Intermittent Exposure of Gravid Rats to 1% Nitrous Oxide and the Effect on the Postnatal Growth of their Offspring

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SUMMARY

Three groups of pregnant rats were exposed to 1% nitrous oxide 6 hours a day, 5 days per week for the first, first and second, and all 3 weeks of gestation. The offspring were weighed and measured at weekly intervals. The following variables were evaluated in control and experimental groups: litter size, body weight, tail length and body length. Litter sizes in animals exposed for the first and second weeks of gestation were significantly smaller than the controls. A two-way nested statistical analysis of the data revealed that over an 8-week period the experimental rats were significantly smaller than the control rats of the same age. Analysis of the 95% confidence intervals revealed no particular pattern related to the timing of the exposure to nitrous oxide.

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There is a growing concern today that anaesthetic gases which escape into the operating theatre may be hazardous to operating room personnel. Nitrous oxide (N_2O) may be one of the anaesthetic agents responsible for spontaneous abortion in female anaesthetists and theatre nurses,[1] possibly owing to the teratogenic effect of the gas.[2,3] The purpose of this study was to determine the effect on the litter size and growth of the offspring when gravid rats had been intermittently exposed to a 1% N_2O atmosphere during various stages of gestation.

MATERIALS AND METHODS

Twelve-week-old female Wistar strain albino rats were obtained from the South African Institute for Medical Research. Rats were mated by placing three female rats with one male rat in a cage in the evening. The following morning the females were examined for copulation plugs and mating was confirmed by examining vaginal smears for the presence of spermatozoa. In this study the time of conception was taken to be midnight on the night of mating. The day of the positive smear was designated day 0, the next day 1, and so on. Pregnant rats were divided into 6 groups of 8 each, of which 3 groups were subjected to N_2O exposure during gestation and 3 groups served as controls. Of the experimental groups, the first group was exposed to N_2O during their entire gestation (i.e. 3 weeks), for 6 hours per day (from 10h00 to 16h00), Monday to Friday. The second group was exposed for the first and second weeks of gestation, while the third group was exposed for the first week of gestation only.

The rats were exposed to N_2O by placing their cages in a specially constructed perspex environmental chamber,[4] into which a mixture of compressed air (9,9 l/min) and N_2O (0,1l/min) was introduced. This flow rate provided for 10 changes of atmosphere per hour. To ensure an even distribution of the gas mixture it was passed through a centrifugal fan for mixing before entering the chamber. The gas mixture was vented through an exhaust duct out of the experimental room. The positions of the cages in the chamber were alternated on a daily rotational basis to provide for any variations in exposure to the gas. The humidity in the chamber ranged between 50% and 60% and no CO_2 build-up was detected. Standard mouse diet (Epol, Vereeniging Consolidated Mills) and water were provided *ad libitum* to all the rats. The control rats were exposed to atmospheric air in the same room as the experimental groups.

Each gravid female was isolated shortly before giving birth. At birth all litter mates were examined for gross defects and the number in each litter was recorded.

At weekly intervals the young rats were weighed, and tail and body length were measured with an apparatus described by Jefferys.[6]

Statistical Analysis

The experimental format was a mixed two-way nested design. The statistical analysis consisted of a nested two-way analysis of variance for a fixed main group effect (control and N_2O exposed groups) and random subgroup effects (rat litters). The former analysis was carried out by means of a modified formula according to Johnson and Leone.[6] The probability values of the calculations were obtained from standard F-value tables.

Finally, the 95% confidence intervals of all the results of all the groups at the various time intervals were examined to see which experimental group was significantly different from the controls.

A one-way analysis of variance and Student's *t* test using the within-groups mean square for pooled variance was applied to test for differences in litter size.

RESULTS

Litter Size

A total of 410 rats was born, of which 248 were control rats and 162 were experimental rats. The mean number

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TABLE I. LITTER SIZE FOR VARIOUS RAT GROUPS

Group	Period of exposure to 1% N_2O/air	Number of litters	Total offspring	Rats per litter (mean ± SD)	Student's *t* test: *t*	Student's *t* test: *P* value
Control	None	8	248	10,4 ± 2,3	—	—
1	3 weeks	8	66	8,3 ± 3,2	6,4	<0,001
2	1st and 2nd weeks	8	50	6,3 ± 2,6	11,2	<0,001
3	1st week	8	46	5,7 ± 1,6	12,4	<0,001

of young rats per litter is shown in Table I. There were significantly fewer rats per litter in all females exposed to 1% N_2O during gestation.

In Table II the mean body weights of both the N_2O-exposed and the control animals are listed, as well as the values obtained through pooling of all the results of all the experimental animals. Experimental animals were significantly lighter than the control rats throughout the 8-week period. At each of the weekly intervals there was a significant difference between the three experimental and the control groups. There were also significant differences between litters at all intervals except 7 weeks.

Table III shows the results obtained in the tail length measurements. At 1 and 2 weeks there were no statistically significant differences between the tail lengths of rats in the control and the N_2O-exposed groups, but thereafter the differences were highly significant.

The growth in body length is listed in Table IV. There were significant differences between the body lengths of rats in the N_2O-exposed and the control groups for the first 6 weeks of the 8-week period.

Examination of the 95% confidence intervals of the data at the various periods revealed no particular pattern of effects related to the timing of the exposure to N_2O. On comparison of the N_2O-exposed groups, it appeared that N_2O had a greater effect on the growth of rats exposed for only the first week of the gestation. This observation was not constant throughout the 8-week period of the study. For body weight this was found during the first, fourth and sixth weeks of growth, for body length during the first, third and fourth weeks of growth, and for tail length during the third, fifth and eighth weeks of growth.

DISCUSSION

The conditions of exposure to mixtures of N_2O and air reported thus far vary considerably. Fink *et al.*[3] exposed

TABLE II. BODY WEIGHT (g)

Weeks postpartum	Control (mean ± SD)	Pooled values for all N_2O-exposed groups (mean ± SD)	Period exposed to N_2O: 3 weeks (mean ± SD)	1st and 2nd weeks (mean ± SD)	1st week (mean ± SD)
1	10,0 ± 1,2	7,0 ± 1,2*	8,1 ± 1,6	8,3 ± 1,4	6,5 ± 0,53
2	17,6 ± 4,7	15,5 ± 4,83*	17,2 ± 2,7	16,1 ± 4,0	13,4 ± 7,8
3	31,6 ± 4,5	24,5 ± 6,13*	27,7 ± 2,8	24,8 ± 4,8	20,9 ± 10,8
4	42,3 ± 3,9	39,1 ± 4,27†	39,4 ± 3,5	41,9 ± 5,6	31,2 ± 13,7
5	54,0 ± 10,3	52,8 ± 10,27*	50,6 ± 5,6	58,0 ± 14,3	50,0 ± 10,9
6	67,0 ± 7,7	65,0 ± 11,23*	64,7 ± 8,7	68,4 ± 10,7	62,3 ± 14,3
7	85,6 ± 9,3	80,2 ± 14,20†	81,5 ± 17,7	85,0 ± 18,3	74,2 ± 6,6
8	107,8 ± 8,10	90,7 ± 15,80*	91,3 ± 21,0	94,3 ± 21,2	86,7 ± 5,2

P values for main group effects: * $P<0.001$, † $P<0.05$.

TABLE III. TAIL LENGTH (mm)

Weeks postpartum	Control (mean ± SD)	Pooled values for all N_2O-exposed groups (mean ± SD)	Period exposed to N_2O: 3 weeks (mean ± SD)	1st and 2nd weeks (mean ± SD)	1st week (mean ± SD)
1	54,0 ± 4,1	23,0 ± 4,6	24,6 ± 4,0	25,4 ± 5,8	20,6 ± 4,1
2	71,0 ± 3,7	36,2 ± 4,6	38,6 ± 2,2	38,6 ± 6,3	28,5 ± 5,3
3	78,1 ± 5,9	43,0 ± 6,6*	48,7 ± 5,8	42,8 ± 6,4	40,1 ± 7,7
4	95,1 ± 14,2	62,0 ± 6,4*	66,9 ± 11,8	68,6 ± 3,5	50,5 ± 3,9
5	118,7 ± 7,0	80,0 ± 10,3*	82,3 ± 13,8	88,1 ± 5,3	67,1 ± 12,8
6	132,4 ± 8,8	89,1 ± 5,8*	80,8 ± 2,5	95,6 ± 13,1	90,8 ± 2,0
7	147,7 ± 9,8	103,9 ± 7,9†	105,6 ± 10,5	108,6 ± 10,7	97,5 ± 2,7
8	159,2 ± 6,9	110,9 ± 10,63*	111,9 ± 12,8	115,0 ± 12,5	105,8 ± 6,6

P values for main group effects: * $P<0.001$, † $P<0.02$.

TABLE IV. BODY LENGTH (mm)

Weeks postpartum	Control (mean ± SD)	Pooled values for all N_2O-exposed groups (mean ± SD)	Period exposed to N_2O: 3 weeks (mean ± SD)	1st and 2nd weeks (mean ± SD)	1st week (mean ± SD)
1	59,5 ± 6,9	54,0 ± 4,9*	59,0 ± 4,4	56,3 ± 5,2	48,1 ± 5,3
2	79,5 ± 8,2	71,1 ± 6,5*	77,8 ± 5,9	76,7 ± 4,9	60,0 ± 8,7
3	88,2 ± 7,3	78,1 ± 16,0*	88,0 ± 4,2	77,8 ± 21,3	68,6 ± 9,2
4	108,8 ± 9,9	95,1 ± 9,8†	101,3 ± 8,3	107,0 ± 13,8	77,0 ± 7,4
5	138,7 ± 7,9	118,7 ± 18,7*	124,8 ± 16,6	131,5 ± 13,1	100,5 ± 26,4
6	148,6 ± 10,5	132,4 ± 15,0*	128,8 ± 11,8	142,6 ± 15,4	125,8 ± 17,8
7	156,7 ± 6,7	147,7 ± 8,0	150,6 ± 8,6	152,4 ± 9,1	140,0 ± 6,3
8	167,3 ± 5,6	159,2 ± 7,0	160,6 ± 8,2	162,0 ± 7,5	155,0 ± 5,5

P values for main group effects: * $P<0,001$, † $P<0,05$.

Sprague-Dawling rats continuously to 45 - 50% N_2O in air from day 8 of the gestation period, for 2, 4, and 6 days, i.e. during the second week of pregnancy, at an unknown number of air changes per hour. Corbett *et al.*[2] in one experiment used a continuous exposure, during the second and third weeks, to 0,1% and 1,5% mixtures of N_2O and air. In a second experiment the exposure was for 8 hours per day to 0,1% and 0,01% N_2O and air. No details were given of the number of air changes per hour. Ramazotto *et al.*[7] used an exposure of 25 minutes per day to 50% N_2O and air during each of the first, second, third and all 3 weeks of pregnancy but gave no further details of their technique.

In this study 1% was chosen as the experimental concentration because this approximates the highest N_2O pollution level reported in a dental surgery by Millard and Corbett.[8] The exposure time of 6 hours a day, 5 days per week, was selected to stimulate N_2O levels which may be encountered during a dentist's working day when N_2O sedation is being used.

The mean litter size of the control animals exposed to atmospheric air in our study, namely 10,4, closely approximates the mean litter size of 11,1 and 10,2 reported in two groups of gravid control rats that breathed compressed air in an environmental chamber and atmospheric air, respectively.[2] In a more recent unpublished study by us, the mean litter size of gravid rats that were exposed to compressed air in an environmental chamber was 11,3.

The reduction in litter size found in our study is in agreement with the results obtained in the other N_2O exposure studies. Fink *et al.*[3] reported a 28% fetal resorption rate, while Corbett *et al.*[2] noted significant reductions in litter size in rats exposed to 1,5% and 0,1% N_2O, but not to 0,01% N_2O. Ramazotto *et al.*[7] reported a significant increase in fetal death in those animals exposed to the anaesthetic during the first, third and all 3 weeks of pregnancy. This confirms that N_2O is toxic to the developing rat fetus.

The present study is the first study of postnatal growth of rats exposed to N_2O during the prenatal period. It has shown that the 1% N_2O and air mixture decreased the postnatal growth of the rats. The offspring that had been exposed during the first week of pregnancy were significantly smaller than those in the other two groups. The reason for this is not clear but may be related to exposure of the fertilized ovum before, during or immediately after implantation on day 5 of pregnancy.

CONCLUSIONS

It is not possible to extrapolate directly the results of this study to man. However, the fact that intermittent exposure to a low concentration of N_2O resulted in retarded postnatal growth suggests that growth studies need to be undertaken in the offspring of female anaesthetists and operating room personnel.

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