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Effects of prenatal androgens on rhesus monkeys: A model system to explore the organizational hypothesis in primates

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Abstract

After proposing the organizational hypothesis from research in prenatally androgenized guinea pigs (Phoenix et al., 1959), the same authors almost immediately extended the hypothesis to a nonhuman primate model, the rhesus monkey. Studies over the last 50 years have verified that prenatal androgens have permanent effects in rhesus monkeys on the neural circuits that underlie sexually dimorphic behaviors. These behaviors include both sexual and social behaviors, all of which are also influenced by social experience. Many juvenile behaviors such as play and mounting are masculinized, and aspects of adult sexual behavior are both masculinized (e.g. approaches, sex contacts, and mounts) and defeminized (e.g. sexual solicits). Different behavioral endpoints have different periods of maximal susceptibility to the organizing actions of prenatal androgens. Aromatization is not important, as both testosterone and dihydrotestosterone are equally effective in rhesus monkeys. Although the full story of the effects of prenatal androgens on sexual and social behaviors in the rhesus monkey has not yet completely unfolded, much progress has been made. Amazingly, a large number of the inferences drawn from the original 1959 study have proved applicable to this nonhuman primate model.

Keywords

Organizational hypothesis; nonhuman primates; rhesus monkeys; prenatal androgens; sex differences; juvenile behavior; adult sexual behavior

Historical Context

During the 1950s, Phoenix, Goy, and Gerall working in W.C. Young's lab at the University of Kansas administered testosterone, a testicular androgen, to pregnant guinea pigs. The dam's female offspring had ovaries and masculinized genitalia and thus were called hermaphrodites (Phoenix et al., 1959). In adulthood, the prenatally testosterone-treated females behaved more like males and less like control females. They were said to have "masculinized" behavior because they showed more male-like mounting behaviors in response to testosterone, and they showed less lordosis behavior in response to the ovarian hormones estradiol and progesterone. The "hermaphrodites" also showed mounting behavior with a shorter latency and in response to lower levels of testosterone than that required to see effects in control females. Furthermore, it appeared that the neural substrate underlying

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"masculinization" of behavior was even more sensitive to prenatal testosterone than was the external genitalia (e.g. some females still showed behavioral masculinization when the dose of testosterone was low and not sufficient to modify the female's genitals). More importantly, these data indicated that sex differences in behavior were not due just to masculinization of the genitalia. In their paper, Young and colleagues (Phoenix et al., 1959) also presented evidence that this effect of testosterone was both specific to the prenatal period and permanent.

From these data Phoenix et al. (1959) hypothesized that during the prenatal period, androgen is a morphogenic substance that has "organizing" or differentiating actions that permanently affect the neural tissues that mediate mating behavior. These "organizational" effects were in addition to the known transient and reversible "activational" effects of hormones on those behaviors in adulthood. They also hypothesized that prenatal androgen permanently changes the responsiveness to activational hormones. Furthermore, they suggested that this organizational effect of androgens might extend to behaviors beyond those that are primarily sexual. They used their guinea pig data to propose an important, encompassing hypothesis; the organizational hypothesis was born.

Aware of the importance of their studies, Young's lab was interested in extending this research to a model more closely related biologically to human beings. At that time, the model of human sexuality emphasized the role of environment and sex of rearing rather than any biological predisposition. Many authors of the time asserted that the human brain was born 'neutral' in regards to sexuality and that sexuality (e.g. gender identity, gender role, sexual orientation, and sexual behavior) was completely malleable in humans until at least 18 months of age (Money 1963; Hampson 1964a; Hampson 1964b). According to Goy (1988), the "dogma regarding human beings was to the effect that prenatal hormonal factors were irrelevant to psychosexual outcomes, and that the primary determinants of human sexuality were experiential." Clearly, their data from the guinea pig suggested that the prenatal hormone environment also played a role, and they wanted to test their hypothesis in a primate model.

Young and his colleagues turned to the rhesus monkey as an attractive model system. Nonhuman primates are more complex, more social, and more physiologically similar to humans than are rodents. W.C. Young told Charles Phoenix (Phoenix, 2008) that although he would be able to obtain funds to build a monkey facility at Kansas that the University of Kansas would not agree to maintain the building once it was built. Another approach had to be taken. So, in August 1961 Phoenix went to Leon Schmidt's lab in Cincinnati, Ohio. Although the lab primarily did malaria research with the primates, Phoenix was allowed to produce some timed, testosterone-treated pregnancies (Phoenix, 2008). At the same time, Goy went to Harry Harlow's lab in Madison Wisconsin and learned about infant and juvenile monkey behaviors (Goy, 1988).

By early summer 1962, Phoenix had 8 pregnant female rhesus monkeys who had been treated with testosterone propionate. Rather than shipping the precious animals by cargo plane, Phoenix obtained a truck and spent an "odoriferous trip" as he "drove the dears to Madison". In September of 1961, Phoenix returned to Kansas and helped manage the remodeling of part of the guinea pig area into a monkey "playroom" and an area to house about 16 rhesus monkeys. When the pregnant females gave birth, the females (called pseudohermaphrodites) and their male counterparts were shipped to Lawrence, Kansas for study (Phoenix, 2008).

In 1963 William Montagna became director of the Oregon Primate Center and offered Phoenix, Goy, and Young appointments to the scientific staff. Young was to establish and

head the Reproductive Biology Division, and Goy and Phoenix were hired as scientists. This was seen as “an opportunity for the three of us who had conceptualized this research effort to stay together and to continue as a team until the work was brought to completion” (Goy, 1988). The pseudohermaphrodites were shipped to Oregon, and Young also insisted on taking guinea pigs from three strains housed in the Kansas lab: two were inbred strains and one, called Topeka, was an outbred strain that Young found on a farm near Topeka Kansas. By August 1963, Phoenix, Goy, and Young relocated to Oregon to work together to explore the organizational actions of androgens on rhesus monkeys (Phoenix, 2008).

With a gestation length of 6 months and 3 years to reach puberty in rhesus monkeys, studies of the effects of prenatal androgens on juvenile and adult behaviors require a large investment of time and resources. But by 1964, Young et al. reported on the early juvenile behaviors of 2 female rhesus monkeys treated prenatally with testosterone. Consistent with their organizational hypothesis, the androgenized female rhesus monkeys showed masculinization of sexually dimorphic social behaviors at 2–5mo of age. Interestingly, Young et al., (1964) noted that it was a volatile time to be studying sex and that it had been recommended that they show restraint in the use of the term, even in institutional records and research proposals.

Although the work on testing the organizational hypothesis in monkeys was well begun, the ‘team’ of Young, Phoenix, and Goy was not able to complete its work together. As Goy said, “fate prevented any joint celebration of the finale” (Goy, 1988), because a short time later, in April 1966, W.C.Young died.

Obviously, work with prenatally androgenized monkeys continued after Young’s untimely death, and it has now included five cohorts of rhesus monkeys with prenatal manipulations. The first full set of prenatally testosterone-treated pseudohermaphrodites was reported on by Phoenix et al. (1968) and Goy (1968). In 1971 Goy moved to the Wisconsin Regional Primate Research Center, succeeding Harlow as director. There, another set of prenatally androgenized females, who were treated with either the aromatizable androgen testosterone (i.e. one that can be made into estrogens) or the nonaromatizable androgen dihydrotestosterone, was developed (first reported by Goy et al., 1977). A third set of prenatally androgenized females, first reported by Goy et al., 1988b, received testosterone either early or late in gestation. A fourth set was developed by Kim Wallen (who had been a graduate student with Goy) at the Yerkes National Primate Research Center. Those animals received the androgen receptor blocker flutamide or a low dose of testosterone either early or late in gestation (first reported on by Herman et al., 2000). Finally, Dave Abbott and colleagues reported on fetal and infant neuroendocrine function in a recent cohort of prenatally androgenized female rhesus monkeys (Abbott et al., 2008a). Numerous articles have been published on each of the older sets of prenatally androgenized females, describing their physiology and/or behavior. Table 1 presents the basic treatment details for the five cohorts along with the articles reporting on each cohort.

In what follows we will look more closely at what has been discovered about the effects of prenatal androgens on rhesus monkeys. The original Phoenix et al. (1959) paper used the term ‘masculinization’ for all of the organizational effects observed in response to prenatal androgens. Subsequent research has indicated that differentiation proceeds in an inherently female direction unless androgens are present during development and it has been proposed that these androgens act to both masculinize and defeminize (Whalen, 1974). Masculinization refers to an increase in male-like structures and behaviors, and defeminization refers to a decrease in female-like structures and behaviors. We will focus on the endpoints of external genitalia, juvenile behaviors, and adult sexual behaviors, with an

eye toward elucidating what is known about some of the core, cross-cutting, conceptual issues related to the organizational hypothesis.

Prenatal androgens and external genitalia

Differentiation of the external genitalia in rhesus monkeys occurs in response to androgens secreted prenatally. Similar to males of other species, rhesus males have higher levels of testosterone than females during much of gestation (Resko and Ellinwood, 1981). Following soon after testis differentiation, androgen levels first rise around day 40, fall briefly around day 75, and then rise again around day 140 of gestation until parturition at approximately 185 days (Resko, 1985). There is also a postnatal rise of testosterone for the first 2–3 months after birth in the rhesus male (Mann et al., 1984). Testosterone levels are low to undetectable during most of the infant and juvenile periods of male rhesus monkeys, including from 6 months to approximately 1.5 years of age (Resko, 1967). In both males and females, neuroendocrine activity and gonadal hormones remain low until the onset of puberty at the age of 2.5–4 years, when testosterone levels increase in males and ovarian cycles begin in females (Plant, 1994; Terasawa and Fernandez, 2001).

Prenatal exposure to testosterone resulted in masculinization of the external genitalia of female rhesus monkeys (e.g. Young et al., 1964) similar to what had been seen in the guinea pig and other species (e.g. Phoenix et al., 1959). Early reports on female rhesus monkeys treated prenatally with testosterone (for 25–50d beginning on d39 for a total of 610–750 mg testosterone propionate) reported that the external genital structures of the resulting offspring were characterized by the formation of a well-developed but empty scrotum (they still had ovaries), the complete lack of a vaginal orifice, and a small, but apparently complete, penis (Young et al., 1964; Goy, 1968; Eaton et al., 1973). Subsequently, it was reported that if females were exposed to 15 mg/d testosterone for 55 or more days beginning between d40–45 of gestation, development of the penis and scrotum in the females were indistinguishable from that of males (Goy and Robinson, 1982). Moreover, either testosterone or dihydrotestosterone caused genital masculinization (Goy, 1981; Goy and Robinson, 1982).

The external genitalia of the rhesus monkey appear to be most sensitive to the masculinizing effects of androgens during the second quarter of the 168d gestation (approximately days 42–84 post-conception). Early reports from the second cohort of androgenized females indicated that as little as 15 days of testosterone treatment that began by day 40 to 60 of gestation had significant masculinizing effects (Goy, 1981). Moreover, although exposure to androgens in the second quarter of gestation (days 40–64 post-conception) fully masculinized female genitalia, androgens later in gestation (during the third and fourth quarter; days 115–139 and days 140–168 post-conception) had no effect (Goy et al., 1988a).

More recent data with prenatal administration of the antiandrogen flutamide are consistent with these prenatal androgen studies. That is, although the effects were varied, numerous males that were exposed to flutamide prenatally during the second quarter of gestation had radically altered genitalia, including a transposed penis and scrotum (Herman et al., 2000). Flutamide treatment later in gestation decreased male penis size, but did not alter the basic pattern of male genital organization. This suggests that flutamide at least partially blocked endogenous androgen action in the males and that blockade of androgen receptors during the second quarter of gestation decreases genital masculinization. The variable, incomplete effect of the flutamide may be because it has a relatively low affinity for the androgen receptor (Eil and Edelson, 1984; Simard et al., 1986) resulting in increased testosterone levels in the mothers, and an incomplete block of androgen action (Herman et al., 2000).

Although most of the cited literature has used the term “masculinization” for the effects of androgen on external genitalia, it might be more accurate to say that androgens both masculinize and defeminize the external genitalia. Embryologically, the external genitalia arise in males and females from one set of undifferentiated structures (e.g. Hadley and Levine, 2007) so that an increase in male-type structures leads to a decrease in female-type structures. Consequently any increase in masculinization is also defeminization. However, the convention is to use the term masculinization.

In summary, androgens masculinize (and defeminize) the external genitalia of rhesus monkeys and the period of maximal sensitivity occurs during the second quarter of gestation. Whether or not this genital masculinization played a role in the masculinization of behavior was a relevant question in the early years after the organizational hypothesis was proposed. However, as described below, this masculinization of the external genitalia is certainly not obligatory for the effects of prenatal androgens on sex differences in behavior.

Prenatal androgens and juvenile behaviors

There are a number of sexually dimorphic behaviors (i.e. behaviors that differ between males and females) in juvenile rhesus monkeys (Harlow, 1965, Phoenix et al., 1968; Goy and McEwen, 1980). These include play behaviors such as rough and tumble play and play initiation behaviors, protosexual behaviors such as mounts, and certain types of vocalizations. Males show higher levels of these behaviors compared to females. On the other hand, certain other types of vocalizations and interest in infants may be shown at higher levels by females (Lovejoy & Wallen, 1988; Tomaszycki et al., 2001, 2005; Herman et al., 2003). In the case of rhesus monkeys, males and females show clear quantitative rather than qualitative differences in these behaviors. Not surprisingly, the first behavioral data to come from the prenatally androgenized female rhesus monkeys were comparisons of their sexually dimorphic juvenile behaviors.

Play and protosexual behaviors

Even from the very first experimental manipulations of prenatal androgens in primates, the organizational effects of prenatal androgens on juvenile sexually dimorphic behaviors were apparent. Young et al. (1964) reported on two prenatally androgenized female infants whose mothers were treated with testosterone propionate daily, from approximately gestational days 40 through 90. These females had masculinized juvenile behavior as early as 6 weeks of age, and a number of social behaviors were affected by prenatal androgen treatments. Between 2 and 5 months of age, the pseudohermaphrodites were similar to males in that they threatened and engaged in rough and tumble play patterns more frequently than did control females. They also withdrew less often from the approaches of other animals, similar to males, and showed more male-typical protosexual behaviors such as mount attempts.

This early report was verified with more animals and more extensive analysis by Phoenix et al., 1968 and Goy, 1968. The female offspring were compared to normally developing males and females and displayed intermediate levels of sexually dimorphic behavior. Pseudohermaphrodites engaged in rough and tumble play more than females and less than males as infants, yearlings, and two year olds (Phoenix et al., 1968; Goy and Phoenix, 1972; Goy and Resko, 1972). Interestingly, later studies (Goy, 1981) indicated that not all components of the sexually dimorphic juvenile play behaviors were affected by prenatal androgens. Although play initiation is sexually dimorphic, with males initiating more play than females, androgenized females did not differ from control females in play initiation. This suggests that specific components of complex social behaviors may vary in the degree to which prenatal hormones bias later behavioral expression. The prenatal androgen treatment also affected mounting behaviors. Typically, developing females almost never

mount their peers, but the frequency of mounting behavior by pseudohermaphrodites during the first year of life was similar to all but the most extreme frequencies observed among normal males (Phoenix et al., 1968). In addition, the average frequency of mounting in the first and second year of life did not differ significantly from that of normal males (Goy, 1970a; Goy and Phoenix, 1972).

Later research has shown that the dimorphisms in juvenile play and mounting behavior are due to prenatal androgens and are not dependent on either the brief postnatal rise in testosterone or on activational/concurrent effects of androgens during the early juvenile period. In particular, sex differences are not due to postnatal testosterone, because blocking the early postnatal rise with a GnRH antagonist has no effect on the sexually dimorphic behaviors of rough and tumble play or mounting (Mann et al., 1984, Wallen et al., 1995). Sex differences are also unlikely to result from the activational effects of circulating androgens, because neuroendocrine activity and gonadal hormone levels are very low in juvenile males and females (Terasawa and Fernandez, 2001). Moreover, neonatal castration had no effects on these behaviors (Goy, 1968, 1970b, 1978).

Both the duration and timing of prenatal androgen exposure affected the organization of juvenile sexually dimorphic behaviors. Although Phoenix and colleagues (1968) varied the duration of testosterone administration, they did not address whether or not there was an association between androgen variation and juvenile behaviors. However, in a second study, Goy (1981) compared 15, 25, and 35 day treatments in a small number of females and found that mounting of peers and rough play were increased in a dose dependent manner with increasing exposure to testosterone during gestation.

The variability observed in these early studies led to an experimental cohort that received short treatments of androgens either early or late in gestation (Goy et al., 1988a). In this study, androgens early in gestation masculinized genitalia without increasing levels of rough and tumble play in juvenile females whereas androgens later in gestation (e.g. days 115–139, 10mg/day) did not affect the external genitalia but did increase rough and tumble play (Goy et al., 1988a). Thus, consistent with findings in the guinea pig (Phoenix et al., 1959), masculinization of the external genitalia did not account for the effects of prenatal androgen administration on sex differences in rhesus behavior. In addition, both prenatal treatment periods increased mounting of peers by young androgenized females relative to control females, suggesting that different behaviors (i.e. play and mounting) may have different developmental periods in which they are maximally sensitive to organizational effects of androgenic hormones.

Extensive research indicates that both non-aromatizable and aromatizable androgens masculinize male-typical behaviors. Prenatal administration of either the aromatizable androgen, testosterone propionate, or a non-aromatizable androgen, dihydrotestosterone propionate, masculinized rough and tumble play behaviors in females (Goy, 1981). Like play behavior, mounting of peers was masculinized in females exposed to either aromatizable or non-aromatizable androgens during gestation. Prenatally androgenized females mounted peers more frequently than control females but significantly less than control males (Goy, 1981). Both testosterone and dihydrotestosterone were seen to be equally effective (Goy, 1981).

A major limitation of the earliest studies of prenatal androgens on the development of behavior was that the experimental subjects were reared with a limited social environment. For example, the first two pseudohermaphrodites were removed from their mothers at birth and raised in a nursery by laboratory technicians (Young et al., 1964 as reported by Phoenix et al., 1968). Behaviors were observed 5 times a week when the experimental females were

paired with control females for 20 minutes in a neutral play arena (Young et al., 1964). The prenatally androgenized females in the cohort described by Phoenix and colleagues (1968) were removed from their mothers at 3 months of age and lived alone except for weekday play sessions with peers. The next set of experimentally manipulated females (described by Goy, 1981) lived with their mothers until 11 months of age in social groups of 5 mother-infant pairs. Although each cohort of females described in these early studies lived in a more socially complex and rich environment than the previous cohort, these early environments did not allow for many socially complex behaviors that are observed in large troops of rhesus macaques (e.g. Altmann, 1962). Rearing conditions and social environment clearly influence the development of social behaviors (Wallen, 1996) and as a result may have confounded interpretation of some of the earlier results. For example, as noted by Wallen (1996), some behaviors such as threat may be enhanced in males more than females when both are raised in less complex social environments making the behavior sexually dimorphic in some but not all rearing conditions. This may explain why an earlier study reported that prenatal androgens increased threat behaviors in prenatally androgenized females (Young et al., 1964), but subsequent studies have not seen sex differences or prenatal androgen effects on threat behavior (Wallen, 1996).

More recently, Wallen and colleagues administered androgens prenatally to female monkeys embedded in large, age-graded, mixed-sex groups living outdoors at the Yerkes National Primate Research Center. In this set of experimentally manipulated females, a smaller dose of androgen was used, which did not masculinize the genitalia of females, even when administered early in gestation (Herman et al., 2000). In addition, androgen treatments did not masculinize play behaviors, as females treated with androgens either early or late in gestation did not differ statistically from control females in the frequency of rough and tumble play (Wallen, 2005). While not statistically significant, yearling control females never showed mounting behavior towards peers, but early and late androgen treated females showed some (Wallen, 2005). Thus, although low levels of prenatal androgen were able to alter neuroendocrine function (Herman et al., 2000), they were not sufficient to produce statistically measurable changes in rough play and mounting like those observed in earlier reports (Goy et al., 1988a).

Vocal behaviors

In natural settings, males and females differ in their vocal behaviors. Working with animals housed in complex, age-graded social groups, Tomaszcyki and colleagues were able to look at the effects of prenatal manipulations on developmental trajectories in vocal communication. Infants vocalize most frequently in response to separation or rejection from their mother and do so with sexually dimorphic patterns of vocal behavior. Separation-rejection vocalizations occur in bouts, with multiple call types occurring in rapid succession. Females have longer bouts, with more calls per bout and more types of calls per bout than males (Tomaszycki et al., 2001). In addition, females use more coos and more arched screams in their separation-rejection vocalizations, and males use more geckers and noisy screams. Early androgen treatment masculinized and defeminized these separation-rejection vocalizations by shifting each of these features toward the male-typical pattern (i.e. increases in percent of geckers and percent of noisy screams) and away from the female-typical pattern (i.e. decreases in bout duration, calls per bout, types of calls per bout, percent of coos, and percent of arched screams; Tomaszycki et al., 2001). In contrast, late androgen treatment altered only a few features of the separation-rejection vocalization bouts, masculinizing and defeminizing the percent of geckers, bout duration and types of calls per bout (Tomaszycki et al., 2001).

Prenatal androgens also masculinize and defeminize the dimorphic pattern of agonistic scream vocalizations. As juveniles, both males and females use screams in agonistic

encounters, but the screams of juvenile males are less like those of adult females than are the screams of juvenile females (Tomaszycki et al., 2005). Adult females, but not males, use a variety of scream types to recruit other members of their social group into agonistic interactions. Agonistic scream types vary not only in general acoustic parameters (e.g. noisy vs. tonal vs. pulsed), but also in the social context in which they are used (Tomaszycki et al., 2005). Androgens administered late in gestation defeminized the agonistic screams of female monkeys by making them less like the adult female screams (Tomaszycki et al., 2005). In contrast, agonistic screams of females treated early in gestation did not differ from control females in their resemblance to adult female screams (Tomaszycki et al., 2005). Sex differences in the contextual usage of agonistic screams were not observed (Tomaszycki et al., 2005). Taken together with the data on separation-rejection vocalizations, these studies show that prenatal androgens acting early or late during gestation masculinize and defeminize several aspects of vocal behavior. Females that have been treated prenatally with dihydrotestosterone have not yet been tested for these behaviors so it is not yet known whether aromatization plays a role in the organization of vocal behavior.

Interest in infants

In contrast to the male-biased behaviors of rough and tumble play and mounting, interest in infants is a female-biased sexually dimorphic behavior in rhesus monkeys (Lindburg, 1971; Lovejoy and Wallen, 1988). When adolescent females treated prenatally with androgen (dihydrotestosterone propionate) were paired with unfamiliar infants in a novel environment, the pseudohermaphrodites approached, groomed, and lipsmacked towards the infants (Gibber and Goy, 1985). These responses did not differ from those observed in control females and suggest that prenatal androgens do not defeminize interest in infants (Gibber and Goy, 1985). Lack of defeminizing effects of androgens were also reported in a more recent cohort of animals from a less restricted social milieu; prenatal androgen (testosterone enanthate) at low doses had no effects on interest in infants by prepubertal females treated either early or late in gestation (Herman et al., 2003). It remains to be seen whether more completely androgenized females that are raised in large social groups would show less interest in infants.

In recent studies, Wallen and colleagues also administered flutamide, an androgen receptor blocker, in an attempt to interfere with prenatal masculinization and defeminization in males. As described previously, flutamide administered early in gestation interfered to some degree with genital differentiation of the male fetus (Herman et al., 2000). Surprisingly, flutamide did not block masculinization of behaviors in males and paradoxically masculinized some sexually dimorphic behaviors in males and females (Wallen, 2005). As infants and yearlings, flutamide-treated males did not differ significantly from control males in their rough play behavior (Wallen, 2005). However, male infants and yearlings who received flutamide treatments late in gestation engaged in more mounting behavior than did control males (Wallen, 2005). Furthermore, females treated with flutamide late in gestation had decreased interest in infants (Herman et al., 2005) and masculinized and defeminized vocal behavior (Tomaszycki et al., 2001; 2005). One possible explanation for the masculinizing and defeminizing effects of flutamide is that flutamide increased circulating testosterone levels in the mothers (Herman et al., 2000). This and other alternatives are described more fully elsewhere (Wallen, 2005).

Summary

Prenatal androgens can both masculinize and defeminize juvenile behaviors in rhesus monkeys. Rough and tumble play, mounting behavior, and separation-rejection vocalizations were masculinized by exposure to androgens during gestation. Although interest in infants was not defeminized, prenatal androgens in late gestation defeminized the

production of agonistic screams and aspects of separation-rejection vocalizations. Furthermore, different behaviors vary in the timing of their sensitivity to the organizational effects of androgens, suggesting that variation in the timing of androgen exposure differentially alters patterns of juvenile behavior. For those behavioral endpoints that have been tested, both aromatizable and nonaromatizable prenatal androgens have been effective.

Prenatal androgens and adult sexual behaviors

Adult male and female nonhuman primates differ considerably in their behavior but, as with juvenile behaviors, adult behaviors are typically not unique to one sex. Both male and female rhesus monkeys can and do display 'male-typical' and 'female-typical' sexual behaviors; they just display them at different frequencies. Moreover, rhesus monkeys are a social species with a flexible repertoire of behavior, and some stereotypical sexual behaviors such as mount and present (see below) are used by both males and females in nonsexual contexts.

Types of male-typical sexual behaviors

'Male- typical' sexual behaviors include both courtship and copulatory components (e.g. Beach, 1956; Dixson, 1998). Courtship behaviors vary by species and generally occur before copulation. Rhesus males may show lip-smacking (Dixson, 1977), purselip gestures (Pomerantz and Goy, 1983), directed approach (Thornton and Goy, 1986), and grooming of the female (Michael et al., 1966). Copulatory behaviors consist of behaviors important for successful mating. Because copulation generally occurs in a dorso-ventral position in rhesus monkeys, copulatory behaviors may include 'sexual contacts' (the male touches the hips of the female with his hands), sex contact to a present (Wallen and Goy, 1977), double foot-clasp mounts, intromissions, and ejaculations (Beach, 1956; Dixson, 1998).

The adult double foot-clasp mount is a complex behavior that develops during the juvenile period and is strongly affected by social experience (Mason, 1960; Harlow, 1965, Goy, 1970a, Wallen, 1996). The age at which the mature mount is first displayed by males varies with rearing conditions and may range from 3 months to 3 years (Goy, 1970a; Wallen, 1996). Young males who are raised in social groups with both their mothers and importantly, their peers, display the adult pattern (a double foot clasp mount) at early ages. In contrast, animals raised under more restricted social conditions tend to show increased aggression and less male-typical sexual behavior as adults (Goy and Wallen, 1979). Although the amount of social experience during development affects the magnitude of the sex difference in mounting in adulthood, sex differences in mounting are seen in most rearing conditions (Wallen, 1996).

Types of female-typical sexual behaviors

Rhesus 'female-typical' sexual behaviors have been conceptualized as falling into a number of categories. Beach (1976) proposed a general schema for female sexuality that included receptive and proceptive behaviors and attractive characteristics. In nonprimate female mammals, receptivity is usually determined by lordosis frequency and quality. Lordosis is a rigid, reflexive behavior that is dependent upon ovarian hormones for its expression (see Pfaff 1980). In the rhesus female, receptivity has been measured by the frequency of present (Carpenter 1942) or present-to-contact or acceptance score (Baum et al., 1977; Wallen and Goy, 1977). In contrast to rodents, the present of female monkeys is not a reflexive posture and is not under rigid hormonal control. Receptivity is neither obligatory nor passive. Moreover, the present may be used to initiate copulation or for sociosexual reasons, and it can also play a role in affiliative and dominance or rank-related behaviors (Dixson, 1998).

Indeed, depending on the developmental and adult social context, the frequency of presenting may be sexually dimorphic or not (Thornton and Goy, 1986; Wallen, 1996).

Proceptive behaviors serve to attract the attention of the male and to promote sexual interaction. Proceptive behaviors tend to be species-specific and have been defined variously in rhesus monkeys by different labs. In studies of female rhesus monkeys, the category of proceptive behaviors has included a) proximity behaviors such as directed approach, sit close, and contact; b) sexual solicitation behaviors such as handslaps, shoulder flex, head bobs and ducks, sidle, and threaten-away; and c) other communicative behaviors such as eye-contact and estrous vocalizations (Ball and Hartman, 1935; Carpenter, 1942; Altmann, 1962; Loy, 1971; Czaja and Bielert, 1975; Pomerantz and Goy, 1983; Dixson, 1998). In captive-group studies of rhesus, more than 90% of all sexual interactions were initiated by females (Wallen et al., 1984).

Although the term attractiveness has been used to characterize female sexuality (Beach 1976), it could easily apply to both males and females. Each sex has characteristics that encourage the approach of the other sex: females are attractive to males and vice versa. In rhesus monkeys, the precise identity of attractive characteristics is unknown, although olfactory cues, sex skin coloration, and others have been strongly implicated (see Czaja et al., 1977, Goldfoot, 1981). Often an individual's attractiveness has been measured indirectly by the amount of courtship or proceptive behavior directed towards the individual.

Activational effects of gondal hormones in adulthood

Even given the flexibility of sexual behaviors in nonhuman primates, adult gonadal hormones have clear activational effects on the sexual behavior of rhesus monkeys. Females show increases in sexual behavior and sexual initiation behaviors around the time of ovulation (Michael and Bonsall, 1979; Wallen, 1982; Wilson et al., 1982; Wallen et al., 1984; Zehr et al., 2000). Gonadectomy of females decreases and replacement estradiol increases both receptive and proceptive behaviors (Michael and Welegalla, 1968; Thornton and Goy 1986; Zehr et al., 1998). Estradiol increases sexual initiation in gonadectomized females independently of male sexual interest and behavior (Zehr et al., 1998). Estradiol also increases the attractiveness of the females to males (Thornton, 1983; Pomerantz et al., 1985; Thornton and Goy 1986). The effect of ovarian hormones on presentations is less clear. In pair tests, estradiol treatment did not increase the number of presents shown by gonadectomized adult females (Thornton, 1983; Thornton and Goy 1986). However, the frequency of presents was high in gonadectomized females and was associated with more male threats towards them. In a study of gonadectomized females living in a social group, estradiol significantly increased the number of presents (Zehr et al., 1998). The differences observed in these studies demonstrate the lability and flexibility of rhesus monkey presenting behavior and may be explained by the use of the presentation in both agonistic/appeasement and sexual contexts.

Gonadectomy of the adult male leads to a decrease in the frequency of male-typical courtship and copulatory behaviors, which are then 'reactivated' by testosterone or dihydrotestosterone (Phoenix et al., 1973; Michael and Wilson, 1974; Phoenix, 1974b; Phoenix, 1978) but not by estradiol (Michael and Bonsall, 1979; Pomerantz et al., 1986). These adult activational androgens also increase the males' attractiveness to females (Phoenix et al., 1973; Michael and Bonsall, 1979).

Gonadectomy and administration of heterologous hormones (e.g. estradiol to males or androgens to females) does not lead to a complete reversal of sexual behavior in rhesus adults. Administration of even large amounts of testosterone to gonadectomized adult female rhesus monkeys did not increase either mounting activity or other male-typical

behaviors (Phoenix et al., 1968; Goy and Resko, 1972; Eaton et al., 1973; Joslyn, 1973). Similarly, injection of the ovarian hormone estradiol did not increase the frequency of most female-typical receptive or proceptive behaviors (Thornton and Goy, 1986) or most measures of the attractiveness of adult gonadectomized males (Thornton, 1983; Pomerantz et al., 1985; Thornton and Goy, 1986). Estradiol did increase the number of presents shown by gonadectomized males, but this may have been in response to threats from the stimulus males (Thornton and Goy, 1986; Wallen, 1996).

These results indicate that, similar to other mammalian species, the activational effects of adult gonadal hormone levels cannot account for all of the sex differences in reproductive behavior shown by male and female rhesus. Consistent with this, prenatal androgens clearly have organizational effects on rhesus adult sexual behavior.

Organizational effects of gonadal hormones: masculinization

Early experiments on the masculinization of adult rhesus sexual behavior by prenatal androgen were somewhat inconclusive. When Eaton and colleagues (1973) tested prenatally testosterone-treated females for masculine behavior in adulthood, they found that although the prenatally androgenized females showed more mounting behaviors in response to adult testosterone treatment than control females, the differences were not statistically significant. Pseudohermaphrodites also showed nonsignificant increases in intromissive behavior with a partner, although three pseudohermaphrodites ejaculated in response to masturbation. When Phoenix and Chambers (1982) retested these same prenatally androgenized females (at the age of 15–17 years), they saw very little mounting and no intromissive behavior. Eaton et al. (1973) suggested that the lack of complete male-typical behavior might be due to social and experiential factors. These particular females were raised under restricted social conditions that produce increased aggression and block the development of masculine behavior in males (Goy and Wallen, 1979).

When more socially adept animals were tested, the organizational effects of androgens on the masculinization of adult sexual behavior became more apparent. Pomerantz et al. (1986; 1988) gave testosterone during adulthood to gonadectomized prenatally androgenized females and tested them with estradiol-primed female partners in pair and trios. The animals had been housed in mother-infant groups during the first year of life, weaned at approximately 1 year of age and then housed with peer groups for most of the next 3 years (see Goy and Wallen, 1979). They compared their adult behavior to that of both gonadectomized males and females who were also testosterone-treated in adulthood and paired with estradiol-primed female partners. They found that gonadectomized adult males and pseudohermaphrodites showed both more mounting behavior than females before testosterone administration in adulthood, and showed an enhanced sensitivity to the activational effects of testosterone on mounting behaviors (Pomerantz et al., 1986). Although adult testosterone administration had relatively little effect on the control females, it increased a number of male-typical behaviors in the prenatally androgenized females, including purselip, approach, sex contact, and mount rate. For most of the behavioral endpoints, the testosterone-treated pseudohermaphrodites resembled testosterone-treated males (although both tended to show fewer male-typical sexual behaviors than gonadally intact males). Prenatal androgens also appeared to increase the attractiveness of the prenatally treated animals to females. Stimulus females showed more proceptive and receptive responses towards prenatally androgenized females than to females not given androgens before birth, although the attractiveness of the pseudohermaphrodites to females did not reach the level of interest shown to males (Pomerantz et al., 1986; 1988).

Organizational effects of gonadal hormones: defeminization

Early experiments that investigated the organizational effects of prenatal androgens on adult female sexual behavior were inconclusive (Phoenix et al., 1983; Phoenix et al., 1984). Phoenix and colleagues (1983) gonadectomized prenatally androgenized and control females, treated them with estradiol in adulthood, and paired them with gonadectomized adult males (the pseudohermaphrodites had displayed aggressive behavior in previous tests so the males were gonadectomized to decrease aggressive interactions). In this study, pseudohermaphrodites and controls showed equivalent proceptive behaviors (as measured by invitation rate and proximity rate) and receptive behaviors (as measured by a present to contact ratio), suggesting that prenatal androgens did not defeminize adult female-typical behaviors (Phoenix et al., 1983). However, two aspects of this study suggest that the results may be open to alternative interpretation. First, females do not approach gonadectomized males as much as intact males (Phoenix et al., 1973). Control females did not display any sexual solicits and had few proximity responses (Phoenix et al., 1983), suggesting that the males used were not attractive enough to elicit typical levels of female sexual behavior. Second, control females produced “threaten-away” behaviors at a much higher rate than the pseudohermaphrodites. This is a behavior that has been considered by others to be a sexual solicitation (e.g. Ball and Hartman, 1935; Michael and Zumpe, 1970; Wallen et al., 1984). If these threats were, in fact, solicitation behaviors then this experiment could be interpreted as showing defeminization of adult sexual behavior. Further work by Phoenix et al., (1984) emphasized that prenatal testosterone can behaviorally masculinize without completely eliminating the capacity to respond as a female under some conditions. They surgically constructed a vagina in a prenatally androgenized female that had previously shown ejaculatory behavior with a female partner (the pseudohermaphrodite had been gonadectomized and treated with testosterone propionate). When treated with estradiol, the pseudohermaphrodite permitted penile intromission from a male partner. Although this shows that prenatal androgens in rhesus monkeys do not eliminate the capacity to show female-typical behaviors, still it is possible that prenatal androgens defeminize by decreasing the *likelihood* that female-typical behaviors will be displayed.

Subsequent studies showed more clearly that prenatal androgens defeminize rhesus adult sexual behavior (Thornton 1983; Thornton and Goy, 1986). Prenatally androgenized and nonadrogenized females were gonadectomized, treated with estradiol in adulthood, and tested with gonadally intact males in pair tests. All animals had been raised in more complex social environments and were less aggressive than the pseudohermaphrodites used by Phoenix et al. (1983), which allowed for the testing with normal (rather than gonadectomized) males. Prenatal androgens had organizational effects on proceptivity, illustrated by a decrease in sexual solicitations by pseudohermaphrodites compared to control females. Although other female-typical behaviors also tended to be lower in the prenatally androgenized females, there were no statistically significant differences in behaviors such as sit close or groom. No clear effect of prenatal androgen on receptivity were seen as measures such as frequency of present or the proportion of sex contacts presented to did not differ significantly between prenatally androgenized and nonadrogenized females. Although there were no differences in receptivity, prenatal androgens may have decreased attractivity slightly. Adult males mounted the pseudohermaphrodites less, and intromissive and ejaculatory behaviors were significantly decreased although the males showed comparable levels of directed approach, sex contact, and proportion of presents contacted towards pseudohermaphrodites and control females (Thornton 1983; Thornton and Goy, 1986). It appeared that the prenatal androgens primarily decreased the sensitivity of the pseudohermaphrodites and males to the activational effects of estradiol in adulthood. There were no clear differences between gonadectomized males and females in female-typical behaviors in adulthood under no-hormone conditions. All

behaviors were shown infrequently by both sexes. In this case, estradiol enhanced female-typical behaviors in gonadectomized females but not in pseudohermaphrodites or males (Thornton and Goy 1986).

Similar effects of prenatal androgens on defeminization were found when pseudohermaphrodites were tested in a situation that allowed the experimental animals more control over sociosexual interactions. Adult pseudohermaphrodites, females, and males were gonadectomized, treated with estradiol, and tested in a relatively large room with a tethered stimulus stud male (Pomerantz et al., 1985). Using slightly different behavioral endpoints, defeminization of proceptive behaviors was again seen. The pseudohermaphrodites showed significantly lower approach rates, solicitation rates, and proximity scores than control females. Their solicitation rates were not significantly different than the low levels shown by gonadectomized males. These differences were apparent both before the first approach and for the whole test (Pomerantz et al., 1985). Prenatal androgen treatment also defeminized the attractivity of the females somewhat. Tethered stimulus-males showed a significantly lower rate of contact and mount to the prenatally androgenized females compared to control females. On the other hand, receptivity was measured using an ‘acceptance score’ (proportion of presentations that resulted in a contact) and did not vary significantly with prenatal treatment.

Role of aromatization in the organization of adult behavior

Aromatization of androgen to estrogen is not critical for differentiation of sexual behavior to occur in the rhesus monkey. Both aromatizable and nonaromatizable androgens masculinize and defeminize the adult sexual behavior of rhesus monkeys (Goy, 1981; Thornton, 1983; Pomerantz et al., 1985, 1986, 1988; Thornton and Goy, 1986). Pomerantz and colleagues (1986, 1988) tested pseudohermaphrodites that had been treated with either dihydrotestosterone or testosterone prenatally and found that both groups showed equivalent levels of masculinization. That is, there were no differences between them when they were injected with testosterone in adulthood and tested with a female for male-type behaviors. Analogously, when females were prenatally androgenized with either testosterone or dihydrotestosterone, given estradiol in adulthood, and tested with a stimulus male for female-type sexual behaviors (Thornton, 1983; Pomerantz et al., 1985; Thornton and Goy, 1986), the two groups of prenatally androgenized females were both defeminized to a comparable extent.

Summary

Prenatal androgen administration both masculinized and defeminized adult sexual behaviors. Courtship and copulatory behaviors were masculinized and proceptive behaviors were defeminized. Attractiveness was also somewhat masculinized and defeminized. Aromatizable and nonaromatizable androgens were equally effective.

Prenatal androgens and rhesus monkeys: Conceptual issues and conclusions

Clearly prenatal androgens have organizational effects on sexually dimorphic behaviors in the rhesus monkey (Table 2). The 1959 paper by Young and colleagues (Phoenix et al., 1959) was extraordinarily prescient. They anticipated much of the research that followed, both in primate and nonprimate species. In that paper, Phoenix and colleagues included a number of related conceptual issues that have continued to be important in the study of prenatal androgens and rhesus monkeys. Continued exploration of these issues in the research described above leads to the following conclusions:

1. The effects of prenatal androgens are permanent. Whether rhesus monkeys that are exposed to prenatal androgens are tested at 2 mo or 15 years of age, behavioral effects continue to be expressed.

2. Androgens act prenatally. Similar to the guinea pig, the most sensitive time in rhesus monkeys for exposure to these morphogenic androgens is during the prenatal period of development. This is not true for many other species, for which the perinatal period is important and species differences may be related to how developed the species is at birth (Wallen and Baum, 2002). Early work used the term “critical period” to describe the time during development in which androgens organize the neural substrate underlying sexually dimorphic behavior. Later work has suggested that the term ‘maximally sensitive period’ is more appropriate for the effects of hormones on neurodevelopment (e.g. Goy and McEwen, 1980), because the brain retains some sensitivity to hormones through later development. More recently it has been suggested that there may be additional times after the perinatal period that are periods of ‘organization’ as steroid hormone exposure during adolescence may further shape the expression of a variety of adult behaviors (for review, Romeo et al., 2002; Sisk and Zehr, 2005; Sisk, 2009 this volume).

3. Behavioral sex differences do not result solely from masculinized genitalia. Theoretically, there are two ways that masculinized genitalia could cause masculinized behavior. One is that other monkeys see male-type external genitalia and treat the pseudohermaphrodite as a male. Consistent with this, prenatal androgens appear to decrease attractivity. Two is that the penis enhances masculine behavior (like mounting and intromission) through sensory feedback. Consistent with this, motor neurons for ejaculation are sexually dimorphic in rodents and humans and require androgens during early development to prevent apoptosis (see Morris et al., 2004). Neither of these ways that genitalia could affect behavior seems to be deterministic: even prenatally androgen-treated females that showed no masculinization of their external genitalia showed masculinization of juvenile behavior. However, it is still possible that the structure and organization of the external genitalia may play a small role in the expression of male-typical adult sexual behavior. Relevant to this, Beach was known to say “You can’t be a carpenter without a hammer”. At least, a hammer may make it easier.

4. Prenatal androgens organize the neural mechanisms that underlie sexually dimorphic behaviors. Although the precise neural effects of prenatal androgens have not been directly tested in rhesus monkeys, there is a plethora of convincing evidence in other species that these hormones act on the brain to affect behavior (e.g. Gorski, 1991; Morris et al., 2004; McCarthy, 2008).

5. Prenatal androgens affect not only sexual but also non-sexual behaviors. Like other species, rhesus monkeys show dimorphisms in many behaviors that are not directly related to reproduction. Indeed, the first behaviors examined in masculinized pseudohermaphrodites were nonsexual play behaviors. Since then, research indicates that many, but not all, sexually dimorphic behaviors in rhesus monkeys are affected by prenatal androgens.

6. Prenatal androgens don’t just affect sensitivity to adult activational hormones. Clearly, differences in rough and tumble play and other juvenile behaviors cannot be due to a change in sensitivity to activational hormones as activational hormones are not needed for the display of the behaviors. For adult male-typical sexual behaviors, prenatal androgens may affect both the behaviors that are shown without hormones and the responsiveness to activational hormones. On the other hand, for

adult female-typical behaviors, prenatal androgens may only affect the sensitivity of the behaviors to adult activational hormones.

7. Prenatal androgens masculinize and defeminize rhesus monkeys. Although Phoenix et al. (1959) used the term “masculinization” for both the increase in male-type and decrease in female-type behaviors in guinea pigs, it was later proposed that these androgens act on independent neural primordia to enhance (masculinize) male-typical behaviors and suppress (defeminize) female-typical behaviors (Whalen, 1974). It was suggested that although generally one neural system develops and the other regresses, they can be affected independently by the type, amount and/or timing of the administration of perinatal androgenic hormones (Goldfoot et al., 1969; Goldfoot and van der Werff ten Bosch, 1975; Davis et al., 1979). Similar to other species, prenatal androgen exposure can both masculinize and defeminize rhesus monkeys. However, some behaviors are more, and others are less dependent on prenatal androgens.

8. The timing of sensitivity to prenatal androgens varies for different behavioral endpoints. During gestation, the basic connections of the neural circuits that contribute to the expression of juvenile and adult behaviors are formed in a complex process that occurs over time. Sensitive periods for the development of different behaviors can be thought of in terms of the development of the underlying neural circuits (Knudsen, 2004). Perhaps instead of two ‘neural primordia’ (one for male-typical behaviors and one for female-typical behaviors), there are many neural circuits, each of which underlies an individual behavior and develops at a different time during gestation. The presence or absence of androgens may act to organize each of those circuits in a male-typical or female-typical direction independently of the others. Alternatively, or perhaps in addition to this possibility, prenatal androgen exposure may act on neural areas responsible for more general brain functions such as motivational or reinforcing circuits that influence a variety of specific behaviors. These then may influence both the type of behavior shown and under what circumstances it is shown. Although the precise mechanism for the neural organization of primate behaviors is currently unknown, it is likely that the androgens modify basic developmental processes such as neuron migration, cell differentiation, apoptosis, and/or synaptic proliferation in this species as in rodent species.

9. Social environment is very important. Hormones do not act in a vacuum. The social environment of both rearing and behavioral testing interacts with the expression of behavior, and thus whether sex differences in behavior are observed in rhesus monkeys. When there is a lack of opportunity for extensive social interactions during development, social and affiliative behaviors tend to decrease and aggressive/threat behaviors increase. Social constraints such as rank or the number of available social partners also affect the expression of behavior during behavioral testing and observation. It is assumed that the effects of these social factors on behavior are mediated by the brain. One interesting possible mechanism for some of the effects of early social rearing on later sexually dimorphic behaviors is epigenetic programming. Recent work in rodents indicates that epigenetic mechanisms may play a role in a diverse set of functions including learning and memory processes, the effect of maternal behaviors on infant stress responsiveness, and circadian rhythms (Weaver et al., 2004; Jiang et al., 2008).

10. Aromatization is not essential in the rhesus monkey. A nonaromatizable androgen such as dihydrotestosterone is as effective as the aromatizable androgen testosterone. This contrasts with many nonprimate species for which aromatization of testicular androgens to estrogens is considered critical for defeminization and/or

masculinization to occur (e.g. Wallen and Baum 2002; McCarthy 2008). Interestingly, the guinea pig is similar to the rhesus monkey in that aromatization may not be essential. For example, prenatal administration of the nonaromatizable androgen dihydrotestosterone masculinizes adult mounting behavior (Goldfoot and van der Werff ten Bosch (1975).

The rhesus monkey has indeed proven to be an attractive, informative model that has provided a wealth of information. Although the rhesus monkey is a good nonhuman primate model, it should be emphasized that it is only a model. Rhesus monkeys are part of a superfamily that has extensive variability. There are six superfamilies of primates that comprise approximately 58 genera with approximately 250 species (see Dixson 1998). Among these species, the mating systems and behaviors are diverse and complex. The mating systems include monogamy, polygyny, polyandry, and promiscuity. In many species, the males and females of a species have mating seasons. Often the females may be sexually receptive for only a brief period. Although sex differences in behaviors have been extensively noted (Dixson, 1998), very little work has been done looking at the effects of prenatal androgens on sexually dimorphic behaviors of nonhuman primates other than the rhesus monkey. However, the small amount that has been done is consistent with the rhesus model (e.g. effects of prenatal androgens on sexually dimorphic play patterns in the Japanese macaque, Eaton et al., 1990).

Final comments

Relevance to humans

Young and his students first extended their studies of the effects of prenatal androgens to rhesus monkeys, because they were interested in a species that is more similar to humans than are rodents. The results from their early primate work were consistent with their organizational hypothesis. When Young et al. published their first results on juvenile behavior in prenatally androgenized rhesus monkeys (1964), they suggested that one implication of their studies was that hormonal actions on the developing brain could "provide a model to which we may look for a reexamination of the psychosexual incongruities" being reported over the previous twenty years in humans (Ellis, 1945; Money et al., 1955, 1957). While acknowledging the importance of experience, they argued that this "need not lead to a rejection of a predetermined psychosexuality for the concept of a psychologic sexual neutrality at birth."

Since then, accumulating evidence has argued for a significant role for organizational effects on both juvenile and adult behavior in humans (see Hines, 2004 for a review). For example, there are numerous sexually dimorphic behaviors in human children, that are similar to behaviors organized by androgens in young rhesus monkeys, including rough and tumble play (Harlow, 1965; DiPietro, 1981; Humphreys and Smith, 1984), playmate choice (Hines, 2004), and interest in young infants (Herman et al., 2003; Leveroni and Berenbaum, 1998). And several of these types of behaviors have been reported to be altered in the direction of male patterns in human females exposed to high levels of androgens prenatally (Ehrhart and Mayer-Bahlburg, 1981, Dittman et al., 1990, Hines, 2004, Pasterski et al., 2008).

Future directions

There are still many exciting avenues to explore related to the organizational role of prenatal androgens on behavior. Not surprisingly, research in nonprimate species such as rodents has progressed much farther than the research on rhesus monkeys. For rodents, we know much more about the brain areas as well as the neurotransmitters and neural pathways involved in sexual differentiation than we know for rhesus monkeys. We are beginning to understand the role of growth factors, apoptosis, neurite growth, and synaptic patterning. The ability to

use techniques on rodents such as antisense technology, conditional knockouts, and other molecular genetic techniques has helped to advance this knowledge. Still there is much to learn. Scientists are continuing to gain important information from rodents and other nonprimate species about the cellular and molecular mechanisms through which perinatal hormones induce the sexual differentiation of the developing brain.

And yet, despite the limitations on what can be done, the nonhuman primate continues to be an important model for translating basic research in rodents to an understanding of normal and atypical developmental processes in humans. Similar to humans, in rhesus monkeys both males and females can and do show the full panorama of sexual and social behaviors. Even so, there are differences between the sexes in how often they show particular behaviors. There are potentially exciting questions to be answered: What brain areas and neurotransmitter systems are sensitive to the actions of prenatal androgens in primates? Do prenatal androgens alter brain plasticity later in life, and if so how? Through what mechanisms do prenatal hormones bias the choices toward showing one set of behaviors more than another? Do they affect sex differences in cognitive and/or affective processes? Can imaging studies be used to identify an anatomical structure or functional response that tracks the developmental emergence of sex differences in behavior? During development, do gonadal hormones alter specific DNA methylation or histone acetylation patterns in the brains of primates? How might experience or social factors interact with the organizational effects of gonadal hormones? The answers to these questions will help us to gain insight into the biological basis and development of sex differences. Once we more fully understand sexual differentiation of brain and behavior, this may also lead to important insights into related areas such as gender-biased susceptibility to certain neurodevelopmental mental disorders (e.g. autism, schizophrenia, etc.). Moreover, clinical research into the etiology of sex-biased psychopathology may provide new developmental hypotheses to be addressed in rodent and primate models.

When discussing claims in science, Wilson (1998) states that they ascend a scale of credibility from “interesting” to “suggestive” to “persuasive” and finally “compelling”, and, that given enough time thereafter, the claims become “obvious”. The work that was set in motion by Phoenix and his colleagues in 1959 with the birth of the organizational hypothesis has had a tremendously large and broad impact on how we view sexuality and the sexually dimorphic behaviors of nonhumans and humans. Textbooks no longer assert that human sexuality is completely malleable. It has become standard for college textbooks on brain and behavior to present the nonprimate work, the early monkey work, and the concordant human data as evidence for the reality and importance of the organizing effects of prenatal androgens on human and nonhuman behavior (e.g. Carlson, 2007; Bear et al., 2007). It has become “obvious”. Yet, more remains to be elucidated. Almost 30 years after the ground-breaking paper by Phoenix et al, (1959), Goy (1988) said “I believe that the full story of the infinite precision of the hormonal control of psychosexuality has not yet unfolded. But I remain optimistic that the full story is worth pursuing.” We believe that this continues to hold true today.

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Table 1

Multiple studies and experiments have been conducted with the five main cohorts of animals with prenatal androgen manipulations. This table lists the first report for each experimental cohort and links subsequent publications to that cohort. Links determined by explicit references within the methods sections, matching treatment periods and doses, and/or by matching animal identification numbers listed in publications. Note that studies using the different cohorts were published concurrently over multiple decades.

Cohort	First Report	Hormone Manipulation	Treatment Period (Gestational Days)	Additional Publications describing Cohort
Proof of Principle	Young et al., 1964	Testosterone Propionate (TP)	d40–90 (n=2)	Phoenix et al., 1968
Cohort 1	Phoenix et al., 1968 and Goy, 1968	Testosterone Propionate	d40–70 (n=3) d40–90 (n=4) d40–111 (n=1)	Goy, 1964† Goy, 1970a Goy, 1970b Eaton et al., 1973 Goy, 1974 Goy & Goldfoot, 1974 Steiner et al. 1976 Wilen et al., 1977 Phoenix et al., 1983 Phoenix et al., 1984
Cohort 2	Goy, 1981	Testosterone Propionate Dihydrotestosterone Propionate (DHTP)	TP: d40-[95–120] (n=9) d40–75 (n=1) d40–55 (n=7) d50–64 (n-3) d60–74 (n=3) d40–65 (n=4) DHTP: d40-[95 to 110] (n=9)	Goy & Robinson, 1982 Gibber & Goy, 1982‡ Thornton, 1983 Pomerantz et al., 1985 Thornton & Goy, 1986 Pomerantz et al., 1986 Goy et al., 1988b Pomerantz et al., 1988 Eisner et al., 2002 Eisner et al., 2003 Zhou et al., 2005
Cohort 3	Goy et al., 1988a	Testosterone Propionate	d40–64 (n=7) d115–139 (n=7)	
Mix of Cohorts 2 and 3	Includes animals created by Goy and colleagues still living in the colony at the Wisconsin National Primate Research Center	Testosterone Propionate	See above, varies by study.	Abbott et al., 1997 Dumesic et al., 1997 Abbott et al., 1998 Eisner et al., 2000 Dumesic et al., 2002 Dumesic et al., 2003 Bruns et al., 2004* Abbott et al., 2005 Zhou et al., 2007 Bruns et al., 2007 Abbott et al., 2008b
Cohort 4	Herman et al., 2000	Testosterone Enanthate (TE) Flutamide DMSO Vehicle	TE: d[35 or 40]-70 (n=6♀, n=6♂) d[110 or 115]-145 (n=7♀, n=5♂) Flutamide: d[35 or 40]-70 (n=7♀, n=7♂) d[110 or 115]-145 (n=7♀, n=6♂) Vehicle: combined d[35 or 40]-70 and d[110 or 115]-145 (n=7♀, n=7♂)	Tomaszycki et al., 2001 Herman et al., 2003 Zehr et al., 2005 Tomaszycki et al., 2005 Herman et al., 2006 McFadden et al., 2006 Herman et al., 2007 Wallen et al., 2005 Wallen & Zehr, 2004§
Cohort 5	Abbott et al., 2008a	Testosterone Propionate Sesame Oil	TP: d40–80 (n=9) Oil: d40–80 (n=6)	

†Review does not describe the cohort, but has a good photograph of female #829.

[‡] Publication reports on DHTP females only.

[*] Publication reports concurrent treatments of prenancies with male offspring, likely from Cohorts 2 & 3

[§] Publication reports on vehicle control females only.

Table 2

Summary of the effects of prenatal androgens on selected behavioral and structural endpoints incorporating the role of aromatization and the timing of the prenatal treatment.

Endpoint	**Organizational Effect Masculinized (M) Defeminized (D) Unaffected (U)**	**Type of Prenatal Androgen Aromatizable (A) Non-aromatizable (N)**	**Effective Prenatal Treatment Period Early - Before d84 Late - After d84**
External Genitalia	M and D	A or N	Early only
Juvenile Behaviors			
Infant Interest	U	A or N	Early or Late
Mounting Behaviors	M	A or N	Early or Late
Rough Play	M	A or N	Late only
Vocalizations	M and D	A (N not tested)	Early or Late
Adult Behaviors			
Mounting Behaviors	M	A or N	Early + Late (not tested separately)
Presenting Behaviors	U	A or N	Early + Late (not tested separately)
Proceptive Behaviors	D	A or N	Early + Late (not tested separately)