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16 September 1983; accepted 22 November 1983

Prenatal Exposure to Carbon Monoxide: Learning and Memory Deficits

Abstract. *Exposing pregnant rats to carbon monoxide (150 parts per million) produced only minor reductions in the birth weights of the pups and gave no evidence of overt teratogenesis. However, behavioral evaluation of learning and memory processes in a two-way avoidance task suggested a functional deficit in the central nervous system of the exposed offspring. Multiple dependent measures and specific control groups confirmed that this deficit was independent of nonassociative or motivational alterations.*

The toxic and physiologic effects of carbon monoxide exposure have been well documented since the pioneering investigations in the late 19th century by Haldane (*1*). However, little is known of the consequences of chronic exposure to low concentrations of CO, particularly with respect to specific populations such as fetuses (*2*). Mild prenatal CO exposure is possible as a result of maternal cigarette smoking or industrial and ambient air sources (*3*). Animal models have revealed that after maternal exposure, CO readily crosses the placenta and decreases fetal oxygen partial pressures (*4*). Prenatal CO exposure throughout gestation increases the incidence of minor skeletal alterations in mice (*5*) and may be teratogenic in rabbits under certain conditions (*6*). Depressions in birth weight and reduced growth patterns may also be observed after CO exposure throughout gestation (*6, 7*). The reduced locomotor activity, an attenuated neurochemical response to L-dopa administration, and delays in development of two landmark behaviors observed in prenatal rats shortly after birth (*7*) represent the only functional evidence of central nervous system toxicity. We now report (i) that chronic exposure to CO restricted to the prenatal period disrupts the acquisition and retention of a conditioned avoidance response in juvenile rats, (ii) that this impairment is associative and does not reflect changes in nonassociative or motivational processes, and (iii) that this effect was observed in the absence of any overt toxicological or teratological effect.

Adult female Long-Evans hooded rats were maintained in the laboratory with continuous access to food and water, a diurnal light cycle (12 hours of light and 12 of darkness), and room temperature of 18° to 22°C. The rats were bred and, after a sperm-positive vaginal smear, transferred to exposure chambers for the duration of gestation. Chamber CO concentrations were monitored electrochemically so that aside from weekly weighing or cage cleaning, or both, the dams were left undisturbed throughout pregnancy (*8*). Average daily chamber CO concentrations (mean ± standard error) were, respectively, 149 ± 2 parts per million (ppm) and 154 ± 2 ppm during the two experiments reported below. In a third experiment, we determined that average daily CO concentrations of 148 ± 2 ppm produce maternal carboxyhemoglobin (HbCO) concentrations of 15.6 ± 1.1 percent (N = 28) relative to control subjects exposed to air (0.5 ± 0.5 percent, N = 20). By comparison, human cigarette smokers show HbCO concentrations ranging from about 1 to 16 percent (*9*).

Within 12 hours after birth, the subjects were removed from the exposure chambers and placed in a normal air environment. All litters were culled to eight pups; an equal number of each sex were left whenever possible.

Among the offspring of 16 dams exposed to CO and 16 to air, we noted a slight but nonsignificant depression in birth weight of experimental animals. We observed no differences in initial growth of the dams, number of pups per litter, sex ratio, or mortality on day 1 (Table 1); no evidence of gross structural deformities was seen in either group.

We investigated the influence of prenatal CO exposure on the functional ontogeny of learning and memory for a two-way conditioned avoidance response (*10*). Three male and three female offspring were randomly chosen from each of eight randomly selected dams from each group. Each pup received 100 acquisition trials in the conditioned avoidance response task at 16, 23, or 30 days of age. After a 24-hour retention interval, subjects were administered a second session of 100 trials (*11*).

The results of the avoidance task (Fig. 1A), analyzed by a 2 by 3 (exposure condition by age) analysis of variance, indicated that acquisition improved with age ($P < 0.001$). However, an interaction of the exposure treatment with age [$F(2, 89) = 3.3$, $P < 0.05$] resulted from an impairment for the prenatal CO-exposed offspring in acquisition of the two-way avoidance contingency. Among the offspring of dams exposed to air, acquisition was minimal in 16-day-old pups but significant by 30 days ($P < 0.01$) (*12*). In contrast, 30-day-old offspring of dams exposed to CO failed to perform the avoidance task any more successfully

Table 1. Consequences of CO (150 ppm) or air exposure on pregnant rats and their offspring. All data are expressed as means ± standard errors.

Prenatal treatment	Litters (No.)	Weight gain during gestation (%)			Gestation period (days)	Litter size (No.)	Sex ratio (M:F)	Birth weight (g)
		Week 1	Week 2	Week 3				
				Experiment 1				
Air	16	8.8 ± 1.1*			21.9 ± 0.2*	12.8 ± 0.7	1.4 ± 0.3	6.30 ± 0.12
CO	16	8.5 ± 1.2*			22.2 ± 0.1*	11.2 ± 0.6	1.0 ± 0.1	5.87 ± 0.10
				Experiment 2				
Air	18	12.7 ± 0.8	13.5 ± 1.0	23.2 ± 0.9	22.0 ± 0.1	13.4 ± 0.5	1.0 ± 0.1	5.91 ± 0.13
CO	18	10.9 ± 0.9	12.8 ± 1.1	23.6 ± 1.2	21.9 ± 0.1	12.4 ± 0.7	1.0 ± 0.1	5.73 ± 0.12

*Data available for only 12 litters.

than their 16-day-old littermates and were also impaired relative to age-matched controls ($P < 0.05$).

This discovery of a deficit in performance of the avoidance task indicated a potential alteration in the associative processes necessary for response learning. Alterations in nonassociative or motivational factors did not readily account for this differential avoidance performance. Specifically, the analysis of intertrial crossings failed to provide any statistically significant evidence for CO-induced alteration of activity during the avoidance session ($F < 1.0$). Similarly, using a measure of average response latency for the first five shock escape trials as an index of the aversive or motivational properties of the footshock stimulus, we found no statistically significant evidence for any treatment-related differences ($F < 1.0$) (*13*).

Reacquistion performance (Fig. 1B), a potential index of retention, was also adversely affected by the prenatal CO exposure. Because assessment of memory is confounded by the difference in acquisition, an analysis of covariance was used to analyze reacquisition with covariates removing the contribution of any treatment-related differences in number of avoidance responses in acquisition and intertrial crossings in reacquisition. A 3 by 2 analysis of covariance indicated a significant age effect ($P < 0.001$) and a CO exposure by age interaction [$F(2, 87) = 4.3$, $P < 0.02$] on avoidance responding in reacquisition. Although the 31-day-old offspring of both treatment groups performed the task more successfully than their respective 17-day-old littermates (P's < 0.01), the 31-day-old pups of CO-exposed dams were impaired relative to age-matched controls ($P < 0.05$).

Because of the unexpected nature and magnitude of the avoidance deficit and our interest in a more definitive assessment of the potential contribution of nonassociative alterations to task performance, we included a pseudo-conditioning control group (*14*) in a replication experiment. To delineate whether both learning and memory processes were affected, a maturation control group was also necessary to indicate whether any differences in reacquisition reflected prior training or simply additional maturation.

Eighteen litters were bred for each condition—CO and normal air. Rate of growth for the dams, number of pups per litter, birth weights, and sex ratio all failed to show any adverse effect of the prenatal CO exposure (Table 1). Again, no evidence of gross teratogenesis was observed in either treatment condition.

Three male and three female pups from eight randomly selected litters representing each treatment condition were evaluated at 30 days of age in the two-way avoidance task. One pup of each sex was assigned to learning-memory, maturation control, and pseudo-conditioning groups. For the learning-memory group, all rats received two consecutive days of avoidance training as in the initial experiment. Those animals assigned to the maturation control group received training only on day 2. Animals in the pseudo-conditioning control group received 2 days of "training" but in a procedure in which the conditional and unconditional stimuli were temporally uncorrelated (*15*).

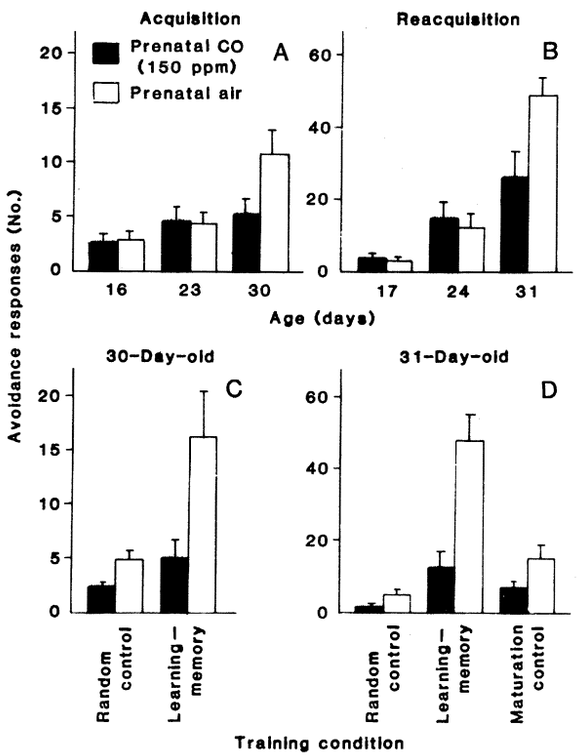

Fig. 1. The mean number of successful avoidance responses by rats exposed before birth to CO (150 ppm) and control rats. (A and B) Ontogeny of avoidance responding for the 100-trial acquisition and reacquisition sessions, respectively. (C and D) Replication of these deficits observed in the acquisition (30 days of age) and reacquisition (31 days of age) of the two-way avoidance task with the addition of pseudo-conditioning and maturation controls.

In agreement with our initial finding, a 2 by 2 analysis of variance on the number of avoidance responses (Fig. 1C) indicated significant effects of the CO exposure and the training contingency [F's(1, 60) > 8.4, P's < 0.005]. The pups of dams exposed to CO were impaired in the acquisition of the avoidance contingency relative to the offspring of dams exposed to air ($P < 0.01$) and did not perform any better than CO-exposed rats in the random control condition. In contrast, air-exposed controls in the learning-memory group were superior to their age-matched counterparts in the random control condition ($P < 0.01$). Again, initial escape latencies failed to provide evidence for a CO-induced differential sensitivity to the mild footshock ($F < 1.0$). Activity during the 5-minute adaptation period was similar across exposure groups ($F < 1.0$). Analysis of intertrial responding revealed a nonsignificant decrease in activity for the CO-exposed animals [$F(1, 60) = 3.2$, $P = 0.08$]; specific planned contrasts were also nonsignificant (*12*).

Retention, as indexed by reacquisition (Fig. 1D), was also impaired as a consequence of the early CO exposure. A 2 by 2 analysis of covariance on the number of avoidance responses with covariates of number of avoidance responses in acquisition and number of adaptation and intertrial responses during reacquisition revealed significant effects of CO exposure [$F(1, 57) = 4.8$, $P < 0.03$], training contingency [$F(1, 57) = 9.8$, $P < 0.003$], and the interaction between the two [$F(1, 57) = 26.9$, $P < 0.001$]. Subsequent analyses confirmed an impairment in avoidance responding within the learning-memory groups as a function of the prenatal CO exposure ($P < 0.01$). Comparison of the learning-memory groups to their respective pseudo-conditioning control siblings indicated an improvement over sessions for the air-exposed ($P < 0.01$) but not CO-exposed animals. Moreover, relative to the most appropriate baseline for evaluation of retention—the maturation control animals—the retention of prior training was significant for air-exposure ($P < 0.01$) but not CO-exposure conditions.

Collectively, these results demonstrate that chronic prenatal CO exposure

may produce a functional deficit in the central nervous system in the absence of any overt toxicity. Compelling preliminary evidence supporting the seriousness of this functional deficit has been noted in adult rats exposed to CO as fetuses (*16*). Although no impairment was observed in avoidance acquisition, young adult rats prenatally exposed to CO demonstrated impaired retention as indexed by reacquisition. While we cannot extrapolate the results to humans, they do resemble the often cited impairment in achievement test scores noted during early childhood in the children of women who were heavy smokers during pregnancy (*17*). Further research identifying the underlying neural substrates for such behavioral alterations may permit extrapolation across species.

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12. Analyses subsequent to a significant F value were performed with the conservative Bonferroni or Dunn's test to maintain the overall alpha level within any set of contrasts at the probability level of $P < 0.05$ [B. J. Winer, *Statistical Principles in Experimental Design*, (McGraw-Hill, New York, ed. 2, 1971)].
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26 September 1983; accepted 13 October 1983

Rapid Mechanical Responses of the Dark-Adapted Squid Retina to Light Pulses

Abstract. *Dark-adapted squid retinas respond to brief light pulses with early and late mechanical responses. These responses are mechanical counterparts of the early and late receptor potentials.*

Photomechanical responses of the retina—movements of visual cells and pigments produced by light—have been known to physiologists for more than a century (*1*). By comparing the photomicrographs of the retinas prepared in light-adapted and dark-adapted states, Young (*2*) showed that the photoreceptors in the octopus and squid eye "contract" when exposed to light. The sensitivity of the microscopic method for detecting mechanical movements in the retina is limited.

We have found that rapid mechanical responses to light stimuli can be detected with a piezoelectric transducer pressing onto dark-adapted squid retina. Previously, rapid mechanical movements were detected by the use of the same transducer in crab nerves, in squid giant axons, and in frog dorsal-root ganglia and spinal cords (*3*).

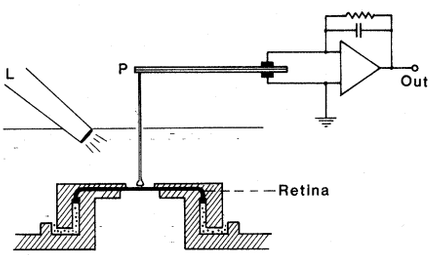

Fig. 1. Schematic diagram of the experimental setup used for measuring photomechanical movements of the squid retina. The retina was clamped between two plastic rings with holes in the middle. The shaded areas represent the partition and the rings dividing the seawater into two parts. The stippled areas represent the space filled with Vaseline. The Ag-AgCl electrode (not shown) immersed in seawater above the partition was grounded; an electrode in the lower compartment recorded the ERG. Abbreviations: L, light-emitting diode operated at 3 V (100 mA); P, piezoelectric transducer connected to an operational amplifier.

Squid, *Loligo pealeii* Lesueur, were used. After about 60 minutes of dark adaptation, the eye was excised under illumination with dim red light and transferred into oxygenated artificial seawater. The cornea and the lens of the eye were then resected with a pair of fine scissors, and a disk of the retina of about 12 mm in diameter was prepared. Finally, the retina was transferred into oxygenated seawater in a black Lucite chamber (Fig. 1). The chamber consisted of two compartments separated by a partition provided with a short tube fixed vertically in the middle. The opening of the tube was completely covered with the retina, whose internal limiting membrane faced upward. The retinal surface was fixed with a separate Lucite ring with a hole 4 mm in diameter in the middle. After the hydrostatic pressure in the closed lower compartment was slightly raised, the stylus of a piezoelectric probe was lowered to make contact with the center of the retina.

The piezoelectric probe was of the lead-zirconate-titanate type (Gulton) connected to an operational amplifier (AD 515); its sensitivity was roughly 30 mV per milligram. The output was amplified by a factor of 100 in the frequency range between 0.1 and 300 Hz and was led to a dual-beam oscilloscope, a signal averager, or both. Variations in the electric potential difference across the retina evoked by light pulses [that is, electroretinogram (ERG)] were recorded with a pair of electrodes, one chloridized silver wire in each compartment. All measurements were carried out at room temperature (19° to 21°C).