References and Notes

1. G. Svärdson, *Oikos* **1**, 157 (1949); R. S. Miller, *Adv. Ecol. Res.* **4**, 1 (1967).
2. D. H. Morse, *Am. Nat.* **108**, 818 (1974).
3. T. Kikuchi, *Sci. Rep. Tohoku Univ. Ser. 4 (Biology)* **31**, 275 (1965); P. R. Grant, *Anim. Behav.* **18**, 411 (1970); R. K. Colwell and E. R. Fuentes, *Annu. Rev. Ecol. Syst.* **6**, 281 (1975); D. W. Inouye, thesis, University of North Carolina, Chapel Hill (1976).
4. I affixed small numbered plastic tags (Opalithplättchen), obtained from Chr. Graze Kg., D-7056 Weinstadt-Endersbach, Postfach 2107, West Germany, to the thoraxes of the bees. These tags and the techniques used in applying them have been described [B. Heinrich, *Ecol. Monogr.* **46**, 105 (1976)].
5. I measured the entire wing, from opposite the distal margin of the tegula of the thorax to the distal extremity of the wing, as described by D. H. Morse [*Ann. Entomol. Soc. Am.* **70**, 311 (1977)]. Wing length is closely correlated with body length [J. T. Medler, *ibid.* **55**, 212 (1962)].
6. D. H. Morse, in preparation.
7. L. K. Johnson and S. P. Hubbell, *Ecology* **56**, 1398 (1975).
8. B. Heinrich, in *Coevolution of Animals and Plants*, L. E. Gilbert and P. H. Raven, Eds. (Univ. of Texas Press, Austin, 1975), p. 141; *Ecol. Monogr.* **46**, 105 (1976).

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Toxicity of Mild Prenatal Carbon Monoxide Exposure

Abstract. *Rats prenatally exposed to a low concentration of carbon monoxide which results in carboxyhemoglobin levels equivalent to those maintained by human cigarette smokers, show reduced birth weight and decreased weight gain. Neurobehavioral and biochemical testing of the offspring reveals lower behavioral activity levels through the preweaning period, altered central catecholamine activity, and reduction in total brain protein at birth.*

Irreversible central nervous system damage reflected in widespread necroses and ultrastructual changes (*1*), alterations in cerebral energy production or metabolism (*2*), and depression of neurotransmitter synthesis or turnover (*3*) have all been reported following acute hypoxic or anoxic exposures. Such findings are useful in predicting the pattern of neurological changes that may result from asphyxia accompanying obstetric procedures and cerebrovascular accidents. Comparatively little is known about the effects of chronic hypoxia at levels relevant to human experience. This may result from natural causes such as high altitude and man-made causes such as chronic carbon monoxide exposure induced by cigarette smoking and industrial sources. We have concentrated on the effects of CO exposure during the prenatal period. Carbon monoxide readily crosses the placenta, and mild maternal exposures in animals result in decreased fetal oxygen partial pressures in the descending aorta and inferior vena cava (*4*). Rats and rabbits prenatally exposed to high-altitude conditions or to CO show a variety of abnormalities at birth, including reduced birth weight (*5–7*), heart hypertrophy (*7, 8*), and reduced brain protein levels suggestive of smaller neurons (*9*). There is evidence, too, that mild prenatal CO exposure in rabbits may be teratogenic (*5*). The long-term consequences of these effects as well as their functional significance have not been elucidated. Moreover, studies of central nervous system toxicity following chronic CO exposure are lacking.

Maternal cigarette smoking and domicile at high altitude are major causes of chronic prenatal hypoxia. Both conditions represent important risk factors, having been associated with excess prenatal mortality (*10, 11*), premature (preterm) birth (*10–12*), and decreased birth weight (*10, 13*). Experimental evidence (*5*) confirms that these sequelae are related to the hypoxic exposure in a dose-related manner regardless of the source of hypoxia (CO or diluting air with nitrogen). We report here that chronic prenatal exposure of rats to CO has behavioral, neurochemical, and physiological consequences which last well beyond birth and that these effects are measureable at CO levels approaching those experienced by offspring of mothers who smoke.

Adult female Long-Evans hooded rats were maintained in the laboratory with continuous access to food and water, a diurnal light cycle (12 hours light, 12 hours darkness), and room temperature at 22°C. The rats were bred and, after a sperm-positive vaginal smear, were transferred to exposure chambers for the duration of gestation. Spectrophotometric measurements (*14*) were used to verify that maternal carboxyhemoglobin (HbCO) concentrations of 15 percent resulted among the subjects exposed to CO. By comparison, human cigarette smokers show HbCO concentrations ranging from about 1 to 16 percent (*15*).

Within 12 hours after birth the subjects were removed from the exposure chambers and placed in a normal air environment. The neonates were counted, weighed, and examined for superficial deformities. Litter sizes were then ad-

Table 1. Growth rate of preweanling rats exposed prenatally to CO [150 parts per million (ppm)] or to air. Values are given as mean ± standard error of the mean (S.E.M.).

Treatment	Body weight (g)				
	Day 1	Day 4	Day 10	Day 14	Day 21
Air	5.77 ± 0.05	9.68 ± 0.2	21.82 ± 0.34	27.16 ± 0.6	45.81 ± 0.77
CO	5.49 ± 0.05	8.16 ± 0.17†	18.19 ± 0.45*	24.40 ± 0.42†	39.90 ± 1.5*

*Significantly different from comparable control group at $P < .01$. †Significantly different from comparable control group at $P < .05$.

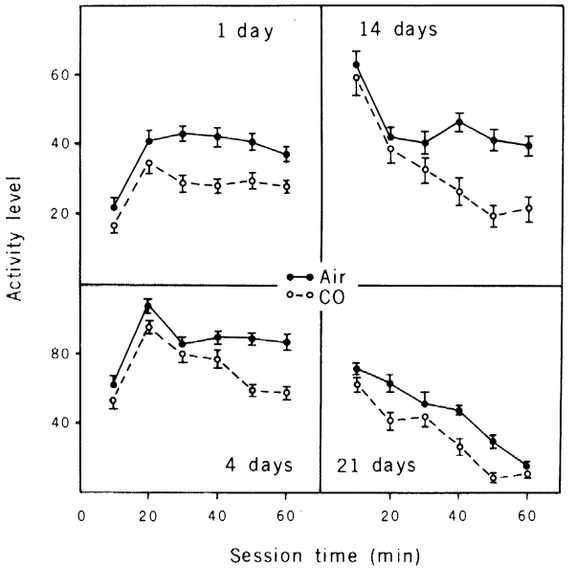

Fig. 1. Open-field activity levels of rats exposed prenatally to CO concentrations of 150 parts per million (ppm) or to room air. Subjects 1 and 4 days old were injected with L-dopa (100 mg/kg) at time zero. Subjects 14 and 21 days old were not injected. Activity was averaged across 10-minute intervals for a 1-hour period.

justed to eight pups per litter and the neonates scheduled for (i) brain protein determination on day 1 ($N = 32$); (ii) behavioral tests on day 1 ($N = 24$), 4 ($N = 20$), or 14 or 21 ($N = 16$); or (iii) assays of whole brain concentrations of the catecholamines dopamine (DA) and norepinephrine (NE) on day 1 ($N = 48$) or 4 ($N = 40$). These assays were of interest because acute hypoxia in adult rats and also neonatal asphyxia have been shown to interfere with monoamine synthesis (*3*).

Offspring of mothers exposed to CO showed slightly (5 percent) although not significantly lower birth weights than normal neonates, but differences in body weight became progressively greater throughout the preweaning period (Table 1) and did reach statistical significance at 4 days of age (Student's t-test, $t = 8.24$, $P < .01$). We did not observe differences in number of pups per litter or mortality rate on day 1 (Table 2); no cases of gross teratogenesis were seen in either group.

Our initial determination of functional consequences of prenatal CO exposure consisted of tests of locomotor activity in an electronic (Stoelting) activity monitor (*16*). We were anxious to examine neonatal animals at the youngest possible age since we did not know whether any behavioral effects of CO might be transitory. To measure activity in 1- and 4-day-old rats, we injected the subjects subcutaneously with the catecholamine precursor L-dopa (100 mg/kg) immediately before the 1-hour session. Earlier reports (*17*) have shown that such treatment greatly increases central DA and NE concentrations and elicits vigorous crawling, head rearing, and swimming-like movements in animals too young to maintain such activity spontaneously. After testing, the subjects were decapitated and the brains rapidly removed and frozen over Dry Ice for subsequent fluorimetric determinations of DA and NE levels (*18*). The assays were performed on four samples of six pooled brains at 1 day of age and on five samples of four pooled brains at 4 days of age. The results were compared with those for the same number of samples taken from saline-injected controls. Subjects 14 and 21 days old were tested without injection and neurochemical data are not included for these subjects.

Activity levels were consistently reduced among the CO-exposed neonates at all ages tested (Fig. 1). Subjects exposed to CO and injected with L-dopa (1 and 4 day olds) showed a similar time course in their behavioral response to the drugs but failed to attain activity levels as high as those of the neonates exposed to air. At 14 days of age, CO- and air-exposed neonates showed similar activity levels during the first 30 minutes of testing. Thereafter, CO subjects showed substantially less activity than controls.

Table 2. Birth weight, litter size, and day-1 mortality of rats prenatally exposed to CO (150 ppm) or to air. Values are given in columns 3 and 4 as mean ± S.E.M.

Treatment	Number of litters	Birth weight (g)	Live pups per litter	Total number of dead pups
Air	27	5.77 ± 0.05	10.6 ± 0.56	17
CO	26	5.49 ± 0.05	10.7 ± 0.59	20

Analyses of variance carried out at each age confirmed that CO- and air-exposed subjects differed in activity levels at 1 ($F = 6.8322$, $P < .05$), 4 ($F = 5.9907$, $P < .05$), and 14 days of age ($F = 4.8224$, $P < .05$). While 21-day-old rats prenatally treated with CO were less active than control subjects, this difference did not reach significance ($F = 2.4563$, $P > .05$).

The activity levels of 1- and 4-day-old rats injected with L-dopa seemed to be related to whole brain DA concentrations. After L-dopa treatment, significantly more DA was found in the brains of air-exposed than of CO-exposed rats at both 1 and 4 days of age ($t = 3.682$, $P < .01$ at day 1; $t = 2.942$, $P < .05$ at day 4). This relationship (Table 3) held even if amine concentrations were calculated with respect to total brain protein concentrations. No differences in whole brain NE, 5-hydroxytryptamine, or 5-hydroxyindoleacetic acid were found between these groups. Among saline-treated rats, no significant differences in monoamine concentrations were found between the CO- and air-exposed subjects at either day 1 or day 4 (Table 3). Further, the differences in amine levels between 1- and 4-day-olds, expressed on a per gram basis, did not reach statistical significance. Thus, observation of neurochemical deficits in our newborn subjects exposed prenatally to low CO concentrations apparently requires the disruption of resting-state conditions. Both the depression in open-field activity and the differences in utilization of exogenous L-dopa in 1- and 4-day-old subjects indicate changes in central nervous system function associated with prenatal CO exposure.

We also compared brain protein levels in 16 newborn rats prenatally exposed to CO or to air. The subjects were decapitated and their brains rapidly removed and frozen at −90°C before spectrophotometric assay by the phenol reagent method (*19*). We found a markedly lower brain protein level in CO neonates than in air controls ($t = 3.867$, $P < .01$), although absolute brain weight did not differ between groups ($t < 1.0$) (Table 4).

To our knowledge, mild CO exposure has not previously been shown to pro-

Table 3. Whole brain catecholamine levels in rats prenatally exposed to CO (150 ppm) or to air. Fluorometric assays were performed on pooled tissue samples from 1- and 4-day-old subjects 60 minutes after saline or L-dopa injection. Statistical comparisons were made between air and CO groups within each age and drug condition. Values are given as mean ± S.E.M.

Treatment	Catecholamine content (μg/g)			
	Day 1		Day 4	
	DA	NE	DA	NE
Saline				
Air	0.158 ± 0.04	0.072 ± 0.02	0.083 ± 0.02	0.045 ± 0.02
CO	0.108 ± 0.02	0.099 ± 0.02	0.133 ± 0.02	0.071 ± 0.02
L-Dopa (100 mg/kg)				
Air	5.47 ± 1.62	0.092 ± 0.02	2.96 ± 0.06	0.127 ± 0.02
CO	3.01 ± 0.81*	0.112 ± 0.03	2.23 ± 0.21†	0.123 ± 0.03

*Significantly different from comparable control group at $P < .01$. †Significantly different from comparable control group at $P < .05$.

Table 4. Whole brain protein concentrations in 1-day-old rats prenatally exposed to CO (150 ppm) or to air. Values are given as mean ± S.E.M.

Treatment	Body weight (g)	Wet brain weight (mg)	Wet brain weight/body weight	Brain protein (mg/g)
Air	5.94 ± 0.17	233.0 ± 6.40	39.98 ± 0.68	81.77 ± 2.46
CO	5.53 ± 0.24	243.9 ± 6.83	43.76 ± 0.80*	63.36 ± 4.08*

*Significantly different from comparable control group at $P < .01$.

duce functional deficits. Our findings indicate, however, that the fetal organism is particularly sensitive to chronic CO exposure and may be impaired at levels of exposure similar to those found among cigarette smokers. Cigarette smoke contains roughly 3 to 5 percent CO (*20*) and inhalation of tobacco smoke is the major source of HbCO in the general population. Cigarette smokers have an average HbCO concentration of approximately 5 percent (*15*), but the level ranges from about 1 to 16 percent in individuals, depending on occupation, smoking habits (frequency and inhalation patterns), and ambient CO concentrations (*15*). In nonsmokers HbCO levels average approximately 0.5 percent and rarely exceed 2 percent (*15*). Carboxyhemoglobin levels also tend to be somewhat elevated during pregnancy, reflecting enhanced endogenous CO production (*4*).

Our results suggest that indices of maternal cigarette smoking such as enhanced neonatal mortality and reduced birth weight reflect only the most readily measurable effects of this toxin; the potentially more serious consequences of altered central nervous system function and biochemistry early in life or, perhaps, permanently are only now beginning to be discovered by use of animal models.

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References and Notes

1. S. Hicks, M. Cavanaugh, E. O'Brien, *Am. J. Pathol.* **40**, 615 (1962); R. Myers, *Am. J. Obstet. Gynecol.* **112**, 246 (1972); M. Ginsburg and R. Myers, *Acta Obstet. Gynecol. Scand.* **53**, 309 (1974); H. Schneider *et al.*, *Acta Neuropathol.* **32**, 287 (1975).
2. Measurement of brain cytoplasmic reduced and oxidized nicotinamide-adenine dinucleotide ($NADH/NAD^+$) and of the adenylate energy charge potential have generally shown these indices to be rather stable across a broad range of arterial oxygen partial pressures. Decreased charge potentials and increased lactate/pyruvate and $NADH/NAD^+$ ratios do appear, however, during severe hypoxia or ischemia [V. MacMillan and B. Siesjö, *Acta Physiol. Scand.* **82**, 412 (1971); L. Drewes, D. Gilboe, A. Betz, *Arch. Neurol. (Chicago)* **29**, 385 (1973); B. Siesjö and B. Ljunggren, *ibid.*, p. 400; F. Yatsu, L. Lee, C. Liao, *Stroke* **6**, 678 (1975); R. Villa, *Farmaco Ed. Sci.* **30**, 561 (1975)].
3. R. Brown and J. Engle, *J. Pharm. Pharmacol.* **25**, 815 (1973); J. Davis and A. Carlsson, *J. Neurochem.* **20**, 913 (1973); *ibid.* **21**, 783 (1973); _____, V. MacMillan, B. Siesjö, *Science* **182**, 72 (1973); N. Simon and L. Volicer, *J. Neurochem.* **26**, 893 (1976).
4. L. Longo, *Ann. N.Y. Acad. Sci.* **174**, 313 (1970); *Science* **194**, 523 (1976); _____ and E. Hill, *Am. J. Physiol.* **232**, H324 (1977).
5. P. Astrup, D. Trolle, H. Olsen, K. Kjeldsen, *Lancet* **1972**, 1220 (1972); *Arch. Environ. Health* **30**, 15 (1975).
6. M. Younoszai, J. Pelosa, J. Haworth, *Am. J. Obstet. Gynecol.* **104**, 1207 (1969); J. Haworth and J. Ford, *ibid.* **112**, 653 (1972).
7. M. Mager, W. Blatt, P. Natale, C. Blatters, *Am. J. Physiol.* **215**, 8 (1968).
8. M. Grandtner, Z. Turek, F. Kreuzer, *Pfluegers Arch. Gesamte Physiol. Menschen Teire* **350**, 241 (1974); L. Fechter, in preparation.
9. E. Petropoulos and P. Timiras, in *Drugs and the Developing Brain*, A. Vernadakis and N. Weiner, Eds. (Plenum, New York, 1974), pp. 429–450.
10. N. Butler and E. Alberman, *Perinatal Problems. The Second Report of the 1958 British Perinatal Mortality Survey* (Churchill Livingstone, Edinburgh, 1969), pp. 72–84.
11. G. Comstock, F. Shah, M. Meyer, H. Abbey, *Am. J. Obstet. Gynecol.* **111**, 53 (1971); *The Health Consequences of Smoking. A Report to the Surgeon General* [Publ. No. (HSM) 71-7513, Department of Health, Education, and Welfare, Washington, D.C., 1971), pp. 387–418]; J. Andrews and J. McGarry, *J. Obstet. Gynaecol. Br. Commonw.* **79**, 1057 (1972); K. Niswander and M. Gordon, Eds., *The Women and Their Pregnancies* (Saunders, Philadelphia, 1972), pp. 72–79; *The Health Consequences of Smoking* [Publ. No. (HSM) 73-8704, Department of Health, Education, and Welfare, Washington, D.C., 1973), pp. 99–149]; M. Meyer, J. Tonascia, C. Buck, *Am. J. Epidemiol.* **100**, 443 (1975); M. Meyer, B. Jonas, J. Tonascia, *ibid.* **103**, 464 (1976).
12. J. Yerushalmy, *Am. J. Epidemiol.* **93**, 443 (1971); J. Fabia, *Can. Med. Assoc. J.* **109**, 1104 (1973).
13. C. Lowe, *Br. Med. J.* **2**, 673 (1959); B. MacMahon, M. Albert, E. Salber, *Am. J. Epidemiol.* **82**, 247 (1965); C. Russell, R. Taylor, C. Law, *Br. J. Prev. Soc. Med.* **22**, 119 (1968).
14. K. Small, E. Radford, J. Frazier, F. Rodkey, H. Collison, *J. Appl. Physiol.* **31**, 154 (1971).
15. J. Goldsmith, *Ann. N.Y. Acad. Sci.* **174**, 122 (1970); P. Lawther and B. Commins, *ibid.*, p. 135; P. Coles, *Nature (London)* **255**, 699 (1975); A. Kahn, R. Rutledge, G. Davis, J. Altes, G. Gantner, C. Thornton, N. Wallace, *Arch. Environ. Health* **29**, 127 (1974).
16. Each subject was placed in a 30-cm^2 plastic box located above tuned oscillator coils. Horizontal movement by the subject resulted in a change in tuning and this event was recorded as an activity count. The sensitivity of the apparatus was set at 90 for 1- and 4-day-old subjects and at 35 for 14- and 21-day-old subjects (uncorrected instrument readings).
17. C. Kellogg and P. Lundborg, *Psychopharmacologia* **23**, 187 (1972); P. Lundborg and C. Kellogg, in *Dynamics of Degeneration and Growth in Neurons*, K. Fuxe, L. Olsen, Y. Zotterman, Eds. (Pergamon, New York, 1974), p. 561; M. Dewyngaert and C. Kellogg, *Brain Res.* **73**, 175 (1974).
18. A. Bertler, A. Carlsson, E. Rosengren, *Acta Physiol. Scand.* **44**, 273 (1958); C. V. Atack and T. Magnusson, *J. Pharm. Pharmacol.* **22**, 625 (1970); C. Atack, *Br. J. Pharmacol.* **48**, 699 (1973).
19. O. Lowry, N. Rosenbrough, A. Farr, R. Randall, *J. Biol. Chem.* **193**, 265 (1951).
20. J. Osborne, S. Adarnek, M. Hobbs, *Anal. Chem.* **28**, 211 (1956); E. L. Wynder and D. Hoffman, *Tobacco and Tobacco Smoke* (Academic Press, New York, 1967), pp. 442–444.
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Developmental Neuroethology: Changes in Escape and Defensive Behavior During Growth of the Lobster

Abstract. *The changes in relative efficacy of two incompatible behaviors was investigated during growth of the lobster,* Homarus americanus. *In larval and early juvenile stages, physiological and morphological factors favor use of the escape response over defensive behavior. In large animals, defensive behavior is preferred almost exclusively to escape behavior unless the claws are lost. The interaction of escape and defensive behavior is modified by neural and morphological factors, which are dependent on the stage in the life cycle of the organism.*

One of the goals of neuroethology (*1*) is to determine the physiological substrates for behavior. Within the past decade, a number of studies have provided an excellent foundation for this emerging field (*1–4*). Most of these studies have been of invertebrates, primarily mollusks and arthropods because these animals have relatively simple nervous systems and relatively simple behaviors. In general, the approach has been to take one or a few simple behavioral acts, determine the neural structures controlling the behaviors, and then determine the interactions among the elements involved. However, these studies have generally focused on the substrates of a simple behavior (*3*) or the interaction of several behaviors (*4*) in adult animals. Other investigators have pursued the changes in a single behavior during growth and development (*2*). However, these studies have rarely been concerned with correlating both neural and morphological substrates with changes in behavior. We have examined both physiological characteristics and allometric relationships among various components of two incompatible behaviors of the lobster—escape and defense—from the larval stages to sexual maturity. During this period of growth, the components of these behaviors develop and differentiate considerably: the animal clearly favors escape early in its life cycle but defensive behavior in the later stages. Furthermore, certain physical characteristics, which determine the efficacy of these behaviors, exhibit transitional stages that occur during the same stage of development.

The primary flight response of the lobster is the well-known tail flip escape response common to the reptantian crustaceans (*5, 6*). The tail flip results from the contraction of the large abdominal flexor muscles and serves to propel the animal backwards. The behavior is mediated by two pairs of giant interneurons, the medial giants (MG) and the lateral giants (LG), as well as by nongiant interneurons (*7–11*). We have studied two aspects of escape behavior during growth of lobsters. We calculated (i) the time for the action potential in the medial giant axon to propagate the length of the ani-