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Effects of prenatal exposure to low concentrations of carbon monoxide on sexual behaviour and mesolimbic dopaminergic function in rat offspring

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1 Inhalation of low concentrations of carbon monoxide (CO) by pregnant rats (75 and 150 p.p.m. from day 0 to day 20 of gestation) leads to changes in mesolimbic dopaminergic transmission associated with an impairment of sexual behaviour in male offspring.

2 Eighty day old males exposed *in utero* to CO (150 p.p.m.) exhibited a significant increase in mount/intromission latency as well as a significant decrease in mount/intromission frequency. A significant decrease in ejaculation frequency was also found in CO (150 p.p.m.)-exposed animals.

3 The acute administration of amphetamine, at a dose (0.5 mg kg^{-1} s.c.) stimulating copulatory activity in control rats, failed to reduce mount/intromission latency and did not increase mount frequency in 80-day offspring exposed to CO (150 p.p.m.) during gestation.

4 These behavioural alterations were paralleled by neurochemical changes (*in vivo* microdialysis) showing that prenatal CO exposure, at concentrations (150 p.p.m.) that did not affect basal extracellular levels of dopamine in the nucleus accumbens, blunted the amphetamine (0.5 mg kg^{-1} s.c.)-induced increase in dopamine release in 80-day old male rats.

5 No significant changes in either behavioural or neurochemical parameters were observed in 10-month old rats exposed prenatally to CO.

6 Since the alterations in sexual behaviour and dopaminergic transmission have been produced by prenatal exposure to CO levels resulting in maternal blood carboxyhaemoglobin concentrations equivalent to those maintained by human cigarette smokers, the present data further point out the large risk that the smoking mother poses for her offspring.

Keywords: Prenatal carbon monoxide; sexual behaviour; nucleus accumbens; dopamine

Introduction

Carbon monoxide (CO), an air pollutant produced by incomplete combustion of carbonaceous materials, represents one of the hundreds of constituents of cigarette smoke (National Research Council, 1977; Longo, 1982).

The developing central nervous system is extremely susceptible to chronic, relatively mild, decrease in oxygen availability induced by CO (Annau & Fechter, 1994). Previous findings have shown that prenatal exposure to CO, at concentrations (75–150 p.p.m.) below those associated with gross malformations and/or overt neurotoxic effects, induces a variety of neurobehavioural abnormalities in rat offspring including lower behavioural activity levels through preweaning, delayed development of homing behaviour and negative geotaxis, altered ontogeny of emotional responsiveness and permanent cognitive deficits (Fechter & Annau, 1977, 1980; Mactutus & Fechter, 1984, 1985; Di Giovanni *et al*., 1993; De Salvia *et al*., 1995).

Furthermore, clinical reports have shown that children born to women who smoked during pregnancy exhibited poorer performance on cognitive tasks and language development (Gusella & Fried, 1984; Fried & Watkinson, 1988, 1990).

Recent findings have demonstrated that chronic prenatal hypoxia (10.5% O_2) alters sexual behaviour of adult male rats (Hermans *et al*., 1993). Since monoamines play an important role in the sexual differentiation of the brain (Gorski, 1973; Sparber, 1974; Reznikov & Nosenko, 1983; Jarzab *et al*., 1986) and developmental hypoxia affects catecholaminergic activity in rat brain (Seidler & Slotkin, 1990; Burke *et al*., 1992; Gross *et al*., 1993), it has been suggested that prenatal hypoxia may exert a 'demasculinizing' effect through an influence on central noradrenergic and/or dopaminergic system (Hermans *et al*., 1993).

However, there is no evidence in the literature that changes in sexual behaviour are produced by prenatal exposure models that simulate the CO exposure observed in human cigarette smokers.

The present study, undertaken to investigate the influence of gestational exposure to low CO concentrations (75 and 150 p.p.m.) on sexual activity of male rat offspring, has revealed an impairment of copulatory activity as well as an altered behavioural response to a challenge dose of amphetamine in CO (150 p.p.m.)-exposed animals.

On the basis of these observations and considering that dopamine plays an important role in the physiologic regulation of male rat sexual behaviour (Gessa & Tagliamonte, 1975), neurochemical experiments (*in vivo* microdialysis) were also carried out to investigate the effects of *in utero* exposure to CO (150 p.p.m.) on extracellular concentrations of dopamine in the nucleus accumbens as well as on biochemical responsiveness to a challenge dose of amphetamine.

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Methods

Animals and exposure conditions

This study was performed in accordance with the Italian ethics legislation governing these experiments (Ministry of Health, D.L. 116/92).

Primiparous Wistar female rats (Morini Laboratories, S. Polo d'Enza, Italy) weighing 250–280 g were used. Animals were allowed free access to food and water, were housed at constant room temperature (20–22°C) and exposed to a light cycle of 12 h/day (08:00 h–20:00 h) for 1 week before the experiment. Pairs of females were placed with single male rats in the late afternoon. Vaginal smears were taken the following morning at 09:00 h. The day on which sperm were present was designated day 0 of gestation. Pregnant rats were then exposed to 0, 75 or 150 p.p.m. of CO mixed with air from day 0 to day 20 of pregnancy.

Exposures were conducted in stainless steel and glass 0.73 m^3 chambers (A.&L.CO. Industries, Segrate, Milan, Italy) with the airflow maintained at 120 l min^{-1}. Concentrations of CO in each exposure chamber were monitored every 15 min for a 5 min period by an infrared CO detector (CO 11M, Environment SA, Poissy, France) at a wavelength of 4.67 μm. Actual concentration was recorded on a digital display every 3 s for the 5 min period. Moreover, CO concentrations were also continuously monitored by a multi-pen recorder. Chamber concentrations of CO deviated by less than 3% from the stated value. Mean temperature values in the exposure chambers ranged from 21–23°C, and the light-dark cycle was 12 h light (08.00 h–20.00 h) and 12 h dark (20.00 h–08.00 h).

All litters were reduced to a standard size of six male pups per litter (when possible) within 24 h after birth. Litters from the control group (0 p.p.m. of CO) or CO-exposed groups (75 and 150 p.p.m.) were then assigned (six pups per litter) to non-exposed mothers whose pups were born on the same day. Data were collected only from male pups whose mothers were exposed either to 0 p.p.m. CO or to CO (75 and 150 p.p.m.) during pregnancy. Pups were weaned at 21 days of age. One pup per litter from different litters per treatment group was used in both behavioural and biochemical experiments.

As observed previously (Di Giovanni *et al*., 1993), prenatal CO (75 and 150 p.p.m.) exposure does not affect dam weight gain, number of dams giving birth, pregnancy length, litter size at birth, pup weight gain, and postnatal mortality (data not shown).

Spectrophotometric measurements of maternal carboxyhaemoglobin (HbCO)

Subgroups of pregnant CO (0, 75 and 150 p.p.m.)-exposed rats were implanted under anaesthesia (Equithesin, 3 ml kg^{-1} i.p.) with catheters in the abdominal aorta. Maternal HbCO was measured by a spectrophotometric method described by Rodkey *et al*. (1979). Briefly, blood samples (10 μl) were taken into a heparinized syringe, diluted about 1000 fold in a deionized solution containing $Na_2S_2O_4$ (2 mg ml^{-1}) and analysed for their absorbance in the Soret region (390–440 nm) using a UV/VIS spectrophotometer (Perkin Elmer). Measurements were performed on gestational day (GD) 10 and 20. HbCO levels were also measured in non-pregnant rats.

Sexual behaviour

The technique has been previously described by Cagiano *et al*. (1988). Heterosexually naive male rats prenatally exposed to 0, 75 and 150 p.p.m. CO were tested for sexual behaviour at 80 days of age (10 min session); thereafter, animals were subjected to a further five 10 min sessions (the inter-session interval was 15 days). Rats achieving the first ejaculation in one of the six 10 min sessions were not retested in the remaining 10 min sessions. The maximum duration of these tests thus was 155 days. Furthermore, rats were tested again at 10 months of age for sexual activity (30 min session). In a further series of experiments, 80-day old control and CO (150 p.p.m.)-exposed rats were given a challenge dose of amphetamine (0.5 mg kg^{-1}) 20 min before the 10 min session. d-Amphetamine sulphate (Sigma Chemical Company, St. Louis, U.S.A.) was dissolved in saline and administered subcutaneously in a volume of 2 ml kg^{-1}. As stimulus females, we used bilaterally ovariectomized female rats in which oestrous had been induced by subcutaneous injections of estradiol benzoate (8 μg/rat) and progesterone (200 μg/rat) dissolved in 0.2 ml of sesame oil, 52 and 4 h before the test sessions, respectively.

Male and female rats were housed under a reversed 12/12 h light-dark cycle (light on: 20.00 h–08.00 h) for 2 weeks before testing. Each male rat was tested for sexual behaviour with a stimulus female under red illumination provided by two 40 W fluorescent lamps. Sexual behaviour was recorded by a JVC video camera connected to a JVC video-tape recorder. The experiments, performed in the central part of the dark period (12.00 h–16.00 h), were carried out in a sound-attenuating cabin (Amplifon G-type cabin).

Each male rat was observed alone for 5 min.; an oestrous female was then introduced into the centre of the arena and the behaviour of the male was then recorded. Video tape-recordings were later replayed and analysed (in slow motion when necessary) and the following parameters were measured: (M/IL) mount-intromission latency (time between the introduction of the female into the mating cage and the first mount or intromission in the first ejaculatory series); (M/IF) mount-intromission frequency (number of mounts or intromissions in each ejaculatory series); (EjL) ejaculation latency (time between the first intromission and ejaculation in each ejaculatory series); (EjF) ejaculation frequency (number of ejaculations in each session); (PEjI) post-ejaculatory interval (interval between each ejaculation and the next intromission in each ejaculatory series); (TM/IF) total mount/intromission frequency (total number of mounts or intromissions during the test session); (ICI) inter copulatory interval (ejaculation latency/intromission frequency in each ejaculatory series).

Microdialysis experiments

Eighty day- and 10 month-old male rats exposed to 0 and 150 p.p.m. CO during gestation were anaesthetized with Equithesin (3 ml kg^{-1} i.p.) and placed on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.).

The skull was exposed and a small hole was drilled. The dura was exposed and removed before inserting the probe at the level of the right shell of the nucleus accumbens (NAC). A vertical dialysis probe (2 mm) was made of copolymer of acrylonitrile sodium metallylsulphonate (AN69 Hospal SpA; 20,000 Daltons cutoff).

Stereotaxic coordinates were as follows: AP = +11.4, L = +1.2, H = +1.8 (80 day-old rats) and AP = +11.8, L = +1.4, H = +1.2 (10 month-old rats) from the interaural line with the incisor bar set at −3.3 according to the Paxinos and Watson atlas (1982).

Experiments were performed 24 h after probe implant on freely moving rats. On the day of the experiment, fibres were

perfused with Krebs Ringer solution containing (mM): NaCl 145, KCl 3, $CaCl_2$ 2.2, $MgCl_2$ 1 in distilled water. The solution was buffered at pH 7.4 with a 2 mM sodiumphosphate buffer, filtered (0.22 μm) and degassed. Fibres were perfused at a constant flow rate of 2 μl min^{-1} with a CMA/100 microinjection pump (Carnegie Medicine, Stockholm, Sweden). After a 60 min wash-out period, consecutive 20 min samples of perfusate were collected and injected into a high performance liquid chromatography (HPLC) equipped with an electrochemical detector (ESA, Coulochem II, Bedford, MA, U.S.A.) in order to quantitate dopamine (DA), according to the technique described by Pozzi *et al.* (1995). The first electrode was set at +300 mV (oxidation) and the second at −225 mV (reduction). Separation was obtained by using a reverse phase column (LC-18 DB, 15 cm, 5 μm particle size, Supelco). The mobile phase, consisting of 13.6 g l^{-1} of sodium acetate, 37 mg l^{-1} of disodium EDTA dihydrate, 80 mg l^{-1} of octyl sodium sulphate, 60 ml l^{-1} of methanol, pH 4.1 with acetic acid, was pumped at a constant flow rate of 1.0 ml min^{-1} (Shimadzu LC-10AD).

Once a stable basal DA output was obtained (no more than 10% difference between three consecutive samples) rats were given a challenge dose of amphetamine (0.5 mg kg^{-1}). d-Amphetamine sulphate (Sigma Chemical Company, St. Louis, U.S.A.) was dissolved in saline and administered s.c. in a volume of 2 ml kg^{-1}. The position of the microdialysis probe was verified by histological procedures at the end of each experiment. Only rats in which the probe track was exactly located in the target area were considered in the results.

Statistical analysis

Blood HbCO levels were analysed by two-tail Student's *t*-test with Bonferroni's correction.

Behavioural data were evaluated first for homogeneity of variances with Bartlett's test. Since the χ^2 value was significant, indicating non-homogeneity of variances, a procedure for data transformation was adopted. However, even after the appropriate transformation of data, Bartlett's test showed a significant heteroschedasticity in all instances. This led to the adoption of a non parametric ANOVA (Kruskal-Wallis ANOVA) followed by two-tail Mann-Whitney *U*-test with Bonferroni's correction as *post-hoc* test. Two-tail Fisher's exact-test was used where appropriate.

Neurochemical data were expressed as fmol/20 min/40 μl. The baseline was defined as the average of at least three consecutive samples with stable levels of DA. Statistical analysis of actual values of basal DA, DOPAC and HVA concentrations (three consecutive samples collected before amphetamine challenge) and of actual DA, DOPAC and HVA changes elicited by amphetamine challenge (with respect to the baseline) was performed using a two-way ANOVA for repeated measures with treatment as the between-subject factor and time as the within-subject factor. *Post-hoc* comparisons were made by two-tail Dunnett's *t*-test.

Results

Maternal HbCO levels

As shown in Figure 1, exposure to CO (75 and 150 p.p.m.) produced a dose-dependent increase in maternal blood HbCO levels on both gestational days (GDs) 10 and 20. HbCO levels in control rats were significantly increased on GD 20 with respect to those found on GD 10. Moreover, rats exposed to 0 p.p.m. CO during pregnancy exhibited higher HbCO levels with respect to those detected in non pregnant females.

Effects of prenatal exposure to CO on sexual behaviour of male offspring

The results indicate that prenatal exposure to CO notably affected copulatory activity of rat offspring. In particular, a significant increase in mount/intromission latency was observed in 80 day old male rats prenatally exposed to CO (150 p.p.m.) with respect to controls (Figure 2).

Moreover, 80 day old CO (150 p.p.m.)-exposed rats exhibited a significant decrease in mount/intromission frequency with respect to control animals (Figure 3).

The percentage of rats (the group sizes for the first 10 min session were as follows: control = 7; CO 75 p.p.m. = 8; CO

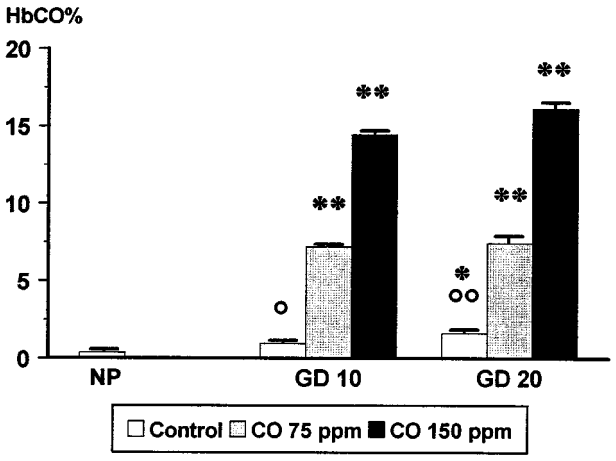

Figure 1 Blood carboxyhaemoglobin (HbCO) levels in pregnant rats exposed to carbon monoxide (CO). Values are means ± s.e.mean of four to six rats; NP: non pregnant rats; GD: gestational day. °$P<0.01$, °°$P<0.001$ vs NP; *$P<0.01$ vs GD 10; **$P<0.001$ vs controls (two-tail Student's *t*-test with Bonferroni's correction).

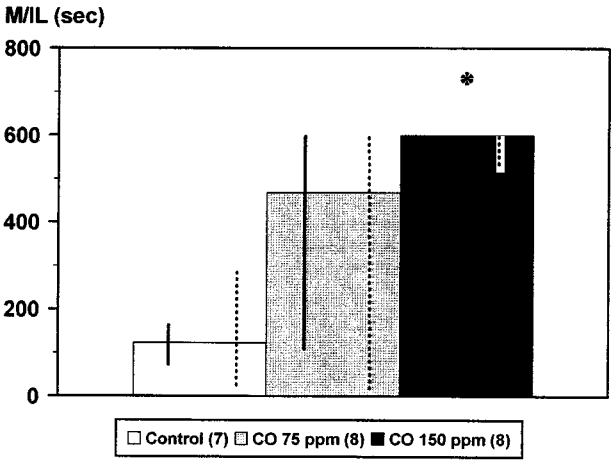

Figure 2 Effects of prenatal exposure to carbon monoxide (CO) on Mount/Intromission Latency (M/IL) in 80 day old rats during a 10 min session. No. of animals in parentheses. Data are expressed as median values, interquartiles (–) and full ranges (- - - - - -). Kruskal-Wallis ANOVA: H = 11.68, *d.f.* = 2, $P<0.005$.*$P<0.001$ vs controls (two-tail Mann–Whitney *U*-test with Bonferroni's correction).

150 p.p.m. = 8) achieving one ejaculation within the 6th 10 min session (155 days of age) was significantly lower ($P<0.01$) in the CO (150 p.p.m.)-exposed group with respect to the control group (0 p.p.m.: 71.4%; 75 p.p.m.: 25%: 150 p.p.m.: 0%).

The administration of a challenge dose of amphetamine (0.5 mg kg^{-1} s.c.) significantly decreased mount/intromission latency (Figure 4) and significantly increased mount frequency in 80 day old control rats (Figure 5). Conversely, acute amphetamine failed to reduce mount/intromission latency (Figure 4) and did not increase mount frequency (Figure 5) in animals exposed to CO (150 p.p.m.) during gestation.

No significant change in copulatory activity was observed in 10 month old rats exposed to CO during pregnancy (Figures 7 and 8).

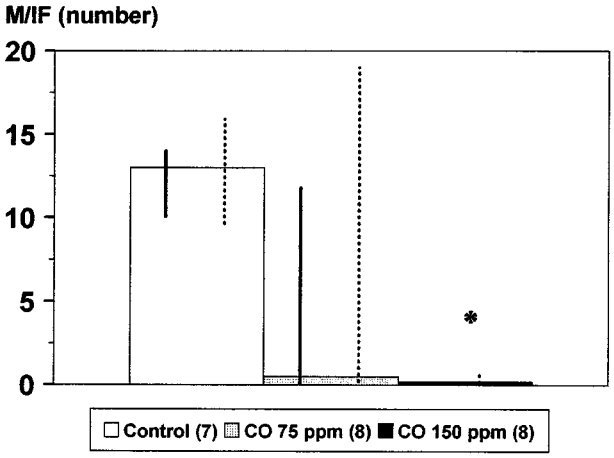

Figure 3 Effects of prenatal exposure to carbon monoxide (CO) on Mount/Intromission Frequency (M/IF) in 80 day old rats during a 10 min session. No. of animals in parentheses. Data are expressed as median values, interquartiles (–) and full ranges (- - - - - -). Kruskal-Wallis ANOVA: H = 11.71, *d.f.* = 2, $P<0.005$. *$P<0.001$ vs controls (two-tail Mann–Whitney *U*-test with Bonferroni's correction).

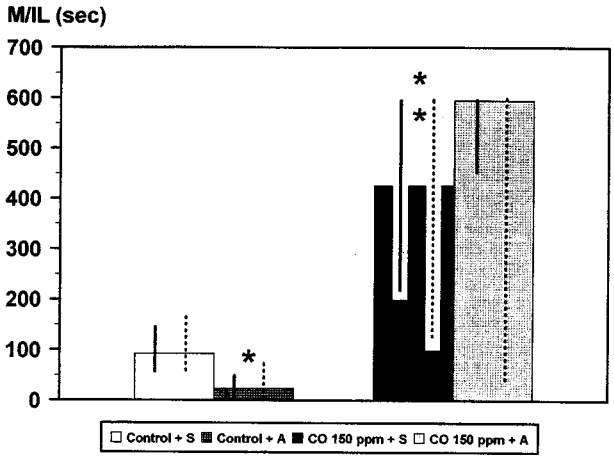

Figure 4 Effects of amphetamine (0.5 mg kg^{-1} s.c.) on Mount/Intromission Latency (M/IL) in 80 day old rats prenatally exposed to 0 p.p.m. CO or 150 p.p.m. CO (10 min session). Each group consisted of six animals. S = Saline; A = Amphetamine. Data are expressed as median values, interquartiles (–) and full ranges (- - - - - -). Kruskal-Wallis ANOVA: H = 16.43, *d.f.* = 3, $P<0.0001$. *$P<0.05$, **$P<0.01$ vs control + S (two-tail Mann–Whitney *U*-test with Bonferroni's correction).

Effect of prenatal exposure to CO on extracellular concentrations of DA, DOPAC and HVA in the NAC of male offspring

Prenatal CO (150 p.p.m.)-exposure did not affect basal DA levels in the NAC of 80 day old rats (Figure 6).

Individual comparisons within groups showed that the administration of a challenge dose of amphetamine (0.5 mg kg^{-1} s.c.) induced a significant increase in extracellular concentrations of DA with respect to the baseline in both control and CO (150 p.p.m.)-exposed rats. However, between groups, comparisons showed that the amphetamine-induced increase in DA levels was significantly attenuated in CO (150 p.p.m.)-exposed rats with respect to control animals (Figure 6).

Prenatal CO (150 p.p.m.) exposure did not affect either basal DOPAC and HVA levels or the amphetamine

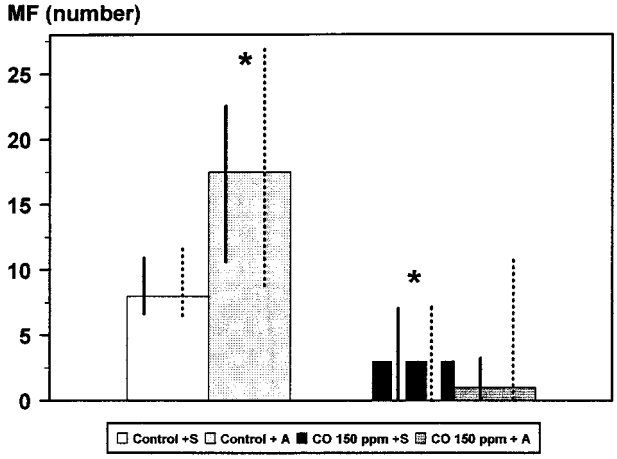

Figure 5 Effects of amphetamine (0.5 mg kg^{-1} s.c.) on Mount Frequency (MF) in 80 day old rats prenatally exposed to 0 p.p.m. CO or 150 p.p.m. CO (10 min session). Each group consisted of six animals. S = Saline; A = Amphetamine. Data are expressed as median values, interquartiles (–) and full ranges (- - - - - -). Kruskal-Wallis ANOVA: H = 15.10, *d.f.* = 3, $P<0.0001$. *$P<0.05$ vs control + S (two-tail Mann–Whitney *U*-test with Bonferroni's correction).

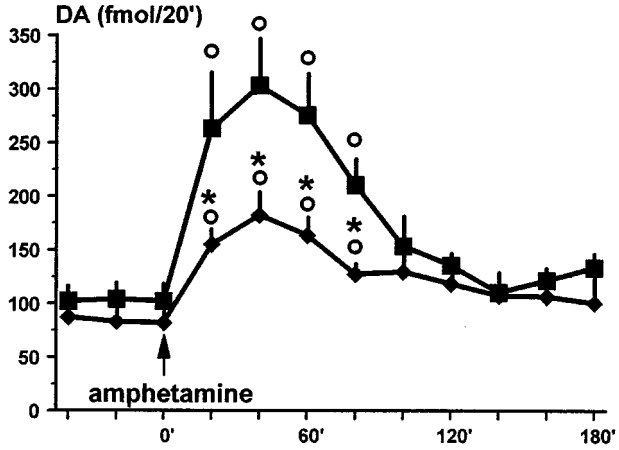

Figure 6 Effects of amphetamine challenge (0.5 mg kg^{-1} s.c.) on extracellular dopamine (DA) concentrations in the nucleus accumbens (NAC) of 80 day old rats exposed prenatally to 0 p.p.m. CO (■) or 150 p.p.m. CO (◆). Data were expressed as means ± s.e.mean of four to five rats. Significant differences (two-tail Dunnett's *t*-test): °$P<0.05$ vs baseline; *$P<0.05$ vs 0 p.p.m. CO-exposed rats.

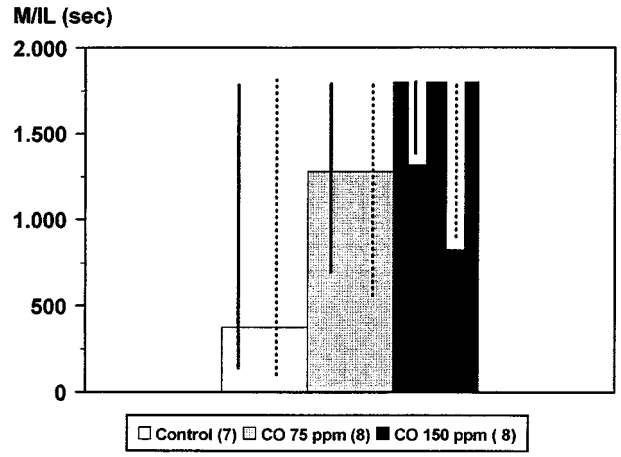

Figure 7 Effects of prenatal exposure to carbon monoxide (CO) on Mount/Intromission Latency (M/IL) in 10-month old rats during a 30 min session. No. of animals in parentheses. Data are expressed as median values, interquartiles (–) and full ranges (- - - - -).

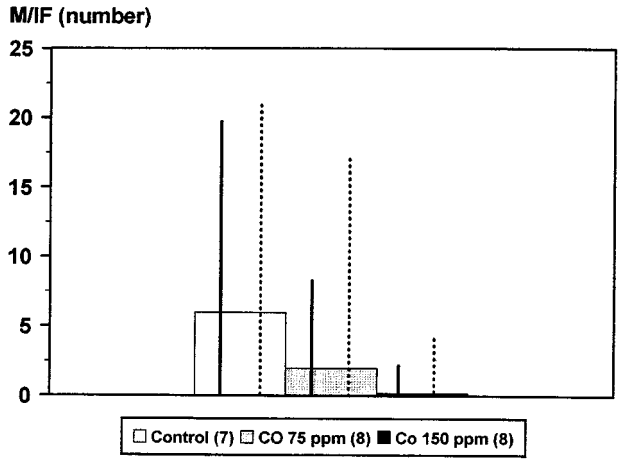

Figure 8 Effects of prenatal exposure to carbon monoxide (CO) on Mount/Intromission Frequency (M/IF) in 10-month old rats during a 30 min session. No. of animals in parentheses. Data are expressed as median values, interquartiles (–) and full ranges (- - - - -).

(0.5 mg kg^{-1})-induced decrease in DOPAC and HVA levels in 80 day old rats (data not shown). Furthermore, no significant changes in neurochemical parameters were observed in 10 month old rats exposed prenatally to CO (Figure 9). Overall two-way ANOVAs for repeated measures are reported in Table 1.

Discussion

The results of the present study provide the first evidence that prenatal exposure to low CO concentrations causes a long lasting (up to 5 months of age) impairment of sexual behaviour and induces subtle changes in mesolimbic dopaminergic function in male rat offspring. Conversely, there is no evidence in the literature that similar alterations in both sexual behaviour and mesolimbic dopaminergic function can be

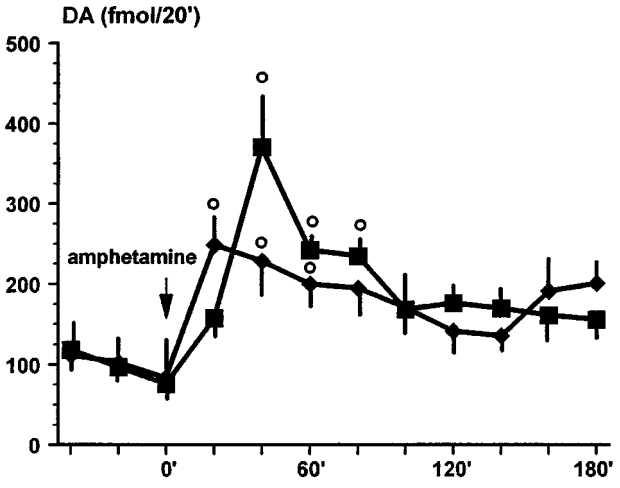

Figure 9 Effects of amphetamine challenge (0.5 mg kg^{-1} s.c.) on extracellular dopamine (DA) concentrations in the nucleus accumbens (NAC) of 10-month old rats exposed prenatally to 0 p.p.m. CO (■) or 150 p.p.m. CO (◆). Data were expressed as means ± s.e.mean of five rats. Significant differences (two-tail Dunnett's *t*-test): $^{\circ}P<0.05$ vs baseline.

Table 1 Overall two-way ANOVAs for repeated measures (neurochemical data)

Rat offspring	*Factors*	*DA*			*HVA*			*DOPAC*		
		F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
80 day old	*Basal levels*[1]									
	Treatments	0.63	1.7	n.s.	1.23	1.7	n.s.	0.63	1.7	n.s.
	Times	0.37	2.14	n.s.	0.40	2.14	n.s.	0.60	2.14	n.s.
	Treatments × times	0.47	2.14	n.s.	0.86	2.14	n.s.	0.01	2.14	n.s.
	Amphetamine challenge[2]									
	Treatments	5.30	1.7	=0.05	0.55	1.7	n.s.	0.21	1.7	n.s.
	Times	15.73	9.63	<0.01	7.83	9.63	<0.001	11.33	9.63	<0.001
	Treatments × times	3.02	9.63	<0.01	1.00	9.63	n.s.	0.55	9.63	n.s.
10-month old	*Basal levels*[1]									
	Treatments	0.03	1.8	n.s	0.16	1.8	n.s.	0.65	1.8	n.s.
	Times	3.21	2.16	n.s.	0.35	2.16	n.s.	0.04	2.16	n.s.
	Treatments × times	0.15	2.16	n.s.	1.09	2.16	n.s.	0.97	2.16	n.s.
	Amphetamine challenge[2]									
	Treatments	0.04	1.8	n.s.	0.02	1.8	n.s.	0.06	1.8	n.s.
	Times	5.08	9.72	<0.001	3.95	9.72	<0.001	1.02	9.72	n.s.
	Treatments × times	0.08	9.72	n.s.	0.38	9.72	n.s.	0.98	9.72	n.s.

[1]Three consecutive samples collected before amphetamine challenge. [2]Baseline and nine consecutive samples collected after amphetamine challenge.

obtained by exposing adult rats to low concentrations of CO, thus suggesting that the developing brain is much more susceptible to the effects of this gas than is the mature brain.

The behavioural and neurochemical changes occurred at maternal HbCO levels equivalent to those found in the blood of cigarette smokers (Goldsmith, 1970; Lawther & Commins, 1970; Kahn *et al*., 1974). Moreover, our data confirm that HbCO concentrations tend to be somewhat elevated during gestation, reflecting increased endogenous CO production (Longo, 1970, 1976; Longo & Hill, 1977).

The behavioural alterations exhibited by CO (150 p.p.m.)-exposed offspring (increase in mount/intromission latency and decrease in mount/intromission frequency in 80 day old rats; ejaculatory abnormalities in 5-month old animals) are consistent with the results of recent findings showing that prenatal exposure to hypoxic hypoxia (10.5% O_2 during the last two trimesters) induces incomplete masculinization of male rat offspring (Hermans *et al*., 1993). However, it should be pointed out that such a severe hypoxia induces a generalized stress syndrome in animals, as evidenced by the significant decrease in birth weight, which by itself can contribute to reproductive dysfunctions. Conversely, CO levels used in our experiments are unlikely to cause the severe stress induced by the hypoxic level reported in the above study.

Interestingly, our findings have also shown that the administration of amphetamine, at a dose (0.5 mg kg^{-1} s.c.) which stimulates copulatory activity in control animals, failed to reduce mount/intromission latency and did not increase mount frequency in 80 day old rats exposed to CO (150 p.p.m.) during gestation.

The impaired behavioural response to amphetamine shown by 80 day old rats exposed to CO (150 p.p.m.) during gestation was paralleled by neurochemical changes in the nucleus accumbens of offspring prenatally exposed to this gas. In particular, *in vivo* microdialysis experiments have shown that prenatal CO (150 p.p.m.)-exposure blunted the amphetamine-induced increase in dopamine release in 80 day old rats.

Therefore, these data suggest that the inhalation of low concentrations of CO by pregnant rats leads to changes in mesolimbic dopaminergic transmission which are associated with an impairment of sexual behaviour in male offspring.

A transiently reduced behavioural and biochemical responsiveness to a pharmacologic challenge affecting dopaminergic system (L-DOPA) has been observed previously in 1 and 4 day old rat pups exposed to CO during gestation (Fechter & Annau, 1977).

At 10 months of age, rats prenatally exposed to CO (75 and 150 p.p.m.) did not exhibit significant changes in copulatory activity and mesolimbic dopaminergic function with respect to controls, even though a trend towards an impairment of some indices of sexual behaviour (i.e., decrease in mount/intromission frequency and increase in mount/intromission latency) was still observed in CO (150 p.p.m.)-exposed animals.

However, since the copulatory activity (mount/intromission frequency) of 10 month old control rats was somewhat low, we cannot exclude that the lack of significant alterations in CO (150 p.p.m.)-exposed rats could be due to a 'floor effect'.

Neurochemical studies have shown that dopaminergic transmission is facilitatory for masculine sexual behaviour (Gessa & Tagliamonte, 1975) and increased release of dopamine in the nucleus accumbens of copulating male rats has been documented (Fumero *et al*., 1994). Moreover, prenatal administration of haloperidol, a dopamine receptor blocking agent, causes incomplete masculinization of male rat sexual behaviour (Hull *et al*., 1984) and alterations in male rat sexual motivation (Cagiano *et al*., 1988).

The present findings, in line with those previously reported in the literature (Fechter *et al*., 1987; Gross *et al*., 1993; Weese-Mayer *et al*., 1994; Nyakas *et al*., 1996), provide further evidence that the developing dopaminergic system seems to be extremely vulnerable to hypoxia. However, since the activity of central dopaminergic system following gestational CO exposure has not been extensively investigated, mechanisms that may be responsible for the observed neurochemical alterations remain elusive.

Furthermore, it has been demonstrated that pharmacologic treatments interfering with testosterone secretion during critical periods of brain development result in incomplete masculinization of male rat behavioural patterns (McGivern *et al*., 1984; Lichtensteiger & Schlumpf, 1985; Raum *et al*., 1990).

Previous studies have shown that chronic prenatal exposure to hypoxia (10.5% O_2) induces incomplete masculinization of rat reproductive behaviour in the absence of overt changes in perinatal testosterone surges (Hermans *et al*., 1993).

Conversely, our recent experiments (unpublished data) have demonstrated that male rats exposed to CO (150 p.p.m.) during gestation exhibited significantly lower concentrations of plasma testosterone with respect to control animals immediately before the pubertal surge (50 days of age). This may suggest that prenatal exposure to CO would interfere with the development of mechanisms regulating gonadotropin secretion. However, no significant changes in plasma testosterone levels were found in 80 day and 5 month old CO (150 p.p.m.)-exposed rats showing notable alterations in sexual behaviour.

Finally, since mice with targeted disruption of the gene for heme oxygenase-2 have recently been reported to exhibit ejaculatory abnormalities (Burnett *et al*., 1998), it is also possible that maternal exposure to CO may influence the development or function of neurones releasing CO locally in the penis.

In summary, the present findings show that prenatal exposure of rats to CO results in functional alterations (impairment of sexual behaviour associated with changes in mesolimbic dopaminergic function) that are measurable at CO levels approaching those experienced by offspring of mothers who smoke during pregnancy.

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