HEALTH EFFECTS INSTITUTE

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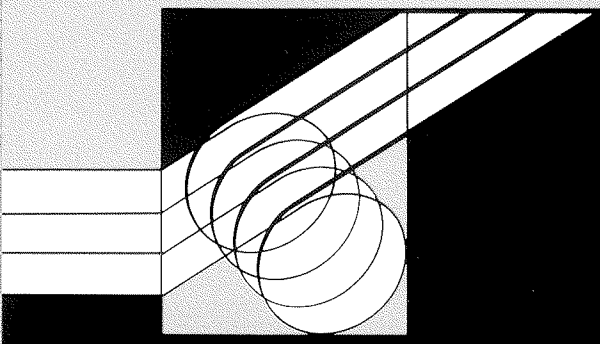
RESEARCH REPORT NO. 12



Neurotoxicity of Prenatal Carbon Monoxide Exposure

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Includes the Report of the Institute's Health Review Committee

The Health Effects Institute (HEI) is a non-profit corporation founded in 1980 to assure that objective, credible, high-quality scientific studies are conducted on the potential human health effects of motor vehicle emissions.

Funded equally by the U.S. Environmental Protection Agency (EPA) and 26 automotive manufacturers or marketers in the United States, HEI is independently governed. Its research projects are selected, conducted, and evaluated according to a careful public process, including a rigorous peer review process, to assure both credibility and high scientific standards.

HEI makes no recommendations on regulatory and social policy. Its goal, as stated by former EPA Administrator William D. Ruckelshaus, is "simply to gain acceptance by all parties of the data that may be necessary for future regulations."

Jessica Schwartz, *Director of Special Projects, was the production consultant for this project.*

Research Agreement No. 83-1
Designed and printed by Prime Communications, Inc.

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ABBREVIATIONS

5 HIAA	5-hydroxyindole acetic acid
5 - HT	serotonin
DA	dopamine
DHBA	dihydroxybenzylamine
DOPA	dihydroxyphenylalanine
DOPAC	dihydroxyindole acetic acid
EGL	external germinal layer
GABA	gamma aminobutyric acid
GAD	glutamic acid decarboxylase
Hb	hemoglobin
HbCO	carboxyhemoglobin
HPLC	high pressure liquid chromatography
HVA	homovanillic acid
MAM	methylazoxymethanol acetate
NE	norepinephrine
PBS	phosphate-buffered saline
PCA	perchloric acid
PCD	Purkinje cell degeneration
PD	postnatal day
TCA	trichloroacetic acid

PREFACE

HEALTH EFFECTS INSTITUTE AND ITS RESEARCH PROCESS

The Health Effects Institute (HEI) is an independent nonprofit corporation which, according to its charter, is "organized and operated...specifically to conduct or support the conduct of, and to evaluate, research and testing relating to, the health effects of emissions from motor vehicles".

It is organized in the following ways to pursue this purpose:

INDEPENDENCE IN GOVERNANCE

HEI is governed by a four-member Board of Directors whose members are William O. Baker, Chairman Emeritus of Bell Laboratories and Chairman of the Board of Rockefeller University; Archibald Cox, Carl M. Loeb University Professor (Emeritus) at Harvard University; Donald Kennedy, President of Stanford University; and Charles Powers, President, Clean Sites, Incorporated. Professor Cox chairs the Board. These individuals, who select their own successors, were chosen initially, after consultation with industry and other individuals, by then Environmental Protection Agency Administrator Douglas M. Costle.

TWO-SECTOR FINANCIAL SUPPORT

The Institute receives half of its funds from the United States government through the Environmental Protection Agency and half from the automotive industry. Twenty-six leading manufacturers of vehicles or engines that are certified for use on U.S. highways contribute to the Institute's budget, in shares proportionate to the number of vehicles or engines that they sell.

RESEARCH PLANNING AND PROJECT EVALUATION

HEI is structured to define, select, support, and review research that is aimed at investigating the possible health effects of mobile source emissions. Its research program is developed by the Health Research Committee, a multi-disciplinary group of scientists knowledgeable about the complex problems involved in determining the health effects of mobile source emissions. The Committee seeks advice from HEI's sponsors and from other sources prior to independently determining the research priorities of the Institute.

After the Health Research Committee has defined an area of inquiry, the Institute announces to the scientific community that research proposals are being solicited on a specific topic. Applications are reviewed first for scientific quality by an appropriate expert panel. Then they are reviewed by the Health Research Committee both for quality and for relevance to the mission-oriented research program. Studies recommended by the Committee undergo final evaluation by the Board of Directors, which also reviews the procedures, independence, and quality of the selection process.

When a study is completed, a draft final report is reviewed by a separate HEI committee, the Health Review Committee. Members are expert scientists representing a broad range of experience in environmental health sciences. The Health Review Committee has no role either in the review of applications or in the selection of projects and investigators for funding. This Committee assesses the scientific quality of each study and evaluates its contribution to unresolved scientific questions.

Each funded proposal is assigned in advance of completion to a member of the Health Review Committee, who acts as "primary reviewer." When the draft report is received, the primary reviewer directs a peer review by technical experts and, when appropriate, by a biostatistician. After the investigator has had a chance to comment on the technical evaluations, the primary reviewer drafts a final report review. This document is sent to the investigator for comment. It is subsequently examined by the full Health Review Committee and revised as necessary. The investigator's final report, as well as the Review Committee's report, are then made available to the sponsors and to the public after evaluation by the HEI Board of Directors.

All HEI investigators are urged to publish the results of their work in the peer-reviewed literature. The timing and nature of HEI report releases are tailored to ensure that the Health Review Committee's report does not interfere with the journal publication process. The report of the Health Review Committee will be as thorough as necessary to evaluate any individual report.

THE CLEAN AIR ACT

Under Sections 202(a)(3) and 202(b)(1) of the Clean Air Act, the Environmental Protection Agency imposes specific requirements for reductions in motor vehicle emissions of carbon monoxide (and other pollutants). The Act also provides EPA with limited discretion to modify those requirements. The determination of the appropriate standards for emissions from mobile sources depends in part on an assessment of the risks to health they present. Research on the effects of automotive emissions on relevant animal models can contribute to such risk assessment and, therefore, to informed regulatory decision-making.

In addition, Section 109 of the Clean Air Act provides for the establishment of national ambient air quality standards to protect the public health. The current standards include carbon monoxide. The legislative history of the Act makes it clear that, in setting ambient air quality standards, EPA is

required to consider the health of particularly sensitive subgroups of the population. The Senate report on the legislation states: "An ambient air quality standard . . . should be the maximum permissible air level of an air pollution agent or class of such agents (related to a period of time) which will protect the health of any group of the population." [U.S. Senate Rep. No. 1196, 91st Cong., 2d Sess. 10 (1970).] The identification of such groups is not clear, but the report does specify that "included among those persons whose health should be protected by the ambient standard are particularly sensitive citizens (such as bronchial asthmatics and emphysematics) who in the normal course of daily activity are exposed to the ambient environment." [U.S. Senate Rep. No. 1196, 91st Cong., 2d Sess. 10 (1970).] The report further states that "in establishing an ambient standard necessary to protect the health of these persons, reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group." [U.S. Senate Rep. No. 1196, 91st Cong., 2d Sess. 10 (1970).] Research on the sensitivity of prenatal and neonatal members of a relevant animal species to carbon monoxide exposure can contribute knowledge useful in determining whether the corresponding human subgroup needs special protection under a national air quality standard.

INTRODUCTION

In July 1982, HEI issued a Request for Application (RFA-3) soliciting proposals on "the models for susceptible populations." Dr. Laurence D. Fechter of Johns Hopkins University, School of Public Health, proposed a project entitled "Neurotoxicity of Prenatal Carbon Monoxide Exposure." HEI approved the three-year project in January 1983 and authorized expenditure of $206,176. This project was initiated on April 1, 1983, and the draft final report was first reviewed by the Health Review Committee in October 1986.

BACKGROUND

Carbon monoxide (CO) is a major product of incomplete combustion. Automobiles are the major sources of outdoor CO. Upon inhalation, 50 to 60% of the CO is rapidly absorbed through the lungs (Root, 1965). The absorbed CO decreases the amount of oxygen available to the tissues of the body in two ways: it displaces one of the two molecules of oxygen from its binding site on hemoglobin, and it enhances the affinity of hemoglobin for the remaining oxygen. Thus, the oxygen level available to tissues is reduced. The tissues that have a high demand for oxygen are, therefore, particularly vulnerable to CO exposure. The cardiovascular system has been the major focus of CO toxicity studies; other organ systems have not received as much attention.

In humans, the brain represents approximately 2% of the body weight but consumes about 20% of the resting oxygen requirement. Hence, the brain is a prime target organ for damage from CO. Several human studies suggest that CO has an effect on behavioral performance, particularly on visual vigilance and complex tracking tasks (Putz et al., 1976, 1979)†. A number of animal studies have demonstrated that CO exposure is associated with a decrement in the response rate of rats trained to respond to a variety of schedules; animal studies also suggest that the visual evoked response, a measure of the electrical activity of the brain cortex, may be disrupted by exposure to CO (Xintaras 1966; Petajan, 1976)†.

There are several physiological reasons to suggest that the fetus may be more susceptible to the effects of CO exposure (Longo, 1976, 1977)†. Oxygen tension in the fetal blood is a constant fraction of the oxygen tension in the maternal blood. In addition, CO can diffuse across the placenta and further reduce the available oxygen. The resulting fetal hypoxia is thought to be especially severe because the normal low oxygen tension in fetal blood is close to the critical levels for adequate oxygenation of fetal tissues; also, the fetus, unlike the adult, cannot increase its cardiac output to compensate for a lack of oxygen since the normal cardiac output in the fetus is believed to be at the maximal levels (Power and Longo, 1975)†.

Children born to mothers who smoke have been reported to have decreased body weight and increased prenatal death (see, e.g., Meyer et al., 1976; D'Souza et al., 1981; U.S. Dept. of Health and Human Services, 1986)†. Cigarette smoke contains approximately 4% (40,000 ppm) CO; however, a causative association cannot be established from such studies because cigarette smoke also contains other components, such as nicotine and tar. Several animal studies of rats and rabbits have demonstrated that prenatal exposure to CO is associated with a decrease in both body weight at birth and fetal survival.

Recently, the effect of CO exposure on the developing nervous system has been investigated. Acute CO exposure of rat fetuses on gestational days 15 or 16, or on neonatal day 5, have resulted in lesions involving the basal ganglia, especially the caudate nuclei, a major component of the striatum,* which has been associated with subsequent hyperactivity (Daughtrey and Norton, 1982, 1983)†. In addition, significant adverse neurobehavioral effects have been observed in rats exposed prenatally to CO levels of 150 ppm and above for prolonged periods. Such effects include decreased learning and reduced memory of two-way avoidance tasks, as well as delays in the development of homing behavior (Fechter and Annau, 1980; Mactutus and Fechter, 1984, 1985)†. The evidence presented in this report further suggests that neurochemical and histopathological measures of the central nervous system in the rat are affected by prolonged exposure to relatively moderate levels of CO.

In the accompanying report, Dr. Fechter has studied the impact of chronic prenatal exposure to moderate levels of CO on the nervous system of fetal rats. These studies focus on histopathological changes in various regions of the brain, and examine the associated neurochemical changes. The experiments were planned as exploratory in nature; further work is required to test rigorously a morphological-neurochemical relationship.

†See References on page 27.

*An explanation of the parts of the mammalian brain, as well as the neurotransmitters, will be found in Appendix A on page 29.

INVESTIGATOR'S REPORT

by Laurence D. Fechter, Ph.D.

Neurotoxicity of Prenatal Carbon Monoxide Exposure

ABSTRACT

Despite the very wide recognition that carbon monoxide (CO) is a significant neurotoxicant, the level at which subtle effects occur, and the existence of sensitive periods in development for such toxicity, has been undetermined. In terms of risk to the fetus, a potentially susceptible sub-population, there is concern, first, that the level of exposure at which neurotoxicity occurs may be different from the adult, and second, that the site of toxic action and subsequent neurotoxic effects of CO may be different in the immature and mature brain. The investigator studied the susceptibility of the developing brain to moderate levels of CO maintained chronically through the period of neuronal proliferation, and into the period of synapse formation.

Carbon monoxide may be thought of as both a prototypical hypoxic agent, and a significant public health hazard in its own right. Carbon monoxide is a ubiquitous toxic agent that accounts for large numbers of deaths and significant morbidity in human populations. Subtle neurotoxic effects of this agent may be even more common, but they may go largely undetected, or fail to be associated with CO exposure. We have shown that prenatal CO exposure at moderate levels can produce significant neurotoxic effects in rats. The data obtained from the cerebellum and neostriatum, in particular, suggest that chronic, moderate perinatal CO exposure may disrupt neuronal proliferation and, perhaps, may disrupt certain markers for neurochemical transmission.

INTRODUCTION

VULNERABILITY OF DEVELOPING BRAIN TO HYPOXIA

Research was initiated based upon theoretical and epidemiological evidence, both of which suggested that the developing organism might be particularly susceptible to CO exposure. On theoretical grounds it is known that CO readily crosses the placenta, and that fetal hemoglobin (Hb) has a higher affinity for CO than Hb found in the adult (Longo, 1970; Roughton and Darling, 1944). It also is known that endogenous production of CO is elevated during pregnancy (Delivoria-Papidopoulous et al., 1974). Finally, it was apparent from oxygen dissociation curves that the leftward shift, resulting from allosteric changes in Hb induced by CO, was particularly pronounced at low oxygen tensions, such as those maintained by the fetus (Longo, 1970).

A major question that remained to be addressed was whether or not moderate perinatal CO exposure could produce neurotoxic effects in the neonate, given the relatively low level of neural activity and oxygen utilization seen in the immature brain. The epidemiological evidence was, at best, indirect, because it came overwhelmingly from the offspring of cigarette smokers who are exposed not only to chronically high levels of CO, but also to nicotine and a great many other compounds. The experimental literature dealing with the neurotoxic effects of CO and hypoxia is based almost entirely on acute, generally life-threatening conditions carried out most frequently in adult subjects, but occasionally in developing subjects as well. This model is clearly appropriate for study, given the clinical cases of trauma, acute fetal asphyxiation, and acute CO intoxication, but there is not necessarily a relationship between the toxic consequences of acute high level CO exposure, which may disrupt a number of different processes, each with its own cascade of effects, and chronic low level CO exposure. The research reported here provides additional opportunities to explore the similarities and differences between the effects of high-dose, acute and low-dose, chronic CO exposures in development.

There is a large body of evidence to suggest that, in more mature subjects that show high levels of neuronal activity, hypoxia may produce its toxic effects on cells, either by disrupting neurochemical transmission (e.g., by disrupting neurotransmitter synthesis), or by altering ion flux across presynaptic membranes. The data obtained chiefly from the cerebellum and neostriatum suggest that chronic, moderate perinatal CO exposure may disrupt neuronal proliferation and, perhaps, as a consequence of this, certain markers for neurochemical transmission. However, comparison of data gathered from the cerebellum, cerebral cortex, hippocampus, neostriatum, and brainstem refute the hypothesis that CO hypoxia produces a single pattern of injury to a category of neuron having a common monoamine neurotransmitter. The selective vulnerability of serotonergic, dopaminergic, and noradrenergic neurons to hypoxia, which others have reported under life-threatening hypoxic conditions, does not appear to occur under the more chronic low level exposures that we have used. Thus, it appears that the neurotoxic effects of CO may be somewhat different in the mature and immature brain.

VULNERABILITY OF THE CEREBELLUM

The cerebellum consists of several different cell types, each with characteristic neurotransmitter, histological, and electrophysiological properties. The cells that make up the cerebellar cortex arise from an active proliferative zone (the external germinal layer) (EGL) according to a rigid timetable. Once the critical time period for generating a neuronal cell type has passed, no additional cells are generated. If the rate of cell proliferation is slowed, fewer cells than normal will be generated. Thus, there are limited possibilities for repair of damage via regeneration.

Postnatal growth of the cerebellar cortex involves the proliferation and subsequent migration of granule, stellate, and basket interneurons from the EGL on the surface of the developing cerebellum to their final location in this structure, and the continued growth and maturation of the earlier proliferating Purkinje and Golgi cells (Altman, 1969, 1972a,b,c). Substantial evidence has further identified the granule neurons as glutaminergic (Young et al., 1974; McBride et al., 1976; Sandoval and Cotman, 1978; Rohde et al., 1979; Sandoval et al., 1984) and all other intrinsic cerebellar cortical neurons as gamma aminobutyric acid (GABA) ergic (Otsuke et al., 1971; Hokfelt and Ljundahl, 1970, 1972; McLaughlin et al., 1974). Alteration in the postnatal development of specific cerebellar cell populations thus can be detected neurochemically as well as histologically, and has been well documented among several mutant mice strains and in studies employing x-irradiation.

Major depletion of cells comprising the EGL, which follows neonatal exposure to methylazoxymethanol acetate (MAM) or to x-irradiation, for example, is accompanied by large decreases in the glutaminergic presynaptic markers, glutamate content, and high affinity uptake, but only mild decreases in GABAergic markers, GABA content, glutamic acid decarboxylase (GAD) activity, and high affinity uptake (McBride et al., 1976; Rohde et al., 1979; Slevin et al., 1982).

Postnatal depletion of Purkinje cells, on the other hand, which occurs among Purkinje cell degeneration (PCD) and "nervous" mutant mice strains, results in decreases of GABA content and GAD activity, and no changes in glutamate concentration (Roffler-Tarlov and Sidman, 1978; Roffler-Tarlov et al., 1979).

In some cases, as with hyper- and hypothyroidism, specific cerebellar populations are not severely depleted, but the rates of proliferation and migration of EGL cells, and the general maturation of other components of the cerebellar cortex, are altered so that the final pattern of cerebellar foliation attained is abnormal (Legrand, 1967; Nicholson and Altman, 1972a,b; Lauder et al., 1974).

The investigator conducted an extensive series of experiments to determine whether or not cerebellar development, as well as the innervation of the cerebellar cortex by extrinsic norepinephrine (NE)-containing neurons, are affected by early CO exposure. Because the rat cerebellum is considerably less mature at birth than the primate cerebellum, two different exposure models, an in utero CO exposure model and a combined in utero plus 10 days of neonatal exposure to CO, were employed. The latter model should yield a rodent cerebellum that has a decreasing EGL, which is a hallmark of the primate cerebellum at birth.

VULNERABILITY OF THE NEOSTRIATUM

The basal ganglia were identified as an important target for damage by acute anoxia with softening, hemorrhage, and necrosis frequently reported (e.g., Ginsberg and Myers, 1974a). Precisely why the basal ganglia appear to be susceptible to anoxia is unclear, although a range of hypotheses has been offered to explain the sensitivity of various neuronal cells to reduced oxygen. These include: the level of cellular metabolic activity; the requirements of neurotransmitter synthesizing enzymes for oxygen; the blood supply to the region; and the accumulation of waste products of anaerobic metabolism, specifically lactic acid (Vannucci and Plum, 1975; Myers, 1979).

The vulnerability of the basal ganglia of the developing brain to hypoxia under a wide variety of acute insults, including ischemia, hypoxic hypoxia, and CO exposure, also was reported, this despite the rather low amount of energy consumption shown by the immature brain. However, these studies generally were conducted either on human autopsy material or on animal survivors following life-threatening hypoxic insult. The clinical reports described necrosis with glial proliferation in the neostriatum following neonatal hypoxia (Schneider et al., 1975; Dambska et al., 1976). CO asphyxiation in the adult often is followed by Parkinsonian-like symptoms, which are consistent with injury to the basal ganglia.

Research on perinatal hypoxia largely has focused on the effects of asphyxiation with measurement either of acute or, less often, chronic histopathologic and neurochemical sequelae. Norton and Culver (1977), for example, asphyxiated rats with 10,000 ppm CO (18% lethality within 24 hours) on postnatal day 5 (PD5), and reported enhanced dendritic spine density in type II Golgi neurons in the caudate nucleus when subjects were sacrificed at two to seven months of age. Such treatment also induced a transient period of behavioral hyperactivity at six weeks of age. Ginsberg and Myers (1974a) exposed pregnant monkeys to 1000 to 3000 ppm CO (carboxyhemoglobin -HbCO- values were above 60%) for one to three hours at term. Among severely exposed fetuses, softening or necrosis of the basal ganglia was observed at the time of sacrifice. The subjects used in that report were maintained under intensive nursing care and were sacrificed for necropsy as they showed signs of expiring. Thus, many of the infants had experienced CO exposures incompatible with survival. Subsequent experiments, in which anaesthetized juvenile monkeys were exposed to similar CO concentrations for 75 to 325 minutes (Ginsberg and Myers, 1974 b,c), resulting in HbCO levels of 62 to 81%, also produced basal ganglia lesions in some subjects.

Because molecular oxygen is necessary for the synthesis and degradation of a variety of neurotransmitters, including the catecholamines dopamine (DA) and NE, and the indoleamine, serotonin (5-HT), several groups have measured neurotransmitter levels and activity during or immediately following hypoxic exposure.

Newby et al. (1978) reported that both neonatal hypoxia (8% oxygen) and CO (1500 ppm) exposure reduced the disappearance of DA in the caudate nucleus, following alpha methyl tyrosine administration in rats. They attributed this effect to decreased DA release. However, Silverstein and Johnston (1984) reported that 8% oxygen and unilateral carotid artery

ligation in PD7 rats resulted in decreased striatal DA levels, and elevation in homovanillic acid (HVA) levels. These neurochemical changes were of increased release, and were particularly noticeable on the ipsilateral side. They may, as the authors suggest, be a specific response to ischemia, as the elevation in HVA was not seen in animals made hypoxic alone. Nevertheless, it is not clear why the results of these two studies are not more coherent. Hedner et al. (1977) exposed rats to 12% oxygen for 30 minutes, and measured accumulations of the catecholamine and 5-HT precursors 3,4 dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan in whole brains, following a blockade of the synthesizing enzymes of l-amino acid decarboxylase. The accumulation of precursors was significantly lower in experimental subjects than in the controls, which suggested that the hypoxia interfered with synthesis of the monoamine neurotransmitters. In four-day-old rats exposed to 6% oxygen for 30 minutes and studied over a recovery period of six hours, a rebound elevation in DOPA levels was observed in the striatum (Hedner and Lundborg, 1980). Shellenberger (1982) reported that surviving five-day-old rats exposed to 4200 ppm CO for two hours (about 25% lethality) showed reduced striatal DA concentrations at 16-to-24 weeks of age.

VULNERABILITY OF THE HIPPOCAMPUS

There are a variety of a priori reasons to believe that the hippocampus, too, might be vulnerable to perinatal CO exposure. The hippocampus, especially the large pyramidal cells of Sommer's sector (CA1), appears to be extremely sensitive to asphyxiation (Brierley et al., 1973; von Lubitz and Diemer, 1983; Vogel, 1979). Moreover, reports of permanent memory deficits after prenatal CO levels in our laboratory (Mactutus and Fechter, 1984, 1985) are consistent with the notion of hippocampal injury.

The hippocampus receives significant cholinergic, noradrenergic, and serotonergic input from several different structures.

AIMS

The objectives of this project were to determine whether or not early CO exposure disrupts the ontogenic development of the brain, and whether or not the damage is quantitatively or qualitatively different from that observed in the adult organism. To accomplish this, neurochemical and histological methods will be used to assess the development of cells intrinsic to targeted brain regions, and the development of neurons that project their terminals into these areas. Specific questions include the following:

(1) Are catecholamine-containing neurons as a class especially vulnerable to CO exposure?

(2) Are 5-HT-containing neurons as a class especially vulnerable to CO exposure?

(3) Are the size and number of cells in the cerebral cortex, neostriatum, brainstem, hippocampus and cerebellum altered by prenatal CO exposure?

(4) Are the effects of CO on neurochemical systems persistent?

(5) What cell types in the cerebellum are vulnerable to CO exposure?

METHODS

SUBJECTS AND EXPOSURE CONDITIONS

Adult female Long Evans hooded rats were bred with males in the laboratory. Pregnancy was determined by vaginal lavage, and sperm-positive females were placed in one of four horizontal-flow, negative-pressure chambers, which were supplied with either air or air diluted with CO to a final concentration of 75, 150, or 300 ppm CO. A complete air change occurred every 11 minutes, and carbon dioxide concentrations measured by a Beckman LB-2 medical gas analyzer did not show accumulations above background. Subjects were maintained in single cages at 22°C, 60% relative humidity, and under diurnal lighting (12:12 hours) with free access to food and water. Chamber CO concentrations were monitored daily by an electrochemical detector (Ecolyzer) with calibrated gas (108 and 308 ppm CO in N_2) used as reference compounds. Spectrophotometric determinations of HbCO concentrations made on adult female rats maintained in the exposure chambers were made with an Instrumentation Laboratory 282 CO. Average HbCO concentrations in adult animals maintained in air alone and in the 75, 150, and 300 ppm chambers were 2.5 ± 0.7%, 11.5 ± 1.6%, 18.5 ± 2.3%, and 26.8 ± 5.4%, respectively. Subtle but permanent behavioral disorders are known to result from an exposure level of 150 ppm. Therefore, doses twice and one-half that amount were also used. CO exposure was maintained until PD1 (i.e. birth) or PD10 for reasons described above. Within 12 hours after parturition, the dams and litters were removed from the exposure chambers, the neonates were counted and weighed, and the litters were culled to eight pups. In experiments employing prenatal and neonatal CO exposures, litters were returned to the same exposure chambers until PD10. Pups remained with their dams for the remainder of the experimental period. Litters were weaned twenty-one days after birth.

CONTROL GROUP

Air control subjects were run concurrently with all CO exposures; the control animals were housed in a separate exposure chamber. Because the experiments to be reported extended over a three-year-period, all statistical analyses were conducted between control subjects exposed at the same time as the experimental subjects.

TISSUE STORAGE AND HANDLING

At various ages (generally PD10, 21, 28, 35, and 42), subjects were weighed and sacrificed by decapitation. The brain was rapidly removed and placed on ice. Brain regions were dissected according to the following method and rapidly placed on dry ice. First, the cerebellum was removed by

severing the cerebellar peduncles. The pons medulla was removed by making a diagonal cut just posterior to the inferior colliculi, and a vertical cut at the level of the obex on the ventral surface. In some experiments, the whole neocortex was removed for study, while in other experiments a portion of neocortex was removed by making vertical cuts on the ventral surface at the anterior limit of the corpus callosum at the level of the optic chiasm, followed by a horizontal cut at the level of the anterior commissure, and removal of septal and striatal tissue. The occipitotemporal cortex was then peeled back from the diencephalon, and the midbrain and hippocampus were removed. Tissue was stored at -80°C until assayed.

MONOAMINE ASSAYS

Brain tissue for endogenous monoamine content was analyzed according to the method of Kontur et al. (1984) by HPLC with electrochemical detection. Briefly, tissue samples were weighed, homogenized in 10 volumes of 0.1 perchloric acid (PCA) containing 0.5 or 1 ng/20 μl dihydroxybenzylamine (DHBA) as an internal standard, and centrifuged at 3000 x gravity for 15 min (2°C). The supernatant fraction was spin-filtered through 0.2-μm centrifugal microfilters (BAS) at 3000 x gravity for five minutes. The pellets were resuspended in 0.1 M NaOH for protein concentration determined according to the method of Lowry et al. (1951). Twenty microliters of the filtered supernatant fraction was injected onto a 5-μm C_{18} reverse-phase column (BAS Biophase ODS 6017). The mobile phase consisted of 650 ml 0.02 M citric acid, 250 ml 0.02 M sodium phosphate with 0.269 mm EDTA, 100 ml acetonitrile, and 85 mg sodium octyl sulfate (pH 3.15). Monoamine neurotransmitters (NE, 5-HT, and DA) and metabolites [5-hydroxyindole acetic acid (5HIAA), dihydroxyindole acetic acid (DOPAC), and HVA] were detected with a TL-5 glassy carbon electrode maintained at a potential of +0.72 V. Sample concentrations were determined with a Hewlett-Packard integrator (HP3390A) and were based on the ratio of the detector response for the endogenous amines to that of the internal standards. The HPLC system was recalibrated each morning and periodically throughout the day. Variability of detector response was 4% or less.

GABA ASSAY

Cerebellar GABA concentrations were determined in offspring at 10 and 21 days after birth, following exposure to either 0, 75, 150, or 300 ppm CO. One or two male pups were removed from each experimental litter, weighed, decapitated, and their cerebella removed within 30 to 40 seconds, rapidly frozen in thoroughly crushed dry ice, and stored at -80°C until assayed. Nine litters are represented in each exposure group at day 10 and seven to nine litters are represented in each exposure group on day 21.

Gamma aminobutyric acid was measured according to a method derived from that of Mailman et al. (1983), which was based on the reverse phase HPLC method of Lindrogh and Mopper (1979). At the time of assay, cerebella were homogenized in 10 parts (100 mg/ml) 0.05M HC1-47.5% ethanol containing 20 μg/ml 5-aminovaleric acid (5-AVA, Sigma Chem. Co.) as an internal standard. Samples were then spun at 3000 x gravity for 15 minutes. Fifty μl of a freshly-made o-phthaldialdehyde (OPT, Sigma)-borate buffer, composed of 0.4M potassium borate (pH 10.4), 5 μl/ml 2-mercaptoethanol and 1 mg/ml OPT, was then added to 50 μl of the sample supernatant. It was allowed to react for exactly 60 seconds, and then 5 μl was injected onto a 10 μm reverse phase C_{18} column (Altex 25 cm x 4.6 mm ID, Ultrasil-ODS) with a mobile phase of 0.02M potassium acetate (pH 5.5) - 48% methanol. Column effluents were monitored with a Schoeffel FS 970 spectroflurometer using an excitation wavelength of 330 nm and 418 nm emission (90% cutoff) filter.

Protein was determined by the method of Lowry et al. (1951), either from aliquots of homogenized samples removed before spinning (day 10) or from pellets obtained after spinning, and resuspended in 1M NaOH (day 21) using bovine serum albumin as the standard.

HIGH AFFINITY UPTAKE

High affinity GABAergic and glutaminergic uptake kinetic parameters (V_{max} and K_m) were determined among 21-day-old offspring that had been exposed to either 0 or 300 ppm CO. Four male pups from each of two litters at each exposure were utilized for GABA uptake studies; and three or four male pups from each of three litters at each exposure level were used for glutamate uptake studies.

Rats were decapitated and their cerebella rapidly removed and placed in 3 mls Hepes-sucrose homogenization medium of 0.32M sucrose and 2.5mM Hepes, pH 7.4 with 1M NaOH. After weighing, they were homogenized in a smooth glass homogenizer with a teflon pestle with 10 even strokes, using a Wheaton homogenizer on setting 3. The homogenate was added to 17 mls Hepes-sucrose homogenization medium and spun at 3000 x gravity for 10 minutes at 4°C. The pellet was discarded and the supernatant fluid was spun at 13000 x gravity for 20 minutes at 4°C. The crude synaptosomal pellets (P_2) were then gently resuspended in 2.8 mls of Krebs-Henseleit buffer, composed of 1.2mM $MgSO_4$, 1.5mM $CACl_2$, 10mM glucose, 2.5mM Hepes, 145mM NaCl, 5mM KCl (and for GABA studies, 10 μm amino-oxyacetic acid, Sigma) adjusted to pH 7.4 with 1M NaOH. Then 50 μl of each resuspended P_2 pellet (about 1 mg protein) was added to each of six tubes and to duplicates containing 1780 μl Krebs-Henseleit buffer plus 20 μl of labeled amino acid of varying concentrations. Uptake of ^{3}H-GABA (34.9 Ci/mM, New England Nuclear (NEN)) was assessed at six concentrations, ranging from 1 x 10^{-7}M to 7.5 x 10^{-9}M, with a specific activity of 0.349 Ci/mM. Uptake of ^{3}H-glutamate (45.8 Ci/mM, NEN) was assessed at six concentrations, ranging from 1 x 10^{-5}M to 7.5 x 10^{-7}M, with each tube containing 0.4 μCi.

In both cases, tubes were incubated in a slowly oscillating water bath for three minutes at 38°C and then immediately placed on ice. The duplicate tubes at each concentration were

kept on ice to control for non-specific uptake. Following incubation, incubated and duplicate tube contents were aspirated onto GF/B Whatman filter strips with a Brandel Harvester, which was then washed three times with ice cold 0.9% saline. The filters were then placed in minivials to which 5 mls Formula 947 (NEN) was added. The samples were counted in a Packard 300-C scintillation counter for 10 minutes after dark and cold adaptation.

Kinetic constants for each cerebellar tissue sample were calculated from individual Eadie-Hoffstee plots. In addition, total ^{3}H-GABA and ^{3}H- glutamate uptake per synaptosomal pellet was determined at the ^{3}H-amino acid concentration closest to the derived K_m.

Portions of the resuspended P_2 pellets were analyzed for protein content according to the method of Lowry et al. (1951), using bovine serum albumin because the standard and protein concentration did not vary between groups.

NUCLEIC ACID ASSAY

Protein and nucleic acid levels were measured to provide an index of average cell size and cell number in several brain regions. The DNA concentration is used as an index of packing density of cells (both neuronal and glial) and the ratio of protein to DNA is used as an indicator of average cell size. RNA and DNA determinations were measured using the method of Schmidt and Thannhauser (1945), as modified by Chandra et al. (1973). Protein levels were assayed by the method of Lowry et al. (1951). Striatal tissue and hippocampal tissue were pooled from two or three subjects from each of nine litters for each exposure condition. All other brain regions were sufficiently large to enable measurements from individual subjects. Ten subjects from different litters were used in determining cerebellum, cortical, and brainstem protein and nucleic acid levels. All tissues were homogenized in ten volumes of distilled water. All tissue samples were run in duplicate, with equal numbers of samples from each exposure condition run in a given assay.

Free nucleotides were extracted using a series of 5% trichloroacetic acid (TCA) washes. RNA was extracted in 0.3 M KOH during a four hour incubation at 37°C. The supernatants were assayed in duplicate for total RNA using the orcinol reaction (Mejbaum, 1939). RNA standards were made up from Bakers yeast. DNA was subsequently extracted twice in 5% TCA at 90° for 15 minutes. The DNA assay was based on the diphenylamine reaction (Burton, 1956). DNA standards were made from calf thymus.

Because protein levels determined in parallel with neurotransmitter determination generally involved the resuspension of a tissue pellet with NaOH, absolute protein levels may vary somewhat from determinations made using the above procedures.

LIGHT AND ELECTRON MICROSCOPY

At twenty-one days of age cerebellar morphology was examined among offspring of dams that had been exposed to either 0 or 300 ppm CO. One to three pups from each of three different litters are represented at each exposure level. Pups were anesthetized with approximately 1 ml/100 g 4% chloral hydrate, and perfused via intracardiac puncture with phosphate-buffered saline, PBS (0.02M KPO_4, 0.9% NaCl), followed by 4% buffered formaldehyde solution (37% formaldehyde solution diluted 1:10 with PBS). Brains were carefully removed and stored overnight in 4% formaldehyde solution and then changed to a 2% formaldehyde solution, in which they were stored until embedded in paraffin.

Comparable 5μm thick near-midline sagittal sections were obtained from each subject and stained with Toluidine Blue for light microscopic observation. A camera lucida tracing was made of each chosen section from each subject. For each subject an electronic graphic calculator (Numonics Corp. Model 1224) was used to count the total number of fissures and the mean depth of five major fissures, the intracentral, preculminate, primary, prepyramidal, and fissura secunda. These were measured as the distance from the fissure concavity to a tangential line drawn between the surface convexities of two adjacent folia. Number of Purkinje cell profiles in the lobulus centralis (Lobule III), which develops most extensively prior to day 10, as well as in the pyramis (Lobule VIII), which develops most extensively between days 10 to 20 (Altman, 1969), also were counted, and the length of the Purkinje cell layer in each of these lobules was determined on the camera lucida tracings.

For electron microscopy, the appearance of Purkinje and granule cells in the cerebellar cortex of subjects exposed to air or 300 ppm CO was studied at PD10 and 22. Because these studies provide qualitative data, a highly experienced electron microscopist evaluated the slides for neuropathology.

STATISTICAL METHODS

In most cases, the data were analyzed using parametric statistics (typically analysis of variance), with the dosage of CO serving as the principal between subjects variable of interest. Other methods used are fully described along with the description of the experiment.

RESULTS AND DISCUSSION

GENERAL FINDINGS

As part of several specific experiments described above, maternal weight gain, birthweights, and growth patterns of pups exposed to CO were measured as an indicator of general toxicity. In all cases, the mean from a given litter, rather than each individual subject, was used as the unit of analysis. Maternal HbCO levels averaged 2.5 $\pm$ 0.1%, 11.4 $\pm$ 0.3%, 1.85 $\pm$ 0.5%, and 26.8 $\pm$ 1.1% in the 0, 75, 150, and 300 ppm CO exposure chambers. Prenatal CO exposure did not significantly disrupt maternal weight gain during pregnancy ($F < 0.5$) (Figures 1 and 2), although dams in the 300 ppm exposure group appeared to gain slightly less weight than those in the other treatment groups. The number of pups born per litter (Table 1) was similarly unaffected by the CO exposure condition. The

birthweights of offspring born to CO-exposed dams were substantially lighter than those of the control offspring (Tables 1 and 2). Pair-wise comparisons between the control pups and the 150 and 300 ppm CO subjects indicated significant differences. Recovery of normal weight tends to occur with development (Tables 2, 3, and 4).

Maternal weight gain is not impaired among CO-exposed dams. This is important because it indicates that maternal toxicity, expressed as suppression of food and water intake, cannot account for the neurobiological effects of CO exposure observed in neonatal subjects. Presumably, the nutritional status of the pups at birth did not vary as a result of maternal nutrition. The source of the reduced birthweight observed in rat pups exposed in utero to CO is difficult to ascertain. At one level it may be consistent with the similar outcome observed in epidemiological studies of the offspring of cigarette smokers, and may be an integral part of a prenatal CO toxicity. Whatever its source, there is clear evidence that it does not reflect such potentially confounding factors as a systematic change in gestation duration or in litter size (Table 1).

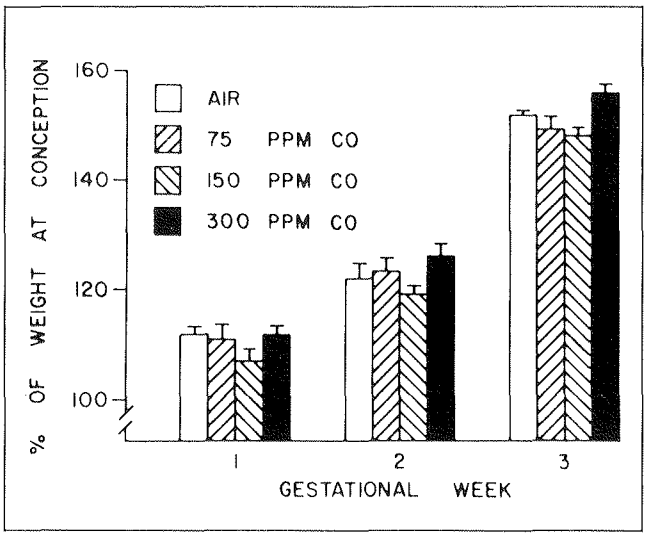

Figure 1: Maternal weight gain in control and CO-exposed dams expressed as a percentage of weight at time of a sperm positive vaginal lavage.

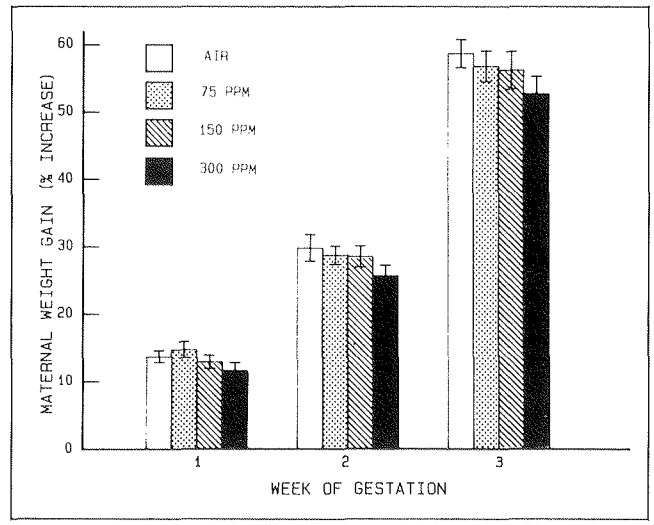

Figure 2: Effects of chronic exposure to air or 75, 150, or 300 ppm CO on maternal weight gain during pregnancy.

Table 2: Postnatal growth in rats exposed prenatally to CO.

		Mean body weight (g) ± SEM		
Exposure	No of litters	*Day 1	Day 21	Day 42
Air	11	6.4 ± 0.1	38.4 ± 1.1	121.2 ± 3.5
75 ppm	14	6.3 ± 0.1	39.6 ± 1.2	127.4 ± 5.8
150 ppm	13	6.2 ± 0.2	39.4 ± 1.3	125.4 ± 3.9
300 ppm	15	5.6 ± 0.2**	38.2 ± 1.1	113.6 ± 3.8

*Day 1 values are derived from Day 1 mean litter values of all male pups; Days 21 and 42 values are only of those male pups used for analyses of brain monoamine content.

**Significantly less than mean air weight by Dunnett's t test, $p < 0.01$.

Table 1: Effects of prenatal CO exposure on litter size, birthweight, and neonatal weight gain.

	CO Exposure ppm			
	0	75	150	300
Total # of Pups[1]	12.9 ± 0.7 (15)	12.5 ± 0.5 (19)	13.2 ± 0.5 (17)	11.7 ± 0.5 (17)
Birthweight (grams)[1]	6.32 ± 0.13 (12)	6.13 ± 0.12 (17)	5.76 ± 0.08* (16)	5.45 ± 0.12* (14)
Postnatal Day 21[1] Weight (grams)	43.6 ± 1.0 (12)	46.8 ± 1.4 (13)	45.8 ± 0.9 (13)	41.4 ± 1.7 † (9)

[1]Numbers given are mean ± SEM. Numbers in parentheses indicate number of litter means used in calculations.

†Significantly different from control and 75 ppm CO group, $p < 0.05$ by Newman Kuels Test.

*Significantly different from 75 ppm CO and 150 CO group, $p < 0.05$ by Newman Kuels Test.

The recovery of weight that occurs perinatally among CO-exposed subjects is viewed as particularly salutary, given the persistence of the specific markers of neurotoxicity described below.

CEREBELLAR EXPERIMENTS USING IN UTERO EXPOSURES

As part of several different experiments, the basic measurements of cerebellar wet weight, nucleic acid content, and protein content were made neonatally. These data are summarized in Tables 3 through 7. Of particular interest were measures of:

(1) cell packing density (DNA concentration);
(2) average cell size (protein/DNA);
(3) total protein concentration; and
(4) regional wet weight.

The data generally show that prenatal CO exposure reduces wet weight in the cerebellum (Tables 3, 4, and 6) in a dose-related fashion (Table 4). Both the total protein content and the DNA content tended to decrease with increasing CO exposure, although these decreases were not sufficiently great to be significant (Table 5). While cerebellar wet weight generally showed a decrease with CO exposures, this value did not always achieve significance.

Table 5: Cerebellar nucleic acid and protein levels at postnatal day 21, following prenatal CO exposure.[1]

	DNA (μg)[2]	RNA (μg)[2]	Protein (mg)[2]
Control	1097.2 ± 51.81	728.80 ± 27.05	23.39 ± 0.70
75 ppm CO	1094.0 ± 35.18	686.80 ± 21.79	22.92 ± 0.53
150 ppm CO	975.9 ± 35.38	716.30 ± 21.53	22.74 ± 0.89
300 ppm CO	987.4 ± 41.40	644.67 ± 24.44*	20.94 ± 0.70

[1]Mean ± SEM.
[2]Tissue from 10 litters was analyzed for this measure.
*Tissue from 9 litters was analyzed for this measure.
No statistically significant differences were found among the groups.

Table 6: Effects of pre- and perinatal CO exposure on high affinity cerebellar ^{3}H-GABA uptake.[a]

	Exposure	
	Air (8)	300 ppm (8)
Cerebellar weight (mg)	226.400 ± 4.500	203.500 ± 4.100**
V_{max} (pm/mg protein/min)	6.400 ± 0.520	6.380 ± 0.400
K_m (μM)	0.023 ± 0.002	0.024 ± 0.002
Total uptake at 0.025 μM GABA (pm/P_2 pellett/min)	21.200 ± 0.800	18.100 ± 0.400**

[a]Determined in crude synaptosomal preparations among rats exposed to air or chronic CO from the day of conception until the tenth day after birth. Numbers in parentheses are numbers of subjects. Data given as $\overline{X}$ ± SEM.
** Significantly less than air, Students *t*-test, $p < 0.01$.

Table 3: Body, cerebellar, and cortical weights in rats exposed to either air or 150 ppm CO prenatally.

	Mean body weight (g) ± SEM		Mean cerebellar weight (mg) ± SEM		Mean cortical weight (mg) ± SEM	
Day after birth	Air	CO	Air	CO	Air	CO
14	27.3 ± 1.2	28.6 ± 1.6	132.0 ± 6.2	133.0 ± 4.2	144.4 ± 10.0	128.0 ± 10.0
21	40.1 ± 1.3	39.4 ± 2.3	195.3 ± 5.6	183.4 ± 5.7	143.8 ± 8.1	165.8 ± 4.8
28	57.8 ± 2.7	60.3 ± 3.7	198.8 ± 5.3	193.9 ± 5.4	138.0 ± 7.4	169.1 ± 10.2
35	86.8 ± 4.7	87.3 ± 3.1	227.7 ± 8.1	215.4 ± 5.9	154.1 ± 6.5	148.2 ± 6.3
42	125.1 ± 5.1	130.5 ± 2.7	237.5 ± 4.7	242.9 ± 5.0	160.7 ± 4.5	176.3 ± 7.6

No significant differences were found between air- and CO-exposed subjects, using ANOVA.

Table 4: Effects of pre- and perinatal CO exposure on cerebellar GABA levels at postnatal day 10 and 21, given as mean ± SEM.[a]

	Exposure	Body[b] Weight (gm)	Cerebellar[c] Weight (mg)	Protein (μg/mg)	GABA (nm/mg)	Total[d] GABA (nm)
Day 10	Air (10)	20.7 ± 0.4	81.9 ± 2.0	124.4 ± 6.4	0.324 ± 0.010	26.54 ± 1.07
	75 ppm (10)	18.5* ± 0.4	75.8 ± 1.9	128.2 ± 5.6	0.310 ± 0.010	23.55 ± 1.09
	150 ppm (10)	16.9* ± 1.0	68.4** ± 2.2	122.8 ± 4.1	0.316 ± 0.010	21.39** ± 0.87
	300 ppm (10)	15.6* ± 0.8	60.5** ± 3.1	124.6 ± 4.2	0.324 ± 0.012	19.68** ± 1.30
Day 21	Air (12)	40.1 ± 1.3	203.2 ± 2.2	121.9 ± 1.4	0.368 ± 0.011	74.75 ± 2.49
	75 ppm (10)	39.3 ± 1.3	189.5** ± 3.9	121.1 ± 2.3	0.360 ± 0.012	68.19 ± 2.30
	150 ppm (11)	39.2 ± 1.5	183.9** ± 3.3	121.4 ± 2.7	0.362 ± 0.011	66.30* ± 1.40
	300 ppm (11)	39.8 ± 1.0	189.7** ± 4.0	119.1 ± 2.0	0.357 ± 0.011	67.66** ± 2.60

[a]Determined among rats exposed to chronic CO from the day of conception until the tenth day after birth. Numbers in parentheses are numbers of subjects.
[b]CO exposure had a significant impact on body weight 10 days after birth. ANOVA $F(3,36) = 8.9$, $p < 0.01$. *Significantly less than air, Dunnetts *t*-test, $p < 0.05$.
[c]CO exposure had a significant impact on cerebellar weight 10 and 21 days after birth. Day 10 ANOVA $F(3,36) = 13.8$, $p < 0.01$, Day 21 ANOVA $F(3,40) = 6.3$, $p < 0.01$. **Significantly less than air, Dunnetts *t*-test, $p < 0.01$.
[d]CO exposure had a significant impact on total cerebellar GABA content 10 and 21 days after birth. Day 10 ANOVA $F(3,36) = 6.6$, $p < 0.01$, Day 21 ANOVA $F(3,40) = 3.0$, $p < 0.05$. **Significantly less than air, Dunnetts *t*-test, $p < 0.01$.

Table 7: Effects of pre- and perinatal CO exposure on high affinity cerebellar ^{3}H-glutamate uptake.[a]

	Exposure	
	Air (10)	**300 ppm (10)**
Cerebellar weight (mg)	238.80 ± 4.100	221.00 ± 4.20**
V_{max} (pm/mg protein/min)	950.00 ± 98.00	932.00 ± 68.00
K_m (μM)	3.01 ± 0.33	2.59 ± 0.12
Total uptake at 1.25 μM glutamate (nm/P_2 pellet/min)	2.45 ± 0.31	2.19 ± 0.26

[a]Determined in crude synaptosomal preparations among rats exposed to air or chronic CO from the day of conception until the tenth day after birth. Numbers in parentheses are numbers of subjects. Data given as $\bar{X}$ ± SEM. **Significantly less than air, Students *t*-test, $p < 0.01$.

These results are consistent with the hypothesis that CO exposure slightly reduces the size and number of cellular elements in the cerebellum. The extent of this loss is not sufficiently large to reliably decrease DNA levels. However, it is possible that a selective loss of large cells in the cerebellum, coupled with an increase in smaller cells, could mitigate against finding a significant shift in this measure. Total DNA is a measure of the total number of cells, not an estimate of the types of cells present. The reduction in cerebellar weight, protein content, and DNA content show internal consistency.

STUDIES OF EXTRINSIC CATECHOLAMINE NEURONS

Based on the evidence cited above, which appears to show a particular vulnerability of noradrenergic neurons to asphyxiation, the development of NE-containing neurons was compared in two brain regions, each of which receives significant NE input from the neurons that have their cell bodies in the brainstem.

The normal innervation of CNS target areas by aminergic neurons during development is characterized by predictable patterns of increases in the concentration of amines within the innervated region (Coyle, 1977). Disruptions of aminergic neuronal development often are reflected in deviations from these normal patterns of amine concentration within affected regions (Johnston and Coyle, 1980; Matsutani et al., 1980; Bartolome et al., 1982; Beaulieu and Coyle, 1983).

One male pup from each of the litters was weighed and decapitated on days 14, 21, 28, 35, or 42 after birth. The entire cerebellum was removed by severing the cerebellar peduncles. A uniform portion of neocortex was removed by making vertical cuts on the central surface at the anterior limit of the corpus callosum, and on the level of the optic chiasm. This was followed by a horizontal cut at the level of the anterior commisure, and the subsequent removal of the septal and the striatal tissue. Both tissues were rapidly frozen over dry ice and stored at -80°C. The NE content was determined according to a method modified from that of Hefti et al. (1980) and described in Storm et al. (1984), which used HPLC with electrochemical detection. All data were analyzed for day and treatment effects using a two-way analysis of variance.

No significant deficits in general body, cerebellar, or cortical growth were evident in the CO-exposed offspring, although the overall cerebellar weights appeared slightly smaller among exposed pups (Table 3). However, the NE concentration was consistently elevated above the control values in the cerebella of CO-exposed offspring (Figure 3A). Further, whereas the air-exposed offspring in this study showed the expected normal maturation pattern of cerebellar NE concentration (Sievers et al., 1981), which increased through the fourth postnatal week and then leveled off, the CO-exposed group showed a pattern of cerebellar NE concentration that continued to increase up to the sixth postnatal week.

When NE was expressed as total content per cerebellum, the difference between the air-exposed and the CO-exposed groups persisted, although the magnitude of the effect was much smaller (Figure 3B). This suggests that slightly decreased cerebellar weights observed among the CO-exposed offspring probably contributed to, but cannot completely explain, the enhanced NE concentration reflected in Figure 3A.

The NE concentration in the cortex and the total NE content per cortical portion for both air- and CO-exposed pups rose gradually and linearly from day 14 to day 42 in a normal maturation pattern (Figure 3C and D), similar to that described by Sievers et al. (1981). No significant differences in cortical NE were observed between the groups.

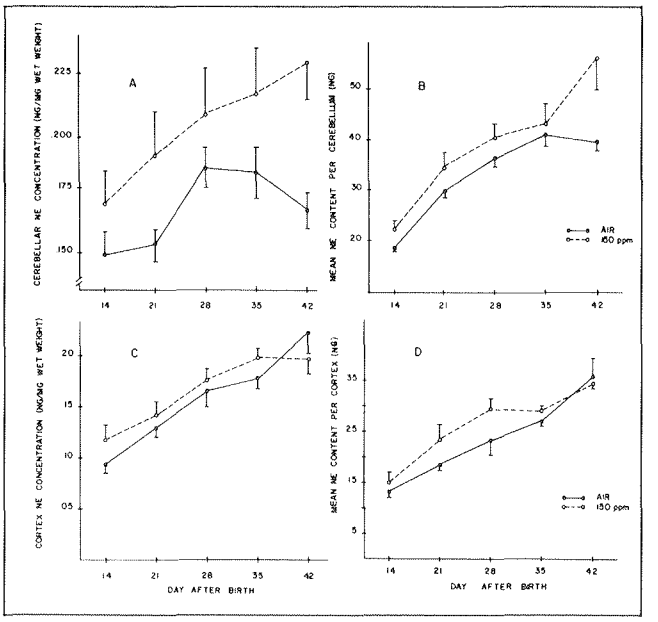

Figure 3: Mean NE concentration and total content (± SEM) from day 14 to day 42 after birth, in cerebellum (A and B) and cortex (C and D) of rats exposed to either air (solid lines) or chronic 150 ppm CO (broken lines) prenatally. Overall cerebellar NE concentration and total NE content among CO-exposed rats are significantly greater than those of air-exposed rats by two-way analysis of variance (treatment effect: $p < 0.01$ and $p < 0.05$, respectively). Cortical NE concentration and total content do not differ between groups.

The small, but significant, increase in the total NE per cerebellum among CO-exposed rats suggests that the cerebellar weight deficits were not entirely responsible for the increased NE concentration. It is possible that the elevated NE concentration also is partly a result of increased storage or decreased turnover, or both, of NE in the affected cerebella. If so, it may reflect altered synaptic relationships that are secondary to subtle deficits incurred by neurons intrinsic to the cerebellum itself. For example, Purkinje cells, the major macroneurons of the cerebellum, are a major target of the noradrenergic nerve terminals (Bloom et al., 1971), whose development has been shown to be sensitive to hypoxic hypoxia in the neonatal period (Yu and Yu, 1980). Also, in PCD-mutant mice, the increases in the cerebellar NE concentrations are associated with small increases in the total amount of NE per cerebellum (Ghetti et al., 1981), a phenomenon similar to that observed here. Moreover, Purkinje cells, as well as the basket, stellate, and Golgi cerebellar interneurons, release the inhibitory neurotransmitter, GABA (Hokfelt and Ljungdahl, 1970, 1972; McLaughlin, et al. 1974). Decreases in the total cerebellar GABA content and the total ^{3}H-GABA high-affinity uptake were observed in the cerebella of 21-day-old rats exposed to 300 ppm CO from the day of conception until the 10th day after birth (Storm et al., in press) (see below). The elevations in NE concentration and the total content reported here may, therefore, be the result of both increased noradrenergic terminal density in a slightly smaller cerebellum, and of alterations in its synaptic connectivity. Neurochemical studies alone cannot adequately address these hypotheses.

To further document the effects of prenatal CO exposure on cerebellar development, an additional dose response study of cerebellar NE and 5-HT levels was conducted at 21 and 42 days of age (Tables 8 and 9). Although NE levels tend to be slightly elevated at both ages relative to control subjects, these differences failed to be significant (Table 8). This finding appears to contradict this laboratory's initial experimental results, but it should be recalled that the statistical elevation of NE concentrations in the cerebellum was observed because of the stability of the elevation across all age groups studied. Taken together, the results of these two experiments suggest a weak but persistent elevation in the NE concentration in the cerebellum of CO-exposed subjects. These results are not consistent with the hypothesis that noradrenergic neurons, as a class, are affected preferentially and in a unitary fashion by CO treatment. No effect of prenatal CO on cerebellar 5-HT was seen at PD21 (Table 9).

Table 8: Regional NE concentration of rat pups 21 and 42 days after birth, following chronic prenatal exposure to CO.[a]

		Mean NE concentration (ng/mg) ± SEM			
	Exposure	**Ponsmedulla**	**Neocortex**	**Hippocampus**	**Cerebellum**
Day 21	Air	0.715 ± 0.200 (8)*	0.140 ± 0.006 (8)	0.245 ± 0.025 (8)	0.185 ± 0.006 (10)
	75 ppm	0.712 ± 0.022 (9)	0.149 ± 0.004 (7)	0.228 ± 0.012 (7)	0.198 ± 0.006 (10)
	150 ppm	0.671 ± 0.022 (7)	0.158 ± 0.008 (6)	0.234 ± 0.017 (7)	0.194 ± 0.006 (10)
	300 ppm	0.665 ± 0.013 (8)	0.146 ± 0.050 (6)	0.237 ± 0.042 (4)	0.201 ± 0.010 (10)
Day 42	Air	0.697 ± 0.015 (8)	0.200 ± 0.008 (7)**	0.258 ± 0.017 (8)***	0.173 ± 0.011 (12)
	75 ppm	0.655 ± 0.032 (8)	0.208 ± 0.007 (8)	0.284 ± 0.026 (8)	0.191 ± 0.014 (12)
	150 ppm	0.687 ± 0.021 (8)	0.218 ± 0.010 (9)	0.286 ± 0.023 (8)	0.192 ± 0.017 (12)
	300 ppm	0.714 ± 0.023 (8)	0.228 ± 0.008 (7)	0.315 ± 0.013 (8)	0.186 ± 0.008 (12)

[a] Numbers in parentheses represent number of subjects.
* Significant linear trend of decreasing NE concentration as prenatal CO exposure concentration increases [$F(1.28) = 4.4$, $p < 0.05$].
** Significant linear trend of increasing NE concentration as prenatal CO exposure concentration increases [$F(1.28) = 5.7$, $p < 0.05$].
*** Linear trend of increasing NE concentration as prenatal CO exposure concentration increases [$F(1.23) = 3.5$, $p < 0.10$].

Table 9: Regional 5-HT concentration of rat pups 21 and 42 days after birth, following chronic prenatal exposure to CO.[a]

		Mean 5-HT concentration (ng/mg) ± SEM			
	Exposure	**Ponsmedulla**	**Neocortex**	**Hippocampus**	**Cerebellum**
Day 21	Air	0.829 ± 0.011 (7)*	0.138 ± 0.013 (8)	0.270 ± 0.010 (8)	0.053 ± 0.007 (7)
	75 ppm	0.805 ± 0.021 (9)	0.151 ± 0.015 (7)	0.277 ± 0.012 (7)	0.064 ± 0.010 (7)
	150 ppm	0.729 ± 0.030 (6)**	0.146 ± 0.012 (6)	0.337 ± 0.027 (7)	0.073 ± 0.009 (7)
	300 ppm	0.750 ± 0.010 (7)**	0.152 ± 0.013 (7)	0.298 ± 0.020 (5)	0.048 ± 0.006 (7)
Day 42	Air	0.764 ± 0.019 (8)	0.175 ± 0.007 (7)	0.305 ± 0.034 (8)	—
	75 ppm	0.737 ± 0.047 (8)	0.187 ± 0.009 (9)	0.306 ± 0.030 (8)	—
	150 ppm	0.704 ± 0.027 (8)	0.187 ± 0.013 (9)	0.311 ± 0.018 (7)	—
	300 ppm	0.758 ± 0.029 (8)	0.202 ± 0.015 (7)	0.360 ± 0.046 (8)	—

[a] Numbers in parentheses represent number of subjects.
* Significant linear trend of decreasing 5-HT concentration as prenatal CO exposure concentration increases [$F(1.25) = 9.9$, $p < 0.05$].
** Significantly less than mean air 5-HT concentration by Dunnett's t test [150 ppm $t(4.25) = 2.9$; 300 ppm $t(4.25) = 3.6$; $p < 0.05$].

PRE- AND NEONATAL CARBON MONOXIDE EXPOSURES: EFFECTS ON INTRINSIC CEREBELLAR NEURONS

1. Determination of Cerebellar GABA Levels

Cerebellar GABA concentrations were determined in offspring at 10 and 21 days after birth, following exposure to either 0, 75, 150, or 300 ppm CO. One or two male pups were removed from each experimental litter, and were weighed and decapitated. Their cerebella were removed within 30 to 40 seconds, rapidly frozen in thoroughly crushed dry ice, and stored at -80°C until assayed.

Information from those pups utilized for cerebellar GABA content is shown in Table 4.

Although body weights were significantly depressed among CO-exposed subjects at 10 days of age, no differences were found to persist at 21 days of age. Cerebellar weight, on the other hand, was significantly reduced at both 10 and 21 days of age at all levels of CO tested despite the fact that CO exposure terminated at 10 days of age. Along with the decrease in cerebellar weight, total GABA levels were also significantly reduced among CO-treated subjects at both ages. This result stands in contradistinction to those described above for NE and 5-HT and below for glutamate. Because the total GABA content per cerebellum was considerably depressed at day 10, and remained depressed at day 21, the results suggest that there was a decrease in the number of GABAergic neurons present at day 10, as a result of CO exposure, that was not made up by day 21. The lack of any effect of CO exposure on the GABA concentration at either age further suggests that the density of GABAergic neurons was unaffected, and that the number of these neurons was decreased in proportion to the decrease in cerebellar weight.

2. Determination of High Affinity GABA and Glutamine Uptake

High affinity GABAergic and glutaminergic uptake kinetic parameters (V_{max} and K_m) were determined among 21-day-old offspring that had been exposed to either 0 or 300 ppm CO. Four male pups from each of two litters at each exposure were used for GABA uptake studies, and three or four male pups from each of three litters at each exposure level were used for glutamate uptake studies.

Cerebellar weight deficits after exposure to 300 ppm CO were observed among those pups utilized for determination of ^{3}H-GABA and ^{3}H-glutamate high affinity uptake parameters, as shown in Tables 6 and 7. CO exposure had no effect on apparent V_{max} or K_m values determined among these offspring for either GABA or glutamate uptake. However, the total amount of ^{3}H-GABA uptake into the synaptosomal preparation was significantly decreased in the CO exposed pups (Table 6), while the total amount of ^{3}H-glutamate uptake was not (Table 7). Because equivalent protein levels were found among control and CO-treated subjects, the difference in total GABA uptake cannot be interpreted as a non-specific consequence of reduced cerebellar weight and, thereby, a smaller P_2 pellet among treated subjects. Further, an intepretation that significant decrements in GABA markers among experimental subjects is an artifact of reduced cerebellar weight is inappropriate, because markers for other neurotransmitters in the cerebellum (NE, 5-HT, glutamate) do not show similar changes.

CEREBELLAR HISTOLOGY

Representative camera lucida tracings of mid-sagittal cerebellar sections of pups exposed to either air alone or 300 ppm CO appear in Figure 4. The cerebella of the exposed pups were uniformly smaller and, as shown in Figure 5, had significantly fewer total fissures. Mean depth of major fissures, however, did not differ significantly between the experimental and control groups (Figure 5). No significant differences were observed between the groups in the total number or density of Purkinje cells (Figure 6), although both lobules contained about 10% fewer cells. Ultrastructural studies showed evidence of degenerating granule cells at PD10 in subjects exposed pre- and perinatally to 300 ppm CO (Figure 7). At 21 days of age, similarly treated subjects showed evidence of PCD in the cerebellum. Figure 8 shows an irregular nucleus and cell membrane, as well as a swollen Golgi apparatus in a CO-treated subject. A typical control subject is shown in Figure 9.

The observation of deficits in the cerebellar weight, and decreases in the total cerebellar content and uptake of GABA, attest to a profound effect of developmental CO exposure upon the postnatal development of the cerebellar cortex. These results further suggest that CO exposure interferes with both specific neurons and the general maturation processes that determine the number of fissures in the maturing cerebellum.

The postnatal growth of the cerebellum is marked by gradual increases in cerebellar weight and by increases in GAD activity, GABA uptake, and GABA content (Coyle and Enna, 1976). Following CO exposure, both the weight and the GABA content were significantly decreased at 10 days of age in a clearly dose-related fashion. The cerebellar weights of pups exposed to 75, 150, and 300 ppm CO were 93%, 85%, and 74%, respectively, of the air-exposed pups. Similarly, the GABA content of the CO-exposed pups was 89%, 81%, and 74% of the air-exposed pups. But, by 21 days of age, 11 days following the cessation of CO exposure, the cerebellar weight, as well as the GABA content among affected CO-exposed groups, was about 90% of that of the air-exposed pups, indicating that some incomplete recovery occurred in all groups (Table 4).

The partial recovery of cerebellar mass may be accounted for by a general recovery of postnatal growth following the cessation of CO exposure, because the body weights were no longer depressed among CO-exposed pups by 21 days of age. The pups' recovery from CO exposure at day 10 also may have been associated with events which normally occur in the cerebellum well into the second and third postnatal week (Altman, 1972b, c). These include increased rates of maturation of those neurons already present and increased rates of cellular proliferation.

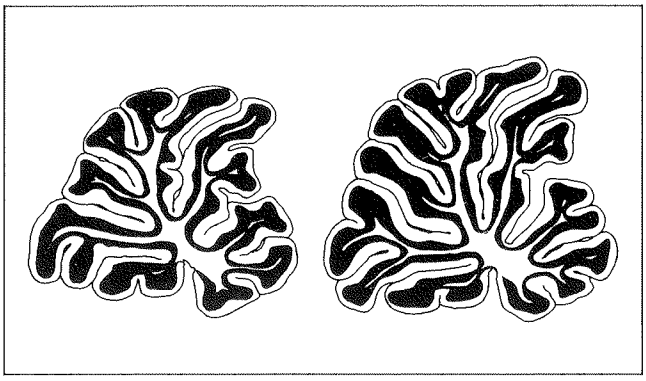

Figure 4: Representative camera lucida tracings of near midline sagittal sections from 21-day-old rats exposed from the day of conception until the tenth day after birth to air (B) or 300 ppm CO (A).

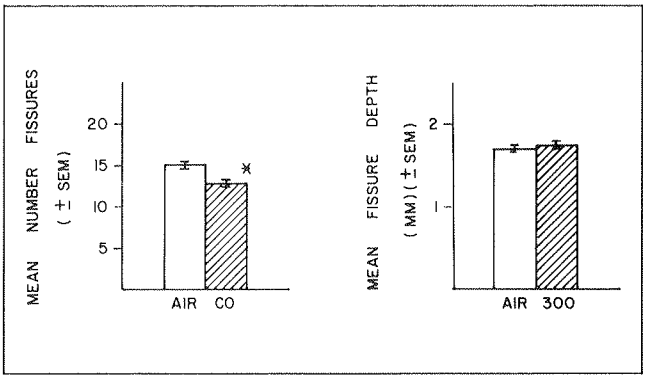

Figure 5: Mean number of fissures and mean fissure depths in cerebellums of 21-day-old rats exposed to air or 300 ppm CO from the day of conception until the tenth day after birth. Mean number of fissures are 15.0 ± 0.6 following air exposure and 12.9 ± 0.5 following CO exposure. Mean fissure depths are 1.70 ± 0.05mm following air exposure and 1.77 ± 0.05 mm following CO exposure. *Significantly less than air, $p < 0.05$, Students t-test.

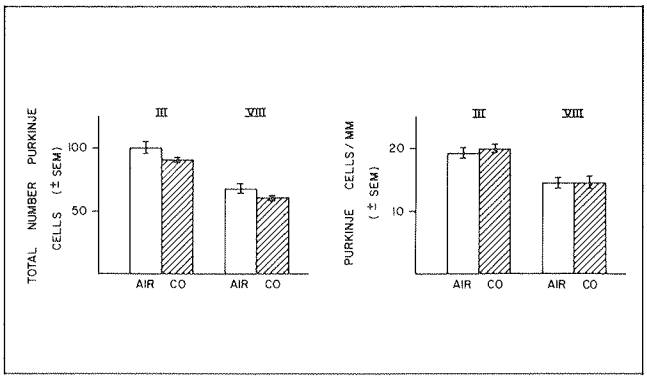

Figure 6: Mean number and density of Purkinje cells in cerebellar lobules III and VIII in 21-day-old rats exposed to air or 300 ppm CO from the day of conception until the tenth day after birth. In lobule III, mean total number of Purkinje cells are 99.7 ± 5.1 following air exposure and 90.7 ± 1.5 following CO. Density of Purkinje cells are 19.3 ± 1.1/mm Purkinje cell layer following air exposure and 19.9 ± 1.3/mm Purkinje cell layer following CO exposure. In lobule VIII, mean total number of Purkinje cells are 67.6 ± 4.5 following air exposure and 60.9 ± 3.0 following CO exposure. Density of Purkinje cells are 14.4 ± 0.9/mm Purkinje cell layer following air exposure and 14.4 ± 1.0/mm Purkinje cell layer following CO exposure.

The decrease in the total cerebellar GABA uptake at day 21, observed among pups exposed to 300 ppm CO, supports this notion (Table 6), despite normal GABAergic uptake kinetic parameters. Finally, the histological data indicate that both Purkinje and granule cells are injured by perinatal CO exposure. A likely hypothesis, given all of the data, is that the disruption in Purkinje cell development is a permanent consequence of early CO exposure, but the injury of granule cells may be rectified either by continued proliferation of these cells, or by curtailment of the normal pairing of cells seen in development.

HIPPOCAMPAL STUDIES

The hippocampus and, in particular, Sommer's Section, is highly susceptible to hypoxic damage. Therefore protein, nucleic acid, and monoamine neurotransmitters, whose terminals lie inside the hippocampus but whose cell bodies are extrinsic to that structure, were studied.

We failed to find evidence that prenatal CO exposure alters hippocampal cell density or cell size, as indexed by nucleic acid and protein levels (Table 10). However, a few neurochemical experiments were conducted. These were justified because they were based on evidence of memory loss in the subjects (Mactutus and Fechter, 1984, 1985), which would be a finding consistent with hippocampal damage.

These experiments consisted of measuring the levels of NE, 5-HT, and the serotonin metabolite, 5HIAA, in rats exposed in utero, as above, and in utero plus neonatally to day 10. Perturbation of these neurotransmitters by CO might occur either in response to damage to the intrinsic hippocampal neurons or, in the case of specific injury to 5-HT and NE neurons, throughout the brain. It was found (Table 11) that the prenatal CO exposure protocol elevated NE levels in the hippocampus at day 42 only, but only with a confidence level of $p < 0.10$. No change in the hippocampal weight or in 5-HT (Table 11) was observed at either 21 or 42 days of age.

As Table 11 shows, there are no clear trends in the data to suggest that CO exposure systematically affected hippocampal NE or 5-HT, tissue weight, or protein concentration levels. In addition, CO exposure did not significantly alter the 5-HT metabolite (5HIAA) levels.

Table 10: Hippocampal nucleic acid and protein levels at postnatal day 21, following prenatal CO exposure ($\bar{X}$ ± SEM).

	DNA (μg)[1]	RNA (μg)[2]	Protein (mg)[1]
Control	165.2 ± 6.3	436.7 ± 20.1	14.91 ± 0.69
75 ppm CO	153.5 ± 3.6	431.0 ± 34.0	13.86 ± 0.80
150 ppm CO	153.6 ± 7.9	432.8 ± 29.9	13.75 ± 0.85
300 ppm CO	145.2 ± 8.5	407.0 ± 27.3	12.15 ± 0.64

[1]Tissue from 12 litters was analyzed for this measure.
[2]Tissue from 10 litters was analyzed for this measure.
No statistically significant differences were found among the groups.

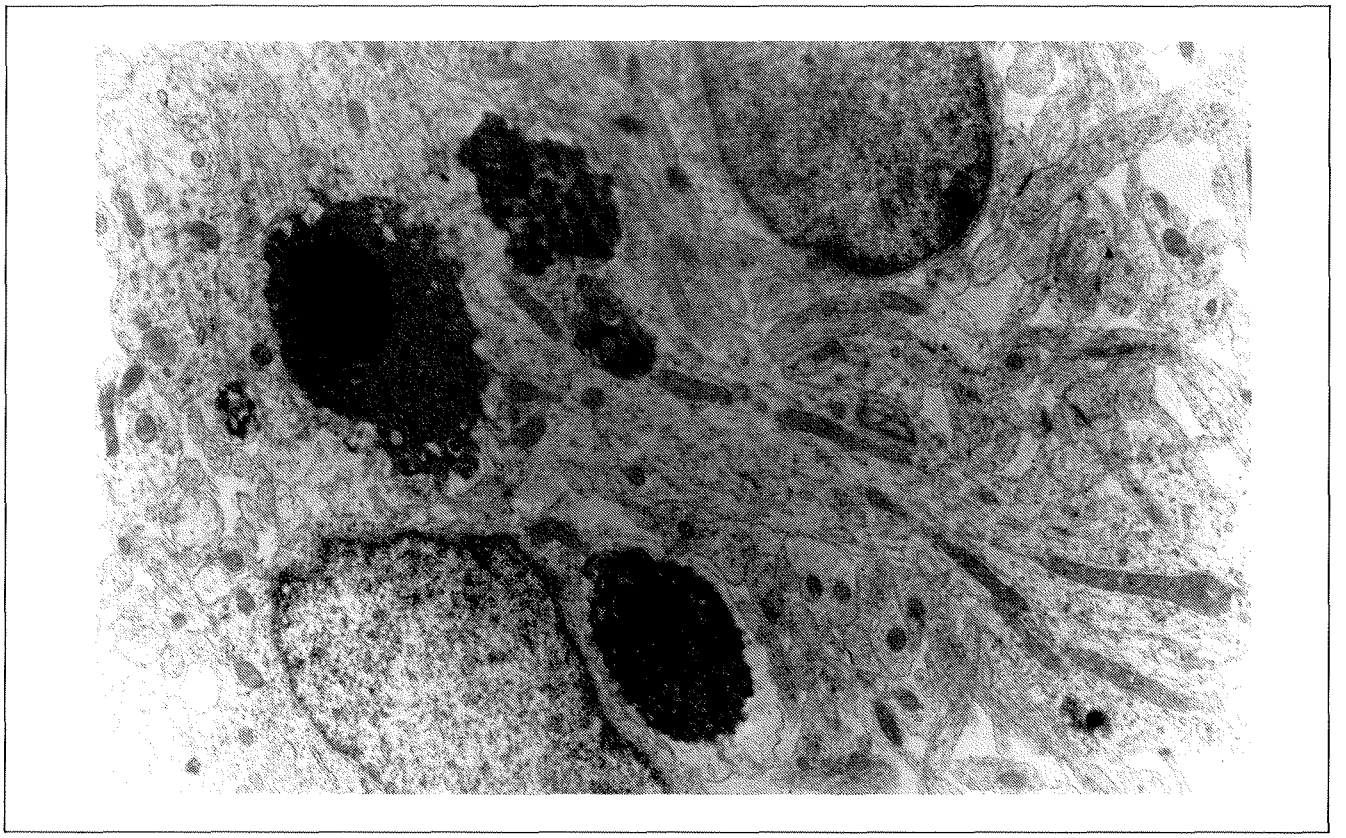

Figure 7: Degenerating granule cells in postnatal day 10 subject exposed to 300 ppm CO. 4420x.

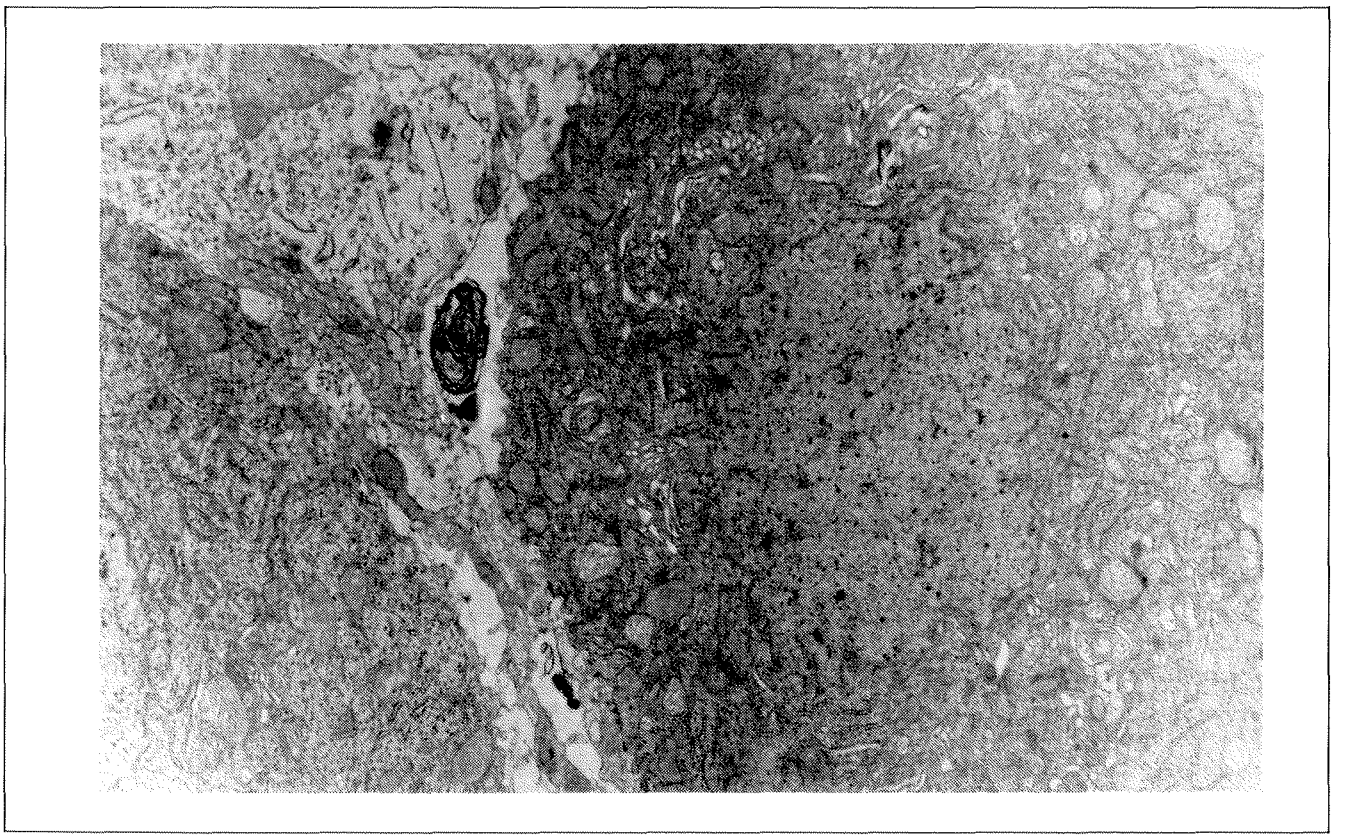

Figure 8: Degenerating Purkinje cell in postnatal day 21 subject exposed to 300 ppm CO. Note irregular nucleus and cell membrane, swollen Golgi apparatus. 2925x.

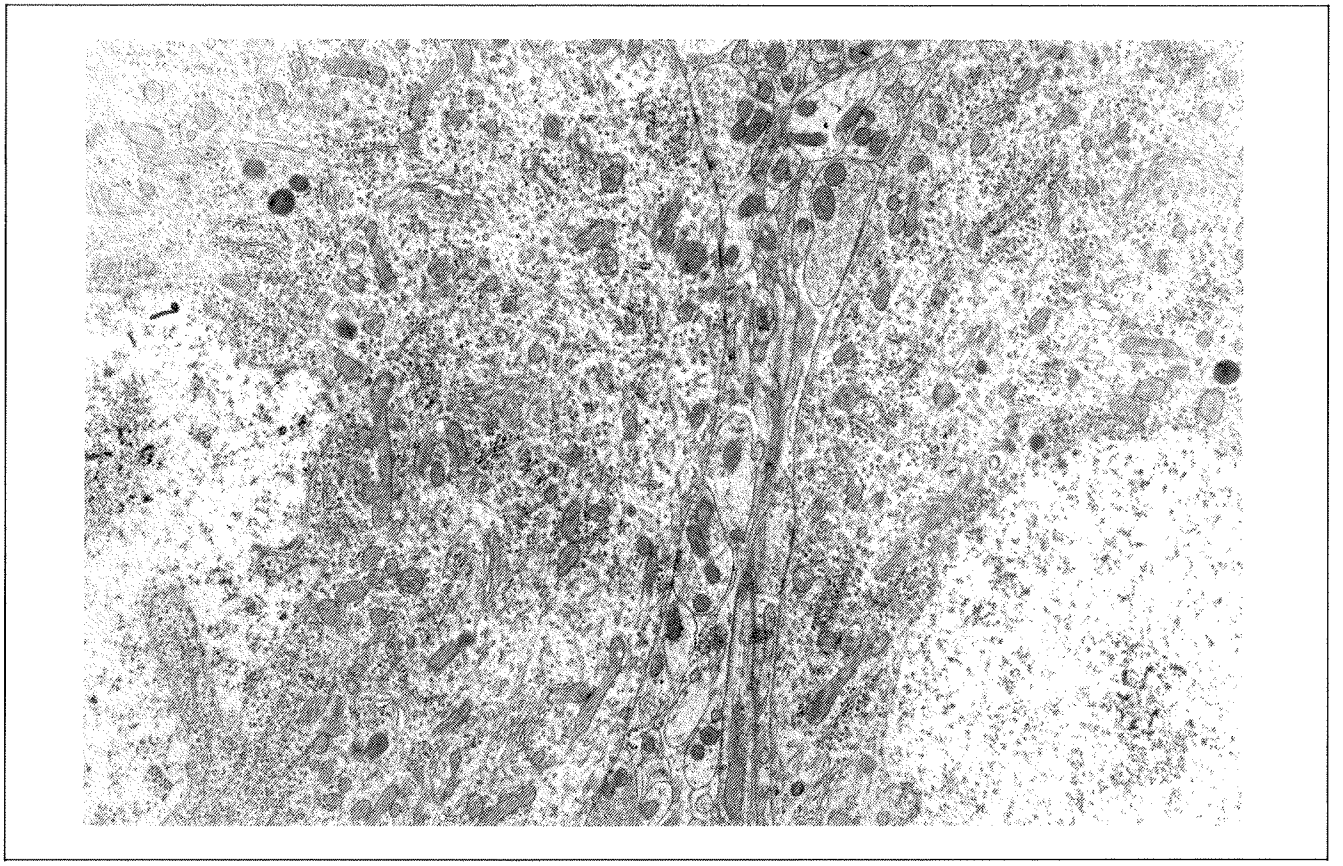

Figure 9: Purkinje cells in postnatal day 21 control subject. Note regularity of cell membranes. 3640x.

There appear to be substantial qualitative differences between the effects of CO in the cerebellum and the hippocampus. Although the cerebellum of exposed subjects was lighter in weight and showed a consistent, though small, elevation of the extrinsic neurotransmitter NE, no such shift in weight was observed in the hippocampus. Further, NE was elevated only at one age level, and was significant only if the confidence limit substantially relaxed the case of the hippocampus. An additional experiment, in which NE and 5-HT levels were measured at PD10, 14, and 21, following combined pre- and neonatal CO exposure, failed to produce positive effects in the hippocampus (Figures 10 to 12).

Table 11: Hippocampal weight, protein, and monoamine neurotransmitter levels at postnatal days 21 and 42, following prenatal CO exposure.[a]

	Exposure	Weight (mg)	Protein (μg/mg)	NE (ng/mg)	5-HT (ng/mg)	5-HIAA (ng/mg)
Day 21	Air	84.6 ± 2.3 (8)	69.2 ± 3.5 (8)	0.245 ± 0.025 (8)	0.270 ± 0.010 (8)	0.419 ± 0.030 (8)
	75 ppm	81.7 ± 2.1 (8)	66.2 ± 5.7 (8)	0.248 ± 0.012 (7)	0.277 ± 0.012 (7)	0.478 ± 0.033 (8)
	150 ppm	85.0 ± 4.3 (7)	66.2 ± 5.0 (7)	0.234 ± 0.017 (7)	0.337 ± 0.027 (7)	0.518 ± 0.039 (7)
	300 ppm	82.8 ± 3.6 (6)	73.7 ± 3.5 (6)	0.237 ± 0.042 (4)	0.298 ± 0.020 (5)	0.462 ± 0.060 (5)
Day 42	Air	93.4 ± 4.0 (8)	79.2 ± 2.5 (8)	0.258 ± 0.017 (8)[b]	0.305 ± 0.034 (8)	0.402 ± 0.042 (8)
	75 ppm	96.2 ± 3.2 (8)	80.2 ± 2.1 (8)	0.284 ± 0.026 (8)	0.306 ± 0.030 (8)	0.385 ± 0.039 (8)
	150 ppm	98.6 ± 3.4 (8)	80.1 ± 4.4 (8)	0.286 ± 0.023 (8)	0.311 ± 0.018 (7)	0.395 ± 0.037 (7)
	300 ppm	92.1 ± 2.9 (8)	77.3 ± 1.2 (8)	0.315 ± 0.013 (8)	0.360 ± 0.046 (8)	0.363 ± 0.048 (8)

[a] Values given are $\overline{X}$ ± SEM. Numbers in parentheses represent the number of subjects.
[b] Linear trend of increasing NE concentration as prenatal CO exposure level increases ($F(1,23) = 3.5$, $p < 0.10$).

NEOSTRIATAL EXPERIMENTS

The effect of perinatal CO exposure on neostriatal nucleic acid and protein levels is shown in Table 12. No differences in these measures are apparent between treatment groups that received CO exposure only during the prenatal period (Table 12A). However, a significant dose-dependent elevation in striatal DNA concentration was observed in 21 day old rats that had been exposed to CO from conception until PD10 ($F = 3.41$, $p < 0.03$) (Table 12B). Subsequent pair-wise comparisons between the groups indicated significant differences in DNA concentration between control and 300 ppm CO-exposed subjects. Neither protein concentrations nor RNA concentrations were significantly affected by CO exposure, although protein levels did tend to increase with the severity of exposure.

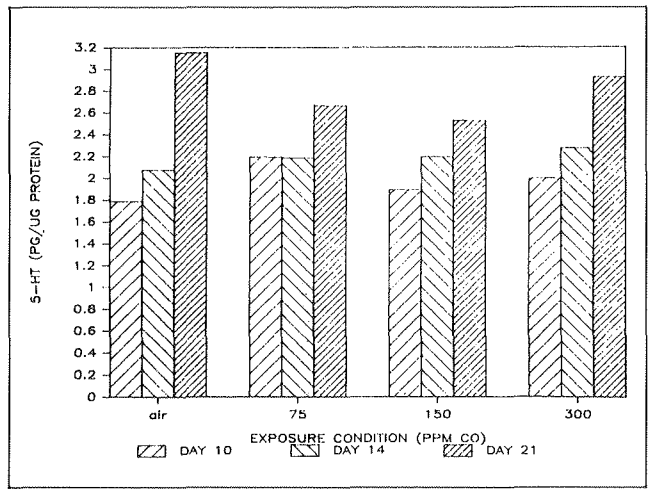

Figure 11: Serotonin concentration in the hippocampus at postnatal days 10, 14, and 21, following CO exposure from conception to postnatal day 10.

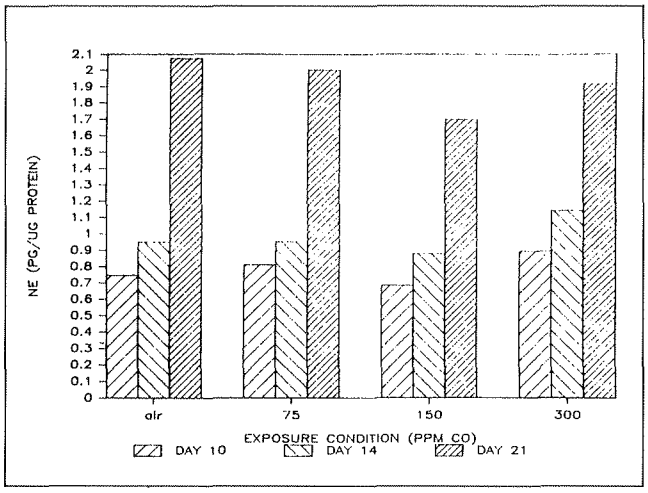

Figure 10: Norepinephrine concentration in the hippocampus at postnatal days 10, 14, and 21, following CO exposure from conception to postnatal day 10.

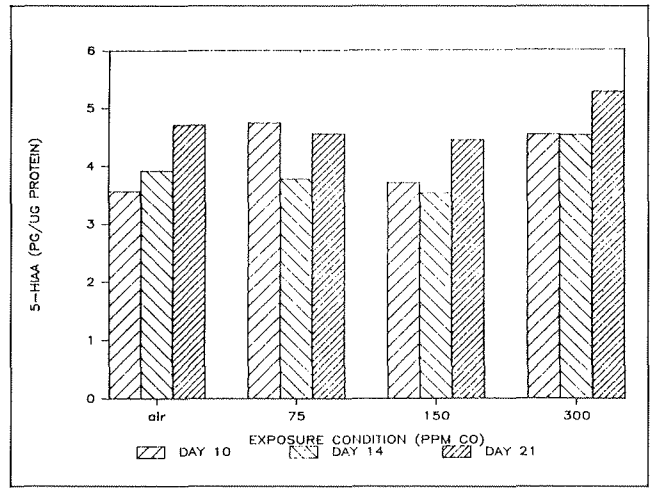

Figure 12: 5-hydroxyindole acetic acid concentration in the hippocampus at postnatal days 10, 14, and 21, following CO exposure from conception to postnatal day 10.

Table 12: Neostriatal nucleic acid and protein concentrations at postnatal day 21, following prenatal and combined pre- and perinatal CO exposure.

A.	Prenatal CO Exposure Only (ppm)			
$\bar{X} \pm$ SEM	0	75	150	300
DNA	1.08 ± 0.02	1.07 ± 0.03	1.10 ± 0.04	1.09 ± 0.02
RNA	2.26 ± 0.16	2.27 ± 0.16	2.19 ± 0.20	2.29 ± 0.16
Protein	109.00 ± 3.00	113.00 ± 3.00	113.00 ± 4.00	109.00 ± 2.00

B.	Pre- and Neonatal CO Exposure ppm to Postnatal Day 10			
$\bar{X} \pm$ SEM	0	75	150	300
DNA	1.05 ± 0.05	1.08 ± 0.05	1.15 ± 0.05	1.23 ± 0.05*
RNA	2.30 ± 0.16	2.16 ± 0.22	2.12 ± 0.30	2.10 ± 0.21
Protein	130.00 ± 4.00	136.00 ± 3.00	138.00 ± 6.00	141.00 ± 2.00

* Significantly different from air controls by Neuman Kuels pair-wise comparison, $p < 0.0292$.

Analysis of DA, HVA, and DOPAC levels in the striatal tissue of subjects receiving CO pre- and neonatally are reported in Figure 13 and Table 13. A substantial increase in DA concentration was observed in 21-day-old subjects exposed to all concentrations of CO, despite the fact that CO exposure ceased on PD10 (Figure 13). The DA increase was particularly pronounced in the 150 ppm CO group. The elevation of DA levels was significant ($F = 7.15$, $p < 0.003$), with both the 150 and 300 ppm CO exposure groups showing a DA level significantly above the control values.

Levels of the DA metabolites were not similarly elevated by perinatal CO exposure (Table 13). Although a slight elevation in HVA values was noted in the 150 ppm CO group, the group that showed the largest elevation in DA, this difference was not sufficiently reliable to be significant.

The present study demonstrates that mild but chronic perinatal CO alters the neostriatal development, so that the neurochemical indices are altered at least 11 days after termination of exposure. This further identifies the neostriatum structure as a sensitive target for hypoxia. The increased DNA levels in the striatum of subjects exposed to CO until PD10, not found in subjects exposed only during gestation, suggest an increased cell number.

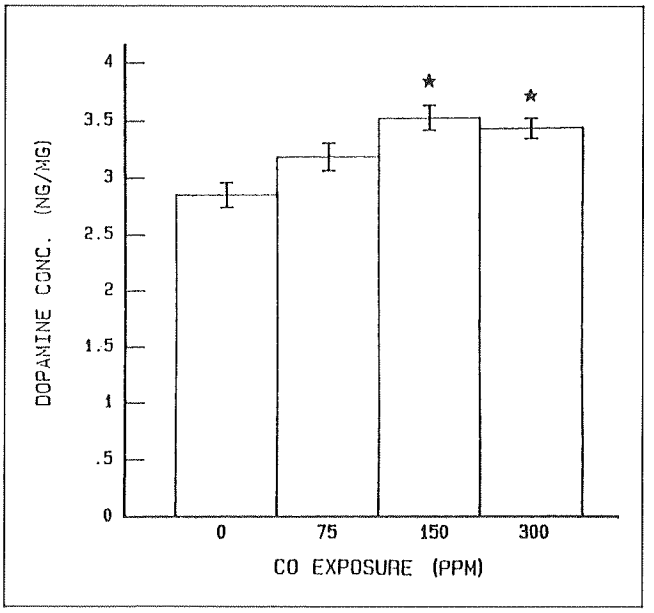

Figure 13: Dopamine concentration in neostriatal tissue of postnatal day 21 rats exposed to air or CO from conception to postnatal day 10. Subjects exposed to 150 and 300 ppm CO have significantly elevated DA levels compared to control subjects ($p < 0.0026$).

It seems unlikely that this increase in DNA concentration reflects enhanced neuronal proliferation, because it occurs with continued exposure after the period of primary neurogenesis, and was absent in subjects exposed to CO only until birth. Rather, it may reflect glial proliferation in response to neuronal injury. Such a result would be consistent both with the results of previous studies carried out in neonatally asphyxiated subjects (e.g., Dambska et al., 1976), and with general responses observed in response to injury. Similarly, the increase in DA levels, not in DA metabolites, also was observed in subjects receiving exposure to CO during the pre- and neonatal period. This may reflect the sprouting of afferent DA neurons in response to damage to neurons intrinsic to the neostriatum.

Although an increase in the number of afferent nerve terminals as a secondary effect of damage to neostriatal neurons appears to be a tenable explanation of the current data, other explanations also might be advanced. Given the lack of increase in DA metabolite levels, one might suggest merely that neuronal activity in DA-containing fibers is reduced. If that were the case, however, one would anticipate reduced synthesis of the neurotransmitter, and thereby a decrease in DA metabolite levels, but no net change in DA levels. Although the present data clearly are inconsistent with that explanation, the additional study of the neostriatum aimed specifically at intrinsic neuron development and synaptogenesis is essential to determine the basis for the current data.

It also might be proposed that hypoxia in general, and CO exposure specifically, disrupts the catecholaminergic neurons and elevates the neurotransmitter concentration. This hypothesis might be appropriate, given the elevations in NE concentrations observed by Storm and Fechter (1985a) in the cerebellum. Potentially, such a change could include increased activity of the rate-limiting synthesizing enzyme, tyrosine hydroxylase, or an increase in the total amount of enzyme. Indeed, rebound increases in tyrosine hydroxylation have been observed, following recovery from very severe hypoxia (Hedner and Lundborg, 1980). However, Storm and Fechter (1985a) failed to observe an increase in the rate of formation of DOPA, the product of tyrosine hydroxylation, following moderate CO exposures equivalent to those reported here. Moreover, increases in catecholamine concentrations do not occur in all brain regions (Storm and Fechter, 1985b).

Table 13: Neostriatal dopamine metabolite concentration and metabolite-to-dopamine ratios on postnatal day 21, following pre- and perinatal CO exposure.

	Pre- and Neonatal CO Exposure to PD10 (ppm)			
SEM	0	75	150	300
DOPAC	1.02 ± 0.10	1.03 ± 0.07	1.02 ± 0.03	1.16 ± 0.20
HVA	0.68 ± 0.03	0.72 ± 0.04	0.78 ± 0.04	0.72 ± 0.05
DOPAC/DA	0.36 ± 0.04	0.32 ± 0.02	0.29 ± 0.01	0.32 ± 0.04
HVA/DA	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.21 ± 0.02

* CO-exposed subjects from PD1 to PD10.
No significant differences were found among groups using ANOVA.

Regardless of the source of this effect, however, these data are important, not only because they document presumed neuropathology resulting from rather mild hypoxic conditions, but also because they reflect changes that persist well beyond the termination of the hypoxic exposure, and must be viewed as a result of injury, rather than as the result of either acute CO intoxication or hypoxia. Although the duration of such changes is presently undetermined, if the hypercellularity observed following pre- and neonatal CO exposure reflects glial proliferation subsequent to neuronal damage, then any recovery certainly would be dependent on processes other than neuronal proliferation.

It is already known from behavioral observation that chronic perinatal CO exposure of the sort employed here can produce permanent effects. Mactutus and Fechter (1984, 1985) reported that perinatal CO exposures at levels used here do produce permanent cognitive disorders. It also was reported that memory disruption becomes more profound with aging (Mactutus and Fechter, 1985). Although the investigators do not propose that the neurochemical changes observed here are responsible for such behavioral changes, it is certain from those behavioral studies that such exposures do permanently alter the central nervous system. The current limited locomotor activity data further suggest that early CO exposure is capable of altering the development of this behavior.

The present data offer interesting comparisons with studies conducted on the cerebellums of litter mates. Storm and Fechter (1985a) showed small elevations in cerebellar NE concentrations in CO-exposed subjects, which appear to reflect normal noradrenergic innervation of a significantly smaller cerebellum.

PONS MEDULLA EXPERIMENTS

A limited number of experiments were undertaken on the pons medulla. These studies consisted of measuring the levels of nucleic acid, protein, NE, 5-HT, and 5HIAA in animals exposed to prenatal CO only. Measurements were restricted to PD21 for the nucleic acids, and to PD21 and 42 for the neurotransmitters. No shift in pons medulla nucleic acid and protein levels was observed at PD21 (Table 14).

In the pons medulla, which contains noradrenergic and serotonergic cell bodies as well as terminals, both NE and 5-HT concentrations tended to decrease linearly with increasing CO exposure level at 21 days of age (Tables 8 and 9). The mean 5-HT concentrations of both the 150 ppm and 300 ppm exposed groups were significantly less than the mean air value (Table 9). Neither of these trends persisted until the pups were 42 days of age. At neither age did the regional weight or protein concentration show a significant linear tendency to increase or decrease with the CO exposure level. No significant trends occurred in the DA concentration or in either of the metabolites measured at either age. HVA was not detected in several tissue samples, so analysis of its concentrations in the pons medulla was not performed.

The decreased NE concentration in the pons medulla at day 21 could reflect interference with the normal development of any one of these noradrenergic parameters, for which the affected offspring had compensated by day 42.

Table 14: Pons medulla nucleic acid and protein levels at postnatal day 21, following prenatal CO exposure.

	DNA (μg)[1]	RNA (μg)[2]	Protein (mg)[2]
Control	330.1 ± 10.63	749.13 ± 80.06	30.18 ± 0.73
75 ppm CO	338.0 ± 10.68	747.13 ± 72.56	30.31 ± 0.87
150 ppm CO	312.9 ± 13.17	722.25 ± 78.86	28.71 ± 0.38
300 ppm CO	334.8 ± 14.50	665.00 ± 58.49	29.21 ± 1.06

[1]Tissue from 10 litters was analyzed for this measure.
[2]Tissue from 8 litters was analyzed for this measure.
No significant differences were found among the groups.

CONCLUSIONS

This investigation focused on the effects of early CO exposure on the development of the brain. The aim of the study was to determine whether or not this environmental agent might irreversibly alter processes of the central nervous system development that occur during a specific and time-limited period. A positive result would indicate a special vulnerability of the developing organism to CO exposure. This would require additional dose-response studies in order to better define the risk of CO to the fetus.

The data support the view that perinatal CO exposure does alter the development of the central nervous system. Parallel studies of adult subjects were not conducted, so comparisons cannot be made directly between the vulnerability of young and adult rats to this toxicant. But some of the data gathered indicate toxic responses that are specific to developmental processes. Such effects suggest qualitative differences in the response of young, as opposed to mature, subjects to CO exposure. Such data are consistent with the notion of the immature organism as a susceptible sub-population.

This study showed alterations in the development of the cerebellum and the neostriatum in neonatal rats exposed perinatally to CO. The description of the injury to these areas will be important as a sensitive indicator of toxic response in subsequent dose-response investigations.

As noted above, reduction in the cerebellar weight and the markers of GABAergic neurons occur with perinatal CO exposure, whereas no change in glutamine, and a slight elevation in NE, was observed. Histopathological examination confirms an early impairment in cerebellar cortex growth (suggested by decreased numbers of fissures) and degenerating Purkinje and granule cells.

This hypothesis could be further tested by determining the rate of neuron proliferation during CO intoxication directly,

to determine whether or not the rate of cell generation is impaired. These data are consistent with the hypothesis that early CO exposure damages developing neurons. Because Purkinje cells develop early in life (prior to birth), disruption in the generation of functional Purkinje cells, either through impaired cell proliferation, unscheduled cell death, or both, is likely to have a profound and permanent effect; deficits in cell number are unlikely to be made up later in life, and the interrelationship among neurons will be affected by a decrease in any given cell population.

Microscopic examination can give only a rough estimate of the loss of functional cells, but the neurochemical data do show that this loss is significant following pre- and neonatal exposure to 150 and 300 ppm CO. This suggests that the effects may result at lower exposure levels as well. Granule cell damage also may occur as a result of CO. It is uncertain whether or not these later developing cells may have time to continue proliferating once exposure ceases. However, because Purkinje cells receive input from the granule cells, as well as from other intrinsic and extrinsic neurons, and because Purkinje cells serve as the only output from the cerebellar cortex to the deep nuclei of this structure and, thence, to other brain regions, the disruption in Purkinje cell development must be viewed as a serious development disorder. Although it is not possible to directly assess the functional significance of these perturbations in cerebellar development, it is important to note that earlier investigations reported that prenatal CO exposure results in the delayed development of various reflexive behaviors that require sensory motor integration and general neurological competency (Fechter and Annau, 1980).

Even though functional (behavioral) consequences of neuron loss may be difficult to isolate when such loss is comparatively mild, it must be understood that any neuronal loss reduces the organism's ability to compensate, and renders the organism less resistent to withstand further loss of cells that may occur through disease, injury, or pathophysiological changes as a result of aging. This principle already has been demonstrated for early CO exposure; cognitive deficits in rats prenatally exposed to this gas become increasingly obvious as the organism passes from a young adult to an aging adult (Mactutus and Fechter, 1984, 1985).

The findings obtained in the neostriatum have not been characterized as well as those in the cerebellum, but they show a significant elevation in innervation of this structure by extrinsic DA-containing neurons, and an increase in cell number, which also may reflect a combination of neuronal loss and glial proliferation. In both the cerebellum and the neostriatum, the extrinsic catecholamine-containing neurons show elevations in density of terminals. In the case of the cerebellum, the elevation in NE is largely a result of the normal innervation of a smaller structure, while in the neostriatum a true increase in DA content is seen among the CO-exposed neonates. Previous work has suggested behavioral and neurochemical abnormalities in CO-treated rats injected with l-DOPA, a precursor for both NE and DA (Fechter and Annau, 1977). It remains to be determined whether or not markers for intrinsic neostriatal neurons (many of which contain GABA or ACh as their neurotransmitter) are reduced, as are GABA-ergic neurons in the cerebellum.

A difficulty that may arise in developmental toxicity studies is the separation of direct toxic effects of the agent being tested from effects of maternal toxicity on the fetus. In particular, the nutritional status of the dam can be expected to have a decided influence on fetal development. For that reason, analysis of the possible role of maternal nutrition has been included in these studies. No evidence that maternal nutrition is compromised by exposure to CO has been observed. Dams placed in CO show normal weight gain during pregnancy, and litter size is normal among their litters. Although this investigation has confirmed earlier evidence of reduced birth weight among offspring of CO exposed dams, there is no evidence that this finding reflects impaired maternal nutrition. Parenthetically, it might be noted that the offspring of human cigarette smokers are also lighter at birth than are the offspring of non-smokers.

SUMMARY

The research presented here represents an initial neurochemical survey of the central nervous system, which was designed to identify brain regions that are sensitive to CO exposure. The investigator has completed several longitudinal experiments in which various neurochemical parameters were studied until postnatal age 42 days. Although this age does not represent a fully mature animal, the neurochemical development of the brain begins to approach adult values by day 42. Thus, it is likely that differences present at that age will persist. In several experiments the investigator noted the emergence of significant neurochemical effects relatively late during development. These late-developing differences may be particularly important because they may represent the appearance of effects that become apparent only as the system approaches maturity. They may also represent a "broadening" of the initial injury that results from the abnormal interaction among cells when a particular cell population is damaged.

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HEALTH REVIEW COMMITTEE'S REPORT

GOALS AND OBJECTIVES

The objectives of Dr. Fechter's study were to determine whether exposure to low levels of CO during gestation, or during gestation plus a 10-day neonatal period, would disrupt the neurochemical development of the brain in rats. Specific hypotheses that were to be tested included: A) Are catecholamine-containing neurons, as a class, especially vulnerable to CO exposure? B) Are seratonin-containing neurons, as a class, especially vulnerable to CO exposure? C) Are the size or the number of cells in the cerebral cortex, neostriatum, brain stem, hippocampus, and cerebellum altered by prenatal CO exposure? D) Are the effects of CO on neurochemical systems persistent? E) What cells in the cerebellum are vulnerable to CO exposure?

The methodologic approach to this project was to use neurochemical and histological methods to assess the development of cells that are intrinsic to the identified target brain regions and the development of neurons that project their terminals into these brain regions. A primary focus of this study (unlike most previous studies on CO, which have used high concentrations with brief exposure) was to determine whether relatively moderate concentrations that were non-toxic to the maternal organism would, with continuous exposure, result in persistent changes in the developing rat brain.

Because the developing rat brain is considerably less mature at birth than the primate brain, two different exposure models were used. One involved exposure of rats in utero only, beginning with the first day of gestation and ending with delivery. The other combined the in utero exposure with an additional 10 days of neonatal exposure.

Using this model, the investigator planned to test any peculiar vulnerability of the developing nervous system under conditions of development of the nervous system which mimicked that which would be present in a human organism during sustained prenatal exposure. By using neurochemical bioassays that are specific to certain cells within the nervous system, coupled with some histological observations, the investigator hoped to determine whether the hypoxia produced by CO would have any specific effects in different regions of the developing rat brain.

SUMMARY OF INVESTIGATOR'S CONCLUSIONS

Pregnant and neonatal rats were exposed either to air or air containing 75, 150, or 300 ppm of CO. These exposures occurred either prenatally during the entire gestation period, or during the gestation period plus 10 days neonatally. The results of the several experiments conducted in this investigation led to the following conclusions by the investigator:

1. No effects on the maternal or the developing organisms were observed at concentrations below 150 ppm.
2. Neither maternal weight gain, nor the number of pups born per litter, was affected by CO exposure.
3. The birth weights of offspring born to the 150 and the 300 ppm CO-exposed mothers were less than those of the control offspring, but recovery of normal weight tended to occur during development.
4. The two areas of the brain that exhibited significant changes in neonatal rats exposed to CO were the cerebellum and the neostriatum.
5. There was a reduction in cerebellar weight, as well as in certain neurochemical markers of GABAergic neurons, in the CO-exposed animals. A slight elevation in NE concentration was also noted. Histopathologic examination also confirmed early impairment at the higher concentration of CO, as indicated by decreased numbers of fissures and degenerating Purkinje and granule cells, which suggested reduced cerebellar cortex growth.
6. The DNA content in the neostriatum of rats exposed to 300 ppm of CO was significantly increased. Accompanying this was a significant increase in the DA content of the neostriatum of rats exposed neonatally and perinatally to 150 and 300 ppm of CO. It was concluded that CO exposure during gestation resulted in increased cell numbers, which the investigator interpreted as a reflection of glial proliferation in response to neuronal injury.
7. When animals exposed to increasing concentrations of CO were examined at 21 days of age, a trend toward decreased NE and 5-hydroxytryptamine concentrations was observed in the pons medulla. However, there was no shift in the pons medulla nucleic acid and protein levels, and the neurochemical changes did not persist at 42 days of age. This observation suggested complete compensation by this age for any effects that had been produced during the gestational period.
8. Prenatal CO exposure produced no effects on hippocampal cell size or cell density. In addition, limited neurochemical measurements indicated no changes in hippocampal NE or 5-hydroxytryptamine levels.

TECHNICAL EVALUATION

ASSESSMENT OF METHODS AND STUDY DESIGN

Overall, the design of the experiments and the methods chosen represent a reasonable approach to addressing the questions stated under the specific objectives. The neurochemical parameters that were selected for measurement are appropriate for determining persistent changes in concentrations of important neurotransmitters in the brain. However, the significance of the changes in concentrations of neurotransmitters is somewhat difficult to assess when the data are obtained at a single point in time. In other words, the function of the nervous system with regard to neurotransmitters is primarily associated with release; the methods used did not obtain direct measurement of rates of release of the transmitters. However, in several of the experimental protocols, measurements of precursors or metabolites of the neurotransmitters were conducted. These measurements may be useful in determining whether there was altered utilization of the transmitter, i.e., increased or decreased synthesis of the transmitter subsequent to increased or decreased release.

Additionally, there were no measurements conducted to reflect the status of the cholinergic neurons, and some alterations, such as the GABAergic neurochemical changes, might have been secondary to the cholinergic changes. Although the initial proposal did not include plans to study cholinergic nerves and fibers, this might be an area for studies in the future. In several cases, the presence of an altered transmitter concentration was interpreted as indicating that there was an altered density of terminals innervated extrinsically. This conclusion was drawn rather indirectly; it would have been interesting to determine actual receptor binding characteristics of the various parts of the brain for the specific transmitters that were studied.

The use of DNA and protein concentrations, and their ratios, to measure cell numbers, has some weaknesses in determining whether there are true reductions in neurons; however, the author has used these in a general sense to provide initial evidence of alteration in the cellular content of the nervous system, and as a guide to determine if further detailed studies of the neurochemistry were warranted. The DNA/protein concentration ratios were probably satisfactory for this general purpose. However, the use of such ratios, and the depth of the fissures in the neocortex of the cerebellum, is not universally accepted as being an accurate measure of altered neuronal development.

The studies do not rigorously test the neurochemical/morphologic correlations. However, the intent of the project was to survey the possibility of specific injury from CO in various regions in the developing brain, and the rigorous correlative experimentation required to establish a morphologic/neurochemical relationship would necessarily require a considerable focus not possible in a survey research program. This could be an area for future research. The fact that measurements were made at 21 and 42 days postnatally allows some opportunity to identify persistent effects. However, some delayed neurochemical effects may not become apparent until later in development.

One other correlative aspect that could have been included in the study design is measurement of HbCO levels in fetal, as well as maternal, blood. This would have allowed a more specific estimation of the relationship between fetal hypoxia and change in neuronal development. Although this would have required development of additional protocols in the investigator's laboratory, it would have made the results of the study more useful.

Several points have been raised regarding analyses of the data during the course of review of this study. One question concerns the statistical treatment of concurrent control values at zero dose of CO. The usefulness of the concurrent control (zero dose of CO) is lost unless it is taken into account as a blocking variable in the analysis. Since the analysis model is only vaguely described, it is not possible to know whether this aspect of the analysis was correctly performed.

Second, the author presents dose-response data in several tables in the report. However, such data have not been formally analyzed to explore the nature of the dose-response relationship. Third, there is a lack of uniformity in calculating multiple comparisons among the means in different tables when the types of inference the analyst wants to make are similar. The Dunnett test, which is appropriate for comparing each of several treatments to a control, is used in Table 2, while the Newman-Kuels test, which is appropriate for all possible pairwise comparisons among the treatments, is used for Table 1.

Furthermore, the mean body weights shown for rats on postnatal day 21 shown in Table 1 differ from those shown for the same age group in Table 2. There are also small differences between the relative maternal weights shown for the control and three treatment groups in Figures 1 and 2. Although it appears that the data in the tables and the figures are each derived from the same set or sets of experiments, there is no explanation of these differences in the investigator's report. The author has explained that the data presented in Tables 1 and 2 were obtained over a long interval of time, and the observed differences represent insignificant experimental variations. He also feels that Figures 1 and 2 are consistent with each other in showing no difference in weight gain among dams from the different treatment groups. This interpretation of the author is unquestioned, but we are left to assume that the apparent relative differences in the two figures are due to some unexplained differences in the data bases from which the two figures are constructed.

Finally, the neurochemical methods used to measure changes in rat brain appear to be state-of-the-art, and have been conducted carefully with appropriate replicate experiments. Because it was frequently necessary, however, to pool tissues

in order to have enough material for measurements, the investigator chose the litter as the statistical unit. In most cases, the number of litters measured were from eight to twelve. Although analysis of litter means is adequate, it would have been desirable to include some weighting to compensate for the variable number of pups within each litter that were sacrificed for different experiments. It is conceivable that such weighting could change the inference where subtle differences are to be detected.

INTERPRETATION OF RESULTS

The investigator's conclusions that exposure to 150 or 300 ppm CO during the prenatal and 10 day postnatal period results in reduced cerebellar weight and reduced total GABA content of the cerebellum are clearly supported by the data obtained. The cerebellum also contained a small, but significantly higher, content of NE in the CO-exposed animals. Since histological studies indicated some impairment of Purkinje cells, the author speculates that the cerebellar neurochemical changes may be associated with the morphologic changes in these cells, as well as changes in the granule cells. The author's interpretation that the increased concentration of NE in the cerebellum of CO-exposed rats is largely a result of normal innervation of a smaller structure appears to be a reasonable conclusion.

However, although the neurochemical analyses for the neostriatum were somewhat less detailed than for the cerebellum, the author indicates that the findings suggest an elevation in the innervation of the neostriatum by extrinsic DA-containing neurons, and an increase in total cell number. It is possible that this increase might reflect a combination of neuronal loss and glial cell proliferation. The author's suggestion that the increased DNA content in the neostriatum, which occurred only during the prenatal exposure and not in the 10 day postnatal period, indicates an increased cell number is a reasonable conclusion.

In several places in the report, the author speculates about possible mechanisms of specific neurochemical changes, but he is quite careful, appropriately, in his discussion and his final conclusions not to overstate the certainty of these mechanisms. There are, of course, other possible interpretations of the data. One important area of uncertainty concerns the relationships between the neurochemical changes and the morphologic changes that were observed, particularly in the cerebellum. A reasonable question to raise is whether the neurochemical changes are merely reflections of morphologic changes in the various brain structures, that is, are they indicative of increased or decreased density of cells or changes in the ratios of different kinds of cells? If that is the case, it would seem that there is a need for research that focuses on the reasons that cell proliferation is altered by CO exposure. In fact, the author indicates that measurements of rates of cell proliferation in CO-exposed fetuses or neonates would be an important area for further research.

Another issue that is not specifically addressed by this study is whether the alterations in the concentrations of neurotransmitters is any reflection of the dynamics of transmitter release and synthesis. It is the release of the transmitter and the binding of the transmitter to the receptors that are the controlling factors in the function of the nervous system; experiments to examine this aspect are not included in this report. However, the author does refer to studies conducted in his laboratory that do show behavioral changes occurring in CO-exposed neonates. These may reflect some of the brain region changes that are reported here. Certainly, more functional correlates of the neurochemical and morphologic changes are necessary to add meaning to any specific change that has been observed.

ATTAINMENT OF STUDY OBJECTIVES

The research reported here reasonably meets the investigator's objectives. However, there remain many unanswered questions about the relationships between neurochemical and morphologic changes that result from prenatal and neonatal exposure to CO. Referring once more to the specific objectives of the investigator, one would have to say that although information has been obtained on each of the specific goals, it is still difficult to give clear, crisp answers to the five straightforward questions that constituted the specific goals.

Nevertheless, this work is important because it provides additional evidence that exposure to CO during development exerts specific effects in the developing nervous system. It further indicates that some specific measures of brain neurochemistry remain altered, after CO exposure ceases, for a period beyond which the initial reduced birth weight is observable. Additional research is required to examine the suggested mechanisms, and to elucidate other possible mechanisms, for the changes that were observed in the study.

REMAINING UNCERTAINTIES AND IMPLICATIONS FOR FUTURE RESEARCH

Possible areas of additional research have been alluded to previously, and include:

1. Additional studies on morphology/neurochemistry correlations, possibly with experiments conducted on a temporal scale. This might permit determination of altered rates of cell turnover and conclusions as to whether the neurochemical changes that were reported here reflect strictly the morphologic changes in a specific brain region.
2. Additional studies to examine the hypothesis that the alterations in the neurotransmitter concentrations that are reported here have significant functional consequences. This might be addressed by experiments specifically designed to measure neurotransmitter rates of release under natural or stimulated situations. It might also include measurements of both the recognition properties and the functional biochemical properties of the neurotransmitters' receptor sites.

3. Studies on correlations of specific behavioral effects in the CO-exposed neonates, conducted over extended periods of their lifetime. These will be necessary for a comprehensive interpretation of the significance of the morphologic and neurochemical changes observed here.
4. In future studies, it would be important to obtain a measure of the HbCO concentrations in the exposed fetuses and in the early postnatally exposed offspring, and to relate this assayable parameter to the brain function changes. Such information might ultimately have significance when extrapolating these findings to human exposure.

SUMMARY AND IMPLICATIONS

The evidence presented in this report underscores the importance of considering the effects of air pollutants on the nervous system. Although the findings are preliminary, they do suggest that the developing brain is vulnerable to CO exposure.

Clearly, the minimal concentration at which effects were observed in this study, i.e., 150 ppm of CO, is high when compared to likely CO exposures resulting from ambient pollution by vehicular exhaust. On the other hand, accidental exposures to CO from automobile exhaust, or a faulty or unventilated heating system, far exceed the concentrations that are reported here. Individuals who smoke are also exposed to elevated levels of CO. Indeed, at 150 ppm CO there might be relatively few other signs of toxicity in humans. No doubt headache, and possibly some other subtle effects, might occur in adult individuals living in such circumstances, but these might not be enough to serve as warnings against the potential of injury to the developing embryo as modeled in this study.

It appears that these studies have established a threshold concentration for exposure of rodent dams and their developing fetuses or offspring below which effects on the offspring would not be detected. We have little information on whether animals with longer gestation periods and, therefore, possibly longer periods of exposure, would be affected by an even lower concentration. If that were the case, then these findings in rodents would become more significant than is apparent with the information available.

The direct application of these findings to risk assessment, or to policy decisions, would be premature. The consequences of exposure of humans to CO, even at the 150 ppm level, will be difficult to assess until enough experimental work has been completed to understand thoroughly the neural impairments and permit more precise functional evaluations.

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APPENDIX A

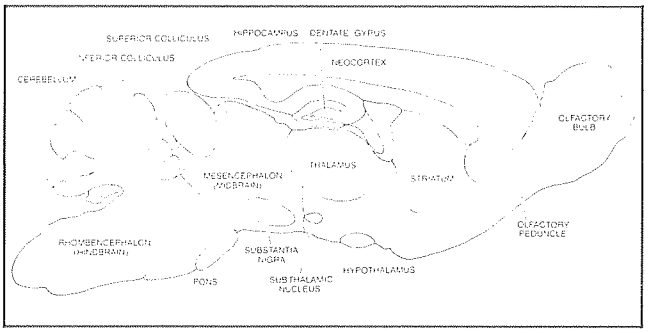

Figure A. Is reproduced from "The Organization of The Brain," W.J.H. Nauta and M. Fiertag. Copyright © 1979 by *Scientific American, Inc.* All rights reserved.

A diagram of the rat brain is given in Figure A. The brain is divided into three sections: the hind brain (which includes the cerebellum), the midbrain, and the forebrain (which includes the hypothalamus, the neocortex and the striatum). Communication between one part of the brain and another, as well as to the rest of the body, occurs via cells called neurons. Structurally, the neuron consists of: a cell body, which incorporates genetic material and the metabolic apparatus; an axon, which is an elongated extension of the cell; and dendrites, which are fibrous branches that emerge directly from the cell body and from the axon terminal. Junctions between the axon terminal on one neuron and the dendrite "tree" around the cell body of a second neuron are called synapses. The axon's terminal branches may form synapses with as many as 1,000 other neurons. Unlike other cells in the body, neurons do not divide after embryonic development is complete.

There are two major modes of communication in the nervous system: electrical and chemical. Signals within the neuron are transmitted as electrical impulses, which are generated in the cell body and propagated along the axon until the terminal is reached. At the nerve terminal, signals between the neurons are transmitted via chemicals called neurotransmitters. Changes in the properties of the pre-synaptic terminal membrane result in the fusion of intracellular synaptic vesicles with the membrane and the release of the neurotransmitters into the synapse. Interaction of the neurotransmitters with the membrane of the post-synaptic cell body leads to the generation of an electrical signal in the post-synaptic cell. After its release, the neurotransmitter is either recycled or degraded. Dr. Fechter has examined levels of neurotransmitters in the brain as a marker of pathological effect.

Until recently, very little was known about the neurotransmitters. These molecules are difficult to study because they are present in minute amounts, and it was difficult to isolate sufficient quantities of a given transmitter system for examination. Major advances since the late 1970s (Nauta and Fiertag, 1979)† have resulted in the identification of more than 30 different types of neurotransmitters in the brain. These fall into two broad categories: amino acids and their derivatives, and neuropeptides. This report is concerned with the monoamines, which belong to the first group and are the best understood.

The monoamine neurotransmitters include dopamine and norepinephrine (derived from tyrosine), serotonin (derived from tryptophan), glycine, glutamate, and the glutamate derivative, gamma-amino-butyric acid (GABA). The chemical structure of tyrosine-derived monoamine transmitters includes a catechol group; hence, these neurotransmitters are collectively called catecholamines. They are also called adrenergic because the adrenal medulla is in part responsible for the synthesis and release of these molecules.

Different neurotransmitters are produced by clusters of neurons that originate in specific regions of the brain. These neurons extend to several other regions of the brain, as well as to the body, in a highly interconnected network. Different neurotransmitter network systems are responsible for different types of behavior.

†See references on page 27.

ABOUT THE AUTHOR

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RESEARCH REPORT No. 12: September, 1987