously in volumes of 0.01 ml/g body weight.

Locomotor Activity

Locomotor activity was determined for twenty eight male mice (7/group) injected with S, P_{20}, P_{40}, or P_{80}. Individual mice were tested between 0830 hr and 1200 hr in one of three transparent Polycarbonate mouse cages (32 cm × 21 cm × 13 cm) enclosed in individual sound attenuated cabinets (80 cm × 56 cm × 40 cm). Exhaust fans provided ventilation and masking noise. Light was provided from 6w bulbs located 35 cm above the middle of the mouse cage. Each cage was divided into quadrants by two photobeams and activity was automatically monitored by counting switch closures of the photocells produced by the mouse interrupting the photobeam directed onto the cell. The counts were cumulated and printed out at minute intervals. On the day of testing, animals were brought into the testing room in groups of three, injected with the appropriate solution and placed immediately into one of the three cages. Activity was then recorded for 60 minutes. Animals in each of the four treatment groups were systematically tested on different activity monitors to eliminate any possible bias which might be introduced by unknown differences in the three recording chambers. The cages were cleaned after each test to eliminate any confounding influence of odor from previously tested mice.

Operant Behavior

Two separate experiments utilizing slightly different methodologies were conducted to determine the influence of phenobarbital on a learned response. For both experiments the animals were individually housed, deprived of food to 80% ± 3% of their ad lib body weight and placed in one of six operant chambers to acquire a lever response for food reinforcement. We have previously reported descriptions of the operant chambers; and the procedures used for food deprivation and response acquisition [9].

In the first operant experiment, the effect of S, P_{20}, P_{40} and P_{80} injections on lever responding maintained by a random interval (RI) schedule of reinforcement was investigated. Six mice were used. After acquisition of the lever response, animals were initially trained 60 min/day on a schedule in which reinforcement was delivered 20% of the time for the first response made one minute after the previous reinforcement (RI 20%, 1 min). After 15 days on this schedule, the testing session was increased to 90 minutes and the minimum time interval between reinforcements was increased to two minutes (RI 20%, 2 min). RI schedules reportedly produce steady response rates across an experimental session similar to the more familiar variable interval schedules [10].

After 40 days on the RI 20%, 2 min schedule, drug testing began. During drug testing one of the drug doses (S, P_{20}, P_{40} or P_{80}) was injected subcutaneously every third day. Each animal received each drug dose, however, dose order was counter balanced such that each animal was exposed to drug doses in a different order. On test days, animals were allowed 30 minutes of responding, then removed and injected with appropriate drug dose. They were immediately replaced in the chamber and responses were recorded at minute intervals for the next hour.

The second operant experiment utilized six additional mice to examine the effect of phenobarbital on lever responding maintained by a fixed interval 60 second (FI60) schedule of reinforcement. It appeared from the RI experiment that the procedure of removing the animal from its daily test for injections was very disruptive and introduced considerable variability. Thus, animals in the present experiment were injected daily with saline prior to each recording session and testing was continuous for 60 minutes. After 35 days of testing to allow stabilization of the response pattern, saline injections were begun. Drug testing began after 20 days of saline injections. Each animal received both drug doses (P_{20} and P_{60}) two times; however, the order of doses was different for each animal. At least three drug free days intervened between drug tests during which time animals continued to receive saline. The total number of responses per session was recorded. In addition, the distribution of responses (in 15 second bins) about reinforcements was determined for the 19th, 20th and 21st minute after each drug injection or each saline injection on the day prior to drug tests.

Phenobarbital Concentrations

Brain concentrations of phenobarbital were determined in 49 (4/group) mice killed 15, 20, 60 and 120 minutes following injections of P_{20}, P_{40} and P_{80}. After decapitation, the brains were removed, weighed, placed on dry ice and then stored at −70°C for three to four days until assayed by high pressure liquid chromatography (HPLC). The brains were homogenized in 10 volumes of 0.1 M potassium phosphate buffer (pH 7.45) using a Brinkman Polytron. Two hundred microliters of the homogenate was then added to a tube containing 50 μl of acetonitrile which in turn contained the internal standard (Primidone). After vortexing, 500 μl of Hexane was added and the samples were again vortexed. KCl (500 μl) was added to the tubes; they were vortexed and 25 μl of the acetonitrile phase was injected into a Waters HPLC system. The samples were chromatographed using a μ Bondapack C_{18} column (Waters Associates, Inc.). The column was eluted with acetonitrile/phosphate mobile phase and the effluent monitored at 195 nm. Concentrations were determined on the basis of peak height ratios. (All reagents were HPLC grade.)

Statistical Analysis

Brain concentration data were initially subjected to a 3 (Dose) × 4 (Time) analysis of variance (AOV; [15] p. 431). Data were further analyzed within each dose across time and within each time period across dose using single factor AOVs, trend analyses and Newman-Keuls tests for multiple comparisons of means ([15], pp. 149, 177, 196).

Behavioral data were initially analyzed according to dose with single factor AOVs using a repeated measures model for data from the operant experiments ([15] pp. 149,261). Differences between means calculated from data under drug conditions vs means from saline data were elevated with Dunnette's tests ([15] p. 20).

Activity data were further analyzed for comparison with brain concentration by separate 2 (Drug) × 8 (Time) AOVs on each drug vs saline condition.

TABLE 1

CONCENTRATIONS OF PHENOBARBITAL (μg/g) IN BRAINS OF C57BL/6 MICE AT INTERVALS FOLLOWING SUBCUTANEOUS INJECTIONS

	Time after Injection (min)			
	15	30	60	120
Dose (mg/kg)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
20*	8.02 ± 0.28	11.08 ± 0.51	11.42 ± 0.32	12.30 ± 0.36
40†	13.68 ± 0.57	21.58 ± 0.80	23.78 ± 0.55	23.60 ± 0.28
80‡	27.28 ± 0.64	40.60 ± 1.42	44.00 ± 1.02	45.85 ± 0.63

*Newman Keuls Test: 15<30, 60, 120 ($p<0.01$).
†Newman Keuls Test: 15<30<60, 120 ($p<0.01$).
‡Newman Keuls Test: 15<30<60, 120 ($p<0.01$).

RESULTS

Brain Concentration of Phenobarbital

Phenobarbital concentration data is summarized in Table 1. Brain concentration of the drug increased as a function of both dose and time. Statistical support for this observation is provided by a significant interaction, $F(6,36)=22.95$, $p<0.01$, obtained from a 3 (Dose) × 4 (Time) AOV. Within each time period, brain concentrations varied as a function of Dose (F values associated with probabilities of 0.01 or less for all time periods). Subsequent trend analyses on each set of data indicated that 97% to 99% of the variance could be accounted for by linear regression. Within each dose, brain concentration varied as a function of Time (F values associated with probabilities of 0.01 or less). The trend analyses on these data established that linear regression could account for only 75%–84% of the variance. The quadratic trend across time was significant for all three doses and accounted for 11%, 24% and 16% of the variance for P_{20}, P_{40} and P_{80}, respectively. The significant quadratic trends suggest that maximum concentrations were obtained prior to the two hour time sample. Comparison of means across time within each dose via Newman-Keuls tests provides confirmation. For the P_{20} dose, brain concentrations did not increace significantly beyond the 30 minute sample; and for the P_{40} and P_{80} doses, no increase beyond the 60 minute sample was observed.

Behavioral Effects of Phenobarbital

Behavioral data obtained during the hour following saline or drug injections are summarized in Fig. 1 for all three experiments. Inspection of the graph indicates that behavioral output compared to saline control levels was clearly increased by the P_{20} dose and decreased by the P_{60} and P_{80} doses. The data points for the locomotor activity and FI experiments are means calculated from raw data. Data points for the RI experiment were normalized due to extreme variability between animals (e.g., from 129–1540 responses over a one hour period for different animals injected with saline). Scores were thus adjusted according to preinjection base rates as previously described [13]. Thus, if response output during a 15 minute interval prior to injection was 50, the number of responses following injection was multiplied by two. If the animal made 200 responses during this period, subsequent scores were divided by two. AOVs on data for all three experiments provided F values associated with probabilities of 0.005 or less indicating a significant effect of phenobarbital dose for all experiments. Dunnette's tests statistically confirmed the following differences between means. Compared to saline control values, P_{20} elevated both locomotor activity

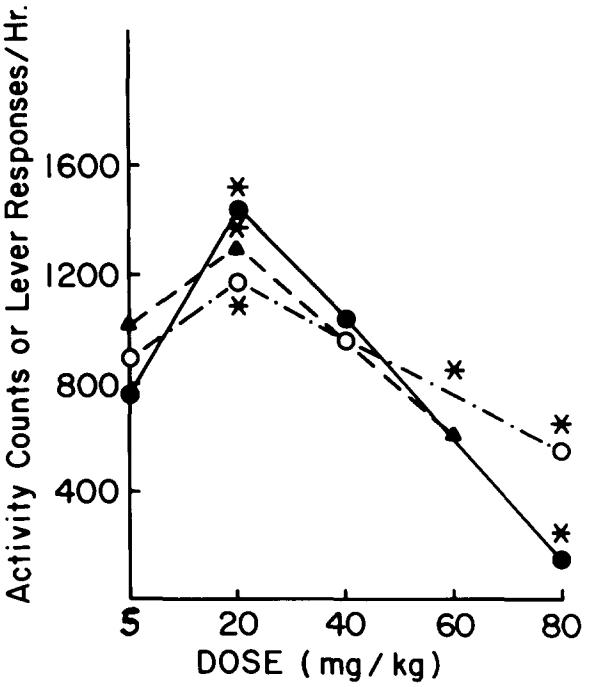

FIG. 1. Locomotor activity (○···○) and lever responses maintained by random (●———●) or fixed (▲———▲) interval schedules of reinforcement for C57 mice at one hour following subcutaneous injections of saline (S) or the doses of sodium phenobarbital indicated. Data points are means for 7 animals per group in the activity experiment and 6 animals for each reinforcement schedule experiment. Asterisks indicate significant increases or decreases from control values via Dunnette's test.

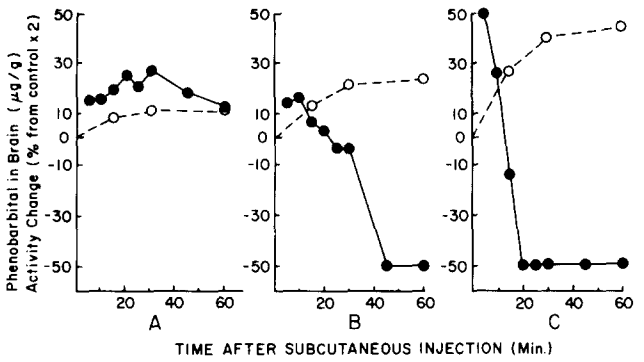

FIG. 2. Brain concentrations on phenobarbital (○– – –○) and percent change in locomotor activity (●– – –●) at intervals following injections of the drug at 20 mg (Graph A), 40 mg (Graph B) or 80 mg (Graph C)/kg.

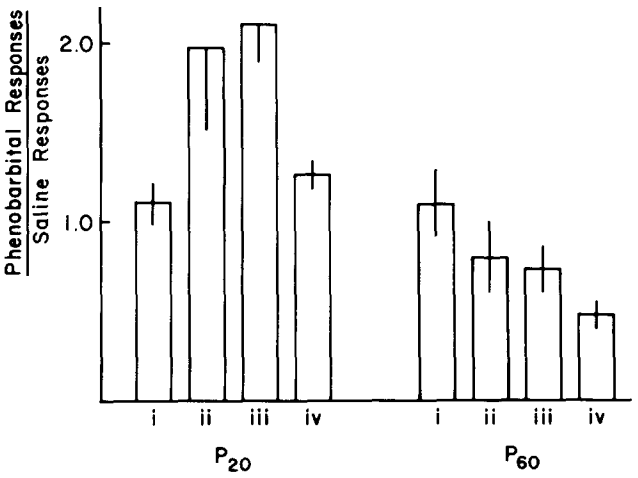

FIG. 3. Distribution of lever responses in 15 sec bins (i:<15, ii: 16–30, iii: 31–45, iv:>46) following reinforcement delivered on an FI 60 schedule. The ordinate indicates ratios of responses made following injections of phenobarbital (20 mg/kg. P_{20}; 60 mg/kg, P_{60}) to responses made during comparable periods following injections of saline. Bars represents means calculated from 6 mice injected on different occasions with each drug dose and vertical lines represent standard errors associated with the means.

(31%, $t(24)=3.390$, $p<0.005$) and lever responding in the RI experiment (102%, $t(15)=3.458$, $p<0.025$). The P_{80} dose reduced both locomotor activity (39%, $t(24)=4.158$, $p<0.005$) and lever responding (80%, $t(15)=2.700$, $p<0.01$) in the two experiments. The two experiments thus clearly establish dose dependent excitatory and depressive effects of phenobarbital on both reflexive and learned behavior. The P_{40} dose produced no reliable change from control values in either experiment suggesting that it may be near a threshold dose for a depressive effect. The results of the FI experiment are consistent with this interpretation in that the P_{60} dose clearly reduced lever responding (38%, $t(10)=5.289$, $p<0.005$). The P_{20} dose in the FI experiment again increased lever responding (34%, $t(10)=36.22$, $p<0.01$) however, not to the extent noted in the RI experiment.

Brain Concentrations and Behavior Comparison

Brain concentrations and changes in locomotor activity across time produced by the various doses of phenobarbital are compared in Fig. 2. Activity change is expressed as percent increase or decrease from saline control values at similar times after injection and is based on a three minute sample of activity surrounding each time point. The values for brain concentrations were obtained from Table 1. Inspection of the three graphs suggests that P_{20} (Graph A) produced only an excitatory effect on activity whereas the P_{40} (Graph B) and P_{80} (Graph C) doses initially elevated then reduced activity. An AOV on saline and P_{20} data (Graph A) indicated an overall Drug effect, $F(1,12)=7.39$, $p<0.01$. Activity was maximally elevated (54%) at 30 minutes after injection. A similar AOV on saline and P_{40} data (Graph B) indicated a Drug × Time interaction, $F(7,84)=2.972$, $p<0.01$. The apparent increase in activity during the initial 10 minutes of recording was not statistically supported. The reduction by 45 minutes after injection, however, was clear since locomotion had virtually ceased. The AOV on saline and P_{80} data (Graph C) was similar to that for P_{40} yielding a highly significant Drug × Time interaction, $F(1,24)=95.44$, $p<0.01$. Data for this analysis was restricted to the first three time points since locomotion had stopped by 20 minutes after injection. The biphasic reaction to this dose was clear. Significant elevation occurred at 5 minutes, $t(12)=2.737$, $p<0.01$, Dunnette's test, and reduction occurred at 20 minutes after injection. For each dose of phenobarbital, brain concentrations were near 10 μg/g tissue when activity was maximally elevated, assuming a linear increase in concentration during the initial 15 minutes after injection for the P_{40} and P_{80} doses. Brain concentration of the drug was 22–27 μg/g tissue at time points where activity was initially reduced. Data from the RI experiment were further analyzed as described above for locomotor activity data. The analysis provided statistical support for the following observations. First, P_{20} elevated lever responding above control values (120%, $t(5)=1.862$, $p<0.05$) by 20 minutes after injection and responding remained above control levels for the rest of the hour. By extrapolation from Fig. 2, Graph A, brain concentration of phenobarbital at 20 minutes is near 9 μg/g. Second, P_{80} reduced lever responding below control values by 10 minutes after injection (−60%, $t(5)=4.886$, $p<0.01$) and the animals completely stopped responding by 15 minutes after injection. Thus, a significant reduction in lever responding occurred at a time when brain concentration of phenobarbital was just under 20 μg/g tissue. Finally, the P_{40} dose produced no reliable behavioral change detected by this analysis.

Effect of Basal Response Rates on Behavioral Effects of Phenobarbital

The effect of the P_{20} and P_{60} doses on the distribution of responses in 15 second bins between reinforcements delivered on the FI 60 schedule is shown in Fig. 3. Data for this figure was collected over a three minute period in the middle of the session on each drug test day and on the preceeding day as described in the Method section. The bars in each graph represent means calculated from ratios of the re-

sponses an animal made in each bin under drug conditions to responses it made during a comparable time under saline conditions. P_{20} increased response output most extensively in bins ii and iii. Responses in the bin just prior to reinforcement (bin iv) was less extensively elevated, and responding in the bin immediately following reinforcement (bin i) was unchanged. P_{60} reliably reduced responding in only bin iv. The tendency toward response reduction noted in bins ii and iii were not statistically confirmed. The number of responses made under saline control conditions were 4, 5, 21 and 49 for bins i–iv respectively. Thus, the excitatory dose (P_{20}) was most effective on moderate response rates and the depressive dose (P_{60}) on high response rates.

DISCUSSION

The present study indicates that phenobarbital can elevate or reduce both reflexive (locomotor activity) and learned (lever responding for food reinforcement) behavior. Whether behavioral output is increased or decreased is related to the concentration of phenobarbital in brain; and also depends on the characteristics of the behavior upon which drug effects are superimposed.

Brain concentration of phenobarbital in the present study increased linearly with injected dose which is comparable with the direct relationship of brain concentrations to injected doses (36.8 mg and 92.0 mg/kg) of sodium barbital [2]. The direct relationship of brain concentration to injected dose of the drugs at all time points indicates that transfer of phenobarbital to brain is along a concentration gradient and is not likely limited by an energy dependent system. Thus, higher doses of the drug produce higher brain concentrations earlier than lower doses as well as producing greater peak concentrations.

Elevated locomotor activity and lever responding produced by the low dose of phenobarbital was due primarily to attenuation of the decline in behavioral output observed under control conditions. A similar phenomenon was recently reported for mice injected with phenobarbital [14] or with ethanol [8]. Thus, in all three experiments, stimulatory effects produced by low doses of phenobarbital or ethanol occurred when basal response rates were low.

The predominant effect of the P_{80} dose was a reduction in both locomotor activity and lever responding. In fact, both types of behavior ceased by 20 and 10 minutes after injection respectively. The effect of this dose on locomotor activity was clearly biphasic. The excitatory phase noted in our study was less prolonged than that reported for injections of 90 mg/kg phenobarbital [13]. In the latter study, maximum elevation was noted at 15 minutes after injection. In addition, Waters and Walczek [14] reported elevated activity on a 30 minute test for doses extending from 20 mg to 80 mg/kg. For comparison, cumulated activity over 30 minutes in the present study was elevated above control values for only the P_{20} and P_{40} doses. Certainly, a major factor accounting for the more rapid onset of depression following P_{80} in the present study compared with the other two is that basal activity in our study was higher because animals were not habituated prior to test. In addition, mice in our study were of a different strain and sex, either of which could contribute to the minor differences noted.

The only reliable effect of the P_{40} dose detected by our procedures was reduced locomotor activity during the last half of testing. Variability of data for this dose was greater than that for the P_{20} and P_{80} doses. Since variability typically increases around threshold levels, P_{40} appears to be near the threshold dose necessary for depression of behavior. The clear reduction in lever responding when the dose was increased to P_{60} is consistent with this interpretation. It is evident from looking at data for individual animals that the P_{40} dose transiently elevated behavioral output, however, the times and degree of elevation differed such that grouped data masked the effect.

The locomotor activity and RI experiments indicate that basal levels of behavior are important in determining whether phenobarbital elevates or reduces behavioral output. A similar dependence on basal response rates is clearly illustrated by the effects of phenobarbital on the distribution of responses about reinforcement in the FI experiment (Fig. 3). P_{20} elevated responses at moderate basal rates much more extensively than at high basal rates. The lack of more extensive elevation at high basal rates is not due to physical limitations of the animal, since response rates during the interval were approximately 81 per minute and mice of the strain used are capable of making at least 240 responses per minute on the apparatuses used in this experiment. The results obtained in this experiment are consistent with an early report [7] that phenobarbital injection into pigeons flattened the "scalloped" appearance of the response pattern characteristic of FI schedules. Thus low doses increase low rate response occurring early in the interval and high dose decrease high rate responding which occurs just prior to reinforcement. It is of interest that P_{20} did not increase responding immediately after reinforcement although basal response rates during this time were similar to the second interval. Certainly, part of this time was utilized to consume the reinforcement. However, on other schedules and in the early phases of FI training, animals require only 2–7 seconds to resume responding after reinforcement, thus time is available during the first 15 seconds to note response elevations. The only explanation we have for the different effects of P_{20} on responding during the two periods is that responding extinguished more rapidly during the period immediately after reinforcement when the response pattern was developing. Thus, as a result of more thorough extinction, responses during this time were less susceptible to manipulation. This interpretation appears reasonable in light of a report [5] that responses with a very low probability, noted during the unreinforced portion of a discrimination problem, were not increased by low doses of phenobarbital.

Certainly, brain concentrations are of primary concern in determining whether phenobarbital increases or decreases behavioral output and the present study provides information about the concentrations associated with behavioral excitation or depression. First of all, excitation for both locomotor activity and lever responding was observed at several times after injection when brain concentration of phenobarbital was near 10 μg/g tissue. Behavioral depression was initially detected by reduced lever responding which occurred at a time when phenobarbital concentration was estimated to be just under 20 μg/g. Locomotor activity was reduced when brain concentration was slightly higher (22 μg/g tissue). The highest brain concentration obtained at one hour was 44 μg/g tissue produced by P_{80}. C57 mice injected with this dose have no locomotor activity at this time, however, the animals do not lose their righting reflex and activity can be provoked by prodding the animal.

In conclusion, the present study demonstrates that a low dose of phenobarbital can exclusively excite both a reflexive (locomotor activity) and a learned (lever responding) behav-

ior. A higher dose clearly evelated locomotor activity prior to depression, hence confirms a biphasic response to the drug. In the present study excitation was noted at several doses and post-injection times when brain concentrations of phenobarbital were 9 μg–12 μg/g tissue. Depressive effects were initially noted at times and doses when concentrations were near 20 μg/g. The highest dose (P_{80}) produced a maximum concentration of 45 μg/g tissue and completely stopped locomotor activity and lever responding, however, left the righting reflex intact. The particular effect of the drug on behavior, regardless of brain concentrations was clearly influenced by the conditions under which the behavior was examined such as basal response level and the behavioral control exerted by environmental stimuli. These findings should be of benefit to those interested in neural mechanisms accounting for excitatory vs depressive effects of barbiturates since brain concentrations and doses associated with each state are provided.

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REFERENCES

1. Browning, R. A. and E. W. Maynert. Phenobarbital, mephobarbital, and metharbital. In: *Antiepileptic Drugs*, edited by D. M. Woodbury, J. K. Penry and R. P. Schmidt. New York: Raven Press, 1972, pp. 345–351.
2. Butler, T. C. The rate of penetration of barbituric acid derivatives into the brain. *J. Pharmac. exp. Ther.* **100:** 219–226, 1950.
3. Dews, P. B. Studies on behavior. I. Differential sensitivity to phenobarbital of pecking performance in pigeons depending on schedule of reward. *J. Pharmac. exp. Ther.* **113:** 393–401, 1955.
4. Dews, P. B. Studies on behavior. II. The effects of phenobarbital, methamphetamine and scopolamine on performances in pigeons involving discriminations. *J. Pharmac. exp. Ther.* **115:** 380–389, 1955.
5. Dews, P. B. Drug-behavior interactions. In: *Behavioral Analysis of Drug Action: Research and Commentary*, edited by Scott Foresman, IL: Glenview, 1971, pp. 10–43.
6. Harvey, S. C. Hyponotics and sedatives: The barbiturates. In: *The Pharmacological Basis of Therapeutics*, edited by L. Goodman and A. Gilman. New York: MacMillan, 1970, pp. 102–123.
7. Herrnstein, R. J. and W. H. Morse. Effects of phenobarbital on intermittently reinforced behavior. *Science* **125:** 929–931, 1957.
8. Middaugh, L. D., B. Read and W. O. Boggan. Effects of naloxone on ethanol induced alterations of activity in C57BL/6J mice. *Pharmac. Biochem. Behav.* **9:** 157–160, 1978.
9. Middaugh, L. D. and C. A. Santos, III. Effects of methadone on behavior maintained by fixed ratio reinforcement schedules. *Pharmac. Biochem. Behav.* **8:** 521–526, 1978.
10. Millenson, J. R. Random interval schedules of reinforcement. *J. exp. Analysis Behav.* **6:** 437–443, 1963.
11. Millichamp, J. G. and P. A. Millichamp. Circadian analysis of phenobarbital-induced hyperkinesia in mice and hansters. *Proc. Soc. exp. Biol. Med.* **121:** 754–757, 1966.
12. Ounsted, C. The hyperkinetic syndrome in epileptic children, *Lancet* **2:** 303–311, 1955.
13. Read, G. W., W. Cutting and A. Furst. Comparison of excited phases after sedatives and tranquilizers. *Psychopharmacologia* **1:** 346–350, 1960.
14. Waters, D. H. and D. Walczak. Cholinergic and dopaminergic involvement in phenobarbital-induced locomotor activity in mice. *Neuropharmacology* **19:** 543–547, 1980.
15. Winer, B. J. *Statistical Principles in Experimental Design*, New York: McGraw-Hill, 1971.