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The biological basis of sexual orientation: How hormonal, genetic, and environmental factors influence to whom we are sexually attracted

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ABSTRACT

Humans develop relatively stable attractions to sexual partners during maturation and present a spectrum of sexual orientation from homosexuality to heterosexuality encompassing varying degrees of bisexuality, with some individuals also displaying asexuality. Sexual orientation represents a basic life phenomenon for humans. However, the molecular mechanisms underlying these diverse traits of sexual orientation remain highly controversial. In this review, we systematically discuss recent advancements in sexual orientation research, including those related to measurements and associated brain regions. Current findings regarding sexual orientation modulation by hormonal, genetic, maternal immune system, and environmental factors are summarized in both human and model systems. We also emphasize that future studies should recognize the differences between males and females and pay more attention to minor traits and the epigenetic regulation of sexual orientation. A comprehensive view of sexual orientation may promote our understanding of the biological basis of sex, and that of human reproduction, and evolution.

1. Introduction

Sexual orientation constitutes the stable sexual attraction toward the opposite sex (heterosexuality), the same sex (homosexuality), or both sexes (bisexuality), or showing no interest in individuals of either sex (asexuality) after maturity (Balthazart, 2011; Rosario and Schrimshaw, 2014). Sexual orientation is conceptually distinguishable from other related phenomena, such as sexual partner preference, gender identity, and sexual behavior (Rosario et al., 2006). Sexual partner preference is highly sexually dimorphic, and refers to the sexual attraction of an animal to a partner of the same or opposite sex when given a choice. Gender identity represents the concept that an individual perceives itself as male, female, a blend of both, or neither, whereas sexual behavior refers to the actual sexual interactions performed by the individual (Bailey et al., 2016; Bailey and Zuk, 2009). Sexual orientation and gender identity refer to only humans, while sexual partner preference and behavior occur in humans as well as other animals. Recent epidemiological surveys of U.S. adults aged ≥18 years reported that 97.6% of individuals self-defined as heterosexual, with the remainder attesting to the other various sexual orientations. Notably, the prevalence of homosexuality is relatively low (1.6%), albeit constant across human populations (Srivastav et al., 2019; Ward et al., 2014). Sexual partner preference and behavior towards members of the same sex have also been observed in a variety of other species, including flies (Napolitano and Tompkins, 1989; Ryner et al., 1996), ants (Matsuura et al., 2002; Zucker et al., 1996), flour beetles (Levan et al., 2009), rodents (Olvera-Hernandez and Fernandez-Guasti, 2015; Slob et al., 1987), flying foxes (Sugita, 2016), ruminant species (Ungerfeld et al., 2014), orangutans (Fox, 2001), and macaques (Gunst et al., 2015; Vasey et al., 2014). Sexual orientation represents one of the basic phenomena in nature; nevertheless, how these diverse traits of sexual orientation develop and evolve remain challenging issues.

The development of sexual orientation involves a complex interplay of diverse factors. Recently, studies have reported extensive findings in the fields of hormonal regulation (Abi Ghanem et al., 2017; Meerts et al., 2017), genetics (Ganna et al., 2019; Sanders et al., 2017, 2015), Y-chromosome immunization (Bogaert et al., 2018), neural correlates (He et al., 2018; Jordan et al., 2018; Safron et al., 2017; Taziaux et al., 2016; Wei et al., 2018), and epigenetics (Ghahramani et al., 2014) with regard to male homosexuality along with bisexuality and female homosexuality (Camperio Ciani et al., 2018). Numerous studies have

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indicated that prenatal exposure to gonadal hormones or endocrine disruptors can lead to irreversible changes in the nervous system as well as sexual behavior and orientation in offspring (Hines, 2011; Schulz et al., 2009). The link between animal and clinical/human literature on the neuroendocrine control of sexual partner preference, behavior, and orientation has previously been reviewed (Baum, 2006; Gooren, 2006). The contributions of genetic factors to sexual orientation have also been investigated through family and twin studies (Alanko et al., 2010; Langstrom et al., 2010), along with segregation and genome-wide association analyses (Mustanski et al., 2005; Ramagopalan et al., 2010; Sanders et al., 2017; Sanders et al., 2015). In particular, a recent genome-wide association study on 493,001 participants identified five autosomal loci significantly associated with same-sex sexual behavior in humans, which also indicate the high polygenicity of sexual orientation (Ganna et al., 2019). Moreover, the role of the immune system in sexual orientation has also been explored (Bogaert and Skorska, 2011; Bogaert et al., 2018). Thus, the goal of this review was to provide an integrative overview of current scientific understanding with regard to sexual orientation. We mainly focused on the biological basis of sexual orientation, including the associated brain regions and the influence of hormones, genetics, and environmental factors. Recent progress in the molecular mechanisms of same-sex preference and behavior in model organisms is also summarized. In addition, we suggest future research perspectives in this area and provide novel insights regarding our understanding of the biological basis of sexual orientation.

2. Measurements of sexual orientation in humans

Although sexual orientation is quite complex, several questionnaire- and psychophysiological-based measurements have been developed to operationally assess sexual orientation. For example, the Kinsey scale, the most widely used questionnaire-based method, is based on descriptions of an individual's gender identity, sexual behaviors, and fantasies related to sexual partners to classify sexual orientation (Kinsey et al., 1948). The Kinsey scale typically conceptualizes sexual orientation on a continuum that possesses seven ordered scores. Men and women exhibit variable distributions of sexual orientation on the Kinsey scale. Men usually fall within a bimodal distribution and rate themselves as predominantly heterosexual (Kinsey score 0–1) or homosexual (Kinsey score 5–6) (Bailey et al., 2016). In contrast, women have lower rates of homosexuality and higher rates of bisexuality, showing a more continuous distribution across the non-heterosexual orientations. It remains unclear how these different patterns developed between men and women and whether innate sex differences in brain circuits and in some peripheral trait (s) contribute to sexual orientation.

The Klein Sexual Orientation Grid (KSOG), another traditional sexual orientation scale, encompasses several modifications of variables related to sexual orientation in the present and past along with ideal choices thereof (Weinrich et al., 2014). In addition, it includes multiple questions relating to mental affective behavior, sexual behavior, social life, and lifestyle, which are not included in the Kinsey scale (Weinrich et al., 2014). Such multi-dimensional information allows the KSOG to measure sexual orientation across several contexts. However, the multiple ratings are not integrated into the final score in the continuum, thereby limiting the interpretation of group differences in the research. In addition, researchers have reported that applying the KSOG may be complicated or confusing (Galupo et al., 2018). The self-report-based measurements mentioned above require individuals to define their gender identity when describing their sexual attraction, which can be also subjective and thus somewhat inaccurate, particularly for individuals who are confused regarding their sexual orientation or those that tend to hide or deny their sexual orientation. In addition to the one-dimensional scale, a two-dimensional conceptualization, Storms' model (1980), sets hetero- and homo-sexuality on two perpendicular axes and can present four types of sexual orientation, including bisexual and asexual. Storms' conceptualization concentrates on sexual attraction and can distinguish between strong attraction to both males and females (bisexual), and little to no attraction to either (asexual). Storms' model can thus accurately describe bisexuality and predict asexuality.

In comparison to questionnaire-based measurements, patterns of sexual arousal can provide direct evidence regarding the sexual self-identification of an individual. Physiological measurements based on sexual arousal, such as penile plethysmography (PPG), which measures blood flow in the penis, have been used to indicate male sexual orientation (Murphy et al., 2015). Generally, heterosexual men are more aroused by female- than male-based stimuli, whereas homosexual men strongly respond to male- rather than female-based stimuli, with adult bisexual men exhibiting an intermediate pattern (Attard-Johnson et al., 2016). Notably, PPG is more commonly used for diagnosing erectile dysfunction and detecting pedophilia than for classification of male sexual orientation (Blanchard et al., 2001; Broderick, 1998). For female sexual arousal, vaginal photoplethysmography (VPP) is commonly used to measure the amount of blood in the walls of the vagina (Huberman and Chivers, 2015; Kukkonen, 2015). However, heterosexual women are aroused approximately equally by male- and female-based erotic stimuli, with homosexual women only showing a slightly stronger genital arousal for female- compared to male-based stimuli, thus differing from the typical category-specific responses of men (Chivers et al., 2004, 2007). Therefore, the sexual orientation of women does not appear to be closely linked to their pattern of sexual arousal.

Other physiological arousal-based methods have been utilized, including evaluation of activated brain regions in response to viewing erotic images of males or females by functional magnetic resonance imaging (fMRI) (Safron et al., 2007), pupil dilation (Attard-Johnson et al., 2016; Rieger and Savin-Williams, 2012), and implicit measurements to reflect immediate automatic reactions (Snowden et al., 2008). However, the extent to which men and women are category-specific in their genital patterns or other physiological characteristics, and the theoretical models that underlie these phenomena remain to be established.

3. Brain regions involved in sexual orientation

During the critical embryonic period of sexual development in humans and rodents, the brain can sense and respond to external hormonal signals owing to the abundant expression of steroid hormone receptors in a subset of brain structures (Celotti et al., 1997; McCarthy et al., 2009; Melcangi et al., 1998). Thus, the brain develops masculinized characteristics when the immature testes secrete testosterone in males, or develops feminized characteristics in females by default, owing to the lack of testes thus failing to effect exposure to high levels of sex steroids (Bao and Swaab, 2011; McCarthy et al., 2009). Moreover, accumulating evidence also suggests the active feminization of the brain by estrogens (Bakker and Baum, 2008; Bakker et al., 2003; Dodd et al., 2019). Multiple brain regions including the preoptic area (POA), the ventromedial nucleus of the hypothalamus (VMH), and the bed nucleus of the stria terminalis (BNST) have established sex dimorphic features in terms of size, cell numbers, neural phenotypes, and synaptic connections (Hines et al., 1985; Jacobson et al., 1981; Jacobson et al., 1980; Madeira et al., 2001; Murray et al., 2009; Roselli et al., 2009). In particular, the POA, a region in the anterior hypothalamus, constitutes a well-known structure in sexual dimorphism and has been found to differ between males and females in almost all examined species to date (Jacobson et al., 1981; Jacobson et al., 1980).

3.1. Brain regions involved in the sexual behaviors of animals

In mice and rats, neural circuits in the brain are sensitive to the effects of gonadal steroids from the late embryonic to the early postnatal period (McCarthy and Arnold, 2011). During this perinatal period, the male brain is exposed to the masculinizing effects of testicular

testosterone, which is driven by kisspeptin, a potent regulator of gonadotropin-releasing hormone (GnRH) secretion, and gonadotropin released by hypothalamic neurons (Clarkson et al., 2014). Notably, brain masculinization in rodents is largely induced by estradiol converted from testosterone by the aromatase (Arom) enzyme, rather than via a direct effect of testosterone as in humans (Matsumoto et al., 2003).

In rats, the POA structure contains two main sexually dimorphic nuclei: the sexually dimorphic nucleus of the preoptic area (SDN-POA, a tiny structure with a volume ranging from 0.001 to 0.007 mm^3 in rats) (He et al., 2012) and the anteroventral periventricular nucleus (AVPV). The typical size of the POA is regulated by testosterone and established around the end of the embryonic period (Roselli et al., 2007). Males and females initially possess the same number of neurons in the SDN-POA; however, apoptotic cell death via caspase-3 activation occurs more frequently in females during the early postnatal period, resulting in more neurons in males than females in adult animals (Tsukahara, 2009; Yoshida et al., 2000). In adulthood, the volume of the SDN-POA in male rats is approximately six-times larger than that in females, and can no longer be altered by castration or treatment with steroid hormones (Jacobson et al., 1981; Jacobson et al., 1980; Roselli et al., 2009). In addition, female rats have a higher cell density in the AVPV, which controls the luteinizing hormone (LH) surge that is essential for ovulation in adult females (Smith et al., 2006).

Similarly, in sheep, the POA of rams (oPOA) is approximately three-times larger and contains approximately four-times more neurons than that of ewes (Roselli et al., 2004). The volume of the oPOA correlates with male-typical partner preference in sheep. For example, 8–10% of domestic rams show spontaneous alterations in sexual attraction and exhibit a sexual partner preference for other males (male-oriented rams) and the volume of the oPOA was shown to be smaller in male-oriented than in female-oriented rams (Roselli et al., 2004). The medial POA also regulates male-specific partner preference in rats and ferrets. For example, male rats and ferrets with an experimental lesion of the medial POA preferred to interact with other males than with sexually receptive females; however, females with a POA lesion maintained the same behaviors as control females (Paredes and Baum, 1995; Paredes et al., 1998), suggesting a sex-specific effect. In addition to male partner preference, the medial POA was recently found to correlate with male-typical mounting and female-typical pup retrieval, and to mediate sexually dimorphic behaviors, regardless of sex, in mice (Wei et al., 2018). Recent studies that begin to explore the neural component of the SDN-POA have found that it encompasses neuronal projections that are interfused with tyrosine hydroxylase-positive projections in rats (He et al., 2018). However, the precise neuronal components and functional connections within the POA remain to be identified, and techniques more specific than structural lesions are needed for future analyses.

The VMH, a region involved in sexual activity, is another structure with sex-typical size, and is larger in males than in females (Madeira et al., 2001; Matsumoto and Arai, 1983). In the VMH of newborn rats, males have twice the number of dendritic spines and more dendritic branches than females (Matsumoto and Arai, 1983, 1986; Todd et al., 2007). This sexual dimorphism can be attributed to the effects of estradiol, as this hormone, centrally aromatized from testosterone, can promote spine formation by enhancing glutamate release (Schwarz et al., 2008). Accordingly, the treatment of female rats with testosterone at birth increases the number of dendritic spines to that observed in males (Todd et al., 2007). However, the correlation of these neural differences with specific sexual behaviors remains to be established.

Additional sex-typical morphological features exist. For example, the principal nucleus of the BNST, a region involved in the control of male sexual behavior (Liu et al., 1997), is larger and contains more cells in male than in female pigs and mice, owing to higher cell death in females (Hines et al., 1985; Murray et al., 2009). In the central nervous system of mice and rats, the density of oligodendrocytes was found to be 20–40% greater in adult males than that in females in the corpus callosum and other white matter tracts (Cerghet et al., 2006). A recent study indicated that these sex differences emerge during the first 10 postnatal days, a period in which a late wave of oligodendrocyte progenitor cells begins to differentiate, and when male mice exhibit higher androgen levels than females (Abi Ghanem et al., 2017). Treating male pups with an androgen receptor antagonist, or female pups with an androgen receptor agonist, persistently affected the density of oligodendrocytes and the structure of the myelin sheaths (Abi Ghanem et al., 2017). However, the correlation of these neural differences with sex-atypical physiological/behavioral traits remains undefined.

3.2. Brain structural and functional differences related to sexual orientation in humans

Similar to the vertebrates mentioned above, steroid receptors are expressed in the human brain; thus, sex steroids control the differentiation of gender-typical external and internal genital structures during embryonic life (Ball et al., 2014). The regional size and cell density in several brain structures have been shown to differ between men and women and between homosexual and heterosexual individuals (Poeppl et al., 2016). A summary of the differences in brain structure and function along with structural connection and functional responses related to sexual orientation is shown in Fig. 1.

The interstitial nuclei of the human anterior hypothalamus (INAH1–4) have been considered as candidates for the potentially homologous SDN-POA nucleus in rats (LeVay, 1991). A sexually dimorphic difference in the INAH was first reported in a cell group in the intermediate nucleus, later known as INAH1 (Swaab and Fliers, 1985). Although subsequent studies failed to verify the sex difference in this nucleus, the volumes of two other nuclei, namely INAH2 and INAH3, were found to be larger in males, particularly the INAH3, which was three-times larger in men than in women (Allen et al., 1989; LeVay, 1991). Subsequent studies confirmed the sex difference in the INAH3 volume and found that presumed heterosexual men possess larger sand more densely packed neurons than presumed heterosexual women (Byne et al., 2000, 2001; Garcia-Falgueras and Swaab, 2008). Moreover, the volume of the INAH3 and the number of neurons in male-to-female transsexuals resemble those of females, suggesting that this area represents the early atypical sexual differentiation of the brain related to gender identity (Garcia-Falgueras and Swaab, 2008). With regard to sexual orientation, the volume of the INAH3 in homosexual individuals that had died of acquired immune-deficiency syndrome (AIDS) has been reported to be smaller than that in heterosexual men who had also died of AIDS (LeVay, 1991). However, subsequent studies only reported a statistical trend for the difference of INAH3 volumes between homosexual and heterosexual men, and found no difference in the number of neurons with regard to sexual orientation (Byne et al., 2000, 2001). Thus, the finding suggesting a key role of the INAH3 in sexual orientation was not well replicated in subsequent studies. Considering that INAH3 is too small to be accurately measured and the sample sizes in the abovementioned studies were small, the contribution of the INAH3 to sexual orientation remains an open question deserving of further exploration.

Several other brain structures have been reported to differ between individuals with different types of sexual orientation. For example, the central clock suprachiasmatic nucleus (SCN) was shown to be larger in homosexual men than that in heterosexual men (Swaab and Hofman, 1990). The size of the anterior commissure in the midsagittal plane (the median vertical longitudinal plane), which is known to be larger in women than in men, was also reported to be larger in homosexual than in heterosexual men (Allen and Gorski, 1992). Moreover, heterosexual men and homosexual women showed larger right cerebral hemispheres and their functional connections were derived from the right amygdala, whereas the volumes of the cerebral hemispheres were symmetrical in homosexual men and heterosexual women, who exhibited more widespread connections from the left amygdala (Savic and Lindstrom,

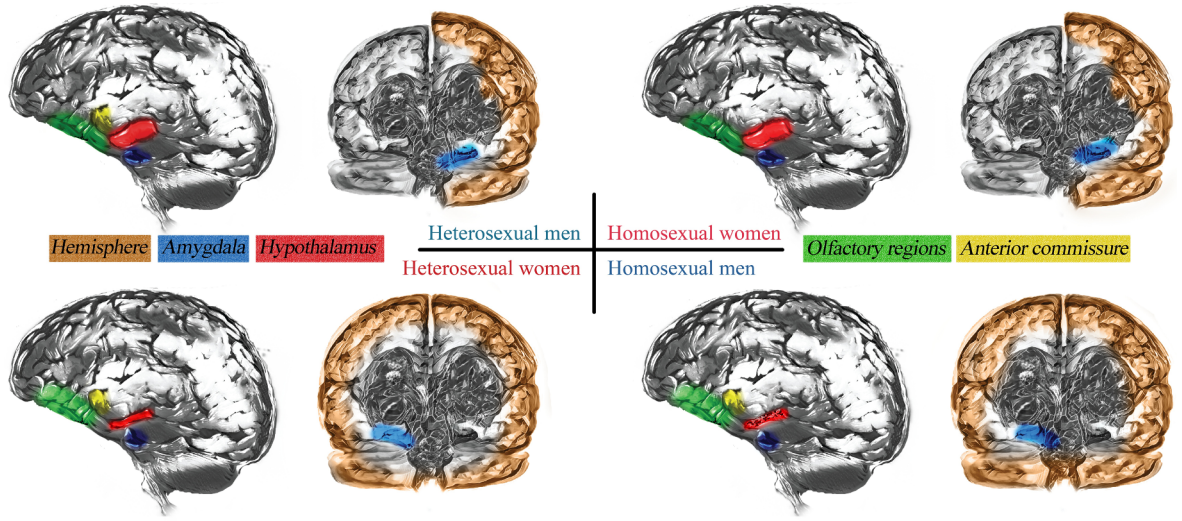

Fig. 1. Brain structural differences between homosexual and heterosexual individuals. The brown region represents the cerebral hemisphere. Heterosexual women and homosexual men have symmetrical hemispheres, whereas heterosexual men and homosexual women possess a larger right cerebral hemisphere (Savic and Lindstrom, 2008). The yellow region highlights the anterior commissure: women possess a larger anterior commissure than men; homosexual men possess a larger anterior commissure than heterosexual men (Allen and Gorski, 1992). The red region highlights the hypothalamus: the sizes of third interstitial nuclei of the human anterior hypothalamus (INAH3) are larger in men than in women (Allen et al., 1989; Garcia-Falgueras and Swaab, 2008), and are larger in heterosexual than in homosexual men (Byne et al., 2001; LeVay, 1991). Green highlights the olfactory regions: different regions are activated in heterosexual and homosexual individuals when smelling pheromones (Savic et al., 2005). Blue highlights the amygdala: the functional connections in the amygdala correlate with different traits of sexual orientation (Savic and Lindstrom, 2008). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2008). In addition, several studies consistently found that women have a greater number of neurons positive for kisspeptin in the human infundibular nucleus (INF) than men (Hrabovszky et al., 2010; Hrabovszky et al., 2011; Rometo et al., 2007). However, no differences in the number of kisspeptin neurons were observed between homosexual and heterosexual men, although female transgender (male sex assigned at birth) individuals exhibited a female-typical number of kisspeptin neurons (Taziaux et al., 2016). Thus, the potential causes of these differences and their potential correlation with sexual orientation, remain to be assessed and interpreted.

Sex-typical brain structures may result in differential processing of sex and gender stimuli associated with sexual attractions. For example, in homosexual men, the preoptic and ventromedial hypothalamic nuclei were activated when smelling progesterone-derivative steroids, and olfactory regions were activated when smelling estrogen-like steroids, which is similar to the results observed in heterosexual women but unlike those in heterosexual men (Savic et al., 2005). In contrast, homosexual women processed progesterone-derivative steroids via the olfactory networks and activated the anterior hypothalamus when smelling estrogen-like steroids, a trait that was partly shared with heterosexual men (Berglund et al., 2006). In addition, homosexual males have been reported to exhibit higher sensitivity to oxytocin, a neural peptide, than heterosexual males (Thienel et al., 2014) and showed the "male-favoring" pattern in brain imaging in response to viewing erotic multimedia, which was unlike the female-favoring pattern observed in heterosexual men (Jordan et al., 2018; Safron et al., 2017). Sex-typical brain structures may also correlate with differential processing of other external stimuli and behaviors in humans. For example, homosexual individuals have been reported to perform similar to their heterosexual opposite-sex counterparts (e.g., homosexual women performed like heterosexual men and homosexual men performed like heterosexual women) in cognitive tests related to spatial cognition and verbal fluency (Xu et al., 2017). Moreover, in a large study investigating the wiring patterns in white matter (referred to by the authors as the "brain structural connectome") using diffusion tensor imaging (DTI), a form of magnetic resonance imaging, Ingalhalikar et al. found that the brains of men exhibit relatively higher connectivity within the hemisphere and within frontal, temporal, and parietal lobes. In contrast, female brains have greater interhemispheric connectivity and greater cross-hemispheric participation (Ingalhalikar et al., 2014). This suggested that the brains of men are optimized for more localized, modular function; in contrast, the brains of women are conferred with connectional, cross-module function. Future study should investigate whether these fundamental sex differences in the brain structural connectome are associated with sexual orientation.

4. Effects of hormones on sexual orientation

Sex hormones, such as androgens and estrogens, have been shown to affect the development of male- and female-specific phenotypes during gestation (Arnold, 2012; Hines, 2011). The question of whether hormone differences underlie sexual orientation has been heatedly discussed. Evidence from studies on animals subjected to prenatal hormonal manipulation (Bakker et al., 1993; Baum et al., 1990) and on humans with atypical hormonal levels during development has indicated that sexual orientation is modulated largely by the hormonal environment at an early, probably perinatal, sensitive period (Cohen-Bendahan et al., 2005). Moreover, environmental factors also exert influence on sexual orientation, possibly through interaction with prenatal endocrine effects.

4.1. Effects of hormones on sexual behavior in animals

Androgens, in particular testosterone, are pivotal for brain masculinization during embryogenesis and maintenance of the male phenotype and sexual behavior in adulthood (Morris et al., 2004; Sato et al., 2004). In diverse mammalian species, including rodents and pigs, exposure to endogenous testosterone during perinatal development increases male-typical behavior and decreases female-typical behavior, and causes alterations in brain regions that mediate male- and female-typical responses (Henley et al., 2011). These effects of testosterone during pre- and postnatal development are often referred to as "organizational effects". Testosterone can also exert activated effects during pubertal periods (Schulz et al., 2009). Moreover, environmental factors, such as prenatal exposure to stress, can lead to irreversible changes in the sexual behavior of offspring by altering the levels of testosterone. In

guinea pigs, for example, prenatal stress resulted in decreased female-typical behavior and increased male-typical behavior in female offspring (Sachser and Kaiser, 1996), but strengthened intercourse behavior in male offspring (Kaiser and Sachser, 2001). The different effects of prenatal stress on sexual behavior between sexes may be due to different responses of males and females to stress-induced changes in testosterone levels (Hines, 2011).

Estrogens play essential roles in the regulation of sexual behavior (Couse and Korach, 1999). In the rodent brain, two types of estrogen receptor (ER) that exhibit similar affinity to estradiol, ERα (encoded by *Esr1*) and ERβ (encoded by *Esr2*), have been identified (Kuiper et al., 1997). ERα activation by estrogen is critical for the expression of normal sexual behavior in both female and male mice. Female mice lacking *Esr1* showed deficient sexual behaviors and repelled normal sexual behavioral interactions by male mice (Ogawa et al., 1998; Ogawa et al., 1996). The development of female-typical sexual behavior also requires the organizing role of prepubertal estradiol (Brock et al., 2011). Although *Esr1* knockout male mice showed normal sexual motivation to females, they achieved less intromissions and almost no ejaculations, and thus failed to exhibit normal masculine sex behaviors (Ogawa et al., 1997). In contrast, male and female mice lacking *Esr2* exhibited sexual behaviors that were generally normal (Ogawa et al., 1999). However, when gonadectomized male *Esr2* knockout mice were treated with female priming hormones, they exhibited enhanced female sex behavior, defined as keeping the lordosis posture and allowing males to mount (Kudwa et al., 2005), suggesting that defeminization of the male brain and behavior is regulated by ERβ. Notably, mice lacking the genes for both *Esr1* and *Esr2* showed no sexual behaviors, including simple mounting behavior (Ogawa et al., 2000), suggesting that the two ERs have redundant function in the expression of sexual behaviors in male mice. In addition to sexual behavior, estrogen, which is synthesized from testosterone by aromatase *in vivo*, is also important for masculinization of the developing brain and neural circuits that control male territorial behaviors (Wu et al., 2009).

4.2. Effects of hormones on sexual orientation in humans

In humans, studies investigating the effects of prenatal hormones on sexual orientation are challenging. An extensively studied model used to address this topic is congenital adrenal hyperplasia (CAH). CAH comprises a group of inherited genetic disorders resulting from mutations in genes encoding enzymes that regulate the biosynthesis of sex steroids, such as the *CYP21A2* gene encoding 21-hydroxylase (White and Speiser, 2000). Male and female fetuses with CAH are exposed to high levels of testosterone prenatally. Most women with CAH are exclusively heterosexual with regard to sexual arousal and behavior; however, 15–30% of women with CAH exhibited greater sexual arousal and fantasies toward women than to men and reported non-heterosexual fantasies and behavior (Frisen et al., 2009; Meyer-Bahlburg et al., 2008; Zucker et al., 1996). These observations suggested a moderate effect size of CAH on female sexual orientation. In addition, considering the high prenatal androgen levels in men with CAH, their core gender identity and sexual orientation are unaffected (Hines et al., 2004).

Testosterone has been proposed to stimulate the prenatal growth of an offspring's fourth finger, whereas estrogen promotes the growth of the second finger (Martin and Nguyen, 2004). The ratio between the second and fourth fingers (2D:4D) has been reported to be larger in women than in men, with a moderate sex difference, especially on the right hand (Honekopp and Watson, 2010). This sex-specific differential ratio emerges in childhood and is negatively correlated with masculinity, but not with femininity (Mitsui et al., 2016). Moreover, women with CAH exhibit smaller 2D:4D ratios (Honekopp and Watson, 2010), suggesting that prenatal androgen levels are associated with this trait. In addition, a meta-analysis on sexual orientation showed that homosexual and heterosexual women differed in their 2D:4D ratios, with a smaller value (that is, more masculine) observed in homosexual women (Grimbos et al., 2010), suggesting that they may have been exposed to higher concentrations of androgens during early development. However, most studies failed to detect a difference in the 2D:4D ratio between heterosexual and homosexual men (Grimbos et al., 2010; Williams et al., 2000), indicating that prenatal androgen may not be responsible for sexual orientation in men. Thus, the validity of the 2D:4D measure as a proxy for relative prenatal androgen exposure is controversial (Berenbaum et al., 2009; van Hemmen et al., 2017; Wallen, 2009).

Handedness is related to innate characteristics of brain sexual differentiation. Men have a higher prevalence (20% greater odds) of left-handedness than women (Papadatou-Pastou et al., 2008). Handedness has been associated with sexual orientation in multiple studies (Blanchard et al., 2006; Bogaert et al., 2007; Kishida and Rahman, 2015; Swift-Gallant et al., 2019). In particular, a meta-analysis of 20 studies including 6987 homosexual and 16,423 heterosexual participants reported that non-right-handedness is more common among homosexual individuals than among heterosexual individuals (Lalumiere et al., 2000). Homosexual participants had a 39% greater chance of being non-right-handed. Specifically, homosexual men had a 34% greater chance of being non-right-handed than heterosexual men, and homosexual women had a 91% greater chance of being non-right-handed than heterosexual women (Lalumiere et al., 2000). These data suggest that the developmental mechanisms underlying handedness may also contribute to some proportion of homosexual behavior.

An extensive morphometric study also examined the relationship between facial structure and sexual orientation (Skorska et al., 2015). Most differences in facial structure have been reported to be sex atypical, with four facial features that differed between heterosexual and homosexual women and three facial features that differed between heterosexual and heterosexual men (Skorska and Bogaert, 2017; Skorska et al., 2015). These seven unique facial features were further validated as multivariate predictors of sexual orientation (Gonzalez-Alvarez, 2017), suggesting that prenatal hormones likely contribute to the facial structures associated with sexual orientation. In addition, an anthropometric analysis indicated that the length of bones that become sexually dimorphic in childhood differed in homosexual and heterosexual individuals (Martin and Nguyen, 2004). In particular, men and women with a sexual partner preference for males had less long bone growth in the arms, legs, and hands compared with those having a sexual partner preference for females (Martin and Nguyen, 2004).

A number of studies have compared the concentrations of sex hormones between homosexual and heterosexual populations to investigate the potential influence of steroids on human sexual orientation. For example, bisexual/homosexual women were found to possess significantly higher levels of cortisol, a hormone regulating the response to environmental stressors, than those heterosexual women after exposure to the stressor; conversely, bisexual/homosexual men displayed lower cortisol levels than did heterosexual men (Juster et al., 2015). Lesbian/bisexual women also had higher overall testosterone and progesterone concentrations than heterosexual women, whereas no differences were found among gay/bisexual men in comparison to heterosexual men (Juster et al., 2016). Conversely, homosexual males exhibited higher sensitivity to oxytocin, another hormone involved in social bonding, than did heterosexual males. In young men, intranasal oxytocin administration increased attractiveness and approachability for male faces among homosexual participants; however, no effects on facial stimuli were observed in heterosexual individuals (Thienel et al., 2014). These results suggest that differences in sexual orientation may indicate specific differences in oxytocinergic signaling.

5. Genetic control of sexual orientation

Genetic studies, in particular twin and family studies, have helped to explain the biological basis underlying many important

Table 1
Genetic evidence of human sexual orientation.

Samples	Methods	Main findings	References
56 male MZs, 54 male DZs, and 57 of adoptive brothers	Population survey	Of the relatives whose sexual orientation could be rated, 52% of MZs, 22% of DZs were homosexual	Bailey and Pillard (1991)
84 homosexual and 79 heterosexual women	Population survey	Homosexual women had a significantly higher proportion of homosexual sisters	Bailey and Benishay (1993)
71 female MZs, 37 female DZs	Population survey	Of the relatives whose sexual orientation could be rated, 48% of MZs, 16% DZs were homosexual	Bailey et al. (1993)
197 homosexual men and 213 unselected subjects	PCR Analysis	The *androgen receptor* gene is not a common determinant of male sexual orientation	Macke et al. (1993)
76 homosexual men and traced out for pedigrees; 40 pairs of homosexual brothers	Paired-sibling analysis with gene markers in the X chromosome	Chromosome *Xq28*	Hamer et al. (1993)
33 new families with homoseuxal brothers	With gene markers in the X chromosome	Chromosome *Xq28*	Hu et al. (1995)
36 lesbian sib-pair families 52 families with two or more homosexual brothers	Microsatellite markers	Not support the role of Xq28 in male and female homosexuality	Rice et al. (1999)
312 male MZs, 182 male DZs, 668 female MZs, 376 female DZs, and 353 opposite-sex DZs in Australian	Population survey	Male homosexual concordance rate for MZs was higher than that for homosexual DZs. Women's same-sex concordance rates did not differ significantly by zygosity.	Bailey et al. (2000)
980 MZs, 928 DZs, and 1085 singles in Australian	Population survey	Heritability estimates of between 50 and 60% for females and approximately 30% for males	Kirk et al. (2000)
763 twins from distinct families and 794 twin pairs in US	Population survey	The rate of nonheterosexual sexual orientation did not differ between the twins and nontwin siblings, between the MZs and DZs, or between the same-sex and opposite-sex dizygotic twins	Kendler et al. (2000)
73 previously reported families and 73 new families with two or more homosexual brothers	Microsatellite markers at 10-cM intervals	D7S798 in 7q36, D8S505 in 8p12, and D10S217 in 10q26; an mlod score of 1.99 for Xq28	Mustanski et al. (2005)
3826 MZs and DZs in Sweden	Population survey	Genetic effects explained 34–39% of male and 18–19% of female homosexual behavior	Langstrom et al. (2010)
91 male MZs, 247 female MZs, 110 male DZs, 270 female DZs, and 203 opposite-sex DZs in Finnish	Quantitative genetic analyses	Childhood gender atypical behavior was a stronger predictor of adult sexual orientation for men	Alanko et al. (2010)
55 Canadian Caucasian families with two or more homosexual siblings	SNP-based screening	A SNP site at *rs760335* on chromosome 14	Ramagopalan et al. (2010)
906 MZs, 806 complete DZs	Multivariate genetic analysis	MZ correlations were consistently higher than DZ correlations	Burri et al. (2011)
409 independent pairs of homosexual brothers in 384 families	Genome-wide linkage analysis	The loci on pericentromeric chromosome 8; linkage to chromosome Xq28	Sanders et al. (2015)
Primarily European ancestry sample of 1077 homosexual men and 1231 heterosexual men	Affymetrix SNP arrays	SNPs on chromosomes 13 (*SLITRK6* is one gene nearest to these peaks), and chromosome 14 (*TSHR* is one gene nearest to these peaks) and on pericentromeric chromosome 8	Sanders et al. (2017)

MZ: onozygotic twin; DZ: dizygotic twin; SNP: single nucleotide polymorphism.

psychological traits, including sexual behavior (Harden, 2014). To date, the contributions of genetic factors to sexual orientation have been investigated through family and twin survey studies (Bailey and Benishay, 1993; Bailey and Pillard, 1991; Bailey et al., 1993; Kendler et al., 2000; Langstrom et al., 2010; Levan et al., 2009; Pillard and Weinrich, 1986; Schwartz et al., 2010), population-based segregation analyses (Bailey et al., 1999; Rice et al., 1999; Schwartz et al., 2010), focused or candidate gene-based approaches (DuPree et al., 2004; Hamer et al., 1993; Hu et al., 1995; Macke et al., 1993; Rice et al., 1999), and genome-wide association studies (GWAS) (Mustanski et al., 2005; Ramagopalan et al., 2010; Sanders et al., 2017; Sanders et al., 2015) (Table 1). Generally, genetic factors may explain 34–39% of the heritability of sexual orientation in men (Langstrom et al., 2010). In addition, female sexual orientation also exhibited moderate heritability (Bailey et al., 2000; Burri et al., 2011).

5.1. Genetic evidence of sexual orientation in humans

5.1.1. Chromosome Xq28

Early family studies reported that male homosexuality was more common among maternal cousins of homosexual individuals, such as the brothers or the sons of the brothers/sisters of their mothers, than among their fathers or paternal relatives (Hamer et al., 1993; Hu et al., 1995; Sanders et al., 2015). Further paired-sibling analysis using gene markers on the X chromosome linked male sexual orientation to a region of chromosome Xq28 (Hamer et al., 1993). However, several subsequent studies failed to identify a link between male homosexuality and Xq28, or an increased maternal relative to the paternal transmission of male homosexuality (Bailey et al., 2000; Bailey et al., 1999; Mustanski et al., 2005; Rice et al., 1999). Recently, a genome-wide screening provided suggestive support for linkage to the previously reported Xq28 region (Sanders et al., 2015), suggesting the etiologic heterogeneity with regard to the proposed Xq28 locus and male homosexuality. Nevertheless, the Xq28 region contains several genes that may bear potential relevance to sexual orientation. For example, arginine vasopressin receptor (AVPR) 2 mediates social and affiliative behavior (Ebstein et al., 2012), and cyclic nucleotide gated channel alpha 2 (CNGA2) has been shown to be critical for the regulation of odor-evoked socio-sexual behaviors in mice (Mandiyan et al., 2005).

5.1.2. Chromosomes 7 and 8

In the first genome-wide screening study of male sexual orientation, Mustanski et al. genotyped 403 microsatellite markers at 10 cM intervals in 146 families with two or more homosexual brothers and reported a significant link between chromosome 7q36 and homosexuality (Mustanski et al., 2005), which, however, lacks follow-up studies to validate. Their results also provided suggestive linkage to the pericentromeric region of chromosome 8 (~60–90 cM, ~8p21–p11) (Mustanski et al., 2005). This locus was further suggested by a genome-wide analysis of 409 independent pairs of homosexual brothers (Sanders et al., 2015) and a recent GWAS of 1077 homosexual men and 1231 heterosexual men (Sanders et al., 2017). Several candidate genes related to the regulation of gonadal hormone levels are located in this region. For example, 8p21 contains the gene *GNRH1*, which is secreted

from hypothalamic neurons and can promote the synthesis and release of LH and follicle-stimulating hormone (FSH), playing a pivotal role in sex-specific sexual development and behaviors (Desaulniers et al., 2017; Takeda et al., 2014). In addition, a gene on 8p11.23 encodes steroidogenic acute regulatory protein (*STAR*), which mediates pregnenolone synthesis and is involved in the hypothalamic-pituitary regulation of adrenal steroid production (Sugawara et al., 1995), playing an important role in the etiology of congenital lipoid adrenal hyperplasia, a potentially lethal form of CAH (Fujieda et al., 1997; Nakae et al., 1997; Sahakitrungruang et al., 2010).

5.1.3. Chromosomes 13 and 14

A region between the genes for SLIT and NTRK-like family member 6 (*SLITRK6*) and *SLITRK5* on chromosome 13 was recently suggested to associate with male homosexual orientation (Sanders et al., 2017). *SLITRK6* and *SLITRK5* are predominantly expressed in neural tissues (Aruga, 2003). In mice, knockout of *Slitrk6* resulted in deafness and myopia; similarly, in humans, *SLITRK6* mutations cause severe myopia and sensorineural deafness (Tekin et al., 2013). *Slitrk5* deficiency impairs corticostriatal neurotransmission and leads to obsessive-compulsive–like behaviors in mice (Shmelkov et al., 2010). However, whether *Slitrk5* or *Slitrk6* deficiency results in altered sexual behaviors in mice and humans requires further study.

Several single nucleotide polymorphisms (SNPs) on chromosome 14 have also been suggested to associate with male homosexual orientation (Ramagopalan et al., 2010; Sanders et al., 2017). On chromosome 14, genetic variation in intron 1 of the thyroid stimulating hormone receptor (*TSHR*) gene was suggested to explain the association of familial atypical thyroid function and male homosexuality. TSHR is essential for thyroid cell metabolism (Kleinau et al., 2013). Notably, homosexual men have been suggested to have an increased incidence of Graves's disease, the primary clinical symptom of which is hyperthyroidism (Frisch et al., 2014). Moreover, a recent retrospective chart review suggested that gestational thyroid dysfunction was associated with homosexual attraction in men (Sabuncuoglu, 2015). TSHR is expressed in both the thyroid gland and neuron-rich areas of the brain, such as the hippocampus (Crisanti et al., 2001), suggesting its potential regulation of complex behavior.

The above family and twin studies, as well as genetic linkage and association analyses, provide evidence for candidate genetic variant(s) in several chromosomes that contribute to the development of male sexual orientation, and serve as the clues supporting later molecular genetic studies. However, specific genes controlling sexual orientation have not been identified. Moreover, genome-wide genetic studies on female sexual orientation are lacking. The conflicting results and limitations of these studies may stem from considerations of statistical power and heterogeneity of human samples, as well as the challenges arising from sexual orientation being a complex trait. For example, bisexual individuals vary with regards to sexual history, arousal, and identity, and even orientation, and have often been indiscriminately or intentionally mixed with other non-heterosexual groups, such as homosexuality, in previous studies (Cerny and Janssen, 2011; Rieger et al., 2005, 2013; Rosenthal et al., 2011). Moreover, some homosexual individuals may have a bisexual history and tend to be sexually attracted to both sexes. Thus, the complexities in interpreting bisexual identities and the specific differences between bisexual and other non-heterosexual traits remain unclear and require further discussion. Furthermore, it is likely that genes involved in homosexuality may exert only modest effects, and that the development of homosexuality involves multiple genes, loci, and environmental factors and their interactions.

5.2. Genetic evidence in animals

Animal models with specific genetic manipulations can help to establish the association of candidate genes or pathways with specific phenotypes, and link genes to specific behavioral traits. For example, several classic behavioral assays, including sterility, courtship preference, and olfactory sensitivity assays, have been used to determine sexual partner preference and behaviors in flies. In particular, the formation of male courtship chains was used to define same-sex partner preference in flies. Similar methods have been used in other insects, such as ants (Matsuura et al., 2002) and flour beetles (Levan et al., 2009). Moreover, multiple behavioral paradigms, including genital odor preference, bedding preference, olfactory learning, and mating choice assays, have been developed to evaluate sexual orientation in rodent models (Hines, 2011; Liu et al., 2011; Mandiyan et al., 2005; Paredes et al., 1998; Zhang et al., 2013). The behavioral experiments performed in other mammals are quite similar to those performed in rodents. In ruminant species, isolated male goats (*Capra hircus*) displayed more frequent homosexual behavior than those housed near females (Ungerfeld et al., 2014). A recent study also reported male homosexual behavior (fellatio/erect penis licking) in flying foxes (*Pteropus pselaphon*) (Sugita, 2016). Homosexual behavior has also been observed in orangutans (*Pongo pygmaeus abelii*) (Fox, 2001) and monkeys (*Macaca fuscata*) (Gunst et al., 2015), two species evolutionarily close to humans. The behavioral paradigms related to same-sex partner preference and behaviors in diverse species are summarized in Fig. 2.

5.2.1. The fruitless (fru) gene in Drosophila

In *Drosophila*, sexual behavior is controlled by a hierarchy of sex-determining regulatory genes (Burtis, 1993; McKeown, 1994). The *fru* gene belongs to a branch of the sex-determination hierarchy and constitutes the first identified gene functioning specifically in the central nervous system to control courtship behavior in *Drosophila* (Ryner et al., 1996). In a *fru* mutant induced by X-rays, male flies with homozygous deletion of *fru* did not respond to female attempts at copulation and courted other males (Gill, 1963). The mutant male flies also stimulated other males to court themselves, resulting in behavioral sterility (Gailey and Hall, 1989). However, mammals use different sex-determination mechanisms to flies, and no genes homologous of *fru* have been reported in mammals. Thus, the gene in mammals controlling sexual orientation requires further investigation.

5.2.2. Olfactory processing signals and neurotransmitters in rodents

Genes regulating olfactory processing in the main sensory organs of mammals, such as the vomeronasal organ (VNO) and the main olfactory epithelium (MOE), have been found to modulate sexual behaviors. For example, the ion channel, transient receptor potential channel 2 (TRP2) belongs to the transient receptor potential family and is expressed in the VNO of the nose (Liman et al., 1999) and also in MOE sensory neurons (Omura and Mombaerts, 2014). Male mice with *Trp2* deficiency failed to fight or discriminate between sexes and displayed sexual and courtship behaviors towards both males and, females (Leypold et al., 2002; Stowers et al., 2002). In contrast, $Trp2^{-/-}$ female mice showed decreased female-specific behavior and displayed male-typical sexual and courtship behaviors indiscriminately towards male and female mice, similar to the phenotypes observed following the surgical removal of VNO in adult animals (Rosario and Schrimshaw, 2014). However, a subsequent study failed to confirm that VNO-mediated neural circuitry repressed the expression of male-typical mating behavior in female mice, suggesting that the effects reported above were not likely due to VNO dysfunction (Martel and Baum, 2009). The cyclic nucleotide-gated channel α2 (CNGA2) is also essential for olfactory signal transduction in mice (Brunet et al., 1996). Mice lacking functional CNGA2 failed to show a preference for female urine odors and showed an overall reduction in sexual behavior (Mandiyan et al., 2005). These results suggested that the sensory system may co-vary with sex-specific behavior, at least in rodents.

Serotonin or 5-hydroxytryptamine (5-HT), a monoamine neurotransmitter, has also been implicated in sexual preference and behavior (Hull et al., 2004). Male mice lacking central serotonergic neurons or

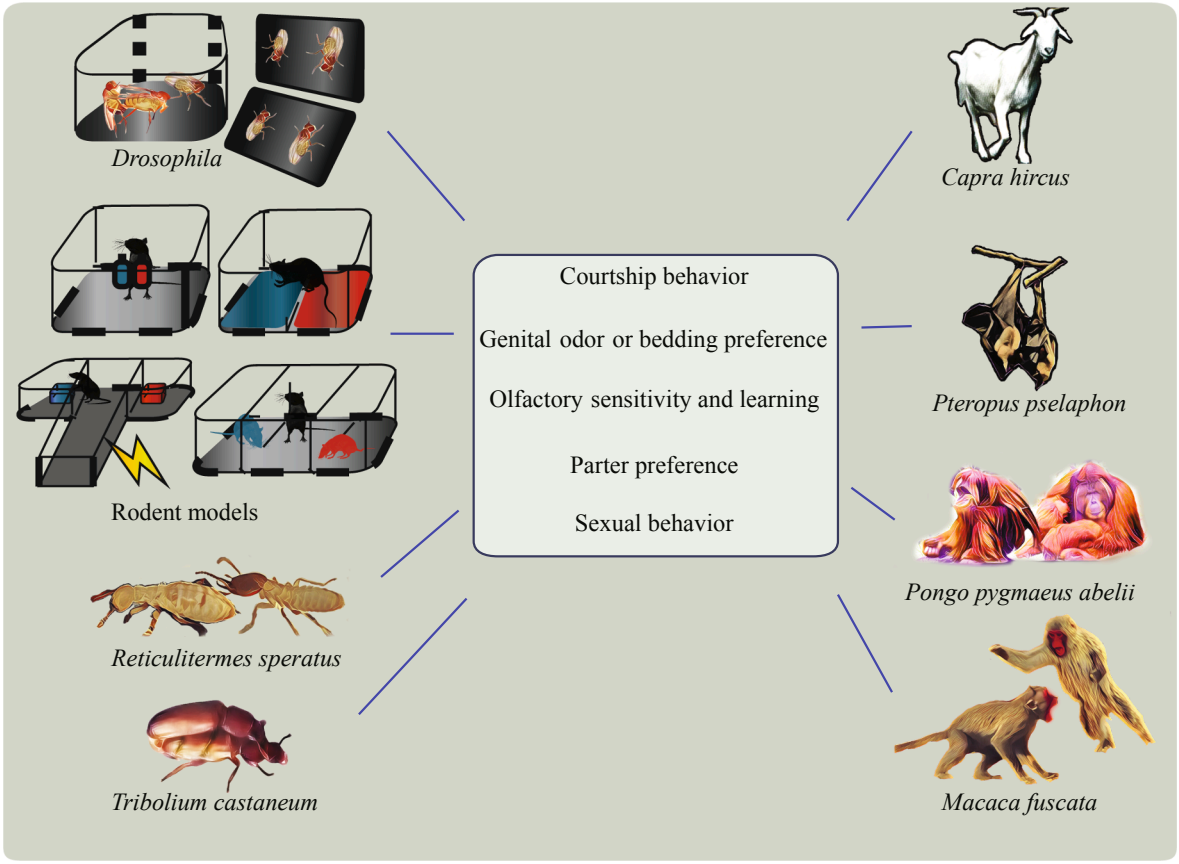

Fig. 2. Homosexual behaviors in diverse species and behavioral paradigms related to sexual orientation. In *Drosophila*, several classic behavioral assays, including the sterility assay, courtship stimulation assay, courtship preference assay, and olfactory sensitivity assay are used to examine sexual behaviors (Gailey and Hall, 1989). In *Reticulitermes speratus*, the tandem running assay is used to examine sexual behaviors (Matsuura et al., 2002). In *Tribolium castaneum*, mating behaviors are observed directly (Levan et al., 2009). In rodent models, multiple behavioral paradigms including genital odor preference, bedding preference, olfactory learning, resident-intruder test, and mating choice assays have been developed to evaluate sexual orientation (Hines, 2011; Liu et al., 2011; Mandiyan et al., 2005; Paredes et al., 1998; Rice et al., 2013; Zhang et al., 2013). In *Pteropus pselaphon*, fellatio or erect penis-licking and allogrooming are measured (Sugita, 2016). In *Capra hircus*, sexual mounts, frequencies of penile display, and flehmen response are recorded (Ungerfeld et al., 2014). In *Macaca fuscata*, sexual behaviors consist of sexual solicitation, sexual mounting, aggressive behavior, non-aggressive interference, sexual coercion, and sexual harassment, which can be easily recorded by observation (Gunst et al., 2015). In *Pongo pygmaeus abelii*, sexual behaviors are also directly observed (Fox, 2001).

tryptophan hydroxylase 2 (Tph2, an enzyme required for the synthesis of 5-HT in the brain), possessed normal pheromone-sensing ability but did not show sexual preference for females (Liu et al., 2011). These mice mounted males and females with similar latencies, frequencies, and durations, and spent equal time on female and male bedding and sniffing female and male genital odors (Liu et al., 2011). In turn, female mice lacking either central serotonergic neurons or serotonin preferred females over males. They sniffed female genitals, heads, and genital odors longer than those of males and displayed an increased incidence of female-female mounting (Zhang et al., 2013). These results suggested that central serotonergic signaling affects sexual preference in mice. Moreover, male rats cohabitating with an almond-scented male under the influence of oxytocin alone or combined with a D2 dopaminergic agonist, developed a same-sex socio-sexual preference (Cibrian-Llanderal et al., 2012; Triana-Del Rio et al., 2015). These results suggested that the D2-type receptor and oxytocin facilitate the development of conditioned same-sex partner preference. However, this effect does not occur in female mice or in the absence of pharmacological treatment. It remains to be determined whether preference for the scented familiar male partner would be generalized to other unfamiliar males as opposed to unfamiliar sexually receptive females. In addition, lesions of the sexually dimorphic POA/anterior hypothalamus in sexually experienced male ferrets caused them to approach body odors emitted from other males, which resembled sham-operated females; in contrast, control males lacking these lesions preferred to approach female as opposed to male body odors (Alekseyenko et al., 2007). The lesion-induced shift in odor preference was correlated with neural Fos responses in the medial POA, suggesting male-typical hypothalamic processing of body odorants (Alekseyenko et al., 2007).

6. Effects of maternal immune system on sexual orientation

The role of the immune system in sexual orientation has been discussed (Bogaert and Skorska, 2011). According to the maternal immune hypothesis, the mother's immune system is progressively immunized to male-specific antigens associated with the Y-chromosome by each successive male fetus. The increasing effects of such immunization would affect sexual differentiation of the brain and subsequently influence the sexual orientation of later-born sons (Blanchard and Bogaert, 1996). Boys with more elder brothers are more likely to develop a homosexual orientation; i.e., the so-called fraternal birth-order effect (Blanchard and Bogaert, 1996; Blanchard, 2018; Bogaert and Skorska, 2011). Each additional older brother may increase the probability of the youngest brother developing a homosexual orientation by approximately 21–33% (Blanchard and Bogaert, 1996; Cantor et al., 2002; Semenyna et al., 2017). Fraternal birth-order effect is one of the best documented correlates of men's sexual orientation; however, its underlying mechanisms have been theoretical. Until recently, it is found that women possess higher immunoreactivity to the Y-linked antigen NLGN4Y, which is important in male fetal brain development,

than men in the blood (Bogaert et al., 2018). Notably, mothers of homosexual sons, in particular those with older brothers, have significantly higher anti-NLGN4Y levels than mothers of heterosexual sons (Bogaert et al., 2018). This finding supports the maternal immune theory to explain the fraternal birth-order effect and the subsequent sexual orientation of male offspring.

7. Effects of environmental and epigenetic factors on sexual orientation

Epigenetic regulation mechanisms, including covalent chemical modifications of DNA (Razin and Riggs, 1980) and histones (Strahl and Allis, 2000), along with the production of non-coding RNAs, allow the stable regulation of gene expression and function induced by environmental factors without changing DNA sequence. Emerging evidence indicates that perinatal environments can affect physiological and behavioral traits in various species via epigenetic regulation (Wang et al., 2017). Whether prenatal and postnatal environments contribute to sexual orientation has attracted considerable attention (Balter, 2015; Rice et al., 2012). Some evidence from animal studies indicates that environmental hormones can affect sexual behavior and partner preference via DNA methylation and histone modification (Crews et al., 2007; Rice et al., 2012, 2013; Skinner et al., 2014; Walker and Gore, 2017). However, the effects of environmental factors on the sexual orientation of humans via epigenetic regulation remain poorly studied.

7.1. DNA methylation

In mammals, DNA methylation occurs mainly at the palindrome dinucleotide sequence 5′C-phosphate-G3′ (5′CpG3′) at the fifth position of cytosine (5mC), and constitutes one of the major epigenetic regulation mechanisms in mammalian cells (Bird, 2002). DNA methyltransferases (DNMTs) comprise the enzymes that establish (i.e., DNMT1) and maintain (i.e., DNMT3a and 3b) DNA methylation levels at individual CpGs (Bergman and Cedar, 2013; Klose and Bird, 2006; Okano et al., 1999). In rodents, the levels of DNA methylation and DNMT enzymatic activity in the neonatal POA differ between sexes, with lower levels observed in males (Nugent et al., 2015). In particular, the methylation levels of *ERα* in the POA of newborn females are higher than those in both males and estradiol-treated females (Nugent et al., 2011). DNA methylation is involved in the feminization of the mammalian brain via the active suppression of masculinization. Blocking DNMT activity or conditional knockout of *Dnmt3a* in the POA region masculinized neonatal gene expression and resulted in male-typical mating behavior in female rats, who engaged in more mounts and thrusts toward a sexually receptive female (Nugent et al., 2015). However, whether DNA methylation contributes to sexual partner preference remains to be investigated.

The levels of DNA methylation respond dynamically to environmental hormones (Ghahramani et al., 2014). For example, treatment of newborn females with a masculinizing dose of estradiol on postnatal day (P) 2 (albeit not P14) decreased DNMT activity and reverse the DNA methylation pattern in rats (Nugent et al., 2015). Similarly, in mice, exposure to a masculinizing dose of testosterone on the day of birth (P0) can result in a late-emerging molecular effect and induce a male-specific increase in DNA methylation in the autosomal genes of females on P60 (Ghahramani et al., 2014). Maternal exposure to bisphenol A, an estrogenic endocrine disruptor widely used in the production of plastics, altered DNMT1 and DNMT3A expression and altered DNA methylation levels and *Esr1* gene expression in the cortex and hypothalamus of juvenile mice (Kundakovic et al., 2013). Notably, daily injection of an endocrine-disrupting chemical vinclozolin on embryonic days (E) 8–14 resulted in a sex-specific effect on mate preference of the third-generation descendants (Crews et al., 2007). Specifically, female rats three generations removed from the exposure, preferred males without a history of exposure, whereas males with similar treatment history did not exhibit such a preference (Crews et al., 2007). This transgenerational inheritance of mate preference behavior correlated with altered sex-specific gene networks in the brain (Skinner et al., 2014), which may result from altered DNA methylation patterns in the sperm of affected animals (Anway et al., 2005; Guerrero-Bosagna et al., 2010). In addition, neonatal disruption of DNA methylation has long-term and sex-specific effects on the numbers of cells expressing ERα in the mPOA (Mosley et al., 2017).

In humans, however, studies regarding the role of epigenetic regulation in the effects of environmental factors on sexual orientation remain limited and primarily consist of mathematical modeling (Rice et al., 2013). For example, an abstract published by Vilain et al. at the 2015 American Society of Human Genetics (ASHG) meeting and discussed in detail by Balter, 2015, reported the DNA methylation levels at 140,000 regions in 37 monozygotic male twins who were discordant for sexual orientation (one being homosexual and the other heterosexual) using the machine-learning algorithm FuzzyForest. They identified five candidate regions linked to sexual orientation and reported an accuracy of 70% to predict sexual orientation. To reach a conclusion regarding the role of DNA methylation in sexual orientation, replication of these findings with a large population of non-overlapping samples is required.

7.2. Histone modification

Histone modifications occur through covalent modifications of the N-terminal tails of histones through acetylation, phosphorylation, and methylation (Jenuwein and Allis, 2001). During histone acetylation, an acetyl group is transferred to the amino group of lysine residues by histone acetyltransferases (Felsenfeld and Groudine, 2003); in contrast, histone deacetylases (HDACs) function as enzymes to remove acetyl groups from acetylated histones (Bolden et al., 2006). In rodents, the levels of certain histone modifications also differ between sexes. For example, the global levels of H3 lysine 9 trimethylation (H3K9me3) and lysine 9 and 14 acetylation (H3K9ac and H3K14ac) in the cortex and hippocampus are higher in males than females on E8, P0, and P8 (Tsai et al., 2009). Similarly, the levels of HDAC2 and 4 at the *Esr1* and aromatase genes are higher in the rat MPOA of males than in females (Matsuda et al., 2011). Testosterone treatment decreased the levels of H3 acetylation in the cortex and hippocampus of females compared with males (Tsai et al., 2009).

Histone modifications and HDAC activity in the early postnatal period are critical for masculinization of the brain and for proper sexual behavior in adulthood (Matsuda et al., 2011). Male rats treated with the HDAC inhibitor trichostatin A (TSA) on P0 and P1 presented a reduced erectile response and longer latencies to the first mount, intromission, and ejaculation (Matsuda et al., 2011). Injection of TSA to the lateral ventricle facilitated the formation of partner preference in both male and female prairie voles, which involved upregulation of the oxytocin receptor in the nucleus accumbens (NAcc) through increased histone acetylation at its promoter (Duclot et al., 2016; Wang et al., 2013). However, the contribution of histone modification to sexual orientation remains unclear and warrants further investigation.

8. Concluding remarks

Sexual orientation in humans represents a highly complex behavioral trait and is the result of multifaceted interactions between endocrine, genetic, and non-socioenvironmental factors. Although research has elucidated some initial insights into the basic mechanisms underlying sexual orientation, our knowledge regarding the full network that contributes to this complex phenomenon remains incomplete; additional interdisciplinary research and critical experiments are therefore required to obtain a better understanding of the biological basis of this basic human characteristic. Considering that the number of quantitative genetic studies related to sexual orientation is relatively

small compared with those addressing other behavioral traits, with most having been conducted using SNP arrays, whole-exome and whole-genome sequencing of larger cohorts of twin and family samples with different sexual orientation will continue to be valuable tools for understanding sexual orientation. Functional verification in animal models is also required for establishing the causal link between a specific gene and particular traits of sexual orientation. Nevertheless, it is likely that the development of sexual orientation involves multiple genes (i.e., Gene 1 × Gene 2), loci (i.e., Locus 1 × Locus 2), and their interactions with hormones and environmental factors.

Current studies have emphasized the important role of prenatal, non-social environments in sexual orientation. However, considering that individuals are very sensitive to external environments at prenatal and early postnatal stages, the question of whether social environment, such as chronic variable stress (Hines et al., 2002; Morgan and Bale, 2011), negative affective states, or parental care (Bai et al., 2017; Farr, 2017) contributes to the development of sexual characteristics by interacting with the in utero environment via epigenetic regulation should be specifically addressed in future studies. Moreover, considering the sensitivity of gonadal hormones to the external environment (Choi and Yoo, 2013), profiling the epigenomes of different sexual traits may provide additional evidence on how environments affect the establishment of sexual orientation.

Human asexuality, usually defined as lack of sexual attraction/eroticism, remains a relatively underrecognized phenomenon. However, increased interest has emerged regarding the definitions and conceptualizations of asexuality as well as its development and variation (Bogaert, 2015; Brotto and Yule, 2017). Several hypotheses have been assumed to explain the biological origins of asexuality. For example, early/prenatal factors have been suggested to exert influences on asexuality (Bogaert, 2004). In particular, non-right-handedness and fraternal birth order were reported to be associated with asexuality (Yule et al., 2014), which are similar to the associations found for homosexual men and women relative to heterosexual people. Considering that asexual individuals show high heterogeneity, a precise definition and conceptualization of this phenomenon will help to elucidate the biological basis of asexuality.

Men and women differ with regard to several aspects of sexual orientation, including the expression pattern, genital arousal, size and cell density of several brain structures, and sensitivity to prenatal hormones. These biological differences require the recruitment of unbiased samples of both genders to study the effects of hormonal, genetic, and environmental factors on sexual orientation. In addition, as sample collection is a limitation for such studies, more efforts are encouraged to eliminate social discrimination toward sexual orientation minorities through policies, public education, and non-profit organizations.

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Declaration of Competing Interest

None.

References

Abi Ghanem, C., Degerny, C., Hussain, R., Liere, P., Pianos, A., Tourpin, S., Habert, R., Macklin, W.B., Schumacher, M., Ghoumari, A.M., 2017. Long-lasting masculinizing effects of postnatal androgens on myelin governed by the brain androgen receptor. PLoS Genet. 13, e1007049.

Alanko, K., Santtila, P., Harlaar, N., Witting, K., Varjonen, M., Jern, P., Johansson, A., von der Pahlen, B., Sandnabba, N.K., 2010. Common genetic effects of gender atypical behavior in childhood and sexual orientation in adulthood: a study of Finnish twins. Arch. Sex. Behav. 39, 81–92.

Alekseyenko, O.V., Waters, P., Zhou, H., Baum, M.J., 2007. Bilateral damage to the sexually dimorphic medial preoptic area/anterior hypothalamus of male ferrets causes a female-typical preference for and a hypothalamic Fos response to male body odors. Physiol. Behav. 90, 438–449.

Allen, L.S., Gorski, R.A., 1992. Sexual orientation and the size of the anterior commissure in the human brain. Proc. Natl. Acad. Sci. USA 89, 7199–7202.

Allen, L.S., Hines, M., Shryne, J.E., Gorski, R.A., 1989. Two sexually dimorphic cell groups in the human brain. J. Neurosci. 9, 497–506.

Anway, M.D., Cupp, A.S., Uzumcu, M., Skinner, M.K., 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308, 1466–1469.

Arnold, A.P., 2012. The end of gonad-centric sex determination in mammals. Trends Genet. 28, 55–61.

Aruga, J., 2003. Slitrk6 expression profile in the mouse embryo and its relationship to that of Nlrr3. Gene Expr. Patterns 3, 727–733.

Attard-Johnson, J., Bindemann, M., ÓCiardha, C., 2016. Heterosexual, homosexual, and bisexual men's pupillary responses to persons at different stages of sexual development. J. Sex Res. 1–12.

Bai, Y., Belin, D., Zheng, X., Liu, Z., Zhang, Y., 2017. Acute stress worsens the deficits in appetitive behaviors for social and sexual stimuli displayed by rats after long-term withdrawal from morphine. Psychopharmacology 234, 1693–1702.

Bailey, J.M., Benishay, D.S., 1993. Familial Aggregation of Female Sexual Orientation. Am. J. Psychiat. 150, 272–277.

Bailey, J.M., Dunne, M.P., Martin, N.G., 2000. Genetic and environmental influences on sexual orientation and its correlates in an Australian twin sample. J. Pers. Soc. Psychol. 78, 524–536.

Bailey, J.M., Pillard, R.C., 1991. A genetic study of male sexual orientation. Arch. Gen. Psychiatry 48, 1089–1096.

Bailey, J.M., Pillard, R.C., Dawood, K., Miller, M.B., Farrer, L.A., Trivedi, S., Murphy, R.L., 1999. A family history study of male sexual orientation using three independent samples. Behav. Genet. 29, 79–86.

Bailey, J.M., Pillard, R.C., Neale, M.C., Agyei, Y., 1993. Heritable factors influence sexual orientation in women. Arch. Gen. Psychiatry 50, 217–223.

Bailey, J.M., Vasey, P.L., Diamond, L.M., Breedlove, S.M., Vilain, E., Epprecht, M., 2016. Sexual orientation, controversy, and science. Psychol. Sci. Public Interest 17, 45–101.

Bailey, N.W., Zuk, M., 2009. Same-sex sexual behavior and evolution. Trends Ecol. Evol. 24, 439–446.

Bakker, J., Baum, M.J., 2008. Role for estradiol in female-typical brain and behavioral sexual differentiation. Front. Neuroendocrinol. 29, 1–16.

Bakker, J., Brand, T., van Ophemert, J., Slob, A.K., 1993. Hormonal regulation of adult partner preference behavior in neonatally ATD-treated male rats. Behav. Neurosci. 107, 480–487.

Bakker, J., Honda, S., Harada, N., Balthazart, J., 2003. The aromatase knockout (ArKO) mouse provides new evidence that estrogens are required for the development of the female brain. Ann. N. Y. Acad. Sci. 1007, 251–262.

Ball, G.F., Balthazart, J., McCarthy, M.M., 2014. Is it useful to view the brain as a secondary sexual characteristic? Neurosci. Biobehav. Rev. 46 (Pt 4), 628–638.

Balter, M., 2015. Can epigenetics explain homosexuality puzzle? Science 350 148–148.

Balthazart, J., 2011. Minireview: Hormones and human sexual orientation. Endocrinology 152, 2937–2947.

Bao, A.M., Swaab, D.F., 2011. Sexual differentiation of the human brain: relation to gender identity, sexual orientation and neuropsychiatric disorders. Front. Neuroendocrin. 32, 214–226.

Baum, M.J., 2006. Mammalian animal models of psychosexual differentiation: when is 'translation' to the human situation possible? Horm. Behav. 50, 579–588.

Baum, M.J., Erskine, M.S., Kornberg, E., Weaver, C.E., 1990. Prenatal and neonatal testosterone exposure interact to affect differentiation of sexual behavior and partner preference in female ferrets. Behav. Neurosci. 104, 183–198.

Berenbaum, S.A., Bryk, K.K., Nowak, N., Quigley, C.A., Moffat, S., 2009. Fingers as a marker of prenatal androgen exposure. Endocrinology 150, 5119–5124.

Berglund, H., Lindstrom, P., Savic, I., 2006. Brain response to putative pheromones in lesbian women. Proc. Natl. Acad. Sci. USA 103, 8269–8274.

Bergman, Y., Cedar, H., 2013. DNA methylation dynamics in health and disease. Nat. Struct. Mol. Biol. 20, 274–281.

Bird, A., 2002. DNA methylation patterns and epigenetic memory. Gene Dev. 16, 6–21.

Blanchard, R., Bogaert, A.F., 1996. Homosexuality in men and number of older brothers. Am. J. Psychiatry 153, 27–31.

Blanchard, R., Cantor, J.M., Bogaert, A.F., Breedlove, S.M., Ellis, L., 2006. Interaction of fraternal birth order and handedness in the development of male homosexuality. Horm. Behav. 49, 405–414.

Blanchard, R., Klassen, P., Dickey, R., Kuban, M.E., Blak, T., 2001. Sensitivity and specificity of the phallometric test for pedophilia in nonadmitting sex offenders. Psychol. Assess. 13, 118–126.

Blanchard, R., 2018. Fraternal birth order, family size, and male homosexuality: meta-analysis of studies spanning 25 years. Arch. Sex. Behav. 47, 1–15.

Bogaert, A.F., 2004. Asexuality: prevalence and associated factors in a national probability sample. J. Sex Res. 41, 279–287.

Bogaert, A.F., 2015. Asexuality: what it is and why it matters. J. Sex Res. 52, 362–379.

Bogaert, A.F., Blanchard, R., Crosthwait, L.E., 2007. Interaction of birth order, handedness, and sexual orientation in the Kinsey interview data. Behav. Neurosci. 121, 845–853.

Bogaert, A.F., Skorska, M., 2011. Sexual orientation, fraternal birth order, and the maternal immune hypothesis: a review. Front. Neuroendocrin. 32, 247–254.

Bogaert, A.F., Skorska, M.N., Wang, C., Gabrie, J., MacNeil, A.J., Hoffarth, M.R., VanderLaan, D.P., Zucker, K.J., Blanchard, R., 2018. Male homosexuality and maternal immune responsivity to the Y-linked protein NLGN4Y. Proc. Natl. Acad. Sci. U S A 115, 302–306.

Bolden, J.E., Peart, M.J., Johnstone, R.W., 2006. Anticancer activities of histone deacetylase inhibitors. Nat. Rev. Drug Discov. 5, 769–784.
Brock, O., Baum, M.J., Bakker, J., 2011. The development of female sexual behavior requires prepubertal estradiol. J. Neurosci. 31, 5574–5578.
Broderick, G.A., 1998. Evidence based assessment of erectile dysfunction. Int. J. Impot Res. 10 (Suppl 2), S64–S73 discussion S77–69.
Brotto, L.A., Yule, M., 2017. Asexuality: sexual orientation, paraphilia, sexual dysfunction, or none of the above? Arch. Sex. Behav. 46, 619–627.
Brunet, L.J., Gold, G.H., Ngai, J., 1996. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. Neuron 17, 681–693.
Burri, A., Cherkas, L., Spector, T., Rahman, Q., 2011. Genetic and environmental influences on female sexual orientation, childhood gender typicality and adult gender identity. PLoS ONE 6, e21982.
Burtis, K.C., 1993. The regulation of sex determination and sexually dimorphic differentiation in *Drosophila*. Curr. Opin. Cell Biol. 5, 1006–1014.
Byne, W., Lasco, M.S., Kemether, E., Shinwari, A., Edgar, M.A., Morgello, S., Jones, L.B., Tobet, S., 2000. The interstitial nuclei of the human anterior hypothalamus: an investigation of sexual variation in volume and cell size, number and density. Brain Res. 856, 254–258.
Byne, W., Tobet, S., Mattiace, L.A., Lasco, M.S., Kemether, E., Edgar, M.A., Morgello, S., Buchsbaum, M.S., Jones, L.B., 2001. The interstitial nuclei of the human anterior hypothalamus: an investigation of variation with sex, sexual orientation, and HIV status. Horm. Behav. 40, 86–92.
Camperio Ciani, A., Battaglia, U., Cesare, L., Camperio Ciani, G., Capiluppi, C., 2018. Possible balancing selection in human female homosexuality. Hum. Nat. 29, 14–32.
Cantor, J.M., Blanchard, R., Paterson, A.D., Bogaert, A.F., 2002. How many gay men owe their sexual orientation to fraternal birth order? Arch. Sex. Behav. 31, 63–71.
Cerghet, M., Skoff, R.P., Bessert, D., Zhang, Z., Mullins, C., Ghandour, M.S., 2006. Proliferation and death of oligodendrocytes and myelin proteins are differentially regulated in male and female rodents. J. Neurosci. 26, 1439–1447.
Cerny, J.A., Janssen, E., 2011. Patterns of sexual arousal in homosexual, bisexual, and heterosexual men. Arch. Sex. Behav. 40, 687–697.
Celotti, F., Negri-Cesi, P., Poletti, A., 1997. Steroid metabolism in the mammalian brain: 5alpha-reduction and aromatization. Brain Res. Bull. 44, 365–375.
Chivers, M.L., Rieger, G., Latty, E., Bailey, J.M., 2004. A sex difference in the specificity of sexual arousal. Psychol. Sci. 15, 736–744.
Chivers, M.L., Seto, M.C., Blanchard, R., 2007. Gender and sexual orientation differences in sexual response to sexual activities versus gender of actors in sexual films. J. Pers. Soc. Psychol. 93, 1108–1121.
Choi, J.H., Yoo, H.W., 2013. Control of puberty: genetics, endocrinology, and environment. Curr. Opin. Endocrinol. Diabetes Obes. 20, 62–68.
Cibrian-Llanderal, T., Rosas-Aguilar, V., Triana-Del Rio, R., Perez, C.A., Manzo, J., Garcia, L.I., Coria-Avila, G.A., 2012. Enhaced D2-type receptor activity facilitates the development of conditioned same-sex partner preference in male rats. Pharmacol. Biochem. Behav. 102, 177–183.
Clarkson, J., Busby, E.R., Kirilov, M., Schutz, G., Sherwood, N.M., Herbison, A.E., 2014. Sexual differentiation of the brain requires perinatal kisspeptin-GnRH neuron signaling. J. Neurosci. 34, 15297–15305.
Cohen-Bendahan, C.C., van de Beek, C., Berenbaum, S.A., 2005. Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. Neurosci. Biobehav. Rev. 29, 353–384.
Couse, J.F., Korach, K.S., 1999. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. 20, 358–417.
Crews, D., Gore, A.C., Hsu, T.S., Dangleben, N.L., Spinetta, M., Schallert, T., Anway, M.D., Skinner, M.K., 2007. Transgenerational epigenetic imprints on mate preference. Proc. Natl. Acad. Sci. USA 104, 5942–5946.
Crisanti, P., Omri, B., Hughes, E., Meduri, G., Hery, C., Clauser, E., Jacquemin, C., Saunier, B., 2001. The expression of thyrotropin receptor in the brain. Endocrinology 142, 812–822.
Desaulniers, A.T., Cederberg, R.A., Lents, C.A., White, B.R., 2017. Expression and role of gonadotropin-releasing hormone 2 and its receptor in mammals. Front. Endocrinol. (Lausanne) 8, 269.
Dodd, L.D., Nowak, E., Lange, D., Parker, C.G., DeAngelis, R., Gonzalez, J.A., Rhodes, J.S., 2019. Active feminization of the preoptic area occurs independently of the gonads in Amphiprion ocellaris. Horm. Behav. 112, 65–76.
Duclot, F., Wang, H., Youssef, C., Liu, Y., Wang, Z., Kabbaj, M., 2016. Trichostatin A (TSA) facilitates formation of partner preference in male prairie voles (Microtus ochrogaster). Horm. Behav. 81, 68–73.
DuPree, M.G., Mustanski, B.S., Bocklandt, S., Nievergelt, C., Hamer, D.H., 2004. A candidate gene study of CYP19 (aromatase) and male sexual orientation. Behav. Genet. 34, 243–250.
Ebstein, R.P., Knafo, A., Mankuta, D., Chew, S.H., Lai, P.S., 2012. The contributions of oxytocin and vasopressin pathway genes to human behavior. Horm. Behav. 61, 359–379.
Farr, R.H., 2017. Does parental sexual orientation matter? A longitudinal follow-up of adoptive families with school-age children. Dev. Psychol. 53, 252–264.
Felsenfeld, G., Groudine, M., 2003. Controlling the double helix. Nature 421, 448–453.
Fox, E.A., 2001. Homosexual behavior in wild Sumatran orangutans (Pongo pygmaeus abelii). Am. J. Primatol. 55, 177–181.
Frisch, M., Nielsen, N.M., Pedersen, B.V., 2014. Same-sex marriage, autoimmune thyroid gland dysfunction and other autoimmune diseases in Denmark 1989–2008. Eur. J. Epidemiol. 29, 63–71.
Frisen, L., Nordenstrom, A., Falhammar, H., Filipsson, H., Holmdahl, G., Janson, P.O., Thoren, M., Hagenfeldt, K., Moller, A., Nordenskjold, A., 2009. Gender role behavior, sexuality, and psychosocial adaptation in women with congenital adrenal hyperplasia due to CYP21A2 deficiency. J. Clin. Endocrinol. Metab. 94, 3432–3439.
Fujieda, K., Tajima, T., Nakae, J., Sageshima, S., Tachibana, K., Suwa, S., Sugawara, T., Strauss 3rd, J.F., 1997. Spontaneous puberty in 46, XX subjects with congenital lipoid adrenal hyperplasia. Ovarian steroidogenesis is spared to some extent despite inactivating mutations in the steroidogenic acute regulatory protein (StAR) gene. J. Clin. Invest. 99, 1265–1271.
Gailey, D.A., Hall, J.C., 1989. Behavior and cytogenetics of fruitless in *Drosophila melanogaster*: different courtship defects caused by separate, closely linked lesions. Genetics 121, 773–785.
Galupo, M.P., Mitchell, R.C., Davis, K.S., 2018. Face validity ratings of sexual orientation scales by sexual minority adults: effects of sexual orientation and gender identity. Arch. Sex. Behav. 47, 1241–1250.
Ganna, A., Verweij, K.J.H., Nivard, M.G., Maier, R., Wedow, R., Busch, A.S., Abdellaoui, A., Guo, S., Sathirapongsasuti, J.F., et al., 2019. Large-scale GWAS reveals insights into the genetic architecture of same-sex sexual behavior. Science 365.
Garcia-Falgueras, A., Swaab, D.F., 2008. A sex difference in the hypothalamic uncinate nucleus: relationship to gender identity. Brain 131, 3132–3146.
Ghahramani, N.M., Ngun, T.C., Chen, P.Y., Tian, Y., Krishnan, S., Muir, S., Rubbi, L., Arnold, A.P., de Vries, G.J., Forger, N.G., et al., 2014. The effects of perinatal testosterone exposure on the DNA methylome of the mouse brain are late-emerging. Biol. Sex Differ. 5, 8.
Gill, K.S., 1963. A mutation causing abnormal courtship and mating behavior in males of Drosophila melanogaster. Am. Zool. 3, 507.
Gonzalez-Alvarez, J., 2017. Perception of sexual orientation from facial structure: a study with artificial face models. Arch. Sex. Behav. 46, 1251–1260.
Gooren, L., 2006. The biology of human psychosexual differentiation. Horm. Behav. 50, 589–601.
Grimbos, T., Dawood, K., Burriss, R.P., Zucker, K.J., Puts, D.A., 2010. Sexual orientation and the second to fourth finger length ratio: a meta-analysis in men and women. Behav. Neurosci. 124, 278–287.
Guerrero-Bosagna, C., Settles, M., Lucker, B., Skinner, M.K., 2010. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. PLoS ONE 5.
Gunst, N., Leca, J.B., Vasey, P.L., 2015. Influence of sexual competition and social context on homosexual behavior in adolescent female Japanese macaques. Am. J. Primatol. 77, 502–515.
Hamer, D.H., Hu, S., Magnuson, V.L., Hu, N., Pattatucci, A.M.L., 1993. A Linkage between DNA Markers on the X-Chromosome and Male Sexual Orientation. Science 261, 321–327.
Harden, K.P., 2014. Genetic influences on adolescent sexual behavior: Why genes matter for environmentally oriented researchers. Psychol. Bull. 140, 434–465.
He, Z., Cui, L., Ferguson, S.A., Paule, M.G., 2018. A working module for the neurovascular unit in the sexually dimorphic nucleus of the preoptic area. Mol. Neurobiol. 55, 156–163.
He, Z., Paule, M.G., Ferguson, S.A., 2012. Low oral doses of bisphenol A increase volume of the sexually dimorphic nucleus of the preoptic area in male, but not female, rats at postnatal day 21. Neurotoxicol. Teratol. 34, 331–337.
Henley, C.L., Nunez, A.A., Clemens, L.G., 2011. Hormones of choice: the neuroendocrinology of partner preference in animals. Front. Neuroendocrinol. 32, 146–154.
Hines, M., 2011. Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior. Front. Neuroendocrinol. 32, 170–182.
Hines, M., Brook, C., Conway, G.S., 2004. Androgen and psychosexual development: core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). J. Sex Res. 41, 75–81.
Hines, M., Davis, F.C., Coquelin, A., Goy, R.W., Gorski, R.A., 1985. Sexually dimorphic regions in the medial preoptic area and the bed nucleus of the stria terminalis of the guinea pig brain: a description and an investigation of their relationship to gonadal steroids in adulthood. J. Neurosci. 5, 40–47.
Hines, M., Johnston, K.J., Golombok, S., Rust, J., Stevens, M., Golding, J., Team., A.S., Avon Longitudinal Study of Parents and Children, 2002. Prenatal stress and gender role behavior in girls and boys: a longitudinal, population study. Horm Behav 42, pp. 126–134.
Honekopp, J., Watson, S., 2010. Meta-analysis of digit ratio 2D:4D shows greater sex difference in the right hand. Am. J. Hum. Biol. 22, 619–630.
Hrabovszky, E., Ciofi, P., Vida, B., Horvath, M.C., Keller, E., Caraty, A., Bloom, S.R., Ghatei, M.A., Dhillo, W.S., Liposits, Z., et al., 2010. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. Eur. J. Neurosci. 31, 1984–1998.
Hrabovszky, E., Molnar, C.S., Sipos, M.T., Vida, B., Ciofi, P., Borsay, B.A., Sarkadi, L., Herczeg, L., Bloom, S.R., Ghatei, M.A., et al., 2011. Sexual dimorphism of kisspeptin and neurokinin B immunoreactive neurons in the infundibular nucleus of aged men and women. Front. Endocrinol. (Lausanne) 2, 80.
Hu, S., Pattatucci, A.M.L., Patterson, C., Li, L., Fulker, D.W., Cherny, S.S., Kruglyak, L., Hamer, D.H., 1995. Linkage between sexual orientation and chromosome Xq28 in males but not in females. Nat. Genet. 11, 248–256.
Huberman, J.S., Chivers, M.L., 2015. Examining gender specificity of sexual response with concurrent thermography and plethysmography. Psychophysiology 52, 1382–1395.
Hull, E.M., Muschamp, J.W., Sato, S., 2004. Dopamine and serotonin: influences on male sexual behavior. Physiol. Behav. 83, 291–307.
Ingalhalikar, M., Smith, A., Parker, D., Satterthwaite, T.D., Elliott, M.A., Ruparel, K., Hakonarson, H., Gur, R.E., Gur, R.C., Verma, R., 2014. Sex differences in the structural connectome of the human brain. Proc. Natl. Acad. Sci. U S A 111, 823–828.
Jacobson, C.D., Csernus, V.J., Shryne, J.E., Gorski, R.A., 1981. The influence of gonadectomy, androgen exposure, or a gonadal graft in the neonatal rat on the volume of the sexually dimorphic nucleus of the preoptic area. J. Neurosci. 1, 1142–1147.
Jacobson, C.D., Shryne, J.E., Shapiro, F., Gorski, R.A., 1980. Ontogeny of the sexually

dimorphic nucleus of the preoptic area. J. Comp. Neurol. 193, 541–548.
Jenuwein, T., Allis, C.D., 2001. Translating the histone code. Science 293, 1074–1080.
Jordan, K., Wieser, K., Methfessel, I., Fromberger, P., Dechent, P., Muller, J.L., 2018. Sex attracts-neural correlates of sexual preference under cognitive demand. Brain Imaging Behav. 12, 109–126. https://doi.org/10.1007/s11682-016-9669-4.
Juster, R.P., Almeida, D., Cardoso, C., Raymond, C., Johnson, P.J., Pfaus, J.G., Mendrek, A., Duchesne, A., Pruessner, J.C., Lupien, S.J., 2016. Gonads and strife: Sex hormones vary according to sexual orientation for women and stress indices for both sexes. Psychoneuroendocrino 72, 119–130.
Juster, R.P., Hatzenbuehler, M.L., Mendrek, A., Pfaus, J.G., Smith, N.G., Johnson, P.J., Lefebvre-Louis, J.P., Raymond, C., Marin, M.F., Sindi, S., et al., 2015. Sexual orientation modulates endocrine stress reactivity. Biol Psychiat 77, 668–676.
Kaiser, S., Sachser, N., 2001. Social stress during pregnancy and lactation affects in guinea pigs the male offsprings' endocrine status and infantilizes their behaviour. Psychoneuroendocrino 26, 503–519.
Kendler, K.S., Thornton, L.M., Gilman, S.E., Kessler, R.C., 2000. Sexual orientation in a U.S. national sample of twin and nontwin sibling pairs. Am. J. Psychiatry 157, 1843–1846.
Kinsey, P., Pomeroy, W.B., Martin, C.E., 1948. Sexual Behavior in the Human Male. W.B. Saunders, Philadelphia, PA.
Kirk, K.M., Bailey, J.M., Dunne, M.P., Martin, N.G., 2000. Measurement models for sexual orientation in a community twin sample. Behav. Genet. 30 (4), 345–356.
Kishida, M., Rahman, Q., 2015. Fraternal birth order and extreme right-handedness as predictors of sexual orientation and gender nonconformity in men. Arch. Sex. Behav. 44, 1493–1501.
Kleinau, G., Neumann, S., Gruters, A., Krude, H., Biebermann, H., 2013. Novel insights on thyroid-stimulating hormone receptor signal transduction. Endocr. Rev. 34, 691–724.
Klose, R.J., Bird, A.P., 2006. Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. 31, 89–97.
Kudwa, A.E., Bodo, C., Gustafsson, J.A., Rissman, E.F., 2005. A previously uncharacterized role for estrogen receptor beta: defeminization of male brain and behavior. Proc. Natl. Acad. Sci. U S A 102, 4608–4612.
Kuiper, G.G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J.A., 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138, 863–870.
Kukkonen, T.M., 2015. Devices and methods to measure female sexual arousal. Sex Med Rev 3, 225–244.
Kundakovic, M., Gudsnuk, K., Franks, B., Madrid, J., Miller, R.L., Perera, F.P., Champagne, F.A., 2013. Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. Proc. Natl. Acad. Sci. USA 110, 9956–9961.
Lalumiere, M.L., Blanchard, R., Zucker, K.J., 2000. Sexual orientation and handedness in men and women: a meta-analysis. Psychol. Bull. 126, 575–592.
Langstrom, N., Rahman, Q., Carlstrom, E., Lichtenstein, P., 2010. Genetic and environmental effects on same-sex sexual behavior: a population study of twins in Sweden. Arch. Sex. Behav. 39, 75–80.
Levan, K.E., Fedina, T.Y., Lewis, S.M., 2009. Testing multiple hypotheses for the maintenance of male homosexual copulatory behaviour in flour beetles. J. Evolution. Biol. 22, 60–70.
LeVay, S., 1991. A difference in hypothalamic structure between heterosexual and homosexual men. Science 253, 1034–1037.
Leypold, B.G., Yu, C.R., Leinders-Zufall, T., Kim, M.M., Zufall, F., Axel, R., 2002. Altered sexual and social behaviors in trp2 mutant mice. Proc. Natl. Acad. Sci. U S A 99, 6376–6381.
Liman, E.R., Corey, D.P., Dulac, C., 1999. TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. Proc. Natl. Acad. Sci. U S A 96, 5791–5796.
Liu, Y., Jiang, Y., Si, Y., Kim, J.Y., Chen, Z.F., Rao, Y., 2011. Molecular regulation of sexual preference revealed by genetic studies of 5-HT in the brains of male mice. Nature 472, 95–99.
Liu, Y.C., Salamone, J.D., Sachs, B.D., 1997. Lesions in medial preoptic area and bed nucleus of stria terminalis: differential effects on copulatory behavior and noncontact erection in male rats. J. Neurosci. 17, 5245–5253.
Macke, J.P., Hu, N., Hu, S., Bailey, M., King, V.L., Brown, T., Hamer, D., Nathans, J., 1993. Sequence variation in the androgen receptor gene is not a common determinant of male sexual orientation. Am. J. Hum. Genet. 53, 844–852.
Madeira, M.D., Ferreira-Silva, L., Paula-Barbosa, M.M., 2001. Influence of sex and estrus cycle on the sexual dimorphisms of the hypothalamic ventromedial nucleus: stereological evaluation and Golgi study. J. Comp. Neurol. 432, 329–345.
Mandiyan, V.S., Coats, J.K., Shah, N.M., 2005. Deficits in sexual and aggressive behaviors in *Cnga2* mutant mice. Nat. Neurosci. 8, 1660–1662.
Martel, K.L., Baum, M.J., 2009. Adult testosterone treatment but not surgical disruption of vomeronasal function augments male-typical sexual behavior in female mice. J. Neurosci. 29, 7658–7666.
Martin, J.T., Nguyen, D.H., 2004. Anthropometric analysis of homosexuals and heterosexuals: implications for early hormone exposure. Horm. Behav. 45, 31–39.
Matsuda, K.I., Mori, H., Nugent, B.M., Pfaff, D.W., McCarthy, M.M., Kawata, M., 2011. Histone deacetylation during brain development is essential for permanent masculinization of sexual behavior. Endocrinology 152, 2760–2767.
Matsumoto, T., Honda, S., Harada, N., 2003. Alteration in sex-specific behaviors in male mice lacking the aromatase gene. Neuroendocrinology 77, 416–424.
Matsumoto, A., Arai, Y., 1983. Sex difference in volume of the ventromedial nucleus of the hypothalamus in the rat. Endocrinol. Jpn. 30, 277–280.
Matsumoto, A., Arai, Y., 1986. Male-female difference in synaptic organization of the ventromedial nucleus of the hypothalamus in the rat. Neuroendocrinology 42, 232–236.
Matsuura, K., Kuno, E., Nishida, T., 2002. Homosexual tandem running as selfish herd in Reticulitermes speratus: novel antipredatory behavior in termites. J. Theor. Biol. 214, 63–70.
Melcangi, R.C., Poletti, A., Cavarretta, I., Celotti, F., Colciago, A., Magnaghi, V., Motta, M., Negri-Cesi, P., Martini, L., 1998. The 5alpha-reductase in the central nervous system: expression and modes of control. J. Steroid Biochem. Mol. Biol. 65, 295–299.
McCarthy, M.M., Arnold, A.P., 2011. Reframing sexual differentiation of the brain. Nat. Neurosci. 14, 677–683.
McCarthy, M.M., Auger, A.P., Bale, T.L., De Vries, G.J., Dunn, G.A., Forger, N.G., Murray, E.K., Nugent, B.M., Schwarz, J.M., Wilson, M.E., 2009. The epigenetics of sex differences in the brain. J. Neurosci. 29, 12815–12823.
McKeown, M., 1994. Sex determination and differentiation. Dev. Genet. 15, 201–204.
Meerts, S.H., Anderson, K.S., Farry-Thorn, M.E., Johnson, E.G., Taxier, L., 2017. Prepubertal ovariectomy modulates paced mating behavior but not sexual preference or conditioned place preference for mating in female rats. Physiol. Behav. 171, 142–148.
Meyer-Bahlburg, H.F., Dolezal, C., Baker, S.W., New, M.I., 2008. Sexual orientation in women with classical or non-classical congenital adrenal hyperplasia as a function of degree of prenatal androgen excess. Arch. Sex. Behav. 37, 85–99.
Mitsui, T., Araki, A., Miyashita, C., Ito, S., Ikeno, T., Sasaki, S., Kitta, T., Moriya, K., Cho, K., Morioka, K., et al., 2016. The relationship between the second-to-fourth digit ratio and behavioral sexual dimorphism in school-aged children. PLoS ONE 11, e0146849.
Morgan, C.P., Bale, T.L., 2011. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. J. Neurosci. 31, 11748–11755.
Morris, J.A., Jordan, C.L., Breedlove, S.M., 2004. Sexual differentiation of the vertebrate nervous system. Nat. Neurosci. 7, 1034–1039.
Mosley, M., Weathington, J., Cortes, L.R., Bruggeman, E., Castillo-Ruiz, A., Xue, B., Forger, N.G., 2017. Neonatal inhibition of DNA methylation alters cell phenotype in sexually dimorphic regions of the mouse brain. Endocrinology 158, 1838–1848.
Murphy, L., Ranger, R., Fedoroff, J.P., Stewart, H., Dwyer, R.G., Burke, W., 2015. Standardization of penile plethysmography testing in assessment of problematic sexual interests. J. Sex Med. 12, 1853–1861.
Murray, E.K., Hien, A., de Vries, G.J., Forger, N.G., 2009. Epigenetic control of sexual differentiation of the bed nucleus of the stria terminalis. Endocrinology 150, 4241–4247.
Mustanski, B.S., DuPree, M.G., Nievergelt, C.M., Bocklandt, S., Schork, N.J., Hamer, D.H., 2005. A genomewide scan of male sexual orientation. Hum. Genet. 116, 272–278.
Nakae, J., Tajima, T., Sugawara, T., Arakane, F., Hanaki, K., Hotsubo, T., Igarashi, N., Igarashi, Y., Ishii, T., Koda, N., et al., 1997. Analysis of the steroidogenic acute regulatory protein (StAR) gene in Japanese patients with congenital lipoid adrenal hyperplasia. Hum. Mol. Genet. 6, 571–576.
Napolitano, L.M., Tompkins, L., 1989. Neural control of homosexual courtship in Drosophila melanogaster. J. Neurogenet. 6, 87–94.
Nugent, B.M., Schwarz, J.M., McCarthy, M.M., 2011. Hormonally mediated epigenetic changes to steroid receptors in the developing brain: implications for sexual differentiation. Horm. Behav. 59, 338–344.
Nugent, B.M., Wright, C.L., Shetty, A.C., Hodes, G.E., Lenz, K.M., Mahurkar, A., Russo, S.J., Devine, S.E., McCarthy, M.M., 2015. Brain feminization requires active repression of masculinization via DNA methylation. Nat. Neurosci. 18, 690–697.
Ogawa, S., Chan, J., Chester, A.E., Gustafsson, J.A., Korach, K.S., Pfaff, D.W., 1999. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. Proc. Natl. Acad. Sci. U S A 96, 12887–12892.
Ogawa, S., Chester, A.E., Hewitt, S.C., Walker, V.R., Gustafsson, J.A., Smithies, O., Korach, K.S., Pfaff, D.W., 2000. Abolition of male sexual behaviors in mice lacking estrogen receptors alpha and beta (alpha beta ERKO). Proc. Natl. Acad. Sci. U S A 97, 14737–14741.
Ogawa, S., Eng, V., Taylor, J., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1998. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. Endocrinology 139, 5070–5081.
Ogawa, S., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1997. Behavioral effects of estrogen receptor gene disruption in male mice. Proc. Natl. Acad. Sci. U S A 94, 1476–1481.
Ogawa, S., Taylor, J.A., Lubahn, D.B., Korach, K.S., Pfaff, D.W., 1996. Reversal of sex roles in genetic female mice by disruption of estrogen receptor gene. Neuroendocrinology 64, 467–470.
Okano, M., Bell, D.W., Haber, D.A., Li, E., 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99, 247–257.
Olvera-Hernandez, S., Fernandez-Guasti, A., 2015. Perinatal administration of aromatase inhibitors in rodents as animal models of human male homosexuality: similarities and differences. Adv. Neurobiol. 10, 381–406.
Omura, M., Mombaerts, P., 2014. Trpc2-expressing sensory neurons in the main olfactory epithelium of the mouse. Cell Rep. 8 (2), 583–595.
Papadatou-Pastou, M., Martin, M., Munafo, M.R., Jones, G.V., 2008. Sex differences in left-handedness: a meta-analysis of 144 studies. Psychol. Bull. 134, 677–699.
Paredes, R.G., Baum, M.J., 1995. Altered sexual partner preference in male ferrets given excitotoxic lesions of the preoptic area/anterior hypothalamus. J. Neurosci. 15, 6619–6630.
Paredes, R.G., Tzschentke, T., Nakach, N., 1998. Lesions of the medial preoptic area/anterior hypothalamus (MPOA/AH) modify partner preference in male rats. Brain Res. 813, 1–8.
Pillard, R.C., Weinrich, J.D., 1986. Evidence of familial nature of male homosexuality. Arch. Gen. Psychiatry 43, 808–812.
Poeppl, T.B., Langguth, B., Rupprecht, R., Laird, A.R., Eickhoff, S.B., 2016. A neural circuit encoding sexual preference in humans. Neurosci. Biobehav. Rev. 68, 530–536.
Ramagopalan, S.V., Dyment, D.A., Handunnetthi, L., Rice, G.P., Ebers, G.C., 2010. A

genome-wide scan of male sexual orientation. J. Homosexual 55, 131–132.
Razin, A., Riggs, A.D., 1980. DNA methylation and gene function. Science 210, 604–610.
Rice, G., Anderson, C., Risch, N., Ebers, G., 1999. Male homosexuality: Absence of linkage to microsatellite markers at Xq28. Science 284, 665–667.
Rice, W.R., Friberg, U., Gavrilets, S., 2012. Homosexuality as a consequence of epigenetically canalized sexual development. Quart. Rev. Biol. 87, 343–368.
Rice, W.R., Friberg, U., Gavrilets, S., 2013. Homosexuality via canalized sexual development: a testing protocol for a new epigenetic model. BioEssays 35, 764–770.
Rieger, G., Chivers, M.L., Bailey, J.M., 2005. Sexual arousal patterns of bisexual men. Psychol. Sci. 16, 579–584.
Rieger, G., Rosenthal, A.M., Cash, B.M., Linsenmeier, J.A., Bailey, J.M., Savin-Williams, R.C., 2013. Male bisexual arousal: a matter of curiosity? Biol. Psychol. 94, 479–489.
Rieger, G., Savin-Williams, R.C., 2012. The eyes have it: sex and sexual orientation differences in pupil dilation patterns. PLoS ONE 7, e40256.
Rometo, A.M., Krajewski, S.J., Voytko, M.L., Rance, N.E., 2007. Hypertrophy and increased kisspeptin gene expression in the hypothalamic infundibular nucleus of postmenopausal women and ovariectomized monkeys. J. Clin. Endocrinol. Metab. 92, 2744–2750.
Rosario, M., Schrimshaw, E.W., Hunter, J., Braun, L., 2006. Sexual identity development among gay, lesbian, and bisexual youths: consistency and change over time. J. Sex Res. 43, 46–58.
Rosario, M., Schrimshaw, E.W., 2014. Theories and etiologies of sexual orientation. In: Tolman, D.L., Diamond, L.M. (Eds.), APA handbook of sexuality and psychology. American Psychological Association, Washington, DC, pp. 556–559.
Roselli, C.E., Estill, C.T., Stadelman, H.L., Stormshak, F., 2009. The volume of the ovine sexually dimorphic nucleus of the preoptic area is independent of adult testosterone concentrations. Brain Res. 1249, 113–117.
Roselli, C.E., Larkin, K., Schrunk, J.M., Stormshak, F., 2004. Sexual partner preference, hypothalamic morphology and aromatase in rams. Physiol. Behav. 83, 233–245.
Roselli, C.E., Stadelman, H., Reeve, R., Bishop, C.V., Stormshak, F., 2007. The ovine sexually dimorphic nucleus of the medial preoptic area is organized prenatally by testosterone. Endocrinology 148, 4450–4457.
Rosenthal, A.M., Sylva, D., Safron, A., Bailey, J.M., 2011. Sexual arousal patterns of bisexual men revisited. Biol. Psychol. 88, 112–115.
Ryner, L.C., Goodwin, S.F., Castrillon, D.H., Anand, A., Villella, A., Baker, B.S., Hall, J.C., Taylor, B.J., Wasserman, S.A., 1996. Control of male sexual behavior and sexual orientation in Drosophila by the fruitless gene. Cell 87, 1079–1089.
Sabuncuoglu, O., 2015. High rates of same-sex attraction/gender nonconformity in the offspring of mothers with thyroid dysfunction during pregnancy: proposal of prenatal thyroid model. Ment Illn. 7, 5810.
Sachser, N., Kaiser, S., 1996. Prenatal social stress masculinizes the females' behaviour in guinea pigs. Physiol. Behav. 60, 589–594.
Safron, A., Barch, B., Bailey, J.M., Gitelman, D.R., Parrish, T.B., Reber, P.J., 2007. Neural correlates of sexual arousal in homosexual and heterosexual men. Behav. Neurosci. 121, 237–248.
Safron, A., Sylva, D., Klimaj, V., Rosenthal, A.M., Li, M., Walter, M., Bailey, J.M., 2017. Neural correlates of sexual orientation in heterosexual, bisexual, and homosexual men. Sci. Rep. 7, 41314.
Sahakitrungruang, T., Soccio, R.E., Lang-Muritano, M., Walker, J.M., Achermann, J.C., Miller, W.L., 2010. Clinical, genetic, and functional characterization of four patients carrying partial loss-of-function mutations in the steroidogenic acute regulatory protein (StAR). J. Clin. Endocrinol. Metab. 95, 3352–3359.
Sanders, A.R., Beecham, G.W., Guo, S., Dawood, K., Rieger, G., Badner, J.A., Gershon, E.S., Krishnappa, R.S., Kolundzija, A.B., Duan, J., et al., 2017. Genome-wide association study of male sexual orientation. Sci. Rep. 7, 16950.
Sanders, A.R., Martin, E.R., Beecham, G.W., Guo, S., Dawood, K., Rieger, G., Badner, J.A., Gershon, E.S., Krishnappa, R.S., Kolundzija, A.B., et al., 2015. Genome-wide scan demonstrates significant linkage for male sexual orientation. Psychol. Med. 45, 1379–1388.
Sato, T., Matsumoto, T., Kawano, H., Watanabe, T., Uematsu, Y., Sekine, K., Fukuda, T., Aihara, K., Krust, A., Yamada, T., et al., 2004. Brain masculinization requires androgen receptor function. Proc. Natl. Acad. Sci. U S A 101, 1673–1678.
Savic, I., Berglund, H., Lindstrom, P., 2005. Brain response to putative pheromones in homosexual men. Proc. Natl. Acad. Sci. U S A 102, 7356–7361.
Savic, I., Lindstrom, P., 2008. PET and MRI show differences in cerebral asymmetry and functional connectivity between homo- and heterosexual subjects. Proc. Natl. Acad. Sci. U S A 105, 9403–9408.
Schulz, K.M., Zehr, J.L., Salas-Ramirez, K.Y., Sisk, C.L., 2009. Testosterone programs adult social behavior before and during, but not after, adolescence. Endocrinology 150, 3690–3698.
Schwartz, G., Kim, R.M., Kolundzija, A.B., Rieger, G., Sanders, A.R., 2010. Biodemographic and physical correlates of sexual orientation in men. Arch. Sex. Behav. 39, 93–109.
Schwarz, J.M., Liang, S.L., Thompson, S.M., McCarthy, M.M., 2008. Estradiol induces hypothalamic dendritic spines by enhancing glutamate release: a mechanism for organizational sex differences. Neuron 58, 584–598.
Semenyna, S.W., VanderLaan, D.P., Vasey, P.L., 2017. Birth order and recalled childhood gender nonconformity in Samoan men and fa'afafine. Dev. Psychobiol. 59, 338–347.
Shmelkov, S.V., Hormigo, A., Jing, D., Proenca, C.C., Bath, K.G., Milde, T., Shmelkov, E., Kushner, J.S., Baljevic, M., Dincheva, I., et al., 2010. Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. Nat. Med. 16, 598–602 591p following 602.
Skinner, M.K., Savenkova, M.I., Zhang, B., Gore, A.C., Crews, D., 2014. Gene bionetworks involved in the epigenetic transgenerational inheritance of altered mate preference: environmental epigenetics and evolutionary biology. BMC Genom. 15, 377.
Skorska, M.N., Bogaert, A.F., 2017. Prenatal androgens in men's sexual orientation: evidence for a more nuanced role? Arch. Sex. Behav. 46, 1621–1624.
Skorska, M.N., Geniole, S.N., Vrysen, B.M., McCormick, C.M., Bogaert, A.F., 2015. Facial structure predicts sexual orientation in both men and women. Arch. Sex. Behav. 44, 1377–1394.
Slob, A.K., de Klerk, L.W., Brand, T., 1987. Homosexual and heterosexual partner preference in ovariectomized female rats: effects of testosterone, estradiol and mating experience. Physiol. Behav. 41, 571–576.
Smith, J.T., Popa, S.M., Clifton, D.K., Hoffman, G.E., Steiner, R.A., 2006. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. J. Neurosci. 26, 6687–6694.
Snowden, R.J., Wichter, J., Gray, N.S., 2008. Implicit and explicit measurements of sexual preference in gay and heterosexual men: a comparison of priming techniques and the implicit association task. Arch. Sex. Behav. 37, 558–565.
Srivastav, A., O'Halloran, A., Lu, P.J., Williams, W.W., Hutchins, S.S., 2019. Vaccination differences among U.S. adults by their self-identified sexual orientation, National Health Interview Survey, 2013–2015. PLoS ONE 14, e0213431.
Storms, M.D., 1980. Theories of sexual orientation. J. Pers. Soc. Psychol. 38, 783–792.
Stowers, L., Holy, T.E., Meister, M., Dulac, C., Koentges, G., 2002. Loss of sex discrimination and male-male aggression in mice deficient for TRP2. Science 295, 1493–1500.
Strahl, B.D., Allis, C.D., 2000. The language of covalent histone modifications. Nature 403, 41–45.
Sugawara, T., Lin, D., Holt, J.A., Martin, K.O., Javitt, N.B., Miller, W.L., Strauss 3rd, J.F., 1995. Structure of the human steroidogenic acute regulatory protein (StAR) gene: StAR stimulates mitochondrial cholesterol 27-hydroxylase activity. Biochemistry 34, 12506–12512.
Sugita, N., 2016. Homosexual Fellatio: erect penis licking between male bonin flying foxes pteropus pselaphon. PLoS ONE 11, e0166024.
Swaab, D.F., Fliers, E., 1985. A sexually dimorphic nucleus in the human brain. Science 228, 1112–1115.
Swaab, D.F., Hofman, M.A., 1990. An enlarged suprachiasmatic nucleus in homosexual men. Brain Res. 537, 141–148.
Swift-Gallant, A., Coome, L.A., Aitken, M., Monks, D.A., VanderLaan, D.P., 2019. Evidence for distinct biodevelopmental influences on male sexual orientation. Proc. Natl. Acad. Sc.i U S A 116, 12787–12792.
Takeda, T., Fujii, M., Hattori, Y., Yamamoto, M., Shimazoe, T., Ishii, Y., Himeno, M., Yamada, H., 2014. Maternal exposure to dioxin imprints sexual immaturity of the pups through fixing the status of the reduced expression of hypothalamic gonadotropin-releasing hormone. Mol. Pharmacol. 85, 74–82.
Taziaux, M., Staphorsius, A.S., Ghatei, M.A., Bloom, S.R., Swaab, D.F., Bakker, J., 2016. Kisspeptin expression in the human infundibular nucleus in relation to sex, gender identity, and sexual orientation. J. Clin. Endocrinol. Metab. 101, 2380–2389.
Tekin, M., Chioza, B.A., Matsumoto, Y., Diaz-Horta, O., Cross, H.E., Duman, D., Kokotas, H., Moore-Barton, H.L., Sakoori, K., Ota, M., et al., 2013. SLITRK6 mutations cause myopia and deafness in humans and mice. J Clin Invest 123, 2094–2102.
Thienel, M., Heinrichs, M., Fischer, S., Ott, V., Born, J., Hallschmid, M., 2014. Oxytocin's impact on social face processing is stronger in homosexual than heterosexual men. Psychoneuroendocrino 39, 194–203.
Todd, B.J., Schwarz, J.M., Mong, J.A., McCarthy, M.M., 2007. Glutamate AMPA/kainate receptors, not GABA(A) receptors, mediate estradiol-induced sex differences in the hypothalamus. Dev. Neurobiol. 67, 304–315.
Triana-Del Rio, R., Tecamachaltzi-Silvaran, M.B., Diaz-Estrada, V.X., Herrera-Covarrubias, D., Corona-Morales, A.A., Pfaus, J.G., Coria-Avila, G.A., 2015. Conditioned same-sex partner preference in male rats is facilitated by oxytocin and dopamine: effect on sexually dimorphic brain nuclei. Behav. Brain Res. 283, 69–77.
Tsai, H.W., Grant, P.A., Rissman, E.F., 2009. Sex differences in histone modifications in the neonatal mouse brain. Epigenetics 4, 47–53.
Tsukahara, S., 2009. Sex differences and the roles of sex steroids in apoptosis of sexually dimorphic nuclei of the preoptic area in postnatal rats. J. Neuroendocrinol. 21, 370–376.
Ungerfeld, R., Giriboni, J., Freitas-de-Melo, A., Lacuesta, L., 2014. Homosexual behavior in male goats is more frequent during breeding season and in bucks isolated from females. Horm. Behav. 65, 516–520.
van Hemmen, J., Cohen-Kettenis, P.T., Steensma, T.D., Veltman, D.J., Bakker, J., 2017. Do sex differences in CEOAEs and 2D:4D ratios reflect androgen exposure? A study in women with complete androgen insensitivity syndrome. Biol. Sex Differ. 8, 11.
Vasey, P.L., Leca, J.B., Gunst, N., VanderLaan, D.P., 2014. Female homosexual behavior and inter-sexual mate competition in Japanese macaques: possible implications for sexual selection theory. Neurosci. Biobehav. Rev. 46 (Pt 4), 573–578.
Ward, B.W., Dahlhamer, J.M., Galinsky, A.M., Joestl, S.S., 2014. Sexual orientation and health among U.S. adults: National Health Interview Survey, 2013. Natl Health Stat Report 2 (77):1–12.
Walker, D.M., Gore, A.C., 2017. Epigenetic impacts of endocrine disruptors in the brain. Front. Neuroendocrin. 44, 1–26.
Wallen, K., 2009. Does finger fat produce sex differences in second to fourth digit ratios? Endocrinology 150, 4819–4822.
Wang, H., Duclot, F., Liu, Y., Wang, Z., Kabbaj, M., 2013. Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles. Nat. Neurosci. 16, 919–924.
Wang, Y., Liu, H., Sun, Z., 2017. Lamarck rises from his grave: parental environment-induced epigenetic inheritance in model organisms and humans. Biol. Rev. Camb. Philos. Soc. 92, 2084–2111.
Wei, Y.C., Wang, S.R., Jiao, Z.L., Zhang, W., Lin, J.K., Li, X.Y., Li, S.S., Zhang, X., Xu, X.H., 2018. Medial preoptic area in mice is capable of mediating sexually dimorphic behaviors regardless of gender. Nat. Commun. 9, 279.
Weinrich, J.D., Klein, F., McCutchan, J.A., Grant, I., and the, H.G. (2014a). Cluster

Analysis of the Klein Sexual Orientation Grid in Clinical and Nonclinical Samples: When Bisexuality Is Not Bisexuality. J. Bisex 14, pp. 349–372.
White, P.C., Speiser, P.W., 2000. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr. Rev. 21, 245–291.
Williams, T.J., Pepitone, M.E., Christensen, S.E., Cooke, B.M., Huberman, A.D., Breedlove, N.J., Breedlove, T.J., Jordan, C.L., Breedlove, S.M., 2000. Finger-length ratios and sexual orientation. Nature 404, 455–456.
Wu, M.V., Manoli, D.S., Fraser, E.J., Coats, J.K., Tollkuhn, J., Honda, S., Harada, N., Shah, N.M., 2009. Estrogen masculinizes neural pathways and sex-specific behaviors. Cell 139, 61–72.
Xu, Y., Norton, S., Rahman, Q., 2017. Sexual orientation and neurocognitive ability: A meta-analysis in men and women. Neurosci. Biobehav. Rev., pii: S0149-7634(0117) 30273–30277.
Yoshida, M., Yuri, K., Kizaki, Z., Sawada, T., Kawata, M., 2000. The distributions of apoptotic cells in the medial preoptic areas of male and female neonatal rats. Neurosci. Res. 36, 1–7.
Yule, M.A., Brotto, L.A., Gorzalka, B.B., 2014. Biological markers of asexuality: Handedness, birth order, and finger length ratios in self-identified asexual men and women. Arch. Sex. Behav. 43, 299–310.
Zhang, S., Liu, Y., Rao, Y., 2013. Serotonin signaling in the brain of adult female mice is required for sexual preference. Proc. Natl. Acad. Sci. U S A 110, 9968–9973.
Zucker, K.J., Bradley, S.J., Oliver, G., Blake, J., Fleming, S., Hood, J., 1996. Psychosexual development of women with congenital adrenal hyperplasia. Horm. Behav. 30, 300–318.