opposite striatum. Another group ($N = 6$) of rats received sham surgery (*14*) in the striatum opposite to the side of the behavioral preference. After recovery from anesthesia, all rats with lesions rotated in the direction ipsilateral to the lesion for several hours after surgery; spontaneous rotation was entirely absent one or more days after surgery. Three days after surgery, rats were tested in the T-maze again. All rats with lesions had side preferences ipsilateral to the lesion, that is, in the same direction as the initial postoperative rotation; all rats with sham surgery had side preferences in the same direction as they had a week earlier. The relation of spatial preferences to rotation was also confirmed in normal rats. Another group ($N = 18$) of naive rats were tested first in the T-maze, then injected with *d*-amphetamine sulfate (1.0 mg/kg), and placed in an apparatus (*15*) designed to measure rotation automatically. When placed in such a spherical rotometer, rats rotate consistently (that is, to the same direction when tested at different times) after this dose of *d*-amphetamine (*5*). In the present case, rats preferentially rotated in the direction of the spatial preferences determined in the T-maze (*16*). These results, as well as others showing that *d*-amphetamine enhances spatial preferences (*17*), suggest that rotation is a stereotyped form of spatial behavior.

In conclusion, the present study provides evidence that rats have a small hemispheric asymmetry in nigrostriatal chemistry which may be related to a behavioral bias of possibly broad significance. This conclusion argues against the generally accepted notion that only man has hemispheric asymmetries (that is, cerebral dominance). The results reported here may eventually be relevant to an understanding of neurological phenomena and disorders in man characterized by unilateral or asymmetric disturbances of function. Regardless of such possibilities, it is important that a relation between a neurochemical index of brain function and behavior can be demonstrated.

BETTY ZIMMERBERG
STANLEY D. GLICK
THOMAS P. JERUSSI

Department of Pharmacology, Mount Sinai School of Medicine, New York 10029, and *H. M. Singer Laboratory of Neurosciences and Addictive Diseases, Beth Israel Medical Center, New York 10003*

References and Notes

1. R. A. Hansing, J. S. Schwartzbaum, J. B. Thompson, *J. Comp. Physiol. Psychol.* **66**, 378 (1968); M. S. Levine, N. Ferguson, C. J. Kreinick, J. W. Gustafson, J. S. Schwartzbaum, *ibid.* **77**, 282 (1971); G. W. Arbuthnott and U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **330**, 117 (1969).
2. E. J. Wyers, H. V. S. Peeke, J. S. Williston, M. J. Herz, *Exp. Neurol.* **22**, 350 (1968); E. J. Wyers and S. A. Deadwyler, *Physiol. Behav.* **6**, 97 (1971); J. W. Haycock, S. A. Deadwyler, S. I. Sideroff, J. L. McGaugh, *Exp. Neurol.* **41**, 201 (1973); S. D. Glick and S. Greenstein, *J. Comp. Physiol. Psychol.* **82**, 188 (1973).
3. U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367**, 49 (1971); J. E. Christie and T. J. Crow, *Br. J. Pharmacol.* **43**, 658 (1971); C. A. Marsden and H. C. Guldberg, *Neuropharmacology* **12**, 195 (1973).
4. U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367**, 69 (1971).
5. T. P. Jerussi and S. D. Glick, *Neuropharmacology* **13**, 283 (1974).
6. S. D. Glick and T. P. Jerussi, *J. Pharmacol. Exp. Ther.* **188**, 714 (1974).
7. The rats (220 to 280 g) were naive female albinos (Sprague-Dawley).
8. The T-maze was constructed of black Plexiglas. The stem was 30 cm long, the arms were each 15 cm long, and the walls were 30 cm high.
9. The floor of the T-maze was a grid of 0.4-cm stainless steel rods, spaced 1.3 cm apart. Scrambled 0.6-ma a-c current was applied to the grid by using a Lafayette A615C shocker.
10. In an initial pilot group of 32 rats, the alternation rate was 72 percent from the first to the second trial and 26 percent from the ninth to the tenth trials, both significantly different from chance ($P < .05$, chi-square tests).
11. Only rats with preferences on the first test were used. Over each time interval, 83 to 85 percent of all rats preferred the same side on subsequent tests as they had on the first test ($P < .005$ in each case).
12. H. Weil-Malherbe, *Methods Biochem. Anal.* **16**, 293 (1968).
13. The skull was inclined according to the atlas of L. J. Pellegrino and A. J. Cushman [*A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967)]. Coordinates were 2.0 mm anterior to bregma, 3.0 mm lateral to the midline, and 5.5 mm from the top of the skull. Lesions were made with a direct 2-ma anodal current for 15 seconds. Methohexital anesthesia was used; all rats were awake within 30 minutes after surgery. All lesions were verified histologically.
14. Sham-operated animals had the electrode lowered but did not receive lesions.
15. The apparatus consisted of a white opaque Plexiglas sphere, 30 cm in diameter, within which the rat rotated. A flexible stainless steel wire, which was connected to a cam positioned on the vertical axis on the outer surface of the sphere, was wrapped around the thorax of the rat. As the rat rotated, the cam closed one of the two microswitches that were positioned so as to indicate left or right turns (*5*).
16. The side preference in the T-maze and the direction of amphetamine-induced rotation was the same in 78 percent of rats ($P < .05$, chi-square test).
17. S. D. Glick, B. Levin, M. E. Jarvik, *J. Comp. Physiol. Psychol.* **73**, 56 (1970); S. D. Glick, *Neuropharmacology* **12**, 43 (1973).
18. Supported by NIMH grant MH25644 and NIMH research scientist development award (type 2) DA70082 to S.D.G. We thank D. H. Waters and L. Manzino for their help in conducting the neurochemical assays.

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Enduring Learning Deficits and Cerebral Synaptic Malformation from Exposure to 10 Parts of Halothane per Million

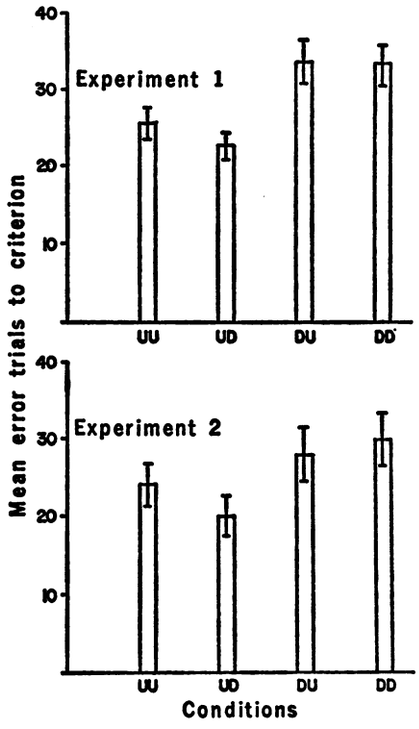
Abstract. *Chronic exposure of rats to 10 parts of halothane per million during early life produced later deficits in learning a shock-motivated light-dark discrimination and a food-motivated maze pattern, correlated with enduring synaptic membrane malformation in cerebral cortex. Adult exposure had no effect. Halothane may provide a useful analytical tool for study of brain. The behavioral-ultrastructural techniques also suggest a standard for assessing the safety of trace toxicants with central nervous system effects.*

The possible toxic effects of anesthetic gases on chronically exposed surgical personnel have been of increasing concern in recent years (*1*). We have chosen to study halothane, which, used in conjunction with nitrous oxide, is the most widely employed inhalation anesthetic in the United States. Ambient concentrations of halothane are found to average 10 parts per million (ppm) in the operating theater. The anesthesiologist, stationed near the exhaust of the patient's breathing circuit, may be exposed to a much higher concentration (*2*).

Two different toxicology questions exist for this situation. First, behavioral deficits might occur during the acute exposure of the operating team to halothane. Bruce *et al.* (*3*) found that 4 hours of exposure to 15 ppm halothane (with 500 ppm nitrous oxide) produces significant deficits in cognition, perception, and motor reaction. Such decreases in capacity during surgery are of obvious concern. The second question, and the one we addressed, was whether chronic exposure to 10 ppm halothane produces lasting behavioral deficits and damage to the central nervous system. Such deficits could place an undue burden upon the lives of the surgical team members. Various studies have suggested that anesthesiologists and operating room nurses may suffer long-term, physiological effects (*4*). However, no data exist on possible behavioral deficits from chronic halothane exposure.

We examined the effects of halothane on rats exposed chronically, either during early development, adult life, or both. To obtain sensitive behavioral measures, we utilized two relatively dif-

Fig. 1. Errors in learning as a function of conditions of exposure to halothane. The UU group was unexposed; the UD group was exposed from day 60 of age on; the DU group was exposed from conception to day 60; the DD group was exposed from conception on. Testing was begun at 130 to 150 days of age.



ficult learning tasks, chosen with regard to the consistent finding that acute halothane exposure produces deficits in memory registration (*5*).

In the present study, Sprague-Dawley rats were divided into one control group (UU) and three experimental groups exposed to a range of 8 to 12 ppm concentrations of halothane/air, 8 hours a day, Monday through Friday of each week. The three experimental groups were as follows: the DU group was exposed throughout early development (from conception to day 60 of age); the UD group was exposed from day 60 of age through the behavioral testing (a period of 75 to 105 days); and the DD group was exposed throughout both age periods. Two controlled environment chambers of the University of Wisconsin Biotron were used, maintaining a constant temperature (26°C), relative humidity (50 percent), and normal oxygen and CO_2 concentrations. In the treatment chamber, during the light phase of a 12-hour light-dark cycle, halothane was introduced into the fresh air supply input via a calibrated Drager vaporizer, and concentrations were monitored by gas chromatography (*6*).

In the first experiment, 12 naive rats from each of the four treatment groups were used. Half the subjects in each group were tested beginning at 130 days of age and half were tested beginning at 150 days of age; results were the same for both age groups. Thirty trials every other day were given on a light-dark discrimination task in an automated Y-maze utilizing electric foot shock escape or avoidance, as described in (*7*). One or more errors on any one trial counted as one "error trial." Criterion was 90 percent correct responses on any one training session. Mean error trials to criterion for each of the four groups were found by a one-way analysis of variance to demonstrate a significant effect ($F = 6.38$, d.f. $= 3/44$, $P < .01$). Subsequent analysis by Fisher's Least Significant Difference, or LSD, test (*8*) revealed that the 30 percent increase in error trials made by the DU and DD groups was significant (Fig. 1).

To test the generality of the effects seen in the first experiment, a spatial discrimination task for appetitive reinforcement was chosen for the second study. This maze, developed by Davenport *et al.*, has been shown to be sensitive in detecting learning deficits in hypothyroid rats (*9*). Ten naive rats from each of the four treatment groups, 140 to 145 days of age at the beginning of the study, were tested on problem T-6 of the test series. Error trials to criterion were scored via closed circuit television. Criterion was four out of five errorless trials, with each subject given a maximum of 48 trials, all run in one testing session. A one-way analysis of variance indicated that the mean error trials to criterion (or for the total of 48 trials for those subjects that failed to reach criterion) for the combined DU and DD groups (mean = 29.50) was significantly higher than for the combined UU and UD groups (mean = 22.05) ($F = 4.61$, d.f. $= 1/36$, $P < .05$). As can be seen in the lower panel of Fig. 1, the mean error trials for the individual groups follow the same pattern as in experiment 1. Furthermore, whereas only one of the 20 subjects in the UU and UD groups failed to reach criterion within 48 trials, nine of the 20 subjects in the DU and DD groups failed to do so. A

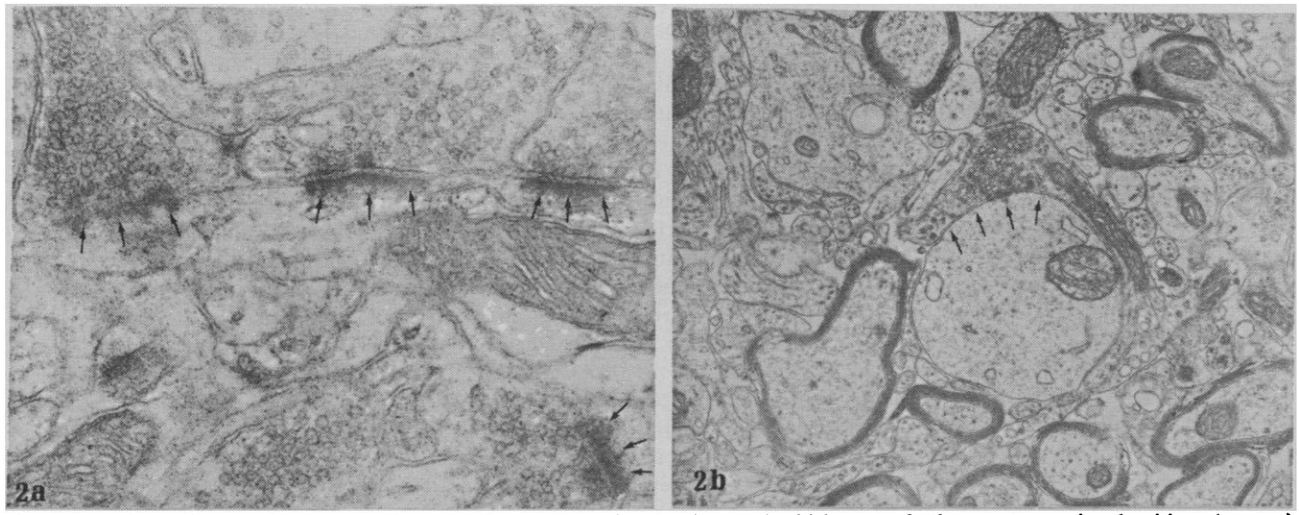


Fig. 2. (a) Synaptic complexes, normal neonatal rat. Note the density and thickness of the postsynaptic densities (arrows) (×45,000). (b) A large synaptic terminal from halothane-treated neonatal rat showing reduction and absence of the postsynaptic densities at the synaptic junction (arrows). The presynaptic vesicles were apparently normal (×20,000).

chi-square analysis revealed this difference as significant ($\chi^2 = 9.06$, $P < .05$).

The data in both experiments indicate that early exposure to halothane in trace amounts causes apparently permanent learning deficits, since these deficits occurred in rats last exposed 75 to 90 days prior to the beginning of behavioral testing (group DU). Because exposure to halothane only after day 60 of age produced no behavioral deficits in either learning task (group UD), the critical exposure period appears to be that of early development. Correlative to the pattern of behavioral deficits, tissue samples from the superior parasagittal cerebral cortex of rats exposed to 10 ppm halothane from conception to parturition showed electronmicroscopic evidence of neuronal degeneration, as well as the permanent failure of formation of the synaptic web and postsynaptic membrane density in 30 percent of the postsynaptic membranes (Fig. 2); only slight neuronal damage was evident at the electronmicroscopic level in rats exposed to 10 ppm halothane as adults (*10*).

The above results reveal that exposure to very low concentrations of halothane throughout the period of major growth in the rat is sufficient to produce subsequent enduring deficits in learning tasks. The nature of the deficit is not revealed by these studies, but these findings raise the question as to whether or not pregnant women should avoid chronic halothane exposure even at trace levels of 10 ppm as a precaution against possible enduring damage to the brain of the fetus.

The present behavioral data provide no indication of damage in the adult rat exposed to 10 ppm halothane. However, the exposure period was only approximately 90 days. Assuming that our electromicroscopic data indicate a susceptibility of developing synapses, and assuming that the plasticity of adult brain in registering new learning involves synaptic changes, then longer exposure times, increased halothane concentration, or behavioral tasks more specific to memory registration might reveal deficits.

The results of this study provide an example wherein exposure to trace levels of a potential toxicant produced no gross evidence of physiological or behavioral damage. Even under light microscopy, various organs, including the brain, appeared normal. Only at the ultrastructural level did morphological damage become manifest. Furthermore, the evidence of synaptic and other neurological damage in the central nervous system may or may not have been accompanied by functional deficits, and only by behavioral testing could this be resolved. It is interesting that behavioral tests have proven here to be as sensitive as electronmicroscopy for detecting damage from exposure to a trace level of toxicant. In assessing the health burden of trace pollutants, at least those potentially affecting the central nervous system, it is clear that ultrastructural and behavioral data will need to be specifically sought as the most sensitive approach to determine exposure levels consistent with health and safety.

KELVIN L. QUIMBY
LEA J. ASCHKENASE
ROBERT E. BOWMAN
Psychology Department and
Regional Primate Research Center, University of Wisconsin, Madison 53706

JORDAN KATZ, LOUIS W. CHANG
Departments of Anesthesiology and
Pathology, University of Wisconsin Medical School, Madison 53706

References and Notes

1. P. Nikky, *Ann. Clin. Res.* **4**, 247 (1972).
2. H. W. Linde and D. L. Bruce, *Anesthesiology* **30**, 363 (1969); C E. Whitcher, E. N. Cohen, J. R. Trudell, *ibid.* **35**, 348 (1971).
3. D. L. Bruce, M. J. Bach, J. Arbit, *ibid.* **40**, 453 (1974).
4. D. L. Bruce, K. A. Eide, H. W. Linde, J. E. Eckenhoff, *ibid.* **29**, 565 (1968); E. N. Cohen, J. W. Bellville, B. W. Brown, *ibid.* **35**, 343 (1971).
5. N. Adam, *J. Comp. Physiol. Psychol.* **83**, 294 (1973); A. L. Porter, *Behav. Biol.* **7**, 291 (1972).
6. Several 500-μl sample injections were made daily, with the use of a Varian Aerograph model 600-D gas chromatograph. The flow rate for the N_2 carrier gas was 25 ml/min. A flame ionization detector was employed. The 5-foot by ⅛-inch column (1.524 m by 0.32 cm), with squaline liquid phase coated to a 5 percent loading on VarAport 30 support, 80 to 100 mesh size (available from Varian), was maintained at 100°C.
7. The procedure was essentially identical to that of R. J. Barrett, N. J. Leith, and O. S. Ray [*Psychopharmacologia* **25**, 321 (1972)]. The maze was symmetrical, with arms 46.5 by 12.0 by 16.0 cm high. A 2.8-watt lamp mounted behind a green convex lens was centered on the end wall of each arm, facing a motorized, plexiglass door 15.5 cm away. The conditioned stimulus was produced by a 4.5-khz audio oscillator (Sonalert) pulsed at 10 per second. Intermittent shock (0.25 ma; 0.5 second on, 1.5 seconds off) was delivered through a grid floor and stainless steel plates that lined the inside walls of the maze. A 25-second intertrial interval was used, and trials consisted of switching the light cue in a predetermined random sequence to one of the previously dark arms. The subject could avoid (within 10 seconds) or escape the shock by interrupting the photocell beam in the lit goal box.
8. H. C. Fryer, in *Concepts and Methods of Experimental Statistics* (Allyn & Bacon, Boston, 1966), pp. 259–270. The significant pairwise comparisons by the LSD test were: UU versus DU ($P < .01$), UU versus DD ($P < .025$), DU versus UD ($P < .001$), UD versus DD ($P < .001$).
9. J. W. Davenport and T. P. Dorcey, *Horm. Behav.* **3**, 97 (1972); J. W. Davenport, W. W. Hagquist, G. R. Rankin, *Behav. Res. Methods Instrum.* **2**, 112 (1970). We used a constant 30-second intertrial interval. The problems, including T-6, consist of a symmetrical arrangement of barriers, requiring the subject to make the same sequence of left and right turns to get from one goal box to the other in a bidirectional shuttling procedure.
10. L. W. Chang, A. W. Dudley, J. Katz, in preparation. Evidence of neuronal degenerative changes in neonatal brain exposed to halothane included such observations as focal weakening and disruption of the nuclear envelope of cortical neurons, vacuolation and myelin-figure formation in many neuronal processes, focal accumulation of vesicular structures, and mitochondrial swelling in neuronal cytoplasm, neuronal necrosis, and edematous changes of the glial cells.
11. Supported by funds from both the University of Wisconsin Graduate School Research Committee and the Medical School Research Committee. We thank Ayerst Laboratories for donation of Fluothane (halothane).

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Interpreting the Failures to Confirm the Depression of Cerebellar Purkinje Cells by Cyclic AMP

Our proposal (*1, 2*) that 3′,5′-adenosine monophosphate (cyclic AMP) might mediate the action of norepinephrine (NE) on cerebellar Purkinje cells has been challenged by Lake and Jordan (*3*). Our proposal evolved from the reported increases in cyclic AMP synthesis produced by NE in cerebellar slices (*4*) and our observations that iontophoretic application of cyclic AMP would mimic the depressant effects of NE on the discharge rate and pattern of Purkinje cells (*1*) and that phosphodiesterase inhibitors would potentiate the depressant actions of NE and of cyclic AMP (*1, 2*). Subsequently, we showed that both NE and cyclic AMP hyperpolarized Purkinje cells (*5, 6*), and that identical hyperpolarizations were produced by stimulating the NE pathway arising in the nucleus locus coeruleus (*7, 8*). In addition, we showed that the proportion of Purkinje cells which reacted to an immunocytochemical method for the detection of cyclic AMP increased five- to ten-fold after stimulation of the locus coeruleus or topical application of NE (*9*).

Lake and Jordan challenged our hypothesis on three grounds. (i) They, like Godfraind and Pumain (*10*), were unable to depress the same frequency of Purkinje cells with cyclic AMP as we were. (ii) They (*3*) discount our