HHS Public Access

Author manuscript

J Neuroendocrinol. Author manuscript; available in PMC 2019 August 02.

Published in final edited form as:

J Neuroendocrinol. 2018 July ; 30(7): e12562. doi:10.1111/jne.12562.

Neurobiology of gender identity and sexual orientation

C. E. Roselli

Department of Physiology & Pharmacology, Oregon Health & Science University, Portland, OR, USA

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, although not always. The present 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 behaviours in animals and humans. One major influence discussed relates to organisational effects that the early hormone environment exerts on both gender identity and sexual orientation. Evidence that gender identity and sexual orientation are masculinised by prenatal exposure to testosterone and feminised 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, although 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 socialisation. 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.

Keywords

brain; foetal development; gender identity; homosexuality; hormones; sexual differentiation; sexual orientation; sexual partner preference; sexually dimorphic nucleus; transsexuality

1 | 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

Correspondence: Charles E. Roselli, Department of Physiology & Pharmacology, Oregon Health & Science University, Portland, OR, USA. rosellic@ohsu.edu.

CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest.

characterised by obvious sex differences. Most genetic females identify as such and are attracted to males (ie, androphilic) and most genetic males identify as males and are attracted to females (ie, 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, behavioural sex differences in other species. Here, a review is provided of the evidence that testosterone influences human gender identity and sexual orientation. The review begins by summarising the available information on sex hormones and brain development in other species that forms the underpinnings of the hypothesis suggesting that these human behaviours are programmed by the prenatal hormone environment, and it will also consider contributions from genes. This is followed by a critical evaluation of the 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.

2 | HORMONES, GENES AND SEXUAL DIFFERENTIATION OF THE BRAIN AND BEHAVIOUR

The empirical basis for hypothesising 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 2 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 2 hormones, testosterone and anti-Müllerian hormone (AMH). In rats, this occurs around day 16–17 of gestation, whereas, in humans, it occurs at about 7–8 weeks of gestation.[6] Testosterone and one of its derivatives, dihydrotestosterone, induce the differentiation of other organs in the male reproductive system, whereas 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 the absence of androgens and later matures under the influence hormones produced by the ovary, in particular oestradiol.

Analogous processes occur during early development for sexual differentiation of the mammalian brain and behaviour. According to the classical or organisational theory,[7,8] prenatal and neonatal exposure to testosterone causes male-typical development (masculinisation), whereas female-typical development (feminisation) occurs in the relative absence of testosterone. Masculinisation 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 oestradiol. In rats, the formation of oestradiol in the brain by aromatisation of circulating testosterone is the most important

mechanism for the masculinisation of the brain;[9] however, as shown below, testosterone probably acts directly without conversion to oestradiol 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 5 days after birth, whereas, in humans, the elevation in testosterone occurs between months 2 and 6 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 foetal and neonatal peaks of testosterone, together with functional steroid receptor activity, are considered to program the male brain both phenotypically and neurologically. In animal models, programming or organising actions are linked to direct effects on the various aspects of neural development that influence 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 assumed to be the major basis of sex differences in a wide spectrum of adult behaviours, such as sexual behaviour, 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 behaviour. 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 behaviour.[12,13] In humans, the genitals differentiate in the first trimester of pregnancy, whereas brain differentiation is considered to start in the second trimester. Usually, the processes are coordinated and the sex of the genitals and brain correspond. However, it is 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. A 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 behaviour 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 that androgen levels differ as a function of gender identity or sexual orientation or that treatment with exogenous hormones alters these traits in either sex.

3 | 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 vs biological (ie, genes and hormones) factors continues to be debated vigorously.[17] The biological basis of gender identity cannot be modelled 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 the current evidence for an array of prenatal factors that influence gender identity, including genes and hormones.[18–20]

3.1 | 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 (ie, 17α-hydroxylase/17, 20 lyase, the enzyme catalysing 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 (ie, 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, oestrogen receptor β and CYP19 (ie, aromatase, the enzymes catalysing oestradiol 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 confirm 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.

3.2 | 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, years later, when 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 as a result of cloacal exstrophy, 5α-reductase or 17β-hydroxysteroid dehydrogenase deficiency and raised as girls from birth.[32,33] All of these individuals were exposed to testosterone prenatally emphasising 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 aromatisation to oestradiol.[34]

3.3 | Neuroanatomy

Further evidence that the organisational 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 neurones and volume of subcortical nuclei such as the bed nucleus of the stria terminalis,[35,36] numbers of kisspeptin and neurokinin B neurones in the infundibulum,[37,38] structural differences of gray[39,40] and white matter microstructure,[41–43] neural responses to sexually-relevant odours[44,45] and visuospatial functioning.[46] However, in some cases, the interpretation of these studies is complicated by hormone treatments, small sample sizes and a 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 raises the possibility that transsexuality is also associated with changes in cerebral networks involved in self-perception.

4 | 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 3 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 (ie, 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.

4.1 | Animal studies

Sexual partner preference is one of the most sexually dimorphic behaviours 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 organising mate choice in several species of animals,

including birds, mice, rats, hamsters, ferrets and pigs. In particular, perinatal exposure to testosterone or its metabolite oestradiol programs male-typical (ie, 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 (ie, 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 organisational theory of sexual differentiation, sheep have an ovine sexually dimorphic preoptic nucleus (oSDN) that is larger and contains more neurones 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 that it could play a causal role in behaviour.[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 foetuses to testosterone can alter oSDN size independently of genetic and phenotypic sex.[13] However, males appear to be resistant to suppression of the action of androgen during gestation because the foetal hypothalamic-pituitary-axis is active in the second trimester (term pregnancy approximately 150 days) and mitigates against changes in circulating testosterone that could disrupt brain masculinisation.[64] These data suggest that, in sheep, brain sexual differentiation is initiated during gestation by central mechanisms acting through gonadotrophin-releasing hormone neurones to stimulate and maintain the foetal testicular testosterone synthesis needed to masculinise the oSDN and behaviour. More research is required to understand the parameters of oSDN development and to causally relate its 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.

4.2 | Human studies

4.2.1 | Genes—Evidence from family and twin studies suggests that there is a moderate genetic component to sexual orientation.[50] One recent study estimated that approximately 40% of the variance in sexual orientation in men is controlled by genes, whereas, in women, the estimate is approximately 20%.[65] In 1993, Hamer et al[66] 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. 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 criticised 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 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, although they 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 that implicated in linkage studies. Other molecular genetic evidence suggests that epigenetic factors could influence male sexual orientation, although this has yet to be demonstrated.[72,73]

4.2.2 | Hormones—The leading biological theory of sexual orientation in humans, as in animals, draws on the application of the organisational 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, although 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 organisational theory. More comprehensive presentations of the clinical evidence on this topic can be found in several excellent reviews.[74–76]

Women born with congenital adrenal hyperplasia (CAH) and exposed to abnormally high levels of androgens in utero show masculinised genitals, play behaviour and aggression.[74,77] 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 appears plausible that these behavioural 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 socialisation.[78] 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 almost all of them were attracted to females (i.e.

gynophilic).[33,50] The outcomes reported for both of these conditions are consistent with the 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.

4.2.3 | Neuroanatomy—Additional evidence that supports a prenatal organisational 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. LeVay[79] 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. 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.[80]

Other neural and somatic biomarkers of prenatal androgen exposure have also been investigated. McFadden[81] 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. OAEs and AEPs are usually stronger in heterosexual women than in heterosexual men and are masculinised in lesbians, consistent with the prenatal hormone theory. However, OAEs were not different in homosexual males and AEPs appear to be hyper-masculinised. The second digit to fourth digit (2D:4D) ratio, which is the length of the second digit (index finger) relative to that of the fourth 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,[82,83] although the validity of this measure as a marker influenced by only prenatal androgen exposure has been questioned.[84] Nonetheless, numerous studies have reported that the 2D:4D ratio is also on average smaller in lesbians than in hetero-sexual women, a finding that has been extensively replicated[85] and suggests the testosterone plays a role in female sexual orientation. Similar to OAEs, digit ratios do not appear to be feminised in homosexual men and, similar to AEPs, may even be hyper-masculinised. The lack of evidence for reduced androgen exposure in homosexual men (based on OAEs, AEPs and digit ratios) led Breedlove[85] to speculate that there may be as yet undiscovered brain-specific reductions in androgen responses in male foetuses that grow up to be homosexual. No variations in the human androgen receptor or the aromatase gene were found that relate to variations in sexual orientation.[86,87] However, Balthazart and Court[88] 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).

4.2.4 | 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.[89] Accordingly, the incidence of homosexuality increases by approximately 33% with each older brother.[90] 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[91] 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 foetal brain development.

5 | CONCLUSIONS

The data summarised in the present 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 modelled by tests of sexual partner preferences, although they 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 that these traits develop independently. This may be a result of differing hormone sensitivities and/or separate critical periods, although this remains to be explored. Most findings are consistent with a predisposing influence of hormones or genes, rather than a determining influence. For example, only some people exposed to atypical hormone environments prenatally show altered gender identity or sexual orientation, whereas 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 suffers from many limitations in the data, such as a 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 categorised and binary. Moreover, none of the biological factors identified so far can explain all of the 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.

ACKNOWLEDGEMENTS

I thank Charles Estill, Robert Shapiro and Fred Stormshak for their thoughtful comments on this review. This work was supported by NIH R01OD011047.

Funding information

This work was supported by NIH R01OD011047 (CER)

REFERENCES

1. Hines M Brain Gender. Oxford: Oxford University Press; 2005.
2. American Psychological Association. Answers to your questions about transgender people, gender identity, and gender expression. Washington, DC: 2011 http://www.apa.org/topics/lgbt/transgender.aspx.
3. American Psychological Association. Answers to your questions: For a better understanding of sexual orientation and homosexuality. Washington, DC: 2008 http://www.apa.org/topics/lgbt/orientation.pdf
4. Jost A, Magre S. Sexual Differentiation In: Thibault C, Levasseur MC, Hunter RHF, eds. Reproduction in Mammals and Man. Paris: Ellipses; 1993:197–212.
5. Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. Physiol Rev. 2007;87:1–28. [PubMed: 17237341]
6. Scott HM, Mason JI, Sharpe RM. Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. Endocr Rev. 2009;30:883–925. [PubMed: 19887492]
7. Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology. 1959;65:369–382. [PubMed: 14432658]
8. Cooke BM, Hegstrom CD, Villeneuve LS, Breedlove SM. Sexual differentiation of the vertebrate brain: principles and mechanisms. Front Neuroendocrinol. 1998;19:323–362. [PubMed: 9799588]
9. MacLusky NJ, Naftolin F. Sexual differentiation of the central nervous system. Science. 1981;211:1294–1302. [PubMed: 6163211]
10. Lenz KM, Nugent BM, McCarthy MM. Sexual differentiation of the rodent brain: dogma and beyond. Front Neurosci. 2012;6:26. [PubMed: 22363256]
11. Nugent BM, Wright CL, Shetty AC, et al. Brain feminization requires active repression of masculinization via DNA methylation. Nat Neurosci. 2015;18:690–697. [PubMed: 25821913]
12. Goy RW, Bercovitch FB, McBrair MC. Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. Horm Behav. 1988;22:552–571. [PubMed: 3235069]
13. Roselli CE, Estill C, Stadelman HL, Meaker M, Stormshak F. Separate critical periods exist for testosterone-induced differentiation of the brain and genitals in sheep. Endocrinology. 2011;152:2409–2415. [PubMed: 21447635]
14. De Vries GJ, Rissman EF, Simerly RB, et al. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. J Neurosci. 2002;22:9005–9014. [PubMed: 12388607]
15. Arnold AP, Chen X. What does the “four core genotypes” mouse model tell us about sex differences in the brain and other tissues? Front Neuroendocrinol. 2008;30:1–9. [PubMed: 19028515]
16. Cox KH, Bonthuis PJ, Rissman EF. Mouse model systems to study sex chromosome genes and behavior: Relevance to humans. Front Neuroendocrinol. 2014;35:405–419. [PubMed: 24388960]
17. Lippa RA. Gender, Nature and Nuture. New York, NY: Psychology Press; 2014.
18. Swaab DF. Sexual differentiation of the brain and behavior. Best Prac Res Clin Endocrinol Metab. 2007;21:431–444.

19. Bao AM, Swaab DF. Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. Front Neuroendocrinol. 2011;32:214–226. [PubMed: 21334362]
20. Savic I, Garcia-Falgueras A, Swaab DF. Sexual differentiation of the human brain in relation to gender identity and sexual orientation. Prog Brain Res. 2010;186:41–62. [PubMed: 21094885]
21. Ngun TC, Ghahramani N, Sanchez FJ, Bocklandt S, Vilain E. The genetics of sex differences in brain and behavior. Front Neuroendocrinol. 2011;32:227–246. [PubMed: 20951723]
22. Segal NL. Two monozygotic twin pairs discordant for female-to-male transsexualism. Arch Sex Behav. 2006;35:346–357.
23. Coolidge FL, Thede LL, Young SE. The heritability of gender identity disorder in a child and adolescent twin sample. Behav Genet. 2002;32:251–257. [PubMed: 12211624]
24. Green R Family cooccurrence of “gender dysphoria”: ten sibling or parent-child pairs. Arch Sex Behav. 2000;29:499–507. [PubMed: 10983252]
25. Bentz EK, Hefler LA, Kaufmann U, Huber JC, Kolbus A, Tempfer CB. A polymorphism of the CYP17 gene related to sex steroid metabolism is associated with female-to-male but not male-to-female transsexualism. Fertil Steril. 2008;90:56–59. [PubMed: 17765230]
26. Bentz EK, Schneeberger C, Hefler LA, et al. A common polymorphism of the SRD5A2 gene and transsexualism. Reprod Sci. 2007;14:705–709. [PubMed: 18000232]
27. Ujike H, Otani K, Nakatsuka M, et al. Association study of gender identity disorder and sex hormone-related genes. Prog Neuropsychopharmacol Biol Psychiatry. 2009;33:1241–1244. [PubMed: 19604497]
28. Hare L, Bernard P, Sanchez FJ, et al. Androgen receptor repeat length polymorphism associated with male-to-female transsexualism. Biol Psychiatry. 2009;65:93–96. [PubMed: 18962445]
29. Henningsson S, Westberg L, Nilsson S, et al. Sex steroid-related genes and male-to-female transsexualism. Psychoneuroendocrinology. 2005;30:657–664. [PubMed: 15854782]
30. Morimoto Y, Ono S, Imamura A, et al. Deep sequencing reveals variations in somatic cell mosaic mutations between monozygotic twins with discordant psychiatric disease. Hum Genome Var. 2017;4:17032. [PubMed: 28765789]
31. Diamond M, Sigmundson HK. Sex reassignment at birth. Long-term review and clinical implications. Arch Pediatr Adolesc Med. 1997;151:298–304. [PubMed: 9080940]
32. Cohen-Kettenis PT. Gender change in 46, XY persons with 5α-reductase-2 deficiency and 17β-hydroxysteroid dehydrogenase-3 deficiency. Arch Sex Behav. 2005;34:399–410. [PubMed: 16010463]
33. Reiner WG, Gearhart JP. Discordant sexual identity in some genetic males with cloacal exstrophy assigned to female sex at birth. N Engl J Med. 2004;350:333–341. [PubMed: 14736925]
34. Wisniewski AB, Migeon CJ, Meyer-Bahlburg HFL, et al. Complete androgen insensitivity syndrome: long-term medical, surgical, and psychosexual outcome. J Clin Endocrinol Metab. 2000;85:2664–2669. [PubMed: 10946863]
35. Zhou JN, Hofman MA, Gooren LJG, Swaab DF. A sex difference in the human brain and its relation to transsexuality. Nature. 1995;378:68–70. [PubMed: 7477289]
36. Garcia-Falgueras A, Swaab DF. A sex difference in the hypothalamic uncinate nucleus: relationship to gender identity. Brain. 2008;131:3132–3146. [PubMed: 18980961]
37. Taziaux M, Swaab DF, Bakker J. Sex differences in the neurokinin B system in the human infundibular nucleus. J Clin Endocrinol Metab. 2012;97:E2210–E2220. [PubMed: 23019350]
38. Taziaux M, Staphorsius AS, Ghatei MA, Bloom SR, Swaab DF, Bakker J. Kisspeptin expression in the human infundibular nucleus in relation to sex, gender identity and sexual orientation. J Clin Endocrinol Metab. 2016;101:2380–2389. [PubMed: 27046106]
39. Simon L, Kozák LR, Simon V, et al. Regional grey matter structure differences between transsexuals and healthy controls - voxel based morphometry study. PLoS One. 2013;8:e83947. [PubMed: 24391851]
40. Hoekzema E, Schagen SEE, Kreukels BPC, et al. Regional volumes and spatial volumetric distribution of gray matter in the gender dysphoric brain. Psychoneuroendocrinology. 2015;55:59–71. [PubMed: 25720349]

41. Rametti G, Carrillo B, Gomez-Gil E, et al. The microstructure of white matter in male to female transsexuals before cross-sex hormonal treatment. A DTI study. J Psychiatr Res. 2010;45:949–954. [PubMed: 21195418]
42. Kranz GS, Hahn A, Kaufmann U, et al. White matter microstructure in transsexuals and controls investigated by diffusion tensor imaging. J Neurosci. 2014;34:15466–15475. [PubMed: 25392513]
43. Rametti G, Carrillo B, Gomez-Gil E, et al. White matter microstructure in female to male transsexuals before cross-sex hormonal treatment. A diffusion tensor imaging study. J Psychiatr Res. 2011;45:199–204. [PubMed: 20562024]
44. Berglund H, Lindstrom P, Dhejne-Helmy C, Savic I. Male-to-female transsexuals show sex-atypical hypothalamus activation when smelling odorous steroids. Cereb Cortex. 2008;18:1900–1908. [PubMed: 18056697]
45. Burke SM, Cohen-Kettenis PT, Veltman D, Klink DT, Bakker J. Hypothalamic response to the chemo-signal androstadienone in gender dysphoric children and adolescents. Front Endocrinol. 2014;5:60.
46. Burke SM, Kreukels BP, Cohen-Kettenis PT, Veltman DJ, Klink DT, Bakker J. Male-typical visuospatial functioning in gynephilic girls with gender dysphoria - organizational and activational effects of testosterone. J Psychiatry Neurosci. 2016;41:395–404. [PubMed: 27070350]
47. Smith ES, Junger J, Derntl B, Habel U. The transsexual brain - a review of findings on the neural basis of transsexualism. Neurosci Biobehav Rev. 2015;59:251–266. [PubMed: 26429593]
48. Savin-Williams RC. Sexual orientation: categories or continuum? commentary on Bailey et al. (2016). Psychol Sci Public Interest. 2016; 17: 37–44. [PubMed: 27113561]
49. Minireview Balthazart J Hormones and human sexual orientation. Endocrinology. 2011;152:2937–2947. [PubMed: 21693676]
50. Bailey JM, Vasey PL, Diamond LM, Breedlove SM, Vilain E, Epprecht M. Sexual orientation, controversy, and science. Psychol Sci Public Interest. 2016;17:45–101. [PubMed: 27113562]
51. Fisher A, Ristori J, Morelli G, Maggi M. The molecular mechanisms of sexual orientation and gender identity. Mol Cell Endocrinol. 2017; 8 25 pii: S0303–7207(17)30430–6. 10.1016/j.mce.2017.08.008.[Epub ahead of print]
52. Adkins-Regan E Sex hormones and sexual orientation in animals. Psychobiology. 1988;16:335–347.
53. Henley CL, Nunez AA, Clemens LG. Hormones of choice: the neuroendocrinology of partner preference in animals. Front Neuroendocrinol. 2011;32:146–154. [PubMed: 21377487]
54. Houtsmuller EJ, Brand T, De Jonge FH, Joosten RNJMA, Van De Poll NE, Slob AK. SDN-POA volume, sexual behavior, and partner preference of male rats affected by perinatal treatment with ATD. Physiol Behav. 1994;56:535–541. [PubMed: 7972405]
55. Olvera-Hernandez S, Tapia-Rodriguez M, Swaab DF, Fernandez-Guasti A. Prenatal administration of letrozole reduces SDN and SCN volume and cell number independent of partner preference in the male rat. Physiol Behav. 2017;171:61–68. [PubMed: 28057567]
56. Paredes RG. Medial preoptic area/anterior hypothalamus and sexual motivation. Scand J Psychol. 2003;44:203–212. [PubMed: 12914583]
57. Bagemihl B Biological Exuberance: Animal Homosexuality and Natural Diversity. New York, NY: St Martin’s Press; 1999.
58. Poiani A Animal Homosexuality. A Biosocial Perspective. New York, NY: Cambridge University Press; 2010.
59. Perkins A, Roselli CE. The ram as a model for behavioral neuroendocrinology. Horm Behav. 2007;52:70–77. [PubMed: 17482616]
60. Roselli CE, Reddy RC, Kaufman KR. The development of male-oriented behavior in rams. Front Neuroendocrinol. 2011;32:164–169. [PubMed: 21215767]
61. Roselli CE, Larkin K, Resko JA, Stellflug JN, Stormshak F. The volume of a sexually dimorphic nucleus in the ovine medial preoptic area/anterior hypothalamus varies with sexual partner preference. Endocrinology. 2004;145:478–483. [PubMed: 14525915]
62. Roselli CE, Stadelman H, Reeve R, Bishop CV, Stormshak F. The ovine sexually dimorphic nucleus of the medial preoptic area is organized prenatally by testosterone. Endocrinology. 2007;148:4450–4457. [PubMed: 17540718]

63. Roselli CE, Estill CT, Stadelman HL, Stormshak F. The volume of the ovine sexually dimorphic nucleus of the preoptic area is independent of adult testosterone concentrations. Brain Res. 2009;1249:113–117. [PubMed: 19007753]
64. Roselli CE, Reddy R, Estill C, et al. Prenatal influence of an androgen agonist and antagonist on the differentiation of the ovine sexually dimorphic nucleus in male and female lamb fetuses. Endocrinology. 2014;155:5000–5010. [PubMed: 25216387]
65. Långström N, Rahman Q, Carlström E, Lichtenstein P. Genetic and environmental effects on same-sex sexual behavior: a population study of twins in Sweden. Arch Sex Behav. 2010;39:75–80. [PubMed: 18536986]
66. Hamer DH, Hu S, Magnuson VL, Pattatucci AML. A linkage between DNA markers on the X chromosome and male sexual orientation. Science. 1993;261:321–327. [PubMed: 8332896]
67. Rice G, Anderson C, Risch N, Ebers G. Male homosexuality:absence of linkage to microsatellite markers at Xq28. Science. 1999;284:665–667. [PubMed: 10213693]
68. Mustanski BS, DuPree MG, Nievergelt CM, Bocklandt S, Schork NJ, Hamer DH. A genomewide scan of male sexual orientation. Hum Genet. 2004;116:272–278.
69. Sanders AR, Martin ER, Beecham GW, et al. Genome-wide scan demonstrates significant linkage for male sexual orientation. Psychol Med. 2015;45:1379–1388. [PubMed: 25399360]
70. Ramagopalan SV, Dyment DA, Handunnetthi L, Rice GP, Ebers GC. A genome-wide scan of male sexual orientation. J Hum Genet. 2010;55:131–132. [PubMed: 20057505]
71. Drabant EM, Kiefer AK, Eriksson N, et al. Genome-wide association study of sexual orientation in a large, web-based cohort. https://blog23andmecom/wp-content/uploads/2012/11/Drabant-Poster-v7pdf 2012.
72. Ngun TC, Vilain E. Chapter eight - The biological basis of human sexual orientation: is there a role for epigenetics? In: Yamamoto D, ed. Advances in Genetics Epigenetic Shaping of Sociosexual Interactions: From Plants to Humans. San Diego, CA: Academic Press; 2014:167–184.
73. Rice WR, Friberg U, Gavrilets S. Homosexuality as a consequence of epigenetically canalized sexual development. Q Rev Biol. 2012;87:343–368. [PubMed: 23397798]
74. Hines M Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior. Front Neuroendocrinol. 2011;32:170–182. [PubMed: 21333673]
75. Berenbaum SA, Beltz AM. Sexual differentiation of human behavior: effects of prenatal and pubertal organizational hormones. Front Neuroendocrinol. 2011;32:183–200. [PubMed: 21397624]
76. Balthazart J, Young LJ. Chapter 48 - Mate Selection, Sexual Orientation, and Pair Bonding In: Plant TM, Zeleznik AJ, eds. Knobil and Neill's Physiology of Reproduction, 4th edn San Diego, CA: Academic Press; 2015:2157–2210.
77. Meyer-Bahlburg HFL, Dolezal C, Baker SW, New MI. Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. Arch Sex Behav. 2008;37:85–99. [PubMed: 18157628]
78. Fetal Pasterski V. androgens and human sexual orientation: searching for the elusive link. Arch Sex Behav. 2017;46:1615–1619. [PubMed: 28660433]
79. LeVay S A difference in hypothalamic structure between heterosexual and homosexual men. Science. 1991;253:1034–1037. [PubMed: 1887219]
80. Poeppl TB, Langguth B, Rupprecht R, Laird AR, Eickhoff SB. A neural circuit encoding sexual preference in humans. Neurosci Biobehav Rev. 2016;68:530–536. [PubMed: 27339689]
81. McFadden D Sexual orientation and the auditory system. Front Neuroendocrinol. 2011;32:201–213. [PubMed: 21310172]
82. Manning JT, Scutt D, Wilson J, Lewis-Jones DI. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Hum Reprod. 1998;13:3000–3004. [PubMed: 9853845]
83. Hönekopp J, Watson S. Meta-analysis of digit ratio 2D:4D shows greater sex difference in the right hand. Am J Hum Biol. 2010;22:619–630. [PubMed: 20737609]
84. van Hemmen J, Cohen-Kettenis PT, Steensma TD, Veltman DJ, Bakker J. Do sex differences in CEOAEs and 2D:4D ratios reflect androgen exposure? A study in women with complete androgen insensitivity syndrome. Biol Sex Differ. 2017;8:11. [PubMed: 28413602]

85. Breedlove SM. Prenatal influences on human sexual orientation: expectations versus data. Arch Sex Behav. 2017;46:1583–1592. [PubMed: 28176027]

86. DuPree MG, Mustanski BJ, Bocklandt S, Nievergelt C, Hamer DH. A candidate gene study of cyp19 (aromatase) and male sexual orientation. Behav Genet. 2004;34:243–250. [PubMed: 14990865]

87. Macke JP, Hu N, Hu S, et al. Sequence variation in the androgen receptor gene is not a common determinant of male sexual orientation. Am J Hum Genet. 1993;53:844–852. [PubMed: 8213813]

88. Balthazart J, Court L. Human sexual orientation: the importance of evidentiary convergence. Arch Sex Behav. 2017;46:1595–1600. [PubMed: 28500563]

89. Bogaert AF, Skorska M. Sexual orientation, fraternal birth order, and the maternal immune hypothesis: A review. Front Neuroendocrinol. 2011;32:247–254. [PubMed: 21315103]

90. Blanchard R, Bogaert AF. Homosexuality in men and number of older brothers. Am J Psychiatry. 1996;153:27–31. [PubMed: 8540587]

91. Bogaert AF, Skorska MN, Wang C, et al. Male homosexuality and maternal immune responsivity to the Y-linked protein NLGN4Y. Proc Natl Acad Sci USA. 2017; doi: 10.1073/pnas.1705895114. In press.