J. A. Nevin, K. Olson, C. Mandell, P. Yarensky, *J. Exp. Anal. Behav.*, **24**, 355 (1975).
8. The nonparametric measures of sensitivity (A) and bias (B) are from J. B. Grier, *Psychol. Bull.* **75**, 424 (1971); they are independent of the nature of the underlying hypothetical distributions. The smooth curves through Fig. 1 are isosensitivity contours based on Gaussian distributions of signal and noise. Data suggest that exponential distributions may be more appropriate.
9. K. A. Lattal [*J. Exp. Anal. Behav.* **23**, 241 (1975)] employed a similar technique and reviews the current experimental work on the discrimination of reinforcement contingencies.
10. W. M. Baum, *ibid.* **22**, 231 (1974); for an analysis of human signal detection performance using similar coordinates, see J. A. Nevin, *ibid.* **12**, 475 (1969); for the application of signal detection analysis to visual generalization gradients, see D. S. Blough, *Science* **158**, 940 (1967).
11. H. H. Kelley, *Attribution in Social Interaction* (General Learning Press, New York, 1971); R. de Charms, *Personal Causation* (Academic, New York, 1968); E. J. Phares, *Locus of Control in Personality* (General Learning Press, Morristown, N.J., 1976).
12. D. Hume, *Enquiries Concerning Human Understanding and Concerning the Principles of Morals* (Clarendon, Oxford, 1777/1975); see also A. Michotte, *The Perception of Causality* (Basic, New York, 1963); J. E. R. Staddon, *Behaviorism* **1**, 25 (1973); T. J. Testa, *Psychol. Rev.* **81**, 491 (1974); H. M. Jenkin and C. W. Ward, *Psychol. Monogr.* **79** (No. 594), 1 (1965).
13. R. D. Luce, *Psychol. Rev.* **70**, 61 (1963).
14. The decision is made more difficult by the existence of plausible threshold theories that invoke multiple thresholds [for example, D. H. Krantz, *Psychol. Rev.* **76**, 308 (1969)] and are therefore more flexible in fitting theory to data. Conversely, the conventional assumption of Gaussian distributions of signal and noise is robust, and it is difficult to discriminate between them and other continuous distributions that might have given rise to the data [I. G. Abrahamson, H. Levitt, L. Landgraf, *J. Acoust. Soc. Am.* **42**, 1195 (1967)]. Such decisions will probably be made on the basis of convergent experimental paradigms, including but not limited to yes-no ROC analysis.
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Phenobarbital: Effects of Long-Term Administration on Behavior and Brain of Artificially Reared Rats

Abstract. *Two doses of phenobarbital were given daily for 2 weeks to infant rats fed by intragastric cannulas. The larger dose (60 milligrams per kilogram of body weight) resulted in decreased spontaneous activity and increased responses to novel stimuli. The smaller dose (15 milligrams per kilogram) resulted in increased spontaneous activity and also an increase of responses to novel stimuli. The larger dose produced a 12 percent reduction in brain growth, while the smaller dose was associated with a 3 percent reduction in brain growth.*

The rapid growth the brain undergoes early in its development causes it to be vulnerable to exogenous insults (*1*). Although a number of centrally acting drugs are commonly used for treating human infants, there is little experimental information regarding the effects of such drugs on brain or behavioral development.

Exposure to drugs during infancy in laboratory animals has been shown to result in behavior and brain alterations later in life (*2, 3*). Drug-induced undernutrition, however, often accompanies neonatal drug treatments, and since early undernutrition itself causes behavioral changes (*4*), it is difficult to interpret these studies. The purpose of the study described here was to examine directly the developmental effects of phenobarbital administered chronically during infancy to artificially reared pups.

Male Wistar rats (4 to 5 days old) were selected for body weight within a range of 6 to 11 g. The animals ($N = 46$) were lightly anesthetized with ether, and intragastric cannulas were permanently implanted by means of a technique similar to that refined by Hall (*5*). Once the cannulas were implanted, the pups were placed in circular plastic dessert cups (12 cm in diameter and 8 cm deep), which floated in a warm water bath (40°C). The cannulas were connected to syringes filled with milk formula (*5*) and mounted on an infusion pump (*5*). The room that housed the water bath was on a 12-hour reverse light cycle. Every morning the animals were disconnected from the pumps, the syringes were washed and refilled, and the cannulas were flushed with saline. Each plastic washer securing the cannula at the stomach was checked and loosened whenever necessary to accommodate the animal's growth.

On day 5, the pups were assigned by weight to control and experimental groups, and for the next 13 days the experimental group ($N = 27$) received daily subcutaneous injections of phenobarbital, while the control group ($N = 19$) received subcutaneous injections of the vehicle. In the first of two studies, the experimental group ($N = 15$) received "high" doses (60 mg/kg) of phenobarbital; in the second study, the experimental group ($N = 12$) received "low" doses (15 mg/kg) of phenobarbital. Immediately after the injections, the animals were placed back in their cups and returned to the water bath. All the cannulas were reconnected to the infusion pump at least 1 hour after the last injection and the pump speed was adjusted to infuse approximately 0.5 ml more milk formula than the previous day.

At 18 or 19 days of age, all the animals were tested in a circular open field enclosure (60 cm in diameter) with the floor divided into 10-cm squares. Each animal was gently placed in the center of the field and immediately covered with a plastic cup (12 cm in diameter, 8 cm deep). After 10 seconds, this cup was lifted and the number of locomotions—that is, squares centered (both forepaws placed into an adjacent square), and the time spent rearing (both forepaws off the floor)—was recorded by two trained observers, one of whom was unaware of the animal's group assignment. The first 5 minutes of the test were conducted under dim illumination (one overhead filtered fluorescent light) with a masking white noise present (approximately 45 db). After 5 minutes, intermittent flashing lights (one overhead 75-watt bulb) and noises (two electromagnetic relays clicking on and off quickly, resembling teeth chattering) were present for an additional minute.

After the last animal was tested in the open field, all the animals were decapitated and their brains were quickly removed. The cerebellum was separated and weighed, and the remaining brain sample was divided by a transcollicular cut into cerebrum and brainstem and weighed. At the time of decapitation,

Table 1. Effects of phenobarbital on brain growth. Data are expressed as means ± standard error. Weights are expressed in grams.

Tissue weighed	Low doses of phenobarbital (15 mg/kg)			High doses of phenobarbital (60 mg/kg)		
	Control ($N = 7$)	Experimental ($N = 12$)	Difference from control (%)	Control ($N = 12$)	Experimental ($N = 15$)	Difference from control (%)
Body	32.1 ± 1.7	30.7 ± 2.7	−4	34.2 ± 2.9	33.8 ± 2.4	−1
Brain*	1.34 ± 0.05	1.101 ± 0.07	−3	1.213 ± 0.05	1.077 ± 0.06†	−12
Cerebrum	0.831 ± 0.03	0.820 ± 0.05	−1	0.907 ± 0.04	0.808 ± 0.04†	−11
Cerebellum	0.146 ± 0.01	0.144 ± 0.01	−1	0.160 ± 0.01	0.147 ± 0.01†	−9

*The brainstem is included. †$P < .01$, t-test.

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blood was collected from the experimental animals to determine blood levels of phenobarbital.

Daily subcutaneous injections of high doses of phenobarbital from day 5 to 18 resulted in a highly significant brain-growth retardation but did not affect body growth. When the dose of phenobarbital was reduced to 15 mg/kg, there were no differences in brain growth between experimental and control animals (*6*) (Table 1).

Serum concentrations of phenobarbital at the time of death (that is, approximately 30 hours after the last phenobarbital injection) ranged from 7 μg/ml to 13 μg/ml in the group given high doses of phenobarbital and from 2 μg/ml to 3 μg/ml in the group given low doses.

A reversal of spontaneous activity patterns was noted as the dosage of phenobarbital was increased. Low doses of phenobarbital resulted in a marked increase of activity within the cups as was apparent by the consistently agitated bobbing of the cups in the water bath. High doses resulted in less agitation in the water bath, suggesting sedation.

When tested in the open field, both experimental groups ambulated in a normal manner, with no indications of any motor deficits. During the first 5 minutes of open field testing, the animals given high doses of phenobarbital locomoted less ($P < .001$, t-test) and spent less time rearing ($P < .005$, t-test) than the control animals (Fig. 1), whereas the animals given low doses were no different from control animals. During the sixth minute of open field testing, the sudden stimulation of flashing lights and noise caused the animals given low doses to locomote significantly more ($P < .05$, t-test) than the control animals (Fig. 1).

Control animals responded to novel stimulation by reducing their locomotor activity as if they were becoming cautious ($P < .05$; Fig. 1). Neither group exhibited this behavior suppression during novel stimulation. In addition, both groups slightly increased their orienting behavior in response to sudden stimulation.

Long-term administration of phenobarbital during infancy in a dose that allows survival and results in normal curves for body growth (*7*) produces marked brain-growth retardation which is independent of nutritional intake. In earlier work (*3*) considerable phenobarbital-induced brain-growth deficits were found, especially in the cerebellum. The rat pups used in the earlier studies, however, were reared normally, and the group injected with phenobarbital had a reduced nutrient intake, as their lower body weights indicate. The resulting undernutrition, which has its own brain-growth retardation effects, may have aggravated a phenobarbital effect on brain growth. In the present study, since nutritional intake was the same for all the animals, there were no differences in body weights between control and experimental animals; moreover, compared to the rest of the brain, the growth-retarding effects of phenobarbital were not excessive in the cerebellum, which is especially vulnerable to undernutrition (*8*).

Long-term phenobarbital administration during infancy produced a marked reduction in spontaneous activity of the group given high doses of phenobarbital. Although the spontaneous activity patterns of this group differed from those of the groups given low doses, both groups exhibited increase responses to high levels of stimulation (flashing lights and noises at the end of the open field test). Thus, spontaneous activity patterns are dissociated in these experiments from stimulus-bound responsiveness. Since the central arousal and inhibitory systems of the rat were still developing during the time of drug exposure (*2, 9*), one may speculate that phenobarbital interferes with the development of inhibitory mechanisms that are responsible for modulating arousal responses.

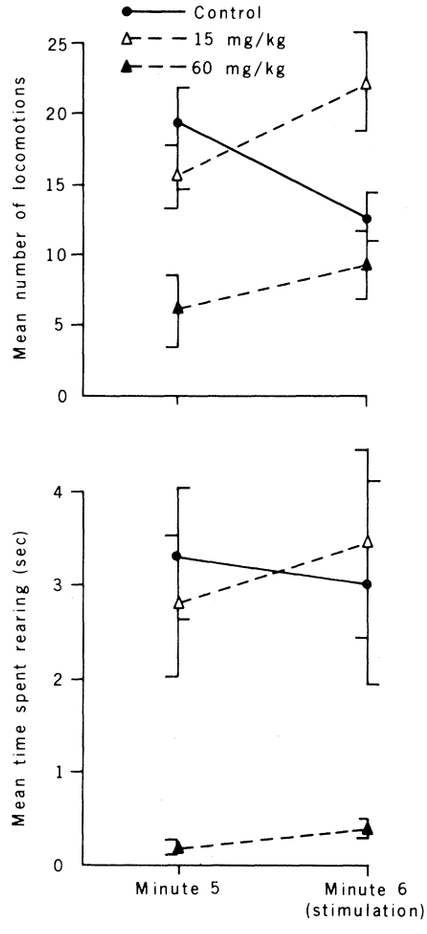

Fig. 1. The behavior of the three groups of rats during the fifth minute (the last minute of habituation) compared to the sixth minute (the 1 minute of stimulation).

A similar paradoxical response to phenobarbital can be seen in human infants. This drug may produce drowsiness or hyperactivity (*10*), depending on dosage and development of tolerance to the drug. When hyperactivity is a problem, some workers recommend an increase in phenobarbital dosage to overcome this effect (*11*). In our studies with rats, we have discovered that when dosage is increased so as to result in reduced activity, the brain-growth retardation in animals given high doses of phenobarbital included a significant reduction of nucleic acid, protein, and cholesterol content of the brain (*12*). Since the maturation of central arousal and inhibitory systems takes place during the time infants are given centrally acting drugs, it becomes a matter of concern to describe the effects of these agents upon brain development and behavior.

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References and Notes

1. J. Dobbing and J. Sands, *Biol. Neonate* **19**, 363 (1971); S. Schapiro and R. Notman, *Science* **155**, 1279 (1967); J. Altman, W. Anderson, K. Wright, *Exp. Neurol.* **17**, 481 (1967); H. Agrawal, M. Fox, W. Himwich, *Life Sci.* **6**, 71 (1967).
2. C. Kornetsky, *Psychopharmacologia* **17**, 105 (1970); R. Young, *Psychol. Bull.* **67**, 73 (1967).
3. R. Schain and K. Watanabe, *Exp. Neurol.* **47**, 509 (1975); *ibid.* **50**, 806 (1976).
4. P. Leathwood, in *Studies of Development of Behavior and the Nervous System*, G. Gottlieb, Ed. (Academic Press, New York, in press).
5. W. Hall, *Science* **190**, 1313 (1975). Hall's method was developed from a method of M. Messer, E. Thoman, A. Terasa, and P. Dallman [*J. Nutr.* **98**, 404 (1969)].
6. None of the animals in either experimental group died because of the phenobarbital injections. The deaths that occurred ($N = 9$) were due to either dislodged intragestive cannulas, causing milk to be infused into the peritoneum, or to unsuccessful attempts to replace the cannulas.
7. Neither of the artificially reared groups differed in their body weight growth curves from their normally reared siblings.
8. J. Dobbing, J. Hopewell, A. Lynch, *Exp. Neurol.* **32**, 439 (1971).
9. B. Campbell, L. Lytle, H. Fibiger, *Science* **166**, 635 (1969); H. Fibiger, L. Lytle, B. Campbell, *J. Comp. Physiol. Psychol.* **72**, 384 (1970); P. Mabry and B. Campbell, *ibid.* **86**, 193 (1974).
10. S. Livingston, *Comprehensive Management of Epilepsy in Infancy and Adolescence* (Thomas, Springfield, Ill., 1972), p. 175.
11. H. Knobloch and B. Pasamanick, Eds., *Gesell and Amatruda's Developmental Diagnosis* (Harper & Row, New York, ed. 3, 1974), p. 280.
12. J. Diaz and R. Schain, *Biol. Neonate*, **32**, 77 (1977).
13. We thank B. G. Bailey, L. Bank, and K. Watanabe for their assistance during this study, and E. van Marthens and J. Garcia for their suggestions on the manuscript. This work was supported by PHS grants HD-05615 and HD-04612 from the NICHHD, NS-07691 from NINCDS, and the Regents of the University of California, Mental Retardation Research Center, Los Angeles.

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