A Critical Review of Recent Biological Research on Human Sexual Orientation

Brian S. Mustanski    Meredith L. Chivers    J. Michael Bailey
Indiana University    Northwestern University    Northwestern University

This article provides a comprehensive review and critique of biological research on sexual orientation published over the last decade. We covered research investigating (a) the neurohormonal theory of sexual orientation (psychoneuroendocrinology, prenatal stress, cerebral asymmetry, neuroanatomy, otoacoustic emissions, anthropometrics), (b) genetic influences, (c) fraternal birth-order effects, and (d) a putative role for developmental instability. Despite inconsistent results across both studies and traits, some support for the neurohormonal theory is garnered, but mostly in men. Genetic research using family and twin methodologies has produced consistent evidence that genes influence sexual orientation, but molecular research has not yet produced compelling evidence for specific genes. Although it has been well established that older brothers increase the odds of homosexuality in men, the route by which this occurs has not been resolved. We conclude with an examination of the limitations of biological research on sexual orientation, including measurement issues (paper and pencil, cognitive, and psychophysiological), and lack of research on women.

Key Words: birth order, developmental instability, genetic, homosexuality, hormones, phallometry, sexual orientation, vaginometry

It has been over a decade since the first review of the evidence for a biological basis of sexual orientation was published in this journal (Gooren, Fliers, & Courtney, 1990). The focus of that review was on the role of hormones in explaining within-sex variations in sexual orientation. More recently Bailey and Pillard (1995) reviewed the extant data regarding genetic influences on sexual orientation. Since these reviews, there has been a considerable expansion in the evidence supporting the existence of biological influences. Such studies have explored genetic, neuroanatomic, endocrine, and morphological differences based on sexual orientation. Given the accumulation of new data since the last reviews, it seems an appropriate time to evaluate the evidence in this area. The focus of this review is on recent evidence (last 10 years)

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regarding the putative role of biological factors influencing sexual orientation. When necessary, less contemporary research will be included to contextualize newer evidence. Articles were identified by searching PubMed and PsychInfo for common terms related to sexual orientation (i.e., homosexuality, gay, etc.) along with relevant biological terms (genetic, brain, etc.), allowing only for the retrieval of articles published in the last 10 years. Authors who presented relevant papers at the International Behavioral Development Symposium convened by Lee Ellis in 2000, which focused on the biological basis of sexual orientation, were asked if they had any recent research that should be included in the review. Finally, a posting was placed on SEXNET, an e-mail listserv for researchers who study sexual behavior, asking for references for recently published or yet-to-be published articles.

Many of the studies that are reviewed in this article have provoked intense interest both in the scientific and popular arenas. Some of this interest likely resulted from the mistaken belief that various etiological accounts of sexual orientation must have differing social and ethical implications (see Greenberg & Bailey, 1993, for a discussion). Such thinking confuses several related but distinct concepts, such as genetic versus environmental, voluntary versus compelled, innate versus acquired, immutable versus changeable, and moral versus immoral (see Bailey & Pillard, 1995, for a discussion). Similarly, the term biological is frequently misunderstood and misused due to it being somewhat amorphous, given that all human behaviors are enacted by the brain and thus are in some sense biological. Rather than asking if sexual orientation is biological, we believe it is more fruitful to consider whether differences in sexual orientation primarily reflect differences in social experiences, differences in biologic factors unrelated to social experiences, or both. The ultimate goal of such research will be to understand the timing and mechanism of various etiological factors that influence sexual orientation.

Empirical researchers exploring the basis of biological influences acting on sexual orientation have traditionally used two approaches. In the first, a wide array of methodology to explore the role that hormones play in influencing sexual orientation has been used. Such research is generally based on the "neurohormonal theory" (Ellis & Ames, 1987) positing that homosexuality is caused by atypical sex hormone levels in utero with concomitant sex-atypical neural differentiation. The second approach, behavioral genetics, is focused on identifying the source and magnitude of genetic influences on sexual orientation. Two more recent approaches are focused on developmental instability as measured by fluctuating asymmetry and nonright-handedness (Lalumière, Blan-
and the relation between sexual orientation and number of older brothers (Blanchard, 1997). There have been relatively few attempts to synthesize the results from these varying approaches, although they are not inherently mutually exclusive. For example, having several older brothers may increase the effects of genes that influence sexual orientation, thereby producing changes in hormone activity in the brain. An inestimable number of additional interactions are imaginable, although at this point insufficient understanding of the biological factors influencing sexual orientation hampers their specification. Consequently, we review each area of study individually, and conclude with a discussion of the limitations of the current research.

Neurohormonal Influences

The most influential theory about the origins of homosexuality implicates sex-atypical androgen action during gestation (Ellis & Ames, 1987), making this a logical starting place for such a review. A now classic paper by Phoenix, Goy, Gerall, and Young (1959), in which the differential organizational and activational influences of sex steroids were demonstrated, initiated this line of research. In this study, Phoenix and colleagues injected pregnant guinea pigs with testosterone propionate during gestation and gonadectomized them after birth. At birth, the treated female offspring had masculinized external genitalia that were nearly identical to those of normal male offspring. When the offspring were sexually mature, they were injected with various doses of estradiol benzoate. Compared to female controls, the prenatally androgenized females showed a reduced duration of lordosis that was not significantly different from male controls. The treated females also were more likely to show more mounting behavior than the female controls. Thus, the findings of Phoenix and colleagues showed that testosterone, when administered during sensitive periods of development, has a masculinizing action on neuronal tissue involved in mating behavior. This organizing effect of sex hormones in males and females seems to hold true for most mammalian species (I. L. Ward & O. B. Ward, 1985).

Although nonhuman animal research is heuristically useful, there are several problems with its use for informing human sexual orientation. Beach (1979) commented that simply because the same descriptive terms are used across species does not guarantee that the underlying concepts are identical. Species-specific behaviors (i.e., lordosis or mounting in rats) fail to capture the full picture of human sexual orientation. A second problem with using animal literature in support of the neurohormonal theory is that the data do not provide unequivocal
support for organizing effects, especially for females (Meyer-Bahlburg, 1984). For example, Dörner (1976) showed that only after gonadectomy and testosterone administration in adulthood do early androgen-exposed female rats demonstrate male-typical sexual behaviors. If the experimental rat’s gonads were not removed, they showed a clear predominance of female sexual behavior and only a slight increase in male-typical behaviors. Similar results have been found for female non-human primates (Eaton, Goy, & Phoenix, 1973). Additionally, the fact that experimental hormone manipulations can influence sexual orientation in a laboratory does not prove that they do so in normal populations. Meyer-Bahlburg (1984) pointed out a final inadequacy of the animal literature: Hormonal manipulations result not only in a shift of sex-dimorphic behavior, but also in alterations of the genitals. In contrast, homosexual people usually have normal genitalia. Based on these limitations, it is clear that although the animal literature can be extremely useful in developing hypotheses and testing ideas not possible with human participants, only data from human studies can definitely establish what role sex hormones play in human sexual orientation.

**Human Studies**

The earliest researchers of the relationship between sex hormones and human sexual orientation explored the hypothesis that some gay men had decreased levels of circulating testosterone. This research has already been extensively reviewed elsewhere (see Meyer-Bahlburg, 1984) and suggests little or no difference in circulating androgen levels between gay and heterosexual men. Additionally, experimental manipulations of male androgen levels appear to affect the magnitude of the libido, rather than the direction of the sexual orientation (Barahal, 1940).

Results from research on activational hormone effects in women have been less consistent. Gartrell, Loriaux, and Chase (1977) and Loraine, Adamopoulos, Kirkham, Ismail, and Dove, (1971) found lesbians to have higher levels of testosterone than heterosexual women. But Dancey (1990) and Downey, Erhardt, Schiffman, Dyrenfurth, and Becker (1987) found no differences. In two studies, Pearcey, Docherty, and Dabbs (1996) and Singh, Vidaurri, Zambarano, and Dabbs (1999) compared two putative subtypes of lesbian women, those who either self-identified or were rated as “butch” with those who were “femme.” “Butch” is a colloquial term used to refer to a masculine woman, and “femme” is the term used for a more feminine lesbian woman. In both studies significant differences were found in salivary testosterone levels among lesbian women, but these studies must be interpreted with caution because of their failure to consistently control for the phase of the men-
strual cycle and their reliance on indirect measures of plasma hormone levels. Thus, research exploring the activational role of hormones on adult sexual orientation, although not directly measuring hormone activity in critical areas, seems to suggest no effect in men. Additional research will be needed examining hormone levels in butch and femme lesbian women and heterosexual women. Future researchers in this area should make every attempt to control for differences in lifestyle and behavior that could explain group differences in hormone activity such as diet, drug use, and exercise.

Another route through which hormones could influence sexual orientation is through prenatal, organizational action. We begin our review of this evidence by suggesting a thought experiment, representing an ideal test of the neurohormonal hypothesis. This thought experiment begins with complete sex reassignment of a random sample of newborn males, along with rearing by adoptive parents unaware of their child's birth sex. Follow up in adulthood indicating that these children were sexually attracted to females would strongly suggest that sexual orientation was programmed prenatally into the developing neural circuitry. Obviously, this study would never be conducted for ethical reasons. Nevertheless, it is worth keeping in mind the ideal experiment in order to judge how closely available data approach it.

The best approximation of our thought experiment is based on studies of children without any known prenatal hormonal abnormalities who, for a variety of reasons, are sexually reassigned shortly after birth. Such procedures rarely occur, but are most often performed for one of two reasons: sufficient damage to a male infant's penis to require its removal (ablation penis), or genetic males born with cloacal extrophy (described below). In neither of these cases is the approximation perfect. First, the child is not adopted by parents unaware of the sex reassignment. This is an important limitation, as the psychosexual developmental effects of parental knowledge of discordance between genetic and rearing sex have not been studied. Second, the sex reassignment is not perfect because of medical limitations. Nevertheless, these cases are the closest approximation of our ideal experiment.

Table 1 contains case reports and studies of prenatally normal males sexually reassigned near birth, and followed up after the onset of sexual maturity. As seen in Table 1, three cases of ablation penis have been reported in the literature, all consequences of circumcision accidents. In only two of these cases was sexual orientation explicitly reported, and in both, sexual orientation was either fully or partially directed toward women. Also reported in Table 1 are 36 cases of genetic males who were sex reassigned because of cloacal extrophy, a disorder of embryogenesis.
that causes, among other problems, poor differentiation of the genitals. In one report (Vates et al., 1999), the patient maintained a female identity and reported sexual attraction toward males. For the larger study (Reiner, personal communication, February 3, 2002) the vast majority of the patients rejected their assigned gender and reported sexual attraction toward females. Interpretation of these data must be accompanied by consideration of the many associated medical issues experienced by these children. Comparisons of the psychosexual outcome between children with cloacal extrophy and a similar medical condition may help control for the possible effects of extensive medical treatment during development. Bladder extrophy-epispadias is a similar disorder of embryogenesis, but the penis is not similarly effected, making sexual

<table>
<thead>
<tr>
<th>Report</th>
<th>Cause for sex reassignment</th>
<th>No. of cases</th>
<th>Age(s) at report</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gearhart &amp; Rock (1989)</td>
<td>Ablatio Penis</td>
<td>1</td>
<td>17</td>
<td>Maintained female identity and sexually active (presumably with males, but not specified)</td>
</tr>
<tr>
<td>Diamond et al. (1997)</td>
<td>Ablatio Penis</td>
<td>1</td>
<td>Adult</td>
<td>Transitioned to male and attracted to females</td>
</tr>
<tr>
<td>Bradley et al. (1998)</td>
<td>Ablatio Penis</td>
<td>1</td>
<td>26</td>
<td>Maintained female identity and identifies as bisexual</td>
</tr>
<tr>
<td>Vates et al. (1999)</td>
<td>Cloacal Exstrophy</td>
<td>1</td>
<td>17</td>
<td>&quot;No doubts&quot; of female identity. Sexual preference reported as &quot;male only,&quot; and &quot;completely satisfied&quot; with sexual life. Has not had intercourse.</td>
</tr>
<tr>
<td>Reiner (personal communication, February 3, 2002)</td>
<td>Cloacal Exstrophy</td>
<td>35\textsuperscript{a}</td>
<td>&gt;14</td>
<td>Of the 79% that have declared themselves or live as male 100% report sexual attraction to females. The other 21% refuse to discuss sex.</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Varying amounts of information on each subject.
reassignment rare. One study of 14 males over the age of 14 reports 100% sexual orientation toward females, but a number of psychosexual and physical dysfunctions (Reiner, Gearhart, & Jeffs, 1999). In both of these approximations of our ideal thought experiment, the results vis-a-vis sexual orientation suggest that whether sex reassigned or not, attraction to females is nearly universal. Such results are consistent with the predictions of the neurohormonal hypothesis because, despite sex reassignment at birth, the male typical hormone levels en utero seem to have produced sexual attraction toward females.

In addition to the evidence provided by hormonally normal male infants assigned to a female gender, several hormonally-induced congenital intersex syndromes have been proposed as models for understanding the organizational role of hormones in sexual orientation differentiation, including congenital adrenal hyperplasia (CAH) (Ehrhardt, Evers, & Money, 1968; Money, Schwartz, & Lewis, 1984; Zucker et al., 1996), androgen insensitivity syndrome (AIS) (Money et al., 1984; Wisniewski et al., 2000), and 5-alpha reductase deficiency (5ARD) (Imperato-McGinley, Guerrero, Gaultier, & Peterson, 1974). Because genetic males with 5ARD have been studied without formal or rigorous assessment of sexual orientation, this condition will not be discussed in this review. Readers interested in further details about these conditions should see one of the above references.

As described by Zucker (1999), CAH is a genetically recessive defect in which the synthesis of cortisol is disrupted and, in its place, the adrenal glands secrete androstenedione, which is metabolized into testosterone and eventually into dihydrotestosterone, both active components of masculinization (Brown et al., 1993). In genetically female fetuses, increased androgen levels result in genital masculinization ranging from mild clitoral enlargement to a fully formed penis and empty scrotum (Migeon, 1979; New, Ghizzoni, & Speiser, 1996). In extreme cases, if the diagnosis is not established neonatally, the child may be assigned a male gender and subsequently raised as a boy (Money & Dalery, 1976). When the condition is correctly diagnosed, the genitals are surgically altered to appear female (Allen, Hardy, & Churchill, 1982), cortisol-replacement therapy is initiated to prevent postnatal virilization (New & Josso, 1988), and a female gender is assigned and the infant is raised as a girl. This second case of CAH has been described as a “model experiment of nature” (Zucker & Bradley, 1995, p. 140) because it allows an examination of the role of prenatal androgens on sexual orientation, while partially controlling for the psychosexual rearing environment.

The sexual orientation of adult women with CAH has been examined
in eight studies with at least 10 participants. Two of these studies took place before the availability of cortisol-replacement therapy, thus the participants did not receive the treatment until late in life (Ehrhardt et al., 1968; Lev-Ran, 1974). Of the remaining studies, only three employed concurrent non-CAH controls (Dittmann, Kappes, & Kappes, 1992; Money et al., 1984; Zucker et al., 1996). Thus, these will be the only ones reviewed.

Money et al. (1984) compared 30 CAH patients to 15 AIS patients and 12 patients with Mayer-Rokitansky-Küster Syndrome (MRKS; the 46, XX counterpart to AIS; see Griffin, Edwards, Madden, Harrod, & Wilson, 1976, for a description). Of those participants who were willing or able to describe their sexual orientation, 48% of the CAH girls reported same-sex arousal imagery and 22% reported same-sex partner sexual contact compared to 7% and 4%, respectively, for the AIS/MRKS groups, and 15% and 10%, respectively, based on the original Kinsey female data (Kinsey, Pomeroy, Martin, & Gebhard, 1953). The relative elevation in bisexual/homosexual fantasy has been replicated by Zucker et al. (1996) in a study of 31 CAH females who were compared to unaffected sisters and cousins (N = 15), and by Dittmann et al. (1992) in a sample of 34 CAH females and 14 control sisters.

The contrast between the AIS and CAH females is particularly powerful, as they represent opposite extremes of androgenization. CAH females have greater-than-typical androgen levels, whereas AIS individuals are, to varying degrees, insensitive to androgens. Because of this X-linked genetic mutation, AIS genetic males possess female external genitalia, undescended testes, and feminine body features, and are typically raised as female (Brinkmann, 2001). Wisniewski et al. (2000), in a sample of 46, XY women (N = 14) with complete AIS, produced results similar to the Money et al. (1984) study mentioned above. Sexual attractions to men were reported by 100% of the women during adolescence, and by 93% during adulthood, suggesting that lack of prenatal androgen action biases sexual attraction toward men. Interpretation of the AIS results is limited by the fact that rearing sex is confounded with hormonal activity. This condition deviates from our thought experiment because AIS children are both reared as female and are insensitive to the masculinizing effects of androgens.

Additional evidence for the role of prenatal excesses of androgens in females comes from the effects of diethylstilbestrol (DES), a nonsteroidal estrogen that has masculinizing effects similar, but not of the same magnitude, as those seen in CAH. DES attained widespread use to treat at-risk pregnancies during the 1940s-1960s until its use was halted because of adverse physical effects such as increased risk for can-
Because DES is a less potent masculinizing agent than CAH, and was administered late in the pregnancy, the genitalia are not masculinized in female children, thus partially ruling out the possibility of differential socialization based on abnormal genitalia. In several studies of DES-exposed women, relative increases in homosexual fantasies have been shown (Ehrhardt et al., 1985; Meyer-Bahlburg et al., 1995), thus providing further support for the role of prenatal androgens in determining sexual orientation.

The results of these various approximations of our thought experiment have been interpreted by some to be consistent with animal research demonstrating the influence of prenatal sex hormones on sexually dimorphic reproductive behaviors. Such interpretations have been met with criticism and attempts at generating alternative explanations to the idea that prenatal hormones hard wire sexual orientation into fetal brains (Bleier, 1984, pp. 97-101; Fausto-Sterling, 1985, pp. 133-138). For example, it has been suggested that parental response to ambiguous genitalia can have uncharacterized psychosocial implications that increase the likelihood of homosexual or bisexual fantasies occurring. Another criticism implicates homosexuality as a possible side effect of one of the many medications that these individuals often take to treat their medical conditions. Money et al. (1984) also present the possibility that a child may avoid developmentally appropriate peer sexual experiences because of internalized anxiety about the appearance of their genitalia, resulting in an altered psychosexual development. Although such alternative explanations are possible, the relatively consistent result across these various conditions, that prenatal androgen activity potentiates attraction to females and the absence of such activity potentiates attraction to males, is strongly suggestive of prenatal neurohormonal effects in determining sexual orientation.

Prenatal Stress

Because the vast majority of homosexual individuals do not have one of the endocrine disorders mentioned above, a major weakness of the neurohormonal theory is the lack of evidence for a proximal mechanism that might impinge on prenatal sex hormone levels. If there is a neurohormonal event linked to sexual orientation, what produces that neurohormonal event? One animal paradigm that has been considered a potential etiological model for this mechanism is based on evidence that maternal stress demasculinizes and feminizes the sexual behavior of male rat progeny (I. L. Ward, 1972; O. B. Ward, Denning, Hendricks, & French, 2002) via a delay of the testosterone surge critical for sexual differentiation of the brain (I. L. Ward & Weisz, 1980). Although external
genitalia remain unaltered, the size of the sexually dimorphic nucleus of the preoptic area (discussed later) is feminized (O. B. Ward, 1992). This model is by no means ideal because of inconsistencies in the animal and human literature. Chapman and Stern (1978) found no morphological or behavioral differences between offspring of stressed and nonstressed rats, whereas Sachar and Clement (1980) stated that the human adrenal response to stress is attenuated in comparison to rats.

Several researchers have explored the effects of prenatal maternal stress on human sexual orientation, with mixed results. One study compared the number of men registered by "venerologists" as homosexual \((N = 865)\) per the total number of male births in East Germany, and found significant differences between cohorts born during and after World War II compared to those before the war (Dörner et al., 1980), the assumption being that women who were pregnant during and immediately after the war were more likely to experience stress. Dörner, Schenk, Schmiederl, and Ahrens (1983) replicated these results by asking 100 bisexual/gay men and 100 heterosexual men about stressful events that might have occurred during their mother's pregnancy. Ellis, Peckham, Ames, and Burke (1988) surveyed a small sample of mothers about stress during each trimester of pregnancy. A marginally significant trend for increased stress during the second trimester was found for mothers of gay offspring. As pointed out by LeVay (1996), the results are difficult to interpret given that a significant difference was also found for a 3-month period a year before they became pregnant. In a more recently reported study conducted between 1988 and 1998 in the U.S., over 7,500 offspring and their mothers provided retrospective information regarding the offspring's sexual orientation and the mother's stressful experiences and use of alcohol and nicotine during pregnancy (Ellis & Cole-Harding, 2001). The inclusion of maternal drug use as a variable in this study is justified, based on animal studies in which it has been demonstrated that the administration of drugs to pregnant females has effects on the sexual behavior of offspring similar to the effects of prenatal stress (O. B. Ward, 1992). Findings from this study suggested that prenatal stress has a small, but significant, effect on sexual orientation in males, alcohol has no effect, and nicotine significantly increases the probability of homosexuality in females.

These studies have been criticized on the methodological grounds that there were no reliability checks for independent and dependent variables, the possibility of demand characteristics (mothers and sons looking for an explanation for homosexuality), and lack of consideration of alternative explanations (e.g., higher paternal absence; Bailey, Willerman, & Parks, 1991). In addition, in one study this effect was not
replicated: Mothers' recall of stress during pregnancy correlated close to zero with their son's Kinsey scale ratings of sexual orientation (Bailey et al., 1991). One possible explanation for the inconsistent results across studies may be the small effect size of the phenomenon, such that it is only detectable with massive samples. Additionally, the reliance on retrospective reporting may blur any detectable effect. Although difficult to perform, prospective studies would provide more convincing evidence of the existence of a maternal stress effect on sexual orientation.

In the absence of technology allowing direct assessment of prenatal, tissue specific hormone action in humans, another research methodology has been the comparison between homosexual and heterosexual subjects on traits thought to be influenced by prenatal sex hormones. This is consistent with one of the main predictions of the neurohormonal hypothesis: that homosexual individuals should have a higher frequency of cross-sex characteristics than heterosexual individuals (Ellis & Ames, 1987). This notion has been explored in a variety of traits measured using biological techniques, including cerebral asymmetry, neuroanatomic structures, otoacoustic emissions, and anthropometric characteristics.

*Neuropsychological Markers of Functional Cerebral Asymmetry*

Functional cerebral asymmetry (FCA) refers to the division of labor between the hemispheres of the brain for the processing of language and spatial abilities. Despite inconsistent results across studies, narrative reviewers of the literature have suggested that men show a modest increase in functional asymmetry compared to women, meaning that the hemispheres of men are more specialized (Hiscock, Inch, Jacek, Hiscock-kalil, & Kalik, 1994; McGlone, 1980). Zucker and Bradley (1995) provided a comprehensive review of studies exploring sexual orientation differences in FCA and reported largely inconsistent results. Given the depth of their review, we focus only on studies published since that time.

Sanders and Wright (1997) found gay men lacked a male-typical left visual field advantage on a dot detection task. In another study, gay men and lesbian women did not show the association between hand preference and the magnitude of perceptual asymmetry on a dichotic listening task found among heterosexuals (McCormick & Witelson, 1994). Reite, Sheeder, Richardson, and Teale (1995) found gay men had a female typical pattern of decreased cerebral laterality when measured with a magnetoencephalograph during an auditory task. Alexander and Sufka (1993) found gay men's EEG patterns resembled the patterns recorded from heterosexual women during various verbal and spatial
tasks (for a replication see Wegesin, 1998). In the only study to include lesbian women Wegesin (1998) found no sexual orientation effects. Although these studies seem to suggest a different pattern of FCA dependent on sexual orientation in men, the limited number of studies and their reliance on small convenience samples require replications with larger samples before any definitive conclusion can be drawn.

One trait shown to be an indirect index of FCA is an individuals handedness (Geshwind & Galabura, 1985). In order for handedness to be relevant to sexual orientation under the prenatal neurohormonal theory, its development should be canalized prenatally and be dependent upon androgens. Little is definitively known about the ontogeny of handedness, but indirect evidence, such as increased rates of nonright-handedness among males, is consistent with both prenatal canalization and hormonal influences (see Lalumière et al., 2000; Mustanski et al., 2002, for a review of the data).

Narrative reviews of the literature on the association between handedness and sexual orientation have disagreed about the existence of a correlation (Halpern & Haviland, 1997; Zucker & Bradley, 1995). A recent meta-analysis of 20 studies examined the rates of nonright-handedness based on sexual orientation (Lalumière et al., 2000). Overall, homosexual participants had 39% greater odds of being nonright-handed than heterosexuals, with 34% odds for men and 91% for women. As mentioned earlier, the most parsimonious neurohormonal hypothesis specifies that male homosexuality depends on low prenatal androgen action and that female homosexuality depends on high prenatal androgen action (Ellis & Ames, 1987). In terms of handedness findings, this theory would predict increased left-handedness in lesbians compared to heterosexual women and decreased left-handedness in gay men compared to heterosexual men (i.e., a sex-atypical pattern for homosexual subjects). Lesbian women demonstrated this pattern, whereas gay men did not. The fact that these data are both consistent with the neurohormonal hypothesis in women but not men, and that women show a larger effect size is suggestive of several considerations. First, there may be different influences impinging on sexual orientation in males and females (we return to this shortly). Second, given that the male data is inconsistent with the neurohormonal hypothesis as specified, alternative theories should be considered.

**Neuroanatomy**

In 1976, Dörner formulated his classic "dual mating center" theory about the role of specific brain regions in sexual behavior. Based on a rat model, Dörner implicated the medial preoptic-anterior hypothalamic
region (mPOA) as being involved in the regulation of male-typical sexual behaviors and the ventromedial nucleus in the regulation of female sexual behavior. The extremely limited research in humans on these areas and how they relate to sex and sexual orientation will be summarized below. Table 2 was created to help organize the findings and clarify that multiple terms have been used for the same structure. To help navigate the pattern of replications and failed replications, the table is laid out to describe which studies have produced positive and negative findings for sex and sexual orientation differences in each brain structure.

Allen, Hines, Shryne, and Gorski (1989) stated that the preoptic area (mPOA) is the most likely to show a sex difference in humans because this area shows the most consistently replicated sex difference in non-humans. Within the preoptic area, a particular region termed the sexually dimorphic nucleus (SDN) was found to be approximately 2.5 times larger in human men than women, and to contain 2.2 times as many

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Sexually dimorphic nucleus (SDN) = INAH-1</th>
<th>Suprachiasmatic nucleus (SCN)</th>
<th>INAH-2</th>
<th>INAH-3</th>
<th>INAH-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive Findings</td>
<td>Negative Findings</td>
<td>Positive Findings</td>
<td>Negative Findings</td>
<td>Positive Findings</td>
</tr>
<tr>
<td>Sexually dimorphic nucleus (SDN) = INAH-1</td>
<td>2 AE</td>
<td>4 DFGH</td>
<td>0</td>
<td>3 FGI</td>
<td></td>
</tr>
<tr>
<td>Suprachiasmatic nucleus (SCN)</td>
<td>0</td>
<td>2 CB</td>
<td>1</td>
<td>0 F</td>
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<tr>
<td>INAH-2</td>
<td>1</td>
<td>2 GH</td>
<td>0</td>
<td>2 GI</td>
<td></td>
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<tr>
<td>INAH-3</td>
<td>3 DGH</td>
<td>0</td>
<td>2</td>
<td>GI</td>
<td></td>
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<tr>
<td>INAH-4</td>
<td>0</td>
<td>3 DGH</td>
<td>0</td>
<td>2 GI</td>
<td></td>
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</tbody>
</table>

Note. Letter corresponds to study as listed below.
A = Swaab & Fliers (1985)
B = Swaab, Fliers & Partiman (1985)
C = Hofman et al. (1988)
D = Allen et al. (1989)
E = Hofman & Swaab (1989)
F = Swaab & Hofman (1990)
G = LeVay (1991)
H = Byne et al. (2000)
I = Byne et al. (2001)
cells (Hofman & Swaab, 1989; Swaab & Fliers, 1985). A difference based on sexual orientation was investigated in a sample of 34 subjects who varied on sex, sexual orientation, and AIDS serostatus (Swaab & Hofman, 1990). Using morphometric analysis of the SDN, no difference in either volume or cell number based on sexual orientation was found. However, another hypothalamic nucleus located in the immediate vicinity of the SDN, the suprachiasmatic nucleus (SCN), was found to be 1.7 times larger in gay versus heterosexual men, and contained 2.1 times as many cells. The fact that the SDN was not found to be associated with sexual orientation is inconsistent with Dörner's hypothesis (1976). The fact that a difference was found in the SCN, a nucleus not known to be sexually dimorphic (Hofman, Fliers, Goudsmit, Swaab, & Partiman, 1988; Swaab, Fliers, & Partiman, 1985), makes interpretation of these data difficult without replication.

Other regions in the preoptic area that have been found to be sexually dimorphic are the second and third interstitial nuclei of the anterior hypothalamic (INAH) (Allen et al., 1989). Allen et al. argued that the region labeled INAH-1 most likely corresponds to the SDN identified by Swaab and Fliers (1985), but methodological differences accounted for the lack of sexual dimorphism in their research. Specifically, Swaab and Fliers (1985) found an interaction between sex and age for the INAH-1 region, introducing the possibility that age differences between the two samples may explain the inconsistent results. However, more recent researchers have failed to replicate this interaction effect, while replicating the sex difference in the INAH-3 region (Byne et al., 2000). Additionally, cytoarchitectonic comparisons between the INAH-3 region in humans with similar hypothalamic structures in monkeys and rats suggest that it may be the best candidate for homology with the mPOA (Byne, 1998).

Based on the reported sex differences in INAH-2 and INAH-3 (Allen et al., 1989), LeVay (1991) measured these regions in the autopsied brains of 19 gay men who died of AIDS, 16 presumably heterosexual men (6 died of AIDS), and 6 presumably heterosexual women (1 had died of AIDS). A heterosexual orientation was presumed in those brains that were not specifically provided by deceased individuals who were known to be gay based on medical records. In this sample, no sex difference in the INAH-1, INAH-2, and INAH-4 was observed. As shown in Table 2, the lack of a sex differences in INAH-1 and INAH-4 is consistent with other studies (Allen et al., 1989; Byne et al., 2000), whereas the lack of a difference in INAH-2 is consistent with the findings of some researchers (Byne et al., 2000), but not others (Allen et al., 1989). The finding of a larger INAH-3 in males compared to females is consis-
tent with all previous studies (Allen et al., 1989; Byne et al., 2000), making it the most theoretically relevant location to look for a sexual orientation difference. This study found a comparable cell volume in the INAH-3 between heterosexual women and gay men, both of which were significantly smaller than the heterosexual men (LeVay, 1991). Comparisons that only included the heterosexual participants who died of AIDS \( n = 6 \) were robust in producing the effect. Recently, Byne et al. (2001) reported a nonsignificant trend toward INAH-3 occupying a smaller volume in gay men \( N = 14 \) than in heterosexual men \( N = 34 \), with no difference in the number of neurons within the nucleus. None of these studies have included lesbian women.

Although no researchers have explored sexual orientation effects in the anatomy of the ventromedial nucleus, the hypothalamic region posited by Dörner (1976) to be related to female sexual behavior, two investigators have explored the anterior commissure (AC). The AC is a fibrous tract located within the hypothalamus connecting the two hemispheres of the brain. In one study, Demeter, Ringo, and Doty (1988) found the cross-sectional area of this tract to be larger in men. In two studies Allen and Gorski (1991, 1992) found it to be larger in women. Lasco, Jordan, Edgar, Petito, and Byne (2002) found no difference and Highley et al. (1999) found no difference in area but did find differences in fiber number and type. The putative increased size of the AC in females has been posited as an explanation for the modest decrease in functional cerebral lateralization (FCL) possibly found in women (Allen & Gorski, 1991). Some evidence for sexual orientation differences in FCL has been shown to exist. Thus, research on this structure seems theoretically appropriate. Allen and Gorski (1992) examined the ACs of 30 gay men, 30 presumably heterosexual men, and 30 presumably heterosexual women, matched for age. The gay men in their sample had ACs that were 34% larger than those of the heterosexual men. Analyses suggested no significant difference between men who did and did not die of AIDS. Lasco et al. (2002), however, failed to find any variation in the size of the AC with sexual orientation in a sample of 120 participants.

Taken together, the results of these studies suggest that brain differences may be related to sexual orientation and implicate brain centers both believed (POA/INAH) and unproven (SCN, AC) to influence sexual behavior. As shown in Table 2, despite some variability in the evidence supporting the existence of sex differences in various hypothalamic nuclei, one consistent finding emerges from these studies: The region that consistently evidences a sex difference, the INAH-3, also shows the best evidence for a sexual orientation effect. One limitation of these studies is the subjective nature of identifying the borders between brain
regions. The fact that in all studies raters blind to the sexual orientation of their subjects were used helps to prevent systematic rater biases. Some studies also included multiple independent raters of the traces of relevant regions, an important step when relying on a subjective measure. Independent replication is essential before any of these findings can be accepted, especially because initial positive reports are likely come from studies in which many areas were examined and several statistical tests were performed on nonindependent measures, usually without a correction for the number of comparisons. Replication is especially important for those areas where there is little reason, a priori, to expect a sexual orientation effect because no sex difference exists (e.g., SCN & AC). Another major criticism is the potential confound of AIDS serostatus as reductions in testosterone levels have been documented in late-stage HIV infections (Byne & Parsons, 1993). The fact that each of these studies included HIV positive heterosexual male controls, and produced no evidence of AIDS having an effect on the results, reduces but does not eliminate this criticism, because different disease courses could vary by sexual orientation. A final limitation of these studies is that they have not included lesbian women.

Otoacoustic Emissions

Click-evoked otoacoustic emissions (CEOAEs) are echo-like waveforms that are emitted by the inner ear in response to brief sounds (McFadden & Pasanen, 1998). CEOAEs show individual differences, are highly heritable, and are generally stable throughout life (McFadden & Pasanen, 1998). Support for a hormonal role in CEOAEs comes from two lines of evidence. First, sex differences exist in both newborns and adults, with women having stronger CEOAEs and both sexes experiencing increased magnitude in the right ear (see McFadden, 2002 for a review). Second, McFadden, Loehlin, and Pasanen, (1996) reported that female dizygotic twins with male cotwins had CEOAEs that are more typical of men. In lower animals, females with male litter mates may be exposed to increased levels of androgens via diffusion across the amniotic fluids (vom Saal, 1989). Evidence for cross-twin hormone exposure in human twin studies is mixed (e.g. Loehlin et al., 2000), but the existence of sex differences does lend support to the hypothesis that androgens may play a role in the development of these emissions. Research examining the CEOAEs of women whose mothers took DES and females affected with CAH is currently underway to further investigate the association between prenatal hormones and CEOAEs (McFadden, 2002).

In a sample of 237 subjects, McFadden and Pasanen (1998) replicated the previously mentioned sex and ear differences. In comparisons based
on sexual orientation, no difference was found in men. For women, a main effect for sexual orientation was demonstrated, with lesbian and bisexual women emitting lower (i.e., more male typical) CEOAEs than heterosexual women. Researchers exploring brain waves in response to click stimuli, called auditory evoked potentials (AEPs), have found similar male-typical results in women (McFadden & Champlin, 2000). For men, these results are less clear, as the nonheterosexual men were shifted even further from the heterosexual women than were the heterosexual men, suggesting to the authors evidence for hypermasculinization of nonheterosexual men and women. McFadden and Pasanen proposed that the most parsimonious and theoretically supported explanation for the above results is a graded progression of exposure to prenatal androgens that influences both sexual orientation and the peripheral auditory system. Sexual orientation differences in behavior that could have confounded the results (e.g., gay men may be more likely to attend loud concerts than heterosexual men) were tested for but are not supported by the currently available evidence (McFadden, 2002). Replications by independent laboratories are needed to substantiate these results.

Anthropometrics

Several anthropometric characteristics have been explored in relation to sexual orientation: finger length, dermatoglyphics, weight, height, body morphology, and penis length. The relation between sexual orientation and these characteristics is hypothesized to be the result of nonspecific effects of prenatal sex-atypical hormonal action. If sexual orientation is related to perturbations in prenatal hormone environments, the effects of these perturbations should be observable in sexually dimorphic traits other than sexual orientation (Money, 1987).

Several lines of evidence suggest that finger length ratio may be a window into the prenatal hormone milieu. The trait is sexually dimorphic; in women the index finger (2D) is nearly equal in length to the ring finger (4D), whereas in men 2D is shorter on average than 4D, and this ratio is established by 2 years of age (Manning, Scutt, Wilson, & Lewis-Jones. 1998). The sex difference in finger length ratio is small, however, approximately one-third of a standard deviation (Williams et al., 2000). According to Williams et al. (2000, p. 445) “because all nongonadal somatic sex differences in humans appear to be the result of fetal androgens masculinizing males (Breedlove, Cooke, & Jordan, 1999), the sex difference in 2D:4D probably reflects the prenatal influence of androgens on males.” Based on this idea, smaller 2D:4D ratios are believed to index greater levels of prenatal androgen exposure. Indeed, the 2D:4D ratio is negatively correlated with adult androgen levels.
(Manning et al., 1998), but the relationship between prenatal and adult androgen levels is unknown at this time. Additionally, differences have been found in finger length ratios in lesbians based on self-identification as "butch" or "femme" (Brown, Finn, Cooke, & Breedlove, 2001), a dichotomy with some evidence of hormonal underpinnings (Singh et al., 1999). Perhaps the strongest evidence that finger length ratios are indicative of prenatal androgen levels comes from a recent study reporting that the 2D:4D ratios in children with CAH were smaller than control subjects, regardless of sex (Brown, Hines, Fane, & Breedlove, 2001).

In a sample of 720 adults attending a public street fair in San Francisco, Williams and colleagues (2000) replicated the higher 2D:4D ratio in women, with the additional observation that the sex difference was greater on the right hand. The authors interpreted this as evidence that the right-hand 2D:4D ratio is more sensitive to fetal androgens than the left. Analyses of these data indicated that the right-hand 2D:4D ratio was significantly smaller in lesbian women (i.e., more masculine) compared to heterosexual women, and not significantly different from that of heterosexual men. The 2D:4D ratio was not significantly different, based on sexual orientation, in men for either hand. However, when the sample was segregated into gay men with and without older brothers, gay men with older brothers had smaller 2D:4D finger ratios (i.e., more masculine). Robinson and Manning (2000) reported lower 2D:4D ratios in a sample of gay men, independent of the number of older brothers. In the largest sample to date (N = 2,000), Lippa (2002) reported significantly more feminine 2D:4D ratios among gay men, a finding in conflict with earlier reports. This finding was also robust against ethnic group differences, a factor known to influence this ratio (Robinson & Manning, 2000). Lippa found no significant difference between lesbian and heterosexual women after controlling for ethnicity. Considering these conflicting results, further research in this area is needed before it will be possible to evaluate its support for the neurohormonal hypothesis. In such research, investigators should make sure to collect a large and ethnically diverse enough sample so that the proven effects of ethnicity on 2D:4D ratio can be controlled. As noted by Lippa, without such controls, difference in ethnic representation across samples could induce spurious results.

Another anthropometric phenomenon that has been studied in relation to sexual orientation is dermatoglyphic asymmetry. Skin ridges, or dermatoglyphics, are found on the palms and soles of all primates, and in humans, are determined between the 8th and 16th week of fetal life (Holt, 1968). After birth, the ridge patterns are not affected by development or the environment, and can only be altered by severe mechanical damage (Cummins & Midlo, 1961), making them a good source of infor-
mation about the timing of prenatal events correlated with their eventual pattern (Mustanski et al., 2002).

In order for dermatoglyphics to be relevant to the neurohormonal hypothesis, their development must be related to prenatal androgens. Jamison, Jamison, and Meier (1994) suggested a mechanism whereby prenatal testosterone might influence dermatoglyphic variation via its relationship with nerve growth factor and epidermal growth factor. Empirical evidence based on studies of nonhuman and human primates provide moderate support for an association between androgens and dermatoglyphic patterns (see Mustanski et al., 2002, for a review).

In a sample of 182 heterosexual men and 66 gay men, Hall and Kimura (1994) reported a significantly greater incidence of leftward asymmetry in gay men, defined as two or more ridges on the left hand. In two more recent studies of the same size or larger, this effect was not replicated (El-Hani et al., in press; Mustanski et al., 2002). In addition, no difference was found for total ridge count or continuous leftward asymmetry (the continuous counterpart of the arbitrary dichotomization used by Hall & Kimura). Several researchers have also explored this phenomenon in transsexuals (Green & Young, 2000; Slabbekoorn, van Goozen, Sanders, Gooren, & Cohen-Kettenis, 2000) and twins (Hall, 2000a, 2000b) with conflicting results. As discussed by Mustanski et al. (2002), large-scale studies that either employed less-reliable dichotomizations of the dependent measure, or reported multiple tests, were the only ones to return positive results. Those studies in which the more reliable and statistically appropriate continuous measure was employed, and in which multiple tests on the data were not conducted, consistently produced no association between sexual orientation and dermatoglyphics (El-Hani et al., in press; Mustanski et al., 2002; Slabbekoorn et al., 2000).

Weight and height, additional sources of anthropometric data, are sex-dimorphic somatic characteristics with men, on average, being taller and heavier than women. Differences between heterosexual and homosexual men and women on these somatic traits could be, under a biological model, attributable to sex-atypical organization of sex-dimorphic brain structures responsible for physical growth (i.e., hypothalamic-pituitary-gonadal axis) (Blanchard & Bogaert, 1996a). In the past, this literature has been problematic to interpret because of small sample sizes, lack of control of confounding variables (e.g., controlling for age and height when examining difference in weight), and the failure to test a directional hypothesis adequately (Blanchard & Bogaert, 1996a). The results of three studies designed to address these factors showed gay men reported being lighter and shorter, compared to heterosexual
men (Blanchard & Bogaert, 1996; Blanchard, Dickey, & Jones, 1995; Bogaert & Blanchard, 1996a), whereas in others with adequate sample sizes no weight (Siever, 1994; Yager, Kurtzman, Landsverk & Wiesmeier, 1988) or height differences (Evans, 1972) have been found.

Studies examining height and weight differences in lesbian and heterosexual women are less numerous. In his analysis of 275 lesbian and 5,201 heterosexual women’s case histories recorded by the Kinsey Institute, Bogaert (1998) showed that lesbians reported being, on average, heavier and taller than heterosexual women did. The weight difference reported by Bogaert replicated differences reported by other researchers (see Bogaert, 1998, for a review). The difference in height reported by Bogaert contradicts that of Perkins (1981), who reported no significant differences between a large sample of lesbian women and population norms.

Factors other than those that are neurohormonal in origin may account for the differences observed in weight. For example, gay men may be relatively more concerned about slimness compared to heterosexual men and thus report being lighter (a similar argument could be made for heterosexual women compared to lesbian women). However, there is little evidence to support these hypotheses (Bogaert, 1998; Bogaert & Blanchard, 1996). One large methodological weakness in this literature is the use of self-reported weight in these studies; objective weight measurement is preferable to eliminate systematic reporting biases.

Examining differences in height as an index of somatic sex-atypicality is particularly appealing because, unlike weight, final adult height is unlikely to be influenced by environmental, psychological, or medical factors (Bogaert & Blanchard, 1996). The differences in height reported in the most rigorous studies to date are small; 1.5 cm for men (adjusted for maternal and paternal height) (Bogaert & Blanchard, 1996), and 89 mm for women (Bogaert, 1998). In both studies analyses were based on self-reported height, reducing the reliability of the values. Again, replication of these effects must be attempted using objective morphometric measures rather than collecting data through self-report.

Related to the issue of weight and height is the study of body morphology. In females, the pubertal surge in estrogens enlarges the pelvis and facilitates fat deposits in the buttocks and thighs. This, in combination with the inhibition of fat deposition in the abdomen, results in a waist-to-hip ratio (WHR) in the range of .67-.80 (Singh, 1993). In men, the pubertal increase in androgen production facilitates fat deposition in a fashion nearly opposite to women, resulting in a WHR in the range of .85-.95 (Singh, 1995). In the only study to-date exploring WHR and sexual orientation, self-described “butch” and “femme” lesbians were compared to each other and a sample of heterosexual women (Singh et al.,

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WHR was measured by the participants based on instructions provided by the researchers. Butch lesbians were found to have higher (i.e., more masculine) WHR than either the femme lesbians or heterosexual women, even after controlling for age and body mass differences. After rejecting lifestyle choices as an explanation for these results, Singh et al. (1999) concluded that the evidence suggests differences in the current hormonal milieu by sexual orientation and degree of masculinity.

The rationale for examining pubertal timing in relation to sexual orientation is similar to that cited for anthropometric measures; sex differences in pubertal onset are evident, with boys reaching puberty later than girls (Reiter & Rosenfeld, 1998). Gay men would resemble heterosexual women (i.e., reach puberty earlier than heterosexual men) because relevant sex-dimorphic brain structures involved in the timing and regulation of puberty would be shifted toward a female-typical pattern (Blanchard & Bogaert, 1996a). Similarly, lesbians would resemble heterosexual men, reaching puberty later than heterosexual women (Bogaert, 1998). Based on multiple physical indices of puberty, (i.e., first ejaculation, pubic hair) gay men have been shown to reach puberty earlier than heterosexual men (Blanchard & Bogaert, 1996a; Bogaert & Blanchard, 1996; Kinsey, Pomeroy, & Martin, 1948) and researchers using behavioral indices of puberty have found similar results (see Bogaert & Blanchard, 1996, for a review). Conversely, using physiological markers for onset of puberty (i.e., age of first menarche), Bell, Weinberg, and Hammersmith (1981), Tenhula and Bailey (1998), and Bogaert (1998) reported no significant difference in pubertal onset between lesbian and heterosexual women. Pubertal timing is measured via retrospective reports of discrete physiological events, but puberty is not a discrete event, it is a series of ongoing physical changes. Therefore, these results should be regarded with some skepticism. Furthermore, it is possible that men's recall of puberty is less reliable than women's because men have no significant discrete event to recall, whereas women do (i.e., menarche).

Putative early androgen differences between gay and heterosexual men could cause subtle differences in penile differentiation, and, thus, penis size and sexual orientation has been examined in two studies. Bogaert and Hershberger (1999) and Nedoma and Freund (1961) have both reported gay men to have larger penises than heterosexual men, both in erect length (Cohen's d = .41 and .78, respectively) and circumference. Although penile length and circumference were self-assessed in Bogaert and Hershberger's sample, their results replicate those of Nedoma and Freund, in which penile measurements were recorded by the researchers. Additionally, Bogaert and Hershberger's sample was
large \( n = 813 \) and \( n = 3,417 \) for gay and heterosexual men, respectively) and analyses included controls for differences in education, height, and weight between gay and heterosexual men. These results have yet to be replicated in a representative sample using objective measures.

The evidence concerning the neurohormonal hypothesis is extremely heterogeneous in several respects. In synthesizing this body of data, it is important to take into consideration that some data are more directly relevant to the hypothesis than other data. For example, the outcome of genetic males with cloacal extrophy who have been reared as girls is almost an ideal test of the neurohormonal hypothesis. In contrast, the ontogeny of other biological markers is not well understood and is imperfectly related to prenatal androgen level. Thus, the rather uniform findings for the cloacal cases, which favor the neurohormonal hypothesis, are more important than the mixed findings for other traits. With this consideration in mind, we review the consistency of different research areas related to the neurohormonal hypothesis.

Three research areas have produced consistent results upon independent replication or meta-analysis: the clinical evidence, pubertal development in men, and FCA/handedness. Within these domains, both the clinical and pubertal development data are supportive of the neurohormonal hypothesis. The recent meta-analysis of the handedness data (Lalumière et al., 2000) produced results that are incompatible for males with the theory as described by Ellis and Ames (1987). The results for females are compatible with the theory. Evidence consistent with the INAH-3 differences described by LeVay (1991) has been reported in one study (Byne et al., 2001), and these results are compatible with the neurohormonal theory. Independent replications have yet to be published for some indices (otocoustic emissions and body morphology), whereas for other areas replication has produced mixed results (prenatal stress, dermatoglyphics, height, weight, and finger length). As in other areas of science, two factors will likely lead to greater understanding of the role hormones play in sexual orientation. Replication by independent laboratories will lead to a clearer picture of the relationship between sexual orientation and many of these putative biological markers of prenatal hormone status. Additionally, technological advances in behavioral endocrinology may allow more powerful tools to be used in the investigation of target tissue hormone sensitivity in humans. Until that time it is difficult to make any definitive conclusions about the soundness of the neurohormonal hypothesis.

**Genetic Influences**

Research hypothesizing genetic influences on sexual orientation
began over 60 years ago, motivated by the idea that gay men are genetically female (Lang, 1937, 1940). Techniques for identifying the sex chromosomes discredited this hypothesis (Pare, 1956). Without evidence for clear chromosomal abnormalities among homosexual subjects, researchers turned to three other methods for exploring the genetic nature of sexual orientation: family studies, to explore the frequency and pattern of the familiality of homosexuality; twin and adoption studies, to partition the population variance in sexual orientation into genetic and environmental components; and molecular genetics studies, to identify specific genes that influence an individual's sexual orientation.

**Family Studies**

Most family studies of sexual orientation have recruited male and female probands through newspaper advertisements that do not mention the nature of the study (Bailey & Bell, 1993; Bailey & Benishay, 1993; Bailey & Pillard, 1991; Bailey, Pillard, Neale, & Agyei, 1993; Bailey et al., 1991; Dawood, Pillard, Horvath, Revelle, & Bailey, 2000; Pillard, 1990; Pillard & Weinrich, 1986). Bailey et al. (1999) used more assiduous ascertainment of two samples, one recruited through consecutive admissions at an HIV outpatient center and the other from a gay pride festival. Such recruitment decreases the chance that siblings will self-select for participation based on their concordance for homosexuality. The median rate of homosexuality in brothers of gay men is approximately 9% (reviewed in Bailey & Pillard, 1995), a figure well above recent population base rates of homosexuality (Laumann, Gagnon, Michael, & Michaels, 1994). Female homosexuality also appears to run in families, although estimates of homosexuality among sisters of lesbians vary widely, ranging from 6% to 25% (Bailey & Pillard, 1995). Although both male and female homosexuality appears to run in families, it remains unclear whether it runs in the same families. When comparing across studies, there is modest evidence that opposite-sex siblings of homosexual probands (i.e., sisters of gay men and brothers of lesbians) have increased rates of homosexuality compared with controls, although statistical genetic techniques have not been utilized to provide a more definitive answer to this question. One major limitation of these family studies is that often the rates of homosexuality are based on the subject's ratings of their siblings' sexual orientation. As discussed later, relying on subjects to identify the sexual orientation of their family members is likely to introduce significant error into the study (see Kirk, Bailey, & Martin, 1999 for discussion).

**Twin Studies**

Family studies cannot disentangle whether a trait's familiality is due
to genetic or common environmental effects because siblings do not just share common genes but usually also share their rearing environment. Twin studies allow the variance in a trait within a population to be partitioned into heritable effects and influences explained by the shared and nonshared sibling environment. Heritability refers to the proportion of variance in a trait within the population that is due to genetic effects. Shared environmental effects, as defined in twin studies, refer to environmental influences that make siblings more similar to each other. Nonshared environmental influences are factors that tend to make siblings different from each other. For a thorough review of these concepts see Turkheimer and Waldron (2000).

The earliest twin studies of sexual orientation reported extraordinarily high heritability estimates (Kallmann, 1952). This and other early studies have been criticized because of their reliance on mentally ill gay men, poor zygosity determination, unusually high MZ concordance rates, and possible ascertainment bias toward concordance (e.g., Rosenthal, 1970). Recent samples of twins ascertained via advertisements in homophile publications or via moderate-sized twin registries suggest that the familial nature of homosexuality is due largely to genetic influences (Bailey & Pillard, 1991; Bailey et al., 1993; Buhrich, Bailey, & Martin, 1991; Whitam, Diamond, & Martin, 1993, but see also King & McDonald, 1992). It is important to note, however, that the validity of these studies is questionable for reasons related to sampling. Identifying participants through homophile publications and word-of-mouth may result in a concordance-dependent ascertainment bias for twins concordant for homosexuality (Kendler & Eaves, 1989). This bias will not likely result in inflated heritability estimates, however. Heritability estimates would be inflated if MZ twins from concordant pairs were more likely to be ascertained than DZ twins from concordant pairs. There is no evidence that this occurred in any study, although the possibility cannot be excluded (Bailey & Pillard, 1995).

All studies before 1991 and by Whitam et al. (1993) only report concordance rates by zygosity. More sophisticated quantitative genetic techniques, which use structural equation modeling with model fit based on maximum-likelihood, allow much more precise estimation of the various sources of variance and also allow significance testing on each parameter (Neale & Cardon, 1992). Additionally, multivariate models have also been used to explore the source of covariation between sexual orientation and related traits (i.e., sex-dimorphic behaviors) while increasing the power of the parameter estimates.

Table 3 contains recent twin studies that have employed genetic modeling techniques to estimate the proportion of variance in sexual orien-
tation due to heritable, as well as environmental sources of variation. The table also shows the method of twin ascertainment and how the dependent variables were measured. One study reported by Hershberger (1997) is not included in the table because an atypical sampling strategy, involving recruiting twins from a volunteer twin registry based on their concordance for being married or never-married, makes it difficult to interpret the parameter estimates. The studies reported by Bailey, Dunne, and Martin, (2000), Kirk, Bailey, Dunne, and Martin, (2000), and Kendler, Thornton, Gilman, and Kessler (2000) deserve special attention because of their population-based ascertainment strategies. Population-based samples of twins overcome many of the selection biases endemic to the previously cited literature. The first of two studies based on the large population-based sample of Australian twins is unique because both univariate estimates of sexual orientation and two covariates, childhood gender nonconformity (CGN) and continuous gender identity (CGI), are included along with a multivariate model incorporating all three (Bailey, Dunne, & Martin, 2000). Univariate analyses of the sexual orientation variable resulted in evidence for familial influences, but genetic and common environmental contributions could not be disentangled without the addition of the previously mentioned covariates. The results of multivariate analyses suggested significant and moderate genetic and nonshared environmental contributions for sexual orientation and its covariates. The second report based on the Australian twin data included a multivariate model that incorporated measures of both behavioral and psychological sexual orientation (Kirk et al., 2000). Significant heritable and nonshared environmental influences were reported for the latent sexual orientation variable indicated by multiple measures of sexual orientation. Kendler et al. (2000) also used a population-based sample, but, because of the low prevalence of nonheterosexuality, they had insufficient power to separately model the data for males and females. Qualitative comparisons of their data indicated that concordance rates were higher in the female-female pairs than the male-male pairs, but the data were not reported in a quantitative fashion. Together, these studies suggest the existence of both genetic and nonshared environmental influences on sexual orientation. As seen in Table 3, and based on the preponderance of evidence, it also appears that genes influence sexual orientation more strongly in males than females.

**Molecular Studies**

In an effort to identify particular genes that contribute to the heritable nature of sexual orientation, a limited number of both linkage and
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<th>Study</th>
<th>Ascertainment</th>
<th>Measurement</th>
<th>N (pairs)</th>
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<td><strong>Male Studies</strong></td>
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<td>Buhrich et al. (1991)a</td>
<td>Australia twin registry</td>
<td>Univariate model of ASO</td>
<td>161</td>
<td>.14</td>
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<td>Multivariate independent factors model including variables of child and adult gender identity. Estimate of ASO</td>
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<td>Bailey &amp; Pillard (1991)</td>
<td>Advertisements</td>
<td>ASO under a range of assumptions about the likelihood of ascertaining a cotwin based on their sexual orientation, and the base rate of homosexuality.</td>
<td>161</td>
<td>.31-.74</td>
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<td><strong>Female Studies</strong></td>
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<td>Bailey et al. (1993)</td>
<td>Advertisements</td>
<td>ASO under a range of assumptions about the likelihood of ascertaining a cotwin based on their sexual orientation, and the base rate of homosexuality.</td>
<td>147</td>
<td>.27-.76</td>
<td>.00-.23</td>
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<td><strong>Combined Male and Female</strong></td>
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<td>Australian Twin Registry</td>
<td>Univariate model of ASO- male</td>
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<td>Univariate mode of ASO- female</td>
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<td>Multivariate common factors model including variables of child and adult gender identity. Estimate of ASO</td>
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</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.51</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.23</td>
</tr>
</tbody>
</table>

(Continued)
Table 3 (continued)

Studies Modeling the Percentage of Variance in Sexual Orientation Attributable to Genetic and Environmental Effects, With 95% Confidence Intervals When Available

<table>
<thead>
<tr>
<th>Study</th>
<th>Ascertainment</th>
<th>Measurement</th>
<th>N (pairs)</th>
<th>$h^2$</th>
<th>$c^2$</th>
<th>$e^2$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirk et al.</td>
<td>Same as above</td>
<td>Multivariate common factors model including ratings of sexual feelings, attitude, and number of partners. Estimate of latent homosexuality variable.</td>
<td>1405</td>
<td>.58</td>
<td>.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2000)a</td>
<td></td>
<td>Male</td>
<td>.48-.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.15-.46)</td>
<td>(.31-.52)</td>
<td>.71</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kendler et al.</td>
<td>National Random Digit Dialing</td>
<td>Self reported sexual orientation</td>
<td>564</td>
<td>.62</td>
<td>.05</td>
<td></td>
<td>.33</td>
</tr>
</tbody>
</table>

Note. $h^2$ = heritability, $c^2$ = shared environment, $e^2$ = nonshared environment, $r^2$ = residual variance in multivariate models not due to variable reported. ASO = Adult Sexual Orientation.

* Indicates that the percentage of variance was calculated for this table based on the data provided in the original article.
association studies have been conducted. Linkage studies look for specific chromosomal regions that are passed down in families, along with a phenotype (i.e., sexual orientation) at probability levels greater than chance (50% for siblings). Association studies look at the relationship between variation at a specific locus of a gene, and phenotypic variation. Hamer, Hu, Magnuson, Hu, and Pattatucci (1993a) reported the first linkage study in 40 families of gay men. In the initial pedigree analysis, the researchers noted increased rates of homosexuality in maternal male relatives. This result implicates the X chromosome because, except in very unusual cases, a male inherits his X chromosome from his mother. Using a genetic linkage methodology, Hamer and colleagues examined 22 DNA markers on the X chromosome. Thirty-three of 40 pairs of gay brothers shared chromosomal region Xq28, which is significantly different from the expected number of 20 (50%) (Hamer et al., 1993a).

The results of the Hamer et al. (1993a) study were questioned, however. Risch, Squires-Wheeler, and Keats (1993) argued that increased rates of gay maternal relatives could simply be due to the decreased rates of reproduction among gay men. In other words, a gay gene is unlikely to be inherited from a gay father, because a gay man is unlikely to have children in the first place. Secondly, Risch et al. disputed Hamer et al.'s (1993a) calculation of the prevalence of gay first-degree family members relative to the general population rate. Although this parameter, \( \lambda_s \), has never been estimated in an epidemiologically valid manner, Risch et al. correctly noted that the larger it is estimated to be, the more significant the results produced by Hamer et al. (1993a). Hamer and his colleagues (1993b) responded to many of these criticisms and concluded that, as with many scientific findings, replication will be the ultimate arbiter.

Three attempted replications of this linkage study have been reported. Hu et al. (1995) reported a successful replication of their earlier Xq28 results and extended the study to include heterosexual brothers. Their report of a 22% Xq28 sharing rate in brothers discordant for sexual orientation was significantly less than the 50% sharing expected by chance. This value, in addition to the 67% marker sharing for the Xq28 region among concordant brothers, represents a positive replication of the earlier results. The study also included 36 pairs of lesbian sisters but no evidence was found for excess marker sharing. A 66% sharing level for Xq28 was also found in an independent sample of 54 pairs of gay brothers (Sanders et al., 1998 [as cited in Hamer, 1999]). However, Rice, Anderson, Risch, and Ebers (1999) report a failed replication of linkage to Xq28 in a sample of 52 gay male sibling pairs from
Canadian families. Unlike Hamer's group, however, they did not select for families consistent with maternal transmission, thus decreasing the likelihood of finding X-chromosome linkage. Further discussion of the methodological issues that may explain the discrepancies in the results, as well as a meta-analysis of results to date, can be found in Bailey (1995), Hamer (1999), and Rice, Risch, and Ebers (1999).

In one candidate gene association study of male sexual orientation, DNA sequence variation in the androgen receptor gene has been explored (Macke et al., 1993). The selection of this gene was based on neurohormonal evidence implicating prenatal androgen levels in determining sexual orientation. No significant differences in the distribution of mutations based on sexual orientation were observed. An additional linkage analysis was performed, demonstrating that sibling pairs concordant for homosexuality did not show increased sharing of specific androgen receptor alleles.

If the previously cited twin studies are correct in that genetic influences do explain a portion of the variance in sexual orientation, then eventually molecular techniques should allow for the identification of specific genes. Recent advances in molecular genetics, along with the added markers provided by the Human Genome Project, should facilitate this search. Notwithstanding these advances, a number of factors limit the applicability of traditional molecular genetic research methodologies for research on complex traits like sexual orientation and other behavioral phenotypes.

First, principles of genetic epidemiology must be considered, such as the fact that familial recurrence patterns of homosexuality are not attributable to common major genes (Bailey & Bell, 1993; Bailey et al., 2000; Bailey & Pillard, 1991; Bailey et al., 1999; Bailey et al., 1993). If sexual orientation is polygenic, many genes exerting a small effect, they will be difficult to identify because of low power (Stoltenberg & Burmeister, 2000).

Second, behavior genetic analyses have found that nonshared environmental influences do play a role in sexual orientation; such factors have not been specifically identified by including them in biometric twin models. The relationship between environmental and genetic factors has also not yet been explicated. Gene X environment interactions are just one likely possibility; in this case the magnitude of genetic effects vary depending on the environment in which they are expressed. For example, genes that predispose toward heterosexuality can only be active in environments in which opposite sex individuals are present. It is also unclear whether nongenetic etiological factors are capable of producing a homosexual orientation on their own. In other words, can envi-
Environmental influences produce homosexuality independently of genetic factors (when this occurs in a trait known to have genetic influences it is known as a phenocopy)? If the answer is yes, the potential for environmentally produced phenocopies exist and, therefore, potentially confound molecular genetic research on this phenotype. Phenocopies reduce the power to detect linkage because, from a genetics perspective, environmentally produced phenotypes are false positives.

Third, traditional approaches to linkage are limited with behavioral phenotypes because of the possibility that some combinations of dispositional genes will not produce effects sufficient to cause the expression of the complete phenotype. In terms of genetic research on sexual orientation, this means that pedigree members may possess dispositional genes in subthreshold numbers, causing them not to express the full homosexual phenotype. If sexual orientation were dichotomized, then such individuals would be labeled heterosexual and would be false negatives from a genetic perspective. Researchers have thus far largely avoided this issue by using “affected” sib-pair designs that only include sib-pairs in which both are gay, but these approaches lose power because the whole pedigree cannot be included and dichotomizations can surrender a lot of information. One partial solution to these problems would be to identify indicators of processes that mediate between dispositional genes and the sexual orientation phenotype. Such factors have been termed “endophenotypes” and are considered more sensitive markers of underlying liability status (Gottesman, McGruff, & Farmer, 1987). Endophenotypes are selected for being closer to the mechanism of gene action than most qualitative categories and thus should be more sensitive to degree of genetic liability among both affected and nonaffected pedigree members. Therefore, endophenotypes would increase power by allowing the inclusion of all pedigree members and also by avoiding the need for dichotomization. Linkage studies of alcoholism that have included both traditional diagnostic information (i.e., DSM diagnosis) and an endophenotype (i.e., P300 amplitude) have provided evidence for the usefulness of such techniques (Lunetta, Wilcox, Smoller, & Neuberg, 1999). Bailey et al. (2000) have suggested childhood gender nonconformity and its adult form, continuous gender identity, as psychometric (meaning not assessed using a biological measure) endophenotypes for sexual orientation. An explicit examination of whether these traits meet the requirements for an endophenotype, as defined by Hesselbrock, Begleiter, Porjesz, O'Connor, and Bauer (2001), has yet to be provided.

Despite all of the limitations mentioned above, the intense interest in uncovering specific dispositional genes for homosexuality will likely lead to additional research efforts. Such research will be aided by the
identification of new markers and genetic techniques identified through the Human Genome Project. The payoff for finding such genes is enormous, but not for social or moral reasons, as we believe such thinking is simpleminded (see Greenberg & Bailey, 1993 for a discussion). Instead, locating such genes will be a large step forward in identifying the more proximal mechanisms through which sexual orientation is determined.

**Fraternal Birth-Order Effect**

Perhaps the most replicated finding in sexual orientation research is that gay men are born later in their sibships, compared with heterosexual men. Furthermore, recent studies have clarified that this effect is primarily due to older brothers, who increase the probability of homosexuality in later-born males (Blanchard, 1997). In at least 14 samples, gay men had significantly more older brothers compared with heterosexual men but did not have a greater number of older sisters, once the number of older brothers has been controlled (see Blanchard, 2001, for a review). This *fraternal birth-order effect* has been shown to be robust beyond gay men attracted to other adult men. The probability of genetic males being attracted to men increases with the number of older brothers in samples of male-to-female transsexuals (Blanchard, Zucker, Cohen-Kettenis, Gooren, & Bailey, 1996; Green, 2000), pedophiles (Blanchard et al., 2000; Bogaert, Bezeau, Kuban, & Blanchard, 1997), and nonpedophilic sex offenders (Blanchard & Bogaert, 1998). The magnitude of this effect may be larger in markedly feminine gay men (Blanchard & Scheridan, 1992). Despite several studies on women, no consistent tendency toward early or late births or atypical sibling sex ratios has been detected (Blanchard, 1997).

Based on statistical analyses of selected samples, Cantor, Blanchard, Paterson, and Bogaert (2002) estimated that between 14.8% and 15.2% of gay men can attribute their homosexuality to the fraternal birth-order effect, based on population base rates of homosexuality of 1% and 4% respectively. Additional statistical evidence suggests that each additional older brother increases the odds of same-sex attraction by 33% (Blanchard & Bogaert, 1996b).

Several hypothetical mechanisms have been posited to explain the fraternal birth-order effect, some psychological and some biological. As the focus of this paper is on biological influences, readers interested in putative psychosocial explanations for this effect should consult Blanchard's (1997) extensive review. In terms of biological theories, traditional genetics cannot explain these results because purely genetic phenomena do not show birth-order effects. Some researchers have suggested the birth-order effect may be an artifact of advanced maternal or paternal age, which then results in increased gametic mutations (Raschka, 1995). Such explana-
tions have been ruled out by multivariate analyses demonstrating the robustness of the effect after controlling for maternal and paternal age (Blanchard & Bogaert, 1996b). Blanchard and Bogaert have hypothesized that a maternal immune response, provoked by male fetuses, becomes stronger with each male pregnancy, thereby allowing the mother's immune system to act as the meter by which fraternal birth order is recorded. The first antigen thought to influence sexual orientation via the maternal antibody response was testosterone (MacCulloch & Waddington, 1981), but this theory was subsequently discredited because steroid hormones are not normally antigenic (Blanchard, 1997). Another proposed antigen is the Y-linked minor histocompatibility antigen (H-Y) (Blanchard, 2001). Thus far, the only empirical evidence for the role of this antigen comes from research demonstrating that male mice whose mothers are immunized to H-Y prior to pregnancy are less likely to successfully mate with receptive females (Singh & Verma, 1987). Use of these data as support of the H-Y antigen hypothesis are limited by the fact that decreased mating among male rats is a poor proxy for homosexuality in human males. Further research should be conducted in this area before maternal immune response to the H-Y antigen can be considered a serious candidate as a mechanism in the fraternal birth-order effect.

Whatever the precise mechanism, recent evidence suggests that the fraternal birth-order effect is related to prenatal events. In two studies, based on large samples, it has been demonstrated that gay males with older brothers weigh less at birth than heterosexual males with older brothers (Blanchard & Ellis, 2001; Blanchard et al., 2002). Because birth weight is clearly a prenatal phenomenon, the fact that it is predicted by the interaction between sexual orientation and the number of older brothers is strongly suggestive of a prenatal determinant for both the fraternal birth-order effect and sexual orientation itself. In another study by Lalumière, Harris, and Rice (1999), it was found that a man's number of older brothers was a significant predictor of fluctuating asymmetry (discussed in greater detail below), one marker of developmental perturbations that could possibly have resulted from a maternal immune response. Although the investigators did not directly assess sexual orientation, the results do provide further evidence that the number of older brothers does have implications for later born male offspring.

**Developmental Instability**

As mentioned in several of the earlier sections of this article, the neurohormonal theory of sexual orientation differentiation does not adequately explain several of the empirical findings (e.g., handedness, finger length). An alternative mechanism, developmental instability
(DI), has recently been suggested as another possibility for explaining these associations (Lalumière et al., 2000; Mustanski et al., 2002). Developmental instability refers to an organism's inability to form an "ideal" (e.g., bilaterally symmetrical) phenotype under varying levels of perturbations (Palmer, 1994). Developmental perturbations during gestation could result from maternal illness, infection, chemical use or exposure, and any number of other random factors. If homosexuality results from a fetus's inability to fully buffer against developmental and/or environmental agents, then homosexual people should show other markers of DI. Two commonly used markers are nonright-handedness (Yeo & Gangestad, 1993; Yeo, Gangestad, & Daniels, 1993) and fluctuating asymmetry (Palmer, 1994).

Nonright-handedness has been established as a marker of DI via its association with a number of traits thought to result from developmental perturbations, such as autism, stuttering, dyslexia (Geshwind & Galabura, 1985), neural tube defects, and schizophrenia (Yeo & Gangestad, 1993, 1998). Fluctuating asymmetry is an index of deviation from bilateral symmetry obtained by measuring the left- and right-side of various physical sites, and taking the absolute value of the difference. High indices indicate greater discrepancy between left- and right-side measurements (i.e., greater asymmetry) and are believed to result when an organism's developmental plan is not expressed equivalently on both sides of the body (Markow & Gottesman, 1989). Dermatoglyphic fluctuating asymmetry, one of the most commonly used indices of developmental instability, is indicated when either a left- or right-hand finger has more ridges than its counterpart on the other hand. Increased levels of dermatoglyphic fluctuating asymmetry have been observed in a variety of disorders believed to be the result of developmental perturbations such as cleft-lip/palate, dyslexia, schizophrenia, and minor physical abnormalities (e.g., Adams & Niswander, 1967; Green, Bracha. Satz, & Christenson, 1994; Qazi, Masakawa, McGann, & Woods, 1980).

DI theorists would predict homosexuality to be the result of a perturbed developmental process because, from the perspective of reproductive fitness, the "ideal" phenotypic outcome of sexual orientation differentiation is likely to be heterosexuality. In evolutionary terms, an organism that includes reproduction in its developmental plan is more "fit." Indeed, gay men have fewer offspring on average than heterosexual men (Bell & Weinberg, 1978). DI theorists would predict that gay men and lesbian women would show characteristics demonstrated to be markers of DI, such as increased nonright-handedness and increased fluctuating asymmetry compared to heterosexual men and women. Support for the DI hypothesis is, at this time, limited. In only one study has
any kind of fluctuating asymmetry in homosexual participants been measured. Mustanski et al. (2002) explored the relationship between dermatoglyphic fluctuating asymmetry and sexual orientation and found no significant effects. Both male and female homosexuality have been linked to increased incidence of nonright-handedness (Lalumiè re et al., 2000), a marker of DI. Therefore, these results could be construed as supportive of the DI model. The application of DI theory to sexual orientation is speculative and certainly less well articulated than the neurohormonal theory. It is possible, however, that the DI and neurohormonal hypotheses are related (e.g., the effects of an altered prenatal hormonal environment may be manifestations of developmental instability). Future research should focus on assessing multiple physical indicators of fluctuating asymmetry in the same participant.

Impediments to Identification of Biological Factors

Despite the extensive research on the biological basis of sexual orientation, methodological limitations impede the clear identification of etiological factors. Many of these issues are relevant to all research on sexual orientation, whereas others are more specific to biological research. General criticisms include lack of a precise and commonly agreed upon definition and measurement of sexual orientation and the exclusion of women from research.

Definition and Measurement of Sexual Orientation

One obstacle in research on sexual orientation is a lack of consensus on the definition of sexual orientation and in methods used to operationalize this construct. Although a myriad of definitions of sexual orientation have been proposed (see Sell, 1997, for a review), most include a psychological (i.e., sexual fantasy/attraction) and/or a behavioral component(s). Self-definition as gay, lesbian, or heterosexual is also used as another method of defining sexual orientation. Therefore, three indices of sexual orientation can be considered: sexual behavior, sexual fantasy and/or attraction, and self-definition.

Paper and pencil measures. The most commonly used instrument for assessing sexual orientation is the Kinsey Scale, unidimensional 0 to 6 scales of attraction, fantasy, and behavior to same- and opposite-sexed persons (Kinsey, Pomeroy, & Martin, 1948). Less frequently used is the Klein Sexual Orientation Grid, a multidimensional scale assessing sexual attraction, sexual behavior, sexual fantasies, emotional preferences, social preferences, self-identification and homosexual/heterosexual lifestyle choice (Klein, Sepekoff, & Wolf, 1985). More recently, researchers have also included items measuring attitudes toward same-
and opposite-sex sexual activity as an additional measure of sexual orientation (Kirk et al., 2000).

What is most problematic about using these measures in research is a lack of consensus on methods of deriving a composite “sexual orientation score” based on the data gathered. In general, there is a strong relationship between sexual attraction/fantasy and behavior in gay men (Diamond, 1993). Thus, collapsing these variables is likely not problematic. Whether this is true for women, however, is doubtful. Bailey et al. (2000) reported correlations between sexual fantasy and sexual attraction for men and women (.92 vs. .67) demonstrating less overlap in women on measures of same-sex interest. Recent research has helped to partially solve this definitional dilemma by demonstrating that additive genetic factors that influence measures of feelings, attitudes, and number of same-sex partners are primarily the same for men and women (Kirk et al., 2000). Additionally, one latent “homosexuality” factor appears to explain most of the variation in these measures (Kirk et al., 2000). Based on these data, it seems appropriate to conclude that all of these measures are tapping into the same construct, indicating they are all appropriate for use in biological research on sexual orientation. However, using certain dimensions of the Kinsey scales, such as sexual behavior, in isolation is unadvisable as factors unrelated to sexual orientation, such as opportunity, may influence the occurrence of same-sex sexual activity.

Assigning individuals to groups based on sexual orientation scores can also be problematic. Typically, Kinsey scores of 0 or 1 are considered heterosexual, 2 though 4 bisexual, and 5, 6 homosexual. Trichotomization, although intuitively appealing, may not be ideal. For example, there is evidence that individuals with composite Kinsey scores of 1 show elevations on traits related to sexual orientation (e.g., childhood gender nonconformity), suggesting that Kinsey 1s should not be considered heterosexual (Bailey et al., 2000). Some researchers, mostly those studying men, may dichotomize the Kinsey scale, including men indicating bisexual interest/behavior in the homosexual group. The rationale for dichotomizing is likely the belief that bisexual men are truly homosexual, but this a priori assumption may be problematic, as varying degrees of same- and opposite-sex interest will likely be represented in a homosexual group. One method of avoiding the conundrum of assigning individuals to groups is to perform analyses using sexual orientation measures as continuous variables.

Defining sexual orientation using self-identity is problematic for women because of the potential heterogeneity in same- and opposite-sex sexual interest amongst women who identify as lesbian. Base-rate estimates of same-sex sexual orientation evinced from large probability sam-
amples have shown exclusive same-sex attraction and self-identification as gay versus lesbian to be less common amongst women (2.4% of men vs. .3% of women; 2.0% in men vs. .9% in women, respectively) (Laumann et al., 1994). These data show a greater percentage of women reporting a lesbian identity versus exclusive same-sex attraction, a pattern that is not seen in men. Similarly, lesbian women consistently report more heterosexual experiences than gay men do (Kinsey et al., 1953) and, after self-identifying as lesbian, report some degree of opposite-sex attraction (Rust, 1992). We advise researchers to use several other indices of sexual orientation, such as attraction and behavior, in addition to self-reported identity as gay, lesbian, bisexual, or heterosexual.

Cognitive and other methods. Recently, psychologists have become interested in applying performance assessment developed by cognitive scientists as a method of measuring various traits. For example, Treat, McFall, Viken, and Kruschke (2001) demonstrated the utility of using such methods to study the role of social information processing in the perpetration of sexually coercive behavior. Recently, Wright and Adams (1999) used reaction time to slides of nude and non-nude males and females in a choice discrimination task as a cognitive measure of sexual orientation. Latency to reaction time in the nude condition was shown to correctly identify 90% of the heterosexual men and 75% of the gay men. Other cognitive paradigms, such as priming and categorization tasks, should be explored for their efficacy in providing information about sexual orientation. If future researchers are able to replicate the results of Wright and Adams (1999), then such cognitive measures may be an appropriate endophenotype for studying the genetic, or more broadly the biological, basis of sexual orientation.

Other methods of assessing sexual orientation, such as responses from family, friends, and sexual partners about the proband’s sexual attractions and behaviors may also be beneficial information, with concordance from cotwin sexual orientation assessments estimated at 97.5% (Bailey & Pillard, 1991; Pillard & Weinrich, 1986). However, such high concordance rates have not been shown in all studies. Kirk, Bailey, and Martin (1999) found concordance rates of 50% for sibling assessment of sexual orientation. Additionally, ethical limitations of this approach may prohibit its use as a method of sexual orientation assessment.

Sexual orientation can be considered a multifaceted construct; assessing several factors in this construct should, in theory, help to define an individual’s sexual orientation more accurately. Information about self-reported attractions and behavior, self-identity, sexual fantasies, adult and childhood gender identity, sexual excitement and disgust at the thought of sex with same and opposite sex individuals,
subjective and genital responses to male and female sexual stimuli, and collateral information from informed individuals could be analyzed to extract a general factor or analyzed to examine the factor components of the construct. In any case, researchers are strongly urged to measure sexual orientation using multiple methods as no single measure gives the "true" answer to an individual's sexual preferences or identity. The magnitude of the agreement between factors in the sexual orientation construct differs for men and for women, as will be further demonstrated in the sections to follow.

**Sexual Arousal Assessment in Men and Women**

*Phallometry.* One very promising objective measure of male sexual orientation is physiological sexual arousal (penile response) to male and female sexual stimuli or, in other words, phallicometric assessment of gender preference. This technique involves measuring changes in penile circumference or volume in response to visual or audiovisual sexual stimuli depicting either men or women, with penile response interpreted as sexual attraction to male or female sexual partners. Both forms of penile plethysmography (i.e., circumferential and volumetric methods) are reliable and valid measures of sexual arousal in men (Janssen, Vissenberg, Visser, & Everaerd, 1997; Zuckerman, 1971). Discriminant validity has been demonstrated for both forms of penile plethysmography; heterosexual and homosexual men are easily differentiated on the basis of penile responses to male and female sexual stimuli (Barr & McConaghy, 1971; Freund, 1963, Freund et al., 1973; Freund, Langevin, Cibiri, & Zajac, 1974; Mavissakalian, Blanchard, Abel, & Barlow, 1975; McConaghy & Blaszczyński, 1991; Sakheim, Barlow, Beck, & Abrahamson, 1985; Tollison, Adams, & Tollison, 1979).

Some researchers dispute the discriminant validity of circumferential measurement of penile responses (McConaghy, 1989, 1999) and psychometric rigor of phallicometric testing in general (Marshall & Fernandez, 2000; see ATSA Professional Issues Committee, 2001, for an opposing view). McConaghy (1989) argued that homosexual and heterosexual men have not been adequately differentiated using circumferential methods of sexual arousal assessment. He noted that circumferential and volumetric methods of penile plethysmography are not equivalent measures of penile erection, although the measures are highly correlated (Kuban, Blanchard, & Barbaree, 1999; Wheeler & Rubin, 1987); circumferential devices assess magnitude of erection, whereas volumetric devices assess haemodynamic changes associated with the early development of erection. This subtle difference is important because of the physiological implications inherent to using either
method. When using a circumferential device to assess sexual orientation, stimuli must be of sufficient length (several minutes, McAnulty & Adams, 1992) and intensity (i.e., audiovisual stimulus vs. visual or audio alone) to provoke significant changes in penile girth. Conversely, volumetric assessment is a more sensitive measure, able to register responses using shorter and less sexually intense stimuli (i.e., still photographs of nudes).

The ability of subjects to manipulate their responses in a phallometric test is problematic and challenges the validity of the measure. There is evidence that individuals can suppress (but not enhance) their response (Money, 1987) presumably through shifting of attentional focus during stimulus presentation. Several researchers have investigated methods of preventing or identifying faking, with mixed results (Freund et al., 1988; Langevin, Stanford, & Block, 1975; Proulx, Cote, & Achille, 1993; Quinsey & Chaplin, 1988; Wilson, 1998). According to McConaghy (1999), penile volume assessment is less prone to response manipulation than circumferential measures because changes in penile volume are unconscious and involuntary. Data are lacking to support this supposition. Concern about response manipulation is greatest under conditions in which subjects are likely to attempt to manipulate test results, such as forensic settings.

Phallometric assessment is, in general, a psychometrically sound and objective measure of sexual preference in men, and because the data are dimensional versus categorical, sexual arousal patterns could be considered viable candidates for an endophenotype for sexual orientation. To date, no researchers have explored this option.

For a more detailed critical review of phallometric assessment in forensic settings see ATSA Professional Issues Committee, 2001. For a critical review of laboratory methods of sexual arousal assessment see Rowland (1999), as well as articles by Seto and by McConaghy previously cited in this review.

Vaginometry. Sexual arousal to male or female sexual stimuli is less promising as an objective measure of sexual orientation for women. The relationship between sexual arousal and sexual orientation in women is less clear, and research is less abundant than for men. Sexual arousal assessment using vaginometry (vaginal photoplethysmography) and/or ratings of subjective sexual arousal to male and female sexual stimuli does not differentiate between heterosexual and lesbian women (Chivers & Bailey, 2000; Laan, Sonderman, & Janssen, 1995). These conclusions are based on two studies (Chivers & Bailey, 2000; Laan, Sonderman, & Janssen, 1995) including approximately 150 women participants. The results have recently been replicated in subsequent
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research based on an additional 90 women (Chivers, Reiger, Latty & Bailey, 2002; Laan, Sonderman, & Janssen, 2002).

Using sexual arousal patterns as an endophenotype for sexual orientation in women seems unlikely and theoretically unsupported, given the data cited above. Moreover, given the lack of discrimination between male and female sexual stimuli in women with adult sexual preferences, vaginometric assessment of women in forensic settings appears equally dubious. To date, research examining vaginometric assessment of women offenders is very rare and includes only one study of seven women offenders against children (Brinton, 1999) and a single case design (Cooper, Swaminath, Baxter, & Poulin, 1990). These two studies provide limited support for using vaginometry in forensic settings. Forensic scientists keen to use the measure are encouraged to consider first conducting research to validate the measure before applying the testing in forensic assessment of women sex offenders. Even so, vaginometric assessment using a photoplethysmograph is impractical with potentially uncooperative or nonadmitting participants (women offenders) as the device is quite sensitive to movement artifacts and could easily be spoiled by voluntary pubococygeal muscle contractions.

Given the seeming “panpotential” nature of genital arousal in women, describing a woman’s sexual orientation based solely upon this aspect of her sexuality would not only contradict her self-report, but would rely on the potentially erroneous assumption that female sexual orientation must be defined in part by genital arousal. Again, we encourage and support multidimensional assessment of the sexual orientation construct in order to avoid making simplistic judgments of an individual’s sexual orientation. Because of the increased flexibility of women’s sexual (compared to that of men), this point becomes even more important. Given that the male model of sexual orientation has been rejected in women for many factors (e.g., lower agreement between sexual attraction and sexual behavior, lack of differentiated sexual arousal patterns to male and female sexual stimuli), the challenge of defining sexual orientation in women and understanding how biological influences function in women remains to be adequately addressed by sexuality researchers.

Limited Research on Women

The body of research examining the biological basis of sexual orientation in women is less extensive than for men. There are approximately twice the number of published articles about sexual orientation that are related to men compared to those related to women. Although it is only a rough estimate, searching MEDLINE for the term “homosexuality”
limited to human research from 1990–2002 returned 5,098 articles when a "male" limit is placed and 2,611 when a "female" limit is placed. Although only a small percentage of these articles are likely to be empirical studies of the etiology of sexual orientation, we believe the magnitude of the difference is likely to be representative.

Some researchers have commented that women have been relatively ignored in research on the biological (as well as psychological) basis of sexual orientation (Mustanski et al., 2002; Peplau, Spaulding, Conley, & Veniegas, 1999). Our review makes apparent that, more often than not, women have been included in preliminary investigations of the majority of biological traits related to sexual orientation. In some cases, as in the investigation of birth order, null effects are observed in women and the line of research is no longer pursued. In other cases, promising insights, such as increased waist-to-hip ratios and circulating testosterone levels in masculine (but not feminine) lesbians (Singh et al., 1999), indicate that meaningful biological markers of sexual orientation may exist for women.

Another possible explanation for the disparity between male and female sexual orientation research may lie in the expectation that biological factors influencing sexual orientation in men and women are essentially mirror opposites. For example, the neurohormonal hypothesis postulates that homosexuality is the result of a similar mechanism in men and women: excesses (in women) or deficits (in men) of androgens in the prenatal environment. Accumulated knowledge about male and female sexual orientation has shown that sexual orientation may not be a parallel phenomenon in men and women. For example, the distribution patterns, bimodal for men and unimodal for women (Bailey et al., 2000), and the prevalence of homosexuality and bisexuality (Laumann et al., 1994) differ between men and women. Additionally, researchers examining the development of sexual identity suggest important gender differences in same-sex attractions and sexual activity; young women typically experienced these milestones in emotionally oriented contexts (i.e., crushes, sexual contact within a romantic relationship) versus the sexually oriented context (i.e., anonymous sexual encounters) experienced by young men (Savin-Williams & Diamond, 2000). Several authors, such as Baumeister (2000) and Peplau et al. (1999), have also commented on the degree of flexibility or plasticity of several facets of female sexuality when compared to male sexuality, including sexual orientation.

As noted by Peplau et al. (1999), the relationship between sexual plasticity and sexual orientation in women has yet to be explored. One potential manifestation of female sexual plasticity is the weak relationship between sexual arousal to male and female sexual stimuli and sex-
ual orientation in women observed by Chivers and colleagues (2000, 2002) and by Laan and colleagues (1995, 2002). Given the differences observed, it may prove more fruitful to examine male and female sexual orientation with separate models and hypotheses in the future (Bailey et al., 2000; Peplau, Garnets, Spalding, Conley, & Veniegas, 1998).

Conclusion

Based on the data summarized in this review, it should be clear that sexual orientation is influenced by biological factors to some degree. Hopefully, we have also made apparent that the degree to which biological factors influence sexual orientation is likely to differ between men and women. The puzzling and outstanding question is how and when these biological factors act and to what degree these factors influence sexual orientation in women and in men.

Although the ontogeny of sexual orientation is not entirely explicated by the extant research, it is possible to make several tentative conclusions based on the evidence reviewed herein. First, biological factors seem to exert a portion of their influence before birth. Evidence from the cloacal extrophy and ablation penis cases, the handedness research, the older brother phenomenon, and several other seemingly correlated biological traits that are canalized prenatally, are all supportive of this conclusion. Secondly, genetic factors appear to explain the familial variance in sexual orientation. Contrary to popular belief, this does not prove that sexual orientation is canalized prenatally, as genes influence a host of traits that are not expressed until after birth. Similarly, it does not preclude the possibility of a prenatal canalization of sexual orientation. Although precise genetic mechanisms have yet to be definitively specified, these are likely to be identified in the future. Third, although further replications are needed, brain anatomy and neuropsychological measures all point to structural and functional brain differences related to sexual orientation in women and in men. Finally, it is apparent from the data summarized that women’s and men’s sexual orientation are very different phenomena. Women’s sexual orientation, and indeed women’s sexuality, must be studied from a perspective that tests the applicability of a male model, rather than assuming it’s applicability, in order to develop a comprehensive model of women’s sexuality.

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