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Effect of Antenatal Exposure to Paroxetine (Paxil) on Growth and Physical Maturation of Mice Offspring

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Objective: Our purpose was to determine, in a placebo-controlled manner, whether antenatal exposure to paroxetine affected long-term growth and physical maturation of mice offspring.

Methods: Forty-one CD-1 mice consumed paroxetine (n = 21) or a placebo (n = 20) for 2 weeks before conception and throughout gestation. The daily dose of paroxetine (Paxil; 30 mg/kg/d) was known to achieve concentrations in the serum equivalent to the upper therapeutic level in humans and in the fetal brain equivalent to that of the adult mouse. Growth and physical maturation of the offspring were compared by paired *t*-test, Welch's corrected test, and Fisher's exact test.

Results: The maternal weight gain, litter sizes, number of fetal resorptions, and gestational age at delivery were not different between the paroxetine and the placebo-exposed offspring. Newborn pups exposed to paroxetine were more likely to have low birthweights (1.65 gm vs. 1.70 gm; $P < 0.05$) and narrower heads (7.7 mm vs. 8.1 mm; $P < 0.05$). Body weight, body length, and head circumference measurements increased in a manner that was indistinguishable between the two groups of offspring, regardless of gender. No differences in achievement of physical milestones (lower incisor eruption, eye opening, and development of external genitalia) were noted between the two groups. The reproductive capability and the perinatal outcomes of the second-generation offspring were unaffected by paroxetine exposure.

Conclusion: A clinically relevant dose of paroxetine, when given throughout gestation, did not affect long-term growth and physical maturation of mice offspring. *J. Matern.-Fetal Med. 2000;9:136–141.*

Key words: paroxetine; mouse behavior; cognition

INTRODUCTION

Psychiatric disorders, especially affective illnesses, are common in women of childbearing age [1]. The prevalence of depression is 8–10% in women of reproductive age [2]. Consequences of uncontrolled depression include vegetative signs and symptoms, family dysfunction, and suicide. The idea that emotional and physiological changes of pregnancy protect against the development or recurrence of depression is generally unfounded [1].

Serotonin uptake inhibitor drugs appear to be the best and most widely prescribed treatment option for depression [2]. Certain of these drugs, such as fluoxetine (Prozac; Eli Lilly, Indianapolis IN) and sertraline (Zoloft; Pfizer, New York, NY), appear to be relatively safe during pregnancy and lactation [2]. Although there are no perfectly designed studies, no greater risk of malformation has been shown with those medications in animal and human studies [3,4]. Information in humans is lacking on long-term growth and

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physical maturation after antenatal exposure to these centrally active medications.

Paroxetine (Paxil, SmithKline Beecham, Pittsburgh, PA), another selective serotonin uptake inhibitor, has become widely prescribed in this country to treat persons with depression, obsessive-compulsive disorders, or panic attacks [5]. Its manufacturer began marketing the drug in 1995 as an alternative to other well-known serotonin uptake inhibitors, because side effects of paroxetine were shown to be less and metabolic effects require less time [6]. When given once daily, paroxetine may lead to improved patient compliance. Information is lacking in humans and animals about paroxetine and its long-term effects from antenatal exposure. The objective of the present investigation was to determine, in a placebo-controlled manner, whether chronic exposure to paroxetine in utero is associated with any alteration in growth and physical maturation of mice offspring.

MATERIALS AND METHODS

Animals and Animal Care

This proposal was approved by our Institutional Animal Care and Use Committee. The CD-1 mouse was chosen because we have shown that, compared with other mouse strains, it has the greatest success with timed conception [7]. We have reported on the normally expected gestational length, litter size, survival, and growth and physical development rates of this mouse strain [7]. On the basis of our experience with this mouse model and with use of a power analysis tool to determine a clear difference between matched samples, it was calculated that at least 10 gravid mice would be necessary in each treatment arm [8,9]. Then, outcomes from these pregnancies would be adequate to demonstrate, with 95% confidence, a 10% change in birthweight, which would reach a statistically significant difference ($P < 0.05$) between the paroxetine group and the corresponding placebo group [power $(1\text{-}\beta) = 0.80$, $\alpha = 0.05$].

Male and female adult mice (Charles River, New Brunswick, NJ) were transferred to our laboratory at 8 weeks old and were allowed 1 week of adjustment to their new environment. The mice were housed in an American Association for the Accreditation of Laboratory Animal Care-approved facility in an isolated animal room with a controlled temperature (72°F), humidity (50 ± 15%), and 12-h light/dark schedule (lights on at 6 AM). The mice were maintained on water and a nutritionally certified breeding diet (5015 PMI Feeds, St. Louis, MO). Mice were housed in polycarbonate cages with hardwood chip bedding (Sanichips, PJ Murphy Forest Products, Montville, NJ) that was changed at least weekly.

Timed mating consisted of one female being matched to a randomly selected experienced male. Food was removed from the male's cage and the female was introduced for 2 h (8:00–10:00 AM) each day for a maximum of 5 consecutive days. The presence of a copulatory plug indicated gestational day (GD) 0. The mice were then housed individually throughout gestation.

Drug Dosing

A clinically relevant dose for rodents often corresponds to the human dose according to body surface area [6]. Paroxetine is almost completely metabolized by the same metabolic pathway in rodents and humans [6]. The recommended daily oral dose of paroxetine, according to the manufacturer, is one 20 mg tablet in the morning [4]. Certain persons may benefit from an increase in dose, in 10 mg/day increments, up to 60 mg daily [4,5]. In a preliminary investigation, we chose an initial dose of 10 mg/kg/d in mice, based on a nongravid mouse weight of 25 gm and on a nongravid human weight of 60 kg taking a 50 mg daily dose [10]. The steady-state serum concentration (about 50 ng/mL) was half of the upper therapeutic range in humans (100 ng/mL[3]) [5]. Paroxetine was administered in food bars either for 2 weeks before mating until GD 16.5 or for 6 weeks if nongravid. Food bars, formed by using a 10 mL syringe with the tip cut off, consisted of a mixture of flour made from regular food, water, and either the pulverized drug or placebo (water). These bars were dried for 48 h at room temperature, then used for a maximum of 4 days. Daily doses of paroxetine, given to adult mice before and during gestation, achieved serum concentrations using a 20 mg/kg/d dose (125 ± 25 ng/mL and 68 ± 18 ng/mL, respectively, in the nongravid and gravid mice) and using a 40 mg/kg/d dose (513 ± 134 ng/mL and 103 ± 32 ng/mL, respectively). Concentrations of paroxetine in amniotic fluid were 17 ± 3 ng/mL using the 20 mg/kg/d dose and 85 ± 20 ng/mL using the 40 mg/kg/d dose. Brain concentrations of paroxetine in the fetus were comparable to the gravid and nongravid adults using the 20 mg/kg/d dose (1.7 ± 0.1 mg/g vs. 1.1 ± 0.4 ng/g and 1.7 ± 0.3 ng/g, respectively) and the 40 mg/kg/d dose (5.9 ± 0.4 ng/g vs. 4.8 ± 0.5 ng/g and 5.8 ± 0.4 ng/g, respectively). Based on these observations, we selected a 30 mg/kg/d dose of paroxetine for the current study, since it would also presumably concentrate in the fetal brain and achieve a serum level in the gravid mouse that was comparable to the upper therapeutic concentration of 100 ng/mL in the human.

Growth and Physical Maturation of Offspring

Beginning on GD 18.5, the dams were checked twice daily for the presence of any litters. Deliveries by 8 AM were designated as postnatal day (PND) 1. The number of live births per litter, birthweights, and sex ratio of the pups were determined. The litter size of each treatment group was reduced to a maximum of eight on PND 5. An equal number of males and females were randomly selected. Growth measurements included the offspring's weight, body length, and head circumference. Head–rump lengths,

TABLE 1. Perinatal Outcomes of Offspring Exposed Chronically In Utero to Either Paroxetine or to a Placebo

	Paroxetine	Placebo	*P*
Birth			
Gestational duration (days)	19.0 ± 0	19.0 ± 0	NS
Live pups/litter			
PND 1	11.2 ± 0.3	11.5 ± 0.4	NS
PND 3	11.0 ± 0.3	11.1 ± 0.5	NS
PND 5	10.9 ± 0.4	11.0 ± 0.5	NS
Sex ratio (% male)	64/123 (52%)	102/196 (52%)	NS
Male offspring			
Body weight (gm)			
PND 1	1.65 ± 0.02	1.74 ± 0.02	<0.01
PND 3	2.22 ± 0.05	2.37 ± 0.04	<0.05
PND 5	3.13 ± 0.08	3.37 ± 0.07	<0.05
Body length (mm)			
PND 1	32.1 ± 0.1	32.4 ± 0.2	NS
PND 3	35.6 ± 0.2	36.4 ± 0.2	NS
PND 5	40.2 ± 0.3	40.7 ± 0.2	NS
Head circumference (mm)			
PND 1	29.2 ± 0.3	29.6 ± 0.2	NS
PND 3	32.0 ± 0.3	32.5 ± 0.2	NS
PND 5	36.4 ± 0.3	37.2 ± 0.2	NS
Female offspring			
Body weight (gm)			
PND 1	1.65 ± 0.01	1.66 ± 0.03	NS
PND 3	2.17 ± 0.05	2.30 ± 0.04	<0.05
PND 5	3.05 ± 0.08	3.27 ± 0.08	NS
Body length (mm)			
PND 1	32.1 ± 0.1	32.3 ± 0.2	NS
PND 3	35.7 ± 0.4	35.9 ± 0.2	NS
PND 5	40.0 ± 0.3	40.3 ± 0.2	NS
Head circumference (mm)			
PND 1	29.6 ± 0.2	29.4 ± 0.2	NS
PND 3	32.0 ± 0.3	32.5 ± 0.2	NS
PND 5	36.3 ± 0.3	36.8 ± 0.2	NS

Mean ± SEM.
NS = not statistically significant.
Litter sizes were 11 in each of the paroxetine—exposed male and female offspring groups and 12 in each of the placebo male and female offspring groups.

body weights, and head circumferences were obtained on PND 1, 3, and 5. Physical development milestones were sought at the following standard times for mice: incisor eruption, PND 9 to 14; eyes opening, PND 14 to 17; and vaginal patency or testis bifurcation, PND 26 to 30. Body weight was assessed regularly until PND 120. Reproductive capability was studied in the adult offspring beginning on PND 90. Two young female offspring were paired with a male offspring from the same litter for a maximum of three estrus cycles. Conception rates during the first estrus cycle and perinatal outcomes (gestational age at delivery, number of live offspring, sex distribution of offspring, body dimensions) were recorded.

Data Reporting and Statistical Analysis

A litter served as the unit of measurement. Data for each gender in each litter were presented either as a mean ± SEM or as a percent of the litter. Comparisons between offspring in the paroxetine- and placebo-exposure groups were conducted using the paired *t*-test or Welch's *t*-test and, in cases of nonparametric comparisons, the Fisher's exact probability test. InStat 2.05 Graphpad Software and Statistix 4.1 Analytical Software (Tallahassee, FL) were used. A *P* value of <0.05 was considered statistically significant. For a significant result to be conclusive, the 95% confidence limits for the two groups were not to overlap.

RESULTS

Drug consumption by the gravid mice was compatible with that anticipated in the formulation of the food bars. The adult mice ingested 30.0 ± 0.9 mg/kg/d during the preconception period and 22.9 ± 0.7 mg/kg/d at GD 11. Maternal weight in both groups increased at a similar rate as gestation advanced.

Table 1 compares the perinatal outcomes between the paroxetine- and placebo-exposed offspring. The mean duration of gestation was 19.0 days for both groups. The number of live pups per litter (11.3 ± 0.3) and the percentage of male pups (52%) did not differ between the paroxetine- and the placebo-exposed offspring. Body weights of the paroxetine-exposed pups were less than those exposed to the placebo both for females (PND 3: 2.17 gm vs. 2.30 gm, $P < 0.05$) and for males (PND 1: 1.65 gm vs. 1.74 gm, $P < 0.01$). The head widths in offspring exposed to paroxetine were narrower than in the placebo group for the males (7.7 mm vs. 8.0 mm; $P < 0.05$) and females (7.7 mm vs. 8.1 mm; $P < 0.001$). This variation in head width was not statistically associated with different head circumference measurements.

No paroxetine-related facial abnormalities were seen. There were no signs of acute central nervous stimulation, including tremors and convulsions. Dam–pup interactions were observed to be indistinguishable between the paroxetine- and placebo-exposed groups. Each dam was able to nurse, and each pup was able to suckle. The dams in each treatment group gained weight appropriately while nursing. There were no differences observed between the two groups as to when the offspring began to grow hair, crawl, or sit. There were no fetal pup losses beyond PND 5.

As shown in Table 2, there were no differences between treatment groups in the onset of lower incisor eruptions, eye openings, and external genital development, regardless of gender. Both groups exhibited lower incisor eruptions by PND 9 and eye openings by PND 14. A delay in the eruption of the upper incisors was present at PND 11 in the male and female offspring exposed to paroxetine ($P <$

TABLE 2. Physical Maturation of Offspring Exposed Chronically In Utero Either to Paroxetine or to a Placebo

	Paroxetine	Placebo	*P*
Male offspring			
Incisor eruption (%)			
PND 9 (lower)	26 ± 6	27 ± 8	NS
PND 11 (upper & lower)	22 ± 11	63 ± 11	<0.02
Eye opening (%)			
PND 14	36 ± 13	14 ± 7	NS
PND 17	100 ± 0	100 ± 0	NS
Testicular bifurcation (%)			
PND 26	51 ± 9	57 ± 10	NS
PND 30	82 ± 6	88 ± 7	NS
Female offspring			
Incisor eruption (%)			
PND 9 (lower)	29 ± 6	30 ± 7	NS
PND 11 (upper & lower)	22 ± 10	56 ± 11	<0.03
Eye opening (%)			
PND 14	23 ± 9	10 ± 6	NS
PND 17	100 ± 0	100 ± 0	NS
Vaginal patency (%)			
PND 26	18 ± 7	17 ± 6	NS
PND 30	46 ± 13	67 ± 6	NS

Mean ± SEM.
NS = not statistically significant.
The litter sizes were 11 in each of the paroxetine—exposed male and female offspring groups and 12 in each of the placebo male and female offspring groups.

0.03). However, all offspring exhibited eruptions of all incisors by PND 14. External genital development was evident by PND 26 regardless of treatment group. Vaginal patency or testicular bifurcation developed in a manner which was indistinguishable between treatment groups.

Figure 1 displays body weights of offspring throughout the lifespan. Female offspring exposed antenatally to paroxetine tended to weigh less than the placebo-exposed group, reaching statistical significance only at PND 3, 45, 75, and 95 ($P < 0.05$). Male offspring in the paroxetine group weighed significantly less than the placebo group at birth, but this difference in body weight was not statistically different after PND 5.

Paroxetine did not impair reproductive capabilities of the offspring. Conception rates during the first estrus cycle were high for the paroxetine-exposed offspring (19 of 20 sibling pairings) and for the placebo-exposed offspring (22 of 22 sibling pairings). The litter size and sex ratio of the pups did not differ significantly between the two treatment groups (Table 3). Birthweights of male offspring were greater in the paroxetine than in the placebo group at PND 1 (1.72 gm vs. 1.65 gm; $P < 0.001$) and at PND 3 (2.12 gm vs. 2.52 gm, $P < 0.001$) but not at PND 5. No significant differences were found in body lengths and in head circumferences measurement between groups.

DISCUSSION

If a woman develops mild depressive symptoms during the first trimester, therapy with nonpharmacologic alternatives may be considered [11,12]. Medications are recommended when symptoms become severe. For those with a history of depression, a previous suicide attempt, or several previous exacerbations of disease, we concur with Kuller et al. [13] that prescription of medications, such as serotonin reuptake inhibitors, is worthy of consideration at any time during pregnancy. A change in pharmacologic management is not routinely recommended either before or during pregnancy without consultation with a member of the health profession who is well versed in affective disorders.

The present placebo-controlled investigation was undertaken to determine whether the widely prescribed serotonin

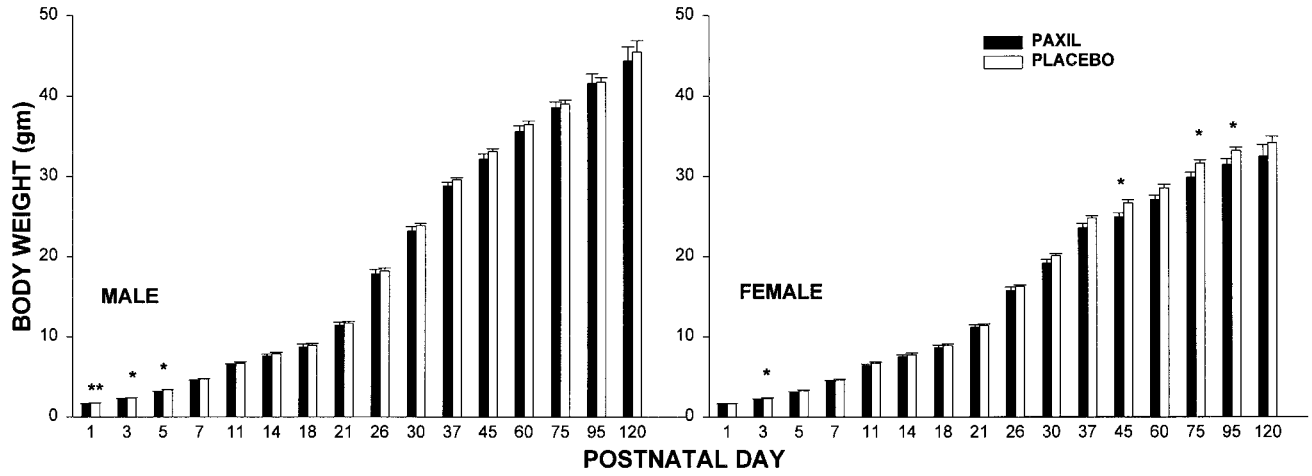

Fig. 1. Body weights of male and female offspring between postnatal day 1 to 120 after chronic in utero exposure either to paroxetine (Paxil; 30 mg/kg/d) or to a placebo. Mean ± SEM. Significant differences between treatment groups for each postnatal day are shown by a *($P < 0.05$) and **($P < 0.01$).

TABLE 3. Physical Outcomes of Second-Generation Offspring

	Paroxetine	Placebo	*P*
Litters mated	19/20	22/22	NS
Live pups/litter			
PND 1	10.9 ± 0.5	10.1 ± 0.6	NS
PND 3	10.0 ± 0.4	8.6 ± 0.6	NS
PND 5	9.8 ± 0.4	8.3 ± 0.5	NS
Sex ratio (% male)	109/218 (50%)	118/222 (53%)	NS
Male offspring			
Body weight (gm)			
PND 1	1.72 ± 0.02	1.65 ± 0.04	<0.001
PND 3	2.12 ± 0.04	2.52 ± 0.08	<0.001
PND 5	3.45 ± 0.06	3.65 ± 0.11	NS
Body length (mm)			
PND 1	32.8 ± 1.2	32.7 ± 0.2	NS
PND 3	36.4 ± 0.2	36.5 ± 0.3	NS
PND 5	41.1 ± 0.3	41.2 ± 0.4	NS
Head circumference (mm)			
PND 1	29.6 ± 0.2	29.4 ± 0.2	NS
PND 3	32.8 ± 0.2	33.3 ± 0.2	NS
PND 5	37.2 ± 0.2	37.4 ± 0.2	NS
Female offspring			
Body weight (gm)			
PND 1	1.67 ± 0.02	1.62 ± 0.03	NS
PND 3	2.36 ± 0.04	2.43 ± 0.07	NS
PND 5	3.32 ± 0.06	3.52 ± 0.11	NS
Body length (mm)			
PND 1	32.5 ± 0.02	32.2 ± 0.2	NS
PND 3	36.0 ± 0.3	36.2 ± 0.3	NS
PND 5	40.2 ± 0.2	40.7 ± 0.3	NS
Head circumference (mm)			
PND 1	29.3 ± 0.2	28.9 ± 0.2	NS
PND 3	32.8 ± 0.2	32.6 ± 0.2	NS
PND 5	37.0 ± 0.2	37.0 ± 0.2	NS

Mean ± SEM.
NS = not statistically significant.
The litter sizes were 11 in each of the paroxetine—exposed male and female offspring groups and 12 each of the placebo male and female offspring groups.

uptake inhibitor paroxetine affected long-term growth and physical maturation of offspring exposed throughout gestation. Animal investigations of effects from antenatal drug exposures have primarily involved the rodent model. Paroxetine is known to cross the placental barrier, and the daily dose used here was similar to that which produced concentrations in the fetal mouse brain that were comparable to those in the adult mouse [10]. Importantly, the dose used here achieved serum levels comparable to those in the upper therapeutic range in humans.

Much of the early work with these serotonin-modulating drugs involved toxic doses and was confined to perinatal survival and to gross physical markers at early postnatal ages. As in similar studies using rodents, we found that paroxetine did not reduce litter size or increase the risk of external anomalies [3,4]. Body dimensions and physical milestones were measured at different times during the life span with a battery of measurements that were conceptually similar to those used in humans. We propose that our observations are especially relevant to humans, because antenatal exposure to paroxetine did not have an impact on the offspring during the prepuberty, juvenile, or adult periods. Any impairment of reproductive development and capability, an important consideration to any obstetrician, is a potential concern with any centrally acting drug. No effect from paroxetine on future reproduction was found in the current investigation.

In this study, the only consistent impacts of paroxetine were limited to lower body weights and narrower head widths among paroxetine-exposed male and female offspring at birth. These findings likely represented a subtoxic effect from the drug. Although the paroxetine-exposed group of offspring had narrower heads, the head circumferences were not smaller. A dysmorphology sequence or crowding effecting may be possible and requires further study. The lower body weight in paroxetine-exposed offspring were temporary and would not be explained by less maternal food ingestion, as weight gain during pregnancy was appropriate. Later, isolated differences in body weights of female offspring between treatment groups were likely only a result of statistical variation and without biological consequence. The delay noted in eruption of upper incisors of the paroxetine-exposed offspring was very short-term. The maturation process was not impaired, since by PND 14 all mice had upper incisors and were able to eat solid food without difficulty.

Despite extrapolation variations between species, it should be reassuring to clinicians that no long-term deficits in growth and physical maturation were observed between the paroxetine- and the placebo-exposed groups beyond the immediate postnatal period. The offspring displayed no loss of reproductive capability. The negative findings from this study are not intended to encourage the prescribing of paroxetine during pregnancy. Further investigation is to be undertaken to determine whether any impairment in neurobehavioral development is possible after chronic in utero exposure to paroxetine.

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