<Further Development 6_09>

Brain, Sex, and Gender

"Brain sex" is a controversial topic, and when it comes to behavior, there may be a great deal of difference between animal models and human subjects. Whereas rodents, fish, and birds appear to have numerous sex-specific behaviors (McCarthy 2015, 2016), most human behaviors overlap between the sexes. Humans do not appear to have "male brains" or "female brains." Moreover, where men and women have brain areas that are structurally different, these regions (as summarized below) generally control the hormones involved in reproductive physiology (such as the menstrual cycle) and gamete production.

Molecular analysis has shown that small sex-related differences exist in most human tissues, and that there is substantial variation between individuals (Oliva et al 2020). Some of the most interesting sex studies, though, have come from brain researchers. Contrary to popular accounts and long-held scientific beliefs, there does not appear to be a "male brain" or a "female brain" (see Rippon 2019). For example, although male and female mice have remarkably different parenting behaviors, their neuroanatomy cannot be told apart. The "circuitry" for both male and female behaviors are present in all mice; the hormones merely activate the genes of one pathway and suppress the other (Dulac and Kimchi 2008). This research "went against the dogma that for decades said that male and female brains are organized differently" (O'Connell 2020).

A recent review of data from the past thirty years found that in human brains, "there is no universal brain features that differ between the sexes" (Eliot 2021). The only statistically significant difference between the sexes is a size differential, and that that is proportional to body size, and it does not appear to make any cognitive difference. (No one is going to argue that smaller people are less intelligent or creative than larger people). And despite a longstanding cultural belief to the contrary, there also doesn't appear to be any evidence that the left and right cerebral hemispheres of women are more holistically integrated than those of men. This study concludes, "Overall, male/female brain differences appear trivial and population-specific. The human brain is not 'sexually dimorphic.'"

This is not to say that the brain doesn't respond to the steroid hormones produced by the gonads. Some of this research may have bearing on the relationships between sex and gender. Neuroanatomist Daphna Joel and her laboratory (2015) have argued that the human brain is not a singular organ and that it is a mosaic of regions, each of which can respond differently to hormones and can develop differently on the spectrum of male and female differences. Their neurological data have shown that even in brain areas where there are statistically significant differences between the brains of men and women, these differences can be altered by environmental agents. Moreover, each man and woman has some areas of the brain tending toward the male-prevalent and some tending to the female-prevalent; and “brains with features that are consistently at one end of the “maleness-femaleness” continuum are rare (Joel et al 2015). Rather, most brains appear to be unique ‘mosaics’ of phenotypes, some more common in females compared with males, some more common in males compared with females, and some common in both males and females. Needless to say, this idea has generated controversy (see Chekroud et al 2016; Rosenblatt 2016; Del Giudice et al 2016; Joel et al 2016).

A similar pattern was seen in mice. Pheromones—sex-specific chemicals secreted into the atmosphere—may play major roles in sexual behaviors in rodents. In mice and many other mammals, the vomeronasal organ (which is not present in humans) is responsible for sensing pheromones. If this organ (or the genes involved in pheromone recognition) are removed from male mice, these mice fail to discriminate between males and females. They attempt to mate with both. If this pheromone recognition system is removed from female mice, they lack certain female behaviors and acquire the full set of male courtship behaviors (including mounting, pelvic thrusting, and solicitation of females).

Thus, it appears that the *neural circuitry* for both male and female behaviors exists in every mouse brain, but the *interpretation* of pheromone signals is what distinguishes male from female brains. In females, the “feminine” pattern of behavior is activated (sexual receptivity to males, lactating behavior with pups), while the “masculine” pattern (if it’s male, fight it; if it’s female, mount it) is repressed. In males, the pheromones activate this “masculine” pattern, while the “feminine” pathway is suppressed (Kimchi et al. 2007). The interpretation of pheromone signals is thought to take place in the medial preoptic area/anterior hypothalamus region of the brain, and we know this region to be sexually dimorphic as a result of prenatal estrogen exposure. Thus, the organizational abilities of testosterone may act largely to effect changes in this small area of the brain, and once this region is organized, it will interpret the pheromone signals to activate either the male or the female sets of neurons (Baum 2009).

Intrinsic sex differences in mouse and human brains

Scientists have known for a long time that the brain, like other tissues, is responsive to the steroid hormones produced by the gonads. In mice, there may even be some sex differences in the brain that become evident *before* the gonads mature. These regions of the brain may experience direct regulation by the X and Y chromosomes (Arnold and Burgoyne 2004). More than 50 genes in the mouse brain are expressed in sexually dimorphic patterns *before* gonad differentiation has occurred (Dewing et al. 2003). Moreover, the mouse *Sry* gene, in addition to being expressed in the embryonic testes, is also expressed in the fetal and adult brain (Lahr et al. 1995; Mayer et al. 1998, 2000).

By creating XX mice with or without an *Sry* transgene attached to an autosome, as well as XY mice with or without *Sry*, Arnold and Chen (2009) were able to determine which sexually dimorphic phenotypes in mice might be caused without hormones and which by other actions of the sex chromosomes. While hormones were seen to be responsible for many of the anatomical and behavioral differences, the chromosomes themselves appeared to have some roles in sexually dimorphic differences in metabolism and in aggressive and pain-sensing behaviors (Chen et al 2013).

In humans, *SRY* is specifically active in the male hypothalamus, where it helps regulate the gene for tyrosine hydroxylase, an enzyme that is critical for the production of the neurotransmitter dopamine (Dewing et al. 2006). *SRY* gene activity in the human hypothalamus also promotes the expression of POMC, a peptide hormone precursor whose products contribute to sexual behaviors in mice (Gangisetty et al 2021) . The possibilities that *SRY* expression in the brain may lead to different gene expression patterns is being considered for several sex-associated normal and disease phenotypes (Sekido 2014; Rosenfeld 2017).

The roles of experience

Usually we think of DNA as controlling neural anatomy, and neural anatomy as controlling behaviors. This is the lesson that genetic mental retardation syndromes have taught us. But new research is claiming that the pathway is not one-way and that behaviors can control both gene expression and nervous system anatomy. One of the most sexually dimorphic regions of the rat central nervous system is the spinal nucleus of the bulbocavernosus (SNB). This controls the pelvic thrusting muscles during mating, and it is larger in the male. The SNB is also testosterone-sensitive, and it shrinks when rats are castrated (unless the rats are given replacement testosterone). Interestingly, the size of SNB neurons changes with sexual behavior, becoming smaller as male rats mate more frequently. “It is possible,” noted Breedlove (1997), “that differences in sexual behavior cause, rather than are caused by, differences in brain structure.”

The roles of experience in causing changes in brain gene expression and behavior were highlighted in a series of studies involving the effects of maternal care on the behaviors of rats. Maternal care during the first week of life involves grooming and licking the young pups. Those female rat pups that experience such maternal care when young will provide such maternal care to their own offspring, whereas female pups that do not receive such maternal attention will not. The licking and grooming responses are largely regulated through the estrogen-responsive neurons of the medial preoptic area (MPOA), a sexually dimorphic region of the brain. When estrogen binds to its receptors in MPOA neurons, these neurons activate the genes that encode receptors for oxytocin, the hormone involved with nursing and grooming.

So how is this trait inherited? It turns out that the key player is the experience of being licked and groomed. Licking and grooming by the mother alters the DNA methylation pattern of brain-specific enhancers in the major estrogen receptor gene (ERa) in the pups (Meaney and Szyf 2005; Champagne et al. 2006). In the MPOA neurons, licking and grooming decreases the amount of DNA methylation. This enables the Stat5 transcription factor to bind and permit the estrogen receptor gene to be transcribed at high levels. This ensures the high levels of estrogen receptors needed to stimulate licking and grooming behaviors. Thus, mothers that lick and groom their offspring tend to have daughters that will lick and groom their offspring. Cross-fostering (giving the newborn pups of "high licking and grooming" mothers to "low licking and grooming mothers" and vice versa) has demonstrated that this neonatal experience does indeed cause the gene expression differences (Cameron et al. 2008).

Interestingly, in another area of the brain, the anterior paraventricular nucleus (PVN), rat pups that experience high levels of licking and grooming have a highly methylated promoter on this gene, thereby downregulating the estrogen receptor in this region. The PVN helps regulate gonadotropins. Rats experiencing low levels of maternal licking and grooming have high levels of estrogen receptors in the PVN and correspondingly high levels of gonadotropins. As they mature, these rats are predisposed to a suite of sexual behaviors that include precocious puberty, heightened sexual activity, and lack of attention to their pups (Cameron et al. 2008). Thus, experience can create changes in gene expression and neuroanatomy. Moreover, inherited variation can come about by experience-induced changes of DNA methylation. The distinction between nature and nurture disappears in this environmental regulation of gene expression.

The human element: gender

It is a very risky business extrapolating from such rodent studies to humans. Human fetuses, for instance, do not make a strong estrogen-binding protein and have a much higher level of free estrogen than do rodent embryos (see Nagel and vom Saal 2003).

Human sexual behaviors differ from those of rodents in many ways, and so does brain development (see Jordan-Young 2010). Outside of physiological events such as ovulation, no sex-specific behavior has yet been identified in humans. Moreover, humans do not use pheromones as a primary sexual attractant (sight and touch being far more critical). The evidence that there are differences in brain anatomy between male homosexuals and heterosexuals has been disputed, and even so, brain anatomy can be altered by experience. No "gay gene" has been discovered, and the concordance of gender identity between identical twins is only 30%—far from the 100% expected if sexual orientation were strictly genetic (Bailey et al. 2000; CRC 2006). Moreover, behaviors that are seen as "masculine" in one culture may be considered "feminine" in another, and vice versa (see Jacklin 1981; Bleier 1984; Fausto-Sterling 1992; Kandel et al. 1995). How humans acquire gendered behaviors appears to involve a remarkably complex set of interactions between genes, hormones, nerves, and environment. As will be discussed in Chapter 26, we inherit a genome that can produce a genetically constrained range of different phenotypes. Indeed, behaviors are the "final phenotype." They have resisted explanation because the link between genotype and behavior is relatively weak, and because behavioral phenotypes are so heavily influenced by environment.

Gender remains a very poorly understood phenotype. While "sex" basically divides reproductively functional mammals into egg-producers (female) and sperm-producers (male), "gender" is far more flexible. Moreover, it can be defined in several ways, depending on which aspect one highlights. Gender has many components, including a **core gender identity** (the sense of self, be it male, female, or intersex), **erotic sexuality** (whether one prefers or fantasizes about male, female or intersex partners), and **performative gender** (how one behaves in larger or smaller social groups.). This lack of clarity is especially true when it comes to sexual orientation. Large studies (Ganna et al 2019) revealed that there are no specific genes whose presence is linked to male homosexuality. Five genes were shown to be statistically more prevalent in gay men than in heterosexual men; but taken together, these five genes could explain less that 1% of the variation in sexual behavior. When all the genetic components were grouped together, DNA differences explained only between 8- 25% of the variation. Not only is there no known "gay gene," there no linear correlation between hormones and sexual orientation. What is known is that “a substantial minority of both sexes have some erotic interest in individuals of their own sex” (Hines 2011). About 5% of men in the United States report having homosexual experiences and about 18% of both men and women report experiencing sexual attraction to individuals of their same sex (Sell et al 1995). Indeed, the terms “homosexual” and "heterosexual" not only differ in different countries, but they depend on the individual context as well. The definition of who is homosexual has also confounded the attempts to find "gay genes" (Hamer et al 2021).

What causes individuals to have erotic feelings towards members of their own another sex? There are no measurable hormonal differences between homosexual and heterosexual men or between homosexual and heterosexual women (Mayer-Bahlberg 1977, 1979). However, hormones obviously do play some role. Those XY women who have complete androgen insensitivity are almost exclusively heterosexual, in that they prefer male sexual partners. Moreover, those women who have congenital adrenal hyperplasia and who were thus exposed in to high levels of testosterone in fetal and neonatal life have a higher percentage of homosexuality than their sisters who lack this condition. Indeed, the levels of androgen production appears to correlate with the probability of homosexuality (Hines 2011.) Yet, the effects are not predictive. The most severe form of CAH is associated with homosexuality only about 50% of the time (Frisén et al 2009). Indeed, the lack of heterosexuality (and sexuality in general) may also be due to the pain and bleeding that can accompany intercourse as well as cosmetic and psychological concerns of the women.

A similar situation appears to exist with those people who have mutations in the genes regulating hormone biosynthesis. Even though raised as girls, about half those individuals, whose testosterone levels rise to new levels and masculinize the body during puberty, make the transition to maleness and form fertile sexual partnerships with women (Wilson et al 1993; Zucker 2002.)

Thus, there are several factors that act to determine sexual orientation. Prenatal gonadal steroids appear to be one of them, and other factors, that are not well characterized, also appear to play roles. Epigenetic alterations of histones and DNA by factors on the X and Y chromosomes may also be important players. Thus, Hines (2011) concludes, “Although a role for hormones during early development has been established, it also appears that there may be multiple pathways to a given social orientation outcome and some of these pathways my not involve hormones.” The causes for concordance or discordance between sex and gender have not been identified .Biochemical or anatomical causes for dissatisfaction with one’s gender identity (either in homosexuality or transgendered manifestations) have still not been found (Mayer-Bahlburg 2013; Ngun and Vilain 2014) and anatomical differences between *cis*-gender and *trans*-gender individuals have also been inconclusive (Frigerio etal 2021).

* The terms estrogen and estradiol are often used interchangeably. However, estrogen refers to a class of steroid hormones responsible (among other functions) for establishing and maintaining specific female characteristics. Estradiol is one of these hormones, and in most mammals (including humans) it is the most potent of the estrogens. The enzyme’s name, aromatase, has nothing to do with aroma (although aromas are certainly crucial to rodent sex), but refers to the destabilization of hydrogen bonds in the steroid ring structure.

Usually, "sex" is defined biologically, while "gender identity" is thought of as psychological phenomena, and "gender performance" as an interaction between gender identity and the particular culture one is in. While the sex of a person is usually a very good clue to their gender identity; this is not always so. Thus, "gender reveal" parties are, at best, "educated guesses as to the baby's sex reveal" parties. A baby assigned "male" at birth because of biological criteria, may decide she is a "woman" as she matures. And a person may take on different aspects of masculine or feminine gender expression patterns that may change at any time (**Figure**).

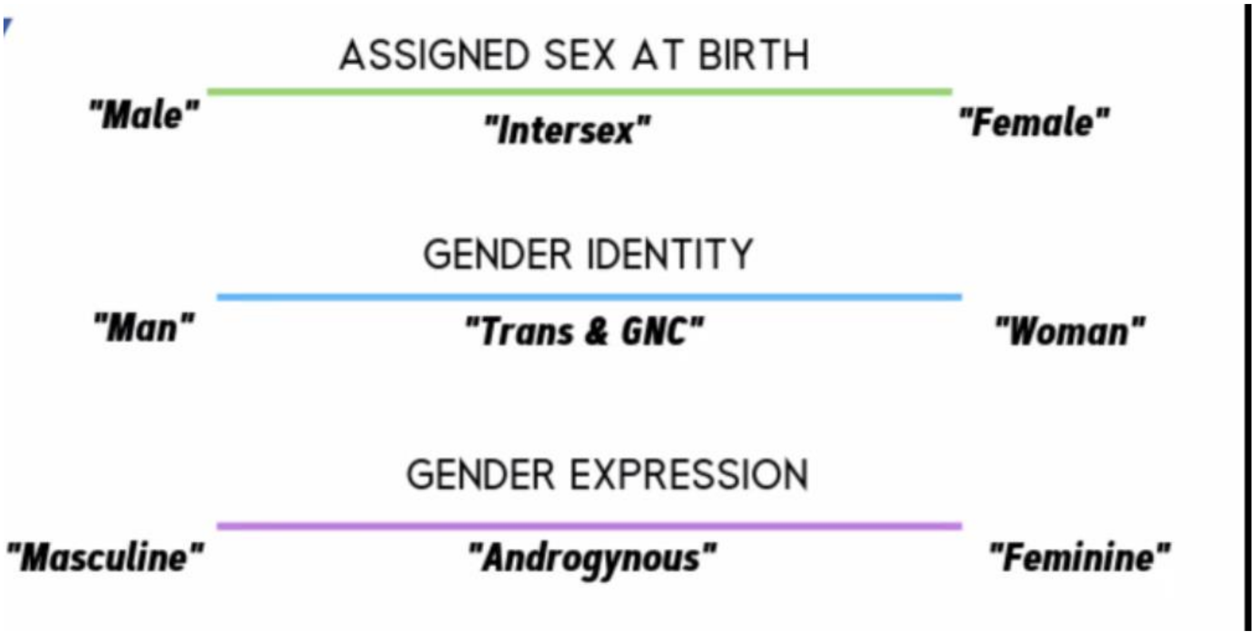

Figure 1. Sex, gender identity, and gender expression are on different axes. Sex concerns the biological assignment (usually at birth) as to whether a person is male or female. However, there are numerous intersex categories between these fates, where a person can be gonadally male and phenotypically female (such as people androgen insensitivity syndrome), genetically female but with male-like external genitalia (congenital adrenal hyperplasia) and other conditions. Gender identity involves assigning one's self the identity of male, female, or a trans-sexual or gender non-conforming identity. Gender expression involves behaviors. Here, the poles are masculine and feminine, but with many possibilities of mixtures between them, including various degrees androgeny, where the culturally expected male and female characteristic are expressed together. Conservative cultural values tend to normalize only the poles of male/female, man/woman, masculine/feminine spectra, while more liberal value systems believe that all regions within these spectra should be considered normal. (After Stayton 2020).

Stayton (2020) finds it useful to talk about "sex roles" and "gender roles", rather than sex and gender "identities." "Sex roles" involve biological functions such as gestation, lactation, egg production, and sperm production that are specific to a sex. (Not all males make sperm and not all women become pregnant, but these are sex-specific biological roles). "Gender roles" concern psychological acts of conforming to the expectations that a culture has for masculine and feminine behaviors. Transgendered people are those whose gender identity or gender expression differs from that of the birth sex as evidenced by their genitalia. As of this writing, we have very little knowledge of what causes such concordance or discordance between sex and gender (see Stayton 2020). The Declaration of Sexual Rights of the World Association for Sexual Health (WAS 2014) asserts that there is a broad spectrum of gender phenotypes throughout the human population and that each is deserving of respect human rights protection. For a discussion of what is "normal," please see Chapter 14.

References

Arnold, A. P. and X. Chen. 2009. What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? *Front Neuroendocrinol.* 30: 1-9.

Chen, X., R. McClusky, Y. Itoh, K. Reue, and A. P. Arnold. 2013. X and Y chromosome complement influence adiposity and metabolism in mice. *Endocrinology* 154: 1092-1104.

Chekroud, A. M., E. J. Ward, M. D. Rosenberg, and A. J. Holmes. 2016. Patterns in the human brain mosaic discriminate males from females. *Proc Natl Acad Sci USA 113: E1968.*

Del Giudice, M., R. A. Lippa, D. A. Puts, D. H. Bailey, M. J. Bailey, and D. P. Schmitt. 2016. Joel et al.'s method systematically fails to detect large, consistent sex differences. *Proc Natl Acad Sci USA* 113: E1965.

Dulac, C. and Kimchi, T. 2008. Neural mechanisms underlying sex-specific behaviors in vertebrates *Current Opinion in Neurobiology* **17**:675–683.

Eliot, L., Ahmed, A., Khan, H., and Patel, J. 2021. Dump the "dimorphism": Comprehensive synthesis of human brain studies reveals few male-female differences beyond size. *Neurosci. Biobehav. Rev.* 125: 667 - 697.

Frigerio, Alberto; Ballerini, Lucia; Valdes-Hernandez, Maria (2021). "Structural, Functional, and Metabolic Brain Differences as a Function of Gender Identity or Sexual Orientation: A Systematic Review of the Human Neuroimaging Literature". Archives of Sexual Behavior. doi:10.1007/s10508-021-02005-9.

Frisén, L., A. Nordenström H. Falhammar, H. Filipsson, G. Holmdahl, P. O. Janson, M. Thorén, K. Hagenfeldt, A. Möller, and A. Nordenskjöld. 2009. Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. *J Clin Endocrinol Metab.* 94(9): 3432- 3439.

Gangisetty O, Mead EA, Sarkar DK. Sex-Determining Region Y Controls the Effects of Fetal Alcohol Exposure on Proopiomelanocortin Gene Expression. Front Neurosci. 2021 Mar 16;15:608102. doi: 10.3389/fnins.2021.608102

Ganna A, Verweij KJH, Nivard MG, Maier R, Wedow R, Busch AS, Abdellaoui A, Guo S, Sathirapongsasuti JF; 23andMe Research Team, Lichtenstein P, Lundström S, Långström N, Auton A, Harris KM, Beecham GW, Martin ER, Sanders AR, Perry JRB, Neale BM, Zietsch BP. 2019. Large-scale GWAS reveals insights into the genetic architecture of same-sex sexual behavior. Science365(6456):eaat7693. doi: 10.1126/science.aat7693.

Hamer D, Mustanski B, Sell R, Sanders SA, Garcia JR. 2021. Comment on "Large-scale GWAS reveals insights into the genetic architecture of same-sex sexual behavior". Science371(6536):eaba2941. doi: 10.1126/science.aba2941

Hines, M. 2011. Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior. *Front. Neuroendocrin*. 32: 170–182.

Joel, D., Z. Berman, I. Tavor, N. Wexler, O. Gaber, Y. Stein, N. Shefi, J. Pool, S. Urchs, D. S. Margulies, F. Liem, J. Hänggi, L. Jäncke, amd Y. Assaf. 2015. Sex beyond the genitalia: The human brain mosaic. *Proc Natl Acad Sci U S A* 112: 15468–15473.

Joel, D, A. Persico, J. Hänggi, J. Pool, and Z. Berman. 2016. Reply to Del Giudice et al., Chekroud et al., and Rosenblatt: Do brains of females and males belong to two distinct populations? *Proc Natl Acad Sci U S A* pii: 201600792.

Meyer-Bahlburg, H. F. 1977. Sex hormones and male homosexuality in comparative perspective. *Arch Sex Behav.* 6: 297–325.

Meyer-Bahlburg, H. F. 1979. Sex hormones and female homosexuality: a critical examination. *Arch Sex Behav.*8: 101–119.

Meyer-Bahlburg, H. F. 2013. Sex steroids and variants of gender identity. *Endocrinol Metab Clin North Am.*42(3):435–452.

Ngun, T. C. and E. Vilain. 2014. The biological basis of human sexual orientation: Is there a role of epigenetics? *Adv. Genetics*. 86: 167–184.

O'Connell, L. 2020. quoted in Discoverer of neural circuits for parenting wins US$3-Million prize. *Nature* 585: 329.

Oliva, M and numerous others. The impact of sex on gene expression across human tissues. Science 369: doi.org/10.1126/science.aba3066

Rosenblatt, J. D. 2016. Multivariate revisit to "sex beyond the genitalia." *Proc Natl Acad Sci USA 113: E1966–E1967.*

Rosenfeld CS. Brain Sexual Differentiation and Requirement of SRY: Why or Why Not?. *Front Neurosci*. 2017;11:632. Published 2017 Nov 16. doi:10.3389/fnins.2017.00632

Sell, R. L., J. A. Wells, and D. Wypij. 1995. The prevalence of homosexual behavior and attraction in the United States, the United Kingdom and France: results of national population-based samples. *Arch Sex Behav.* 1995 Jun;24(3): 235–48.

Sekido, R. 2014. The potential role of SRY in epigenetic gene regulation during brain sexual differentiation in mammals. *Adv Genet.* 86: 135–165.

Stayton, W. R. 2020. Sinless Sex: A Challenge to Religion. Luminaire Press, Eugene, OR.

WAS (World Association of Sexual Health) 2014. Declaration of Sexual Rights. https://worldsexualhealth.net/wp-content/uploads/2013/08/Declaration-of-Sexual-Rights-2014-plain-text.pdf

Wilson, J. D., J. E. Griffin, and D. W. Russell. 1993. Steroid 5-alpha reductase-2 deficiency. *Endocr. Rev.* 14: 577–593.

Zucker, K. J., S. J. Bradley, G. Oliver, J. Blake, S. Fleming, and J. Hood. 1996. Psychosexual development of women with congenital adrenal hyperplasia. *Horm Behav.* 30: 300–318.