subunits. Similarly, neurons did not bind antibodies to muscle-specific (desmin), epithelium-specific (cytokeratin), or astrocyte-specific (GFA) IF subunits (data not shown). The binding of antibodies to vimentin to the nonneuronal cells in spinal cord cultures suggests that these cells contain a protein similar to vimentin. Additional evidence for this was obtained by analyzing the detergent-insoluble residue of such cultures on sodium dodecyl sulfate gels (Fig. 2E). The major band was one that migrated with chick vimentin.

GUDRUN S. BENNETT
STEPHEN J. TAPSCOTT
FREDRIC A. KLEINBART
PARKER B. ANTIN
HOWARD HOLTZER
*Department of Anatomy, University of Pennsylvania Medical School, Philadelphia 19104*

### References and Notes

1. P. N. Hoffman and R. J. Lasek, *J. Cell Biol.* **66**, 351 (1975).
2. W. W. Schlaepfer and L. A. Freeman, *ibid.* **78**, 653 (1978).
3. R. K. H. Liem, S.-H. Yen, G. D. Salomon, M. L. Shelanski, *ibid.* **79**, 637 (1978).
4. M. S. Runge, H. W. Detrich III, R. C. Williams, Jr., *Biochemistry* **18**, 1689 (1979).
5. L. F. Eng, J. J. Vanderhaegen, A. Bignami, B. Gerstl, *Brain Res.* **28**, 351 (1971).
6. W. W. Schlaepfer and S. Micko, *J. Cell Biol.* **78**, 369 (1978); W. A. Day, *J. Ultrastruct. Res.* **70**, 1 (1980).
7. H. Holtzer, S. Fellini, N. Rubinstein, J. Chi, K. Strahs, in *Cell Motility*, R. Goldman, T. Pollard, J. Rosenbaum, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1976), p. 823; J. V. Small and A. Sobieszek, *J. Cell Sci.* **23**, 243 (1977); S. Brown, W. Levinson, J. A. Spudich, *J. Supramol. Struct.* **5**, 119 (1976); R. O. Hynes and A. T. Destree, *Cell* **13**, 151 (1978); J. Starger and R. D. Goldman, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2422 (1977); A. Eriksson and L.-E. Thornell, *J. Cell Biol.* **80**, 231 (1979); P. Cooke, *idid.* **68**, 539 (1976).
8. E. Lazarides and B. D. Hubbard, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4344 (1976).
9. G. S. Bennett, S. A. Fellini, J. M. Croop, J. J. Otto, J. Bryan, H. Holtzer, *ibid.* **75**, 4364 (1978).
10. S. A. Fellini, G. S. Bennett, Y. Toyama, H. Holtzer, *Differentiation* **12**, 59 (1978); S. A. Fellini, G. S. Bennett, H. Holtzer, *Am J. Anat.* **153**, 451 (1978).
11. W. W. Franke, E. Schmid, M. Osborn, K. Weber, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5034 (1978); *J. Cell Biol.* **81**, 570 (1979).
12. C. Wang, D. J. Asai, E. Lazarides, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1541 (1980).
13. W. W. Schlaepfer, *J. Cell Biol.* **74**, 226 (1977); ——— and R. G. Lynch, *ibid.*, p. 241.
14. D. Dahl and A. Bignami, *J. Comp. Neurol.* **176**, 645 (1977).
15. M. Schachner, C. Smith, G. Schoonmaker, *Dev. Neurosci.* **1**, 1 (1978).
16. S. H. Blose, M. L. Shelanski, S. Chacko, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 662 (1977).
17. S.-H. Yen, D. Dahl, M. Schachner, M. L. Shelanski, *ibid.* **73**, 529 (1976).
18. A. Eriksson, L. E. Thornell, E. Lundgren, T. Stigbrand, *FEBS Lett.* **102**, 177 (1979).
19. H. Holtzer, G. S. Bennett, S. J. Tapscott, J. M. Croop, A. Dlugosz, Y. Toyama, in *International Cell Biology, 1980–1981*, H. G. Schweiger, Ed. (Springer Verlag, Berlin, in press).
20. G. S. Bennett, S. A. Fellini, H. Holtzer, *Differentiation* **12**, 71 (1978).
21. E. Schmid, S. Tapscott, G. S. Bennett, J. Croop, S. A. Fellini, H. Holtzer, W. W. Franke, *ibid.* **15**, 27 (1979).
22. Our procedure was based on those described in (*2*, *4*). Cervical spinal cord, after being freed of meninges, was homogenized either in buffer A (100 m*M* sodium phosphate, 3 m*M* $Na_2$-EDTA, and 2 m*M* dithiothreitol, *p*H 7.2) or in buffer B (10 m*M* tris, 3 m*M* $Na_2$-EDTA, and 2 m*M* dithiothreitol, *p*H 6.8). The supernatant obtained after centrifugation at 25,000*g* for 30 minutes was centrifuged at 270,000*g* for 60 minutes. The resulting pellet was washed twice and suspended in buffer A. This suspension, when examined by electron microscopy after negative staining, contained 10-nm filaments and membranous debris. Peripheral nerve filaments were obtained by osmotic shock of desheathed, minced sciatic nerve in buffer B, followed by centrifugation as above. The pellet was washed and suspended in buffer A.
23. G. S. Bennett, S. J. Tapscott, H. Holtzer, in *Changing Concepts of the Nervous System*, A. R. Morrison and P. L. Strick, Eds. (Academic Press, New York, in press).
24. The report by H. Czosnek, D. Soifer, and H. M. Wisniewski [*J. Cell Biol.* **85**, 726 (1980)], which appeared after submission of this report, provides different evidence that the three NF proteins are not synthesized as larger precursors.
25. Production and characterization of rabbit antiserum to chick vimentin (previously termed the 58-kd protein) is described in (*9*, *10*, *20*). More recent estimates of the size of chick vimentin give a lower value of 53 kd (*19*). We also used a monoclonal mouse antibody to chick vimentin (S. J. Tapscott, P. Antin, G. S. Bennett, R. H. Kennett, H. Holtzer, in preparation).
26. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
27. J. Renart, J. Reiser, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3116 (1979).
28. W. S. Adair, D. Jurivich, U. W. Goodenough, *J. Cell Biol.* **79**, 281 (1979).
29. Supported by NIH grants HL18708, HL15835, HD07152, and CA 18194 and by the Muscular Dystrophy Association. S. J. T. is the recipient of a Medical Science Insurance Scholarship from the Nationwide Insurance Co.

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## Drug Discrimination Learning in Lead-Exposed Rats

Abstract. *Lead acetate (0.02 or 0.5 percent) was administered to dams throughout the lactation period with half of the litters continuing on lead after weaning. Drug thresholds for* d*-amphetamine were determined by using the drug-discrimination learning paradigm. All the offspring that had been exposed to lead were less sensitive to the stimulus properties of* d*-amphetamine irrespective of whether or not they had continued on lead after weaning.*

Studies of the neurobehavioral effects of long-term exposure to lead show consistently that lead-exposed rat pups exhibit altered drug responsivity. Responsivity to both agonists and antagonists of putative neurotransmitters has been examined, but most studies have been concerned with the effects of *d*-amphetamine sulfate. The altered response to an injection of *d*-amphetamine may be expressed as an attenuated increase (*1*) or paradoxical decrease (*2*) in locomotor activity relative to uninjected controls. Lead-exposed offspring have also shown altered drug responsiveness on active avoidance tasks, spontaneous alternation, and drug-elicited aggression (*3*). However, the direction of the drug response relative to controls has varied and appears to depend on such factors as the nature of the task, the dose, and the duration of exposure. Conflicting theories of a hyposensitive or hypersensitive catecholaminergic system have therefore been postulated [see (*4*)].

To address this question we used a drug-stimulus discrimination paradigm that permits assessment of drug-response thresholds (*5*). The task presented to the animal is analogous to a simultaneous discrimination problem with the only cue for directing response selection being the endogenous drug state, that is, whether the animal has received a drug or "no-drug" (vehicle) injection. We hypothesized that if a lead-exposed animal had a decreased sensitivity to amphetamine then it would have an elevated drug-response threshold relative to the controls. In contrast, increased drug sensitivity would shift the drug discriminability threshold to below that of the controls.

Twelve female Long-Evans hooded rats (Charles River; four per group) were mated at 90 days of age. Within 12 hours after parturition and for the duration of lactation they were exposed to 0, 0.02, or 0.5 percent lead acetate in their drinking water. Litters were culled to ten pups at birth and eight pups at day 3. At weaning (day 21), two males were randomly selected from each of the litters from lead-exposed dams, one male pup being exposed to the lead treatment of its dam, the other to distilled water. In addition, one male pup was randomly selected from each of the four control litters and exposed to distilled water. Remaining pups in the litters were assigned to other studies. All offspring were individually housed and maintained on Purina Lab Chow 5001. We thus had five treatment groups (four rats per group) for subsequent experimentation—namely, group 0/0, the controls; groups 0.02/0.02, offspring exposed to 0.02 percent lead acetate during lactation via the dam and after weaning; group 0.02/0, offspring exposed to lead during lactation only; and group 0.5/0.5 and group 0.5/0, offspring exposed to 0.5 percent lead acetate during the same periods as their 0.02 counterparts.

The litters exposed to 0.5 percent lead acetate had significantly depressed weights at weaning (42.41 ± 4.6, mean ± standard deviation) compared to the controls (48.6 ± 3.9) and litters exposed to 0.02 percent lead acetate (49 ± 4.4).


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Table 1. Concentrations of lead in the blood of rats exposed to lead acetate during lactation or in their drinking water, or both. The data are expressed as micrograms of lead per 100 milliliters of blood, and show group means ± standard deviations.

| Treatment | Day 10* | Day 21* | Day 30 | Day 90 |
|---|---|---|---|---|
| 0/0 | < 4 | 5.7 ± 3.5 | < 4 | 5.5 ± 1.0 |
| 0.02/0.02 | 16.5 ± 5.3 | 25.6 ± 5.0 | 27.4 ± 98.0 | 29.3 ± 11.2 |
| 0.02/0 | 16.5 ± 5.3† | 25.6 ± 5.0† | 5.9 ± 2.2 | 4.3 ± 1.4 |
| 0.5/0.5 | 50.0 ± 8.8 | 180.2 ± 25.5 | 223.5 ± 55.0 | 117.4 ± 25.0 |
| 0.5/0 | 50.0 ± 8.8† | 180.2 ± 25.5† | 28.5 ± 5.5 | 7.8 ± 6.7 |

*Calculated with the litter being used as a unit of measurement. †Values are the same for the different groups because it was not until weaning that the treatment of the groups changed.

This difference persisted for the duration of the experiment in the offspring continued on 0.5 percent lead acetate, whereas the littermates placed on distilled water were no different from the controls by 30 days of age. Lead concentrations in the blood were determined periodically and these values are reported in Table 1 (*6*).

At 90 days of age the animals were gradually reduced to 80 percent of their weight. They were then trained to bar press in a two-bar Colbourne operant chamber. Reinforcement was available on one level after a drug injection was given and on the "no-drug" bar after a saline injection was given. Reinforcement was 0.1 ml of 33 percent sweetened condensed milk solution. The conditions of drug or no-drug were alternated over days during the initial shaping procedures in moving the animals from a continuous reinforcement schedule to a schedule on which the animals were required to press the correct bar ten times to obtain one reinforcement [fixed ratio 10 (FR 10)] (*7*). During training *d*-amphetamine sulfate (1 mg/kg) or saline (controls) was injected subcutaneously 30 minutes before the start of each 30-minute test session. An animal was judged to have discriminated accurately between saline and amphetamine injections if it pressed the incorrect bar five or fewer times before pressing the correct bar ten times during the first 15 bar presses of a session. If this criterion was met on eight out of ten consecutive sessions, then the animal's drug dose was lowered by approximately 30 percent. This criterion and procedure are similar to that recently described by Overton (*8*). If criterion was not attained on eight out of ten consecutive sessions, the dosage was raised by 30 percent for the next ten sessions. This decreasing and increasing of the dose was done over successive ten-session blocks with testing being terminated when the animals failed to meet criterion when tested twice (in two ten-session blocks) at a particular dose.

The data were analyzed in terms of the lowest dosage preceding each animal's initial failure to meet the criterion on eight out of ten consecutive sessions as well as the dosage where the final threshold level was set. To contrast the control (0/0) group's performance with that of the groups that received lead during lactation only (0.02/0 and 0.05/0) and the groups that were exposed to lead continuously (0.02/0.02 and 0.5/0.5), we used a repeated measures analysis of variance (ANOVA) with one between-factor (treatment) test and one within-factor (original versus final threshold) test. We found that the controls had significantly lower drug thresholds (0.14 ± 0.07, mean ± standard deviation) than group 0.02/0 (0.29 ± 0.04), group 0.5/0 (0.26 ± 0.05), and the two groups continued on lead after weaning: group 0.02/0.02 (0.23 ± 0.04) and group 0.5/0.5 (0.3 ± 0.04) (*9*). There were no significant differences between original and final thresholds or any significant interactions.

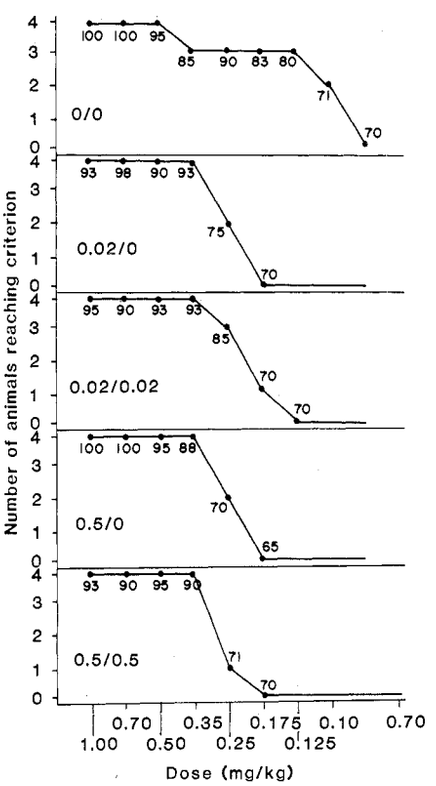


Fig. 1. The number of animals in each treatment group reaching criterion during training. Numbers on the lines represent the mean percentages of trials on which criterion was met at each dose.

The dose response curves in Fig. 1 reflect the number of animals in each group meeting criterion in eight out of ten consecutive sessions at each dose level. Figure 1 also shows the performance of each group at each dose as the mean percentage of trials within a ten-block session at which criterion was met. The large number of control animals at the lower doses indicates their superior discriminative ability.

We also examined the tendency of lead-exposed animals to emit extraneous bar presses. Such random responding could produce a background level of errors that would summate with "true" errors of discrimination at low dose levels, yielding an artificially elevated drug threshold. We therefore determined (i) the number of correct and incorrect responses made before the first reinforcement was given as well as afterward for the entire session; (ii) the number of reinforcements received per session; (iii) the latency (in seconds) to the first reinforcement per session; and (iv) the number of responses above FR 10 prior to the animal's disengaging the bar to obtain each reinforcement. Statistical analyses run on these measures revealed no significant treatment effects. Although all animals tended to show increased errors as the drug dose was lowered, this tendency was not treatment-related. Thus the lead-exposed animals did not exhibit a higher background error rate. Moreover, the absence of differences in measures of latency and total reinforcements suggest that the lead-exposed offspring were as well motivated as controls in the performance of this task. This last finding is in agreement with earlier work (*10*) that indicated no performance differences under an FR 10 schedule between lead-exposed animals and control offspring.

These data substantiate the hypothesis of an amphetamine-hyposensitivity in the lead-exposed animal and suggest that the development of such hyposensitivity may depend primarily on the animal receiving neonatal exposure to lead since the deficit was seen in all lead-exposed animals whether or not they were continued on lead after weaning.

Several lines of evidence suggest that the altered sensitivity may in part reflect an altered dopaminergic system. This

contention is supported indirectly by data from drug-discrimination studies in which attempts were made to delineate the neurochemical substrate of the amphetamine cue. Schechter and Cook (*11*) examined a number of specific biogenic amine depletors (for example, α-methyl-*p*-tyrosine and disulfiram) and selective receptor antagonists (for example, haloperidol, phenoxybenzamine) for their effects on the discriminative properties of amphetamine. Their data suggested that dopaminergic systems mediate the interoceptive cue produced by amphetamine. Others have reported similar findings (*12*).

Lead has been reported to affect many of the putative neurotransmitter systems. According to Shih and Hanin (*4*), studies in the rat have revealed either no change or decreases in dopamine levels. However, more recent reports show consistently that lead induces alterations in the dopaminergic system, and studies by Govani and colleagues (*13*) show that lead modifies dopamine synthesis in various directions according to the region examined. Moreover, no modification of the dopamine receptors (measured as either dopamine-sensitive adenylate cyclase or as [$^3$H]spiroperidol binding) was observed.

Theories abound regarding the neurochemical substrate involved in lead toxicity. The results of the present study suggest that with the appropriate manipulation of antagonists, depletors, and agonists, the drug-discrimination paradigm can provide insight into the nature of the increased drug thresholds observed in the lead-exposed animals in this study.

HAROLD ZENICK
MARSHALL GOLDSMITH
*Department of Environmental Health, Kettering Laboratory, University of Cincinnati, Cincinnati, Ohio 45267*

### References and Notes

1. L. W. Reiter, G. E. Anderson, J. W. Laskey, D. F. Cahill, *Environ. Health Perspect.* **12**, 119 (1975); L. S. Rafales, R. L. Bornschein, I. A. Michaelson, R. K. Loch, G. F. Barker, *Pharmacol. Biochem. Behav.* **10**, 95 (1979).
2. E. K. Silbergeld and A. M. Goldberg, *Exp. Neurol.* **42**, 146 (1974).
3. T. J. Sobotka and M. P. Cook, *Am. J. Ment. Defic.* **79**, 5 (1974); J. Kostas, D. J. McFarland, W. G. Drew, *Pharmacology* **16**, 226 (1978); W. G. Drew, J. Kostas, D. J. McFarland, S. E. DeRossett, *ibid.* **18**, 257 (1979).
4. T. M. Shih and I. Hanin, *Life Sci.* **23**, 877 (1978).
5. B. T. Ho, D. W. Richards, D. L. Chute, *Drug Discrimination and State Dependent Learning* (Academic Press, New York, 1978).
6. Samples were collected from tail veins and analyzed by Environmental Science Associates, Bedford, Mass. Lead values of blood collected in this manner agreed highly with those of blood obtained by cardiac puncture.
7. This introduction of drug condition during shaping appears to speed up the acquisition of the discrimination as contrasted with more traditional approach of familiarizing the animal with both levers in the no-drug state prior to introduction of the drug condition.
8. D. A. Overton, *Science* **205**, 720 (1979).
9. The $F$ values for two ANOVA tests were $F(2, 9) = 8.16$, $P \leq .01$, and $F(2, 9) = 8.84$, $P \leq .01$, respectively.
10. R. Padich and H. Zenick, *Pharmacol. Biochem. Behav.* **6**, 371 (1977).
11. M. D. Schechter and P. G. Cook, *Psychopharmacologia* **42**, 285 (1975).
12. B. T. Ho and J. T. Huang, *Pharmacol. Biochem. Behav.* **3**, 1085 (1975); M. D. Schechter, *Eur. J. Pharmacol.* **44**, 51 (1977); D. M. Kuhn, J. B. Appel, I. Greenberg, *Psychopharmacologia* **39**, 57 (1974).
13. S. Govani, O. Montefusco, P. F. Spano, M. Trabucchi, *Toxicol. Lett.* **2**, 333 (1978); S. Govani, M. Memo, P. F. Spano, M. Trabucchi, *Toxicology* **12**, 343 (1979).

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## Eusociality in a Mammal: Cooperative Breeding in Naked Mole-Rat Colonies

Abstract. *Laboratory observations on a field-collected colony of 40* Heterocephalus *have shown that only a single female breeds. The remaining individuals constitute two or three castes, each containing both sexes and distinguishable by size differences and the tasks they perform. These features, together with long life-spans, overlap of generations, cooperative brood care, and possible age polyethism provide parallels with the eusocial insects.*

Naked mole rats (*Heterocephalus glaber*: Rodentia, Bathyergidae) are found in the hot, arid regions of Kenya, Somalia, and Ethiopia. They live entirely underground in a system of extensive foraging tunnels running at root or tuber level and a deeper nest area (*1*). They feed largely on bulbs and tubers. Unlike other completely subterranean mammals, they live in large colonies (*2*).

Mixed colonies (*3*) of mole rats have been under laboratory observation for 6 years. In October 1977 an almost complete colony (*4*) of 40 individuals was collected at Mtito Andei, Kenya. There were 16 females and 24 males, of whom 4 males and 1 female were clearly juveniles and probably represented the most recent litter born in the colony. The breeding female was not caught (*5*). The mole rats were marked individually, established in an artificial burrow system (*6*), and given a year to adjust to captivity and for a new breeding female to establish herself. Then, after 100 hours of observation, the mole rats were rated according to their reproductive roles and the frequency with which they performed various tasks in the colony. In eusocial insects, a caste is any set of individuals of a particular morphological type or age group (or both) that performs specialized labor in the colony (*7*). By these criteria, *Heterocephalus* colonies appear to have a system of castes (Fig. 1).

"Frequent workers" are mole rats that frequently perform tasks associated with nest building and foraging. Included in the broad category of foraging are digging and transporting soil and the carrying of small items of food to the communal nest. During the latter activity, the frequent workers make repeated trips with food without pausing to eat. "Infrequent workers" show role overlap with the frequent workers, but perform tasks in the colony at less than half the rate of the frequent workers (25 percent of 616 observations). The mean weight of this group is significantly greater than that of the frequent workers. Further research is needed to determine whether this is a separate permanent caste. "Nonworkers" are usually the largest mole rats in the colony. They very rarely dig or transport materials. Their role is not easy to define, but appears to be partly reproductive in that males of this caste are the most likely to mate with the breeding female. Sleeping nonworkers are often joined by other individuals and their huddling significantly decreases the energy requirements of the colony (*8*). Nonworkers assist in the care of the young.

The female mole rats in all these castes are nonbreeding, and probably most will never breed. Histology of the ovaries of nonbreeding females shows them to have many primordial and primary follicles, but few secondary and tertiary follicles (*9*). The females are probably not sterile, but the ovaries appear to be quiescent. Spermatogenesis was evident in males from all the castes, suggesting that all are potentially able to inseminate the breeding female. Small males, however, have difficulty in copulating with the larger breeding female.

The breeding female resembles the nonworkers in size and does not perform tasks in the colony. She is the only female in the colony to breed and, in the laboratory, produces one to four litters of up to 12 young a year. Prominent teats and a perforate vagina are characteristic of the breeding female. Histology of the


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