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Effects of Prenatal Δ^9-Tetrahydrocannabinol on the Development of Rat Offspring[1]

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BORGEN, L. A., W. M. DAVIS AND H. B. PACE. *Effects of prenatal Δ^9-tetrahydrocannabinol on the development of rat offspring.* PHARMAC. BIOCHEM. BEHAV. 1(2) 203–206, 1973.–Pregnant rats were injected SC with 10 mg/kg Δ^9-tetrahydrocannabinol (Δ^9-THC) or vehicle solution on Days 10–12 of gestation. The course of pregnancy and parturition was unaffected by this drug treatment. The litter size, sex ratio, average birth weight and external appearance of the progeny did not differ from normal. Twenty-four male pups were selected from each of 6 Δ^9-THC litters and 7 control litters for observations of physical maturation and for testing of reflexive and exploratory behavior development from birth to weaning. Cross fostered controls were employed. Offspring of Δ^9-THC treated females showed delayed incisor eruption and retarded development of cliff avoidance and visual placing reflexes. Delta^9-THC progeny were significantly hyperactive in an open field arena at 9 days of age. Decrements in rearing and grooming behavior were found at 13 and 17 days of age. Differences in open field exploration had disappeared by weaning age. Although no differences in body weight were present at birth, Δ^9-THC exposed pups showed retarded growth from the fourth day through weaning. The failure of cross fostering procedures to reduce any of these effects indicates a direct, prenatal drug action on the developing fetus.

Δ^9-Tetrahydrocannabinol Marihuana Prenatal drug administration Reflex development Open field behavior Neonatal physical development

A SERIOUS concern arising from the current widespread use of marihuana is the possibility of its having adverse effects on fetal or postnatal development of offspring if used during pregnancy. Animal experimentation in this regard thus far has produced somewhat conflicting results. Initial investigations with crude *Cannabis* preparations gave evidence of both congenital malformations and fetal death in laboratory animals [6, 7, 15, 16]. In later studies from our laboratories, using pure Δ^9-Tetrahydrocannabinol (Δ^9-THC, the primary psychoactive constituent of marihuana), no congenital defects were observed following doses of 0.01–200 mg/kg of Δ^9-THC given throughout gestation [2]. Fetal and neonatal mortality as well as signs of maternal toxicity (weight loss, organ weight changes) were present following higher dosages of Δ^9-THC. Additional studies using a standardized marihuana extract (marihuana extract distillate, MED, containing 17.1% Δ^9-THC) likewise have failed to produce evidence of frank teratogenesis [14]. Prenatal and postnatal lethality were again observed with higher doses of the MED. Other workers have reported similar effects in mice following 200 mg/kg Δ^9-THC during Days 8–13 of pregnancy [8].

To date, however, no data have been presented to indicate whether the physical or behavioral development of the offspring are affected by prenatal exposure to *Cannabis* compounds at lower dosages corresponding more nearly to possible levels of human exposure. The present experiment was designed to study the physical maturation and the development of reflexive and exploratory behaviors in neonatal rats following prenatal exposure to a subfetocidal dose of Δ^9-THC during a portion of major organogenesis. The experimental approach used was patterned after that described by Smart and Dobbing [7] in studying effects of maternal undernutrition on offspring behavior and development.

METHOD

Drug Conditions

Synthetic Δ^9-tetrahydrocannabinol supplied by the National Institute of Mental Health was suspended with polyvinylpyrolidone (PVP) in physiological saline according to the procedure of Fenimore and Loy [5]. At a concentration of 10 mg/ml, the Δ^9-THC was injected in a volume of 1.0 ml/kg body weight for a dosage of 10 mg/kg. A 100 mg/ml solution of PVP in 0.9% saline served as the vehicle control. Injections were given subcutaneously at the back of the neck.

Animals

Experimentally naive, female, Wistar rats (200–240 g) which had previously produced a normal litter were used.

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Groups of three females were caged with a known fertile male which was removed after all 3 females were mated. Copulation was ascertained by daily vaginal smears, the presence of sperm being designated as Day 0 of gestation. On Days 10, 11 and 12, which is the period of organogenesis in the normal 21--22 day gestation period, half of the gravid females were injected with 10 mg/kg of Δ^9-THC, while the other half received only the drug vehicle on the same days. On Day 20 of pregnancy, the females were placed in individual littering boxes with appropriate bedding material and allowed to deliver undisturbed. Rat chow and water were continuously available. Animals were housed in air-conditioned quarters with an automatically timed cycle of 14 hr light and 10 hr darkness.

Within 8 hr after parturition, the pups were individually weighed and examined as to sex and physical abnormalities. The day of birth was referred to as postnatal Day 0. Litters were reduced to a total of 8 pups so as to include a preponderance of males, up to a maximum of six when possible. To control for possible postpartum differences in maternal behavior, offspring of half the Δ^9-THC treated females were cross fostered to a control female while half the control litters were fostered by Δ^9-THC exposed females. On the day following parturition, 3 or 4 pups were arbitrarily selected as test animals from the litters of each of 6 Δ^9-THC treated females and 7 control females. After reflex testing on Day 1, each pup tested was marked for identification by snipping a single digit from one forepaw. Thus, 48 male progeny in total were selected for daily examination and testing from birth to weaning at 21 days of age.

Test Procedures

The selected male offspring were weighed daily and inspected for maturation of three physical features: incisor eruption (appearance of the upper incisors, which always preceded the lower), eye opening (any visible break in the membrane of either eye) and ear unfolding (complete unfolding of both pinnae).

Testing of the ontogenesis of five reflexes was done in the manner described by Smart and Dobbing [17] except that responses were scored quantally, not graded. All testing was done on surfaces covered with cheesecloth. A brief summary of the eliciting stimuli and the positive response for each reflex is presented in Table 1.

Exploratory behavior was observed in an open field arena when the pups were each 9, 13, 17 and 21 days of age. The arena was constructed of plywood with interior surfaces painted in black enamel. Having floor dimensions of 90x90 cm with a 15 cm wall, the arena was demarcated by dark blue lines into 81 squares, each 10 cm square. A 20 W fluorescent light was suspended 45 cm above the field and a masking white noise (70 db) was present. Following reflex testing on test days, each test pup was placed in the center square of the arena, and the following parameters were recorded during a 2 min observation period: latency to leave the center square, latency to reach the peripheral wall, number of squares crossed, number of rearings, and the time spent in grooming behavior. The frequency of defecation or urination was found to be too low in animals this young to be a usable measure of emotionality. The field was sponged clean and dried before each test period to minimize variability in olfactory cues.

RESULTS

The reproductive success of female rats following Δ^9-THC treatment or vehicle injection is shown in Table 2. In agreement with our previous studies, 10 mg/kg of Δ^9-THC injected SC on Days 10--12 of gestation did not produce observable morphological defects in offspring or increase the frequency of stillbirths. Neither the average litter size nor the mean birth weight were altered. Length of gestation was also unaffected. Thus, by all major overt criteria, the pregnancy and progeny were normal following this dosage and duration of prenatal Δ^9-THC administration.

Initial statistical analysis of the data revealed no significant difference between the normal fostered and cross

TABLE 1

SUMMARY OF REFLEX TESTS

Reflex	Eliciting stimuli	Positive response*
Righting	Rat placed on back on flat surface	Turns over with all legs free from under body
Freefall righting	Rat dropped, upside down from 30 cm unto cotton pad	Lands on all four feet
Negative geotaxis	Rat placed head down on a 20° slope	Turns at least 135° to face up the slope
Cliff avoidance	Rat placed on edge of bench, with nose and forefeet just over edge	Withdraws head and both forefeet from edge
Visual placing	Rat held by tail, head 2--3 cm from edge of bench	Lifts head and extends forelegs toward the bench

*A maximum time limit of 60 sec allowed for responding.

TABLE 2

REPRODUCTIVE SUCCESS FOLLOWING PRENATAL ADMINISTRATION OF Δ^9-THC SUBCUTANEOUSLY ON DAYS 10 TO 12 OF GESTATION

Drug Treatment	Litter N	Pups (Male: female) Born Alive	Pups Born Dead	Pups Malformed	Average Litter Size	Average Birth Weight (g)
Control (PVP)	7	76 (40:36)	0	0	10.8	6.53
Δ^9-THC (10 mg/kg)	6	62 (34:28)	0	0	10.3	6.42

fostered subgroups for either Δ^9-THC or control progeny. Therefore, the data for the fostering subgroups were pooled, and a final analysis between Δ^9-THC exposed and vehicle control pups was done using the two-tailed *t*-test for independent samples [4].

The average ages for maturation of the reflexes and the physical features are presented jointly in Table 3. As indicated, the development of both cliff avoidance and visual placing responses was significantly delayed in the Δ^9-THC offspring. Additionally, tooth eruption was retarded for the cannabinoid-treated group. Other reflexes and physical features were not significantly affected.

TABLE 3

MATURATION OF REFLEXES AND PHYSICAL FEATURES OF RAT OFFSPRING FOLLOWING PRENATAL Δ^9-THC ADMINISTRATION

		Prenatal Drug Treatment	
		Control	Δ^9-THC
Age at Reflex Ontogeny (days)	Righting reflex	1.0 ± 0.0*	1.0 ± 0.0
	Negative geotaxis	2.4 ± 0.2	2.4 ± 0.3
	Cliff avoidance	2.9 ± 0.3	3.9 ± 0.2†
	Freefall righting	14.7 ± 0.2	14.7 ± 0.3
	Visual placing	16.9 ± 0.2	17.6 ± 0.3†
Age at Maturation (days)	Incisor eruption	8.4 ± 0.2	9.8 ± 0.3†
	Eyes open	14.5 ± 0.2	14.8 ± 0.2
	Ears unfolded	15.9 ± 0.3	15.7 ± 0.2

*Mean ± S.E. (N = 24/group)
†$p<0.05$

Results of the four measurements of exploratory behavior for the 48 pups tested are shown in Table 4. In their initial exposure to the test arena at 9 days of age, the progeny of Δ^9-THC injected females were markedly hyperactive, showing a shorter latency to leave the starting square, a shorter latency to reach the periphery, and an increase in the number of squares traversed. In testing at 13 days of age, the Δ^9-THC exposed offspring showed a marked reduction in the incidence of rearing and grooming behavior and an increase in the time to reach the peripheral wall. The frequency of rearing continued to be reduced at the 17-day observation. By weaning age of 21 days all five open field measures of Δ^9-THC pups were indistinguishable from those of the vehicle control pups.

TABLE 4

EFFECTS OF PRENATAL Δ^9-THC ON THE OPEN FIELD BEHAVIOR OF RAT OFFSPRING

		Prenatal Drug Treatment	
		Control	Δ^9-THC
Age 9 days	Leave center (sec)	24.1 ± 1.9*	17.1 ± 2.4†
	Reach periphery (sec)	61.7 ± 7.0	39.0 ± 8.6†
	Areas entered	9.5 ± 0.8	13.9 ± 1.0†
	Rearings	0.3 ± 0.1	0
	Grooming (sec)	0	0
Age 13 days	Leave center (sec)	12.4 ± 1.0	11.7 ± 1.1
	Reach periphery (sec)	33.1 ± 2.8	50.1 ± 4.6*
	Areas entered	27.8 ± 2.2	32.3 ± 2.8
	Rearings	1.7 ± 0.3	0.8 ± 0.2*
	Grooming (sec)	2.3 ± 0.3	0.7 ± 0.2*
Age 17 days	Leave center (sec)	8.5 ± 0.5	7.6 ± 0.7
	Reach periphery (sec)	15.6 ± 2.9	16.4 ± 1.4
	Areas entered	61.6 ± 7.3	70.5 ± 6.0
	Rearing	5.0 ± 0.5	2.5 ± 0.4*
	Grooming (sec)	4.0 ± 0.5	3.9 ± 0.6
Age 21 days	Leave center (sec)	6.1 ± 0.5	5.2 ± 0.6
	Reach periphery (sec)	11.7 ± 1.0	9.9 ± 0.7
	Areas entered	67.8 ± 8.6	61.0 ± 7.7
	Rearing	3.5 ± 0.5	3.1 ± 0.3
	Grooming (sec)	7.9 ± 0.8	9.8 ± 1.4

*Mean ± S.E. (N = 24/group)
*$p<0.05$

Although there was no difference in birth weight between Δ^9-THC and control progeny, a significant retardation in growth was evident during the neonatal period. The mean body weight of prenatal drug treated offspring was significantly below that of control pups from the fourth day postpartum through weaning (Table 5). As with the other parameters, the crossfostering procedure did not alter this growth depressant effect.

DISCUSSION

Relatively few experiments have studied the effects of prenatal drug administration upon the behavior of the developing offspring. Psychopharmacological agents such as chlorpromazine, meprobamate, and morphine have been

TABLE 5

EFFECTS OF PRENATAL Δ^9-THC ON THE GROWTH OF MALE NEONATAL RATS

Age (days)	Body Weight (g) Control	Δ^9-THC
0	6.5 ± 0.1*	6.4 ± 0.1
1	7.9 ± 0.2	7.7 ± 0.1
4	13.0 ± 0.3	11.5 ± 0.2†
7	18.0 ± 0.4	16.3 ± 0.4†
10	24.8 ± 0.5	22.0 ± 0.4†
13	32.4 ± 0.7	29.3 ± 0.6†
17	41.2 ± 0.7	37.9 ± 0.6†
21	58.6 ± 1.1	52.5 ± 1.0†

*Mean ± S.E. (N = 24/group)
†$p<0.05$

found to modify various behaviors of progeny following administration to the pregnant female [3, 9, 10, 12, 13, 18, 19, 20]. In the open field arena chlorpromazine and meprobamate exposed offspring showed decrements in locomotor activity [10,18]. Morphine progeny, on the other hand, were found to be hyperactive in the same test [3]. Performance in learning situations was also adversely affected [9,20]. It should be noted that in each of these studies there was evidence of overt fetal or neonatal toxicity (e.g., reduced birth weight, decreased litter size, high postpartum mortality). Also, the cross fostering control procedure was used in only one experiment [13]. Therefore, a possible contribution of postnatal maternal influences to the production of behavioral changes in offspring of drug treated females has not been eliminated for most studies of this sort.

The data presented demonstrate that prenatal exposure to Δ^9-THC, the major psychoactive substance in marihuana, can produce changes in the physical and behavioral development of neonatal rats at a dose level which neither kills or stunts the fetus, nor causes any observable change in the morphology of the newborn. The differences observed between control and Δ^9-THC offspring were not ameliorated by cross fostering. Thus, it appears that the alterations in physical, reflexive and exploratory development observed in the present test situation were related to the prenatal drug treatment and cannot be attributed to postnatal influences such as poor maternal care or inadequate lactation. That such a direct drug action on the fetus is possible is supported by findings of placental transfer of Δ^9-THC or its metabolites in rodents [8, 11, 14]. There is at present little or no significant basis for speculation on the mechanism by which such an action is exerted.

It has been shown repeatedly that the drug metabolizing capacity of the young organism is much inferior to that of the mature adult. Also, the rate of metabolism and elimination of Δ^9-THC in the adult rat may under some conditions be quite prolonged, with one-half of an intravenous dose remaining in the body after one week [1]. Thus, in the present study, it is conceivable that significant blood levels of Δ^9-THC could have been present in the offspring at birth, 10 days after the last drug injection. If so, the significant differences in the drug offspring could have resulted from a direct action of Δ^9-THC. Such a possibility cannot be excluded pending a direct experimental test.

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