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REVIEW

Review of Experimental Male-Mediated Behavioral and Neurochemical Disorders

B. K. NELSON, W. J. MOORMAN AND S. M. SCHRADER

Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, Cincinnati, OH 45226

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NELSON, B. K., W. J. MOORMAN AND S. M. SCHRADER. *Review of experimental male-mediated behavioral and neurochemical disorders.* NEUROTOXICOL TERATOL **18**(6) 611–616, 1996.—Paternal exposures to exogenous agents have been reported to produce a variety of developmental defects in the offspring. In experimental animals, these effects include decreased litter size and weight, increased stillbirth and neonatal death, birth defects, tumors, and functional/behavioral abnormalities—some of these effects being transmitted to the second and third generations. This article reviews the exogenous agents that have reportedly caused behavioral or neurochemical alterations in offspring of experimental animals following paternal exposures, including advanced age, alcohols, cyclophosphamide, ethylene dibromide, lead, opiates, and a few miscellaneous chemicals. Based upon the consistency of effects in several of these agents in a variety of studies in experimental animals, the conclusion is that paternal exposures may contribute to the incidence of neurobehavioral disorders in humans. *Copyright © 1996 Elsevier Science Inc.*

Paternal exposures Neurobehavioral disorders Neurochemical effects Offspring
Experimental animals Male-mediated effects

AS is well established, developmental toxicity (manifested by embryo/fetal death, malformations, growth impairment, and functional deficits) can be produced by exposure of the maternal organism to exogenous agents during development. A recent review indicated that 3% of all children born in the United States have a major malformation detectable at birth; some 6–7% of infants have developmental disorders detectable by 1 year of age; and 12–14% of children have developmental disorders detectable by school age (41). A separate survey suggests that about 17% of children have had one or more developmental disabilities up through age 17 (17). About 70% of these developmental disorders are of unknown etiology. Historically, embryos prior to implantation were thought to be relatively immune to the production of developmental disorders (16,66). Evidence suggested that exposure of this early conceptus to an adverse influence either killed the conceptus or the embryo withstood this influence with no detectable damage. It is now apparent, however, that exposure during preimplantation periods to certain agents can produce developmental defects in the offspring (37,41,55,59). Various agents can produce preimplantation effects, including opiates (26), nickel chloride (60), medroxyprogesterone acetate (23), methylnitrosurea (36,62), ethylene oxide (32), nonionizing (radiofrequency) radiation (42), and ionizing radiation (51, 55). On a related, yet very different, note, a recent report indicated that ethylnitosourea administered during embryogenesis to female mice produced mutations in the primordial germ cells of the male offspring (65). These mutations manifested as reduced fertility when these males were mated with untreated females, and increased malformations in the next generation.

The mechanism(s) by which most developmental toxicants function are unknown. It is apparent, however, that genetic or epigenetic mechanisms may be operational, particularly for those agents that act prior to organogenesis. Consequently, it is not surprising that paternal exposures may also induce developmental defects in the offspring (e.g., embryo/fetal death, malformations, growth impairment, and functional deficits).

Requests for reprints should be addressed to B. K. Nelson, NIOSH C-24, 4676 Columbia Parkway, Cincinnati, OH 45226. Tel: (513) 533-8178; Fax: (513) 533-8596.

In human or animal studies, developmental defects reported to result from paternal exposures include decreased litter size and weight, increased stillbirth and neonatal death, birth defects, tumors, and behavioral/neurochemical abnormalities—some of these effects being transmitted to the second and third generations (10,15,20,21,29,38,40,49,53,54,64). Several possible mechanisms by which exposure of the male may result in disorders in the offspring have recently been reviewed (20,56). Postulated mechanisms range from genetic (germ cell) alterations, toxic or epigenetic effects, seminal fluid transfer of toxicants, and hormonal alterations in the males, potentially leading to infertility and germ cell loss.

In addition to advanced age, a number of chemicals have been tested for their ability to produce neurobehavioral and/or neurochemical alterations in offspring of experimental animals following paternal exposures. [It should be noted that the present review focuses on chemical agents because these have been reported in the literature, but physical agents such as radiation have been shown to produce adverse developmental outcomes (50).] Some chemicals have only single studies, whereas others have several studies, thus strengthening the hypothesis that behavioral/neurochemical changes can be produced in offspring by paternal exposures. To provide the reader a synthesis of the literature in this area, a brief review of these agents and studies follows. Where provided in the various reports, the number of exposed animals and offspring tested are provided, along with the methods of statistical analyses, to enable readers to sense the reliability and strength of reported effects. Readers should use typical standards for toxicological studies (e.g., reproducability within and between laboratories, dose–response relationships, appropriateness of statistical techniques with the litter used as the unit of comparison, etc.) to assess the strength of individual studies. Perhaps more importantly, the reader is encouraged to step away from the tunnel vision often used to focus on individual studies and to gain a broader perspective of patterns that the combination of studies reveal (i.e., overview the forest, rather than focus on individual trees). When this is done, the reader will likely conclude that, in spite of limitations in some studies, the "forest" of studies indicates that paternal exposures can produce behavioral/neurochemical effects in offspring—at least in experimental animals. Wyrobek (69) presents the case strongly by stating that "extensive human investigations are warranted because the (experimental animal studies) have demonstrated conclusively that exposure of the male before mating can lead to developmental and behavioral abnormalities in the offspring."

AGE

Fifteen Wistar rats at 2.5, 6, 10, 14, 18, and 22 months were mated with females at 2.5 months (12). Between 10 and 13 weeks of age, the offspring (10 offspring/litter) were evaluated for open field behavior and two-way avoidance conditioning. Data were analyzed using analysis of variance (ANOVA) or χ^2. No effect was seen in open field behavior, but impaired learning was seen in the acquisition of a conditioned avoidance response in the offspring of the oldest males tested, $\chi^2(1) = 2.56$, $p < 0.05$. Although the number of males mated and offspring tested should provide good statistical power to assess the effects of age, this study needs replication before firm conclusions can be reached.

ALCOHOLS

Different investigators have documented the paternally mediated behavioral effects of ethyl alcohol in experimental animals. Abel and his colleagues have conducted several studies in which males were exposed until immediately prior to mating, and have typically reported behavioral changes in the absence of weight effects (6). Groups of 18–20 male Long–Evans rats were given 0%, 17.5%, or 35% ethanol-derived calories (EDC) in liquid diet for a minimum of 52 days (7). One female and one male/litter were tested between 17–20 days of age for spontaneous alternation, passive avoidance, and activity, and on days 31–32 for active avoidance. Data were analyzed using multivariate analysis of variance (MANOVA) or ANOVA. Spontaneous alternation and passive avoidance were unaffected by paternal alcohol exposure. In contrast, various components of activity were decreased, $F(26, 152) = 1.70$, $p < 0.03$, some in a dose-related manner. Also, alcohol offspring made fewer avoidance responses than control, $F(2, 46) = 3.53$, $p < 0.04$, but no consistent dose–effect relationship was observed.

The effects of liquid alcohol diets containing 0%, 17.5%, or 35% EDC were compared in Long–Evans and Sprague–Dawley male rats ($N = 20$–40/group) (1). After 3 or 4 weeks of diet consumption, these males were bred to females of the same strain. Pregnant females were divided into similarly treated alcohol groups and were fed these diets beginning on gestation day 8, thus creating a factorial study with strain, paternal, and maternal alcohol consumption as main factors. From 8–20 offspring/sex/group were tested for passive avoidance at 17 days of age and for activity at 20 days of age (infrared monitor); at 59 days of age, one offspring of each sex per group was sacrificed and blood analyzed for testosterone and estradiol. Data were analyzed by ANOVA. Long–Evans males consumed an average of 10.0 and 4.9 g/kg/day in the 35% and 17.5% EDC groups, respectively, compared with 8.3 and 4.4 g/kg/day for Sprague–Dawley rats. Peak blood alcohol levels were 144 and 5 vs. 190 and 13 mg%, respectively. Paternal alcohol consumption was associated with decreased litter size, $F(2, 303) = 11.6$, $p < 0.001$, decreased testosterone levels, $F(1, 245) = 5.22$, $p < 0.03$, and a strain-related effect on offspring activity. Activity decreased for offspring sired by 17.5% EDC Sprague–Dawley fathers, $F(1, 309) = 4.65$, $p < 0.03$, but increased for those sired by 35% EDC fathers, $F(1, 309) = 6.51$, $p < 0.01$. Paternal alcohol consumption did not affect postnatal mortality or passive avoidance learning of offspring.

Following up on the hyperactivity observed in Sprague–Dawley rats, the same investigators administered 0, 2, or 3 g/kg ethanol to groups of 20 male rats of this strain for 9 weeks (4). The males were mated with untreated females. Between 13 and 23 offspring/group were tested for activity at 21, 42, and 90 days of age. Just before the 60-min activity testing, the offspring were injected with 0.5, 1.0, or 2.0 mg/kg/ml of *d-amphetamine*. Data were analyzed by ANOVA. The higher dose of ethanol led to hyperactive offspring on days 21, $F(2, 89) = 4.95$, $p < 0.01$, and days 42, $F(2, 42) = 5.11$, $p < 0.01$, with marginal effects on day 90, $F(2, 116) = 3.11$, $p < 0.05$. The hyperactivity was manifest only when rats were challenged with 2 mg/kg amphetamine.

EDC of 0%, 5%, 10%, 15%, 20%, or 25% was administered to groups of 22–30 male Swiss Webster mice for 7 weeks (2). After being mated with untreated females, the males were continued on the same diet for 7 additional weeks. One female and one male/litter were tested for activity on day 16, days 17–20, and day 75. Data were analyzed by MANOVA. After 7 weeks of exposure, offspring from alcohol-exposed males were less active than controls on several measures of activity at the younger ages [e.g., $F(5, 193) = 5.32$, $p < 0.009$]. However, after 14 weeks of exposure, there were no differences from control

in the activity measures. A follow-up study found that offspring from male mice (N = 22–30/group) given 10% or 25% EDC were more immobile than controls in a swimming test at 75 days of age regardless of duration of paternal alcohol consumption (5). When offspring of alcohol-consuming rats were tested, their swimming behavior was opposite to that of mice (i.e., rats were less immobile).

Four 30-day-old male rats were maintained on a 35% EDC liquid diet for 39 days (67). Following a 2-week drug-free period, the males were mated with untreated females. Offspring (N = 15/group) were evaluated on several developmental indices and on various learning/memory tasks to assess functional deficits in adulthood. Data were analyzed using MANOVA. Offspring of alcohol-treated males did not differ from control offspring on several developmental landmarks, or on tests of sensorimotor development. As adults, male offspring groups did not differ on tests of activity or on an object exploration/recognition task. However, male offspring of alcohol-treated males demonstrated impaired acquisition performance in an eight-arm radial maze, $F(4, 25) = 4.37$, $p < 0.005$, and on T-maze discrimination, $F(1, 28) = 6.28$, $p < 0.019$.

Groups of 18 male rats were exposed to 10,000 or 16,000 ppm ethanol via inhalation for 7 h/day for 6 weeks (45). After 3 days of nonexposure, males were placed with unexposed females until mating occurred (up to 10 days after exposure ended). One female and one male/litter were tested on a variety of tasks from days 10 to 80. Data were analyzed by MANOVA, but no behavioral changes were detected in the offspring. A naive pair from each litter was sacrificed by focused microwave irradiation at 21 days of age, and brain regions were assayed for several neurotransmitters. Differences between exposed and control groups were reported in some transmitters and brain regions, but statistical details were not reported. Neurochemical changes (decreased body weight, cerebral weight, and cerebral DNA, RNA, and leucine incorporation into protein without a decrease in the litter size) were also reported in fetal brains after paternal exposure of rats to 30% ethanol in drinking water (Ns = 4–5/group) (63). However, no significant behavioral or neurochemical changes were found after paternal exposure to *n*-propanol (46), *n*-butanol (47), or *tertiary*-butanol (48).

The relatively large number of studies reviewed above make a strong case for the ability of high levels of ethyl alcohol to produce male-mediated disorders in the offspring of experimental animals. Hyperactivity has been reported in most of the studies reviewed. Although additional research should be conducted to characterize the mechanism of action in experimental animals, epidemiological studies should also be conducted to determine the effects of paternal ethanol exposure on children. [Some studies, focusing on the confounding effects of paternal alcoholism on offspring development in Fetal Alcohol Syndrome, have been reviewed (11).]

CYCLOPHOSPHAMIDE

Several investigators have reported behavioral deviations in offspring following paternal exposure of rats to cyclophosphamide (CP). Injection of 10 male rats (five of which were mated with CP-treated females, and five mated with control females) with 10 mg/kg/day, 5 days/week for 5 weeks (mating begun 3 days after treatment ended) was reported to produce adverse effects on fertility (8). In the few offspring that survived, developmental delay (as measured in cliff avoidance and swimming ontogeny) and hyperactivity (open field) were observed (method of statistical analysis not reported). Subsequently the same investigators reported that either the chronic dosing (described above) or an acute dose of 10 mg/kg CP to five male rats (mating 7–9, 14–16, or 28–30 days after dosing) produced hypoactivity in the open field and impaired acquisition and extinction of a one-way active avoidance procedure in the 14–16 day dose group (statistical procedures not reported) (9). Further testing suggested that behavioral impairment induced by CP could be detected in the second generation (neither numbers of animals or statistical procedures reported) (10).

CP (10 mg/kg) was administered for 15 days to 16 male Wistar rats (13). At 100 days after treatment, these males were mated with unexposed females. Offspring (number unclear) between 10 and 14 weeks of age were tested in an open field and two-way active avoidance. Data were analyzed by ANOVA or χ^2. Neither open field activity nor learning ability of the offspring was impaired, although the percent of unsuccessful responses of males in the conditioning trials was higher in the treated group than in controls, $F(1, 48) = 7.93$, $p < 0.01$. Subsequently, this study was replicated (14) using 18 males, with greater effects on learning ability [$\chi^2(1) = 8.04$, $p < 0.01$ for males, and $\chi^2(1) = 12.45$, $p < 0.001$], and open field activity was reduced in the offspring of CP-exposed offspring [$F(1, 138) = 4.13$, $p < 0.05$ for males, and $F(1, 138) = 6.30$, $p < 0.02$ for females]. However, a combination of CP with vinblastine did not alter the effects seen with CP alone. Second (15) and third generation (22) effects have also been reported for CP.

Adult F344 rats (N = 6–10/treatment group) were exposed to CP for either 5 days per week for 5 weeks or a single injection (10 mg/kg, IP) (34). Three to 14 days after the chronic treatment or 14–16 days after the acute treatment, the males were mated with untreated females. At 90 days of age, six female and six male offspring/group were sacrificed. Brains were separated into cerebellum, corpus striatum, hippocampus, hypothalamus, and temporal cortex, and aliquots of the homogenates were assayed for choline acetyltransferase (ChAT), acetylcholine esterase (AChE), and glutamic acid decarboxylase (GAD), along with protein. Data were analyzed using the Mann–Whitney U-test. The investigators reported increases or decreases in these enzymes in several of the brain regions (statistical details not reported).

Studies investigating the mechanism of action of CP in producing male-mediated developmental toxicity have recently been reviewed (33). Although these studies have not included neurobehavioral developmental end points in the offspring, they have elucidated many details as to how CP may act to produce neurobehavioral deficits. This work, together with the other articles reviewed above, makes a strong case for the ability of CP to contribute to the incidence of neurobehavioral deficits in children.

ETHYLENE DIBROMIDE

Ethylene dibromide (EDB) at 1.25, 2.5, 5, and 10 mg/kg (IP) was administered to groups of four to five male Fisher 344 rats for 5 consecutive days (24). Beginning 4 weeks after the last injection, the males were mated with untreated females. On postnatal day 3, the offspring (number not specified) were begun on a series of testing of preweaning behaviors. Data were analyzed using ANOVA. Surface righting and negative geotaxis were not affected, but cliff avoidance, $F(4, 70) = 12.3$, $p < 0.01$, swimming ontogeny, $F(4, 32) = 3.1$, $p < 0.05$, and open field activity, $F(4, 24) = 7.62$, $p < 0.01$, were altered by EDB.

These same investigators administered 1.0 mg/kg EDB to Fisher 344 rats (number not reported) for 5 successive days

(35). One week after the last injection, the males were mated with untreated females. Offspring (4–9/group) were sacrificed at 7, 14, 21, and 90 days of age. Brain regions (cerebellum, corpus striatum, frontal cortex, hippocampus, and hypothalamus) were analyzed for ChAT, AChE, and GAD, along with protein. Data were analyzed using the Mann–Whitney U-test. Age- and region-specific alterations in these enzymes were observed (details of the statistical analyses not reported).

Although these studies on EDB are suggestive of effects in offspring following paternal exposures, only one laboratory has studied the effects. Replication would greatly strengthen our confidence in these studies.

LEAD

Lead has been investigated for effects on the male, as well as male-mediated effects on the offspring, in several studies [e.g., (31,43,52,61)]. An example of paternally mediated behavioral effects is provided (18). Male and female rats (numbers not specified) were intubated with 500 mg/kg lead acetate from 30–90 days of age. At 90 days of age, these males and females were mated together or to control males and females; lead-exposed females continued exposure throughout gestation and lactation. Offspring from these matings (17 pups/group) were tested for learning in a water T-maze beginning at 30 days of age. Data were analyzed using ANOVA. The three lead-exposed groups (viz., maternal only, paternal only, or maternal plus paternal) made more errors in the maze than the controls [e.g., $F(1, 64) = 19.54, p < 0.01$], but did not differ from one another. However, offspring in the maternal plus paternal exposure group had longer swimming times than those in either maternally or paternally exposed groups, and those in the latter groups had longer swimming times than the controls. Thus, either maternal or paternal exposure exerted deleterious effects on the offspring, but maternal plus paternal exposure had the most severe effects.

More recently, male-mediated developmental disorders in rats have been reported, including lead-induced changes in early gene expression in early embryos, as well as alterations in hippocampal development of late fetal and early neonatal rats (57).

Taken together, these studies suggest the need for additional research on paternally mediated effects of lead—particularly because lead is such a ubiquitous pollutant.

OPIATES

Pregestational female or paternal exposure of mice to morphine (5–10 males/group; 120 mg/kg on the first day increasing to 420 mg/kg on the fifth day) or to methadone (10 mg/kg on the first day, increasing to 30 mg/kg on the fifth day) can produce behavioral deficits in the offspring (6–8 mice from four litters, with mating 6 days following the final injection) (27,28,30). Water maze performance was adversely affected by the paternal exposures (t-test $p < 0.001$).

Morphine pellets were implanted in 44 "adolescent" male rats (postnatal days 27–40), with the intent of maintaining "relatively high blood and brain levels of morphine through out the period of puberty and early adolescence" (19). These males, 8 weeks after initiating morphine treatment (a time when blood morphine levels were undetectable), were mated with untreated females. Developmental landmarks were monitored in the offspring up to 60 days of age when they were sacrificed and various reproductive organs collected for hormone analyses. Data were analyzed using ANOVA (although details of the results are not reported). Male offspring (N = 29 50/group) had lower serum LH and testosterone levels, along with altered organ weights in seminal vesicles, testes, and adrenals. Female offspring (N = 18–34/group) also had some endocrinological deviations from the placebo-derived offspring, but not the same as in the males.

Methadone (10 mg/kg, SC) was administered to 15 rats for up to 12 days 6–8 h prior to mating. Testing involved two animals/sex/litter. Data were analyzed using ANOVA. Neither open field activity nor active avoidance conditioning was altered by the paternal treatment, but "emotionality" was affected [decreased defecations, $F(1, 150) = 8.33, p < 0.01$]. Treatment for longer durations increased the number of defecations (58). Six rats were injected with 5 mg/kg methadone for 4 days immediately prior to mating to untreated females (39). At 100 days of age, the offspring (N = 2 females and 2 males/litter) were found to have performance alterations on all tests (deviations on certain test days): decreased open field activity, $F(1, 20) = 10.3, p < 0.01$; increased activity in an electronically monitored activity cage, $F(1, 16) = 4.72, p < 0.05$; decreased latencies to reenter the shock compartment in passive avoidance (suggestive of poorer retention), $F(1, 40) = 4.58, p < 0.05$; increased avoidances in two-way active avoidance, $F(1, 40) = 10.33, p < 0.01$; and reduced performance on the rotorod, $F(1, 40) = 4.82, p < 0.05$.

COCAINE

Adult male Long–Evans rats (N not specified) were injected with 0, 15, or 30 mg/kg cocaine for 72–92 days (3). These males were then mated with untreated females (time between injection and mating unclear). The offspring (one per litter) were tested at 16 days of age for activity in an automated activity monitor. At 17 days of age, offspring were tested for passive avoidance learning. At 33–35 days of age, offspring were tested for spontaneous alternation. Statistical procedures were not discussed. Abel reported that the 15 mg/kg offspring were hyperactive, and that the 15 and 30 mg/kg offspring had an increase in the number of trials to alternation.

Although some experimental details are lacking in certain studies cited above, the preponderance of evidence suggests that cocaine and several opiates may produce adverse neurodevelopmental effects in the offspring of experimental animals, and suggests the need for epidemiological research to determine the extent to which opiates may contribute to the incidence of neurobehavioral disorders in humans.

MISCELLANEOUS

Many details of the studies were not reported, but one study suggested that CP, ethyl methanesulfonate, mitomycin C, and procarbazine each produced behavioral effects in offspring after paternal exposure (25). A small number of behavioral and neurochemical changes were reported in offspring following inhalation exposure to the glycol ether 2-methoxyethanol in rats (44). A group of 18 males was exposed to 25 ppm 2-methoxyethanol for 7 h/day, 7 days/week, for 6 weeks. After 3 nonexposure days, the males were mated to untreated females, which were allowed to litter and rear their own offspring. Data were analyzed using MANOVA or the Wilcoxon two-sample (nonparametric) test. In the battery of behavioral tests given from days 10–75, the only test to show differences from control was two-way active avoidance conditioning, where the offspring of exposed males had less time shocked than controls (statistical details not presented). Several neurochemical deviations from control were observed in brains from 21-day-old offspring.

Because the studies just cited have not been reported in detail or not replicated, one has less confidence in the results. Future research will be necessary to address the ability of these chemical agents to produce neurobehavioral disorders in offspring following paternal exposure.

COMMENT

The studies cited above illustrate the complexity of conducting these kinds of studies. Obviously, such studies require investigators with expertise in reproduction, reproductive toxicology, behavioral toxicology, statistics, and potentially other fields (especially when mechanisms are being investigated). When designing studies, investigators must address questions such as: 1) most appropriate species; 2) dose ranges and duration of treatment, overlaying the sperm cycle of the species being used; 3) the time between dosing and mating; 4) the sexual maturity and age of the exposed male; 5) the number of exposed males, as well as of mated females, based on power calculations; 6) the number and age of offspring to be tested, together with the established behavioral test battery (and/or other observations) to be used; 7) a number of statistical questions in addition to the methods for data analyses, such as is the exposed male treated as the "*n*" or can one male be mated with two females and use the litter number as the "*n*"? (Clearly, the individual offspring would not be treated as the unit of statistical comparison.)

SUMMARY

As described above, several chemicals have reliably demonstrated paternally mediated neurobehavioral and neurochemical effects in experimental animals. These chemicals include ethyl alcohol, cyclophosphamide, lead, and several opiates. Although we are not yet in position to describe the mechanism of action, the number of investigations that produced positive results in experimental animals makes a strong case for the likelihood of such effects in humans [cf. (68)]. These data also suggest the need for more research into paternally mediated effects on offspring, in experimental animals as well as in humans.

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