



Sexual partner preference in animals and humans

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ABSTRACT

Sex differences in brain and behavior of animals including humans result from an interaction between biological and environmental influences. This is also true for the differences between men and women concerning sexual orientation. Sexual differentiation is mediated by three groups of biological mechanisms: early actions of sex steroids, more direct actions of sex-specific genes not mediated by gonadal sex steroids and epigenetic mechanisms. Differential interactions with parents and conspecifics have additionally long-term influences on behavior. This presentation reviews available evidence indicating that these different mechanisms play a significant role in the control of sexual partner preference in animals and humans, in other words the homosexual versus heterosexual orientation. Clinical and epidemiological studies of phenotypically selected populations indicate that early actions of hormones and genetic factors clearly contribute to the determination of sexual orientation. The maternal embryonic environment also modifies the incidence of male homosexuality via immunological mechanisms. The relative contribution of each of these mechanisms remains however to be determined.

1. Introduction

I began investigating the endocrine controls of behavior in the early 1970ies using mainly avian species (ducks first, and then chicken, ring doves, Japanese quail, and various species of songbirds) (Balthazart, 2017) and quickly became interested in the topic of sex differences and their development during ontogeny. Following the seminal paper of Phoenix and collaborators (Phoenix et al., 1959) establishing the notion of organizing effects of sex steroids, research coming from several laboratories had clearly demonstrated that in rodents (mostly rats) the early action of testosterone respectively masculinizes and defeminizes the expression of male- and female-typical behaviors (Forger et al., 2015; Gerall et al., 1992; McCarthy et al., 2009). Elizabeth Adkins-Regan and her collaborators at Cornell University were at that time establishing the model of sexual differentiation in quail by demonstrating that contrary to what is observed in rats, estrogens demasculinize the expression of male typical copulatory behavior in this species (for review (Balthazart et al., 2017)).

These studies provided a clear conceptual framework integrating the various mechanisms that contribute to generate adult subjects of two sexes that differ in multiple ways related to morphology, physiology and behavior (see Fig. 1). This information is available in many textbooks and reviews. I shall just provide here a brief overview mostly to define key concepts and the way I will use them since some of them

are sometimes employed with slightly different meanings in the literature.

In a nutshell, sex in mammals is essentially determined at the time of ovum fecundation by whether a sperm is bearing an X or a Y chromosome. In XY embryos resulting from fecundation by a Y sperm, the SRY gene located on the Y chromosome will determine the differentiation of the germinal ridge into testes while in the absence of SRY, in an XX embryo, this tissue will differentiate into an ovary. This determines the gonadal sex (Arnold, 2017). During the second part of fetal life, the testes will then produce testosterone (T) while the ovaries will remain essentially quiescent. Testosterone and/or its metabolites estradiol (E2) and 5 α -dihydrotestosterone (DHT) organize the morphology of the external genitals (DHT) as well as the structure and physiology of the brain (T and E2). These organizing effects are for the most part irreversible and take place during limited periods of increased sensitivity often called critical periods. After puberty, the adult gonads produce sex steroids (schematically T in males and E2 plus Progesterone, P in females) that will continue to modify the body structure (hip width, muscle mass, jaw width, etc.) and activate a variety of physiological and behavioral traits, obviously including sexual behaviors (McCarthy, 2012, 2008). Together, all these characteristics compose the phenotypic sex defining males and females.

This organizing role of sex steroids was until the end of the 20th century considered as the main factor organizing sex differences.

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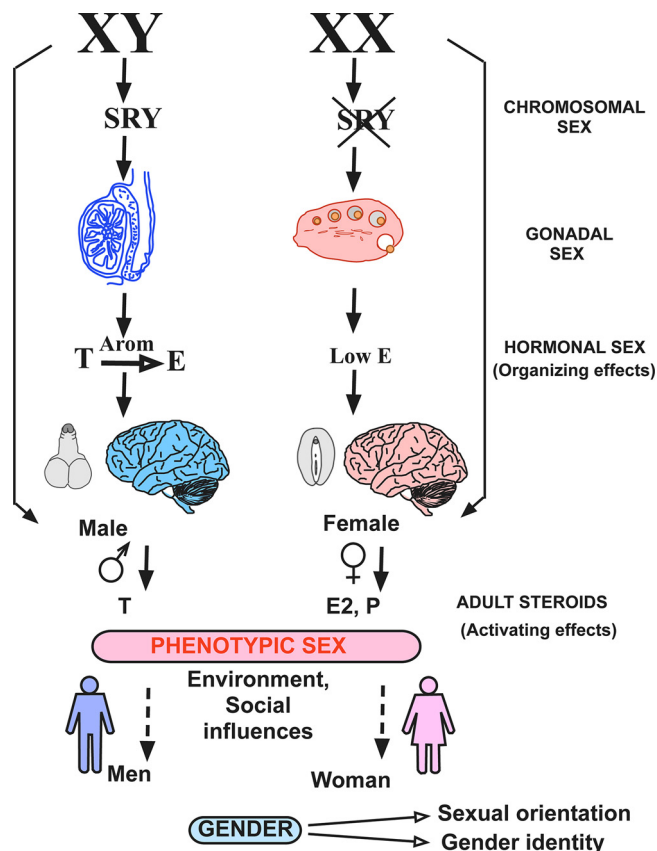


Fig. 1. Schematic presentation of the mechanisms that mediate the development of sex differences in mammals including humans. The SRY gene on the Y chromosome induces the formation of testes that will secrete testosterone (T) that will be acting by itself or via aromatization (Arom) into estradiol (E2) to masculinize the genital structures and the brain. Activational effects of sex steroids will then complete the differentiation of the adult male and female phenotype. Genes can additionally induce sex differences in a more direct manner that is not mediated via the action of sex steroids. Environmental and social influences will additionally modulate this sexual phenotype to determine the gender of an individual including his/her sexual orientation and gender identity.

However, investigations of a gynandromorphic zebra finch, *Taeniopygia guttata* (Agate et al., 2003) followed by extensive studies of the four core genotypes in mice in which gonadal sex is disconnected from chromosomal sex (Arnold, 2017; Arnold and Chen, 2009) clearly established that genes located on the sex chromosomes (or genes located on autosomes but differentially expressed in the two sexes) can also, independent of gonadal steroids, affect the sexual differentiation of an individual. These effects are often called “direct genetic effects” even if “effects not mediated via gonadal steroids” would be more appropriate.

Even more recently, evidence has accumulated supporting a role for inflammatory signaling molecules and for immune cells in the process of brain masculinization and its behavioral consequences (McCarthy, 2019; McCarthy et al., 2017). This work will be reviewed in a separate chapter prepared by McCarthy for this special issue.

Additionally, in humans but also to some degree in other animals (e.g., See (Meaney and Szyf, 2005; Moore, 1984, 1992; Moore and Power, 1992; Weaver et al., 2004)) various factors of the physical and social environment affect in a more or less enduring manner some behavioral traits. These factors determine what is, in humans specifically, called the gender, which I consider here as a partially social construct adding a layer of social influences on the sex, envisioned essentially as the result of various biological influences.

2. Sexual orientation as a sexually differentiated trait

Most adult humans are sexually attracted by members of the other sex and are thus heterosexual. There is however a significant minority estimated between 3 and 10 % depending on the sex of the subjects and on the study methods, who are attracted and interested in having sexual interactions with persons of the same sex; they often identify as gay/lesbian (Kinsey et al., 1953, 1948; Mosher et al., 2005). In the context of the sexual differentiation theory, homosexuality can be viewed as the reversal of sexual attraction observed in the largest fraction of the population. It is thus tempting to assume that sexual orientation is determined by the same mechanisms that control the differentiation of other sexually differentiated traits. Schematically, attraction to females (gynephilia) would be determined by early exposure to the action of testosterone while attraction to men (androphilia) would result from the absence of exposure to this hormone, although alternate views have been proposed (see (Jordan-Young, 2010), p 159).

My interest for human sexual orientation relates to a quest animating many basic scientists for identifying the translational value of research in animals. The prominent sex differences affecting expression of sexual behavior in rodents (and birds) that have been the object of intense investigations are not present in humans. Male rats mount, intromit and ejaculate following standardized behavior patterns and females reliably adopt the lordosis posture whereas human sexual behavior takes a variety of forms that are not specifically associated with one sex or the other (see (Nelson and Kriegsfeld, 2017)). In contrast, two dimensions of sexuality, namely sexual orientation and gender identity, are clearly differentiated in humans. The sex difference affecting these two dimensions of sexual behavior are associated with an effect size larger than 6 while most other behavioral sex differences in humans are associated with an effect size smaller than 1 (Hines et al., 2016b).

The origins of the sex difference in sexual orientation (homosexual or heterosexual, this is in fact the same question) have been the topic of heated debates. Some researchers, usually psychologists or sociologists, consider that the orientation is essentially learned or imposed by raw models in the society (behaviorism and constructivism) while Freud and his followers have popularized theories indicating that homosexuality would reflect some form of arrest in psycho-sexual development caused by inadequate interactions with one or both parents (Bailey et al., 2016).

There is however far more evidence supporting nonsocial causes than social causes for sexual orientation (Bailey et al., 2016) and like most biologists, I believe that sexual orientation is largely influenced, if not entirely determined, by biological factors acting during the fetal or early post-natal life, largely independently of social influences (Balthazart, 2012a; Balthazart and Young, 2014).

3. Sexual orientation in rodents

Unlike other aspects of human sexuality that have no equivalent in animals (e.g., gender identity), sexual orientation can easily be studied in non-human mammals by giving them a choice between a male or female sexual partner and recording toward which of these partners the animal will orient his/her sexual behavior.

Lesion and stereotaxic implantation studies demonstrated that the sexual orientation of males, more commonly called sexual partner preference for animals, is controlled, like male copulatory behavior, by the medial part of the preoptic area. Lesions of this brain area switch the preference of a male from a female to a male partner (Paredes and Baum, 1995; Paredes et al., 1998).

This preference for a partner of the same or the other sex is also reversed in both sexes by the early hormonal treatments that have been shown to sex-reverse copulatory behavior. Studies of Julie Bakker and Kos Slob at the University of Rotterdam (NL) initially demonstrated that inhibition of aromatase (the transformation of T into E2) during the two weeks surrounding birth in male rats largely switches their preference

from a female partner to a male partner (Bakker et al., 1993a, b, 1996). Similar effects of neonatal endocrine manipulations were later confirmed in male (Olvera-Hernandez et al., 2015) and female rats (Bodo and Rissman, 2008; Henley et al., 2009). Importantly the effects of these early hormonal manipulations seem to last the entire life of the subjects, they have to take place during a critical period of development, and the same treatments in adulthood have no effect on sexual partner preference. Recent work has additionally identified interactions between these early hormonal treatments and the sexual experience that subjects receive (Olvera-Hernandez et al., 2019).

Although same-sex sexual behavior is common in the animal kingdom (males or females mounting or being mounted by a same-sex conspecific) (Bagemihl, 2000; Poiani, 2010), it is often when partners of the opposite sex are not available (biased sex ratio, females monopolized by a dominant male, captivity, etc.). This behavior potentially serves as an outlet for sexual motivation in the absence of an appropriate sexual partner or fulfills other functions not directly related to sexuality (e.g., social appeasement, relations of dominance / subordination, etc.). A recent paper suggested that same-sex sexual behavior might be the ancestral conditions in all animals and this behavior has been lost during evolution in some but not other phyla (Monk et al., 2019). This is quite plausible but this topic is largely unrelated to the present review. Exclusive homosexual behavior and absolute same sex preference have rarely been observed in any animal species while this review concerns the mechanisms potentially controlling the more or less exclusive homosexual preference specifically observed in humans.

An animal model of spontaneous exclusive homosexuality has however been described in sheep. About 8% of the males in a population studied in the western United States were shown to mate exclusively with other males, even when the choice was given between a male or female partner (Perkins and Roselli, 2007; Roselli et al., 2011b). This homosexual behavior was correlated with the presence of a sexually dimorphic nucleus (ovine SDN) of the preoptic area that had a reduced size compared to heterosexual males. This ovine SDN is about three times larger in males than in females and contains about four times more neurons in males than in females. The ovine SDN of males attracted by other males has a structure quite similar to what is observed in females in terms of volume, numbers of neurons and also expression of aromatase (Roselli et al., 2004).

Hormonal manipulations further showed that the size of this nucleus is determined by the action of testosterone during embryonic life and is no longer modified in adulthood by the same hormone (Roselli et al., 2011a, b; Roselli and Stormshak, 2009). The small size of the ovine SDN in "homosexual" sheep is therefore probably determined before birth and before sheep have the opportunity to express their sexual orientation. Given that this nucleus is located in the medial preoptic area, a region involved in the control of sexual orientation, it appears likely that the small ovine SDN of homosexual sheep is the (one) cause of their atypical sexual orientation, resulting from an inadequate masculinization by testosterone during embryonic life.

Taken together these data indicate that sexual partner preference is largely determined in rodents and sheep by the early hormonal environment in particular by testosterone, eventually acting via its estrogenic metabolites. Clinical and epidemiological data indicate that the same mechanism is still present in humans and that gynephilia is enhanced by the fetal or early neonatal action of testosterone while androphilia would result from the absence of such androgenic effects. These endocrine effects however only explain part of the human sexual orientation. Genetic and immunological influences have also been clearly identified and they do not appear, based on current knowledge, to depend only on changes in the early endocrine environment. Together these hormonal, genetic and immunological effects seem to play a major role in the determinism of human sexual orientation. These effects have been reviewed multiple times by me (Balthazart, 2011, 2012a, b, 2016; Balthazart and Young, 2014) and others (LeVay, 1997, 2010; LeVay and Hamer, 1994). We will therefore only briefly

summarize these data and then focus on a few studies that were recently published that add critical evidence for the role of these biological mechanisms in the control of sexual orientation.

4. Is human sexual orientation reflecting organizing effects of sex steroids?

Two types of experimental evidence, clinical and epidemiological studies, indicate that hormones have an influence on adult sexual orientation.

4.1. Clinical studies

Three human pathologies support the notion that significant modifications of the embryonic endocrine environment affect the incidence of homosexuality independent of the sex of rearing. Firstly, girls suffering from congenital adrenal hyperplasia (CAH) are exposed *in utero* to abnormally high levels of androgens that masculinize their genital structures. Even if their endocrine condition is normalized by pharmacological means, their genital morphology is surgically "corrected" soon after birth and they are subsequently raised as girls, these girls will later on demonstrate masculinization of a variety of behavioral traits (e.g., aggressive play and toy selection) (Berenbaum, 1999; Berenbaum and Hines, 1992; Hines, 2004, 2006). They will also display a significantly increased incidence of homosexual (or at least not strictly heterosexual) orientation (up to 40 % compared to less than 10 % in control populations; See Fig. 2 (Hines, 2006; Meyer-Bahlburg et al., 2008; Money et al., 1984).

Secondly, girls born to mothers who had been treated with the synthetic estrogen diethylstilboestrol (DES) in the hope of preventing undesired abortions were shown to display a significant increase in non-heterosexual (bi- or homo-sexual) fantasies and sexual activities (Ehrhardt et al., 1985; Meyer-Bahlburg et al., 1995) (see however (Titus-Ernstoff et al., 2003) for a failure to replicate this effect). This suggests that estrogens might also be implicated in the differentiation of human sexual orientation, contrary to the conclusion of other studies

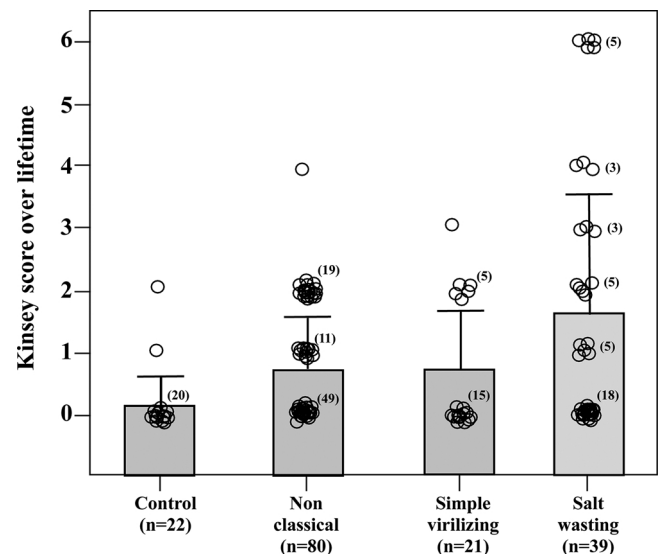


Fig. 2. Sexual orientation of women affected by congenital adrenal hyperplasia (CAH) as a function of the severity of the disease. The figure shows the individual Kinsey scores on a scale from 0 (completely heterosexual) to 6 (completely homosexual) evaluated for the entire lifetime as well as the means and SD of these scores in each group. The severity of CAH (increased prenatal exposure to androgens) increases from the non classical to the simple virilizing to the salt wasting form. When multiple subjects have the same Kinsey score in one group, the number of subjects is indicated next to the cluster of points. Redrawn from data in (Meyer-Bahlburg et al., 2008).

rather indicating that, in humans, androgens themselves play the major role in sexual differentiation.

Thirdly, in males, the study of patients suffering from cloacal exstrophy further supports a role for prenatal testosterone in the development of gynephilia. Cloacal exstrophy is a rare genito-urinary malformation resulting in the birth of XY males who, in addition to various malformations of the pelvis, have no penis. These subjects have normal testes and were thus presumably exposed to a male-typical pattern of androgen secretions before birth. The detailed follow-up of 7 of these subjects who had been submitted to vaginoplasty at birth ($n = 5$) or after 7–17 months of life ($n = 2$) and raised as girls revealed that in all cases but one these subjects reverted to a male gender identity in adulthood and were exclusively ($n = 6$) or predominantly ($n = 1$) sexually attracted to women (Data compiled in (Bailey et al., 2016); see also (Meyer-Bahlburg, 2005; Reiner, 2004; Reiner and Gearhart, 2004)).

Even if alternative explanations can and have been proposed for some of these observations, the most parsimonious explanation remains that embryonic hormones play a substantial role in the control of adult sexual orientation and that the sex of rearing is unable to completely counter the endocrine influences experienced prenatally.

4.2. Indirect markers of the prenatal endocrine milieu

On another hand, epidemiological studies indicate that homosexual and heterosexual populations differ in a number of established sex differences that are known, or at least strongly suspected, to be controlled by prenatal testosterone in animal species and often also in humans. This approach provides indirect information about the hormonal milieu to which an individual with a given sexual orientation was exposed during his/her embryonic life, given that it is nearly impossible, for practical reasons, to obtain this information in a direct manner.

These sex differences concern morphological, physiological and behavioral traits that are too numerous to be reviewed here in detail (See (Balthazart, 2012a, b; Balthazart and Young, 2014; LeVay, 2010) for detail and references). Morphological differences include 1) the relative length of the index (D2) to the ring finger (D4) (shorter, masculinized ratio in lesbians compared to heterosexual women), 2) the relative length of long bones in the legs, arms, and hands (shorter bones in gay men and women who are attracted by men compared to men and lesbians who are attracted by women), and the volume of several brain structures including 3) the surrachiasmatic nucleus (larger in gay than in heterosexual men), 4) the anterior commissure (larger in gay than in heterosexual men) and finally 5) the Interstitial Nucleus of the Anterior Hypothalamus number 3 (INAH3; 2–3 times larger in heterosexual men than in gay men (LeVay, 1991) and with a trend to a higher density of neurons in gay than in heterosexual men (Byne et al., 2001)).

Functional differences relate to 6) aspects of the inner ear physiology, the so-called oto-acoustic emissions (less frequent and of lower amplitude in lesbians compared to heterosexual women; see Fig. 3), 7) the feedback of steroids on the secretion of luteinizing hormone (presence of a weak positive feedback after injection of a large dose of estrogens in gay but not in heterosexual men), and 8) the brain activation as detected by positron emission tomography (PET) in response to male or female typical odors (reaction of gay men to male odors contrary to heterosexual men and lack of reaction of lesbians to male odors contrary to heterosexual women). A number of behavioral traits related to visuo-spatial (males [M] > females [F]) and verbal (F > M) tasks, aggressiveness (M > F), and lateralization (M more often left-handed than F) that are putatively influenced by prenatal testosterone have also been reported to be different between homosexual and heterosexual populations and also indirectly support a role of prenatal sex hormones but data are less conclusive and will not be reviewed here (see (Balthazart, 2012a, b; LeVay, 1997, 2010) for more detail).

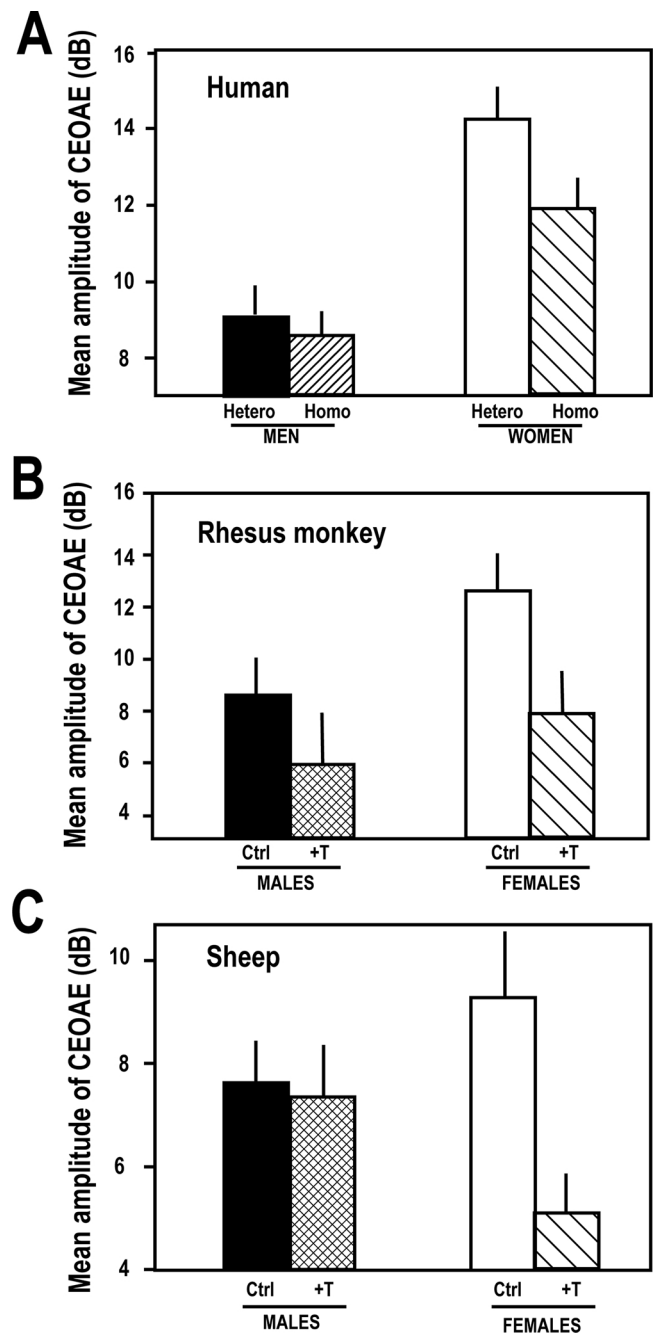


Fig. 3. Amplitude of click-evoked otoacoustic emissions (CEOAE) observed in male and female humans (A), rhesus monkeys (B) and sheep (C). Data refer to the left ear except in monkeys where the mean responses in both ears are shown. In humans CEOAE have a larger amplitude in heterosexual women than in heterosexual men but they are more masculine in lesbian women. The same sex difference is observed in both monkeys and sheep (females > males) and prenatal treatment with testosterone decreases CEOAE amplitude in females to male levels or below. This suggests that the sex difference observed in control (Ctrl) subjects is due to the early exposure of males to testosterone and that lesbians were similarly exposed to an excess of testosterone during fetal life. Redrawn from data in (McFadden and Pasanen, 1998; McFadden et al., 2006, 2009).

4.3. Intrasex phenotypic diversity

The endocrine theory of sexual differentiation would predict that if a sex difference is affected in one direction in lesbians compared to heterosexual women due to excessive prenatal exposure to androgens,

then a modification in the other direction should be present when comparing gay to heterosexual men. Interestingly this is not always the case and some of the sex differences that are modified in lesbians suggesting they have been exposed to an excess of androgens early in life are not affected in gay men. This is namely the case for the 2D:4D ratio. While meta-analyses consistently indicate a masculinized (smaller) ratio in lesbians compared to heterosexual women (Breedlove, 2010; Grimbos et al., 2010), the expected reverse effects (lack of masculinization or feminization) has not been consistently found in gay men. Either no difference is found or differences in both directions (gay > straight and straight > gay) are reported (reviewed in (Gooren and Byne, 2017)). This led some authors to speculate that the hormonal theory of sexual orientation could apply to women only but not to men (e.g., (Breedlove, 2017)).

This result is quite surprising since the 2D:4D ratio is clearly sexually differentiated and affected by prenatal testosterone in mammals (Bailey et al., 2005; Brown et al., 2002a; Roney et al., 2004; Zheng and Cohn, 2011), birds (Burley and Foster, 2004; Lombardo et al., 2008; Romano et al., 2005), reptiles (Rubolini et al., 2006) and amphibians (Kaczmarek et al., 2015), even if this is a very imprecise marker of prenatal androgenization. This ratio is for example masculinized in CAH girls who have been exposed to an excess of testosterone in utero (Brown et al., 2002c; Okten et al., 2002) and it is usually feminine in genetic males bearing a mutation of the androgen receptor, preventing all effects of the steroid, the complete androgen insensitivity syndrome (Berenbaum et al., 2009). The 2D:4D ratio is admittedly a noisy reporter of prenatal androgen exposure with a limited effect size for the sex difference (Cohen *d* around 0.5, see (Breedlove, 2017)) that does not permit to predict the sexual orientation of a specific individual but it is reasonably reliable at the population level. It has a number of significant drawbacks (see (Balthazart and Court, 2017) for detail) but why it is not feminized in gay men raises the question of whether male homosexuality is or is not influenced by the prenatal androgen action.

An abundant literature, some of it quite old, indicates that circulating concentrations of testosterone are higher than necessary to activate male sexual behavior in adulthood although they are needed to promote the full spermatogenesis (Damassa et al., 1977; Grunt and Young, 1953). Full copulatory behavior is activated in castrated males by administration of doses of testosterone that restore plasma concentrations equivalent to only about 10 % of concentrations present in sexually mature male (Damassa et al., 1977). One might speculate that, conversely, testosterone concentrations during ontogeny in males are higher than concentrations required to masculinize morphology (genitals and the 2D:4D) but are needed to masculinize sexual orientation. A small decrease in concentration could thus interfere with the development of gynephilia without leaving any trace in the periphery.

It is also possible that male homosexuality results from a deficit in androgen action specifically in the brain while androgens circulating concentration and action in the periphery on the growth of long bone (and thus the 2D:4D ratio) are left intact. But then why would this discrepancy between central and systemic action of androgens not be present also in females?

A more likely explanation in my eyes, relates to the phenotypic heterogeneity of male and female homosexual subjects. It has been reported that the 2D:4D ratio is more reliably masculinized in “*butch*” (more masculine) than in “*femme*” (more feminine) lesbians (Brown et al., 2002b). One could thus wonder whether a same dichotomy also exists in gay men and explains why variable 2D:4D ratios are reported based on differences in the degree of masculinization in the sampled population.

In the Anglo-saxon culture, gay men identify themselves as belonging to two categories: the more feminine, self-identified as “twinks” and the more masculine or hyper-masculine (hairier and heavier), self-identified as “bears” (Blankenship, 2013; Hennen, 2005; Moskowitz et al., 2013). A similar distinction has been described in the Zapotec population of the Istmo region of Oaxaca (Mexico) where more

feminine male androphiles are identified as *muxe gunaa*, whereas more masculine male androphiles are labeled as *muxe nguii* (Gomez et al., 2017, 2018). If two such subpopulations do exist among gay men and one has putatively been exposed to high androgen action during fetal life while the other has not, a variable mix of these two phenotypes in a population would explain the absence or variability of the difference in 2D:4D ratio between gay and straight men. Until recently, no research had attempted to better characterize the phenotype of the gay men that were studied, in particular those analyzed for the 2D:4D ratio, in order to determine whether a mix between high and low 2D:4D ratios in two more feminine and more masculine sub-populations is responsible for the failure to find a reliable difference associated with sexual orientation.

Ashlyn Swift-Gallant at the Memorial University of Newfoundland recently reviewed literature indicating that, in both mice and human, the organizing effects of androgens are not necessarily linear. It is classically admitted that exposure to androgens during development promotes male-typical behaviors and male-typical sexual preference (gynephilia) whereas lack of such exposure or exposure to very low levels promote female-typical behaviors and androphilia (Swift-Gallant, 2019). Studies in mice have however accumulated showing that when androgen stimulation is very high, due to injection of large doses of exogenous steroid or to androgen receptor overexpression, male-typical behavior is still expressed but sexual preferences may be altered and same-sex attraction is enhanced (e.g., (Dela Cruz and Pereira, 2012; Henley et al., 2010; Swift-Gallant et al., 2016a, b); see (Swift-Gallant, 2019) for a general review of this evidence). She also presented evidence that homosexual sexual orientation in men might result from an early developmental exposure to both lower and also higher androgenic stimulation as compared to heterosexual men (Swift-Gallant, 2019). This raises the possibility that early exposure to high androgenic stimulation may contribute to explain the homosexual orientation of gay men who prefer an insertive anal role (called tops) while those who prefer receptive anal sex (bottoms) or show no preference (versatiles) would have been exposed to low androgen stimulation, based on indirect retrospective markers of androgen exposure. This conclusion is however not based on measures of the 2D:4D ratio as a marker of prenatal androgen exposure but rather to a differential incidence of left-handedness.

Handedness is another marker of the sexual differentiation process that is largely determined prenatally (Hepper, 2013; Hepper et al., 1991). Men have on average a higher preference for using the left hand for various tasks as compared to women (Papadatou-Pastou et al., 2008) and this seems to be influenced by prenatal androgens given that some studies identified an increased left handedness in CAH girls, even if independent genetic, epigenetic and immunological factors are also implicated. Handedness has also been associated with sexual orientation but results have been inconsistent like for the 2D:4D ratio. Some studies showed that non-right-handedness is more frequent in gay than in heterosexual men but other studies failed to replicate this difference. Swift-Gallant investigated whether anal sex role in homosexual men (top vs. bottom) could be associated with retrospective measures of androgen exposure. They found that gay men with a bottom anal sex role have, counter-intuitively, an increased left-hand preference compared to heterosexual men but this difference was not found in tops. These two groups of gay men also differed by a number of other measures including the recalled childhood gender non-conformity, height, body hair and pubertal onset (Swift-Gallant, 2019).

In addition, the study of a Chinese population of gay men indicated that there is a significant difference between tops and bottoms in the empathizing-systemizing cognitive scale with tops scoring higher on the systemizing than bottoms and versatiles, and vice versa (Zheng et al., 2015). Given that systemizing has been associated with prenatal exposure to high androgen concentrations while empathizing would rather be linked to a lower prenatal androgen action (Auyeung et al., 2012; Chapman et al., 2006; Greenberg et al., 2018), all these data

suggest that androphilic attraction in men could result from both high and low early exposure to androgens while the gynephilic attraction would result from a mid-range exposure to the steroid. This hypothesis should now be tested using several of the best established markers of early androgen exposure such as the 2D:4D ratio or the rate of oto-acoustic emissions. If confirmed it would explain the discordant results previously obtained in studies of these markers that did not take into account the phenotypic diversity among gay men.

5. Genetic contributions

Taken together, the data discussed in the previous section support the notion that prenatal androgenization plays a significant role in the control of human sexual orientation. However changes in sexual orientation as a result of prenatal endocrine disorders always concern only a fraction of affected individuals (usually a maximum of 30–40%). Furthermore, all markers of the prenatal hormone milieu that have been associated with homosexuality are noisy markers that only explain a limited part of the variance in orientation. In addition, if variations in endocrine exposure during ontogeny explain differences in sexual orientation, the origin of these variations needs to be identified.

It was suggested that a decreased androgenization might be the consequence of intense stress in pregnant women based on the fact that retrospective studies indicated an increased incidence of male homosexuality in men born in Berlin during the last two years of World War II (Dörner et al., 1980, 1983). However, even if this potential mechanism is supported by a host of studies in rodents (Ward, 1972, 1984; Ward and Ward, 1985; Weisz et al., 1982), no additional evidence based on human studies has since been collected.

Individual genetic differences could obviously explain differences in steroid production or action. In addition, animal research has during the last two decades demonstrated that genes can affect the adult phenotype in a “direct” manner i.e., by mechanisms that do not involve changes in steroid exposure (see Section 1 and 3). A substantial body of research has therefore been dedicated to the potential genetic bases of homosexuality.

5.1. Heritability within families and twins comparisons

Epidemiological studies have clearly established that concordance in sexual orientation correlates to some extent with genetic relatedness. If a son is homosexual in a family, there is an increased probability that some of his brothers will share this orientation as compared to the general population. The incidence of homosexuality in brothers of homosexual males is evaluated at 9%, which clearly exceeds the incidence in the general population (Bailey and Pillard, 1991). Similarly sisters of lesbians have an increased probability of being homosexual (Diamond, 1993; Rahman and Wilson, 2003). This concordance could obviously reflect educational/social factors but the study of twins allows to a large extent to reject this interpretation.

Homosexual twins separated at birth are too rare to allow drawing firm conclusions (Eckert et al., 1986) but the comparison of monozygotic versus dizygotic twins (“true” versus “false” twins) provides key information about the genetic bases of homosexuality. Monozygotic twins indeed share essentially the same genetic material while dizygotic twins are not genetically more similar than brothers issued from different gestations.

A first study reported a perfect concordance (100 %) of sexual orientation in monozygotic twins as compared to only 12 % in dizygotic twins (Kallmann, 1952) but these extreme values were never reproduced. A review of multiple studies indicated an average concordance of 68 % in monozygotic twins versus 16 % in dizygotic twins (Diamond, 1993) leading to speculate that 50–60 % of the variance in sexual orientation in men has a genetic basis (Swaab, 2007). Importantly all studies reported a higher concordance in true twins even if the magnitude of the difference with false twins was variable

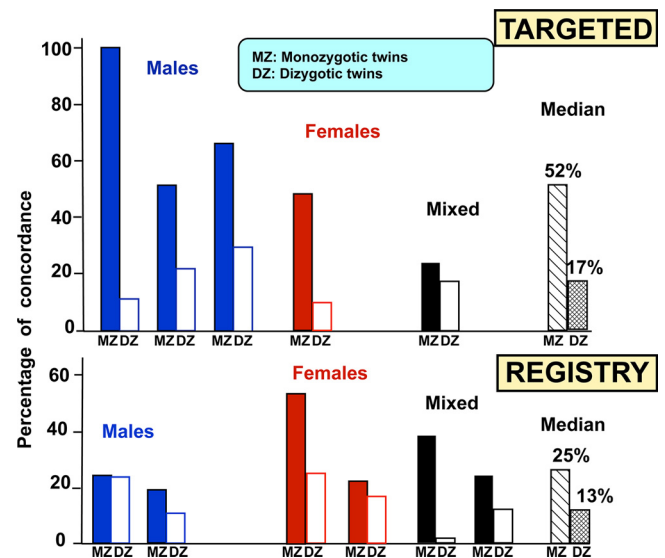


Fig. 4. Percentage of concordance in sexual orientation of monozygotic or dizygotic twins with results separated according to whether subjects were recruited by the targeted or registry method (see text). Studies were dedicated to males or females or to mixed-sex populations. The medians of all results are displayed in the rightmost columns. Redrawn from data compiled in (Bailey et al., 2016).

(Diamond, 1993).

Variability between studies could come from random fluctuations or bias in sampling but could also relate to the degree of morphological *in utero* interaction between the twins, a factor that has, to my knowledge, never been considered in these studies. Monozygotic twins can indeed develop with two separate chorions and two separate amniotic sacs, or with two amniotic sacs but one single chorion or with one amniotic sac and one chorion for both (Bermudez et al., 2002; Cordero et al., 2005). The degree of interactions between these types of twins and the possibilities of exchange of biological material (hormones, cells) are therefore quite variable and might impact the degree of concordance in sexual orientation.

More recent work has revised these estimates towards lower values (Bailey et al., 2016) but clearly confirms the systematic difference in concordance of sexual orientation between the two types of twins (See Fig. 4). Some studies have also extended this difference to lesbians.

Two types of studies based on different methodologies to recruit subjects have addressed this question. Targeted studies recruit homosexual populations explicitly via advertisements or direct contacts. In contrast in registry studies, the population of interest is randomly selected based on preexisting registries. The first of these two approaches is vulnerable to sampling bias because potential subjects will presumably decide to participate or not based in part on their twin's orientation, which could lead to an overrepresentation of concordant pairs (participating may create tension between twins if the pair is discordant) (Kendler and Eaves, 1988). In addition, for purely statistical reasons, pairs of homosexual twins are more likely to be contacted than twins with a different orientation (Torrey, 1992), which could also inflate their representation.

The observed results clearly confirm these suspicions: the percentage of concordance is almost always higher in targeted than in registry studies and the difference between monozygotic and dizygotic twins is larger (52 versus 17 % compared to 25 versus 13 %). This highlights the challenges of studies analyzing such a complex phenomenon in humans. This being said, it has to be noted that the difference between the two types of twins always goes in the same direction; this is true in studies of males, of females and in studies analyzing both sexes. These data therefore support the idea that sexual orientation has, at least in

part, a genetic basis unless one postulates effects on sexual orientation of difference in education between the two types of twins, but such effects have never been reported to this date.

5.2. The linkage to Xq28

Although solid data support the conclusion that genes significantly influence sexual orientation, the search for the specific genes that are implicated has proved extremely difficult. A pedigree study identified an excess of gay uncles or first cousins (13.5 %) in the maternal lineage of homosexual men, as compared to the paternal lineage or to the general population (Hamer et al., 1993). This heritability was pointing to a control based on genes located on the X chromosome that are specifically inherited from the mother, even if alternative mechanisms are possible. Hamer and collaborators therefore recruited pairs of homosexual brothers without evidence of paternal inheritance (subjects were excluded if the father was homosexual) and examined by linkage analysis 22 markers distributed along the X chromosome. Pairs of homosexual brothers were found to share markers located in the terminal region of the X chromosome called Xq28 (Hamer et al., 1993). This linkage was significant (33 pairs out of 40) but based on a relatively limited sample size.

Until recently, three studies had attempted replicating this finding, some with positive (Hu et al., 1995; Sanders and Dawood, 2003), some with negative (Rice et al., 1999) results. The analysis of these combined results still yielded a significant probability of this association with Xq28 but there was only a statistical trend if the initial positive study is excluded (see (Bailey et al., 2016; Bocklandt and Vilain, 2007; Dawood, 2015)).

With the advances in sequencing techniques, data related to this question began to accumulate around the beginning of this century. In 2005 Mustanski and collaborators published a first full genome analysis of 456 individuals from 146 families with two or more gay brothers (Mustanski et al., 2005a). They reported associations of homosexuality with loci located on chromosomes 7, 8 and 10 but failed to replicate a clear linkage with Xq28. As discussed in that paper, this discrepancy could be due to methodological problems.

In 2015, Sanders and collaborators published a DNA linkage analysis based on more than 400 homosexual brothers (908 individuals) confirming the Xq28 linkage (Sanders et al., 2015) suggesting that this chromosomal region still represents an interesting target for future investigations.

To date, no investigation has related a specific gene located in Xq28 to homosexuality. However, the human genome has now been fully sequenced and this work has identified a few interesting candidate genes (see (Sanders et al., 2015) for discussion). For example, the arginine-vasopressin (AVP) receptor 2 (AVPR2) is located in Xq28 and AVP is known to play a key role in the control of social and affiliative behaviors (Balthazart and Young, 2014). Modifications in the AVPR2 gene or in its expression could therefore have an impact on sexual orientation, although expression of this gene is limited in the brain and more present in peripheral structures such as the kidney.

Another Xq28 gene mentioned by Sanders and collaborators is the *cyclic nucleotide gated channel alpha 2* (CNGA2), a gene that is expressed in the human brain and is critical in mice for the control of odor-evoked sociosexual behaviors, including odors related to the Major Histocompatibility Complex (MHC) (Mandiyan et al., 2005; Spehr et al., 2006). The contribution of olfaction to human sexual orientation is not firmly established but differential hypothalamic responses to olfactory compounds with presumed pheromonal activity have been identified in gay compared to straight men (Savic et al., 2005) and in lesbian compared to heterosexual women (Berglund et al., 2006) as well as in gender dysphoric children, a condition associated with a high probability of being homosexual when adult (Burke et al., 2014). Odors, in particular MHC-related odors, and the CNGA2 gene located in Xq28 may thus have relevance to sexual orientation in humans (Havlicek and

Roberts, 2009; Milinski et al., 2013; Wedekind et al., 1995).

Interestingly, genes of the *melanoma-associated antigen* (MAGE-A) family are also located in Xq28. MAGE-A11, a gene in this family, encodes for a protein that has been recognized as a co-activator for the androgen receptor and is namely transcribed in the prostate (Karpf et al., 2009). MAGE-A11 prostatic expression is markedly up-regulated by castration (100–1500 fold) by an epigenetic mechanism based on the hypomethylation of a CpG island in the promoter of the gene. This provides a mechanism potentially explaining the increased androgen sensitivity in prostate cancers after prolonged androgen deprivation (Karpf et al., 2009). This Xq28 gene that is also expressed in multiple parts of the human brain including the olfactory bulbs, the hypothalamus, amygdala and prefrontal cortex (see: <http://biogps.org/#goto=genereport&id=4110>) could help to explain homosexuality. A mutation or an epigenetic modification of its expression taking place in the brain should indeed result in a decreased brain androgen sensitivity and thus androgen action, a factor that has been postulated to take place during early development in gay males (see Section 4).

This mechanism would additionally provide a “bridge” between studies that tried to explain homosexuality by genetic or by hormonal factors (see Sections 4 and 5 of the present review) (Bocklandt and Vilain, 2007; Ngun and Vilain, 2014). To represent a viable mechanism explaining male homosexuality, a change in MAGE-A11 expression would, however, have to change sensitivity to testosterone specifically in the brain, since no decrease in somatic androgen-sensitive structures, such as penile size, is systematically associated with masculine homosexuality (if anything a larger penis is present in gay men but the interpretation of these data is questionable; (Bogaert and Hershberger, 1999)). Such a mechanism would also beg the question of how this change in expression of the androgen receptor co-regulator can be heritable. A mutation would obviously be transferred to the next generation but this is less clear for an epigenetic modification of expression as observed in the prostate after androgen deprivation, even if recent work has identified trans-generational inheritance of epigenetic marks and correlated physiological changes (Klosin et al., 2017; Morgan and Bale, 2011) (see also next section).

It should also be noted that MAGE-A11 has not been identified in the rat or mouse genome. This might help to explain why there is no spontaneous (and exclusive) homosexual behavior in these species, even if same sex preference can be induced in males of these species by perinatal manipulations of androgens action (e.g., (Bakker et al., 1993a; Bodo and Rissman, 2008; Henley et al., 2009)). Taken together these data support the notion that MAGE-A11 could contribute to explain human homosexuality and would therefore deserve additional research on the controls of its expression in the brain.

5.3. Other loci associated with male homosexuality

Besides Xq28, the different studies performed so far have identified linkages to several other loci located at least on 6 different chromosomes (Ganna et al., 2019; Mustanski et al., 2005b; Sanders et al., 2017, 2015). The large linkage study of Sanders and colleagues (908 individuals) namely confirmed a previously identified (Mustanski et al., 2005b) linkage to the peri-centrometric region of chromosome 8 with a multipoint support peaking at 8q12 (Sanders et al., 2015). The association of this locus with homosexuality was actually more significant than for Xq28. This chromosomal region contains several genes that could in theory be functionally linked to the homosexual preference. These genes include micro RNAs, transcription factors or genes known to be expressed in the brain including genes coding for factors related to neurotransmission, neuroendocrine function or brain development but no specific functional link can be highlighted at this point.

Genetic analyses of complex traits often have a limited power due to insufficient sample size. Given the complexity of homosexuality, this also applied to some extent to the Sanders et al. (Sanders et al., 2015) study despite the inclusion of more than nine hundred subjects. In the

summer 2019, an international collaboration between over 20 researchers published a much larger Genome Wide Association Study (GWAS) based on five cohorts of subjects totaling 492,678 genotyped individuals that should potentially not be subjected to this kind of limitation (Ganna et al., 2019). However, given this large sample size, the phenotypic characterization of the subjects was limited to self-reports by the participants indicating whether or not they ever had same-sex sexual behavior, an obvious limitation but balanced by the extremely large sample size.

The study first confirmed the heritability (percentage of the trait attributable to genetic variations) of male and female homosexuality to be around 32 % consistent with the results of previous twins studies (Langstrom et al., 2010); related individuals were more likely than expected by random sampling to display concordant behavior.

The analysis of small nucleotide polymorphism (SNP) in these genomes discovered five loci that correlated with same-sex behavior. Two loci concerned both sexes while two were specific to males and one to females, suggesting that the genetic bases of homosexuality are different in men and women, which was indeed anticipated based on previous phenotypical studies (Bailey et al., 2016). These five loci contain genes associated with olfaction (several olfactory receptor genes) and others related to the production of sex steroids, including TCF12, a heterodimerization partner for TCF21, a transcription factor critical for gonadal development in mice (Ganna et al., 2019). These genes could represent links to homosexuality that seem related both to detection of sexual olfactory signals and alterations of the early steroid milieu but no specific link has been established to date.

Unexpectedly this study did not identify links with loci on the X chromosome but it must be remembered that the selection of subjects was here based on the mere presence/absence of same-sex behavior, not of a (more or less) exclusive homosexual preference and it is well-known that same-sex behavior can have other origins and is more widely distributed than exclusive homosexual preference. In addition, there was in the Ganna et al. study (Ganna et al., 2019) no additional selection of the subjects who experienced same-sex behavior while Hamer and collaborators performed their study specifically on a population of subjects with no paternal (presumed maternal) inheritance (Hamer et al., 1993). These methodological differences potentially explain the discrepancy in results.

The contribution of each of these SNP (SNP-based heritability) to the variation in female or male same-sex behavior was estimated to range between 8–25 %. Furthermore variance explained by the polygenic score was smaller than 1 % indicating that same-sex behavior, like any other complex trait is modulated by small additive effects of a large number of multiple gene variants. This is however not necessarily surprising given that it is widely accepted since the work of Alfred Kinsey that there are various degrees of homosexual preference (the 7 points Kinsey scale). There is also individual variance in the phenotype of homosexual subjects within each sex (see Section 4.3) and male and female homosexuality clearly differ in multiple aspects (Bailey et al., 2016; Herek et al., 2010; Kinsey et al., 1953, 1948).

The limited magnitude and observed variability of these associations with genes (see (Ngun and Vilain, 2014) for additional descriptions of these associations) indicate that each of these genes only has a limited effect. The control of sexual orientation is, as might be expected for such a complex behavioral trait, associated with an oligogenic inheritance, i.e. a stochastic variation in the linkage with a specific locus while multiple loci are implicated. Practically this means that although the heritability of homosexuality is unquestionable and probably explains around 30 % of the variance in sexual orientation, the idea of a prenatal genetic test for homosexuality is impossible since the small magnitude of effect of each single locus would make such a test essentially useless. There is therefore no reason to ban research on the genetic bases of homosexuality and it should even be encouraged since there is factual evidence showing that there is a positive correlation between the individual's opinion on the causes of sexual orientation

and their view about homosexuality: people who accept the idea that biological factors are important more often hold positive attitudes and believe there is nothing wrong about same-sex sexual orientation (Bailey et al., 2016).

6. Epigenetic effects and the older brother effect

Together, data reviewed in the two previous sections provide evidence for biological controls of sexual orientation including hormonal and genetic factors. The contribution of these factors is however only part of a multifactorial causation that has not been fully uncovered at this time. Hormonal factors only explain part of the variance (Section 4) and the same is true for genetics (Section 5). This is clearly illustrated by the fact that monozygotic twins who share the same genome are only concordant for sexual orientation in at best 50–60 % of the cases and usually much less (see Fig. 4). Additional causal factors thus need to be identified.

6.1. Epigenetic modulation of androgen action

At the endocrine level, it has been pointed out that in humans (Bocklandt and Vilain, 2007; Mustanski et al., 2005b) and even in rats (Rahman and Wilson, 2003; Weisz and Ward, 1980) that are best studied, during most of the embryonic life testosterone concentrations overlap between males and females even if males have on average higher concentrations. The difference in plasma testosterone concentrations is therefore an ambiguous sexual signal that cannot explain alone the sex-specific phenotypes. For example, the undifferentiated genital tubercle develops into a phallus under the influence of testosterone while a vulva and clitoris will develop in the absence of this steroid but this sexual differentiation of the external genitalia takes place in rats and humans during a period of embryonic life when testosterone concentrations overlap between the sexes (Mustanski et al., 2005b; Perera et al., 1987; Reyes et al., 1974). Given that a discordance between genetic sex and sex of the genitalia is extremely rare, it becomes necessary to invoke additional factors that should be in action to organize the sexually differentiated phenotype.

In this context, Rice and colleagues described complex genetic and epigenetic mechanisms acting very early during fetal development that contribute to increase the sensitivity to testosterone in males and decrease it in females (see Fig. 5) (Rice et al., 2012).

Evidence accumulated during the last 15–20 years indicates that genes located on sex chromosomes epigenetically regulate the expression of a variety of autosomal genes in a “direct” manner independent of sex hormones and this might explain the differential sensitivity to sex steroids in males and females. It was also demonstrated that expression of large panels of genes is sexually differentiated even before the gonads develop (Dewing et al., 2003; Eakin and Hadjantonakis, 2006; Ngun and Vilain, 2014) (see also Section 1). The male and female embryos are therefore already somewhat differentiated at the blastocyst stage (Rice et al., 2012), way before androgen production begins, and epigenetic marks are likely controlling this differentiation.

Specifically, testosterone action is modulated by a variety of mechanisms at multiple levels in the circulation and at the level of target cells. First, the sex hormone binding globulin (SHBG) that binds testosterone in the blood and therefore restricts its uptake by target cells is approximately 50 % more concentrated in female than in male human fetuses (Hammond et al., 1983) and this should decrease the sensitivity to testosterone in the XX subjects (Hammond, 1995). Second, expression of the 5 α -reductase-2 gene coding for the enzyme that catalyzes the transformation of testosterone into 5 α -dihydrotestosterone is three times higher in the urogenital swelling and genital tubercle that will produce the penis of XY than in XX fetuses (Wilson et al., 1993) and there is evidence that this enzymatic difference is not controlled by androgens (Boehmer et al., 2001). Since it is firmly established that the sexual differentiation of the genital structures in males depends on the

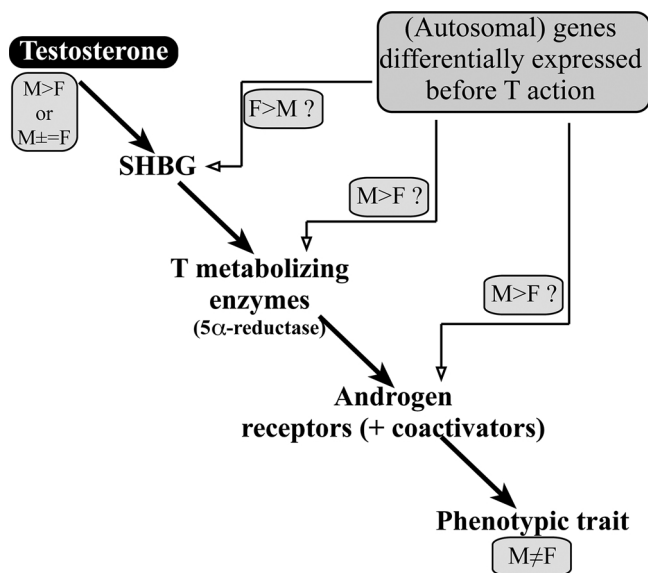


Fig. 5. Mechanisms potentially modulating in a sex-specific manner testosterone action during fetal development in mammals. Large panels of (autosomal) genes are expressed in a sexually differentiated manner, presumably via epigenetic control mechanisms, even before the gonads develop. Testosterone action is modulated by a variety of mechanisms at multiple levels in the circulation and at the level of target cells. The effects of marginally different concentrations of testosterone in males and females can therefore be canalized to produce sexually differentiated phenotypes. Drawn from information in (Rice et al., 2012).

5 α -reduction of testosterone - mutations of the enzyme result in intermediate or feminine genital structures (Imperato-McGinley, 1994; Imperato-McGinley and Zhu, 2002) - this enzymatic sex difference clearly contributes to the differentiation of the genital phenotype even in the presence of overlapping concentrations of circulating testosterone in male and female fetuses. Sex differences in androgen receptors and in their co-activators and co-repressors could additionally modulate responses to androgens in the target cells, including in neuronal structures that mediate sexual orientation or gender identity. Some studies actually identified differences in the gene structure of sex steroid receptors (Fernandez et al., 2014; Henningson et al., 2005) in transgender individuals and the same might be true in relationship with sexual orientation. These changes in gene structure likely modify the expression of the corresponding receptors and thus the sensitivity of target structures to circulating steroids. Sex steroid receptors also use different co-activators and co-repressors in different organs and thus in the brain to control transcription (Charlier, 2009; Chen, 2000; Shibata et al., 1997) and it is conceivable that different epigenetic marks transmitted across generations potentially affect subsets of sexually dimorphic traits. This could explain why a control by androgens of sexual orientation could be effected in the absence of any effect of the genitalia.

The organizational actions of androgens on sexual orientation could thus be canalized towards a specific endpoint by the cooperation of multiple mechanisms enhancing or decreasing androgen action in specific targets. This canalization (*sensu* (Waddington, 1942)) is potentially mediated by micro RNAs that are known to modulate sex differences in mRNA concentrations in the mouse brain (McCarthy and Nugent, 2015) and by epigenetic marks (Nugent et al., 2015) that have now been demonstrated to be heritable across one and possibly more generations (Bale, 2015; Klosin et al., 2017; Morgan and Bale, 2011; Rodgers et al., 2015) (See (Kaiser, 2014)). This idea is supported by the observation that, in most cases, girls affected by congenital adrenal hyperplasia are born with genital structures are only partly masculinized (Hall et al., 2004) and although they display an increased

incidence of homosexuality, almost 50 % of them are still heterosexual (Money et al., 1984). All this despite the fact that they have been exposed throughout most of their fetal life starting at the seventh week of gestation to an excess of adrenal androgens sometimes equivalent to the concentrations observed in fetal males (New, 2004; Speiser and White, 2003; Trakakis et al., 2009).

Rice and collaborators have presented a mathematical simulation demonstrating the feasibility of the control of sexual orientation via sexually antagonistic epigenetic marks modulating androgen-sensitivity in a sex- and tissue-specific manner (see (Rice et al., 2012) for a full presentation). By sex-reversing brain sensitivity to androgens epigenetic marks could sex reverse sexual orientation. These marks may or may not escape erasure in the primordial stem cells and zygote, and, if occasionally inherited, they would explain the limited but clearly demonstrated heritability of homosexuality, as well as the difficulty in identifying clear genetic markers of homosexuality (see Section 5).

6.2. The asymmetrical inactivation of the X chromosome

Several studies actually tried to identify the postulated epigenetic marks that would control the homosexual orientation and its heritability in the absence of widespread genetic markers (Ngun and Vilain, 2014). Some experimental support for this idea was obtained by the analysis of the X chromosome. Males (XY) have one copy of this chromosome while females (XX) have two copies. To avoid generating a huge number of sex differences linked to the overexpression in females of the numerous genes located on X, a mechanism has evolved by which each female cell randomly inactivates via epigenetic marks one X chromosome during early development and this inactive chromosome will remain inactive in daughter cells (Brown and Robinson, 2000). In principle this inactivation randomly concerns either the paternal or the maternal X (50 % of each). By comparing X inactivation in a sample of 97 mothers of homosexual men and in 103 age-matched control mothers without gay sons, Bocklandt, Vilain and collaborators showed that the number of women with extreme skewing in X inactivation (inactivation of a given X in more than 90 % of the cells) was significantly larger in mothers of gay men (13/97 = 13.4 %) than in control mothers (4/103 = 3.9 %) (Bocklandt et al., 2006). This further suggests an implication of the X chromosome in the control of homosexuality and that a mechanism influencing homosexual orientation can be detected in the cells of their mother (see for review (Ngun et al., 2011)).

Work from the Vilain lab was also presented in 2015 at the meeting of the American Society of Human Genetics indicating the existence of 5 epigenetic markers that were differently present in gay and straight men (Ngun et al., 2015) (see DOI: 10.1126/science.aad4686 or <https://www.sciencemag.org/news/2015/10/homosexuality-may-be-caused-chemical-modifications-dna>). The study was based on the screening of methylation patterns in 140,000 regions of DNA in 37 pairs of monozygotic twins who displayed a different sexual orientation (one was straight and the other gay) compared to 10 pairs of monozygotic twins who were both gay. These methylated sites concerned one gene implicated in nerve conduction and others related to immune function. To our knowledge this study however remains unpublished, suggesting that additional work and verifications based on a larger sample are still ongoing. If confirmed, these findings could help to explain the frequently discordant sexual orientation of monozygotic twins. They could also relate to the older brother effect discussed in the next section.

6.3. The older brother effect

More than 20 years ago, Ray Blanchard and Anthony Bogaert reported, based on the study of 302 homosexual and 302 matched heterosexual men, that for each older brother born from a given women the incidence of male homosexuality increases by about 33 % (Blanchard and Bogaert, 1996). This phenomenon was called the older brother effect or fraternal birth order (FBO) effect. It has since been

confirmed in a large number of studies totaling over 10,000 subjects in various cultures (Blanchard, 2004; Nila et al., 2019; VanderLaan and Vasey, 2011). A meta-analysis indicates that between 15 and 29 % of gay men owe their sexual orientation to this FBO effect (Blanchard, 2004). Until recently the biological bases of this phenomenon had however remained quite speculative.

Thanks to the extremely large sample size in these aggregate studies, the authors were able over the years to rule out by multiple regression analyses the most obvious biological factors that were potentially implicated such as the age of the mother or of the father, the social influence of other boys living in the family or other peculiarities in the education within a family (Bogaert and Skorska, 2011). The FBO effect ended up being the best-documented phenomenon associated with male homosexuality but its bases remained unclear.

The effect was clearly related to the multiple male pregnancies experienced by a woman since it was not present for example in a family where the father had divorced and remarried a woman who had no male children before. The fact that it was associated with a decreased body and brain weight at birth suggested that the developmental pathway to homosexuality triggered by older brothers was in fact initiated during prenatal life (Blanchard, 2004). Based on the available evidence, the authors came to the conclusion that the only possible mechanism had to be based on a progressive immunization against a male antigen of the mother bearing male embryos. In this scenario, antibodies would accumulate in the mother's blood over successive pregnancies and increasingly interfere with the development of the male embryos, similar to what happens in the hemolytic disease of the newborn where a rhesus negative (Rh-) mother mounts an immune response against the Rh factor if gestating to a Rh + fetus and eventually causes hemolytic disease in subsequent Rh + fetuses (see (Bogaert and Skorska, 2011)). To be viable, this maternal immune hypothesis (MIH) had to fulfill several conditions: a) some embryonic material should enter the mother circulation and this material should cause immune responses in females against male proteins, b) these proteins should be important for brain development and this role should be affected by specific antibodies against them, and finally c) antibodies to these male proteins should persist in the mother's blood so that an incremental immune response could build up after multiple male pregnancies.

These very specific predictions allowed selecting, based on existing biomedical knowledge, a list of 4 male-specific proteins fulfilling these criteria (Bogaert and Skorska, 2011) and recently a study experimentally tested the hypothesis by quantifying in the blood of women with diverse reproductive histories antibodies directed against the two most likely candidate proteins namely protocadherin 11 Y-linked (PCDH11Y) and neuroligin 4 Y-linked (NLGN4Y). These antibodies were quantified in blood samples coming from 54 mothers of gay sons, including 23 who had previously given birth to a straight son, and control samples of 72 mothers of heterosexual sons, 16 women with no sons and 12 men (Bogaert et al., 2018).

This research demonstrated that overall, women have a higher blood concentration of anti-NLGN4Y antibodies than men but more importantly that, after controlling for the number of pregnancies, mothers of gay sons, especially those with older brothers, had significantly higher concentrations of antibodies against NLGN4Y levels than control women, including mothers of heterosexual sons (i.e., women with no sons < mothers of heterosexual sons < mothers of gay sons with no older brothers < mothers of gay sons with older brothers) (Fig. 6).

After an initial antigen exposure antibodies often disappear relatively rapidly from the blood with a half-life of just a few weeks (Crotty and Ahmed, 2004). Interestingly, the antibodies and NLGN4Y that were quantified here were still present many years after the last pregnancy and birth of a son. This should be made possible due to the existence of long-lived plasma cells that replenish the supply of antibodies or to memory B cells that could be re-stimulated by the antigen via male microchimerism (see (Bogaert et al., 2018) for discussion).

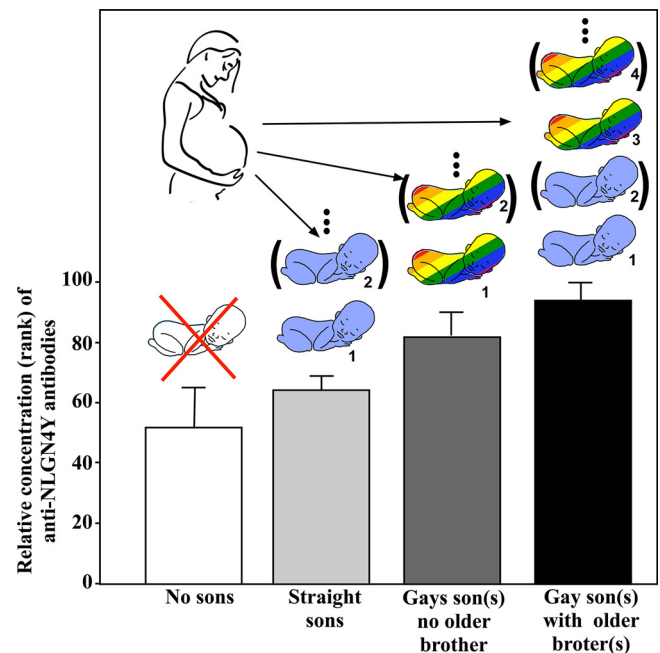


Fig. 6. Mean rank of concentration (from 1 = lowest to 142 = highest) of antibodies against neuroligin 4Y-linked (NLGN4Y) in different groups of women who either had no son, or only had heterosexual son(s), or had at least one gay son who had no older brother or who had one or several older brothers. The concentration of these antibodies in the mother's blood significantly increases across these 4 categories. Numbers indicate the sequence of birth from a same mother and parentheses indicate the presence one or more sons of a given category. Heterosexual (straight) sons are illustrated in blue and gay sons with the rainbow flag associated with homosexuality. Redrawn from data in (Bogaert et al., 2018).

NLGN4Y interacts with neuroligin at the cell membrane and in this way plays a role in synaptic functioning (Jamain et al., 2003; Skaletsky et al., 2003). Antibodies against this protein may alter this interaction with neuroligin during development thus modifying the role of synapses implicated in the masculinization of brain structures. Although many mechanistic questions remain open at this level, the recent study of Bogaert et al. brings for the first time direct experimental support to the maternal immune theory that has been evoked to explain a fraction of male homosexuality via the FBO effect (29 % or possibly a bit more if one assumes that a fraction of the mothers unknowingly had previously miscarried a male embryo (Bogaert et al., 2018)).

7. Social factors

We focused in this review on the biological mechanisms that have been shown to contribute to the determinism of sexual orientation. In contrast, it is often claimed that sexual orientation, and in particular homosexuality, is controlled by the social environment during childhood and this view is broadly shared in the general public. This approach has been described and evaluated in detail by a recent review (Bailey et al., 2016).

In this context, psychoanalytical theories have constantly held dysfunctional relationships between the parents and their child (emotionally distant fathers and/or overbearing mothers) responsible for the development of homosexuality. These hypotheses are however only based on speculative interpretations of anecdotal evidence. Scientific evaluation of these theories has returned little positive evidence (Bailey et al., 2016) except for the fact that pre-homosexual children tend to have more distant relationships with their parents, especially their father. It should be noted that these children also often tend to display gender nonconforming behavior, which may likely be the cause, not the

consequence, of the difficult relationship with their father (Kane, 2006).

This being said, it is admittedly difficult to conceptualize how the action of a single hormone or few genes could control a trait as complex as sexual orientation. One partial possible solution to this problem is suggested by a study of Melissa Hines and colleagues on young girls suffering from congenital adrenal hyperplasia (CAH). She found that these girls exposed prenatally to an excess of androgens due to their genetic condition are less responsive than control girls to information indicating that particular objects are for girls or for boys (Hines et al., 2016a). CAH girls who were repeatedly told or shown, via short video clips, that a given object or toy is for girls or for boys did not use this information later to select a given object or toy nor to state what they prefer while control girls did. This experiment therefore indicates that prenatal testosterone modifies processes involved in socialization of gendered behaviors. Whether this effect expands to sexual orientation remains however to be evaluated.

8. Conclusions

Science has during the last 30–50 years identified a variety of biological factors acting prenatally that converge to influence and possibly determine sexual orientation. Many holes admittedly remain in this knowledge and it is difficult at this time to present a fully deterministic model of the control of sexual orientation. Hormonal mechanisms only explain a fraction of the variance in sexual orientation and thus presumably a limited percentage of homosexual cases (Section 4). As reviewed in Section 5, although sexual orientation clearly has a heritable component, no single gene is responsible; multiple markers have been identified but each of them only explains a limited part of the variance. Finally the FBO effect (Section 6) potentially explains a maximum of about 30 % of male homosexual cases.

Additional investigations will be needed before we can provide a more complete and unifying theory of sexual orientation. At present, we are only left to speculate that either the different biological mechanisms (hormonal, genetic and immune) each explain a fraction of the cases and male homosexuality is a heterogeneous phenomenon that has multiple independent origins in different subjects, or alternatively that these different mechanisms interact and complement each other in some way to control a phenotype that is otherwise essentially homogeneous. Evidence has been presented indicating that this heterogeneity in the mechanisms influencing sexual orientation (hormones, genes, FBO) is indeed present (Swift-Galland et al., 2019). It has also been suggested that male homosexuality could be the result of opposite influences at the endocrine level and would be induced or at least favored by both lower and higher than usual exposure to androgens during prenatal life (Swift-Gallant, 2019). This possibility should clearly be investigated further. It is intellectually easy to draw links between the endocrine and genetic mechanisms: a genetic mutation or variant can for example modify the secretion or action of sex steroids in the brain (e.g., *MAGE-A11* gene located in Xq28 that would modify androgen action in the brain). The interaction between the immune mechanisms discussed here in the context of the FBO effect and the endocrine-genetic mechanisms is in contrast less obvious.

It may alternatively be hypothesized that even taken together these prenatal biological factors do not determine the sexual orientation but that they only control predispositions that must interact with specific aspects of the post-natal environment to fully crystallize a given sexual orientation. These postnatal factors remain to this date completely unidentified but the research of Melissa Hines on CAH girls (Section 7) suggests interesting tracks for future research.

To some extent, the Nature-Nurture debate thus remains open even if a lot of data indicate that sexual orientation is an essential trait of a person (Nature) while almost no scientific evidence has been collected demonstrating a role of the postnatal environment (Essentialism versus Existentialism or Constructivism). These two approaches however do

not need to be mutually exclusive and any trait in an adult phenotype always results from an interaction between genetic or pre/perinatal biological influences and post-natal experiences. Furthermore, these two types of effects are not independent: the postnatal environment experienced by an individual is not shared with anybody including his/her brothers and sisters or even his twins. Based on a variety of determinisms, each individual lives into his/her own environment and is actively seeking specific stimuli and interactions (the “*Umwelt*” of Jakob von Uexküll). There is therefore no Nature-Nurture dichotomy in the mechanisms that control the development of an adult phenotype and this also presumably applies to sexually differentiated traits such as sexual orientation.

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