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Neurobiology of Gender Identity and Sexual Orientation

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Abstract

Sexual identity and sexual orientation are independent components of a person's sexual identity. These dimensions are most often in harmony with each other and with an individual's genital sex, but not always. This review discusses the relationship of sexual identity and sexual orientation to prenatal factors that act to shape the development of the brain and the expression of sexual behaviors in animals and humans. One major influence discussed relates to organizational effects that the early hormone environment exerts on both gender identity and sexual orientation. Evidence that gender identity and sexual orientation are masculinized by prenatal exposure to testosterone and feminized in it absence is drawn from basic research in animals, correlations of biometric indices of androgen exposure and studies of clinical conditions associated with disorders in sexual development. There are, however, important exceptions to this theory that have yet to be resolved. Family and twin studies indicate that genes play a role, but no specific candidate genes have been identified. Evidence that relates to the number of older brothers implicates maternal immune responses as a contributing factor for male sexual orientation. It remains speculative how these influences might relate to each other and interact with postnatal socialization. Nonetheless, despite the many challenges to research in this area, existing empirical evidence makes it clear that there is a significant biological contribution to the development of an individual's sexual identity and sexual orientation.

Introduction

Gender identity and sexual orientation are fundamental independent characteristics of an individual's sexual identity (1). Gender identity refers to a person's innermost concept of self as male, female or something else and can be the same or different from one's physical sex (2). Sexual orientation refers to an enduring pattern of emotional, romantic, and/or sexual attractions to men, women, or both sexes (3). Both gender identity and sexual orientation are characterized by obvious sex differences. Most genetic females identify as such and are attracted to males (i.e., androphilic) and most genetic males identify as males and are attracted to females (i.e., gynophilic). The existence of these dramatic sex differences suggest that gonadal hormones, particularly testosterone, might be involved, given that testosterone plays an important role in the development of most, behavioral sex differences in other species. This article will review evidence that testosterone influences human gender identity and sexual orientation. It will begin by summarizing information on sex hormones and brain development in other species that forms the underpinnings of the hypothesis that these human behaviors are programmed by the prenatal hormone environment and will also consider contributions from genes. It will then critically evaluate evidence in humans and relevant animal models that relates sexual identity and sexual orientation to the influences that genes and hormones have over brain development.

Hormones, genes and sexual differentiation of the brain and behavior.

The empirical basis for hypothesizing that gonadal hormones influence gender identity and sexual orientation is based on animal experiments involving manipulations of hormones during prenatal and early neonatal development. It is accepted dogma that testes develop from the embryonic gonad under the influence of a cascade of genes that begins with the expression of the sex-determining gene *SRY* on the Y chromosome (4,5). Before this time, the embryonic gonad is "indifferent," meaning that it has the potential to develop into either a testis or an ovary. Likewise, the early embryo has two systems of ducts associated with urogenital differentiation, Wolffian and Müllerian ducts, which are capable of developing into the male and female tubular reproductive tracts, respectively. Once the testes develop, they begin producing two hormones, testosterone and anti-Müllerian hormone, or AMH. In rats this occurs around day 16 to 17 of gestation, while in humans it occurs at about 7 to 8 weeks of gestation (6). Testosterone and one of its derivatives, dihydrotestosterone, induce differentiation of other organs in the male reproductive system, while AMH causes the degeneration of the Müllerian ducts. Female ovaries develop under the influence of a competing set of genes that are influenced by expression of *DAX1* on the X chromosome and act antagonistically to SRY. The female reproductive tract in the embryo develops in absence of androgens and later matures under the influence hormones produced by the ovary, in particular estradiol.

Analogous processes occur during early development for sexual differentiation of the mammalian brain and behavior. According to the classical or organizational theory (7,8), prenatal and neonatal exposure to testosterone causes male-typical development (masculinization), whereas female-typical development (feminization)

occurs in the relative absence of testosterone. Masculinization involves permanent neural changes induced by steroid hormones and differs from the more transient activational effects observed after puberty. These effects typically occur during a brief critical period in development when the brain is most sensitive to testosterone or its metabolite estradiol. In rats, the formation of estradiol in the brain by aromatization of circulating testosterone is the most important mechanism for masculinization of the brain (9), but, as seen below, testosterone probably acts directly without conversion to estradiol to influence human gender identity and sexual orientation. The times when testosterone triggers brain sexual differentiation in different species correspond to periods when testosterone is most elevated in males compared to females. In rodents and other altricial species this occurs largely during the first five days after birth, while in humans the elevation in testosterone occurs between 2nd and 6th months of pregnancy and then again from 1 to 3 months postnatally (6). During these times testosterone levels in the circulation are much higher in males than in females. These fetal and neonatal peaks of testosterone, together with functional steroid receptor activity are thought to program the male brain both phenotypically and neurologically. In animal models, programming or organizing actions are linked to direct effects on various aspects of neural development that influences cell survival, neuronal connectivity and neurochemical specification (10). Many of these effects occur well after the initial hormone exposure and have recently been linked to epigenetic mechanisms (11).

The regional brain differences that result from the interaction between hormones and developing brain cells are thought to be the major basis of sex differences in a wide spectrum of adult behaviors, such as sexual behavior, aggression and cognition as well as gender identity and sexual orientation. Factors that interfere with the

interactions between hormones and the developing brain systems during gestation may permanently influence later behavior. Studies in sheep and primates have clearly demonstrated that sexual differentiation of the genitals takes places earlier in development and is separate from sexual differentiation of the brain and behavior (12,13). In humans, the genitals differentiate in the first trimester of pregnancy while brain differentiation is believed to start in the second trimester. Usually the processes are coordinated and the sex of the genitals and brain correspond. However, it hypothetically possible that in rare cases these events could be influenced independently of each other and result in people who identify with a gender different from their physical sex. Similar reasoning has been invoked to explain the role of prenatal hormones on sexual orientation.

Although the role of gonadal steroids in the sexual differentiation of reproductive brain function and behavior is undeniable, males and females also carry a different complement of genes encoded on their sex chromosomes that also influence sexual differentiation of the brain (14-16). As will be discussed, family and twin studies suggest that there is a genetic component to gender identity and sexual orientation at least in some individuals. However, the nature of any genetic predisposition is unknown. The genetic component could be coding directly for these traits or, alternatively, could influence hormonal mechanisms by determining levels of hormones, receptors or enzymes. Genetic factors and hormones could also make separate yet complementary or antagonistic contributions. It should be noted that although the early hormone environment appears to influence gender identity and sexual orientation, hormone levels in adulthood do not. There are no reports indicating androgen levels differ as a function of gender identity or sexual orientation or that treatment with exogenous hormones alters these traits in either sex.

Gender identity

The establishment of gender identity is a complex phenomenon and the diversity of gender expression argues against a simple or unitary explanation. For this reason, the extent to which it is determined by social versus biological (i.e. genes and hormones) factors continues to be debated vigorously (17). The biological basis of gender identity cannot be modeled in animals and is best studied in people who identify with a gender that is different from the sex of their genitals – in particular transsexual people. Several extensive reviews by Dick Swaab and coworkers elaborate current evidence for an array of prenatal factors that influence gender identity, including genes and hormones (18-20).

Genes. Evidence of a genetic contribution to transsexuality is very limited (21). There are few reports of family and twin studies of transsexuals but none offer clear support for the involvement of genetic factors (22-24). Polymorphisms in sex hormone-related genes for synthetic enzymes and receptors have been studied based on the assumption that these may be involved in gender identity development. An increased incidence of an A2 allele polymorphism for CYP17A1 (i.e., 17α-hydroxylase/17, 20 lyase, the enzyme catalyzing testosterone synthesis) was found in female-to-male (FtM), but not in male-to-female (MtF) transsexuals (25). No associations were found between a 5α-reductase (i.e., the enzyme converting testosterone to the more potent dihydrotestosterone) gene polymorphism in either MtF or FtM transsexuals (26). There are also conflicting reports of associations between polymorphisms in the androgen receptor, estrogen receptor beta and CYP19 (i.e., aromatase, the enzymes catalyzing estradiol synthesis) (27-29). A recent study using deep sequencing detected three low allele frequency gene mutants i.e., *FBXO38* (chr5:147774428; T>G), *SMOC2* (chr6:169051385; A>G)

and *TDRP* (chr8:442616; A>G) between monozygotic twins discordant for gender dysphoria (30). Further investigations including functional analysis and epidemiological analysis are needed to verify the significance of the mutations found in this study. Overall, these genetic studies are inconclusive and a role for genes in gender identity remains unsettled.

Hormones. The evidence that prenatal hormones affect the development of gender identity is stronger, but far from proven. One indication that exposure to prenatal testosterone has permanent effects on gender identity comes from the unfortunate case of David Reimer (31). As an infant, Reimer underwent a faulty circumcision and was surgically reassigned, given hormone treatments and raised as a girl. He was never happy living as a girl and when years later he found out what happened to him, he transitioned to living as a man. However, for at least the first 8 months of life, this child was reared as a boy and it is not possible to know what impact rearing had on his dissatisfaction with a female sex assignment (1). Other clinical studies have reported that male gender identity emerges in some XY children born with poorly formed or ambiguous genitals due to cloacal exstrophy, 5α-reductase or 17∃-hydroxysteroid dehydrogenase deficiency and raised as girls from birth (32,33). All of these individuals were exposed to testosterone prenatally emphasizing a potential role for androgens in gender development and raising doubts that children are psychosexually neutral at birth (20). On the other hand, XY individuals born with an androgen receptor mutation causing complete androgen insensitivity are phenotypically female, identify as female and are most often androphilic indicating that androgens act directly on the brain without the need for aromatization to estradiol (34).

Neuroanatomy. Further evidence that the organizational hormone theory applies to development of gender identity comes from observations that structural and functional brain characteristics are more similar between transgender people and control subjects with the same gender identity than between individuals sharing their biological sex. This includes local differences in the number of neurons and volume of subcortical nuclei such as the bed nucleus of the stria terminalis (35,36), numbers of kisspeptin and neurokinin B neurons in the infundibulum (37,38), structural differences of gray (39,40) and white matter microstructure (41-43), neural responses to sexually-relevant odors (44,45) and visuospatial functioning (46). However, in some cases the interpretation of these studies is complicated by hormone treatments, small sample sizes and failure to disentangle correlates of sexual orientation from gender identity (47). The fact that these differences extend beyond brain areas and circuits classically associated with sexual and endocrine functions raise the possibility that transsexuality is also associated with changes in cerebral networks involved in self-perception.

Sexual orientation

Research over several decades has demonstrated that sexual orientation ranges along a continuum, from exclusive attraction to the opposite sex to exclusive attraction to the same sex (48). However, sexual orientation is usually discussed in terms of three categories: heterosexual (having emotional, romantic or sexual attractions to members of the other sex), homosexual (having emotional, romantic or sexual attractions to members of one's own sex) and bisexual (having emotional, romantic or sexual attractions to both men and women). Most people experience

little or no sense of choice about their sexual orientation. There is no scientifically convincing research to show that therapy aimed at changing sexual orientation (i.e., reparative or conversion therapy) is safe or effective (3). The origin of sexual orientation is far from being understood, although there is no proof that it is affected by social factors after birth. On the other hand, a large amount of empirical data suggests that genes and hormones are important regulators of sexual orientation (49-51). Useful animal models and experimental paradigms in animals have helped frame questions and propose hypotheses relevant to human sexual orientation.

Animal studies. Sexual partner preference is one of the most sexually dimorphic behaviors observed in animals and humans. Typically males choose to mate with females and females choose to mate with males. Sexual partner preferences can be studied in animals by using sexual partner preference tests and recording the amount of time spent alone or interacting with the same or opposite sex stimulus animal. Although imperfect, tests of sexual partner preference or mate choice in animals have been used to model human sexual orientation. As reviewed comprehensively by Adkins-Regan (52) and Henley et al.(53), studies demonstrate that perinatal sex steroids have a large impact on organizing mate choice in several species of animals, including birds, mice, rats, hamsters, ferrets and pigs. In particular, perinatal exposure to testosterone or its metabolite estradiol programs male-typical (i.e., gynophilic) partner preferences and neonatal deprivation of testosterone attenuates the preference that adult males show typically. In the absence of high concentrations of sex steroid levels or receptor-mediated activity during development a female-typical (i.e. androphilic) sexual preference for male sex partners develops.

Sexually dimorphic neural groups in the medial preoptic area of rats and ferrets have been associated with sexual partner preferences. In male rats, a positive correlation was demonstrated between the volume of the sexual dimorphic nucleus of the preoptic area (SDN) and the animal's preference for a receptive female (54), although this was not replicated in a recent study (55). Furthermore, in both rats and ferrets destruction of the SDN caused males to show either neutral or androphilic preferences (56).

Naturally occurring same-sex interactions involving genital arousal have been reported in hundreds of animal species, however they often appear to be motivated by purposes other than sex and may serve to facilitate other social goals (57,58). Exclusive and enduring same-sex orientation is, however, extremely rare among animals and has only been documented conclusively and studied systematically in certain breeds of domestic sheep (59,60). Approximately 6 to 8% of Western-breed domestic rams choose to exclusively court and mount other rams, but never ewes, when given a choice. No social factors, such as the general practice of rearing in same sex groups or an animal's dominance rank, were found to affect sexual partner preferences in rams. Consistent with the organizational theory of sexual differentiation, sheep have an ovine sexually dimorphic preoptic nucleus (oSDN) that is larger and contains more neurons in female-oriented (gynophilic) rams than in male-oriented rams (androphilic) and ewes (androphilic) (61). Thus, morphological features of the oSDN correlate with a sheep's sexual partner preference. The oSDN already exists and is larger in males than in females before sheep are born suggesting it could play a causal role in behavior (62). The oSDN differentiates under the influence of prenatal testosterone after the male genitals develop, but is unaffected by hormone treatment in adulthood (63). Appropriately timed

experimental exposure of female lamb fetuses to testosterone can alter oSDN size independently of genetic and phenotypic sex (13). However, males appear to be resistant to suppression of androgen action during gestation because the fetal hypothalamic-pituitary-axis is active in the second trimester (term pregnancy ~150 days) and mitigates against changes in circulating testosterone that could disrupt brain masculinization (64). These data suggest that in sheep, brain sexual differentiation is initiated during gestation by central mechanisms acting through GnRH neurons to stimulate and maintain fetal testicular testosterone synthesis needed to masculinize the oSDN and behavior. More research is needed to understand the parameters of oSDN development and to causally relate it's function to sexual partner preferences in sheep. Nonetheless, when considered together the body of animal research strongly indicates that male-typical partner preferences are controlled at least in part by the neural groups in the preoptic area that differentiate under the influence of pre- and perinatal sex steroids.

Human studies.

Genes. Evidence from family and twin studies suggests that there is a moderate genetic component to sexual orientation (50). One recent study estimated that ~40% of the variance in sexual orientation in men is controlled by genes, while in women the estimate is ~20% (65). In 1993, Hamer published the first genetic linkage study that suggested a specific stretch of the X chromosome called Xq28 holds a gene or genes that predispose a man to being homosexual (66). These results were consistent with the observations that when there is male homosexuality in a family, there is a greater probability of homosexual males on the mother's side of the family

than on the father's side. The study was criticized for containing only 38 pairs of gay brothers and the original finding was not replicated by an independent group (67). Larger genome wide scans support an association with Xq28 and also found associations with chromosome 7 and 8 (68,69), although this has also been disputed (70). Scientists at the personal genomics company 23andme performed the only genome-wide association study (GWAS) of sexual orientation that looked within the general population (71). The results were presented at the Annual Meeting of the American Society for Human Genetics in 2012, but have not yet been published in a peer-reviewed journal. Although no genetic loci reaching genome wide significance for homosexuality among men or women, the genetic marker closest to significance was located in the same region of chromosome 8 in men as was implicated in linkage studies. Other molecular genetic evidence suggests that epigenetic factors could influence male sexual orientation although this has yet to be proven (72,73).

Hormones. The leading biological theory of sexual orientation in humans, like in animals, draws on the application of the organizational theory of sexual differentiation. However, this theory cannot be directly tested because it is not ethical to experimentally administer hormones to pregnant women and test their effect on the sexual orientation of their children. Naturally occurring and iatrogenic disorders of sex development that involve dramatic alterations in hormone action or exposure lend some support to a role for prenatal hormones, but these cases are extremely rare and often difficult to interpret (74). Despite these limitations, two clinical conditions are presented briefly that lend some support for the organizational theory. More comprehensive presentations of the clinical evidence on this topic can be found in several excellent reviews (75-77).

Women born with congenital adrenal hyperplasia (CAH) and exposed to abnormally high levels of androgens in utero show masculinized genitals, play behavior and aggression (74,78). They also are less likely to be exclusively heterosexual and report more same-sex activity than unaffected women, which suggests that typical female sexual development is disrupted. Although it seems plausible that these behavioral traits are mediated through effects of elevated androgens on the brain, it is also possible that the sexuality of CAH women may have also been impacted by the physical and psychological consequences of living with genital anomalies or more nuanced effects of socialization (79). There is also evidence for prenatal androgen effects on sexual orientation in XY individuals born with cloacal exstrophy. It was reported originally that a significant number of these individuals eventually adopt a male gender identity even though they had been surgically reassigned and raised as girls. Follow up studies found that nearly all of them were attracted to females i.e., gynophilic (33,50). The outcomes reported for both of these conditions are consistent with idea that prenatal testosterone programs male-typical sexual orientation in adults. However, effects on sexual orientation were not observed across the board in all individuals with these conditions indicating that hormones cannot be the only factor involved.

Neuroanatomy. Additional evidence that supports a prenatal organizational theory of sexual orientation is derived from the study of anatomical and physiological traits that are known to be sexually dimorphic in humans and are shown to be similar between individuals sharing the same sexual attraction. Neuroanatomical differences based on sexual orientation in human males have been found. Le Vay reported that the third interstitial nucleus of the anterior hypothalamus (INAH3) in homosexual men is

smaller than in heterosexual men and has a similar size in homosexual men and women (80). Based on its position and cytoarchitecture INAH3 resembles the sheep oSDN, which has similar differences in volume and cell density correlated with sexual partner preference. This similarity suggests that a relevant neural circuit is conserved between species. A recent review and meta-analysis of neuroimaging data from human subjects with diverse sexual interests during sexual stimulation also support the conclusion that elements of the anterior and preoptic area of the hypothalamus is part of a core neural circuit for sexual preferences (81).

Other neural and somatic biomarkers of prenatal androgen exposure have also been investigated. McFadden reported that functional properties of the inner ear, measured as otoacoustic emissions (OAEs) and of the auditory brain circuits, measured as auditory evoked potentials (AEPs) differ between the sexes and between heterosexual and homosexual individuals (82). OAEs and AEPs are usually stronger in heterosexual women than in heterosexual men and are masculinized in lesbians, consistent with the prenatal hormone theory. However, OAEs were not different in homosexual males and AEPs appear to be hyper-masculinized. The 2nd digit to 4th digit (2D:4D) ratio, which is the length of the 2nd digit (index finger) relative to that of the 4th digit (ring finger), is another measure that has been used as a proxy for prenatal androgen exposure. The 2D:4D ratio is generally smaller in men than in women (83,84), although the validity of this measure as a marker influenced by only prenatal androgen exposure has been questioned (85). Nonetheless, numerous studies have reported that 2D:4D ratio is also on average smaller in lesbians than in heterosexual women, a finding that has been extensively replicated (86) and suggests the testosterone plays a role in female sexual orientation. Similar to OAEs, digit ratios do not appear to be feminized in

homosexual men and, like AEPs, may even be hyper-masculinized. The lack of evidence for reduced androgen exposure in homosexual men (based on OAEs, AEPs and digit ratios) led Breedlove to speculate that there may be as yet undiscovered brain-specific reductions in androgen responses in male fetuses that grow up to be homosexual (86). No variations in the human androgen receptor or the aromatase gene were found that relate to variations in sexual orientation (87,88). However, Balthazart and Court (89) provided suggestions for other genes located in the Xq28 region of the X-chromosome that should be explored and it remains possible that expression levels of steroid hormone response pathway genes could be regulated epigenetically (11).

Maternal immune response. Homosexual men have, on average, a greater number of older brothers than do heterosexual men, a well-known finding that has been called the fraternal birth order (FBO) effect (90). Accordingly, the incidence of homosexuality increases by ~33% with each older brother (91). The FBO effect has been confirmed many times, including by independent investigators and in non-Western sample populations. The leading hypothesis to explain this phenomenon posits that some mothers develop antibodies against a Y-linked factor important for male brain development, and that the response increases incrementally with each male gestation leading, in turn, to the alteration of brain structures underlying sexual orientation in later-born boys. In support of the immune hypothesis, Bogaert et al. demonstrated recently that mothers of homosexual sons, particularly those with older brothers, have higher antibody titers to neurolignin 4 (NLGN4Y) an extracellular protein involved in synaptic functioning and presumed to play a role in fetal brain development (92).

Conclusion

The data summarized in this review suggest that both gender identity and sexual orientation are significantly influenced by events occurring during the early developmental period when the brain is differentiating under the influence of gonadal steroid hormones, genes and maternal factors. However, our current understanding of these factors is far from complete and the results are not always consistent. Animal studies form both the theoretical underpinnings of the prenatal hormone hypothesis and provide causal evidence for the effect of prenatal hormones on sexual orientation as modeled by tests of sexual partner preferences, but do not translate to gender identity.

Sexual differentiation of the genitals takes place before sexual differentiation of the brain making it possible that they are not always congruent. Structural and functional differences of hypothalamic nuclei and other brain areas differ in relation to sexual identity and sexual orientation indicating these traits develop independently. This may be due to differing hormone sensitivities and/or separate critical periods, but has not yet been explored. Most findings are consistent with a predisposing influence of hormones or genes, rather than a determining influence. For instance, only some people exposed to atypical hormone environments prenatally show altered gender identity or sexual orientation, while the many do not. Family and twin studies indicate that genes play a role, but no specific candidate genes have been identified. Evidence that relates to the number of older brothers implicates maternal immune responses as a contributing factor for male sexual orientation. All of these mechanisms rely on correlations and our current understanding suffer from many limitations in the data, such as: reliance on retrospective clinical studies of individuals with rare conditions, small study populations sizes, biases in recruiting

subjects, too much reliance on studies of male homosexuals and the assumption that sexuality is easily categorized and binary. Moreover, none of the biological factors identified so far can explain all variances in sexual identity or orientation, nor is it known whether or how these factors may interact. Despite these limitations, the existing empirical evidence makes it clear that there is a significant biological contribution to the development of an individual's sexual identity and sexual orientation.

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