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Changes in Developing Behavior Following Prenatal Administration of Imipramine

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COYLE, I. R. *Changes in developing behavior following prenatal administration of imipramine.* PHARMAC. BIOCHEM. BEHAV. 3(5) 799–807, 1975. – Female rats were given oral doses of Imipramine (5mg/kg) from 14–21 days prior to mating to conception or Day 19 of gestation and the physical maturation and behavioral development of their offspring was compared with that of controls. There were significant differences between the weights of the Imipramine and control animals at 21 days and the appearance of some reflexes was delayed. Behavior in an open field was observed when the rats were 9, 13, 17 and 21 days of age and it was found that exploratory responses were less frequent in the drug exposed offspring. In contrast there were no obvious physical anomalies and the adult behavior of the Imipramine animals on a spontaneous alternation task and a swimming maze did not differ from that of controls.

Pregnancy Imipramine Behavioral abnormalities Reflex ontogenesis Open field behavior

PREVIOUS studies have shown that administration of Imipramine prior to and during pregnancy causes an increase in fetal and neonatal mortality in the rat [13,25]. Although none of these studies reported any overt teratogenic effects it can be argued that the observed increase in mortality is an indicator of teratogenic potential [18,30]. Thus, it has been suggested [13] that the increased neonatal mortality they noted may have been due to the offspring being anomalous in some subtle way, as it is well known that anomalous infant rats are usually destroyed by the mother.

There is an increasing amount of evidence which suggests that sub-teratogenic doses of pharmacological agents can cause enduring behavioral anomalies in the offspring of prenatally treated females [7, 11, 16, 18, 22]. These data suggest the hypothesis, tested in the present study, that prenatal exposure to Imipramine may affect the behavior of the offspring. Since previous studies have shown that performance in a swimming maze is a good indicator of neurological impairment [7,28] this task was used to evaluate the behavior of the drug-exposed offspring. The offspring were also evaluated on a spontaneous alternation task since performance on this task reflects the ability to acquire and process sensory information [20,21].

EXPERIMENT 1

Method

Animals. The animals were the offspring of 25 naive Wistar rats which were 90–100 days old at the beginning of the experiment. A total of 72 offspring, 36 males and 36 females, survived weaning and were tested.

Apparatus. The apparatus consisted of a swimming maze (a multiple T-maze) and a T-maze. The general structure of the swimming maze, which was constructed of grey p.v.c., was taken from a previous study [5]. The sides of the maze were 920 X 920 cm and each alley was 8 cm wide X 32 cm high. The straight alley, which was attached to one side of the swimming maze, was 920 cm long X 8 cm wide X 32 cm high. A platform constructed of the same material as the maze and measuring 8 cm wide X 10 cm long was located 22 cm above the floor of the maze and served as a goal platform for all testing in the maze. The maze was enclosed by a transparent acrylic cover which permitted clear observation of the animals position at all times. Entry and exit holes were provided in this cover and fitted with moveable lids which allowed easy placement and removal of the animals. During testing the maze was placed in a large stainless steel tank containing water at 25–27°C. Although no attempt was made to maintain this temperature, it was found to remain within these limits for the duration of each testing session.

Each arm of the T-maze measured 100 cm long, 12 cm wide and 12 cm high and a guillotine door was located 25 cm along the start alley. The apparatus was designed with a swing-away lid to permit easy placement and removal of the animals. All interior surfaces were matt black and the apparatus was covered with transparent acrylic so as to permit clear observation of the rats' position. During testing the apparatus was placed in a room where the only illumination came from a 40 W incandescant red lamp located 40 cm above the choice point of the apparatus.

Procedure. Prior to mating, the females were housed 2–3 to a cage and kept in an air conditioned laboratory which was maintained at approximately 22°C. The daily light cycle was 12 hr light, starting at 7:00 a.m. and 12 hr dark. The females were matched on the basis of weight and

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assigned to either a drug or placebo group. The drug group was administered a 5 mg/kg dose of Imipramine-HCl (0.15 g/100 ml dissolved in 0.9 percent saline). The Placebo group was administered a 1.00 ml dose of 0.9 percent saline. All drugs were administered orally, at about the same time each day, by means of a specially modified brass-beaded 19 ga needle. After 14–21 days of drug administration each female was placed in a mating cage together with a male rat of proven fertility. The female was removed to a breeding cage when a vaginal plug was observed and this was taken as Day 0 of gestation. Drug treatment was continued daily until Day 19 of gestation.

On the day of birth, the litters were randomly reduced to 8 offspring which were fostered to an untreated female of the same age as the experimental female and which had delivered pups on the same day if possible. If the offspring could not be fostered within 24 hr of birth they were discarded. This fostering procedure was used in preference to the more common cross-fostering technique since postnatal maternal influences on behaviour were of minor importance to the present study. After fostering, the animals were left undisturbed, apart from routine cage maintenance, until weaning at 25 days of age. Those litters which had less than 5 animals alive at weaning were discarded. At weaning, the animals were marked for identification by toe and ear clipping. So that the number of animals in the drug and control groups would be equal, a number of offspring were randomly discarded at this time. Each of the two treatment conditions comprised 18 males and 18 females at the completion of testing. After weaning, the animals were housed singly and left undisturbed, apart from routine cage maintenance, until they were 60–61 days old when behavioral testing was commenced. The sequence of testing was as follows: testing of spontaneous alternation; initial testing of swimming ability in the straight alley; pretraining in the swimming maze; training in the swimming maze; final testing in the swimming maze. This testing procedure was chosen in an attempt to differentiate between motor performance, maze learning ability and retention. At the conclusion of behavioral testing all animals were weighed.

For the testing of spontaneous alternation each animal was placed in the starting alley of the T-maze and the guillotine door was removed. When the animal had made a four footed entry into one of the arms of the maze it was immediately removed, replaced in the start alley and given a second trial using the same procedure and criterion. The time taken to make an entry into one of the arms, together with the arm entered was recorded on each trial. If the animal entered the same arm of the maze on both trials it was classified as having perseverated. If it entered a different arm on both trials, it was classified as having alternated. If the animal did not completely enter either arm within 5 min on both trials, it was classified as having failed to respond. After each animal had been tested, the paper covering the floor of the maze was replaced so as to minimize variability in olfactory cues.

During the testing of swimming ability and pretraining in the swimming maze the water level was kept slightly below the height of the goal platform; during subsequent training and final testing, the maze was completely filled with water so that no air spaces remained under the acrylic lid. The water in the maze was changed daily during testing in the straight alley and after every two trials in the maze. To evaluate the animals swimming ability the time taken to swim along the straight alley over 5 consecutive trials a day for two days was recorded. On the first day of pretraining the animals were given 2 trials and on the next two days 4 trials a day. When the animals were 65–66 days old training was commenced. The animals were given 2 trials on the first day of training and 4 trials on each of the two following days. The number of errors defined as a head and shoulder entry into an incorrect alley were recorded on each trial. If an animal had not escaped from the maze within 50 sec it was removed. Trials were separated by at least 30 min and the animals were dried and returned to their cage between trials. When the animals were 125–126 days old they were again tested in the maze. The procedure and criterion were identical to that employed during training.

Results

The reproductive success of drug and control animals is shown in Table 1. The median number of live offspring in the Imipramine group was significantly smaller than in the Placebo group. (Fisher's Exact Probability Test, one tailed, $p<0.05$). In addition, the number of stillborn offspring in the Imipramine group was significantly higher than in the Placebo group as was neonatal mortality during weaning (Fisher's Exact Probability Test, one tailed, $p<0.05$ and 0.025 respectively). There was no significant difference between the Imipramine and Placebo groups with respect to median length of gestation (Fisher's Exact Probability Test, one tailed, $p>0.05$).

The drug exposed offspring which survived weaning displayed no obvious physical abnormalities. A two factor analysis of variance revealed that there was no significant drug effect on weight, $F(1,70) = 2.28$. The sex by drug interaction also failed to reach significance, $F(1,70) = 0.78$, although there was a significant effect due to sex, $F(1,70) = 731.52$, $p<0.01$.

A breakdown of the behavioral data by sex indicated that males and females were distributed almost exactly according to chance expectation and so this data was combined in subsequent analyses.

The number of Imipramine and Placebo animals alternating was 15 (42 percent) and 17 (47 percent) respectively and there was no significant difference between these frequencies ($\chi^2 = 0.02$, $df = 1$). The failure to find any difference between drug and control animals could not be attributed to an increased incidence of freezing in either group as the proportion of non-responding control animals was identical to that in the Imipramine group. In both groups, 14 animals failed to respond within the specified time.

The analysis of swimming ability in the straight alley prior to pretraining was measured over the last 3 trials so as to minimize any habituation effect. There were no significant differences between drug and control animals with respect to swimming performance in the straight alley, ($t = 0.75$, $df = 70$).

Performance in the swimming maze as reflected in the number of errors made during training and testing was analyzed by a two factor repeated measures analysis of variance procedure. There was no significant effect due to prenatal drug treatment, $F(1,70) = 0.42$, nor was the drug-group by trials interaction significant, $F(1,70) = 1.32$. There was a highly significant trials effect with both groups of animals showing improvement, $F(1,70) = 44.42$, $p<0.01$.

TABLE 1

SUMMARY OF REPRODUCTIVE SUCCESS

	Median Number of Offspring		
	Alive at Birth	Stillborn	Alive at Weaning*
Imipramine	11	1	5
Placebo	14	0	8

*Excluding litters which had less than 8 animals alive at birth.

Discussion

It has been argued that teratogenic studies which have utilised interperitoneal or subcutaneous administration of Imipramine are invalid because of the more complete absorption of the drug when given by these methods as compared with oral administration [2]. Other authors [25] have suggested that this is not an important consideration since the metabolic patterns following interperitoneal and oral administration of Imipramine are essentially similar [8]. In this respect, it is important to note that the increase in foetal and neonatal mortality in the present study is in agreement with previous studies [13,25] which have utilised different routes of drug administration.

As has been pointed out [18,30], any agent which causes increased fetal and neonatal mortality must be considered potentially teratogenic. Considering this argument, the failure to find any effects on gross morphology or behavior following prenatal exposure to Imipramine is somewhat surprising. Three explanations seem tenable. In the first place it could be argued that Imipramine's effect on fetal and neonatal mortality is divorced from any teratogenic action. This seems unlikely in the light of previous studies [18,30]. Secondly, there is the possibility that the tests used to evaluate behavior were insensitive to drug-induced anomalies. Since previous studies have found these tests to be sensitive to neurological impairment and teratogenic effects on behavior [7,28] this possibility can be reasonably discounted. Thirdly, it is possible that prenatal exposure to Imipramine produces some developmental effect on the offspring's behavior which is compensated for or masked in the mature animal. Prenatal exposure to Δ^9-THC results in delayed development of reflexive and exploratory behavior in the neonate [6]. Physical maturation was also significantly retarded in the cannabinoid-treated neonatal animals. However, by weaning, most of the differences between drug and control animals had disappeared. A similar finding has been reported with the offspring of rats who were nutritionally deprived during pregnancy. The manner in which behavioral anomalies in the neonate was compensated for, or masked, in the more mature animal is not clear. It is probable however, that developmental delays in behavior are indicative of neurological impairment [27].

EXPERIMENT 2

A second experiment was carried out to study the effect of prenatal exposure to Imipramine on physical maturation and behavioral development. The experimental approach used was derived from previous studies of prenatal influences on developmental behavior [27]. Spontaneous alternation was also tested since this has been shown to be a sensitive measure of maturation [20,21].

Method

Animals. The animals were the offspring of 23 naive Wistar rats which were 90–100 days old at the beginning of the experiment. A total of 68 offspring, 34 males and 34 females, survived weaning and were tested.

Apparatus. The apparatus consisted of a T-maze and a circular open field. The T-maze was of similar construction to that described previously but with smaller dimensions. Each arm of the maze measured 40 cm long, 8 cm wide and 10 cm high and a guillotine door was located 10 cm along the start alley. The open field was 84 cm in diameter and 66 cm high. The floor in the center of the apparatus was demarcated into 30 squares, each 10 cm square; the remainder of the floor was marked into 16 approximately equal areas. The interior surfaces of the apparatus were matt black and illumination was provided by a 40 W incandescent light centered 66 cm over the floor.

Procedure. The drug administration and fostering procedure were the same as for Experiment 1. On Day 0, (the day of birth) all offspring were marked for identification by clipping a single joint from one of the forefeet. Toe marking was carried out at the completion of testing on Day 0.

The animals were weighed on the day of birth and on Day 1 and, thereafter, every second day until weaning. In addition they were inspected daily for the maturation of three physical features, incisor eruption (appearance of the upper incisors), eye opening (any visible break in the membrane of either eye) and ear unfolding (complete unfolding of both pinnae). Testing of the ontogenesis of six reflexes was done in the manner described by Smart and Dobbing [27], except that the responses were scored quantally, not graded. A summary of the eliciting stimuli and the positive response for each reflex is given in Table 2. Five min before testing, which was conducted between 12:00 a.m. and 4:00 p.m., the rats were removed from their home cage and placed in a holding cage where the temperature was maintained at 33°C by means of two 40 W incandescent red lights suspended over the cage.

When the animals were 9, 13, 17 and 21 days old exploratory behavior was observed in the circular open field. Prior

TABLE 2

DESCRIPTION OF REFLEX TESTS

Reflex	Eliciting Stimuli	Response
Righting	Rat placed on back on a flat surface	Turns over onto ventral surface
Free fall righting (acceleration righting)	Rat dropped, back downwards, from 35 cm onto cotton wool pad	Turns in mid-air to land on all fours
Negative geotaxis	Rat placed, head downwards, on a 20° slope	Turns to face up the slope
Cliff avoidance (cliff drop aversion)	Rat put on edge of bench, with nose and forefeet just over edge	Moves away from 'cliff'
Auditory startle	Sound stimulus: snap of mouse trap closing on wooden base	Sudden, brief extension of hind limbs (which raises hindquarters)
Visual placing	Rat held upside down near edge of bench	Lifts head and extends forelegs in direction of bench

From Smart and Dobbing [27].

to reflex testing, each animal was placed in the center of the open field and the following behaviors were recorded during a 2 min observation period: number of areas entered; frequency of head lifting with both forelegs on the ground; frequency of head lifting with one foreleg on the ground (half-rearing) frequency of rearing on the hind legs either against a vertical surface or unsupported; frequency of turning (defined as a change in direction of 180° in a radius not exceeding one body length); and frequency of grooming. The apparatus was sponged clean and dried after each animal had been tested. On Day 21 after the completion of all other testing the animals were given a 5 min rest at 33°C and spontaneous alternation was tested. The procedure and criterion were identical to those employed in Experiment 1.

Results

In contrast to the findings reported in Experiment 1 the reproductive success of females administered Imipramine during pregnancy was not different from that of control animals. As can be seen from Table 3 there was no significant difference between the Imipramine and Placebo animals with respect to the number of live or stillborn offspring at birth (Fisher's Exact Probability Test, one tailed, $p>0.05$). Similarly there was no significant difference between the Imipramine and Placebo animals with respect to length of gestation ($p>0.05$). However there was a significant increase in neonatal mortality in the Imipramine group (Fisher's Exact Probability Test, one tailed, $p<0.05$).

Since this result was contradictory to the findings of previous studies (see above) and Experiment 1, which were identical to Experiment 2 so far as drug administration procedure and dosage were concerned, the data on fetal mortality from Experiment 1 and Experiment 2 was combined and reanalysed. Although the cell frequencies of the combined data were large enough to permit use of a more powerful test Fisher's Exact Probability Test was again used since it was felt that the nature of the data (live or dead offspring) reflected a discrete rather than a continuous underlying distribution. With the combined data it was found that the number of live offspring in the Imipramine groups was significantly smaller than in the Placebo groups (Fisher's Exact Probability Test, one tailed, $p<0.025$). There was no significant difference between the Imipramine and control groups with respect to the number of stillborn offspring (Fisher's Exact Probability Test, one tailed, $p>0.05$) or length of gestation (Fisher's Exact Probability Test, one tailed, $p>0.05$). Although statistically significant the difference in litter size between the Imipramine and Placebo treated females was small. Thus, the median number of live offspring in the Imipramine groups was 10 as compared to 13 in the Placebo groups.

In the present study the animals were derived from a colony where systematic outbreeding was employed. Under these conditions it is reasonable to consider measures of individuals within a litter as independent, ([17], p. 100; Coyle, in preparation). Accordingly, within litter correlation of individual offspring [1] was ignored in all analyses of developmental behavior and physical maturation.

There were no obvious physical abnormalities in the Imipramine offspring which survived weaning. However, there was a drug effect on weight. Since a trend analysis [12] showed no significant sex effects these groups were

TABLE 3

SUMMARY OF REPRODUCTIVE SUCCESS

	Median Number of Offspring		
	Alive at Birth	Stillborn	Alive at Weaning*
Imipramine	12	0	5
Placebo	12	0	7

*Excluding litters which had less than 8 animals alive at birth.

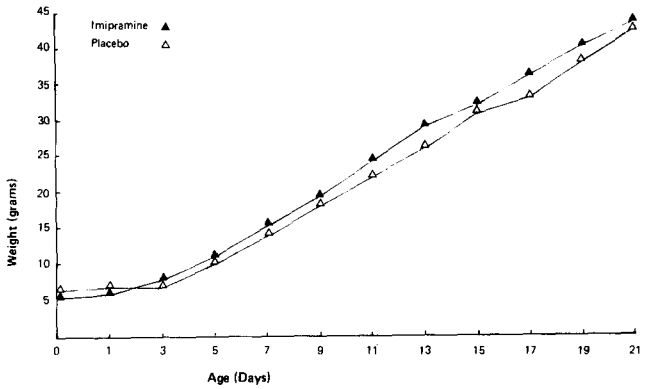

FIG. 1. Mean weight from birth to weaning.

combined in subsequent analyses of the data by a test for trend [12]. The mean weight of the Imipramine and Placebo animals is shown in Fig. 1.

The Imipramine animals mean weight was significantly greater than that of the control animals, $F(1,66) = 6.76$, $p<0.05$. In addition, there was a significant overall between groups trend, $F(11,726) = 4.49$, $p<0.01$. The significant overall between groups trend was mainly attributable to significant differences between the Imipramine and Placebo Ss in linear and quadratic trends, $F(1,66) = 4.48$ and 13.28 respectively, $p<0.05$ and 0.01 respectively).

Because of the limited range of scores and the large number of tied observations Rodgers' [23] generalized median procedure was used to evaluate the physical maturation of the animals. A separate analysis was performed for each measure of physical maturation. The scores for males and females in both groups were pooled and dichotomized according to the following criterion. Animals whose physical features developed later than the majority of all animals were assigned to one group, all other animals were assigned to another group. In most cases this effectively discriminated the animals according to whether they were above or below the mode. There was no significant difference between the Imipramine and Placebo animals with respect to time of unfolding of the external pinnae or eye opening, $F(1,\infty) = 2.27$ and 2.15 respectively. However incisor eruption was significantly retarded in the Imipramine treated offspring, $F(1,\infty) = 7.07$, $p<0.01$.

Reflex ontogenesis was evaluated according to the same procedure used to analyze physical maturation. There were no significant differences between the Imipramine and Placebo animals with respect to righting, auditory startle, visual placing and free fall righting, $F(1,\infty) = 0.26$, 0.16, 1.57 and 1.54 respectively. The Imipramine group was significantly retarded in the development of cliff avoidance and negative geotaxis, $F(1,\infty) = 3.98$ and 5.95 respectively, $p<0.05$ in both cases.

A trend analysis [12] of behavior in the open field indicated that there were no significant sex effects and so this factor was disregarded in subsequent analyses. The open field data were analyzed by a test for trend [12]. Since the frequency of head-lifting, half-rearing and rearing proved too low to be analyzed meaningfully, these responses were grouped together as upward responses for the purpose of analysis. These are shown in Fig. 2. The mean number of upward responses exhibited by the Imipramine group was significantly less than that of the Placebo group, $F(1,66) = 38.33$, $p<0.01$. There were also significant differences between the Imipramine and Placebo animals with respect to overall trend, $F(3,198) = 10.34$, $p<0.01$. This difference was mainly attributable to significant between group differences in linear trends, $F(1,66) = 24.06$, $p<0.01$. There were no significant differences between the Imipramine and Placebo animals with respect to number of areas entered when group means $F(1,66) = 2.13$, and between group overall trends were considered, $F(1,198) = 1.97$. However, there was a significant between group difference in quadratic trends, $F(1,66) = 5.32$, $p<0.05$. When body turns were analyzed, it was found that the Imipramine group made significantly more turns than the Placebo group with respect to group means, $F(1,66) = 6.36$, $p<0.05$ and to cubic trends, $F(1,66) = 4.22$, $p<0.05$. There were no significant differences between the groups with respect to overall group trends, $F(3,198) = 1.32$, linear, $F(1,66) = 0.31$, or quadratic trends, $F(1,66) = 0.04$. The incidence of grooming proved to be too low to be analyzed meaningfully.

The number of Imipramine and Placebo animals alternating was 23 (67 percent) and 24 (70 percent) respectively and there was no significant difference between these frequencies ($\chi^2 = 0.00$, $df = 1$). (The percentage of animals alternating in these groups was higher than the 53 percent that would be expected in rats of this age [20]. This discrepancy was probably caused by the handling and testing procedures consequent upon reflex testing and open field

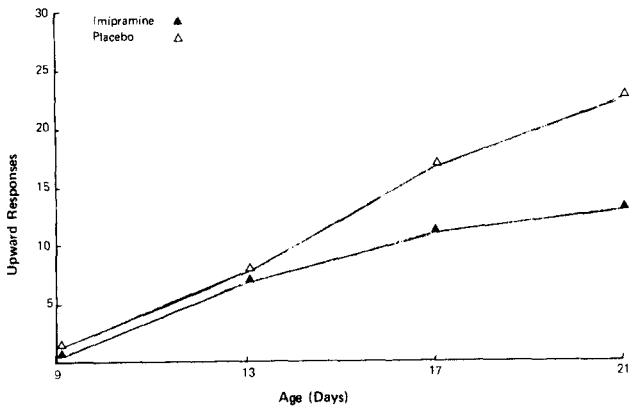

FIG. 2. Mean number of upward responses in the open field.

testing). Since only a very small proportion of animals failed to respond, due no doubt to the handling and testing procedure employed during weaning, the failure to find any difference between drug and control animals could not be attributed to an increased incidence of freezing in either group.

Discussion

The failure to find any effects on litter size following prenatal exposure to Imipramine is surprising in view of the findings of Experiment 1 and previous studies [13,25]. This is emphasised by the observation that neonatal mortality was increased in the Imipramine exposed offspring in the present study. It is likely that the failure to find any effect on fetal mortality in Experiment 2 is due to the subtle (in a statistical sense) nature of the effect. The results of the combined analysis of fetal mortality in Experiments 1 and 2 support this argument.

The data on reflex ontogenesis and open field behavior demonstrate that prenatal administration of Imipramine can produce changes in the physical and behavioral development of neonatal rats at a dose level which does not have any observable effects on the morphology of the newborn. Since fostering procedures were employed it seems reasonable to conclude that the alterations in physical maturation, reflex ontogeny and open field behavior were due to prenatal drug treatment and not to postnatal influences such as poor maternal care. However, before such a conclusion can be reached there are a number of factors which must be evaluated.

It has been repeatedly demonstrated that the drug metabolizing capacity of the neonate is markedly inferior to that of the mature animal [10,31]. Thus, in the present study, it is conceivable that significant levels of Imipramine may have been present in the drug exposed offspring at birth. If this was so, then the retarded physical and behavioral development of the Imipramine animals may have been due to a direct drug effect. This is unlikely to be the case however. Equilibrium between the fetal and maternal systems in the rat is reached within 3 minutes of an intramuscular injection of Imipramine [9]. Metabolism and excretion of Imipramine in the rat and man is similarly rapid with over 90 percent of the drug being metabolized within 24–48 hours of administration [4, 8, 15]. Accordingly, by the time of parturition in the present study, significant metabolism and excretion of Imipramine and its metabolites would have occurred. Further, it has been demonstrated that the fetus is capable of metabolizing tricyclic antidepressants [26] and it is possible that the neonatal rats would at least have partially metabolized any residual levels of Imipramine.

EXPERIMENT 3

While it is unlikely that the developmental delays observed in Experiment 2 were due to a direct drug effect on the neonatal animals, it is arguable that these delays may have been due to an indirect drug effect in the neonate rather than a teratogenic effect on the fetus. Imipramine's antidepressant action may be due, in part, to interference with the production of some enzyme which, eventually, is sufficiently lacking to produce the clinical effects [8]. This suggestion is in agreement with the observation that Imipramine takes an average of 10–18 days to produce improvement from psychiatric depression and continues to have its antidepressant action for some time after cessation of treatment [3,19]. Thus, in Experiment 2, residual effects on an enzyme system could have caused the behavioral anomalies in the Imipramine-exposed offspring. In order to investigate this possibility, a further experiment was carried out.

Method

Animals. The animals were the offspring of 13 naive female Wistar rats which were 90–100 days old at the beginning of the experiment. A total of 68 offspring, 34 males and 34 females, survived weaning and were tested.

Apparatus. The apparatus was the same as for Experiment 2.

Procedure. The procedure was the same as for Experiment 2 with the exception of the duration of drug administration. This was discontinued immediately after mating instead of Day 19 of gestation.

Results

As is shown in Table 4 the reproductive success of females administered Imipramine prior to pregnancy was not detectably different from that of the control animals. There was no significant difference between the Imipramine and Placebo groups with respect to length of gestation, the number of live and stillborn offspring or with respect to neonatal mortality during weaning (Fisher's Exact Probability Test, one tailed, $p>0.05$ in all cases).

There were no obvious physical abnormalities in the Imipramine offspring which survived weaning. However, there was a significant drug effect on weight. As was the case in Experiment 2, a preliminary trend analysis [12] revealed no significant sex effects and so these groups were combined prior to subsequent analysis. In general, the results were opposite to those of Experiment 2. (See Fig. 3). The mean weight of the Imipramine animals was significantly less than that of the control animals, $F(1,66) = 6.87$, $p<0.05$. In addition, there was a significant overall between groups trend, $F(11,726) = 4.82$, $p<0.01$. The significant overall between groups trend was attributable to differences between the Imipramine and Placebo animals in linear trends, $F(1,66) = 6.34$, $p<0.05$.

Physical maturation and reflex ontogenesis were evaluated by the same procedure used in Experiment 2. There was no significant difference between the Imipramine and

TABLE 4

SUMMARY OF REPRODUCTIVE SUCCESS

	Median Number of Offspring		
	Alive at Birth	Stillborn	Alive at Weaning*
Imipramine	11	0	6
Placebo	12	0	7

*Excluding litters which had less than 8 animals alive at birth.

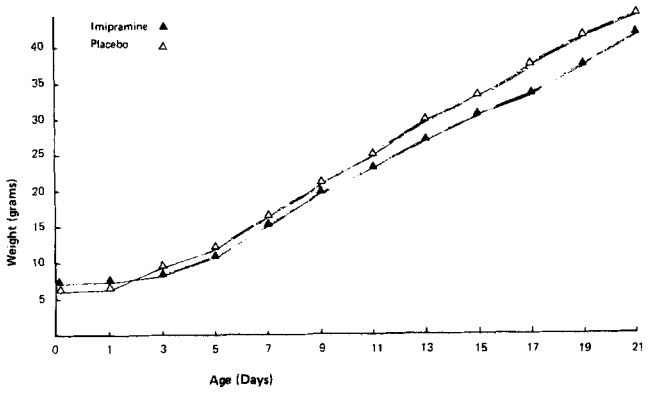

FIG. 3. Mean weight from birth to weaning.

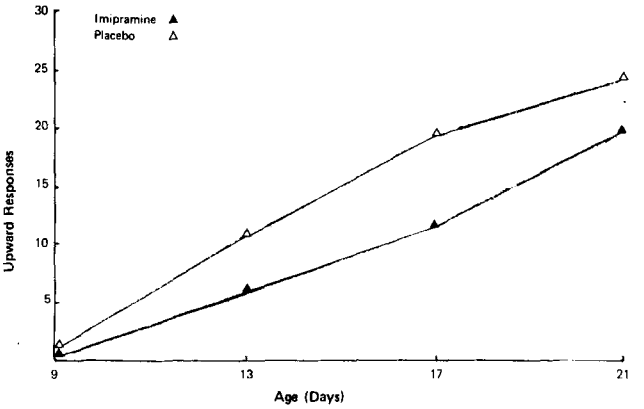

FIG. 4. Mean number of upward responses in the open field.

Placebo animals with regard to eye opening, $F(1,\infty) = 0.00$. Pinnae unfolding and incisor eruption were significantly retarded in the Imipramine treated offspring, $F(1,\infty) = 4.21$ and 14.23 respectively, $p<0.05$ and 0.01 respectively. There were no significant differences between the Imipramine and control animals with respect to righting, cliff avoidance and negative geotaxis, $F(1,\infty) = 1.83$, 0.05 and 3.78 respectively. The Imipramine group was significantly retarded with respect to the appearance of the auditory startle reflex, visual placing and free fall righting, $F(1,\infty) = 4.21$, 6.34 and 5.95 respectively, $p<0.05$ in all cases.

Analysis of behavior in the open field again failed to reveal any significant sex effects and, accordingly, this factor was not considered in subsequent analyses. The open field data were analyzed by a test for trend [12]. Head lifting, half rearing and rearing were grouped together as upward responses as in Experiment 2. These are shown in Fig. 4. The trend of upward responses exhibited by the Imipramine and Placebo animals was similar to that reported in Experiment 2. There was a significant difference between the Imipramine and Placebo animals with regard to group means, $F(1,66) = 31.83$, $p<0.01$, and overall trend, $F(3,198) = 4.58$, $p<0.05$. The difference in overall trend was mainly attributable to significant differences in linear and quadratic trends, $F(1,66) = 5.50$ and 6.54 respectively, $p<0.05$ in both cases. There were no significant differences between the number of areas entered by the Imipramine and Placebo animals with respect to group mean, $F(1,66) = 0.10$, overall group trends, $F(3,198) = 0.62$, or linear, quadratic and cubic trends, $F(1,66) = 0.18$, 0.75 and 1.21 respectively. Locomotor activity as measured by the number of body turns was, in general, not detectably different in the Imipramine and Control animals. There was no significant difference between the drug and control offspring with respect to group means, $F(1,66) = 1.10$, overall group trend, $F(1,198) = 3.52$, quadratic, $F(1,66) = 2.92$, and cubic trends, $F(1,66) = 1.06$. However, there was a significant difference between the Imipramine and Placebo groups in linear trends, $F(1,66) = 5.06$, $p<0.05$. As was the case in Experiment 2, the incidence of grooming proved to be too low to be meaningfully analyzed.

The number of Imipramine and Placebo animals alternating was 22 (65 percent) and 29 (85 percent) respectively and there was a significant difference between these frequencies ($\chi^2 = 5.02$, $df = 1$; $p<0.05$).

Discussion

The failure to find any effects due to administration of Imipramine on fetal or neonatal mortality is noteworthy. In Experiments 1 and 2 and in previous experiments where the drug has been administered throughout pregnancy, decreased fetal viability and/or increased neonatal mortality have been consistently reported [13,25]. Although little is known about the precise mechanisms of teratogenesis, it is possible that this increased fetal and neonatal mortality is

TABLE 5

SUMMARY OF MORPHOLOGICAL AND BEHAVIORAL MEASURES OF IMIPRAMINE OFFSPRING IN EXPERIMENTS 2 AND 3

Observations	Experiment 2	Experiment 3
MORPHOLOGICAL		
Fetal mortality	NS	NS
Neonatal mortality	*	NS
Pinnae unfolding	NS	*
Incisor eruption	*	*
Eye opening	NS	NS
Weight	*	*
BEHAVIORAL		
Righting	NS	NS
Negative geotaxis	*	NS
Cliff avoidance	*	NS
Auditory startle	NS	*
Visual placing	NS	*
Free fall righting	NS	*
Spontaneous alternation	NS	*
Upward responses	*	*
Areas entered	NS	NS
Body turns	*	NS

*Indicates a significant difference between Imipramine and Placebo animals. With respect to the open field observations, only indicates a significant difference between group means and/or overall trend of drug and control animals. The significant differences in weight gain in Experiments 2 and 3 were in opposite directions.

related to the Imipramine-induced blockade of catecholamines [29]. In Experiment 3 administration of Imipramine prior to pregnancy would not have affected catecholamine reuptake in the fetus. In the other experiments, where the drug was administered during pregnancy, catecholamine reuptake in the developing organism would have been blocked. It may then be argued that the effect of Imipramine on fetal and neonatal mortality is a consequence of its effect on catecholamines. At present there is little evidence to either confirm or disprove this hypothesis.

The retarded physical and behavioral development exhibited by the offspring of Imipramine treated females in Experiment 3 cannot be attributed to an indirect drug effect upon some enzyme system since drug administration was discontinued some 21–23 days prior to birth. The retarded development of exploratory behavior, physical maturation and reflex ontogeny would therefore seem to be an indication of a teratogenic effect. This conclusion is given additional support by the observed decrease in the rate of spontaneous alternation among Imipramine treated offspring.

There is little basis for speculation as to the mechanism by which Imipramine exerts its teratogenic effect. This is emphasized by the observation that the behavioral and physical anomalies observed in the experimental offspring in Experiment 3 differed markedly from those observed in Experiment 2 where the drug was administered throughout pregnancy. Thus, the Imipramine treated animals weighed significantly more than the control animals in Experiment 2 but significantly less than the control animals in Experiment 3. There were similar discrepancies between Experiments 2 and 3 with respect to the other developmental

measures, as is shown in Table 5. Clearly the teratogenic action of Imipramine is a complex one.

The offspring of rats treated with Imipramine during and/or prior to pregnancy develop more slowly than normal rats. They show delays in physical maturation, in the appearance of reflexes and exploratory behavior. Most of these characteristics eventually develop and by adulthood there is no detectable difference between drug exposed offspring and control animals on sensitive measures of neurological impairment. The implications of these findings are difficult to ascertain. It could be argued that developmental delays are of minimal adaptive significance. Conversely, it is possible that developmental delays are indicative of significant neurological impairment which is masked or compensated for in the adult animal. In this regard, it seems important to note that normal laboratory rearing procedures may mask drug effects on behavior [24]. In an analogous experiment it has been found that genetic influences on the behavior of mice can be obscured by laboratory rearing [14]. Work in progress in our laboratories indicates that a similar mechanism may explain the failure to find any effects due to prenatal Imipramine treatment in adult animals in the present series of experiments (Coyle and Singer, in press).

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