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ORGANIZATIONAL EFFECTS OF HORMONES ON SEXUAL ORIENTATION

Kevin A. Rosenfield, Khytam Dawood, and David A. Puts

Introduction

Sexual orientation refers to relative sexual attraction to men (androphilia), women (gynephilia), or both (Bailey et al., 2016) and is one of the most sexually dimorphic human psychological traits. In most Western surveys, more than 95% of genetic (XY) males report exclusive or near-exclusive gynephilia, and a similar proportion of women report exclusive or near-exclusive androphilia (Bailey et al., 2016), resulting in a sex difference of approximately six standard deviations (Hines, 2011). However, despite this large sex difference, non-trivial proportions of both sexes report not only non-heterosexual orientations, but also non-heterosexual behavior (e.g., Grulich et al., 2014; Wienke & Whaley, 2015) and identity (e.g., Pakula & Shoveller, 2013). How and why variations in sexual orientation arise is a matter of fascination and debate within and outside of the scientific community. What are the genetic, environmental, and neurophysiological factors responsible for variation in sexual orientation? Researchers in the social and life sciences have made significant progress toward answering these questions (Hill, Dawood, & Puts, 2013), and much evidence suggests that activities of the neuroendocrine system during prenatal or early postnatal development exert an influence on sexual orientation later in life.

In this chapter, we review this evidence. After exploring how sexual orientation is measured, we turn our attention to studies relating variation in sexual orientation to sex hormone signaling, by which we mean both production of and sensitivity to sex hormones. We consider cases of gender reassignment during infancy or early childhood, followed by evidence regarding specific classes of hormones: androgens and estrogens. We review data from studies examining hormone levels during fetal development, medical conditions associated with atypical hormone levels or sensitivity, normal variation in hormone sensitivity, manipulations of hormone levels via pharmaceutical treatment or castration, as well as endogenous hormone levels in adulthood. We also examine indirect evidence – hormone biomarkers, such as finger length ratios. Finally, we consider possible targets of hormonal signaling: how sexual orientation is represented in the brain. Throughout, we incorporate relevant data from animal models. Whenever possible, we have indicated the sample sizes and effect sizes associated with the studies cited in the text. While some of these are reported in the text itself, for ease of comparison we have presented the vast majority of these figures in several tables, along with information on the study variables, predicted associations, and statistical significance.

Measuring sexual orientation

Whereas some researchers simply ask study participants whether they consider themselves heterosexual, homosexual, or bisexual (Sell, 2007), perhaps the most widely used instruments for measuring sexual orientation are the Kinsey Scale (Kinsey, Pomeroy, & Martin, 1948) and Klein Sexual Orientation Grid (Klein, Sepekoff, & Wolf, 1985). The Kinsey Scale measures sexual orientation along four dimensions: attraction, fantasy, behavior, and identity using 7-point scales ranging from exclusive heterosexual orientation to exclusive homosexual orientation. The Klein Grid evaluates sexual orientation along dimensions of attraction, behavior, fantasy, social and emotional preferences, self-identification, and heterosexual/homosexual lifestyle, also using 7-point scales. Within each dimension, subjects are asked to report their past, present, and ideal behavior/preferences. Responses to the 21 questions of this instrument loaded onto a single factor in a factor analysis, suggesting considerable redundancy (Weinrich et al., 1993). However, it is important to note that the various dimensions sometimes measured under the rubric of sexual orientation are not perfectly correlated. For example, one may identify as heterosexual and engage only in heterosexual sex, yet be attracted to and sexually fantasize only about members of one's own sex. In such cases, most researchers would say that one has a homosexual orientation. This is because sexual behavior and identity can be constrained by local culture, and because sexual attraction motivates behavior and identity, not vice versa (Bailey et al., 2016). In both sexes, self-report measures of sexual orientation generally correlate well with more objective measures, such as reaction time (Wright, 1994), viewing time (Israel & Strassberg, 2009), genital arousal (Chivers, Rieger, Latty, & Bailey, 2005), and pupil dilation (Rieger & Savin-Williams, 2012; Rieger et al., 2015; Watts, Holmes, Savin-Williams, & Rieger, 2017) in response to male vs. female stimuli, although these relationships are often stronger in men than in women. This may be related to increased plasticity of sexual orientation in women compared to men (Bailey, 2009), but there remains disagreement as to whether this difference in plasticity results in part from sex differences in gender socialization (Baumeister, 2000; Shibley-Hyde & Durik, 2000).

Gender reassignment

Among vertebrates, sex differences in the brain and behavior emerge when androgens divert development onto a masculine trajectory (Morris, Jordan, & Breedlove, 2004). This cascade of events commences with the differentiation of the gonads, which is triggered by the expression of sex-determining region Y (*Sry*) gene as early as gestation day 10 in mammals (Wilhelm, Palmer, & Koopman, 2007). It is thus logical to hypothesize that the human sex difference in gynephilic vs. androphilic orientation also arises from males' higher androgen levels. However, the different social environments of males and females, namely, their upbringing as boys and girls respectively, may also contribute to sex differences in sexual attraction. If one could design the ideal experiment for disentangling the role of intrinsic physiological sex differences from gender socialization it would likely resemble the following: From infancy, expose a randomly selected cohort of children of one genetic sex (male: XY, female: XX), who were exposed to sex-typical hormonal regimes prenatally, to a social environment typical of the opposite sex (i.e., raise XY individuals as girls and XX as boys), including by changing the child's appearance so that others would perceive the child as the opposite sex. Later, ascertain sexual orientation (Bailey et al., 2016). The larger the role of gender socialization, the more typical the child should be of the reassigned gender.

Such manipulations are obviously unethical, but several cases of gender reassignment at birth have nevertheless occurred for various reasons. Gender was reassigned in several hormonally typical males whose penises were accidentally destroyed during circumcision, or were

malformed due to cloacal exstrophy, in which the abdomen and penis develop abnormally. The medical community once recommended reassigning these males as female, both surgically (via castration, penectomy, and vaginoplasty) and socially (e.g., encouraging female-typical play, playmates, and dress behavior). While castration necessarily leads to male-atypical hormone levels subsequently, the organizational effects of prenatal hormones on the developing brain will remain. Data on adult sexual orientation are available for seven such natal males whose gender was reassigned as described above between birth and 17 months (Bailey et al., 2016). In all cases, adult sexual attraction was gynephilic (Figure 15.1), an extremely improbable outcome (less than 2 in 100 trillion) if prenatal development were irrelevant (Bailey et al., 2016). Thus, postnatal gender socialization appears generally insufficient to produce androphilia in individuals with male-typical early androgen exposure. We now turn our attention to evidence concerning such androgenic influences on sexual orientation.

Androgens

Androgen signaling in genetic females

Findings from studies measuring fetal hormones are mixed. However, studies of genetic or endocrine disorders that lead to atypical production of or sensitivity to gonadal hormones (disorders of sexual development) indicate that androgens promote gynephilia in genetic females (summarized in Table 15.1).

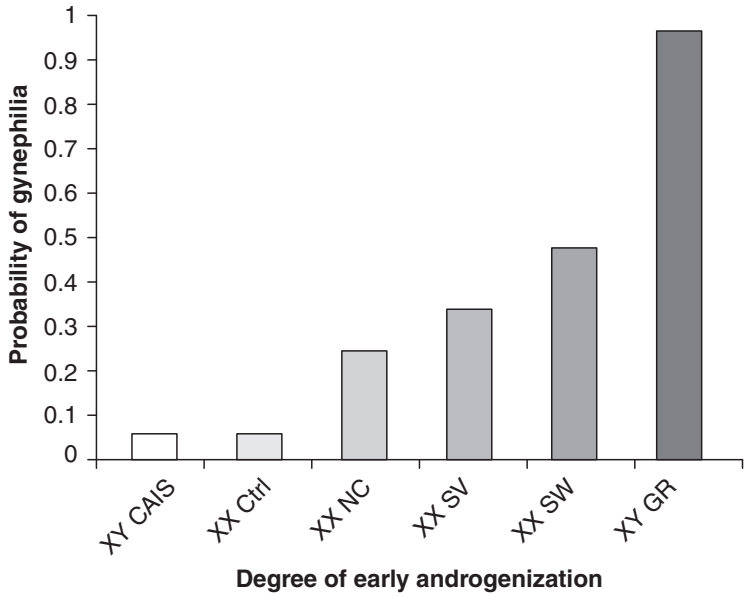

Figure 15.1 Proportion of individuals experiencing gynephilia as a function of early androgen signaling in individuals raised as females. Gynephilia is defined here as Kinsey 2–6 (regular attractions to and fantasies about women). Degree of androgenization refers to both exposure and sensitivity to androgens. XY = genetic male, XX = genetic female. Categories are rank-ordered: CAIS = complete androgen insensitivity syndrome (sexual orientation does not differ from female controls; Hines, Ahmed, & Hughes, 2003), Ctrl = women recruited without regard to diagnosis or sexual orientation (Gangestad, Bailey, & Martin, 2000), NC = non-classical CAH, SV = simple virilizing CAH, SW = salt-wasting CAH (Meyer-Bahlburg, Dolezal, Baker, & New, 2008 for all CAH), GR = gender-reassigned natal males (Bailey et al., 2016). In general this indicates that increased androgen signaling is related to increasing incidence of gynephilia.

Fetal hormone levels

The relationship between fetal hormones and sexual orientation itself has not been systematically studied. However, several groups have tested the hypothesis that children's gender-typical play behavior, a correlate of adult sexual orientation (Cohen, 2002; Rieger, Linsenmeier, & Bailey, 2008), is associated with fetal hormone levels. While one study found a relationship between male-typical behavior and testosterone sampled from amniotic fluid in genetic females (Auyeung et al., 2009), two others did not (Knickmeyer et al., 2005; van De Beek, Van Goozen, Buitelaar, & Cohen-Kettenis, 2009). van De Beek et al. (2009) also observed no relationship between amniotic progesterone or estrogen and girls' female-typical play behavior, or between any of the three steroids. In addition, maternal blood testosterone assayed once between weeks 5 and 36 of pregnancy predicted male-typical play behavior in preschool girls (Hines, Golombok, Rust, Johnston, & Golding, 2002). These studies indirectly hint at a relationship between maternal hormone levels and sexual orientation, but they did not measure sexual orientation directly and only sampled testosterone during small developmental windows. Experimental manipulation of fetal hormones would be conclusive but unethical in humans. However, several disorders of sexual development are associated with altered androgen signaling and hence can serve as "natural experiments" – or quasi-experiments, as "treatments" are not randomly assigned.

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder of sexual development characterized by underproduction of one of the five enzymes responsible for glucocorticoid synthesis in the adrenal cortex. Unable to synthesize glucocorticoids, the adrenal cortex converts a portion of the unusually abundant glucocorticoid precursors to androgens, leading to excess androgen (hyperandrogenism) (Merke & Bornstein, 2005; Speiser & White, 2011). During early development, this hyperandrogenism may masculinize brain structures underlying sexually differentiated cognitive and behavioral traits. Despite postnatal hormone replacement therapy, females with CAH sometimes present with more male-typical toy choice, play behavior, vocational preferences, maternal behavior (Meyer-Bahlburg, 2001), and cognition (Puts, McDaniel, Jordan, & Breedlove, 2008).

Published evidence overwhelmingly suggests that CAH also leads to an increased chance of non-heterosexuality in women (Hines, 2011). Ten studies with age-matched (or in one case, unaffected sister-matched) control groups have reported that women with CAH are more likely to identify as non-heterosexual, have a history of engaging in homosexual relationships, or report attraction to members of the same sex (Dittmann, Kappes, & Kappes, 1992; Frisén et al., 2009; Gastaud et al., 2007; Hines, Brook, & Conway, 2004; Johannsen, Ripa, Mortensen, & Main, 2006; May, Boyle, & Grant, 1996; Meyer-Bahlburg et al., 2008; Money, Schwartz, & Lewis, 1984; Zucker, Bradley, Oliver, Blake, & Fleming, 1996). Four of these studies found that CAH severity (assessed by hormonal assay, specific mutation of the 21-hydroxlase gene, or degree of genital virilization at birth) positively predicted the likelihood of non-heterosexuality (Figure 15.1). Additionally, two studies reported a high incidence of homosexual behavior or fantasies, but did not use a control group for comparison (Ehrhardt, Evers, & Money, 1968; Khorashad et al., 2017). Three studies failed to replicate a relationship between CAH and female non-heterosexuality Kuhnle & Bullinger, 1997: no data provided; Lev-Ran, 1974: 0 of 18 patients reporting non-heterosexual behavior or attraction, with no control group; Slijper et al., 1992: 0 of 18 patients reporting non-heterosexual behavior or attraction, with no control group. Several authors have questioned the methodologies employed in these unsupportive studies (e.g., Hines, 2011), but their criticisms,

Table 15.1 Summary of results for studies linking androgen levels and receptors to sexual orientation in genetic females (see section "Androgen signaling in genetic females")

Reference	*Predictor variable*	*Dependent variable*	*Predicted association*	*Significant?*	*N*	*T*	*C*	*ES*	*Stat*	*Method*
Auyeung et al., 2009	Testosterone in amniotic fluid	Male-typical play behavior	Positive	Yes	100	n/a	n/a	0.42	*r*	From paper
Knickmeyer et al., 2005	Testosterone in amniotic fluid	Male-typical play behavior	Positive	No	21	n/a	n/a	−0.19	*r*	From paper
Van De Beek et al., 2009	Testosterone in amniotic fluid	Male-typical toy preference	Positive	No	63	n/a	n/a	0.00	*r*	From paper
Van De Beek et al., 2009	Estradiol in amniotic fluid	Male-typical toy preference	Positive	No	63	n/a	n/a	−0.04	*r*	From paper
Van De Beek et al., 2009	Progesterone in amniotic fluid	Male-typical toy preference	Positive	No	63	n/a	n/a	0.07	*r*	From paper
Van De Beek et al., 2009	Testosterone in amniotic fluid	Female-typical toy preference	Positive	No	63	n/a	n/a	0.03	*r*	From paper
Van De Beek et al., 2009	Estradiol in amniotic fluid	Female-typical toy preference	Positive	No	63	n/a	n/a	0.14	*r*	From paper
Van De Beek et al., 2009	Progesterone in amniotic fluid	Female-typical toy preference	Positive	No	63	n/a	n/a	0.00	*r*	From paper
Van De Beek et al., 2009	Testosterone in maternal blood	Male-typical toy preference	Positive	No	58	n/a	n/a	−0.01	*r*	From paper
Van De Beek et al., 2009	Estradiol in maternal blood	Male-typical toy preference	Positive	No	58	n/a	n/a	0.06	*r*	From paper
Van De Beek et al., 2009	Progesterone in maternal blood	Male-typical toy preference	Positive	No	58	n/a	n/a	0.22	*r*	From paper
Van De Beek et al., 2009	Testosterone in maternal blood	Female-typical toy preference	Positive	No	58	n/a	n/a	0.09	*r*	From paper
Van De Beek et al., 2009	Estradiol in maternal blood	Female-typical toy preference	Positive	No	58	n/a	n/a	−0.05	*r*	From paper
Van De Beek et al., 2009	Progesterone in maternal blood	Female-typical toy preference	Positive	No	58	n/a	n/a	−0.05	*r*	From paper
Hines et al., 2004	CAH vs. non-CAH unrelated	Gynephilia	Higher in CAH	Yes	31	16	15	0.82	*d*	From paper
Johannsen et al., 2006	CAH vs. non-CAH unrelated	Gynephilia	Higher in CAH	Yes	110	40	70	0.24	*V*	Calculated
Meyer-Bahlburg et al., 2008	CAH vs. non-CAH unrelated	Gynephilia	Higher in CAH	Yes	167	143	24	0.60	*d*	Calculated
Money et al., 1984	CAH vs. CAIS (XX) or MRKS (XX)	Gynephilia	Higher in CAH	Yes	57	30	27	1.03	*d*	Calculated
Zucker et al., 1996	CAH vs. non-CAH unrelated	Gynephilia	Higher in CAH	Yes	45	30	15	0.47	*d*	From paper
Dittmann et al., 1992	CAH vs. non-CAH sisters	Gynephilia	Higher in CAH	Yes	48	34	14	1.03	*d*	Calculated
Frisén et al., 2009	CAH vs. non-CAH unrelated	Gynephilia	Higher in CAH	Yes	124	62	62	0.59	*d*	Calculated
Gastaud et al., 2007	CAH vs. non-CAH unrelated	Gynephilia	Higher in CAH	Yes	104	35	69	0.45	*d*	Calculated
Khorashad et al., 2017	CAH with no control group	Gynephilia	High in CAH	45% gynephilic	18	18	0	n/a	n/a	n/a
Ehrhardt et al., 1968	CAH with no control group	Gynephilia	High in CAH	48% gynephilic	23	23	0	n/a	n/a	n/a
May et al., 1996	CAH vs. non-CAH w/ diabetes	Gynephilia	Higher in CAH	Yes	36	17	19	0.43	*V*	Calculated
Agrawal et al., 2004	Homosexual vs. heterosexual	Incidence of PCO	Higher in homosexual	Yes	618	254	364	1.07	*d*	Calculated
Agrawal et al., 2004	Homosexual vs. heterosexual	Incidence of PCOS	Higher in homosexual	Yes	618	254	364	0.58	*d*	Calculated
Smith et al., 2011	Homosexual vs. heterosexual	Incidence of PCO	Higher in homosexual	No	211	114	97	0.16	*d*	Calculated

Smith et al., 2011	Homosexual vs. heterosexual	Incidence of PCOS	Higher in homosexual	No	211	114	97	0.16	*d*	Calculated
Loraine, Ismail, Adamopoulos, & Dove, 1970	Homosexual vs. heterosexual	Testosterone in urine	Higher in homosexual	Yes	18	4	14	n/a	n/a	n/a
Griffiths et al., 1974	Homosexual vs. heterosexual	Testosterone in urine	Higher in homosexual	n/a	42	42	0	n/a	n/a	n/a
Downey, Ehrhardt, Schiffman, Dyrenfurth, Becker, 1987	Homosexual vs. heterosexual	Testosterone in plasma	Higher in homosexual	No	14	7	7	0	*d*	Calculated
Dancey, 1990	Homosexual vs. heterosexual	Testosterone in urine	Higher in homosexual	No	20	10	10	−0.39	*d*	Calculated
Singh, Vidaurri, Zambarano, & Dabbs Jr., 1999	Butch rating	Testosterone in saliva	Positive	Yes	33	33	0	0.59	*r*	From paper
Pearcey, Docherty, & Dabbs, 1996	Within-couple butch rating difference	Within-couple salivary T difference	Negative	No	26	n/a	n/a	−0.35	*r*	From paper
Pearcey et al., 1996	Within-couple butch rating difference	Within-couple salivary T difference	Above omitting outlier	Yes	25	n/a	n/a	−0.54	*r*	From paper
Pearcey et al., 1996	Butch rating	Testosterone in saliva	Higher in butch	No	54	n/a	n/a	−0.03	*r*	From paper

Note: N: Total sample size, T: Treatment group sample size, C: Control group sample size, ES: Effect size, Stat: Effect size statistic used, Method: Method used to obtain effect size.

such as "inadequate assessments of sexual orientation" (Meyer-Bahlburg, 2001), could also be applied to many studies supporting the relationship. Overall, 11 of 14 published studies (summarized above) report increased (or in the case of Ehrhardt et al., 1968 and Khorashad et al., 2017, substantially higher than the general population) incidence of non-heterosexuality in female CAH patients. Given these results (see also Table 15.1), the relationship between CAH and sexual orientation in women is ripe for meta-analysis, so that more general conclusions can be drawn. To reduce the chance of publication bias, such an analysis should include any unpublished results that can be obtained, as these would be more likely to be unsupportive of the hypothesized relationship.

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women (Barry et al., 2010). Symptoms appear around menarche or slightly before and may include polycystic ovaries, hyperandrogenism, and irregular or infrequent menstrual cycling (Silfen et al., 2003). Genetic and prenatal environmental causes of PCOS have been suggested (Abbott, Barnett, Bruns, & Dumesic, 2005; Barry et al., 2010; Bronstein et al., 2011; Goodarzi, Guo, Yildiz, Stanczyk, & Azziz, 2007), and some evidence implicates elevated early androgens. For example, female rhesus macaques (*Macaca mulatta*) and sheep (*Ovis aries*) exposed to exogenous androgen during fetal development present with PCOS-like symptoms, including hypersecretion of luteinizing hormone, oligomenorrhoea (infrequent menstrual cycles), irregular follicular development, and insulin resistance (Franks, 2002). In addition, androgen levels in umbilical vein blood were elevated in daughters of women with PCOS (Barry et al., 2010), who themselves are more likely to have PCOS, given its high heritability (approximately 70%; Vink, Sadrzadeh, Lambalk, & Boomsma, 2006). If women with PCOS were exposed to excess androgen during early development, and if prenatal androgens contribute to later gynephilia, then the incidence of non-heterosexuality might be elevated in PCOS women. In fact, a far higher prevalence of both PCO (polycystic ovaries with or without other PCOS symptoms; Cohen's $d = 1.07$) and PCOS (Cohen's $d = 0.58$) was observed in 254 self-identifying lesbians than in 364 non-lesbians attending a fertility clinic (Agrawal et al., 2004). In a smaller sample of 114 lesbian and 97 heterosexual women, only 13 of whom were diagnosed with PCOS, non-significant trends were observed toward increased PCOS (Cohen's $d = 0.16$), polycystic ovaries (Cohen's $d = 0.16$), hirsutism, testosterone, and androstenedione in lesbians compared to heterosexual women (Smith et al., 2011). Additional studies are needed before any firm conclusions regarding the relationship between PCOS and sexual orientation can be drawn, although given their general consistency, meta-analysis of the studies already conducted may yield significant results.

Studies of PCOS thus suggest that hyperandrogenism and/or exposure to elevated prenatal androgens from hyperandrogenic mothers increases the probability of gynephilia. The late emergence of observable symptoms in PCOS helps rule out differential rearing as an explanation for elevated homosexuality in women with PCOS. However, mothers of women with PCOS may exhibit different rearing practices regardless of daughters' condition, so it would be useful to compare sexual orientation in sisters with and without PCOS, but to our knowledge, no sibling study relating PCOS and sexual orientation has yet been undertaken.

Adult hormone levels

Some evidence indicates that homosexual women exhibit higher androgen levels than heterosexual women in adulthood (Griffiths et al., 1974; Loraine et al., 1970); one of these studies

reported no statistical results, while the other compared testosterone levels of only four lesbians to a "normal" population range in four separate statistical tests. In addition, several studies have found no differences (Dancey, 1990; Downey et al., 1987). However, homosexual women self-identifying as "butch" had higher salivary testosterone that those identifying as "femme" in two samples (Pearcey et al., 1996; Singh et al., 1999). Very little has been published on the effects of testosterone treatment on sexual orientation in women; however, a case report of an androphilic female-to-male transsexual indicates that testosterone treatment did not produce gynephilic interests (Blanchard, 1990).

Androgen signaling in genetic males

Studies of reduced androgen signaling or production suggest that androgens promote gynephilia in genetic males (summarized in Table 15.2).

Fetal hormone levels

Four studies have investigated the relationship between prenatal hormone levels and childhood male-typical play behavior, reaching contradictory conclusions. Auyeung et al. (2009) found that testosterone levels in amniotic fluid were positively associated with male-typical play behavior in genetic males, while Knickmeyer et al. (2005) and van De Beek et al. (2009) found no such relationship. In addition, maternal blood testosterone assayed once between weeks 5 and 36 of pregnancy did not predict male-typical play behavior in preschool boys (Hines et al., 2002). The methods employed in these studies differ substantially, which may account for the difference in findings. More studies utilizing these and other methodologies are needed before any firm conclusions can be drawn.

Adult hormone levels

In contrast to evidence that prenatal or early postnatal androgens promote androphilia in genetic males, research on adult hormones and sexual orientation has produced mixed but generally null results (Meyer-Bahlburg, 1977). An early study found that plasma testosterone levels were significantly lower in exclusively homosexual (Kinsey 6; $N = 8$), and almost exclusively homosexual (Kinsey 5; $N = 7$) men than heterosexual men (Kolodny, Masters, Hendryx, & Toro, 1971). Meyer-Bahlburg (1977) notes that a disproportionate number of homosexual subjects were drug users, suggesting that sexual orientation could have been confounded by drug use. However, all 13 of these men reported marijuana use and virtually no experience with other drugs. Analogous numbers are not reported for the heterosexual controls. Loraine et al. (1970) reported lower average testosterone levels in three homosexual men compared to 14 heterosexual controls, but the small sample size impedes generalization of these results. Two other studies found relationships between adult testosterone and sexual orientation in men, but were criticized on the basis of methodological problems such as small samples and inappropriate controls (reviewed by Meyer-Bahlburg, 1977), and six studies found no relationship between adult hormones and sexual orientation (see Table 15.2 in Meyer-Bahlburg, 1977).

In addition, testosterone treatment in adulthood did not produce gynephilic interests in homosexual men (Barahal, 1940), and removal of testicular androgen via castration appears not to produce androphilic interests. Of 36 men who reported being mostly or exclusively attracted to women prior to voluntary castration without hormone replacement, none reported a change to being mostly or exclusively attracted to men after castration, and three reported

Table 15.2 Summary of results for studies linking androgen levels and receptors to sexual orientation in genetic males (see section "Androgen signaling in genetic males")

Reference	*Predictor variable*	*Dependent variable*	*Predicted association*	*Significant?*	*N*	*T*	*C*	*ES*	*Stat*	*Method*
Van De Beek et al., 2009	Testosterone in amniotic fluid	Male-typical toy preference	Positive	No	63	n/a	n/a	0.11	*r*	From paper
Van De Beek et al., 2009	Estradiol in amniotic fluid	Male-typical toy preference	Positive	No	63	n/a	n/a	−0.05	*r*	From paper
Van De Beek et al., 2009	Progesterone in amniotic fluid	Male-typical toy preference	Positive	Yes	63	n/a	n/a	0.30	*r*	From paper
Van De Beek et al., 2009	Testosterone in amniotic fluid	Female-typical toy preference	Positive	No	63	n/a	n/a	0.01	*r*	From paper
Van De Beek et al., 2009	Estradiol in amniotic fluid	Female-typical toy preference	Positive	No	63	n/a	n/a	0.04	*r*	From paper
Van De Beek et al., 2009	Progesterone in amniotic fluid	Female-typical toy preference	Positive	No	63	n/a	n/a	−0.19	*r*	From paper
Auyeung et al., 2009	Testosterone in amniotic fluid	Male-typical play behavior	Positive	Yes	112	n/a	n/a	0.20	*r*	From paper
Knickmeyer et al., 2005	Testosterone in amniotic fluid	Male-typical play behavior	Positive	No	31	n/a	n/a	0.00	*r*	From paper
Van De Beek et al., 2009	Testosterone in maternal blood	Male-typical toy preference	Positive	No	57	n/a	n/a	0.09	*r*	From paper
Van De Beek et al., 2009	Estradiol in maternal blood	Male-typical toy preference	Positive	No	57	n/a	n/a	−0.13	*r*	From paper
Van De Beek et al., 2009	Progesterone in maternal blood	Male-typical toy preference	Positive	No	57	n/a	n/a	0.12	*r*	From paper
Van De Beek et al., 2009	Testosterone in maternal blood	Female-typical toy preference	Positive	No	57	n/a	n/a	−0.17	*r*	From paper
Van De Beek et al., 2009	Estradiol in maternal blood	Female-typical toy preference	Positive	No	57	n/a	n/a	0.05	*r*	From paper
Van De Beek et al., 2009	Progesterone in maternal blood	Female-typical toy preference	Positive	No	57	n/a	n/a	−0.07	*r*	From paper
Kolodny et al., 1971	Kinsey rating	Testosterone in plasma	Lower in Kinsey 5 and 6	Yes	65	15	50	−2.26	*d*	Calculated
Loraine et al., 1970	Homosexual vs. heterosexual	Testosterone in urine	Higher in heterosexual	Yes	17	3	14	n/a	n/a	n/a
Hines et al., 2003	CAIS vs non-CAIS women	Gynephilia	Higher in CAIS	No	44	22	22	−0.10	*d*	Calculated

Note: N: Total sample size, T: Treatment group sample size, C: Control group sample size, ES: Effect size, Stat: Effect size statistic used, Method: Method used to obtain effect size.

equal attraction to men and women after castration (E. Wibowo, T. Johnson, and R. Wassersug, personal communications; September 3–5, 2017; data from Handy, Jackowich, Wibowo, Johnson, & Wassersug, 2016). These results are consistent with no overall activational effect of androgens on sexual orientation, but a depressed libido leading to more equal (reduced) attraction to women and men following castration (Handy et al., 2016; Wassersug, Westle, & Dowsett, 2017).

Androgen sensitivity

Complete androgen insensitivity syndrome (CAIS) is a genetic disorder in which XY individuals (i.e., genetic males) have a nonfunctional androgen receptor. Individuals with CAIS are born with undescended testes and produce normal-to-high male levels of circulating androgen, but their androgen insensitivity results in a female appearance. Likewise, individuals with CAIS have a female gender identity and are female-typical psychologically: No significant differences were found between XX female controls and XY women with CAIS in aspects of gender identity, gender role, childhood play behavior, or sexual orientation (Hines et al., 2003). Other studies have found extremely low levels of non-heterosexuality in women with CAIS (Money et al., 1984; Wisniewsky et al., 2000; Figure 15.1).

Like CAIS, partial androgen insensitivity syndrome stems from mutations in the androgen receptor gene. However, there is wide variation in the extent of androgen insensitivity across patients with partial androgen insensitivity syndrome (PAIS) and some (Quigley, 2002; Oakes, Eyvazzadeh, Quint, & Smith, 2008), but not all (Bouvattier, Mignot, Lefèvre, Morel, & Bougnères, 2006), evidence indicates increased androphilia in XY individuals with partial androgen insensitivity syndrome compared to genetic males with fully functional androgen receptor genes, perhaps reflecting the variability in insensitivity (Oakes et al., 2008).

Studies relating sexual orientation to either normal variation in androgen receptor gene sequence (Macke et al., 1993; $N = 410$) or androgen receptor immunoreactivity (a measure of androgen receptor density) in the brain (Kruijver, Fernández-Guasti, Fodor, Kraan, & Swaab, 2001; $N = 33$) have produced null findings. Why typical variation in androgen receptor function is not reliably associated with sexual orientation has yet to be resolved. Perhaps such variation has modest effects on function or is compensated by feedback on circulating androgen levels or androgen receptor transcriptional activity or distribution.

α-reductase deficiency

The enzyme 5α-reductase-2 converts testosterone into the more potent androgen dihydrotestosterone. A number of genetic mutations lead to 5α-reductase-2 deficiency, resulting in a significant shortage of dihydrotestosterone in males (Imperato-McGinley & Zhu, 2002). At birth, XY males born with 5α-reductase-2 deficiency present with ambiguous external genitalia and reduced prostate volume, but remaining internal structures are consistent with genetic sex. Until puberty, affected individuals have often been raised as girls and identified as girls. However, at the onset of puberty, genital masculinization occurs, along with male-typical increases in muscle mass and decreases in vocal pitch. The majority of affected individuals have identified as men after this transition (Imperato-McGinley & Zhu, 2002) and are gynephilic in adulthood (Garcia-Falgueras & Swaab, 2009). Thus, in general, sexual orientation is more consistent with early testosterone exposure than gender of rearing in this population. Although effects of pubertal or adult androgens on the brain, as well on appearance and hence social interactions, cannot be ruled out, cases of gender reassignment at birth (Section 3) suggest that these influences are minimal.

Estrogens

Despite their common characterization as "female" hormones, estrogens exert masculinizing effects in some mammals, including rats and mice (McEwen, Lieberburg, Chaptal, & Krey, 1977; Wu et al., 2009). In these species, most brain masculinization is accomplished through the conversion via the enzyme aromatase of androgens to estrogens and subsequent binding of estrogen to estrogen receptors (Naftolin, Ryan, Davies, Petro, & Kuhn, 1975). It has been hypothesized that estrogens also play a part in masculinizing the human brain, but little evidence supports this hypothesis (Motta-Mena & Puts, 2017; Puts & Motta-Mena, 2017; Zuloaga, Puts, Jordan, & Breedlove, 2008).

Diethylstilbestrol

Diethylstilbestrol (DES) is a synthetic estrogen that was administered to pregnant women during the mid-twentieth century with the intended effect of reducing the risk of abnormal pregnancy and miscarriage, but with many unintended negative consequences (Titus-Ernstoff et al., 2003). For women exposed to DES in utero, these include increased incidence of breast cancer, structural abnormalities of the reproductive tract, infertility, and abnormal pregnancies (Schrager & Potter, 2004).

A sample of women exposed prenatally to DES scored higher on several dimensions of same-sex orientation (including behavior and attraction variables) than both an unrelated control group and their unexposed sisters (Ehrhardt et al., 1985; $N = 60$), and a follow-up study (Meyer-Bahlburg et al., 1995; $N = 217$) replicated some associations but not others. However, these results were not replicated in subsequent larger studies (Newbold, 1993). In by far the largest study of this type ($N > 5{,}500$), women exposed to DES were significantly *less likely* than controls to report homosexual behavior (Titus-Ernstoff et al., 2003).

Other evidence that sexual orientation is not estrogen-mediated

Additional data cast further doubt on the role of estrogens in masculinizing the human brain (see Puts & Motta-Mena, 2017 for a more complete review). First, dihydrotestosterone has similar effects to testosterone on female sexually differentiated behavior in rhesus macaques (Wallen, 2005). Because dihydrotestosterone cannot be aromatized into estrogen, this means that estrogen-to-estrogen-receptor binding must not be essential to brain and behavior masculinization in a close human relative. Second, eight case studies reviewed in Cooke, Nanjappa, Ko, Prins, and Hess (2017) found no differences in self-reported sexual orientation between men with and without functioning aromatase genes, and an additional case study reported male-typical gender identity and sexual orientation in a man lacking a functional estrogen receptor (Smith et al., 1994). Although larger samples are clearly desirable, these results provide evidence that men can have masculine behavior despite the inability to aromatize testosterone to estrogen. This indicates that estrogen-to-estrogen-receptor binding is not essential for masculinizing the human brain in general and sexual orientation in particular.

Hormone biomarkers

Researchers have also used several morphological or behavioral traits as putative biomarkers of prenatal sex hormone exposure (e.g., Rahman & Wilson, 2003). In general, these data indicate increased masculinization in gynephilic females, but mixed results in males (summarized in Table 15.3).

Table 15.3 Summary of results for studies linking hormonal biomarkers to sexual orientation (see section "Harmone biomarkers")

Reference	*Predictor variable*	*Dependent variable*	*Sex*	*Predicted association*	*Significant?*	*N*	*T*	*C*	*ES*	*Stat*	*Method*
McFadden & Pasanen, 1998	Sexual orientation	Otoacoustic emissions	F	Stronger in heterosexual	Yes	94	37	57	0.37	*d*	From paper
McFadden & Champlin, 2000	Sexual orientation	Auditory evoked potentials	F	Different in two samples	Yes (5 of 19)	105	57	49	0.37–0.62	*d*	From paper
McFadden & Champlin, 2000	Sexual orientation	Auditory evoked potentials	F	Different in two samples	No (14 of 19)	105	57	49	0.01–0.30	*d*	From paper
Martin & Nguyen, 2004	Andro- vs. gynophilia	Bone growth/body size	Both	Androphilia = female-typical	Yes (7 of 10)	412	228	184	0.20–0.38	*d*	From paper
McFadden & Champlin, 2000	Sexual orientation	Auditory evoked potentials	M	Different in two samples	Yes (5 of 19)	103	53	50	0.38–0.47	*d*	From paper
McFadden & Champlin, 2000	Sexual orientation	Auditory evoked potentials	M	Different in two samples	No (14 of 19)	103	53	50	0.03–0.26	*d*	From paper
Bogaert & Hershberger, 1999	Sexual orientation	Penile length (flaccid)	M	Different in two samples	Yes	4230	813	3417	0.65	*d*	Calculated
Bogaert & Hershberger, 1999	Sexual orientation	Penile length (erect)	M	Different in two samples	Yes	4230	813	3417	1.28	*d*	Calculated
Valentova & Havlíček, 2013	Sexual orientation	Ratings of SO from vocal recordings	M (F raters)	Ratings match actual orientation	Yes	20	n/a	n/a	0.92	*d*	From paper
Valentova & Havlíček, 2013	Sexual orientation	Ratings of SO from vocal recordings	M (M raters)	Ratings match actual orientation	Yes	19	n/a	n/a	0.61	*d*	From paper
Valentova & Havlíček, 2013	Sexual orientation	Ratings of SO from facial images	M (F raters)	Ratings match actual orientation	Yes	20	n/a	n/a	0.32	*d*	From paper
Valentova & Havlíček, 2013	Sexual orientation	Ratings of SO from facial images	M (M raters)	Ratings match actual orientation	Yes	19	n/a	n/a	0.58	*d*	From paper
Valentova, Kleisner, & Havlíček, 2014	Sexual orientation	Ratings of SO from facial images	M (F raters)	Ratings match actual orientation	No	20	n/a	n/a	0.31	*d*	Calculated
Valentova et al., 2014	Sexual orientation	Ratings of SO from facial images	M (M raters)	Ratings match actual orientation	No	20	n/a	n/a	−0.10	*d*	Calculated

(*Continued*)

Table 15.3 (Continued)

Reference	*Predictor variable*	*Dependent variable*	*Sex*	*Predicted association*	*Significant?*	*N*	*T*	*C*	*ES*	*Stat*	*Method*
Valentova et al., 2014	Ratings of masculinity	Ratings of SO from facial images	M	Positive – feminine & gynephilia	Yes	66	n/a	n/a	0.46	*r*	Calculated
Hall & Kimura, 1994	Sexual orientation	Asymmetry of dermal ridge direction	M	Higher in homosexuals	Yes	248	66	182	0.37	*d*	Calculated
Forastieri et al., 2017	Sexual orientation	Asymmetry of dermal ridge direction	M	Higher in homosexuals	No	136	60	76	n/a	n/a	n/a
Mustanski et al., 2002	Sexual orientation	Asymmetry of dermal ridge direction	M	Higher in homosexuals	No	333	169	164	−0.28	*d*	Calculated

Note: N: Total sample size, T: Treatment group sample size, C: Control group sample size, ES: Effect size, Stat: Effect size statistic used, Method: Method used to obtain effect size.

Biomarkers in women

The ratio of the lengths of the 2nd digit (index finger) to the 4th digit (ring finger; 2D:4D) is a widely used biomarker. Males have lower ratios than do females from the end of the first trimester through adulthood (Galis, Ten Broek, Dongen, & Wijnaendts, 2010; Malas, Dogan, Evcil, & Desdicioglu, 2006). A more masculine 2D:4D has been associated with testosterone relative to estradiol in amniotic fluid (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004; Ventura, Gomes, Pita, Neto, & Taylor, 2013), as well as CAH (Brown, Hines, Fane, & Breedlove, 2002; Ciumas, Hirschberg, & Savic, 2009; Okten, Kalyoncu, & Yaris, 2002: but see Buck, Williams, Hughes, & Acerini, 2003), and XY androgen insensitive individuals have a feminine 2D:4D (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009). Thus, 2D:4D is utilized as a proxy for prenatal androgen signaling, and meta-analysis of 18 studies indicated that non-heterosexual women indeed have more masculine 2D:4D than do heterosexual women (Grimbos, Dawood, Burriss, Zucker, & Puts, 2010; total N = 2,707).

Because left-handedness is more common in males, it may also reflect prenatal androgen. In a meta-analysis of 20 studies comprising over 20,000 participants, homosexual women were 91% more likely than heterosexual women to be left-handed (Lalumiere, Blanchard, & Zucker, 2000). It is possible that this difference reflects greater developmental instability in homosexual individuals rather than hormonal influences (Mustanski, Bailey, & Kaspar, 2002; see Conclusion), but Martin, Puts, and Breedlove (2008) found little evidence of increased developmental instability in homosexual individuals.

Also used as a biomarker for early androgen signaling are the extremely subtle sounds produced by the inner ear in response to short click sounds known as click-evoked otoacoustic emissions (henceforth: otoacoustic emissions). Otoacoustic emissions are stronger in women than in men (McFadden & Pasanen, 1998), and stronger in women without male co-twins than in those with male co-twins, whose androgen may have masculinized their sisters in utero (McFadden, Loehlin, & Pasanen, 1996). In one study, non-heterosexual women had masculinized otoacoustic emissions compared to heterosexual women (McFadden & Pasanen, 1998).

Like otoacoustic emissions, auditory evoked potentials can be elicited by click stimuli, and some of their features are sexually dimorphic. Auditory evoked potentials are measured via electrodes attached to subjects' heads and ears, resulting in a series of peaks and waves that are thought to represent neuronal firing in response to acoustic stimuli. Some of these features have been found to be masculinized in homosexual women (McFadden & Champlin, 2000).

Finally, Martin and Nguyen (2004) found that long bone growth in the arms, hands, and legs, another sexually dimorphic putative biomarker for early steroid exposure, was more masculine (greater) in homosexual women compared to heterosexual women and homosexual men.

Biomarkers in men

Some putative biomarkers of early androgen signaling have been reported to be more masculine in homosexual men compared to heterosexual men. For example, meta-analysis of 20 studies (total N = 20,990) homosexual men were 34% more likely than heterosexual men to be left-handed (Lalumiere et al., 2000). In addition, features of auditory evoked potentials that differed by sexual orientation were more masculine in homosexual men (McFadden & Champlin, 2000), homosexual men have been reported to have hypermasculinized penis size (Bogaert & Hershberger, 1999), and homosexual men's faces were rated as more masculine than heterosexual men's faces (Valentova & Havlíček, 2013; Valentova et al., 2014).

Other biomarkers show mixed or no associations with men's sexual orientation. For instance, sexual orientation was unrelated to both 2D:4D in a meta-analysis of 18 studies (Grimbos et al., 2010; total $N = 3{,}121$) and otoacoustic emissions (McFadden & Pasanen, 1998; $N = 108$, $d = 0.07$). Moreover, although the shapes of several facial features differed between homosexual and heterosexual men, about half of these features were more masculine, and half more feminine, in homosexual men (Valentova et al., 2014). Fingertip dermal ridge count is also sexually dimorphic: Women are likelier than men to have more ridges on the left than right hand. Hall and Kimura (1994) found that dermal ridge asymmetry was more feminine in homosexual men, but Forastieri et al. (2017) and Mustanski et al. (2002) failed to replicate this, and neither sexual orientation nor genetic sex was related to dermal ridge asymmetry in male-to-female transsexuals (Slabbekoorn, van Goozen, Sanders, Gooren, & Cohen-Kettenis, 2000).

Finally, Martin and Nguyen (2004) found that homosexual men were more feminine in long bone growth of the arms, hands, and legs.

Neurobiology of sexual orientation

Research into the neurobiology underlying sexual orientation has benefited from both nonhuman animal models and comparisons of sexually dimorphic brain regions in people of different sexual orientations (Rahman, 2005). In many cases, the finding that certain brain regions are sexually dimorphic has led researchers to posit that prenatal hormones play a part in determining their size or neuronal density, which would account for sex – and potentially sexual orientation – differences in these characteristics (summarized in Table 15.4).

SDN-POA/INAH-3

The sexually dimorphic nucleus of the preoptic area (SDN-POA) is a cluster of cells in the hypothalamic medial preoptic area of rats. It is larger in males than in females, and this difference is abolished by neonatal castration and restored by administration of exogenous androgen (Arnold & Gorski, 1984). Lesions to the SDN-POA of male rats reduced male-typical sexual behavior, including mounting, intromission, and ejaculation (De Jonge et al., 1989). In sheep, Roselli, Larkin, Resko, Stellflug, and Stormshak (2004) demonstrated that rams displaying sexual partner preference for other rams had feminized (smaller) sexually dimorphic nuclei than female-oriented rams. The closest homologue in humans to the SDN-POA of rats and the sexually dimorphic nucleus of sheep may be the third interstitial nucleus of the anterior hypothalamus (INAH-3; e.g., Allen, Hines, Shryne, & Gorski, 1989). In support of a connection between INAH-3 size and sexual orientation in humans, LeVay (1991) found that it is on average twice as voluminous in heterosexual men as it is in heterosexual women and homosexual men. Another study failed to reproduce the significant relationship between sexual orientation and INAH-3 volume in men (Byne et al., 2001). However, the large effect size calculated from Byne et al.'s (2001) data ($d = 1.22$; see Table 15.4) suggests that a larger sample may have produced a result supportive of the hypothesized relationship.

Suprachiasmatic nucleus

The suprachiasmatic nucleus is a cell group located in the hypothalamus. It is primarily responsible for maintenance of circadian rhythms (Marieb & Hoehn, 2010) but may also be related to reproduction (Södersten, Hansen, & Srebro, 1981). Swaab and Hofman (1990) found that the suprachiasmatic nucleus was on average 1.7 times as large and contained 2.1 times as many

Table 15.4 Summary of results for studies linking neurobiology to sexual orientation (see section "Neurobiology of sexual orientation")

Reference	*Predictor variable*	*Dependent variable*	*Sex*	*Predicted association*	*Significant?*	*N*	*T*	*C*	*ES*	*Stat*	*Method*
Byne et al., 2001	Genetic sex	INAH-3 volume	Both	Higher in males	Yes	65	31	34	2.62	*d*	Calculated
Byne et al., 2001	Sexual orientation	INAH-3 volume	Male	Higher in heterosexuals	No	45	31	14	1.22	*d*	Calculated
LeVay, 1991	Genetic sex	INAH-3 volume	Both	Higher in males	Yes	22	16	6	3.2	*d*	Calculated
LeVay, 1991	Sexual orientation	INAH-3 volume	Male	Higher in heterosexuals	Yes	35	19	16	5.63	*d*	Calculated
Swaab & Hofman, 1990	Sexual orientation	Suprachiasmatic nucleus volume	Male	Higher in heterosexuals	Yes	28	10	18	4.72	*d* (non-par)	Calculated
Swaab & Hofman, 1990	Sexual orientation	Suprachiasmatic nucleus # of neurons	Male	Higher in heterosexuals	Yes	28	10	18	5.76	*d* (non-par)	Calculated
Allen & Gorski, 1991	Genetic sex	Area of midsagittal plane of anterior commissure	Both	Higher in males	Yes	60	30	30	n/a	n/a	n/a
Allen & Gorski, 1991	Sexual orientation	Area of midsagittal plane of anterior commissure	Male	Higher in heterosexuals	No	60	30	30	n/a	n/a	n/a
Lasco et al., 2002	Genetic sex	Area of midsagittal plane of anterior commissure	Both	Higher in males	No	101	58	43	1.02	*d*	Calculated
Lasco et al., 2002	Sexual orientation	Area of midsagittal plane of anterior commissure	Male	Higher in heterosexuals	No	78	20	58	-0.42	*d*	Calculated
Ponseti et al., 2007	Genetic sex	Grey matter volume	Both	Higher in males	Yes	49	24	25	1.99	*d*	Calculated
Ponseti et al., 2007	Sexual orientation	Grey matter volume	Male	Higher in heterosexuals	No	30	16	24	0.19	*d*	Calculated
Ponseti et al., 2007	Sexual orientation	Grey matter volume	Female	Higher in heterosexuals	No	40	15	25	0	*d*	Calculated
Ponseti et al., 2007	Sexual orientation	Perirhinal cortex volume	Male	Higher in homosexuals	No	30	16	24	n/a	n/a	n/a
Ponseti et al., 2007	Sexual orientation	Perirhinal cortex volume	Female	Higher in homosexuals	Yes	40	15	25	n/a	n/a	n/a
Abé et al., 2014	Sexual orientation	Cortical thickness	Male	Higher in heterosexuals	Yes for 6 of 8 regions	40	19	21	n/a	n/a	n/a
Abé, Johansson, Allzén, & Savic, 2014	Sexual orientation	Cortical thickness	Male	Higher in heterosexuals	No for 2 of 8 regions	40	19	21	n/a	n/a	n/a

Note: N: Total sample size, T: Treatment group sample size, C: Control group sample size, ES: Effect size, Stat: Effect size statistic used, Method: Method used to obtain effect size.

neurons in homosexual compared to heterosexual men. The authors to posited that the differences found between heterosexual and homosexual men were hormonally mediated. To test this, the same research group treated male rats with an aromatase-inhibitor. Rats treated pre- and postnatally possessed on average 59% more vasopressin-expressing neurons in the suprachiasmatic nucleus than untreated controls and expressed bisexual social and sexual partner preference. Groups that were untreated or treated only prenatally developed sexual and social partner preference toward females (Swaab, Slob, Houtsmuller, Brand, & Zhou, 1995).

Anterior commissure

The anterior commissure is one of several white matter brain regions connecting the hemispheres of the cerebral cortex (Marieb & Hoehn, 2010). It is not implicated in the regulation of sexual behavior, but the size of its midsagittal area was found to be larger in women than men, and also larger in homosexual men than in both heterosexual men and heterosexual women (Allen & Gorski, 1991). Allen and Gorski speculated that (1) size differences in this structure may be related to differences between homosexual and heterosexual men in cerebral lateralization, given that homosexual men are more likely than heterosexual men to be left-handed, and (2) anterior commissure size is mediated by levels of steroid hormones such as testosterone. While the latter hypothesis has not been tested, Lasco, Jordan, Edgar, Petito, and Byne (2002) failed to replicate the relationship between anterior commissure size and sexual orientation in men; this association has yet to be investigated in female subjects. Finally, there is some evidence that two thyroid hormones, thyroxin and triiodothyronine, play a part in determining the size of the anterior commissure in rats (Ferraz, Escobar, & De Escobar, 1994). These hormones have not been studied in the context of sexual orientation, but if their levels differ in homosexual and heterosexual populations, then this may help to explain the sexual orientation-based difference in size.

Perirhinal cortex

Using structural magnetic resonance imaging (sMRI) and voxel-based morphometry, Ponseti et al. (2007) investigated sex and sexual orientation differences in gray matter concentrations in the brains of living people. While they found sex differences in global gray matter concentrations, heterosexual men and women did not differ from their homosexual counterparts on this global measure. However, heterosexual women showed higher gray matter concentrations than homosexual women in several regions, including the perirhinal cortex (this was not the case for heterosexual vs. homosexual men). The perirhinal cortex is a cell group located in the medial temporal lobe and is associated with olfactory and spatial processing, as well as memory encoding. Although the authors note that spatial processing may differ according to sexual orientation, there is no direct evidence of a functional link between the perirhinal cortex and sexual orientation. With regard to a hormonal mechanism, neither hormone levels, nor the expression of hormone receptors has been studied in the context of perirhinal cortex dimorphism. However, the sex difference in its size suggests that the development of this region may be influenced by steroid hormones, as has been found in several of the other brain regions discussed in this section.

Cortical thickness

Finally, it has been shown that cortical thickness is sexually dimorphic and that this difference may be related to androgen exposure (Bramen et al., 2012). The relationships are complex;

cortical thickness in pubescent boys and girls was inversely related to androgen levels (Bramen et al., 2012), and the cortices of men were thicker than women's in some regions and thinner in others (Lv et al., 2010). Abé et al. (2014) hypothesized a difference in cortical thickness between heterosexual and homosexual men. Using sMRI, they found that heterosexual men had larger thalamus volumes and thicker cortices in several areas than both homosexual men and heterosexual women. Whether cortical thickness has any causal connection to sexual orientation has yet to be determined, but relationships between cortical thickness and androgens suggest that prenatal hormonal environment may play a role.

Conclusion

Although research into the endocrinology of human sexual orientation is almost entirely correlational, and much work has yet to be done, some tentative inferences are possible. First, androgen signaling at or below female-typical levels leads to androphilia. Both typical genetic females and genetic males with no androgen signaling due to complete androgen insensitivity syndrome are highly likely to be androphilic.

Second, individuals whose androgen signaling exceeds female-typical levels are more likely than typical females to be gynephilic. Whether they are raised as boys or girls, natal males with male-typical prenatal androgen signaling are nearly always gynephilic. Genetic males exposed to androgen signaling that is intermediate between typical males and females due to partial androgen insensitivity syndrome or 5α-reductase-2 deficiency are intermediate in their probability of gynephilic orientation. Likewise, genetic females exposed to intermediate androgen signaling are intermediate in their probability of gynephilic orientation, as indicated by studies of congenital adrenal hyperplasia, polycystic ovary syndrome, 2D:4D, handedness, otoacoustic emissions, auditory evoked potentials, and long bone growth.

Third, androgens act on the neural substrates underlying sexual orientation primarily by binding directly to androgen receptor rather than being aromatized into estrogen and binding to estrogen receptor. Prenatal exposure to a synthetic estrogen has little or no effect on women's sexual orientation, and genetic males with nonfunctional aromatase and estrogen receptor genes apparently have male-typical sexual orientation. By contrast, studies of partial and complete androgen insensitivity syndrome indicate that androgen receptor function is critical to sexual orientation.

Fourth, the influence of androgens on sexual orientation is organizational rather than activational. Adult hormone concentrations are weakly correlated, if at all, with sexual orientation within men. Moreover, testosterone treatment does not produce gynephilia in androphilic men, and removal of androgen signaling due to castration does not produce androphilia in gynephilic men. Although homosexual women, especially those identifying as "butch" lesbians, may have higher testosterone levels than heterosexual women, this may be explained by the higher incidence of polycystic ovary syndrome in lesbians. The scanty available evidence indicates that testosterone treatment does not produce gynephilia in androphilic women.

Fifth, most variation in sexual orientation among genetic males is not due to chronic, systemic differences in androgen signaling. Disorders of sexual development such as complete androgen insensitivity syndrome, partial androgen insensitivity syndrome, and 5α-reductase-2 deficiency demonstrate that decreases in androgen signaling increase the probability of androphilia in genetic males, but little evidence links such global alterations in androgenization to sexual orientation in males without disorders of sexual development. Indeed, handedness, auditory evoked potentials, penile size, and facial appearance have been found to be more masculine in homosexual compared to heterosexual men, contrary to the prediction that lower overall

androgenization produces androphilia in homosexual men. Other biomarkers such as 2D:4D, click-evoked otoacoustic emissions, objective facial measures, and fingertip dermal ridge asymmetry have shown mixed or no associations with men's sexual orientation, or have been found to be more feminine in the case of long bone growth.

Perhaps acute decreases in androgen signaling during a developmental window when sexual orientation is organized lead to compensatory increases in androgen during other developmental windows, hypermasculizating some biomarkers (McFadden, 2017). Or perhaps elevated androgens when some biomarkers develop leads to compensatory decreases in androgenization when sexual orientation differentiates. Perhaps the mixed associations reflect varied androgen signaling across anatomical regions of the brain and body, with lower androgenization in brain regions associated with sexual orientation as well as the epiphyses of the long bones in homosexual men, and higher androgenization in regions associated with handedness and auditory evoked potentials, for example. More research is needed to discriminate between these possibilities.

Finally, more work is needed to clarify the neural substrates of sexual orientation. For example, although the perirhinal cortex has been found to be masculinized in lesbians, this region is not directly related to sexual attraction, and no research has compared the third interstitial nucleus of the anterior hypothalamus or nearby nuclei in homosexual women to heterosexual women or men. Some brain regions and attributes such as interstitial nucleus of the anterior hypothalamus, suprachiasmatic nucleus, and anterior commissure size, and cortical thickness have been found to vary with sexual orientation in men, but the identification of causal relationships to sexual orientation require further investigation, as does how development in these regions may be influenced by sex hormones.

Overall, however, a wide variety of evidence compellingly indicates that androgens acting during the pre-and perinatal periods of human development influence sexual orientation in adulthood.

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