

## Effects of Neonatal Testicular Suppression with a GnRH Antagonist on Social Behavior in Group-Living Juvenile Rhesus Monkeys

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Twenty-four male and eight female 1-year-old rhesus monkeys (*Mucaca mulatta*) were observed for social interaction with other yearlings and with their mothers. The males comprised three groups which differed in the level of neonatal androgen exposure. One group received the GnRH antagonist Antide during their first four neonatal months (*Antide*  $n = 8$ ), which suppressed pituitary LH secretion resulting in peak neonatal T levels  $<0.7$  nmol/liter. A second group received Antide treatment combined with a long-lasting testosterone replacement (*Ant/And*  $n = 8$ ), which resulted in peak neonatal T levels of  $29.1 \pm 3.8$  nmol/liter. The third group (*Vehicle*  $n = 8$ ) received the Antide and androgen vehicles and had intermediate peak T levels of  $5.2 \pm 1.0$  nmol/liter. Behavior of males was compared to that of unmanipulated control females living in the same social group (*Control Female*  $n = 8$ ) when androgen levels were uniformly low ( $<0.7$  nmol/liter) in all male groups. Subjects received 12 weekly 30-min focal observations by an observer blind to the neonatal treatments. Marked sex differences were found in several categories of sociosexual behavior. All three groups of males engaged in significantly more sexual and play behavior than females, with the exception of quiet solitary play, which females exhibited significantly more frequently. In addition, females exhibited significantly more interest in infants than did any male group. There were no differences between groups in agonistic behavior or time spent in contact with other individuals, but females spent significantly more time than any male group in proximity to other animals. Both females and *Antide* males initiated proximity and followed animals significantly more frequently than *Ant/And* males, but not *Vehicle* males. Proximity durations with mothers initiated and terminated by yearlings were longer for females than for any male group and for *Antide* males than for *Ant/And* males. *Antide* males were groomed significantly longer than any other group. These results demonstrate effects of neonatal testosterone exposure on social behavior in yearling rhesus. Suppression of neonatal T did not affect sexually dimorphic patterns of play and sexual behavior, but altered the character of interactions with their mothers. Whether this reflects a delay in the development of maternal independence or a fundamental alteration in patterns of social interaction remains to be resolved. © 1995 Academic Press, Inc.

Sex differences in juvenile macaque behavior are well established in both experimentally created laboratory groups (rhesus: Harlow and Harlow, 1965; Goy, 1978; Goy, Wallin, and Goldfoot, 1974) and in stable captive and free-

ranging groups (rhesus: Berman, 1982; Lovejoy and Wallen, 1988; stumptail, *Macaca arctoides*: Niewenhuijsen, Slob, and van den Werff ten Bosch, 1988; Japanese macaque, *Macaca fuscata*: Hanby and Brown, 1974; Eaton, Johnson, Glick, and Worlein, 1985; Glick, Eaton, Johnson, and Worlein, 1986; Nakamichi, 1989; bonnet, *Macaca radiata*: Handen and Rodman, 1980). Studies of rhesus infant development which compared a variety of rearing environments, demonstrated that some behavioral sex differences varied with the social environment. For example, sex differences in agonistic behaviors such as threatening and withdrawal were found when males and females had a very limited time for social interaction, or when adults were not present during development (Harlow and Harlow, 1965; Wallen, Goldfoot, and Goy, 1981). In contrast, these agonistic behaviors were not sexually dimorphic when rhesus were studied in groups of five to six infants and mothers (Goy and Wallen, 1979) or in large long-term heterosexual age-graded captive groups (Lovejoy and Wallen, 1988). However, the effect of social context did not affect the expression of all sexually dimorphic behavior since in all study environments males and females consistently differed in patterns of play and in the occurrence of mounting behavior, with males displaying high energy-expenditure play and mounting more frequently than females (Harlow and Harlow, 1965; Goy, 1978; Goy and Wallen, 1979; Lovejoy and Wallen, 1988). Thus these patterns of behavior are reliable developmental indicators of behavioral masculinization in rhesus monkeys.

Play and mounting behavior in genetic females can be influenced by exogenous prenatal androgen exposure (Goy and McEwen, 1980). Administration of exogenous testosterone (T) to pregnant females carrying a female fetus from prenatal Days 40 through 110 or longer produced substantial virilization of the female offspring's genitalia and resulted in masculinization of juvenile behavior as well (Goy, Uno, and Sholl, 1989). However, the behavioral masculinization was unlikely to be a consequence of genital masculinization as exposing the developing female fetus to androgen for 25 days during the last third of gestation did not alter the female genitalia, but did result in the display of masculine levels of play and mounting (Goy, Bercovitch, and McBair, 1988). Thus, there is a period when the developing female brain is sensitive to the organizing actions of prenatal androgen occurring after genital differentiation is completed. Whether this sensitivity in the female reflects the normal timing of masculinization in the male is unknown, but raises the possibility that the action of androgens on the developing nervous system may extend for a substantially longer period than is commonly believed. It is known that fetal males secrete androgen during the period when the behavior of genetic females can be masculinized by exogenous androgen (Resko, 1985). Thus, it is likely that similar mechanisms may pertain in males and females.

In addition to the prenatal secretion of androgens, rhesus males, like human males (Forrest, 1990), are exposed to elevated androgens produced by their testes immediately after birth and for the first 2–3 months postnatally (Fraw-

ley and Neill, 1979; Mann *et al.*, 1984). Blockade of this period of neonatal secretion in rhesus with a GnRH agonist delayed, but did not prevent the onset of puberty (Mann, Gould, Collins, and Wallen, 1989). Furthermore, suppression of neonatal testicular function was associated with alterations in CNS centers regulating gonadotropin secretion (Mann *et al.*, 1993), and with lower sex drive after puberty (Eisler, Tannenbaum, Mann, and Wallen, 1993). However, there is little evidence that lowering neonatal androgen alters juvenile behavior (Mann, Gould, and Wallen, 1992). This may reflect the fact that neonatal androgen secretion has little influence on the development of gender-specific behaviors. Alternatively, it may be because the GnRH agonist used to suppress neonatal testicular secretion was not administered until 10–14 days postnatally (Mann *et al.*, 1989). Thus, although GnRH agonist treatment suppressed a major portion of neonatal androgen secretion, it did not block the first two postnatal weeks making the failure to detect changes in juvenile behavior more difficult to interpret.

We have recently used a long-lasting GnRH antagonist (Antide) to suppress neonatal androgen secretion in male rhesus monkeys (Mann *et al.*, 1994). Due to this compound's long duration of action when injected, treatments could be started during the first three postnatal days producing immediate suppression of LH secretion. Thus males treated neonatally with this compound were exposed to, at most, a single day of elevated T during their first 6 months of life. In addition to males whose neonatal testosterone was suppressed, we created a replacement T group which received both Antide and a long-lasting testosterone preparation, CDB. Due to the nature of this compound, these males actually received supranormal levels of T (Mann *et al.*, 1994). Thus within this study group we had males with suppressed neonatal T, elevated neonatal T, and normal neonatal T, in addition to females, who also have low levels of neonatal T. We report here sex differences in sexual, play, and affiliative behavior and provide evidence that neonatal T influences the nature of the interactions between rhesus males and their mothers during the second year of life when concurrent T levels were uniformly low in all groups.

## METHODS

### *Subjects and Housing*

Subjects were 24 yearling male and 8 yearling female rhesus macaques belonging to two long-term captive social groups housed in 25 × 25-m outdoor compounds with attached indoor quarters at the Field Station of the Yerkes Regional Primate Research Center in Lawrenceville, Georgia. Each social group was composed of 3–5 adult males and 30–35 adult females with their juvenile and infant offspring. All juvenile subjects were born in their respective group and had been living with their mothers since birth. Group composition remained constant during the study period. The monkeys were fed between 0700 and 0800 h with monkey chow, and again at 1500 h with fresh fruit or vegetables. Water was continuously available.

### *Neonatal Treatment*

At birth, male infants were randomly assigned to one of three treatment conditions. The hormonal preparations employed, the timing of treatments, dosages, and protocols have been previously described (Mann *et al.*, 1994). Briefly, 8 animals were treated with the GnRH antagonist (Ac-D-2-Nal<sup>1</sup>-D-4-Cl-Phe<sup>2</sup>-D-3-Pal<sup>3</sup>-Nic-Lys<sup>5</sup>-D-Nic-Lys<sup>6</sup>-I-Lys<sup>6</sup>-D-Ala<sup>10</sup>-GnRH; *Antide* group), 8 with Antide plus a long-acting testosterone preparation (testosterone-trans-4-*n* butylcyclohexancarboxylate, CDB; *Ant/And* group), and 8 with the Antide and CDB vehicle preparations from birth (Days 1–3) until 4 months of age (*Vehicle* group). Eight females were used as controls for behavioral observations and received no neonatal manipulations (*Control Female* group). In male subjects, serum LH and T concentrations were monitored on a weekly basis during the 4-month treatment period and monthly through the first year of life. Antide effectively blocked neonatal activation of the pituitary–testicular axis in the present subjects (Mann *et al.*, 1994) with peak serum T levels during Antide treatment of  $<0.7$  nmol/liter compared to  $5.2 \pm 1.0$  nmol/liter in *Vehicle* males. Peak serum T levels were  $29.1 + 3.8$  nmol/liter in *Ant/And* animals. By the 6th month of age, serum T levels in all groups of males had fallen to near limits of detection (Mann *et al.*, 1994) where they remained until the age of 2.5 years.

### *Procedure*

Behavioral data were collected from April to September with observations for each subject started when the subject reached one year of age. Each subject was focally observed for 30 min weekly for 12 weeks. A total of 192 h of observation (6 h for each subject) were collected. Observation sessions were randomly distributed between 0800 and 1900 hr. During an observation period all animals were locked out of the indoor housing area to provide constant visual access. All data were collected by the same observer using a portable computer (Radio Shack Model 102) programmed to allow the collection of true frequencies, durations and sequences of behavior. The observer was blind to the treatment conditions of the experimental animals.

At 19, 22, and 25 months of age, after the completion of the behavioral data collection, the males were anesthetized with ketamine to obtain measures of bone density, skeletal growth, and penile development. Penile length was obtained by gently stretching the penis of the anesthetized male and measuring its length from the tip of the glans penis to the opening of the foreskin.

### *Behavioral Categories*

The following behavioral categories were used:

*Contact.* The number of contacts made and broken by the focal subjects and the time spent in contact with other individuals, and in particular, with the subjects' mothers. A contact was scored when any approach resulted in body contact between two individuals except brief contact in passing.

*Proximity.* The number of approaches and leaves made by the focal subjects and the time spent in proximity to other individuals, and in particular, to the subjects' mothers. Approaches were defined as movements that brought the actor within arm's reach (approximately 60 cm for adults) of the recipient without making contact. Leaves were defined as movements that caused an increase in distance between two individuals to greater than arm's reach. Time in contact and proximity were analyzed in relation to who initiated and who terminated the contact. Thus it was possible to assess responsibility for maintaining contact and proximity.

*Following.* The number of times a focal subject was observed in persistent pursuit of another individual without aggression. This measure did not include chasing, but only walking after another individual.

*Grooming (Altmann, 1962).* The number and duration of grooming bouts between the focal subjects and other individuals. A 10-s pause in grooming ended a grooming bout.

*Agonistic behavior.* The number of episodes of aggression (*Threats*, Altmann, 1962; and *Chases*, Altmann, 1962), submissive gestures (*Fear Grimace*, Altmann, 1962), and spatial displacements (*Avoidance* or *Withdrawal* in response to approach) involving the focal subjects as actors or recipients.

*Play.* Four categories of play were distinguished: *Quiet Solitary* (Solitary play without a vigorous physical component, e.g., solitary hand play or picking up objects), *Brief-Contact* (Goy, Wallen, and Goldfoot, 1974), *Rough-and-Tumble* (Goy, Wallen, and Goldfoot, 1974), and *Chase play* (active chasing of another animal when not in an agonistic context). The number of play events initiated and received by the focal subjects in each category was recorded. The latter three categories of social play were differentiated from agonistic behavior when the animal clearly displayed the "play-face" (van Hooff, 1967).

*Sexual behavior.* The number of episodes of *Present* (Altmann, 1962) and *Hiptouch* interactions (Altmann, 1962), and three types of mounts initiated and received by the focal subjects were recorded: *No-Footclasp* (Wallen, Bielert, and Slimp, 1977), *Footclasp without Pelvic Thrusts* (Wallen, Bielert, and Slimp, 1977), and *Footclasp with Pelvic Thrusts* (Wallen, Bielert, and Slimp, 1977).

*Infant-directed behavior.* The number of times a focal subject was observed touching, carrying, or grooming an infant. During the period of behavioral observations infants would have ranged in age from newborn to 5 months of age.

### *Statistical Analysis*

Data are expressed as means per subject per 6 h of observation. Duration measures reflect the total time during the 6 h of observation that the selected behavior occurred. Differences between treatment groups were detected using a one-way factorial ANOVA followed by a *t* test for multiple means (TTFM) which uses the MS-error term from the ANOVA to correct for multiple

comparisons (Bruning and Kintz, 1977). All tests are two-tailed with a  $p \leq 0.05$  considered significant.

## RESULTS

Table 1 presents the data for all significant differences in sexual, play, and agonistic behavior and selected nonsignificant differences in behaviors often considered to vary between juvenile male and female monkeys. For 10 of the 12 patterns of play and sexual behavior, consistent sex differences were found between the control females and all three groups of males, but none were found for any of the measures of agonistic behavior. Though females differed from the males on many behavioral measures, there were no significant differences among the three groups of males. Males in each treatment condition initiated more hiptouches and mounts of all types than did females. There were, however, no differences among the four groups of subjects in presents displayed or in hiptouches or mounts received (data not shown). Males in all three groups initiated and received Rough-and-Tumble Play, and Chase Play significantly more frequently than did females. Although males in all groups initiated more Brief Contact Play than did females, only *Ant/And* males received more Brief Contact Play. Females engaged in Quiet Solitary Play significantly more frequently than did males. There were no sex differences in any agonistic behavior, either aggressive or submissive. Though only initiation of agonistic behavior by the yearlings are presented here, the findings are the same for receiving agonistic behavior, with no differences between the sexes or among the groups of males.

Table 2 presents data on the frequency and duration of various affiliative behaviors reflecting patterns of association between subjects and other group members. There were no differences among the four groups of subjects in any measure of physical contact between subjects and their mothers or other members of their social group.

In contrast, proximity measures differed significantly between the groups. Females and *Antide* males initiated proximity significantly more often than did *Ant/And* males, but not *Vehicle* males. Females differed significantly from *Ant/And* and *Vehicle* males, but not *Antide* males in the duration of the proximities they initiated. Similarly, females spent significantly more time than did males from any of the three treatment groups in proximity to other individuals and, in particular, to their mothers. *Antide* males spent significantly longer than *Ant/And*, but not *Vehicle* males in these two types of proximity.

Interaction with the mother characterizes the clearest difference in affiliative behavior among the groups. Figure 1 illustrates the percentage of total proximity time spent as proximity to a subject's mother for the four groups of subjects. This measure varied significantly among the four groups of subjects ( $F(3, 28) = 5.0, p = 0.007$ ). *Ant/And* males spent a significantly smaller percentage of their proximity time with their mothers than either *Antide* males or females, but not *vehicle* males. The means for this measure appear inversely related to the level of neonatal T. Thus *Ant/And* males who had the highest

TABLE I  
 Mean Frequency of Sexual, Agonistic, and Play Behavior in Yearling Male Rhesus Exposed  
 to Different Neonatal Androgen Levels in Comparison  
 to Untreated Female Controls

Behavioral measure	Ant/And male (mean $\pm$ SE)	Antide male (mean $\pm$ SE)	Vehicle male (mean $\pm$ SE)	Female (mean $\pm$ SE)	F, p (df = 3, 28)
	Sexual behavior				
Hiptouch	4.0 $\pm$ 0.5	4.8 $\pm$ 0.7	3.7 $\pm$ 1.0	0.0 $\pm$ 0.0†§‡	10.0, p < 0.001
Noncontact present	1.9 $\pm$ 0.9	2.6 $\pm$ 0.6	2.1 $\pm$ 0.9	1.9 $\pm$ 0.5	0.2, p = 0.876
No-footclasp mount	2.1 $\pm$ 0.6	1.6 $\pm$ 0.6	2.2 $\pm$ 0.5	0.0 $\pm$ 0.0†§‡	4.1, p = 0.015
Footclasp mount without thrusts	8.0 $\pm$ 2.5	7.5 $\pm$ 1.3	8.6 $\pm$ 1.6	0.0 $\pm$ 0.0†§‡	6.3, p = 0.002
Footclasp mount with thrusts	9.2 $\pm$ 3.1	15.2 $\pm$ 2.8	13.0 $\pm$ 4.8	0.0 $\pm$ 0.0†§‡	4.5, p = 0.011
	Play behavior				
Quiet solitary play	2.1 $\pm$ 0.8	3.1 $\pm$ 0.8	2.0 $\pm$ 0.7	6.6 $\pm$ 1.9†§‡	3.6, p = 0.026
Brief-contact play (initiated)	23.2 $\pm$ 3.3	25.2 $\pm$ 3.1	24.5 $\pm$ 3.6	11.6 $\pm$ 2.2†§‡	4.3, p = 0.013
Brief-contact play (received)	26.6 $\pm$ 3.7	22.6 $\pm$ 2.1	20.1 $\pm$ 2.5	14.7 $\pm$ 2.4§	3.3, p = 0.037
Rough-and-tumble play (initiated)	39.4 $\pm$ 6.6	33.5 $\pm$ 3.2	44.1 $\pm$ 8.9	5.8 $\pm$ 2.1†§‡	8.5, p < 0.001
Rough-and-tumble play (received)	39.3 $\pm$ 7.4	37.7 $\pm$ 3.9	35.2 $\pm$ 5.9	10.2 $\pm$ 2.1†§‡	6.9, p = 0.001

TABLE 1—Continued

Behavioral measure	Ant/And male (mean $\pm$ SE)	Antide male (mean $\pm$ SE)	Vehicle male (mean $\pm$ SE)	Female (mean $\pm$ SE)	F, p (df = 3, 28)
Play behavior—(Continued)					
Chase play (initiated)	21.7 $\pm$ 5.5	20.4 $\pm$ 3.1	19.8 $\pm$ 3.5	6.1 $\pm$ 2.4†§‡	3.7, p = 0.024
Chase play (received)	23.4 $\pm$ 5.2	18.6 $\pm$ 2.1	18.0 $\pm$ 3.3	6.9 $\pm$ 2.0†§‡	4.1, p = 0.015
Agonistic behavior					
Threat	0.9 $\pm$ 0.3	0.5 $\pm$ 0.3	2.0 $\pm$ 0.6	2.1 $\pm$ 0.6	2.7, p = 0.062
Attack	2.6 $\pm$ 1.0	3.6 $\pm$ 1.6	3.4 $\pm$ 1.7	3.1 $\pm$ 0.9	0.1, p = 0.958
Fear grimace	4.4 $\pm$ 1.6	4.6 $\pm$ 1.7	4.0 $\pm$ 0.4	2.3 $\pm$ 0.5	0.8, p = 0.531
Withdrawal	10.9 $\pm$ 2.2	5.4 $\pm$ 1.2	7.5 $\pm$ 2.2	9.8 $\pm$ 2.7	1.3, p = 0.294

† Differs significantly from Vehicle Male group.

§ Differs significantly from Ant/And group.

‡ Differs significantly from Antide Group.



TABLE 2  
 Mean Frequencies and Durations (Minutes) of Affiliative Behaviors Displayed and Received by Yearling Rhesus Males Exposed to Different Neonatal Androgen Levels in Comparison to Untreated Female Controls

Behavioral measure	Anti/And male (mean $\pm$ SE)	Antide male (mean $\pm$ SE)	Vehicle male (mean $\pm$ SE)	Female (mean $\pm$ SE)	F, p (df = 3,28)
Affiliative behavior					
Contacts initiated	25.7 $\pm$ 3.5	27.1 $\pm$ 3.9	26.5 $\pm$ 4.1	37.4 $\pm$ 2.8	2.3, p = 0.097
Contacts received	5.5 $\pm$ 1.6	6.2 $\pm$ 1.0	7.0 $\pm$ 1.7	8.1 $\pm$ 1.4	0.6, p = 0.623
Contact duration	82.0 $\pm$ 12.6	92.7 $\pm$ 9.54	83.9 $\pm$ 14.0	95.3 $\pm$ 9.1	0.3, p = 0.812
Contact duration with mother	56.8 $\pm$ 14.6	74.8 $\pm$ 13.7	71.4 $\pm$ 14.8	81.8 $\pm$ 9.2	0.6, p = 0.623
Proximity frequency yearling initiated	62.4 $\pm$ 3.5	76.8 $\pm$ 4.2§	71.8 $\pm$ 3.8	81.0 $\pm$ 4.7§	3.9, p = 0.019
Proximity frequency received	55.6 $\pm$ 2.7	53.8 $\pm$ 3.3	52.6 $\pm$ 4.7	64.6 $\pm$ 4.2	2.0, p = 0.134
Proximity duration total	107.2 $\pm$ 10.3	122.9 $\pm$ 18.1	120.2 $\pm$ 12.4	171.1 $\pm$ 9.7†§‡	4.6, p = 0.010
Proximity duration yearling initiated	59.0 $\pm$ 6.9	76.3 $\pm$ 11.4	65.5 $\pm$ 7.2	91.9 $\pm$ 5.2†§	3.2, p = 0.032
Proximity duration with mother	24.4 $\pm$ 3.4	46.7 $\pm$ 4.6§	40.3 $\pm$ 8.3	75.3 $\pm$ 5.9†§‡	13.0, p < 0.001
Following frequency	8.1 $\pm$ 2.2	16.6 $\pm$ 2.9§	14.4 $\pm$ 2.6	21.9 $\pm$ 3.2§	4.3, p = 0.013
Grooming frequency yearling initiated	5.8 $\pm$ 1.9	4.3 $\pm$ 1.7	4.6 $\pm$ 0.9	6.8 $\pm$ 1.5	0.5, p = 0.665
Grooming duration yearling initiated	8.2 $\pm$ 3.4	4.7 $\pm$ 2.6	3.2 $\pm$ 1.1	6.5 $\pm$ 1.5	0.8, p = 0.491
Grooming frequency received	10.1 $\pm$ 3.1	19.5 $\pm$ 4.9	17.8 $\pm$ 5.8	16.6 $\pm$ 3.6	0.8, p = 0.491
Grooming duration received	5.4 $\pm$ 1.5†	20.4 $\pm$ 3.5	10.6 $\pm$ 2.6†	10.7 $\pm$ 4.0†	4.2, p = 0.014
Infant-directed behavior	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.6 $\pm$ 0.4	6.0 $\pm$ 1.8†§‡	9.1, p < 0.001
Genital inspection by mother	0.9 $\pm$ 0.3	0.1 $\pm$ 0.1§	0.2 $\pm$ 0.2§	0.0 $\pm$ 0.0§	3.7, p = 0.024

† Differs significantly from Vehicle Male group.

§ Differs significantly from Anti/And group.

‡ Differs significantly from Antide Group.

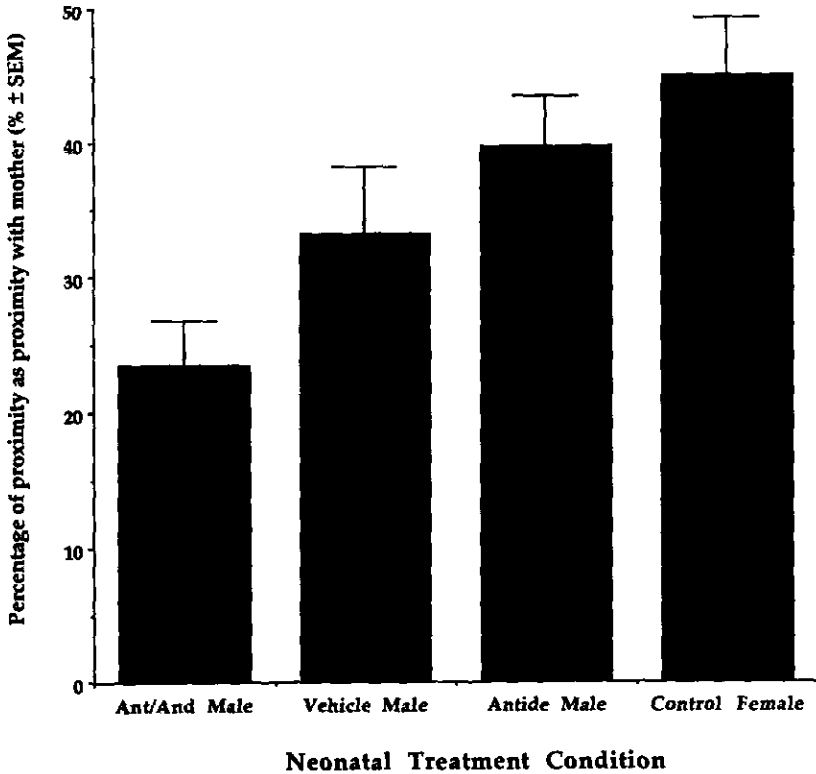


FIG. 1. Percentage of total proximity spent as proximity to the yearling's mother for males exposed to different neonatal levels of androgen and unmanipulated female control rhesus monkeys. *Ant/And* males differ significantly from both *Antide* and control females.

neonatal T levels showed the lowest percentage of time spent in proximity to their mothers, whereas females, with the lowest neonatal T levels, showed the highest percentage of time in proximity to their mothers.

This difference in proximity to the mother is not due to the mother's differential treatment of the yearlings as a result of their neonatal treatment. Figure 2 presents the mean duration of infant-controlled proximity with their mother; those proximity bouts which were both initiated and ended by the infant. The overall difference in this measure was highly significant ( $F(3, 28) = 9.6, p < 0.001$ ) with *Ant/And* males producing significantly shorter proximity durations than both *Antide* males and females. In contrast, *Vehicle*, but not *Antide*, males differed significantly from females in proximity duration to their mother. *Antide* males and females did not differ in this measure.

Both females and *Antide* males followed other animals significantly more frequently than *Ant/And* males, but not vehicle males (Table 2). Since 92% of the 490 following episodes were directed towards the subject's mother, this measure essentially reflects the yearling's pattern of maternal association.

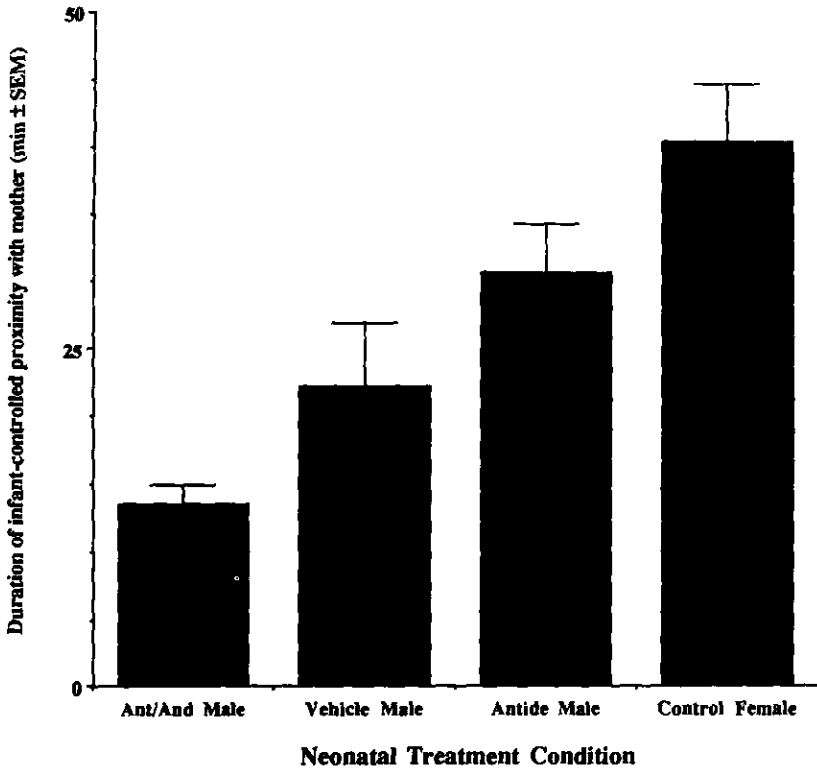


FIG. 2. Mean total duration (minutes per 6-h observation) per subject of infant-controlled proximity to mother in yearling rhesus monkeys. *Ant/And* males differed significantly from *Antide* males and control females. *Vehicle* males differed significantly from control females. *Antide* males did not differ significantly from either *Vehicle* males or control females.

Only for the measure of maternal inspection and manipulation of genitals did *Ant/And* males show a significantly greater interaction with their mother than any other subject group (Table 2). Although this behavior was relatively infrequent, mothers of *Ant/And* males inspected their son's genitals significantly more often than occurred for any other group. Maternal inspection of male genitalia may have been related to the effects of neonatal androgen levels on genital development. Figure 3 illustrates the mean penis length of males from 19 to 25 months of age and shows that the penises of *Ant/And* males were significantly longer than those of males in the other two groups ( $F(2, 21) = 6.4, p = 0.007$ ). At 19 months of age, all three groups differed significantly from each other (TTFM;  $p < 0.05$ ). The correlation between mean male penis length and the frequency of maternal genital grooming approached, but was not significant ( $r = 0.38, p = 0.07$ ).

The groups generally did not differ in the receipt of grooming from their mothers with the exception that the mothers of *Antide* males groomed their

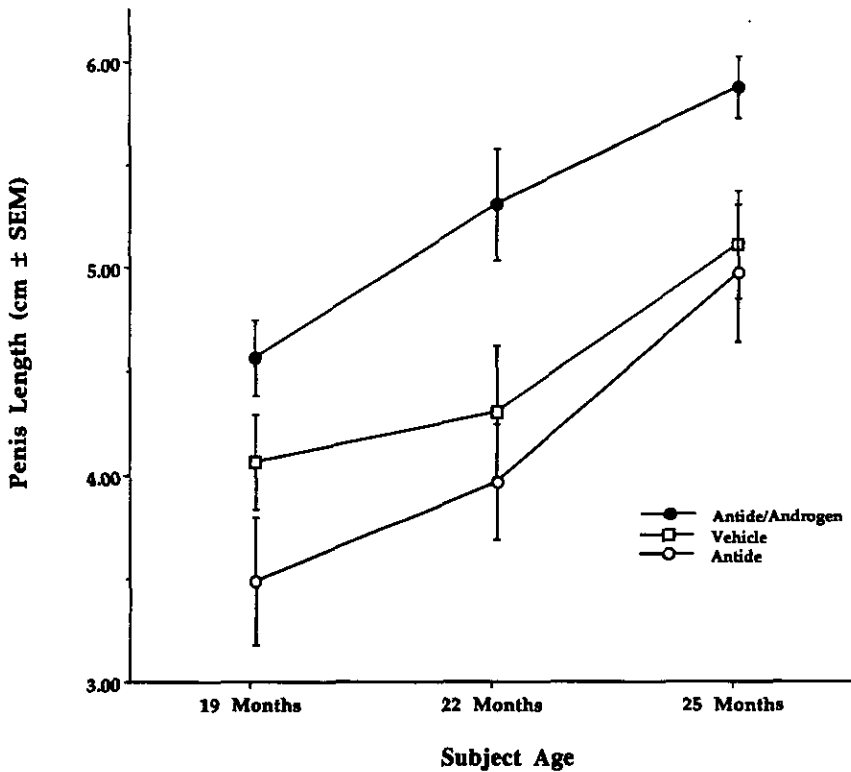


FIG. 3. Mean stretched penis length for juvenile rhesus males at three prepubertal ages following different neonatal androgen exposures during the first 3 months of life. At 19 months, penis lengths differed significantly between the three groups of males. At the next two time points, *Ant/And* males had a significantly longer penis than did *Ant* or *Vehicle* males.

sons significantly longer than did the mothers of the other three groups of subjects (Table 2).

Males in all three treatment groups rarely interacted with infants, whether siblings, other relatives, or strangers. In contrast, females displayed a 10-fold higher interaction with infants than the highest male group (Table 2).

#### DISCUSSION

These results replicate and extend the previously reported sex differences in social behavior in yearlings in a large long-term social group of rhesus monkeys (Berman, 1982; Lovejoy and Wallen, 1988). In addition to the expected differences in rough play and mounting, as well as interest in infants we also found a sex difference in association with mothers. There was also evidence that neonatal testosterone influences patterns of social interaction, but does not affect the most dramatic sex differences in sexual behavior, play patterns, and interest in infants. What is particularly striking about these

results is that the clearest sex differences occurred in patterns of juvenile play and sexual behavior, with less striking differences in juvenile affiliative behavior. In contrast, though neonatal androgen manipulations had no apparent effect on juvenile play, sexual, or agonistic behavior, they altered the character of the interactions between males and their mothers. Females spent more time in proximity, but not in contact, with their mothers and the yearlings were primarily controlling this interaction. In contrast, males neonatally exposed to elevated T spent significantly less time in maternal proximity. Males with normal neonatal T spent intermediate times and males with suppressed neonatal T spent more time than elevated T males, but still spent less time than did females. In addition, males with suppressed neonatal T were groomed significantly more by their mothers than any other group of subjects. Thus 8 months after manipulating male T levels, the effects were detectable in the interactions between males and their mothers. The finding that the differences are as pronounced when analysis is limited to maternal proximity controlled by the infant suggests that these differences reflect a change in the character of the infant's interaction with the mother, rather than changes in maternal response to the infants. The full explanation for these findings is not immediately apparent since there is much unknown about the development of males and females during these first two years.

During the first year of life, rhesus males spend more time away from their mothers than females, a finding that could be conceptualized as an earlier development of infant independence in males (Simpson, 1983). The present results suggest that neonatal T may mediate this development of independence, which is delayed when T is suppressed, or accelerated when T is increased above physiological levels. Alternatively, the proximity relations with mothers may not reflect a developmental lag in neonatally suppressed males, but instead indicate an alteration in their patterns of association. We previously reported that yearling females initiated proximity with other adult females and infants more than did males, suggesting a female attraction to mothers and other adult females. In the present study both females and *Antide* males initiated proximity to other animals significantly more frequently than did *Ant/And* males. Thus for the most extreme contrast in neonatal T, the results are consistent with neonatally suppressed males showing a pattern of proximity initiation more similar to that of yearling females than high T males.

These results suggest that the effect of neonatal suppression of T or elevated T levels is not to define the fundamental sexually dimorphic behavioral characteristics of the male; these clearly appear related to prenatal hormonal events. Instead, it appears that neonatal T influences the dynamics of social interaction, with T-suppressed males showing more maternal dependence and T-augmented males showing less maternal interaction and more independence. Importantly, these differences were displayed long after the treatment period and at a time when T levels were the same, and very low, in all three groups of males. The present developmental results are consistent with our findings in adult males who experienced neonatal T suppression, where the effect

of the neonatal treatment was not to eliminate any gross aspect of adult male sexual or agonistic behavior (Eisler *et al.*, 1993); neonatally suppressed males mated competently and competed successfully with other males for social rank. They did, however, display differences in the degree of their sexual motivation and seemed to be more affected both by the hormonal state of the females in their group and by the season of the year (Eisler *et al.*, 1993).

It now seems likely that prenatal androgens influence the differentiation of different physiological and behavioral endpoints depending upon when, during gestation, androgen exposure occurs (Goy, Bercovitch, and McBair, 1988). The current results, in concert with our previous findings on adult behavior and evidence from human development (Swaab and Hofman, 1988) support the idea that the period of neonatal androgen secretion is another time when the developing nervous system is sensitive to the organizational effects of androgen. In contrast to their prenatal effects, androgens during the neonatal period don't determine fundamental sexually dimorphic characteristics, but instead "fine-tune" the specific character of male social interactions.

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