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The Effect of Phenobarbital Dose Upon A Variety of Drinking Related Response Measures

HANS SCHMIDT, JR.

Department of Psychology, Xavier University, Victory Parkway, Cincinnati, OH 45207

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SCHMIDT, H., JR. *The effect of phenobarbital dose upon a variety of drinking related response measures. PHARMAC. BIOCHEM. BEHAV.* 11(2) 145–149, 1979.—Amount of water ingested, total laps, duration of drinking, amount per lap, laps per minute, and running velocity were investigated as a function of phenobarbital dosage (0 to 60 mg/kg). Twenty-three and a half (23 1/2) hour water deprived females rats served in the experiment. Amount of water ingested, total laps, and duration of drinking all responded similarly to phenobarbital all rose and subsequently fell as a function of phenobarbital dose on the day of drug treatment, rose as a linear function of dose a day later, and had no significant relation with dose 2 days after drug administration. These measures significantly intercorrelate with each other on the day of drug treatment and the day thereafter though not 2 days thereafter. Running velocity largely declines as a function of drug dosage on the day of treatment but is unaffected by the drug thereafter. The other measures show no definite trend. However these measures, running velocity and amount/lap and laps/minute, intercorrelate significantly with each other on the day of treatment and not thereafter. The first group of response measures and the latter group do not consistently correlate with each other. It was concluded that there are two identifiable classes of variables: one motor, which is largely a decremental function of dose, the other uncharacterized, initially rising, then falling as a function of phenobarbital dose on the day of drug treatment.

Phenobarbital Drinking Response measures

THE customary measure of drinking employed in drug studies has been the total amount ingested in some fairly prolonged period of time. This measure is accurately determined under such circumstances and has produced reliable results with respect to a number of independent variables. However, subdivision of the time period using water ingestion as a measure can be severely inaccurate, especially when drinking is proceeding rapidly, unless relatively expensive equipment is used. Alternatives to water ingestion as a measure are provided by drinkometers and associated recorders whether cumulative or not. Hill and Stellar [4], for example, reported a high positive correlation between number of laps and amount ingested for a wide range of degrees of water deprivation. Hulse and his associates [5], reported that there is more variation as a function of treatment than did Hill and Stellar, inferring that licking is an operant.

The present study attempts to relate several measures obtained from drinkometer records with water ingestion in rats treated with varied amounts of phenobarbital. Moreover, running velocity was obtained for further comparative purposes. Another aim of the reported investigation was to identify functional relations between phenobarbital dose and the measures of drinking behavior employed.

METHOD

Animals

Twelve female albino rats of approximately 100 days of age served in this experiment. These animals were bred in our laboratory. One animal failed to survive the experiment. Consequently, data are presented for only 11 rats.

Apparatus

The apparatus consisted of 3 drinking boxes 35 cm long by 10 cm wide, and 10 cm high. In these boxes, a drinking spout was to be found approximately 2.5 cm above the hardware cloth floor. A door separated the spout from the main body of the chamber. A 6.3 mm plywood panel was placed immediately in back of the door. A slot 1 cm wide, and about 2 1/2 cm long was cut in the panel in the vertical plane. The drinking spout was about 5 mm in back of the plywood panel. This panel fixed in place, restricted the access of the rat to the spout and allowed only tongue contact. When the tongue contacted the water in the glass spout, completing a circuit, a 12 μA current (AC) went through the rat's tongue and feet closing a relay, pulsing a counter and deflecting an Esterline Angus event recorder pen (Model AW). Paper speed in the recorder was 2 cm/min.

Another apparatus employed in this investigation was a straight alley. The alley was 10 cm wide by 15 cm high in cross section. The length of the main alley was 120 cm. A start box was to be found at one end of the alley, a goal box at the other. The cross section of the start and goal boxes was the same as the alley; each was 30 cm long. The floors and walls were made of wood painted gray. The roof of the alley and end boxes was made of hardware cloth. Guillotine doors separated the end boxes from the straight alley. Photocells and lamps were placed 2.5 cm from each end of

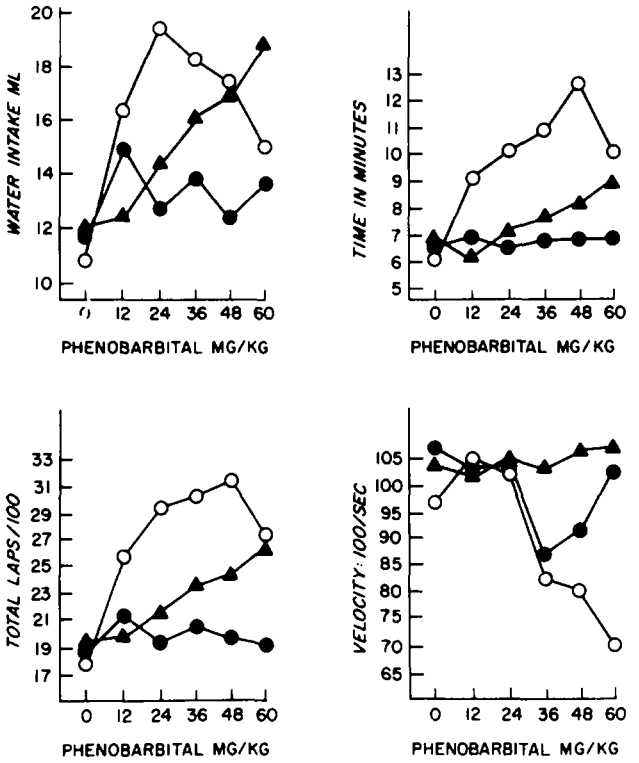

FIG. 1. Mean water intake, duration of drinking, total laps, and running velocity as a function of phenobarbital dose. The open circles indicate the level of responding 45 min after drug injection: the triangles indicate the response a day later; the filled circles indicate responding 2 days later. Water intake and total laps as represented in Figs. 1A and 1C are simply ascertaining the volume of water removed from the tube and taking a number from a counter. Duration of drinking, Fig. 1B, represents the time spent drinking with no more than 3 to 5 sec between laps. Running velocity, Fig. 1D, is simply the reciprocal of the time to traverse the alley times 100.

the alley. The photocell nearest the start box started a 0.01 sec Standard Timer, the photocell at the goal box had stopped it. A 2.5 cm high pedestal with a 2 cm diameter drinking cup attached to the top was placed at the back wall of the goal box. A tube connected to an automatic pipetting device fed into the cup.

Procedure

Rats were placed upon a 23 1/2 hr water deprivation schedule. Shortly before the 23 1/2 hr had elapsed, each rat was given 5 trials in the straight alley; each trial consisting of running down the alley and drinking from the cup. On the first two days of the deprivation schedule, 1.0 ml of water was placed in the cup on each trial. On subsequent occasions, 0.2 ml of water was to be found in the cup. Immediately after the 5 trials in the alley had been completed, the animals were given 30 min in the drinking apparatus. During the drinking period in the drinking boxes, counters and event recorder made their usual sounds familiarizing the animals with such sounds. Upon the completion of drinking, the animals were removed to their home cages and given free access to food. Administration of the drug treatments started only after 10 days of training in straight alley and drinking boxes. The same schedule was maintained throughout the course of the experiment with respect to running in the straight alley and drinking in the boxes.

On the eleventh day and every third day thereafter, phenobarbital was administered. Each rat received each dose once during the course of the experiment as determined by a 6×6 Latin Square. The range of doses varied from 0 to 60 mg/kg in 12 mg/kg steps. The solvent for the drug was 0.9% saline, the same solution used for the 0 mg/kg treatment. Solutions were adjusted so that 1 ml/kg was administered by subcutaneous injection. On those days on which drugs were administered, each rat was removed from its home cage 45 min before the 23 1/2 hr water deprivation period had elapsed, was weighed and injected with the appropriate drug solution in the prescribed amount and placed in another cage without food or water. After the 45 min had elapsed, the procedure for running in the straight alley and drinking boxes described above began.

RESULTS

Five measures of drinking response and one measure of running were obtained. The drinking measures were volume ingested, number of licks, time spent drinking, amount per lick, and licks per minute of drinking. The running time measure was the median time of the 5 trials for a given drug dosage.

Figure 1 presents the results of 4 measures of those evaluated: volume ingested, duration of drinking, total laps, and the reciprocal of median running time. The reciprocal was used so that higher values indicated greater performance in line with the other measures.

The volume of water ingested on the day of treatment first rises then falls, $F(5,45)=6.15$, $p<0.001$. There is a significant quadratic component of the variance, $F(1,45)=23.58$, $p<0.001$; the best fitting quadratic function accounting for 94% of the dosage variance. The only noticeable departure from previous findings being that the estimated dosage producing maximum drinking is somewhat lower than found in earlier studies (33.1 mg/kg in this experiment vs approximately 38 to 40 mg/kg in earlier investigations). Water intake rises on the day following injection, $F(1,44)=6.66$, $p<0.001$. The best fitting linear function accounts for 98% of the dosage variance. There is no significant effect of phenobarbital dose two days following drug administration.

The time actually spent drinking during the half hour drinking period varies significantly 45 min after drug administration, $F(5,45)=8.37$, $p<0.001$. The amount of time spent drinking rises with low doses and falls with higher doses in a quadratic function of the form $Y=A+BX-CX^2$, $F(1,45)=13.55$, $p<0.001$, similar to that obtained for water ingestion. The best fitting quadratic function accounts for 94% of the dosage variance. A day following drug administration, the time spent drinking also varies as a function of dose, $F(1,45)=14.41$, $p<0.001$, accounting for 87% of the dosage variance. No significant effect of phenobarbital dose is found two days after injection.

The effect of phenobarbital dose upon total number of laps is also significant upon the day of drug administration, $F(5,45)=4.77$, $p<0.01$. Similar to both amount of water ingested or duration of drinking, the downturn at the higher dosages of phenobarbital is significant. In contrast to those measures a day later, there is a quadratic rise in number of laps as a function of dosage, $F(1,45)=12.14$, $p<0.001$. Two

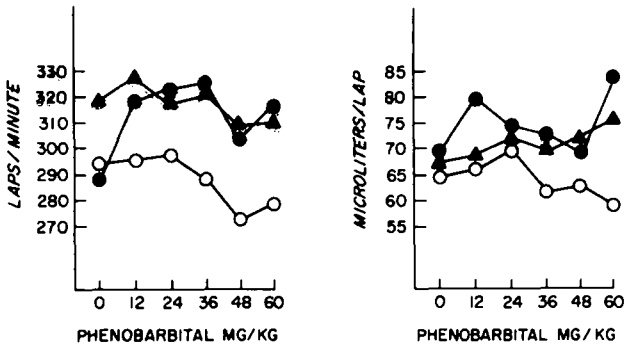

FIG. 2. Mean laps per min and intake per lap as a function of phenobarbital dose. The open circles indicate the level of responding 45 min after drug injection; the triangles indicate the response a day later; the filled circles indicate responding 2 days later. Laps per minute is total laps divided by the number of minutes the rat actually spends drinking. Amount per lap is the result of dividing water intake by the total number of laps.

days following drug treatment, no effect of dose is manifest with respect to number of laps.

The reciprocal of running time follows a different course than do the previous measures. Examination of Fig. 1D indicates a definite decline in running velocity as a function of dose 45 min after phenobarbital has been administered. The best fitting function describing this relationship is a linear decline, $F(1,45)=17.36$, $p<0.001$. The curvature is insignificant. On the two days following drug administration, no significant relationship in running times can be related to phenobarbital dose.

Two derivative measures of drinking behavior were obtained as well as the measures repeated above, namely, average laps per minute and average amount per lap. These data are summarized in Fig. 2. With respect to laps per minute, there is no significant overall effect of phenobarbital dose on the day of drug treatment ($F=1.79$). However, there is a significant linear decline in laps per minute as a function of dose, $F(1,45)=6.90$, $p<0.05$. The day following drug administration, no significant relationship was observed relating dose to laps/minute. In contrast to all other measures used in this investigation, laps/minute is significantly affected by phenobarbital dose two days after drug administration, $F(5,45)=3.41$, $p<0.05$. The sole significant component of dosage variance is the quadratic, $F(1,45)=10.26$, $p<0.001$, yielding an initially rising then falling function. The other derivative measure, amount per lap, failed to yield any significant relation between dose and the measure at any time examined.

Another approach which may reveal significant relationships in these data is via correlations. One approach used was to intercorrelate mean responses as a function of dose so as to get at construct validity rather than to correlate the behavior of individual animals to ascertain predictability for individual animals [3]. Table 1 summarizes the findings of such a correlational approach. On the day of drug treatment there are several significant ($p<0.05$) correlations. These appear to be separable into two groups: (1) duration, total laps, and amount ingested are related while (2) the reciprocal of running time, amount per lap, and laps per minute are related. The measures in Group 1 do not correlate significantly with those in the second group on the day of treatment.

The relations between the correlations of the various measures are much less clear a day after drug treatment. The relations obtained on the day of injection persist with respect to laps, duration, and amount of water ingested. These again significantly intercorrelate which should not be especially surprising in the light of the increase in all of those measures as a function of dose. However, there is not a clear separation of independent groups of measures comprising laps/min, amount/lap, and the reciprocal of running time, again perhaps not surprising in the face of no significant functional relation between dose and those measures. Amount per lap correlates significantly and positively with amount ingested,

TABLE 1

CORRELATION COEFFICIENTS RELATING THE MEAN RESPONSES AS A FUNCTION OF DOSAGE FOR THE 3 TEST DAYS

	Laps	Running Velocity	Duration of Drinking	Amount/Lap	Laps/Minute	
H_2O Intake	0.882*	−0.036	0.743	−0.286	−0.204	Day 1
Laps		−0.411	0.991*	−0.331	−0.621	
Running Velocity			−0.515	0.941*	0.890*	
Duration of Drinking				−0.449	−0.718	
Amount/Lap					0.943*	
H_2O Intake	0.999*	0.782	0.965*	0.958	−0.760	Day 2
Laps		0.784	0.968*	0.947*	−0.753	
Running Velocity			0.874*	0.782	−0.953*	
Duration of Drinking				0.893*	−0.873*	
Amount/Lap					−0.747	
H_2O Intake	0.782	−0.132	0.313	0.739	0.689	Day 3
Laps		−0.310	0.418	0.212	0.446	
Running Velocity			0.346	0.206	−0.391	
Duration of Drinking				0.145	−0.453	
Amount/Lap					0.558	

*Significant at the 5% level of confidence, df=4 [2].

TABLE 2

CORRELATION COEFFICIENTS FOR INDIVIDUAL RATS INDEPENDENT OF DRUG DOSAGE FOR THE 3 TEST DAYS

	Laps	Running Velocity	Duration of Drinking	Amount/Lap	Laps/Minute	
H_2O Intake	0.802*	−0.327	0.836*	0.081	−0.071	Day 1
Laps		−0.606*	0.950*	−0.500	0.163	
Running Velocity			−0.688*	0.418	0.343	
Duration of Drinking				−0.299	−0.129	
Amount/Lap					−0.520	
H_2O Intake	0.528	−0.107	0.610*	0.643*	−0.258	Day 2
Laps		−0.221	0.964*	−0.296	0.010	
Running Velocity			−0.374	0.145	0.317	
Duration of Drinking				−0.197	−0.151	
Amount/Lap					−0.324	
H_2O Intake	0.323	−0.084	0.396	0.494	−0.230	Day 3
Laps		−0.260	0.952*	−0.613	0.195	
Running Velocity			−0.405	0.050	0.452	
Duration of Drinking				−0.468	−0.111	
Amount/Lap					−0.514	

*Significant at the 5% level of confidence, df=9 [2].

duration, and total number of laps but not laps per min, nor the reciprocal of running time. Laps/minute correlates negatively and significantly with the reciprocal of running time and drinking duration. The reciprocal of running time correlates positively with drinking duration. On the final day of the 3 day cycle, no significant correlations were obtained.

In contrast to the correlations of mean responses to various phenobarbital doses, one may correlate the behavior of individual rats independent of dose with respect to the various measures. This latter procedure yields a different pattern of correlation than did the process of correlating mean responses to various doses. It is clear from examination of Table 2 that duration of drinking and total laps are measures of the same thing within very small limits of error. No especially convincing evidence relates specific motor performance measures such as running velocity, laps per minute, and amount per lap. Except for the first day the correlation between water intake and total laps or duration is not very large though on the second day water intake correlates with duration of drinking significantly.

DISCUSSION

Three measures of drinking behavior are clearly related with respect to their variation as a function of phenobarbital dose, namely, volume of water ingested, duration of drinking behavior, and total number of laps. O'Kelly and Weiss observed that Dial, diallylbarbituric acid, prolonged the initial burst of drinking [7]. Furthermore, Hill and Stellar found that amount ingested correlated highly with number of laps for a fixed spout aperture [4]. These same regularities obtained with respect to phenobarbital induced alterations of drinking. Both F tests and correlation coefficients relating mean response clearly are indicative of these relations on the day of drug treatment.

The other measures of drinking related behavior, namely, the reciprocal of running time, laps per minute, and amount per lap do not follow the same pattern. Both the reciprocal of running time and amount per lap show a significant reduction in responding as a function of phenobarbital dose with little, if any, rise at low doses. This reduction of amount per lap should be approached cautiously, however, since the effect observed is small and the linear decline in drinking as a function of dose was obtained in the face of no significant overall effect of phenobarbital dose. It is possible that the purpported effect cannot be reproduced. The reciprocal of running time does not fully correspond to the earlier findings of Schmidt and Stewart [10] who observed that quite low doses of phenobarbital reduced running time with a sharp increase of the measure as dosage was increased. The very small increase in velocity observed in this experiment while in the appropriate direction is not significant. This could reflect the use of female rats in the present study in contrast to the use of male animals in the earlier study. Equally deserving of consideration is the possibility of pressing the animals to what is nearly their mechanical limit of running so that differences are not easily observed. A reduction in magnitude of deprivation would seem to be a worthwhile avenue of exploration with respect to such relationships.

The data obtained on the day of treatment show that there are two groups of measures: (1) total laps, duration of drinking, and volume of water ingested which rise as a function of low doses of phenobarbital and then fall as phenobarbital dose is increased and (2) the other measures which largely fall as a function of increasing phenobarbital dose. The latter relationship appears to involve no more than the expected reduction of motor activity by phenobarbital, terminating finally in hypnosis. That leaves the explanation of the other group of measures. The most obvious approach is the suggestion of some direct effect upon regulation of ingestion followed by inhibition of the urge to drink as dose further increases [8]. Falk and Burnidge, however, together with a pointed and appropriate criticism of the data base underlying a regulation approach to barbiturate facilitation of drinking,

indicate similarity between such facilitation and that of fixed ratio responding for food in the pigeon [1]. The present data do not afford support to such a notion nor do they refute it. Another approach worthy of consideration is that there are meaningful taste alterations so as to make some solutions more palatable [9].

Volume of water ingested, total laps, and duration of drinking a day after drug treatment all show a substantial increase in water ingested as a function of dose. This is in contrast to observations that low doses of phenobarbital administered a day previously inhibit drinking while higher doses facilitate it [8]. It seems likely that sex differences in the experiment are relevant here since Moir demonstrated longer barbiturate sleep times in female rats than males [6], which is probably indicative of slower rates of metabolism of those drugs in females.

Conceptually, a distinction can be made between amount of behavior and rate of behavior. In this experiment, amount per lap, laps per minute, and running velocity can be regarded as indices of rate of behavior. These measures either decline as a function of phenobarbital dose or show negligible effects as a result of acute drug treatment. These decrements largely, if not entirely, dissipate within 24 hr. In contrast, water ingested, total laps, or duration of drinking seem to measure amount of behavior, in this case thirst related behavior. The effect of low doses of phenobarbital is to increase total responding with a diminution of responding at higher doses as the acute drug effect. Moreover, these effects do not dissipate within less than 24 hr though are diminished in amount. Consequently, it is likely that these data are produced by 2 actions of phenobarbital. One, the sedative-hypnotic, producing decrements in the rate of responding and reducing total responding only at high doses. The other, best described as stimulant, increasing total responding of one kind or another, in this case water ingestion related indices.

The positive correlations between amount per lap and duration of drinking, volume ingested, and total laps is not easily explained at this time. One possibility which needs further examination is that there was a chance configuration of the amount per lap obtained in conjunction with the very high intercorrelations between the other measures produced a number of positive correlations. Reproduction of the data obtained here would be evidence for a definite phenomenon rather than a chance constellation.

Correlation of individuals for the various response measures indicates limited prediction from one response measure to another for a given animal with the exception of total laps and duration of drinking. This latter seems scarcely surprising in that more laps must take more time and variation in laps per minute while present is relatively small. Neglecting the small sample size as a factor in the correlations, apparatus needs consideration. A glass drinking spout may not be sufficiently subject to standardization in aperture to yield altogether reliable results. Furthermore, the distance between rats' noses and drinking spouts could be critical. Too short a distance and the rats will not make and break circuits to be counted. Too great a distance and the task may impose motor difficulties which could be markedly exacerbated by even moderate barbiturate doses. Sadly, rats with a standardized tongue length are not available.

A final consideration is the differences between correlations of individual rats for the various measures and mean responses of the various doses for the various measures. Rats differ markedly in at least 3 significant ways: (a) initial ingestion after $23^1/_2$ hr deprivation; (b) degree of facilitation of drinking by the drug; (c) sedative and hypnotic effectiveness of the drug. These variables are not correlated and may be differentially related to the measures used in this study.

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