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Neurochemical, but not Behavioral, Deviations in the Offspring of Rats Following Prenatal or Paternal Inhalation Exposure to Ethanol[1]

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NELSON, B. K., W. S. BRIGHTWELL, D. R. MACKENZIE-TAYLOR, J. R. BURG AND V. J. MASSARI. *Neurochemical, but not behavioral, deviations in the offspring of rats following prenatal or paternal inhalation exposure to ethanol.* NEUROTOXICOL TERATOL **10**(1) 15–22, 1988.—In addition to its widespread social use, ethanol is used extensively as an industrial solvent. Inhalation exposures to ethanol which produce narcosis in maternal rats are not teratogenic. The present study sought to extend the previous research by including offspring from paternal exposures, and testing for behavioral disorders in the offspring following maternal or paternal exposures. Groups of 18 male (approximately 450 g) and 15 female (200–300 g) Sprague-Dawley rats were exposed 7 hours/day for six weeks or throughout gestation to 16000, 10000, or 0 ppm ethanol by inhalation and then mated with untreated rats. Litters were culled to 4 males and 4 females, and were fostered within 16 hours after birth to untreated dams which had delivered their litters within 48 hours previously. Offspring from paternally or maternally exposed animals performed as well as controls on days 10–90 in tests of neuromotor coordination (ascent on a wire mesh screen, rotorod), activity levels (open field, modified-automated open field, and running wheel), and learning ability (avoidance conditioning and operant conditioning). In addition, brains of 10 21-day-old pups were analyzed for neurochemical differences from controls in concentrations of protein and the neurotransmitters acetycholine, dopamine, norepinephrine, 5-hydroxytryptamine, substance P, Met-enkephalin, and β-endorphin. Levels of acetylcholine, dopamine, substance P, and β-endorphin were essentially unchanged in the offspring of rats exposed to ethanol. Complex, but significant changes in levels of norepinephrine occurred only in paternally exposed offspring. 5-Hydroxytryptamine levels were reduced in the cerebrum, and Met-enkephalin levels were increased in all brain regions of offspring from both maternally and paternally exposed rats.

Behavioral teratology Developmental neurotoxicology Neurochemistry Neurochemicals
Reproductive toxicology Ethanol Alcohols

MOST studies on prenatal ethanol have utilized oral administration, aimed at a chronic ingestion paradigm similar to that encountered in human alcoholism [1, 2, 14, 18–20, 44, 48]. However, ethanol is also widely used as an industrial solvent for fats and oils, in the manufacture of artificial silk, in lacquers and varnishes, in fuels, and as an intermediate in the production of a variety of industrial and commerical products. We previously administered concentrations of 0, 10000, 16000, and 20000 ppm ethanol by inhalation to groups of 15 pregnant rats for 7 hr/day throughout gestation [34]. Despite severe maternal toxicity, with blood alcohol levels approaching 200 mg/dl (0.2%), we observed no teratogenic effects. Blood levels at the lower concentrations were 50 and 3 mg/ml for 16000 and 10000 ppm, respectively, values consistent with low susceptibility of rats to inhalation exposure to ethanol [35]. The present study sought to extend this work to both paternal exposure and functional assessments in the offspring. As 20000 ppm ethanol had previously been found to produce complete narcosis and reduce feed intake in the exposed animals, we selected the two lower concentrations (16000 and 10000 ppm) for the present study. The higher concentration, 16000 ppm, produces slight maternal toxicity (reduction in feed intake) and 10000 ppm produces no discernable maternal toxicity.

[1]This research was conducted under the Good Research Practices program of our Division, which is based upon the guidelines of Good Laboratory Practices issued by the Food and Drug Administration in 1978. Also, during the conduct of this study, our facility received accreditation by the American Association of Accreditation of Laboratory Animal Care (May 1, 1984). Mention of product or company names does not constitute endorsement by NIOSH.

METHOD

Compound Administration

Inhalation exposures were conducted in 0.5 m^3 Hinners exposure chambers (Charles Spengler and Associates, Cincinnati, OH) as described previously [34]. Briefly, a micrometering pump controlled the injection of a specified amount of reagent grade ethanol (absolute-200 proof; AAPER Alcohol and Chemical Co., Louisville, KY) into one inlet of a three-way valve. Through the second inlet heated, compressed air was introduced and the liquid/air mixture was forced through the outlet into a Greensmith impinger for evaporation and mixing. The concentration was controlled by flow of the chemical and heat of the compressed air. This vapor/air mixture was introduced into the exposure chamber air flow upstream from an orifice plate, and the resulting turbulence provided uniform mixing of the vapor and air before it entered the chamber. Air flow through the chamber provided approximately one air change/minute.

The concentration within the chamber was monitored by a Miran 1A infrared analyzer (Wilkes/Foxboro Analytical, South Norwalk, CT) that had been calibrated within the concentration range being administered, and was connected to a stripchart recorder. Daily mean, range, and time-weighted average concentrations were calculated. These daily values were averaged for an overall study mean for each concentration. In addition, the analyzer was interfaced with an Apple II+ computer which recorded five-minute means of the ethanol concentration.

Samples of ethanol were analyzed by gas chromatography, and found to be of greater than 95% purity [34]. Target concentrations were 16000 and 10000 ppm ethanol, with the lower exposure (including their own control group) prepared approximately four months following the higher one. For independent verification of exposure chamber concentrations, charcoal tube samples were collected from the chamber atmosphere. Sampling times varied from 10–30 minute duration, and samples were collected at the rate of approximately 13 samples/week of exposures. These charcoal tubes were analyzed [11] by Arthur D. Little, Inc., Cambridge, MA, to provide an independent measure of chamber concentration.

Experimental Animals

Virgin female Sprague-Dawley rats (VAF/plus; 176–200 g, Charles River Breeding Laboratories, Wilmington, MA) were acclimated to a 12 hr light-dark cycle and to a temperature of 24±2° during quarantine for one–two weeks. The humidity was generally about 40% (range 20–70%). Males over 300 g from the same source were housed individually under similar conditions in suspended steel wire mesh cages equipped with automatic water dispensers (Hoeltge Inc., Cincinnati, OH). Virgin females were housed three/cage in similar cages. Rodent pellets (NIH-07; Zeigler Bros., Inc, Gardner, PA) and tap water were available ad lib, except when animals were in the exposure chambers.

For breeding, females were placed individually with males. Each morning, the paper under each male's cage was examined for sperm plugs. If no plugs were detected, vaginal smears were taken to determine stage of estrus and to be certain the animals were cycling. Females with sperm (gestation day 0) were placed individually into polycarbonate shoe box cages with filter covers. Bedding was hardwood sawdust (Absorb-dri; Zeigler Bros., Inc., Gardner, PA).

Maternal weights, feed intake, and water intake were measured at weekly intervals (i.e., on gestation days 0, 7, 14, and 21). On gestation days 1–19, exposed and control females (target Ns=15/group; individual Ns given in the Results section) were placed in the exposure chambers for 7 hr/day, and the animals were left in the chambers for degassing for approximately 1/4 hr after vapor generation terminated. Controls were placed in an adjacent exposure chamber for the same hours as the exposed animals.

Males (N=18/group, 430±42 g for the high exposure group of 16000 ppm, 498±44 g for the low exposure group of 10000 ppm) were exposed to the same concentrations of ethanol in the same chamber with the females for 7 hr/day, 7 days/week for 6 weeks, and were weighed weekly. After two non-exposure days, each male was mated (1:1) with an exposed virgin female for a maximum of 5 days. The males were then discarded, and the females were handled in the same manner as the controls. (Thus, there was no group of males specifically exposed to 0 ppm; the stock males were sires for the controls.)

On gestation day 21, females were placed in clean bedding, given a paper towel for nest construction, and left undisturbed until after parturition. Within 16 hr after parturition, the dam was weighed, as was her litter. In most cases, four female and four male pups (arbitrarily selected from those that had nursed) were fostered to untreated dams which had delivered within the preceding 48 hr. All exposed dams and extra pups (including those from the foster dams) were discarded at this time. Litters not having at least three pups of each sex were discarded (this was rare). Rats were weaned at 25 days of age. Offspring were weighed on days 7, 14, 21, 28, and 35 (birth=day 0), and were observed for abnormalities.

Behavioral Testing

Seven behavioral tests (Table 1) have been utilized in our laboratory to evaluate various functions of the central nervous system (neuromuscular ability, activity, and learning) at several stages of development [29,33]. Behavioral testing occurred on days 10 through approximately 90. Female and

TABLE 1

BEHAVIORAL TESTS AND DAYS OF TESTING IN BEHAVIORAL TERATOLOGY STUDY

Behavioral Tests	Group*	Function Tested	Days of Age
1. Ascent on wire mesh	1	Neuromuscular	10, 12, 14
2. Rotorod	1	Neuromuscular	21, 25, 29
3. Open field and 4. Automated open field	2	Exploratory activity	16, 17, 18; 30, 31, 32; 44, 45, 46; 58, 59, 60
5. Activity wheel	2	Circadian activity	32–33
6. Avoidance conditioning	1,2	Aversive learning	Begun days 34, 60
7. Operant conditioning	3	Appetitive learning	Begun day 40

*Rats in the same group were administered the tests in the order shown.

(See text for explanation.)

male pups were selected randomly, ear marked, and assigned to test groups on postpartum day 10. For each test, one female and one male were used from each litter. Those involved with animal testing were not aware of the treatment groups to which subjects belonged. Details of six of the seven test procedures have recently been published [33].

1. Ascent on a 6 mm mesh screen inclined 70° from horizontal was administered for a maximum of 60 sec on days 10, 12, and 14.

2. The rotorod consisted of a rod 9 cm in diameter and 10 cm long, and the surface was rough with sand. The test was administered to the same animals used for the ascent test, with the rpms increased until the animals had five unsuccessful trials on days 21, 25, and 29.

3. The open field was 1 m in diameter, with an enclosure wall 0.5 m high. Animals were tested for 3 min/day on days 16, 17, 18; 30, 31, 32; 44, 45, 46; and 58, 59, 60.

4. An addition to our test battery was an optical digital animal activity monitor (Opto-Varimex; Columbus Instruments International Corp., Columbus, OH). The animal test area was a 40×40×20 cm Plexiglas cage which had 30 photodiodes per side. Two such units were housed in an acoustically shielded audiometric chamber (Eckel Industries, Inc., Cambridge, MA). Immediately following the open field test, the same rats used in the open field were placed in the center of the Opto-Varimex, and the activity recorded for 3 min. Activity scores were summed over the 3 days of testing at each age. Floors were cleaned with a detergent solution and dried with paper towels after each rat.

5. Running wheel activity was measured for approximately 24 hr on days 32–33. Two Wahman activity wheels were placed in a sound-attenuated chamber, and data were separated into day and night activity scores.

6. Two BRS/LVE shuttle boxes were housed in sound-attenuated chambers. A partition 4 cm high was placed in the center of each box. Scrambled electric shock (0.7 mA) could be administered to either side of the grid floor. The warning stimulus was a 5-sec tone. Times between trials varied between 15 and 45 sec, with a mean of 30. Two sets of animals were used for this test, one beginning on day 34, and the other on day 60. Animals were given 20 trials/day for a maximum of 14 days for learning (defined as reaching a criterion of $\leq$4 shocks/day for two consecutive days) and 10 days for extinction (two consecutive days of $\geq$16 nonresponse trials).

7. Operant conditioning was conducted in test cages having a response lever on either side of a centrally-located water dipper and trough and were housed in sound-attenuated cubicles. The water dipper was activated by a response, providing the animal access for 3 sec. Animals were magazine trained for 30 min/day on days 40 and 41, and autoshaped overnight on day 41. The reinforced response lever was alternated on successive days. Rats were on a progressive fixed ratio schedule of reinforcement for 1.5 hr/day until they no longer responded sufficiently to receive reinforcement.

Neurochemistry

One female and one male, which had received no prior testing, from 5 litters were sacrificed on day 21 for analysis of concentrations of protein and the neurotransmitters acetylcholine, dopamine, norepinephrine, 5-hydroxytryptamine, substance P, β-endorphin, and Met-enkephalin. Individual pups were placed into a small animal holder, inserted into a focussed microwave oven (model 4104 Metabostat; Gerling Moore Inc., Santa Clara, CA) and irradiated for 1.0 sec between 1300 and 1400 hours, thus sacrificing them while preserving neurotransmitters by inactivating brain synthetic and degradative enzymes [6,26]. The brain was separated into the four general brain regions of cerebrum, cerebellum, brainstem (medulla-pons), and midbrain [33]. Samples were frozen at −80° until assayed.

Brain samples were homogenized by sonication in 8 ml of 0.1 N HCl. Aliquots were then removed for determination of (a) protein (2.0 μl) [21]; (b) acetylcholine (ACh, 25 μl) [12], as modified [17,22]; (c) dopamine (DA, 50 μl) [10]; norepinephrine (NE, 50 μl) [10]; 5-hydroxytryptamine (5-HT, 10 μl) [39]; substance P (sub P, 20 μl) [28]; β-endorphin (β-End, 200 μl) [13]; and Met-enkephalin (m-Enk, 75 μl) [13]. Samples were randomized for processing sequence, and the individual performing the assays was unaware of the group to which the sample belonged.

Statistical Analyses

Behavioral data were analyzed using multivariate analysis of variance (MANOVA) where the data fit normal distributional assumptions required for parametric analyses. In the majority of cases, however, nonparametric tests were thought to be more appropriate, and an m-ranking procedure was used [46]. Repeated measures analyses were conducted where appropriate. All groups at 16000 ppm were included in one analysis and all at 10000 ppm were included in a separate analysis; there were no direct comparisons between groups at 16000 and 10000 ppm. In all cases, the level of significance was $p \leq 0.05$. When the same group of animals was used for multiple comparisons, corrections were made to adjust the probabilities required for significance [8]. Neurochemical data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range post-hoc tests where a significant ANOVA was found.

RESULTS

The exposures were close to the desired concentrations, with hourly means equal to the target concentrations of 16000 (±200) ppm and 10000 (±200) ppm. Means from the continuous (5-min) analysis were 16300±300 and 10700±500 ppm, respectively. During 65 exposure days at the higher concentration, 84 charcoal tube samples were collected, with a mean of 14300±1600 ppm. During 84 exposure days at the lower concentration, 59 charcoal tubes were collected, with a mean of 8900±500 ppm.

The number pregnant/number mated for maternal exposed, paternal exposed, control, and foster were 16/16, 16/16, 19/19, and 53/55 for the 16000 ppm exposures and 17/22, 16/17, 17/18, and 45/50 for the 10000 ppm exposure groups. Thus, there was no effect on fertility. Although we did not include a group of sham-exposed males with which to compare paternal weight gain, it appeared that weight gain was retarded during the first week of exposure, but proceeded normally thereafter. Maternal weight data were compared using ANOVA and postpartum weight using analysis of covariance with initial weight as the covariate. At 16000 ppm, mean gains from days 0–22 for maternally exposed, paternally exposed, control, and foster were 124, 143, 128, and 134 g, respectively. Weight gain was not significantly affected by ethanol exposures, although feed intake (m-ranking procedure) during the first week was reduced relative to controls (124±16 vs. 144±12 g) after exposure to

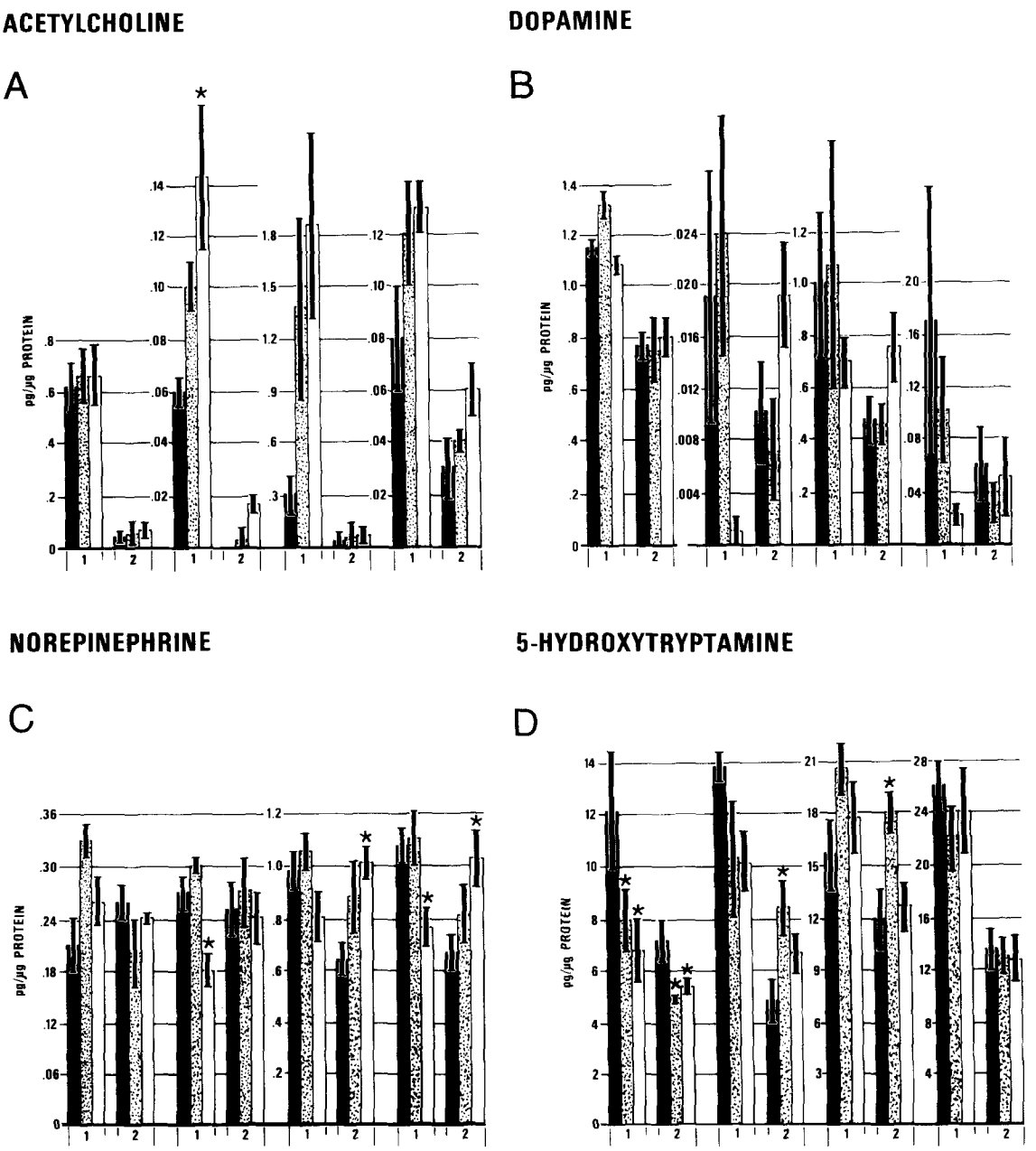

FIG. 1. Effects of maternal or paternal inhalation of ethanol on regional brain concentrations of the four monoaminergic neurotransmitters acetycholine (in pmole/μg protein, panel A), dopamine (in pg/μg protein, panel B), norepinephrine (in pg/μg protein, panel C) and 5-hydroxytryptamine (in pg/μg protein, panel D) in the offspring. Data represent means from 10 rats/group. *Indicates a significant difference at $p<0.05$ from the appropriate control group. Control: dark column, maternal: dotted column, paternal: open column. 1: 16000 ppm ethanol, 2: 10000 ppm ethanol.

160000 ppm ethanol. Water consumption (compared using the m-ranking procedure) was not affected, with means ranging from 237 to 351 g/week. At the lower concentration, neither maternal weight gain (order same as above=129, 150, 125, and 145 g, respectively), feed intake (means ranging from 122–162 g/week), nor water consumption (means from 245–361 g/week) were significantly affected by the exposures. No group differences were found for litter size, number of dead pups, or length of pregnancy. Litter sizes typically ranged from 11–15 pups, with very few dead pups, and gestation averaged 22 days. Offspring survival and weight gain did not differ among groups for either the 16000 or 10000 ppm ethanol exposures.

Since no significant effects were observed in the behavioral data, and data from the controls were published previously in comparing a series of control groups [35], the behavioral data are not presented here (but can be obtained from the authors). Neither test of neuromuscular ability detected effects as a result of alcohol exposures. The ascent data were analyzed in several ways, including contingency table analysis, but no significant differences among groups were detected. Rotorod data were analyzed using the m-ranking

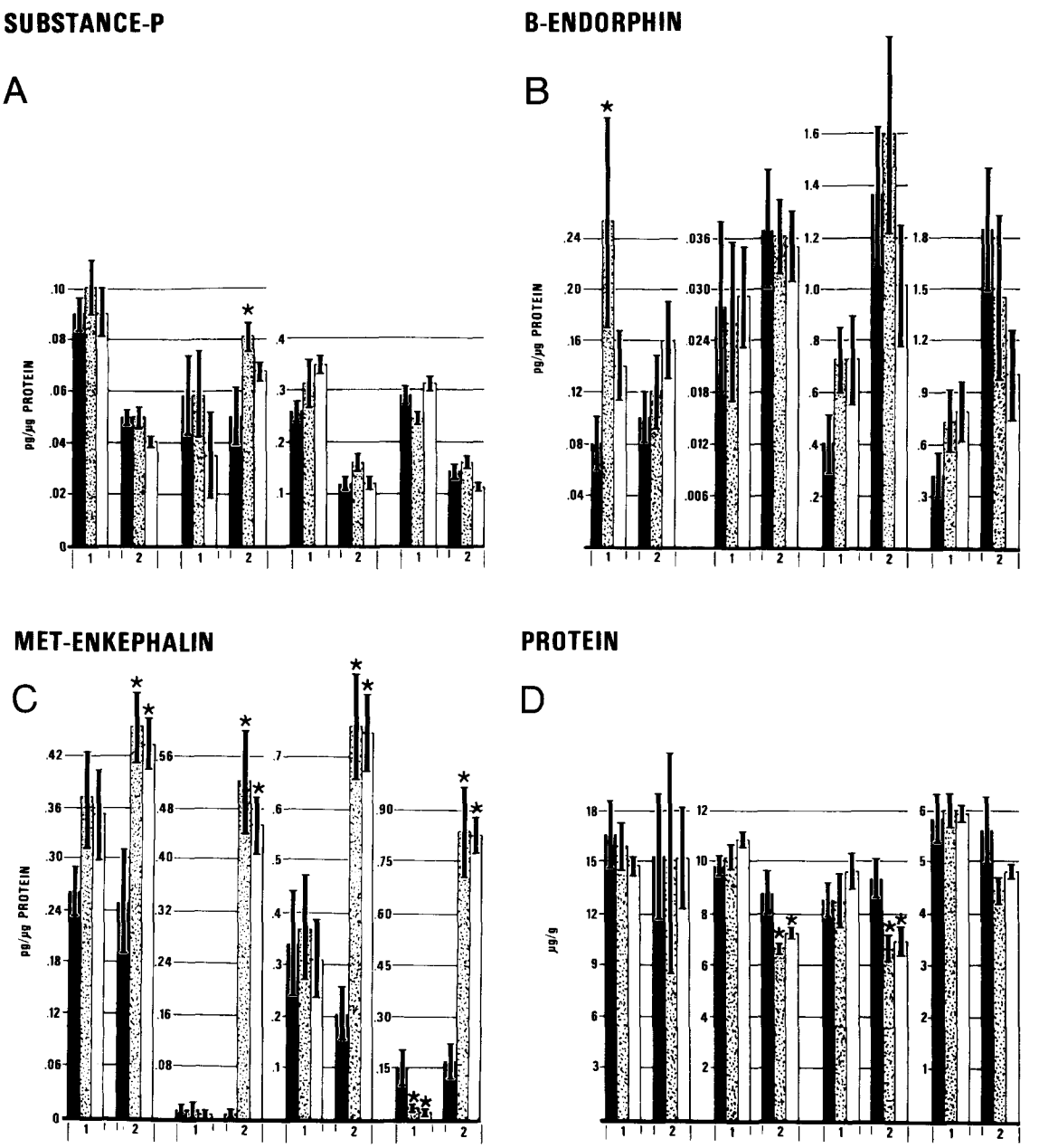

FIG. 2. Effects of maternal or paternal inhalation of ethanol on regional brain concentrations of the three neuropeptide transmitters substance P (in pg/μg protein, panel A), Met-enkephalin (in pg/μg protein, panel B), and β-endorphin (in pg/μg protein, panel C) and protein (in μg, panel D) in the offspring. Data represent means from 10 rats/group. *Indicates a significant difference at $p<0.05$ from the appropriate control group. Control: dark column, maternal: dotted column, paternal: open column. 1: 16000 ppm ethanol, 2: 10000 ppm ethanol.

procedure, but no differences were detected. Means for day 21 ranged from 11 to 15 rpm, for day 25 from 22 to 25 rpm, and on day 29 from 13 to 24 rpm. We did not see a strong correlation (Spearman Rank) between performance for the three test days on the rotorod.

The data from all three activity tests were analyzed using the m-ranking procedure, and also showed no differences among groups. Neither open field latency (sums of the three days ranging from 136 to 256 sec), activity levels (sums for the three days at the various ages ranging from 24 to 51, 101 to 137, 83 to 240, and 106 to 240 sections), or number of fecal boluses were significantly different among groups on any of the days of testing. Activity levels, as measured in the optical activity measure (sums for the three days at each age ranging from 2843 to 3540, 3293 to 4209, 2818 to 5381, and 3013 to 5502 counts), were also unaffected by prenatal treatment. For both of these measures, sex differences were consistently observed in the oldest animals tested and some groups showed sex differences on days 44–46 as well. Running wheel activity did not differ among groups (means for the 12 hours during daylight hours ranged from 261 to 518 revolutions, and those for the 12 hours of darkness ranged

from 404 to 750 revolutions).

The two learning measures did not detect differences among groups. In avoidance conditioning from the 16000 ppm ethanol groups there were two statistically significant differences, but the differences were no longer significant after correcting for multiple comparisons. Means for young vs. old rats for the following variables were: number of crosses in the 5-min adaptation period 9 to 15 vs. 9 to 15; mean crossings in the 20-trial learning phase 21 to 28 vs. 22 to 26; mean number of escapes 13 to 17 vs. 10 to 18; and for seconds shocked 20 to 55 vs. 18 to 43. Variability was high in the operant conditioning data and no group differences were found.

Among the monoamines, levels of DA and ACh were essentially unchanged by exposure to ethanol (Fig. 1A and B). However, NE and 5-hydroxytryptamine (5-HT or serotonin) levels were significantly altered (Fig. 1C and D). Changes in NE concentration were seen in both the low and high concentration exposure groups. However, the magnitude and direction of changes seen were not linearly correlated with the exposure concentrations. Significant effects on NE occurred only in the paternally exposed groups. Both concentrations of ethanol significantly reduced 5-HT levels in the cerebrum of offspring from both maternally and paternally exposed rats (Fig. 1D). However, 5-HT levels were increased in the midbrain and cerebellum of rats whose mothers had inhaled the lower concentration of ethanol.

Among the peptidergic neurotransmitters, levels of sub P and β-End were not significantly different from control (Fig. 2A and C) in both groups. The lower concentration of ethanol increased m-Enk levels significantly in all brain regions of offspring from both maternally and paternally exposed animals (Fig. 2B). Surprisingly, this effect was not seen at the higher dose of ethanol. Rather, a decrease in m-Enk was observed in the brainstem from both exposure groups.

DISCUSSION

There were no indications of maternal toxicity in this study of ethanol (10000 and 16000 ppm) administered by inhalation to rats. Nor were there any consistent treatment-related effects in the offspring on the behavioral tests, either after maternal or paternal exposures. We previously reported that concentrations of ethanol which produced narcosis in the dams did not produce gross malformations in rats [34]. Thus, our studies lend support to the suggestion that the rat is resistant to many alcohol-related teratogenic effects [20].

Although the effects of maternal ethanol consumption on offspring development have dominated the research on alcohol, there have been a number of studies which indicate that paternal exposure to ethanol may be hazardous [3]. Several studies have been completed in experimental animals in which exposure of males to ethanol produced adverse effects. For example, Mankes *et al.* [24] administered 10% ethanol in the drinking water to 10 male Long-Evans rats for 60 days, and these males were then mated weekly for three weeks to untreated rats. Pregnant dams were sacrificed and their offspring were examined for defects in growth rate, skeletal ossification, and soft-tissue anomalies. Five of the ten males had an increased incidence of resorptions and malformations. No recovery in function was noted over the three weeks of matings. Anderson *et al.* [4] administered 5% or 6% ethanol in a liquid diet to groups of approximately 10 male mice (C57Bl/6J) for 5, 10, or 20 weeks. They then assessed the quantity, motility, and morphology of epididymal spermatozoa, ability to fertilize in vitro, and pathological changes in the reproductive tract [49]. Dose- and duration-dependent effects were noted on all parameters examined.

The lack of behavioral effects after prenatal inhalation exposure is not surprising in view of the low blood levels achieved. Our levels of 50 mg/dl after exposure to 16000 ppm ethanol were much lower than the peak levels often achieved after experimental studies investigating Fetal Alcohol Effects (which are often $\geq$200 mg/dl). Such lack of effects after low blood levels is similar to some of our previous research in which ethanol was administered in the drinking water. As part of a study investigating the prenatal interaction effects of ethanol with an industrial solvent, 2-ethoxyethanol, we found that administration of ethanol in the drinking water (10% wt./wt., likely producing very low blood levels) during gestation days 7–13 or 14–20 produced very few changes in offspring behavior [30], although in combination with the solvent, ethanol did influence behavioral outcome [32]. Exposure to ethanol on days 14–20 reduced maternal weight gain, but not offspring weights. Only two of six tests administered to the pups at various ages produced any changes, and these were in young animals, and were in opposite directions. Since the pups were not fostered, it is possible that the effects were maternally-mediated and transitory in nature. Our observations that steady-state concentrations of several neurotransmitters were reduced in newborn animals but not in 21-day-old animals [31] supports the hypothesis of a transitory effect. Other investigators found no long-term effects on cholinergic or serotonergic receptor binding in adult female rats exposed prenatally to ethanol [7].

The present data reveal that maternal or paternal inhalation exposure to ethanol can cause significant biochemical effects in the offspring, as has been reported after other routes of exposure [5, 25, 37, 38, 47]. Changes were noteworthy in noradrenergic, serotonergic, and m-enkephalinergic brain systems. NE changes were seen exclusively in the offspring of paternally exposed rats. The 5-HT changes were seen more frequently in the offspring of maternally exposed rats. Significant m-Enk effects were seen in the offspring of both maternally and paternally exposed rats, but only for the lower concentration of ethanol. The magnitude of effects seen in a given brain region was seldom linearly related to the concentration of ethanol used, and neurotransmitter changes were often not consistent across brain regions.

These neurotransmitter results are consistent with previous neurochemical data showing substantial changes in levels of NE, DA, 5-HT, and ACh in specific brain regions of the offspring of male or female rats exposed to vapors of the solvent 2-methoxyethanol [33]. In the present study, one of the most dramatic effects was elevation in levels of enkephalin throughout the brain in offspring of both maternally or paternally exposed rats. McGivern *et al.* [23] have also reported that in utero exposure to ethanol caused an increase in Met- and Leu-enkephalin in the basal ganglia (blood levels not reported). In contrast, Shoemaker *et al.* [42] reported changes in β-End but not m-Enk in newborn rats following prenatal exposure to ethanol, with maternal blood levels exceeding 100 mg%. Ethanol is able to influence opiate receptors both in vivo and in vitro [15,36]. Enkephalins are widely distributed neurotransmitters and neurohormones, being found in the brain, spinal cord, pituitary, adrenal medulla, autonomic nervous system, and gastrointestinal tract [27]. Consequently, ethanol-induced

changes in enkephalins may be expected to influence many functions. Further studies are needed to evaluate this possibility, particularly studies of the responsiveness to painful stimuli, since enkephalins can produce analgesia under appropriate conditions [27]. Although no significant changes in avoidance conditioning were observed in the present study, this test is not suitable for discerning changes in pain threshold. In future experiments to determine the mechanisms underlying the actions of ethanol on neurotransmitters, it would be helpful to combine microdissection of individual brain nuclei with several measures of the dynamics of neuronal function (i.e., transmitter turnover as well as changes in receptors).

Our research indicates that industrial inhalation exposures to ethanol may not be expected to produce alarming blood ethanol levels, and did not find consistent behavioral effects, although several neurochemical changes were noted from prenatal administration of ethanol on the offspring. In addition, the potential of ethanol to modify the adverse effects of other chemicals should be noted. The ability of ethanol to modify the effects of industrial agents in adult animals is well-recognized [9, 16, 43, 45], with studies of behavioral effects reported for 1,1,1-trichloroethane [50], styrene [40], and isopropanol [41]. Further, as discussed previously, we found that prenatal administration of ethanol altered the behavioral teratogenic effects of 2-ethoxyethanol [32].

In summary, most studies have administered ethanol by an oral route, while we have investigated exposure by inhalation. Such exposure was not found to produce behavioral teratogenic effects in rats, but it did produce neurochemical alterations. Moreover, existing evidence points to the likelihood that ethanol can interact with other prenatally administered agents.

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