sprouts with an electron microscope. Figure 3A shows a labeled retinogeniculate terminal from a previously denervated lamina *A*. Although the cytoplasm of this labeled profile is filled with the dark reaction product, the marker has not crossed the axonal plasmalemma, nor has it crossed the membranes of the synaptic vesicles or mitochondria. Thus, it is possible to see that the synaptic vesicles are round and that the mitochondria have a pale appearance due to their widely spaced cristae and pale intercristal substance. These features are characteristics of normal retinogeniculate axon terminals (*15, 16, 19*).

The labeled profile also forms synaptic contacts like those described previously for normal retinogeniculate axons (Fig. 3A). It is surrounded by an arrangement of profiles in a configuration resembling a synaptic glomerulus in a normal nucleus. Some of these profiles contain pleomorphic synaptic vesicles. In this micrograph the labeled terminal is presynaptic to one of these profiles (arrow) but is not postsynaptic to any of them. Thus, even though this terminal is from an abnormally grown axon branch, it has formed patterns of synaptic contacts that appear to be quite normal (*15, 16, 19*).

Figure 3B shows another example from denervated layer A in the same animal. Profiles labeled with HRP are involved in a more complex synaptic zone. These profiles contact dendrites, terminals with pleomorphic vesicles and spinelike processes. In addition, a single dendritic profile is contacted by two labeled profiles, both from the same axon. Although this last feature differs somewhat from the usual concept of the lateral geniculate glomerulus, we have observed similar clusters of retinogeniculate terminals in normal adult cats [(*19*); see also (*16*)].

We have demonstrated that a single axon can innervate its normal field and also an abnormally invaded field. The pattern of axonal branching indicates that the axon goes to the correct site first and then sends terminal branches to the new site. The classical picture that sprouts are induced to form from intranodal axonal segments (*21*) does not appear to apply to this interlaminar sprouting in the lateral geniculate nucleus. Instead, it would appear that terminal growth has continued beyond the stage at which one eye was removed and that some of the newly grown terminals invaded the denervated sites.

The phenomenon is probably growth that is abnormal in direction but not necessarily abnormal in amount. That is, the growth of the axons would have occurred even without the eye removal, but the denervation of some geniculate layers has allowed the axons to grow into inappropriate layers (*2, 4, 7, 21*). This interpretation assumes that, at the time of enucleation, the retinogeniculate axons are segregated into their normal layers. Axonal degeneration and autoradiographic studies (*22*) at early postnatal ages show that this is the case. Furthermore, in Golgi material from kittens as young as 8 days, O'Leary (*13*) never saw an axon that sent branches to both laminae *A* and A_1.

In a normal cat, one retinogeniculate axon does not invade two adjacent geniculate laminae (*13, 14*). The crossed and the uncrossed axons have distinct and well-specified terminal fields (*12, 22*). On this basis one might have anticipated that an axon with terminals in lamina *A* would not also develop terminals in lamina A_1. Our results show that the normal selectivity has been destroyed by the early eye removal. That is, either the specific character of the denervated layer is lost after the eye removal, so that inappropriate axons can invade it, or the formation of these specific connections depends entirely upon the properties of the retinogeniculate axons. Further studies with our method of the early development of retinogeniculate axons in normal and monocular kittens may show how the retinogeniculate axons define their terminal fields.

JOHN A. ROBSON
CAROL A. MASON
R. W. GUILLERY
Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, Illinois 60637

References and Notes

1. For reviews, see F. W. L. Kerr (*2*) and G. S. Lynch, R. L. Smith, and C. W. Cotman [in *Neurophysiologic Aspects of Rehabilitation Medicine*, A. A. Buergher and J. S. Tolnar, Eds. (Thomas, Springfield, Ill., 1976), p. 280.]
2. F. W. L. Kerr, *Exp. Neurol.* **48**, 16 (1975).
3. C. N. Liu and W. W. Chambers, *Arch. Neurol. Psychiatry* **79**, 46 (1958); D. C. Goodman and J. A. Horel, *J. Comp. Neurol.* **127**, 71 (1966); G. S. Lynch, S. Mosko, C. W. Cotman, *Brain Res.* **50**, 174 (1973); G. E. Schneider, *Brain Behav. Evol.* **8**, 73 (1973).
4. R. W. Guillery, *J. Comp. Neurol.* **146**, 407 (1972).
5. R. E. Kalil, *Anat. Rec.* **175**, 353 (1973).
6. M. Murray and M. E. Goldberger, *J. Comp. Neurol.* **158**, 19 (1974).
7. T. L. Hickey, *ibid.* **161**, 359 (1975).
8. R. Y. Moore, A. Bjorklund, V. Stenevi, *Brain Res.* **33**, 13 (1971); G. S. Lynch *et al.*, *ibid.* **42**, 311 (1972).
9. G. Raisman, *Philos. Trans. R. Soc. London Ser. B* **278**, 349 (1977); *Brain Res.* **14**, 25 (1969); ——— and P. M. Field, *ibid.* **50**, 241 (1973); R. D. Lund and J. S. Lund, *Science* **171**, 804 (1971).
10. L. Guth and J. J. Bernstein, *Exp. Neurol.* **4**, 59 (1961); P. D. Wall and D. Eggers, *Nature (London)* **232**, 542 (1971).
11. J. C. Adams and W. B. Warr, *J. Comp. Neurol.* **170**, 107 (1976); H. Vanegas, H. Holländer, H. Distel, *ibid.* **177**, 193 (1978); D. A. Keefer, W. B. Spatz, V. Misgeld, *Neurosci. Lett.* **3**, 233 (1976); E. Proshansky and M. D. Egger, *ibid.* **5**, 103 (1977).
12. W. R. Hayhow, *J. Comp. Neurol.* **110**, 1 (1958); A. M. Laties and J. M. Sprague, *ibid.* **127**, 35 (1966); R. W. Guillery, *ibid.* **138**, 339 (1970); T. L. Hickey and R. W. Guillery, *ibid.* **156**, 239 (1974).
13. J. L. O'Leary, *J. Comp. Neurol.* **73**, 405 (1940).
14. C. A. Mason and J. A. Robson, *Soc. Neurosci. Abstr.* **3**, 569 (1977); in preparation.
15. J. Szentágothai, J. Hámori, T. Tömböl, *Exp. Brain Res.* **2**, 283 (1966); R. W. Guillery, *Z. Zellforsch. Mikrosk. Anat.* **96**, 1 (1969).
16. E. V. Famiglietti and A. Peters, *J. Comp. Neurol.* **144**, 285 (1972).
17. J. C. Adams, *Neuroscience* **2**, 141 (1977).
18. C. L. F. Woodcock and P. R. Bell, *J. R. Microsc. Soc.* **87**, 485 (1967).
19. J. A. Robson and C. A. Mason, *Soc. Neurosci. Abstr.* **3**, 574 (1977); in preparation.
20. J. Szentágothai, *Acta Anat.* **55**, 166 (1963); R. W. Guillery, *J. Comp. Neurol.* **128**, 21 (1966).
21. D. Barker and M. C. Ip, *Proc. R. Soc. London Ser. B* **163**, 538 (1966).
22. W. Richards and R. Kalil, *Brain Res.* **72**, 288 (1974); R. Kalil, unpublished observations.
23. We thank P. Spear and B. Kofron for their help with the HRP-injection procedure. We also thank A. Lysakowski for helping to prepare the electron microscopic material and C. Klisiak for typing the manuscript. Supported by NIH postdoctoral fellowship NS-05407 to J.A.R. and NIH research grants NS-11869, NS-14271, NS-14283, and EY-02374.

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Lead Exposure During Infancy Permanently Increases Lithium-Induced Polydipsia

Abstract. *Lead (200 milligrams per kilogram) was administered daily by intubation to Long-Evans rats on days 3 through 30 of life. Thirty to 180 days after cessation of lead administration, the lead-treated rats were consistently more polydipsic after lithium administration (2 millimoles per kilogram per day) than were pair-treated controls. Lithium increased the plasma renin activity equally in both the lead-treated and the control groups. These data are evidence that there may be permanent neural changes induced by postnatal exposure to lead that are manifested by pharmacological challenge with lithium.*

Acute lead intoxication (plumbism) is a clinical entity with defined physiological changes and concomitant diagnostic signs (*1*) which are usually ameliorated by cessation of exposure to lead and chelation therapy (*2*). However, it has been extremely difficult in both humans and laboratory animals to define the effects

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of lead at exposures below those resulting in overt toxicity. Presumably, this is a major public health problem because certain infants, who may be more or less sensitive to lead exposure, have significantly increased concentrations of lead in their blood (*3*). Because exposure to lead during infancy and childhood occurs concomitantly with many developmental changes, the possibility of lead causing permanent alterations is one of paramount importance.

Several reports have suggested that accumulations of lead in the body during developmental periods may cause permanent changes in the central nervous system. David and co-workers (*4*) and others (*5*) reported that incidences of various cognitive or behavioral disabilities in humans are correlated with increased exposure to lead. Rats treated with lead during neonatal periods exhibit increased motor activity (*6–8*). In addition, Overman (*8*) demonstrated several subtle behavioral deficits in rats that had been exposed to lead. However, not all investigators have been able to demonstrate similar behavioral deficits in animals treated with lead. Such discrepancies, which could depend on factors such as lead dose, route, and time of administration as well as individual variability, illustrate the inherent difficulties that have been encountered with animal models of developmental lead exposure.

Several coincidental factors suggested that lithium-induced polydipsia (LIP) might be altered in lead-treated rats. The condition was first noted in clinical trials, and recent studies in our laboratory have shown that central catecholamine neurons are important to LIP (*9*). The renin-angiotensin system has been directly linked to LIP (*10*), and other studies on drinking mechanisms have suggested that angiotensin-induced polydipsia has similar physiological and pharmacological characteristics to LIP (*11*). We hypothesized that since exposure of animals to lead has been correlated with physiological changes that may involve central catecholamine systems (*6, 7*) as well as renin-angiotensin systems (*12*), lead may cause alterations that will be manifest after administration of lithium. We chose a model of lead exposure during development that has temporal features comparable to lead exposure in children and that produces little effect on somatic growth, survival, or viability.

Long-Evans rats were treated once daily with lead (200 mg/kg; administered in the form of lead acetate, 0.01 ml of solution per gram of body weight). Control rats received equimolar sodium acetate. The solutions were administered by gastric intubation from day 3 to day 30 of life, day 1 being the day of birth. Each of the matched lead-treated and control litters was handled by a single individual. Litters were randomly culled to ten at birth and eight at day 3, with filler pups used to maintain the litter size until weaning. Most litters used in these experiments were adjusted to six to eight males at day 3. A tail blood sample obtained precisely 24 hours after cessation of treatment indicated that lead concentrations were 123 ± 17 μg/dl (mean ± standard error) for lead-treated animals as opposed to 7.7 ± 0.7 μg/dl for controls. The lead treatment caused no increase in mortality, differences in body weight, or change in food consumption. Treated and control rats were individually housed at the initiation of LIP experimentation and daily water consumption was measured by weighing the water bottles. After 4 days of obtaining baseline measurements, we began to give the rats daily intraperitoneal injections of lithium (2 mmoles per kilogram, as 0.4*M* LiCl); water consumption was monitored daily for an additional 10 days. In certain experiments, rats were decapitated and trunk blood was collected into 0.2 ml of 0.4*M* sodium ethylenediaminetraacetate buffer, *p*H 7.4. The blood was immediately centrifuged at 9000*g* for 45 minutes at 4°C, and the plasma was removed and stored at −20°C. Plasma renin activity was determined by means of commercially available reagents for the angiotensin I radioimmunoassay (*13*), with a plasma renin activity standard (New England Nuclear) being used as a control. Lead content in the femur was determined by flameless atomic absorption spectroscopy after the bone was digested in concentrated nitric acid and the solution cleared with hydrogen peroxide, whereas the amount of lead in the blood was determined by the Delves cup method (*14*). The amounts of lithium in the plasma were determined by atomic absorption spectroscopy of 1:20 dilutions of plasma with deionized water (*15*).

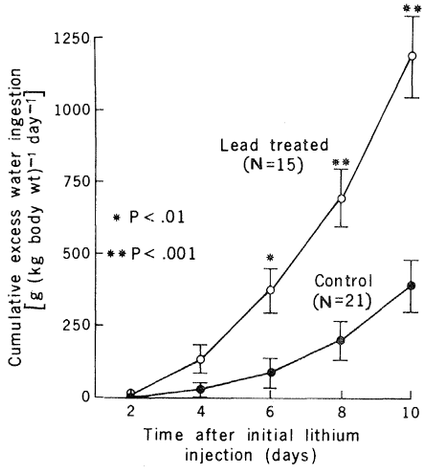

Fig. 1. Excess water consumption in rats injected with lithium chloride. The excess water consumption is calculated by subtracting the average baseline consumption (per kilogram of body weight) from the daily consumption after initiation of lithium treatment. These differences are summed through the test period.

With the paired litters of male control and lead-treated rats, we observed no differences in the baseline (before lithium administration) water consumption between the two groups, consistent with other studies with this model of lead administration (*8*). However, the administration of lithium caused the lead-treated rats to become significantly more polydipsic than the control rats (Fig. 1). During the 9 days after lithium administration, lead-treated rats increased their consumption of water nearly three times more than did controls (*16*). Female rats treated with lead during early postnatal development also exhibited increased LIP ($P < .05$). We also tested several litters of rats 6 months after the cessation of lead treatment. The lead-treated rats were still significantly more polydipsic after lithium administration than were the controls ($P < .005$).

Since the amounts of lead in the brain and blood were indistinguishable between the control and lead-treated groups (*17*) at times when significant differences were observed in LIP, the change could not be attributed to the presence of the presumed causative agent, lead. Further, the lead-induced increases in LIP persisted through at least 6 months of age indicating that the alterations responsible for increased LIP are probably permanent. Plasma renin activity (PRA) in lead-treated and control animals was measured before or after lithium administration and no significant differences were found. Lithium administration caused the PRA to increase after 8 days to 11.2 ± 1.7 ng of angiotensin I per milliliter per hour in 19 lead-treated animals as opposed to 14.0 ± 3.8 in 16 matched controls. Further, there was no correlation ($r < .1$) between the PRA of individual animals and the relative increase in drinking after lithium administration (*18*). This evidence indicates that circulating renin-angiotensin levels are not responsible for the increased lithium-induced polydipsia caused by the administration of lead during development.

These experiments demonstrate that, in rats, lead administration during neonatal periods causes permanent physio-

logical changes that are latent until an agent such as lithium is administered (*17*). The failure to observe a change in renin activity in peripheral plasma suggests that a central mechanism may be responsible for the lead-induced increase in LIP. Thus, the observed lead-induced alteration may provide an excellent model for further study of developmental changes caused by lead, because LIP occurs consistently and is easily quantified. Further studies are needed to elucidate the mechanisms responsible for increased LIP in rats treated neonatally with lead, as well as to determine if these mechanisms are associated with pathological conditions linked to lead exposure, such as hypertension (*12*).

RICHARD B. MAILMAN
Department of Psychiatry and
Biological Sciences Research Center,
University of North Carolina School of Medicine, Chapel Hill 27514
MARTIN R. KRIGMAN
Department of Pathology and
Biological Sciences Research Center,
University of North Carolina
ROBERT A. MUELLER
Departments of Anesthesiology and
Pharmacology and *Biological Sciences Research Center,*
University of North Carolina
PAUL MUSHAK
Department of Pathology and
Biological Sciences Research Center,
University of North Carolina
GEORGE R. BREESE
Departments of Psychiatry and
Pharmacology and *Biological Sciences Research Center,*
University of North Carolina

References and Notes

1. R. K. Byers and E. Lord, *Am. J. Dis. Child.* **66**, 471 (1943); R. K. Byers, *Pediatrics* **23**, 585 (1959).
2. R. G. McAllister, A. M. Michelakis, H. Sansteud, *Arch. Intern. Med.* **127**, 919 (1971).
3. M. W. Oberle, *Science* **165**, 991 (1969); J. J. Chisholm, *Clin. Chem.* **23**, 252 (1977).
4. O. David, S. Hoffman, B. McGann, J. Svend, J. Clark, *Lancet* **1976-II**, 1376 (1976).
5. R. Baloh, R. Sturm, B. Green, G. Gleser, *Arch. Neurol.* **32**, 326 (1975); B. de la Burde and M. Choate, *J. Pediatr.* **81**, 1088 (1972).
6. E. Silbergeld and A. Goldberg, *Life Sci.* **13**, 1275 (1973); M. W. Sauerhoff and I. A. Michaelson, *Science* **182**, 1022 (1973).
7. M. Golter and I. A. Michaelson, *Science* **187**, 359 (1975).
8. S. R. Overman, *Toxicol. Appl. Pharmacol.* **41**, 459 (1977).
9. M. Schou, *Pharmacol. Rev.* **9**, 17 (1957); R. B. Mailman, T. S. Barlow, G. R. Breese, *Neurosci. Abstr.* **2**, 495 (1976).
10. Y. Gutman, F. Benzaken, P. Livneh, *Eur. J. Pharmacol.* **16**, 380 (1971); T. A. Perumal and J. P. Rao, *Br. J. Pharmacol.* **51**, 107 (1974).
11. J. T. Fitzsimons and P. E. Setler, *J. Physiol. (London)* **250**, 613 (1975).
12. D. G. Beevers *et al.*, *Lancet* **1976-II**, 1 (1976).
13. E. Haber, T. Koemer, L. B. Page, B. Kliman, A. Pernode, *J. Clin. Endocrinol. Metab.* **29**, 1349 (1969).
14. H. T. Delves, *Analyst* **95**, 431 (1970); R. D. Ediger and R. L. Coleman, *At. Absorpt. Newsl.* **11**, 33 (1972).
15. "Analytical methods for atomic absorption spectrophotometry" (Perkin-Elmer Corporation, Norwalk, Conn., 1976).
16. To date (15 months of experimentation), these experiments have encompassed 20 pairs of lead-treated and control litters totaling 97 lead-treated rats and 95 controls given lithium at least once. In every experiment the lead-treated rats exhibited significantly greater LIP than did controls for days 7 through 9 after lithium administration.
17. R. B. Mailman, M. Krigman, R. A. Mueller, P. Mushak, G. R. Breese, in preparation. In these animals the amounts of lead in the brain and blood decreased rapidly after cessation of lead treatment. Brain levels decreased from a maximum of 0.60 to 0.80 μg of lead per gram of tissue to levels indistinguishable from controls (<0.10 μg/g) by 60 days after treatment, whereas the amount of lead in the blood decreased from greater than 100 μg/100 ml 24 hours after treatment to control levels (<10 μg/100 ml). This animal model has been examined for possible neurochemical or behavioral alterations induced by lead. For example, we found no significant changes in concentrations or turnover of catecholamines or serotonin, in basal food or water consumption, somatic growth, or in basal locomotor activity ("doughnut" cage). A decreased locomotor response after injections of L-dihydroxyphenylalanine (L-dopa), but not after injections of amphetamine or scopolamine, has been observed, but unlike the LIP, does not occur consistently from litter to litter.
18. The relative increase in water consumption of each rat (lead-treated and control) was plotted against the PRA when it was killed. No correlation was found for treated or control rats, alone or separately. This suggests that LIP is not mediated directly by circulating PRA. Measurements of circulating angiotensin II were made by direct radioimmunoassay of both fresh and stored plasma. Though no significant differences were again observed between lead-treated and control rats (both demonstrating similar increases in immunoreactivity after lithium administration) the technique of direct assay without removal of competing materials or separation from degrading enzymes probably necessitates reinvestigation [K. J. Kosunen, *Scand. J. Clin. Lab. Invest.* **36**, 467 (1976)] of the role of angiotensin II in this phenomenon. In one experiment in which we used an average of two animals from each of six different pairs of lead-treated and control animals, plasma lithium levels were determined when the animals were killed approximately 24 hours after the last LiCl injection. No significant differences were found between lead-treated and control rats.
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Human Auditory Frequency-Following Responses to a Missing Fundamental

Abstract. *Both a complex tone perceived as a 365-hertz "missing fundamental" and a 365-hertz pure tone evoked 365-hertz far-field frequency-following responses. Narrow-band masking noise centered at 365 hertz attenuated the responses to the pure tone but not to the complex tone. Results support the concept that perception of the missing fundamental is based on periodic neural activity.*

A complex tone composed of harmonics related to a low fundamental frequency can produce a sensation of pitch one octave or more below the lowest frequency present in the stimulus complex. The perception of a pitch in the absence of spectral energy at that frequency has been of interest to auditory researchers since the experiments of Seebeck in 1843 (*1*). Seebeck's findings challenged the prevailing concept that pitch perception was determined solely on the basis of a sinusoidal stimulus component corresponding to each pitch perceived (*2*). Subsequently, this phenomenon has been viewed as an example of an alternative form of pitch coding in which the auditory system responds to the inherent periodicity of a stimulus pressure wave in addition to its spectral composition. Perceptual phenomena related to those observed by Seebeck have been described more recently under several different labels. Among these are "the missing fundamental" (*3*), "residue pitch" (*4*), and "periodicity pitch" (*5*).

Although the stimulus conditions yielding missing fundamentals are widely known, the neural basis by which their pitch is perceived remains largely unexplored, save for a few studies of single units in animals. Such studies have shown that auditory neurons at various brainstem levels can phase-lock to low tones or, in some cases, to difference tones derived from higher partials, even when phase-locking to the partials themselves fails to occur (*6*). Electrophysiological data obtained with two other methods further suggest that low-frequency "volley coded" (*7*) pitch information might be carried in the ensemble characteristics of groups of phase-sensitive auditory neurons. One of these methods, the multiple-unit record (*8*), like single-unit recording, lends itself only to animal preparations. The other, the auditory frequency-following response (FFR), has recently been obtained from human subjects through the use of far-field procedures (*9*).

The FFR is a low-voltage neuroelectric wave whose period corresponds precisely to that of a low-frequency tonal stimulus. Such FFR activity, evoked by acoustical stimulation, is generally considered to be the aggregate envelope of the action potentials of a large group of phase-locking auditory neurons concentrated within major brainstem auditory nuclei (*10–12*). Although in the past the FFR has often been confused with

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