PEER AND MATERNAL SOCIAL INTERACTION PATTERNS IN OFFSPRING OF RHESUS MONKEYS TREATED CHRONICALLY WITH DELTA-9-TETRAHYDROCANNABINOL*

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I. INTRODUCTION

Behavioral teratology studies assess the impact of developmental treatments on subsequent behavioral competence (1-3). The goal of such studies is to identify a detrimental effect on functional maturation of the nervous system and ultimately to define the mechanism by which this effect occurs.

Primate models in behavioral teratology are particularly valuable for identifying toxicity in adaptive systems relevant to humans, such as complex intellectual and social skills. These skills impact heavily on behavioral symptom complexes for which children are referred to physicians (developmental delay, learning disability, attention deficit disorder). The present study used a primate model (the rhesus monkey, Macaca mulatta) to assess the influence of chronic, low-level maternal delta-9-tetrahydrocannabinol (THC) treatment on early social behavior. In general, social behavior toward mother and peers was found to be adequate and within the range of the untreated control group. Some differences between THC offspring and untreated controls were identified in mother-infant interactions of late infancy and in the initial responseto entry into a social group with unfamiliar peers.

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II. METHODS

A. Subjects

Five offspring (3 male and 2 female) of THC-treated rhesus dams (Macaca mulatta) were compared to nine controls (3 male, 6 female). Infants were born during two successive annual breeding seasons (See Table I for pregnancy outcome data). All dams were 5-8 years old and had been maintained according to the same experimental protocol during gestation and lactation and for at least two years prior to the study.

B. Housing and Maintenance

Dams and infants were maintained in individual indoor cages in the same cageroom. Care and maintenance met the guidelines of the Federal Animal Welfare Act and the Institute of Laboratory Animal Resources. Details of breeding procedures have been described (4). At 3.5 mos of age, infants were weaned and separated from the dam and began a behavioral test series (5). At 6 mos of age, infants were formed into small living groups of three or four animals each with one THC-treated animal per group.

C. Drug Treatment

A daily dose of 2.4 mg/kg THC was administered orally in a preferred food item. Alcohol solutions of THC (200 mg/ml)

TABLE I. Pregnancy Outcome in THC-treated Female Monkeys and Non-treated Controls in Two Successive Breeding Seasons*

	Non-treated		THC-treated	
	Year 1	Year 2	Year 1	Year 2
Liveborn, accepted by dam	5	5	3	2
Liveborn, nursery reared	1	2	0	1
Fetal death	1	0	1	1
Abortion	0	0	0	1

*Liveborn infants accepted by the dam served as subjects in the present experiment.

were obtained from the National Institute on Drug Abuse and injectedin to the food item. Independent studies have indicated that peak plasma concentrations of 200 to 300 ng/ml THC are reached within one to three hours of administration using this administration technique. Daily drug treatment began at least two years prior to conception and continued throughout gestation and lactation. Control dams received an undrugged food item according to the same schedule.

D. Behavioral Measures

Mother-infant behavior measures were obtained from five 20-min videotape sessions recorded during early infancy (10-20 days) and late infancy ((90-100 days of age). Tapes were scored for the duration of four arousal activity states and for the quality of mother-infant interaction (Table II) by trained observers who were unaware of the group assignments of the animals (see 4).

Peer interaction was recorded using a continuous, focal animal sampling technique with a 10-min daily sampling period per animal. For each social interaction, initiator, behavior and response were recorded (6).

E. Plasma Cortisol

Blood samples (0.5 ml) were obtained from the cephalic or femoral vein of the conscious, restrained animal within three minutes of capture. Samples were centrifuged immediately and plasma removed and frozen for subsequent determination of plasma cortisol using a protein binding assay (7).

III. RESULTS AND DISCUSSION

Various measures of maternal and infant behavior (Table II) were initially examined with analysis of variance using maternal drug treatment and sex of infant as independent variables. Measures reflecting a possible influence of the drug treatment variable ($(p < 0.10)$ were then examined in a multiple regression that included twelve other predictor variables relevant to perinatal influences on behavior. This procedure helps prevent false identification of the drug effect via confounding of drug with other factors affecting mother-infant behavior.

Measures of activity and arousal were not apparently affected by drug treatment. This is in agreement with other

TABLE II. Variables Considered in Regression Analysis

Major Independent Variables

- Drug treatment of mother
- Sex of infant

Background Variables

- Age of mother
- Social experience of mother
- Mother bred in indoor or outdoor caging
- Weight of mother prior to conception
- Maximum weight gain of mother during pregnancy
- Parity of mother
- Infants raised previously by mother
- Infants rejected previously by mother
- Year of birth
- Month of birth
- Birth weight of infant
- Weaning weight of infant

Dependent Variables (behavioral measures)

1. Duration of activity/arousal states
   a. Percentof total observation time of activity/arousal states
2. Frequency of occurrence of nonsocial behaviors
   a. Frequency of initiation
      1. social
      2. explore environment
      3. locomotion
      4. disturbance
3. Quality of mother-infant interaction
   a. Frequency of social categories
      1. minutes infant restrained by mother
      2. positive proximity behaviors by infant
      3. negative proximity behaviors by infant
      4. positive proximity behaviors by mother
      5. negative proximity behaviors by mother
   b. Relative proportion of all categories scored
      1. positive behaviors by infant
      2. positive behaviors by mother
   c. Totalnumberof social behaviorsrecordedin mother-infant pair
   d. Maternal rejection score

data suggesting that full behavioral tolerance to arousal-altering effects of this dosage level had developed in the chronically treated animals.

Several measures reflecting changes in the quality of mother and infant interaction from early to late infancy were associated with the drug treatment variable (Table III). Negative proximity behaviors, including primarily rejection of contact with the infant, increased more from early to late infancy in the THC-treated dams. At the same time, THC-infants increased the frequency of their attempts to gain contact with the dam. Such interaction patterns normally signal the onset of increased separation of mother and infant (8,9) and apparently were more intense, or earlier in appearance in THC-treated group. It should be noted that instances of neglect or physical abuse of infants were not recorded in either group. What is the potential significance of these alterations in mother-infant interaction in late infancy? Table IV demonstrates the intercorrelation in our data set

TABLE III. Behavioral Measures Associated with Maternal Drug Treatment[a]

	Predictor Variable	F	df	P
Decrease in maternal positive proximity behaviors (%)[b]	Maternal drug	5.01	1,12	< 0.05
	Weaning weight	6.96	1,11	< 0.025
Increase in infant positive proximity behaviors (%)[c]	Maternal drug	5.49	1,12	< 0.05
	Prior infants rejected	9.54	1,11	< 0.025
Increase in maternal negative proximity behaviors (%)[d]	Maternal drug	6.65	1,12	< 0.025
	Parity of mother	17.58	1,7	< 0.005
	Infant's birth weight	9.31	1,5	< 0.05

[a]"Maternal drug" entered the stepwise multiple regression first and accounted for a significant amount of variability in these measures. Other predictor variables with significant partial regression coefficients are also listed.

[b]Includes approach, retrieval, groom, restrain. Change from early to late infancy was measured.

[c]Includes approach, contact initiation, groom, play.

[d]Includes break contact, reject contact, punish.

between behavioral measures affected by drug and other measures reflecting mother-infant independence, such as amount of time spent by infant away from mother. These variables, as might be expected, increase from early to late infancy (Fig. 1). More detailed information about the growth of infant independence have been provided by Jensen and Bobbitt (8). Typically there is a dramatic increase in the level of infants physical activity while with the dam, followed by an increase in negative proximity behaviors by the dam and finally by an increase in physical separation of mother and infant. Apparently, in the present experiment, THC treatment influenced this transition between early dependence and later independence. It is not apparent whether the drug acted primarily on the dam or the infant in intensifying these transition behavior patterns.

Further information about mother-infant relationship was obtained by examining the response to separation. Plasma cortisol response to short-term separation from mother and to

TABLE IV. Product-moment Correlations Between Drug-related Variables and Variables Reflecting Strength of Mother-Infant Bond[a]

	Drug-related			Mother-infant Bond		
	1	2	3	4	5	6
1. Dam's negative behavior	-	.85[e]	.58[c]	.48	.88[e]	.66[d]
2. Dam's negative behavior (%)	-	-	.83[e]	.67[d]	.65[c]	.88[e]
3. Infant positive behavior (%)	-	-	-	.59[c]	.41	.84[e]
4. Infant's time away from mother	-	-	-	-	.34	.84[e]
5. Dam's behavior directed at infant	-	-	-	-	-	.42
6. Dam's "rejection score"[b]	-	-	-	-	-	-

[a]Based on 15 mother-infant pairs.
[b]Based on Hinde & Atkinson (2).
[c]$p < 0.05$
[d]$p < 0.01$
[e]$p < 0.001$

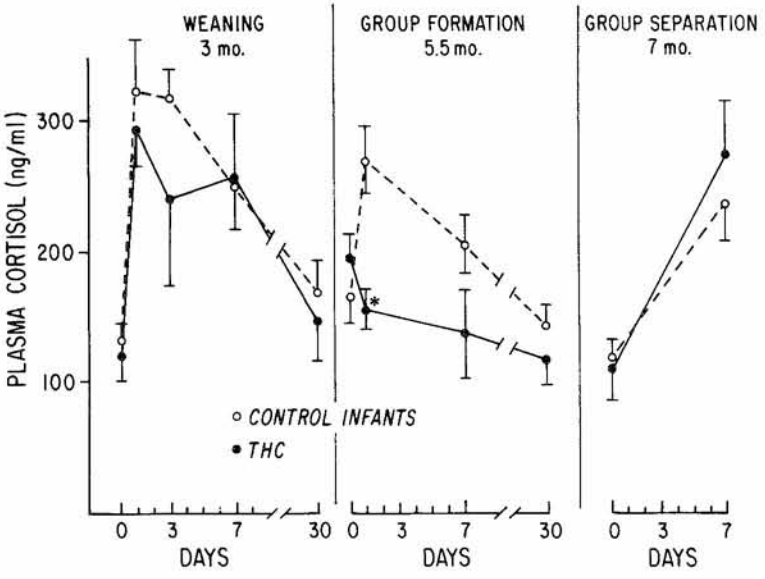

FIGURE 1. Changes in mother-infant behavior patterns from early to late infancy. Significant group differences are illustrated in Panel A. Related measures of activity level and mother-infant dependence are shown in Panels B and C.

weaning (Fig. 2) did not differ in THC and control offspring. This measure of response to separation is frequently used to assess the degree of mother-infant attachment in primates (10,11). Similarly, measures of heart rate, activity and vocalizations during a short term separation were similar in the two groups. This indicates that a normal degree of attachment had formed in the THC-treated mother-infant pairs. Group differences were apparent in the plasma cortisol response of THC infants to peer grouping (Fig. 2). While controls showed the expected increase in cortisol levels in response to this situation (12), THC infants maintained baseline values. Behavioral data supported a relative lack of stress-induced behavioral inhibition in THC offspring. Although the degree and type of social interaction varied widely from group to group, THC offspring consistently initiated more social interactions than any of their cagemates during the first three days of social grouping (Sign test, $p < 0.003$). Subsequently, initiation of social interaction was not associated with prior drug exposure, and plasma cortisol responses to later separation from the peer group were similar in the THC-treated and non-treated animals.

Taken together, these observations indicate that chronic low-level THC affects behavioral development on a relatively subtle level under controlled experimental conditions. This is consistent with our other evaluations of this sample of infants (4,6,13,14) and with the limited data available from other nonhuman primates (15) and from human infants (16). Similarly, behavioral evaluations of rats and mice exposed developmentally to THC and other cannabis preparations have not shown a clear cut pattern of severe developmental toxicity in terms of growth retardation, developmental delay or cognitive disorder (see review by Abel, 17). Social behavior was included in only one such evaluation. In an experiment using

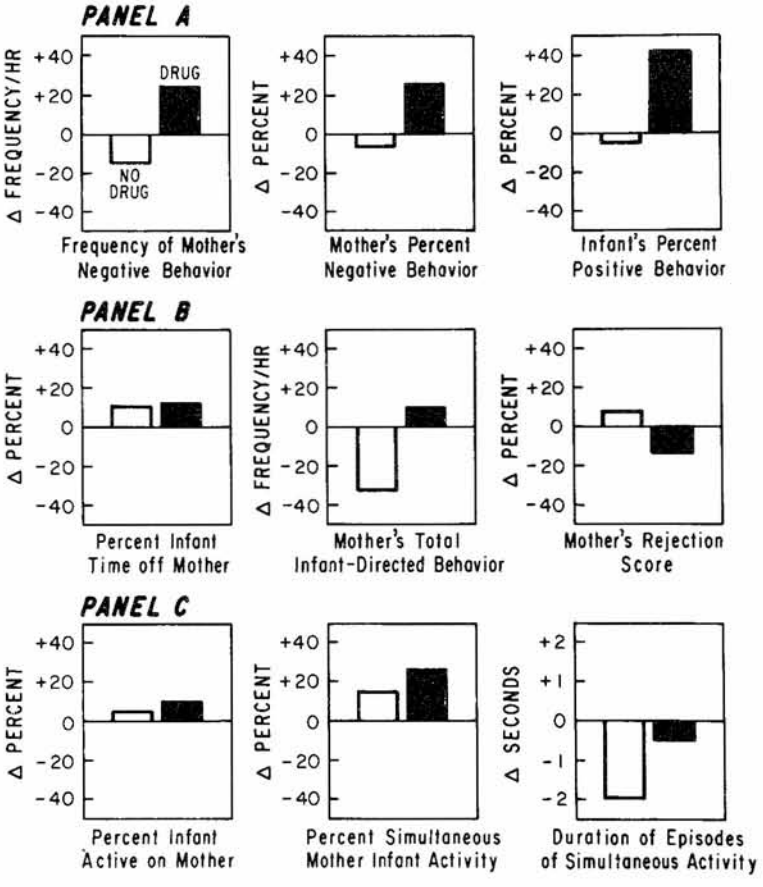

FIGURE 2. Plasma cortisol response of THC and control offspring to weaning (separation from dam), peer group formation and separation from peer group.

chronic, low-level treatments (2 mg/kg/day) throughout gestation, Vardaris et al. (18) demonstrated an increase in the number of treated rats gaining control of limited space in competition with another animal. This finding seems consistent with an interpretation of relative lack of inhibition in response to negative social cues in THC-treated monkey infants.

Certain precautions are appropriate in interpreting an experiment such as the one presented here. Behavioral changes in THC-treated infants are not necessarily attributable to an interaction between THC and developing neural tissues; indirect drug effects can occur via maternal behavior, maternal nutrition and physiology or environmental conditions created by the presence of the drugged dam. More complex designs involving fostering and artificial rearing would be necessary to establish these points.

Also, in a small sample experiment with a genetically heterogeneous population a chance confounding of drug treatment with some other relevant variable can occur. Careful subject selection and group assignment and detailed environmental control protocols are helpful in safeguarding against this possibility. In addition, the screening of identified drug effect with multiple regression, as illustrated above, is also useful.

With these precautions in mind, primate studies offer the exciting opportunity of examining a perinatal system very similar to humans in a controlled experimental setting. This is particularly important in the case of an agent, which like THC, cannot be evaluated for developmental toxicity in humans using a prospective design with random group assignment.

In conclusion, the present data, together with other studies, fails to support the hypothesis that severe morphological or functional disorders are likely to be identified after chronic low level developmental exposure to THC. However, limited behavioral changes may be detected under these circumstances.

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