Sex Hormones and Brain Development

What goes on early in development when sex hormones act on the brain?

A recent symposium on sex hormones and behavior was opened by E. J. Sachar of Columbia University, who posed what he described as "the most naïve and simpleminded questions." To what extent, he asked, and by what mechanisms and under what conditions do hormones play a role in determining human sexual behavior? And if we do not know exactly, how can we find out?

Sachar's questions are, of course, politically loaded. The arguments over sex hormones and human behavior have been compared to the arguments over race and I.Q. But because it is impossible to do definitive experiments with humans, all speculations that sex hormones influence human sexual behavior ultimately are defended by analogies with animals.

A major problem in making these analogies is that it is very hard to say just what is "masculine" or "feminine" human behavior. For example, psychologists have defined masculine girls as those who are "tomboys" and want careers rather than marriage, a definition that seems not only derogatory but highly dubious as well. In contrast, animal sexual behavior is well defined. It includes copulatory posture in rodents, urination posture in rodents, dogs, and monkeys, juvenile play patterns in monkeys, and maze learning and avoidance learning in rats.

The animal studies indicate that there is a period early in life, termed the critical period, when exposure to sex hormones irreversibly alters the animals' brains and subsequent sexual behavior. For example, it was found 20 years ago that an adult female rat who was given male sex hormones in the first few days of life will not exhibit the typical mating stance, with arched back, when she is approached by a male. She will not ovulate, and, if given the male sex hormone testosterone, she will mount other females. An adult male rat who was castrated early in life (and thus deprived of male sex hormones) will assume the female mating posture when given estrogen and will not mount females even when he is given testosterone.

Many investigators believe that humans, too, go through a period early in development when sex hormones act on their brains to irreversibly alter behavior. The suspicion is that if humans do have such a period, it occurs before birth. The critical period for differentiation of animal brains ends at ages apparently unique to each species. In rats this occurs within the first week of life and in monkeys, whose development more closely resembles that of humans, before birth.

This is the third of a series of occasional articles on recent developments in neurobiology. The first story dealt with the search for new psychoactive drugs (24 August, p. 774). The second dealt with brain peptides (31 August, p. 886). A future article will discuss the development of the nervous system.

What is known about sex hormones and human sexual behavior comes from a few medical experiments and so-called experiments of nature—studies of biochemical abnormalities that cause fetuses to have unusual hormonal exposures. For example, some women took sex hormones, such as diethylstilbestrol (DES) or progestins, early in pregnancy in the hope of preventing spontaneous abortions. This treatment, which turned out to be medically ineffective, seems to have had no effect on the children's sex-specific behavior.

Some of the experiments of nature seem more likely to indicate whether sex hormones alter human behavior. One of the best-studied of these is congenital adrenal hyperplasia, an enzyme defect of the adrenal cortex that results in the production of large amounts of androgens. Affected persons are exposed to enormous quantities of male sex hormones during fetal life. After birth, the child can be medically treated to correct the hormonal abnormalities.

John Money of Johns Hopkins University and Anke Ehrhardt of Columbia University have followed children with congenital adrenal hyperplasia from infancy to puberty. They conclude that girls born with this disorder are extreme tomboys, more masculine than expected in their play and in their aspirations. They spend little time at maternal role rehearsal—playing with dolls, for example. They do ovulate, however, and they do consider themselves female. Since the oldest of the girls are still teenagers, it is not known whether they will marry or have children. Boys with this syndrome are not significantly different from other boys in their behavior.

These studies of girls with adrenal hyperplasia seem to agree with studies of female monkeys exposed to male sex hormones during fetal life. About 10 years ago, Robert Goy of the University of Wisconsin found that these monkeys behaved much like male monkeys, going in for the kind of wrestling and jumping on each other that is called rough-and-tumble play and that is typical of males. The female monkeys ovulated but could not mate because they had no vaginas. (Girls with adrenal hyperplasia have very small vaginas that must be enlarged before they can have intercourse.) The conclusion from this research is that the female monkeys are "masculinized" but not "defeminized" by the hormones. That is, a male behavior pattern is superimposed on a female one.

But Goy cautions that hormones can have different effects depending on when and in what concentration they are present. The monkey critical period lasts for several months of fetal life. Goy found previously, for example, that if he treated female fetuses for 2 months to masculinize them, the monkeys, when they were born, would exhibit certain masculine behaviors such as mounting their mothers and their peers. (Mounting their mothers is a way of expressing emotional ties. Mounting peers is a way of asserting position in a dominance hierarchy.) But he now finds that if he treats the female fetuses for only 15 days, they will mount their mothers but not their peers. The point of all this is that until it is known what effects the hormones have at different times during the critical periods, it may be misleading to compare monkeys and humans.

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Until recently, the psychologists' interpretation of a few case histories was that even if sexual behavior is influenced by hormones, sexual identity is socially determined. A child will identify with whatever sex he or she is reared to be. But results from a recent study led by endocrinologist Julianne Imperato-McGinley of Cornell Medical School are causing some researchers to question this belief.

Imperato-McGinley studied 38 males in two small towns in Santo Domingo who have a peculiar enzyme deficiency. Because they cannot metabolize testosterone to dihydrotestosterone, their external genitals looked female at birth. (Testosterone must be metabolized in the skin of the precursors of the fetal genitals in order for the male external genitals to be formed.) At puberty, however, the boys make testosterone normally. Their testes descend, their clitorises grow into penises, their voices deepen, and they develop male musculature. Seemingly, they are transformed from "girls" to men.

This very rare genetic disorder is fairly common in the two Santo Domingo towns because the populations are inbred. Sporadically, according to Imperato-McGinley, cases of the disorder have turned up in other societies, including the United States. Then doctors have castrated the boys at puberty and hormonally and surgically changed them to "girls," assuming that their sexual identities would be female because they were reared as girls. In Santo Domingo, such sex-change operations are not available. What Imperato-McGinley found is that the affected children in these towns took on male sexual identities at puberty, even though they were reared as girls. She postulates that their prenatal exposure to testosterone irreversibly shaped their sexual behavior.

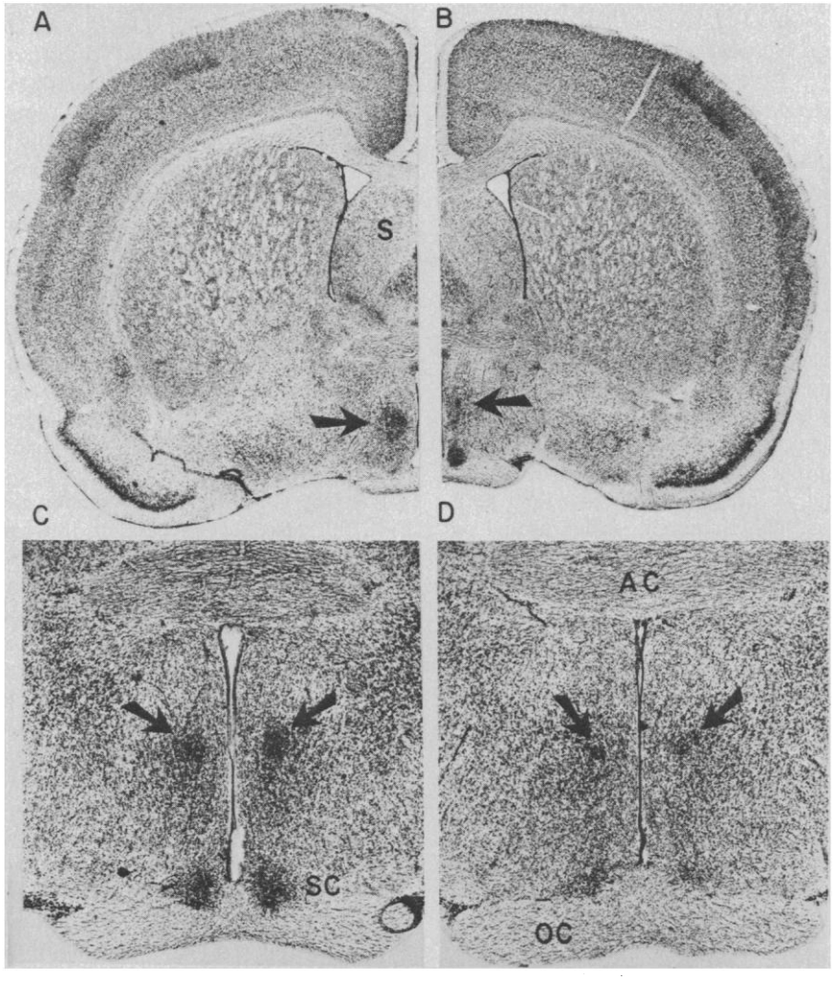
These conclusions are still controversial. Ehrhardt and others have criticized them on the grounds that the children and their families may have had some doubts about the childrens' sexual identities when they were growing up. Thus the children may not have felt themselves to be normal girls.

First of all, the children have enlarged clitorises, which may make them and their families question their sexual identities. Second, the families who tend to have children with this disorder are known in the communities, which may make any of their female children suspect.

Imperato-McGinley responds by saying that she and her associates interviewed older people who have the genetic disorder and who were born before the disorder was recognized in the town. They, too, have male sexual identities. Neither they nor their parents said they suspected any abnormalities when these people were growing up. And, she says, there are a few people from other countries, including France, England, and Italy, who have the disorder, whose sex was not surgically changed at puberty, and who have male sexual identities. Thus the social factors in Santo Domingo may not have determined the behavior of the people Imperato-McGinley studied.

It is a large jump from animal studies to studies of human behavior, especially since the human studies involve such imprecise measures as personality. Yet researchers hope to someday make a connection, suspecting that some basic principles may universally apply. Roger Gorski, an animal physiologist at the University of California at Los Angeles, says he believes the central fact is that mammals, birds, and almost certainly humans go through a critical period when sex hormones exert permanent effects on brain structure and function. So by understanding what goes on during this period in animals, researchers may have some hope of understanding human sexual behavior.

Since effects of sex hormones on behavior were first recognized in animal experiments of the 1950's and 1960's, biologists have suspected that male and female brains differ chemically as a result of these hormones. But it was not until 1973 that Geoffrey Raisman and Pauline Field of the National Institute of Medical Research at Mill Hill, England, reported differences in the nerve connections between male and female animal brains. Using an electron microscope, Raisman and Field found sex-related differences in the distribution of synaptic connections of nerve cells in the preoptic



Visible differences in the brains of male and female rats. Arrows indicate the sexually dimorphic nucleus in brains of male (A and C) and female (B and D) rats. Sections are 60 μm thick and are stained with thionine. AC, anterior commissure; OC, optic chiasm; S, septum; SC, suprachiasmatic nucleus. [Source: R. A. Gorski, Neurosciences Research Program Bulletin, *vol. 17, No. 5, in press]*

area of rat brains, which is adjacent to the hypothalamus. Then they showed that when newborn rats are castrated during the critical period, but not later, they develop a female pattern of synaptic connections. And when newborn females are given testosterone during the critical period, they develop a male pattern of synaptic connections.

Shortly after this discovery, William Greenough and his associates at the University of Illinois also reported similar differences in the preoptic area and in the suprachiasmatic nucleus of male and female hamster brains. They saw these differences with a light microscope. Recently, L. W. Christiansen and Gorski found sex differences in the preoptic area of rat brains that are visible to the naked eye. Part of this area, which they call the sexually dimorphic nucleus, is five times larger in the male than in the female. If males are castrated during the critical period, their sexually dimorphic nuclei are much smaller. If females are given testosterone as newborns, their nuclei increase in volume.

"It is ironic that these studies [of morphological sex differences in the brain] went from the most complicated and laborous technique [electron microscopy] and then became progressively simpler," says animal physiologist Bruce McEwen of Rockefeller University. "Maybe people were afraid to look and didn't believe they could see anything."

Now that it is clear that there are morphological differences in the brains of male and female rats, it is not surprising that these differences are seen in the preoptic area, which is full of receptors for estrogen. Donald Pfaff of Rockefeller University and Walter Stumpf of the University of North Carolina found that radioactively labeled estrogen binds mainly to the preoptic area, the hypothalamus, and the amygdala in rat brains. Then Pfaff and Joan Morrell showed that the pattern of estrogen binding to brain neurons is remarkably constant in amphibians, fish, birds, and mammals. The areas where estrogen binds have long been known to play a role in sexual behavior.

In addition to knowing that there are sex differences in brains, researchers want to know which hormones cause these differences and how. In rats, at least, there is good evidence that the key hormone is estrogen. But, surprisingly, estrogen causes male, not female, brain development. This is because newborn female rats are protected from the effects of estrogen in their blood by α-fetoprotein, a protein made by the fetal liver and present in diminishing quantities for the first 3 weeks of life. The protein binds estrogen and prevents it from reaching the animal's tissues. But α-fetoprotein does not bind testosterone, which the newborn male rats synthesize. Thus testosterone reaches the brain cells of newborn males, where it is converted to estrogen and dihydrotestosterone. The estrogen alters the animal's brain to permit male sexual behavior; the dihydrotestosterone seems not to alter brain development. For example, if female rats are given high doses of estrogen during the critical period, they will be masculinized. But they will not be masculinized if they are given dihydrotestosterone.

There is still some question about whether the rat results extend to other species. Animals whose critical periods for brain sexual differentiation end before birth have α-fetoproteins that do not bind estrogens. No one knows what, if anything, protects those animals from estrogen. McEwen points out that there is a placental barrier to estrogen and speculates that it might serve the same function as α-fetoprotein in rats. Or it could be that testosterone and estrogen separately affect these animals' brains.

To understand how estrogen causes sexual differentiation in rat brains, neurobiologists are looking at where the estrogen receptors are and how they may function during the critical period. Apparently, there are only small numbers of estrogen receptors in the brains of both male and female fetal rats until a few days before birth. The estrogen receptors increase rapidly in number about 2 days before birth and continue to increase until 4 or 5 days after birth (the end of the critical period).

According to McEwen, the estrogen receptor system in rat brains may not be hooked up to normal cell functions during the critical period. For example, he finds that he cannot induce progestin receptors on estrogen-containing nerve cells during that time. Normally, when nerve cells containing estrogen receptors are exposed to estrogen, progestin receptors subsequently appear on their surfaces.

McEwen also finds that there is a high concentration of estrogen receptors in the cerebral cortex during the critical period but that these receptors start to disappear after 14 days. This finding is especially intriguing because of the function of the cerebral cortex: it is the highest neural center, the area where speech, hearing, thinking, and consciousness are controlled. The possibility exists that sex differences in learning, at least in rats, and cognitive functions may have their origins in effects due to these transient estrogen receptors, since only in male rats is estrogen accessible to these receptors.

These estrogen receptors also are intriguing precisely because they are transient. "It would be interesting to know whether the estrogen receptors in the cerebral cortex disappear because the cells that contain them disappear or because the receptors disappear from the cells," McEwen says. During fetal life, far more neurons are produced than survive. A fundamental process in brain development is the selective death of nerve cells.

Gorski speculates that hormones may prevent cell death during the critical period. Investigators who studied the "birth dates" of neurons (the days when particular neurons stop dividing) found that cells of the preoptic and hypothalamic area of rat brains have birth dates 10 days before the critical period. Gorski is now determining the birth dates of neurons in the sexually dimorphic nuclei of rat brains, which are contained in the preoptic areas. He says he expects that the nerves of the sexually dimorphic nuclei will also have birth dates 10 days before the critical period. If so, estrogen probably does not cause these nerves to divide. An obvious alternative is that it prevents cell death.

It is thought that nerve cells die when they fail to contact other cells during brain development. Estrogen may increase nerve cells' contacts by causing the cells to enlarge and making it more likely that they contact other cells. Dominique Toran-Allerand took tissue from the preoptic area and the hypothalamus of fetal mice and cultured it in the laboratory for several weeks. She and McEwen found that the cells containing estrogen receptors respond to estrogen and testosterone during a period corresponding to the critical period for sexual differentiation. And the cells respond by enlarging and sending off tendrils.

The result of this neurobiology research is that a clearer picture is beginning to emerge of how the sex-related differences in rat brains, at least, occur. Whether these exact mechanisms have counterparts in human brain development is still up in the air. But many investigators would agree with Gorski, who says, "I always take the position that if we can understand sexual differentiation in the rat, certain concepts will apply to humans."

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Additional Readings

1. *Neurosci. Res. Program Bull.* **17**, No. 5, in press.
2. *Sex, Hormones and Behavior*, Ciba Foundation Symposium 62 (Excerpta Medica, Amsterdam, 1979).