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Hormones and Behavior 49 (2006) 405–414
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Interaction of fraternal birth order and handedness in the development of male homosexuality

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Received 2 May 2005; revised 8 September 2005; accepted 12 September 2005
Available online 24 October 2005

Abstract

The present study investigated evidence for an interaction between two of the best established etiologic factors, or markers of etiologic factors, in the literature on male homosexuality: fraternal birth order and hand preference. By combining five samples, the authors produced study groups of 1774 right-handed heterosexuals, 287 non-right-handed heterosexuals, 928 right-handed homosexuals, and 157 non-right-handed homosexuals. The results showed a significant ($P = 0.004$) handedness by older brothers interaction, such that (a) the typical positive correlation between homosexuality and greater numbers of older brothers holds only for right-handed males, (b) among men with no older brothers, homosexuals are more likely to be non-right-handed than heterosexuals; among men with one or more older brothers, homosexuals are less likely to be non-right-handed than heterosexuals, and (c) the odds of homosexuality are higher for men who have a non-right hand preference or who have older brothers, relative to men with neither of these features, but the odds for men with both features are similar to the odds for men with neither. These findings have at least two possible explanations: (a) the etiologic factors associated with non-right-handedness and older brothers–hypothesized to be hyperandrogenization and anti-male antibodies, respectively–counteract each other, yielding the functional equivalent of typical masculinization, and (b) the number of non-right-handed homosexuals with older brothers is smaller than expected because the combination of the older brothers factor with the non-right-handedness factor is toxic enough to lower the probability that the affected fetus will survive.

Keywords: Androgen; Antibody; Birth order; Handedness; Homosexuality; Sexual orientation

Introduction

Most researchers who study the origins of sexual orientation believe that homosexuality in human males has multiple causes. Some researchers also acknowledge the possibility that the various etiologic factors that contribute to homosexuality may interact with each other (e.g., Mustanski et al., 2002b)—that the effect of two or more factors together may be quite different from the sum of their effects in isolation. Although such interaction is recognized as a theoretical possibility, it has never been demonstrated empirically in a large-scale study with variables having well established individual effects. The present study therefore investigated evidence for interaction between two of the best established etiologic factors, or markers of etiologic factors, in the literature on male homosexuality: fraternal birth order and hand preference.

A meta-analysis of aggregate data from 14 samples representing 10,143 male subjects has shown that homosexuality in human males is predicted by higher numbers of older brothers, but not by higher numbers of older sisters, younger brothers, or younger sisters (Blanchard, 2004). The relation between number of older brothers and sexual orientation holds only for males. This phenomenon has therefore been called the *fraternal birth order effect*.

Blanchard (2004) included in his meta-analysis only studies in which he had participated and in which there was an appropriate control group. The fraternal birth order effect has also been demonstrated in a number of other studies (Bogaert, 2003; Poasa et al., 2004; Purcell et al., 2000; Zucker and

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0018-506X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2005.09.002

Blanchard, 2003; Zucker et al., 1997), including six by investigators working independently of Blanchard and his colleagues (Camperio-Ciani et al., 2004; Green, 2000; King et al., 2005; Rahman, 2005; Robinson and Manning, 2000; Williams et al., 2000). One methodologically similar study (Rahman et al., 2004) did not confirm the effect, perhaps because of inadequate statistical power. The bulk of studies outside Blanchard's (2004) meta-analysis, therefore, bolster the conclusion that the fraternal birth order effect is a reliable one.

Blanchard and Bogaert (1996) hypothesized that the fraternal birth order effect reflects the progressive immunization of some mothers to male-specific antigens by each succeeding male fetus and the concomitantly increasing effects of anti-male antibodies on the sexual differentiation of the brain in each succeeding male fetus. This notion has been called the *maternal immune hypothesis*. In later articles (Blanchard and Klassen, 1997; Blanchard, 2004), Blanchard speculated on the mechanisms by which anti-male antibodies might block full masculinization of the fetal brain, for example, by binding to, and thus inactivating, male-specific molecules located on the surface of fetal brain cells. The recent finding that biological brothers increase the odds of homosexuality in later-born males, even if they were reared in different households, whereas stepbrothers or adoptive brothers have no effect on sexual orientation (Bogaert, 2005) reinforces the notion that the fraternal birth order effect, whatever its precise mechanism, relates to changes in the uterine environment.

A meta-analysis by Lalumière et al. (2000) has established hand preference as another reliable correlate of sexual orientation. That study was carried out to resolve the conflicting conclusions that had been produced by narrative reviews of the relation between handedness and homosexuality. Lalumière et al. computed the rates of non-right-handedness (i.e., preferential use of the left hand, or equal use of both hands, in common tasks) in 20 comparisons of homosexual and heterosexual men. The odds of non-right-handedness were 34% higher for homosexual than for heterosexual men.

Lalumière et al. considered several possible explanations for why homosexual men show an excess of non-right-handedness despite that non-right-handedness is associated with masculinization (Oldfield, 1971) and that male homosexuality is commonly associated with undermasculinization (e.g., Bailey and Zucker, 1995). One explanation was developmental instability: a compromised ability to compensate for perturbations of development. According to this theory, homosexuality and non-right-handedness co-occur in the same individuals because susceptibility to one perturbation implies susceptibility to others. Lalumière et al. (2000) also considered Geschwind and Galaburda's (1985) modification of Dörner's (1972) prenatal androgen hypothesis: homosexual men are exposed to elevated levels of testosterone during some developmental periods in utero but to reduced levels during others. According to Geschwind and Galaburda, elevated testosterone occurs during a critical period for the development of handedness, and reduced testosterone occurs during a critical period for sexual orientation. An alternate formulation of the prenatal androgen hypothesis was suggested by Lindesay (1987): that homosexuality results from elevated levels–and not from reduced levels–of testosterone, and that elevations in testosterone in utero increase the probability both of homosexuality and of non-right-handedness.

Research published after the Lalumière et al. meta-analysis has continued the pattern of inconsistent results from individual studies, with the preponderance of evidence for higher non-right-handedness in homosexual men. Mustanski et al. (2002a) found very similar rates of non-right-handedness for homosexual and heterosexual men, whereas Lippa (2003) found that the odds of non-right-handedness were 82% higher for homosexual men. Zucker et al. (2001) found higher rates of non-right-handedness in boys with gender identity disorder than in control boys; the former group may, for present purposes, be considered a subtype of homosexual males (see Bailey and Zucker, 1995). The relation between handedness and sexual orientation may therefore also be regarded as reliable.

The specific objective of this study was to determine whether fraternal birth order and hand preference interact in their effects on sexual orientation. This question might be stated more concretely in several ways. For example, do older brothers have a greater, lesser, or equal effect on sexual orientation in right-handed and non-right-handed men? The study required a large number of subjects in order to obtain a sufficient number of men in the rarest category: non-right-handed homosexuals. The present authors therefore pooled their data from several studies that happened to include the variables needed for this analysis.

Method

Subjects

The all-male subjects comprised 1774 right-handed heterosexuals, 287 non-right-handed heterosexuals, 928 right-handed homosexuals, and 157 non-right-handed homosexuals. These came from five extremely diverse samples, here called Ellis, Breedlove, Blanchard, Bogaert (Non-biological Families), and Bogaert (Other). These samples represent a mixture of archival data from published studies, unpublished data from studies currently in preparation, and data retrieved specifically for the present study. The samples will be described separately. Each of the samples was collected with approval from the original investigator's institutional research review board. The sizes and demographics of the samples are presented in Table 1.

In this article, we use the word *subject* rather than *participant*, for two reasons: (a) in many cases, the person who actually participated in the study was not the subject but instead his mother, and (b) it is confusing, in the case of re-analyzed archival data, to use language implying that the subjects were recruited to participate in the later study.

Ellis

A detailed description of this sample is given in Ellis and Blanchard (2001), among other places. The sample was collected to investigate numerous potential prenatal and perinatal influences on adult human behavior. The homosexual subjects were university students and sons of P-FLAG members (Parents and Friends of Lesbians and Gays). The heterosexual subjects were university students. The respondents were recruited through numerous universities and P-FLAG chapters in the USA and Canada from 1988 to 1997. Respondents were not given any reward for participating.

Table 1
Number of homosexual and heterosexual men, age, education, and race for subjects from the five original samples

Sample	*N*	Age (mean and standard deviation)	Education (median)	Race (% White)
Ellis [a]				
Homosexual	168	31.88 (7.93)	15.5 years completed	98
Heterosexual	915	21.89 (4.38)	14.0 years completed	95
Breedlove				
Homosexual	278	40.18 (11.38)	Not available	Not available
Heterosexual	104	41.51 (14.72)	Not available	Not available
Blanchard				
Homosexual	92	41.24 (13.63)	High school grad.	89
Heterosexual	672	38.68 (13.31)	High school grad.	75
Bogaert (Non-biological families)				
Homosexual	280	40.00 (9.67)	Some postsecondary	78
Heterosexual	222	37.55 (11.41)	Some postsecondary	72
Bogaert (Other)				
Homosexual	267	35.74 (12.42)	Some postsecondary	85
Heterosexual	148	20.82 (3.94)	Some university	84

[a] For Ellis's sample, *N* and Age refer to the subjects. Education and race describe the educational level and race/ethnicity of the subjects' mothers.

Breedlove

The original study, which investigated the relations among second-to-fourth finger length ratio, birth order, and sexual orientation, was reported by Williams et al. (2000). The subjects were adults who were approached at public street fairs in the San Francisco area during 1999, and who were offered lottery "scratcher" tickets for their participation in that study.

Blanchard

This sample was retrieved specifically for the present study from a cumulative database used in an ongoing neuropsychological research program (e.g., Cantor et al., 2004, 2005). The subjects were patients referred during the years 2000 to 2004 to a hospital unit in Toronto, Ontario, Canada, for clinical assessment of their sexual feelings or behaviors. The subjects were asked, after completion of their assessments, for permission to use their test data in research studies; they were not offered any payment or other incentive in return for this.

Bogaert (Non-biological Families)

These data were collected to compare biological with non-biological older brothers in the prediction of sexual orientation (Bogaert, 2005). The subjects were men (e.g., adoptees) who had been reared in environments other than intact biological nuclear families. These were recruited in 2001–2004 through targeted newspaper advertisements in various Canadian cities. Subjects were paid $20 (Canadian) for their participation.

Bogaert (Other)

This sample comprised three subsamples. The primary purpose of two was to investigate variables that predict homophobia in heterosexual men (Bogaert, 2000, 2001), and that of the third was to investigate variables related to gay men's sexual development and health (Hafer et al., 2001). The heterosexual subjects were students at Brock University (St. Catharines, Ontario, Canada), who participated for Introductory Psychology course credit in 2000 and 2001. The homosexual subjects were mostly community adults who were recruited in 1999 through newspaper advertisements and who were paid $20 (Canadian) for their participation.

Materials and procedure

Ellis

Paired self-administered questionnaires (for mothers and children) were distributed in university classes; the mothers' questionnaire was distributed through P-FLAG chapters without the accompanying children's questionnaires. Thus, the P-FLAG mothers provided all information about their offspring. The mothers of university students provided all information except the subject's sexual orientation, which was taken from the questionnaire completed by the subject.

Parallel items in the mothers' and children's questionnaires asked if the subject (son or self) was heterosexual, bisexual, or homosexual. In the present study, as in Ellis and Blanchard (2001), the bisexual subjects were included with the homosexual ones. One item in the mothers' questionnaire asked the respondent to rate the subject's handedness on a 5-point scale. Subjects described by their mothers as extremely or generally right-handed were classed as right-handed for this study; those described as ambidextrous, generally left-handed, or extremely left-handed were classed as non-right-handed. Information on the subject's sibship came from a section of the mothers' questionnaire in which the respondent was requested to list all her known pregnancies. Maternal half siblings were counted the same as full siblings.

Breedlove

The subjects completed a self-administered questionnaire as part of their examination. One questionnaire item asked the subject how he identified himself with regard to sexual orientation. Subjects who identified themselves as exclusively or predominantly heterosexual were classified as heterosexual in this study (as in Williams et al., 2000); subjects who identified themselves as bisexual, predominantly homosexual, or exclusively homosexual were classed as homosexual. Another item asked the subject which hand he usually used for writing. Subjects who indicated the right hand were classified as right-handed for the present study; subjects who indicated the left hand or both hands were classified as non-right-handed. Sibship information came from questionnaire items that asked the subject how many male and how many female babies his biological mother had carried before him; information on younger siblings was not collected. Full siblings were not differentiated from maternal half siblings. Subjects were not asked their degree of certainty that they knew of all children born to their mother.

Blanchard

The information used in this study came from variety of sources: (a) a structured sexual history interview, which covered the patient's self-reported erotic preferences as well as his history of criminal and non-criminal sexual behaviors, (b) clinical and legal documents pertaining to the patient's charges and convictions for sexual offenses, (c) a self-administered questionnaire that included the patient's personal and family demographics, (d) a structured interview that accompanied his neuropsychological testing and that included a standard handedness inventory, and (e) phallometric testing. Phallometric testing is a psychophysiological method for the assessment of erotic preferences in human males. In this procedure, a man's penile blood volume changes are monitored as he experiences a standard, prerecorded set of potentially erotic stimuli. The phallometric data used in the present study came from a test described in detail by Blanchard et al. (2001); the stimuli were depictions of prepubescent, pubescent, and physically mature males and females. The examinee's penile responses to the various gender–age categories were ipsatized by *z*-scoring, that is, standardized within subjects.

The patient's sexual orientation was classified as heterosexual or homosexual while ignoring the age of the males or females he found most attractive. Reasons for ignoring age-preference have been presented elsewhere (e.g., Blanchard et al., 2000; Bogaert et al., 1997). Classification was accomplished by sieving the patient through a fixed sequence of diagnostic criteria. The first criterion was based on his history of sexual offending. If the patient's maximum number of victims in any one category of males (prepubescent, pubescent, teenaged, or adult) was greater than his maximum number of victims in any one category of females, and if that maximum number was greater than or equal to four victims, then the patient was classified as homosexual. If the patient satisfied the reverse criterion (female victims greater then male victims and greater than or equal to four), then he was classified as

heterosexual. If the patient satisfied neither sexual-offense criterion, then he was evaluated according to his phallometric test results. If the patient's penile response to any age-category of males exceeded his maximum response to any age-category of females by 0.25 *z*-units, then he was classified as homosexual. If he satisfied the reverse criterion (maximum penile response to females), he was classified as heterosexual. If he satisfied neither phallometric criterion, he was next evaluated according to his (qualified) self-report. If the patient stated that his sexual attraction to any age-category of males was greater than his strongest attraction to any age-category of females, then he was classified as homosexual, provided that he had no sexual offenses against females of any age. If he met the reverse criterion, he was classified as heterosexual. The reason that these patients' sexual orientations were not classified on the basis of their self-reports alone, as was done with the other samples, is the well-known unreliability of self-reported erotic interests in sexual offenders (see, for example, Blanchard et al., 2001).

As in most of the other samples, the assessment of handedness was based solely on writing hand. If the patient told the interviewer that he wrote with his right hand, he was classified as right-handed. If he said the left hand or both hands, he was classified as non-right-handed. Items on the personal and family demographics questionnaire asked the patient how many boys and girls his mother delivered before and after she gave birth to him and whether he felt sure that he knew of all children delivered by his mother. A subject was excluded if his mother had any children by any man other than the subject's own father—the usual practice in the first author's laboratory when numbers of subjects permit (e.g., Blanchard and Bogaert, 1996). Thus, "siblings" for this sample meant "full siblings".

Bogaert (Non-biological Families)

Subjects completed and mailed in a self-administered questionnaire. For the present study, the subject's sexual orientation was classified on the basis of two items. One asked the subject to rate his sexual behavior, and the other to rate his sexual thoughts and feelings, on identical 7-point scales. The end-points of these scales were labeled "exclusively heterosexual" and "exclusively homosexual," and the mid-point was labeled "equally homosexual and heterosexual." Scores on these items were averaged. Subjects whose averaged scores fell in the range from "exclusively homosexual" to "equally homosexual and heterosexual" were classified as homosexual; the remainder was classified as heterosexual. Another item asked the subject to indicate his writing-hand preference on a 5-point scale. Subjects who indicated that they always or usually wrote with their right hands were classified as right-handed; those who indicated that they wrote with both hands or that they usually or always used the left were classified as non-right-handed. Sibship information was collected with a variety of items. The present study used the number of biological siblings known to the subject (whether he ever resided with them or not). Maternal half-siblings were not differentiated from full siblings.

Bogaert (Other)

The subjects completed self-administered questionnaires that included the items described in the previous paragraph. In the case of the paid sample, the questionnaires were mailed in to the investigators. Quantification of sexual orientation, handedness, and sibship composition was similar to that for the other Bogaert sample.

Results

The data analysis was conducted from two different perspectives: one taking sexual orientation as the dependent variable and one taking handedness as the dependent variable. This was done partly because it facilitated comparison of the present results with those of earlier studies, which have varied in their designation of the dependent variable, depending on their focus. It was also done because interaction effects–the topic of the present research–can be viewed from different perspectives, and the conclusions suggested by one view are not necessarily redundant with the conclusions suggested by another.

Sexual orientation as the dependent variable

Most recent statistical analyses of sexual orientation and fraternal birth order have treated sexual orientation as the dependent variable (e.g., Bogaert, 2005). This approach follows directly from the assumed causal model–a man's number of older brothers might influence his sexual orientation, but not vice versa–and it also enables quantitative estimation of the impact of each older brother. The present question, "Do older brothers have the same effect on sexual orientation in right-handed and non-right-handed men," was therefore investigated in a series of logistic regression analyses, with sexual orientation, coded 0 for heterosexual and 1 for homosexual, treated as the criterion (i.e., dependent) variable.

The first analysis used only one predictor (i.e., independent) variable: the subject's original sample (hereafter, *source*). Source was deviation-coded, with Ellis's sample as the reference category. In this context, the choice of deviation coding (weights = −1, 0, or 1) for this categorical variable was arbitrary, as was the designation of Ellis's sample as the reference category (the category with weight = −1).

The first analysis was actually just a preliminary step in graphing the statistical relations of primary interest. Its sole purpose was to control for between-samples differences–specifically, the large differences in the proportion of homosexual subjects–in the visual representation of the main findings, and thus to partly parallel our control for these differences in the statistical analysis of those findings. To this end, sexual orientation was regressed on source, and the standardized differences between the observed and expected probabilities of homosexuality were saved as a new variable, the residual probability of homosexuality.

Fig. 1 shows the residual probability of homosexuality as a function of handedness and number of older brothers. Negative values along the ordinate denote probabilities of homosexuality lower than the mean for all subjects, and positive values denote probabilities higher than the mean. The figure shows the usual association between increasing numbers of older brothers and increasing probabilities of homosexuality, but only for right-handed men. For non-right-handed men, the curve relating older brothers to homosexuality appears quite different, perhaps even opposite. It should be noted that the capping of the older brothers variable at "three or more" was done solely for the purpose of tidying the graphical display. In all statistical analyses, numbers of older brothers and older sisters were analyzed exactly as reported.

Analogous data are shown in Fig. 2 for number of older sisters. There is, again as usual, little evidence of any association between a man's number of older sisters and his likelihood of homosexuality. The curve for right-handers is virtually flat, and the curve for non-right-handers does not depart markedly from that.

The second logistic regression analysis investigated whether regression lines fitted to the data shown in the figures would in fact differ significantly in slope between right-handed and non-right-handed men. There were six predictors, four of which represented main effects: older brothers, older sisters, source,

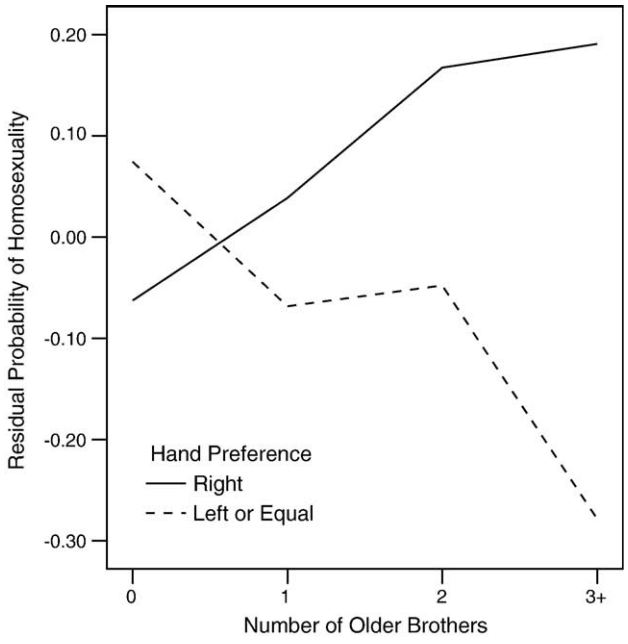

Fig. 1. Standardized residual probability of homosexuality as a function of older brothers and handedness. Higher values indicate greater probabilities of homosexuality. See text for explanation.

and handedness. Number of older brothers and number of older sisters were treated as continuous variables. Handedness (right or non-right) and source (the five samples of origin) were treated as categorical variables. These were deviation-coded; the reference categories were right-handed for the handedness variable and Ellis's sample for the source variable. The other two predictors were the product of handedness and older brothers and the product of handedness and older sisters. These terms carried the interactions of handedness with older brothers and older sisters, respectively. The results are shown in Table 2. The results confirmed that handedness interacts significantly with older brothers (see Fig. 1) but not with older sisters.

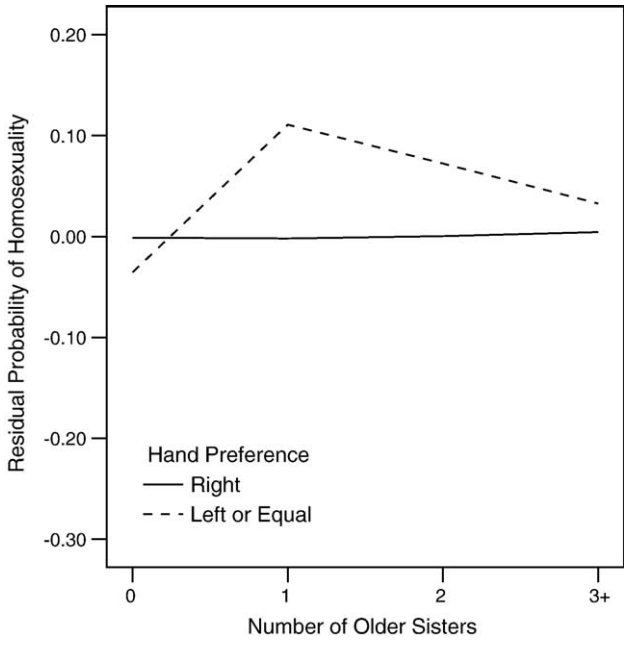

Fig. 2. Standardized residual probability of homosexuality as a function of older sisters and handedness.

Table 2 shows that the main effects for older brothers and older sisters were not significant when the full group of subjects was used and the terms for the interactions of handedness with older brothers and older sisters were included in the model. The third and fourth analyses tested whether the relations between older brothers and sexual orientation (Fig. 1) would be statistically significant within groups who had the same hand preference.

The third analysis was carried out on the 2702 right-handers only. The predictors were source, older brothers, and older sisters. The results (Table 3) indicate a significant odds ratio of 1.24 for older brothers, which means that each older brother increased the odds of homosexuality by about 24%. The relation between older sisters and homosexuality was not statistically significant and was not even in the same direction. It must be stressed that this result cannot be considered a replication because the combined sample includes previously published data. The odds ratio for older brothers (1.24) was a little lower than usual. This may relate to the considerable heterogeneity of the subjects or to the fact that we did not fully control for family size (younger brothers and younger sisters were not available for all subjects and could not be included in the analysis). It does not appear to be related specifically to the inclusion of Blanchard's sample (patients rather than community volunteers) because the same odds ratio computed on right-handed subjects from that sample alone was virtually identical: 1.25.

The fourth analysis was similar to the third one, except that it was carried out on the 444 non-right-handers only. The results are shown in Table 4. Both the relation between sexual orientation and older brothers and the relation between sexual orientation and older sisters were opposite in direction to those observed among the right-handers; in this group, older brothers lowered the odds of homosexuality and older sisters raised them. However, neither of these relations was statistically significant.

In summary, we could reject the hypothesis that the slope of the line relating older brothers to sexual orientation is zero for right-handed subjects, and we could reject the hypothesis that the slope is the same for right-handed and non-right-handed

Table 2
Logistic regression of sexual orientation on source, number of older brothers, number of older sisters, and hand preference, using all subjects

Predictor	*B*	SE	Wald	*df*	*P*	e^B
Source			724.44	4	<0.0001	
Older brothers	0.02	0.07	0.13	1	0.72	1.02
Older sisters	0.03	0.08	0.17	1	0.68	1.03
Handedness	0.07	0.08	0.84	1	0.36	1.08
Handedness by older brothers	−0.19	0.06	8.47	1	0.004	0.83
Handedness by older sisters	0.12	0.08	2.42	1	0.12	1.13

Note. Contrasts for levels of the *source* variable are not presented because the differences in the proportion of homosexual subjects in the five samples are not "findings" and have no importance in themselves.

Table 3
Logistic regression of sexual orientation on source, number of older brothers, and number of older sisters, using right-handed subjects

Predictor	*B*	SE	Wald	*df*	*P*	e^B
Source			623.18	4	<0.0001	
Older brothers	0.21	0.05	18.93	1	<0.0001	1.24
Older sisters	−0.09	0.05	3.05	1	0.08	0.92

subjects. We could not reject the hypothesis that the slope is zero for the non-right-handed subjects, despite the negative-tending curve suggested by Fig. 1.

The foregoing findings (especially Fig. 1) prompted the hypothesis that the etiological factors associated with non-right-handedness and fraternal birth order might cancel each other out. This hypothesis generated the testable prediction that the odds of homosexuality among non-right-handed men with older brothers will be similar to the odds among right-handed men without older brothers. The data, re-cast in a form that corresponded to this question, are shown in Fig. 3. The raw data appeared consistent with the prediction.

The hypothesis was formally tested in the logistic regression analysis reported in Table 5. A new variable, handedness–brothers, was created for this analysis. This variable represented the subject's assignment to one of four groups, according to his hand preference and his number of older brothers: (a) no older brothers and right-handed (n = 1630), (b) no older brothers and non-right-handed (n = 248), (c) one or more older brothers and right-handed (n = 1072), and (d) one or more older brothers and non-right-handed (n = 196). Handedness–brothers was indicator-coded, with the first group (no older brothers and right-handed) as the reference category.

The results were similar with and without source and number of older sisters added to the regression equation as control variables. We will therefore comment only on the results with the control variables (bottom panel of Table 5). The odds of homosexuality were 41% higher for men who had a non-right hand preference, and 50% higher for men who had older brothers, relative to men with neither of these features. As we predicted, however, the odds for men with both features were similar to the odds for men with neither.

Handedness as the dependent variable

All previous studies of handedness and sexual orientation have treated handedness as the dependent variable and compared rates of non-right-handedness in heterosexual and homosexual men (See Lalumière et al., 2000). Our second set of analyses estimated rates of non-right-handedness that could be compared directly with those calculated in previous studies and investigated how differences in fraternal birth order affected these rates.

Table 4
Logistic regression of sexual orientation on source, number of older brothers, and number of older sisters, using non-right-handed subjects

Predictor	*B*	SE	Wald	*df*	*P*	e^B
Source			102.61	4	<0.0001	
Older brothers	−0.12	0.12	0.97	1	0.32	0.89
Older sisters	0.15	0.14	1.09	1	0.30	1.16

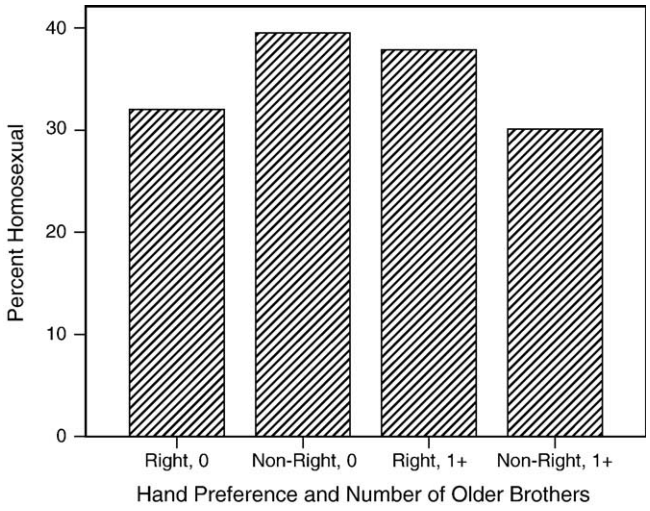

Fig. 3. Percentage of homosexual subjects in each of four groups: right-handed and no older brothers (Right, 0), non-right-handed and no older brothers (Non-Right, 0), right-handed and one or more older brothers (Right, 1+), and non-right-handed and one or more older brothers (Non-Right, 1+).

A preliminary comparison of the five samples showed that the rates of non-right-handedness varied little among them: 13% to 16%. The differences in rates were not statistically significant, likelihood ratio $\chi^2(4, N = 3146) = 1.59$, $P > 0.80$.

The first analysis was a straightforward methodological replication of previous studies. This analysis, which used all subjects, found that the rates of non-right-handedness in the

Table 5
Logistic regression of sexual orientation on subjects grouped by hand preference and existence of any older brothers (handedness–brothers), source, and number of older sisters, using all subjects

Predictor	*B*	SE	Wald	*df*	*P*	e^B
Model 1						
Group (handedness–brothers)			14.22	3	0.003	
Right-handed, 0 older brothers vs.						
Non-right-handed, 0 older brothers	0.33	0.14	5.43	1	0.02	1.39
Right-handed, ≥1 older brothers	0.26	0.08	9.79	1	0.002	1.29
Non-right-handed, ≥1 older brothers	−0.09	0.16	0.30	1	0.59	0.91
Model 2						
Group (handedness–brothers)			19.16	3	0.0003	
Right-handed, 0 older brothers vs.						
Non-right-handed, 0 older brothers	0.34	0.16	4.37	1	0.04	1.41
Right-handed, ≥1 older brothers	0.41	0.10	15.88	1	<0.0001	1.50
Non-right-handed, ≥1 older brothers	−0.06	0.19	0.11	1	0.75	0.94
Source			725.24	4	<0.0001	
Older sisters	−0.04	0.05	0.62	1	0.43	0.96

homosexual men (14%) and the heterosexual men (14%) were not significantly different, $\chi^2(1, N = 3146) = 0.17$, $P > 0.60$. The interaction detected in the first phase of our data analysis, however, suggested that one might find handedness differences between homosexual and heterosexual men if one examined subjects with older brothers and subjects without older brothers separately. This proved so. There were 620 homosexual and 1258 heterosexual men who had no older brothers. The rate of non-right-handedness was 16% for the homosexuals and 12% for the heterosexuals. This result, expressed differently, means that the homosexual subjects had 39% greater odds of being non-right-handed. A χ^2 test showed that the difference between groups was statistically significant, $\chi^2(1, N = 1878) = 5.33$, $P = 0.02$.

Contrary results were found for the 465 homosexual and 803 heterosexual men who had one or more older brothers. For these men, the rate of non-right-handedness was 13% for the homosexuals and 17% for the heterosexuals. This result means that the homosexual subjects had 29% lesser odds of being non-right-handed. The difference between these groups was also statistically significant, $\chi^2(1, N = 1268) = 4.41$, $P = 0.04$. Neither of the foregoing findings was substantially altered when we examined the relation between handedness and sexual orientation in a logistic regression set-up and controlled for the variables, source, and number of older sisters.

The last analysis focused on the question: do older brothers influence handedness itself, besides interacting with handedness to influence sexual orientation? The question was addressed with a logistic regression analysis similar to that presented in Table 2, with the exception that the roles of sexual orientation and hand preference were reversed. In this analysis, hand preference, coded 0 for right and 1 for left or equal, was the criterion variable. Sexual orientation took the place of handedness among the predictors. Sexual orientation was deviation-coded, with the heterosexuals as the reference category. The analysis was carried out on all 3146 subjects.

The results (Table 6) did not support the notion that older brothers affect handedness itself. There were only two statistically significant findings. One was the sexual orientation by older brothers interaction. This is simply the handedness by older brothers interaction (Table 2), viewed from a different perspective. The other significant finding was the main effect for older sisters: each older sister decreased the odds of non-right-handedness by 15%.

Table 6
Logistic regression of hand preference on source, number of older brothers, number of older sisters, and sexual orientation, using all subjects

Predictor	*B*	SE	Wald	*df*	*P*	e^B
Source			1.20	4	0.88	
Older brothers	0.02	0.06	0.15	1	0.70	1.02
Older sisters	−0.17	0.07	6.49	1	0.01	0.85
Sexual orientation	0.04	0.07	0.30	1	0.59	1.04
Sexual orientation by older brothers	−0.13	0.06	5.57	1	0.02	0.88
Sexual orientation by older sisters	0.11	0.06	2.99	1	0.08	1.12

Discussion

The study produced three main findings. All argue that handedness and fraternal birth order interact with regard to sexual orientation. First, the positive correlation between homosexuality and greater numbers of older brothers appears to hold only for right-handed males. The best evidence at this time suggests that the correlation is zero for non-right-handed males. Second, the relative rates of non-right-handedness in homosexual and heterosexual men depend on their numbers of older brothers. Among men with no older brothers, homosexuals are more likely to be non-right-handed than heterosexuals; among men with one or more older brothers, homosexuals are less likely to be non-right-handed than heterosexuals. Third, the odds of homosexuality are higher for men who have a non-right hand preference or who have older brothers, relative to men with neither of these features, but the odds for men with both features are similar to the odds for men with neither. It goes without saying that these findings should be regarded as tentative unless and until they are confirmed in additional samples. In the meantime, one can consider how our data might be explained, and how they might explain the data of others.

The three main findings may be interpreted as follows: some factor associated with non-right-handedness increases the odds of homosexuality in first male births. This same factor, however, prevents older brothers from increasing the odds of homosexuality in later male births. If that interpretation is correct, the problem becomes one of identifying the postulated factor. One possible candidate is fetal testosterone.

As stated in the Introduction, Lindesay (1987) hypothesized that males exposed to higher levels of fetal testosterone are more likely to become non-right-handed and to become homosexual. This hypothesis was advanced to explain the correlation between non-right-handedness and homosexuality, but it has also been used to account for other findings (Alias, 2004; Bogaert and Hershberger, 1999; McFadden and Champlin, 2000). A variant form of this hypothesis, which could explain much the same data, would be that males who are more sensitive to fetal testosterone are more likely to be non-right-handed and homosexual. In either form, this hypothesis requires the auxiliary proposition that hyperandrogenization of the male fetus may paradoxically produce the same outcome as hypoandrogenization: atypical sexual differentiation in the fetal brain and a future preference for male rather than female sexual partners. Such paradoxical effects have never been demonstrated directly in humans, but some animal research suggests that they may be possible (Baum and Schretlen, 1975; Clark et al., 1996; Diamond et al., 1973; Pollak and Sachs, 1975).

The hyperandrogenization hypothesis can obviously be applied to the present finding that, among men with no older brothers, non-right-handedness is more common in homosexuals than in heterosexuals, because the handedness–homosexuality correlation is one of the findings that it was advanced to explain in the first place. There is no scientific consensus that the hyperandrogenization hypothesis is correct

(e.g., James, 2001), and even the notion that high levels of testosterone produce non-right-handedness has been contested (e.g., Previc, 1994). Our present purpose, however, is not to undertake a general evaluation of the hypothesis but rather to consider whether it could also explain the finding that older brothers do not increase the odds of homosexuality in non-right-handed males.

The hyperandrogenization hypothesis implies that high rates of non-right-handedness may be interpreted as evidence of high concentrations of fetal testosterone, which–in more moderate quantities–masculinizes the brain. The maternal immune hypothesis implies that older brothers may be interpreted as evidence of anti-male antibody, which feminizes the brain. If both factors (hypermasculinization and feminization) are present in the same fetus, they may cancel each other out, yielding the functional equivalent of typical masculinization. Thus, one might expect to find higher rates of homosexuality among men who have a non-right hand preference, or who have older brothers, but not among men who have both. This hypothesis predicts that the rate of homosexuality among non-right-handed men with older brothers should be similar to the rate among right-handed men without older brothers. This prediction proved consistent with our data.

Another possible explanation has no direct precedent in the sexual orientation research literature but is much simpler: the number of non-right-handed homosexuals with older brothers is smaller than expected because the combination of the older brothers factor with the non-right-handedness factor (be it hyperandrogenization, developmental instability, or something else) is toxic enough to lower the probability that the affected fetus will later be available for research. This could happen if the combination lowers the fetus's chances of survival, or if it predisposes the individual to some condition (e.g., mental retardation) that makes him less likely to be sampled than other members of the community.

This alternative explanation is consistent with prior findings that older brothers decrease the birth weight of subsequent male fetuses (Blanchard and Ellis, 2001; Côté et al., 2003), especially prehomosexual male fetuses (Blanchard and Ellis, 2001; Blanchard et al., 2002), and that non-right-handedness also correlates with low birth weight (e.g., O'Callaghan et al., 1987; Powls et al., 1996). Low birth weight is generally disadvantageous for fetal health, and it is associated with lower IQ in fetuses born alive (e.g., Matte et al., 2001). Such findings constitute reason to speculate that the combination of older brothers and non-right-handedness might differentially affect the probability of homosexual males' inclusion in survey research, and that this produced the lower than expected percentage of homosexuals among our non-right-handed subjects with older brothers (Fig. 3).

The alternative explanation of the present data fits more naturally with the developmental instability hypothesis than with the hyperandrogenization hypothesis of the non-right-handedness/homosexuality correlation. The notion of increased vulnerability to environmental perturbation (developmental instability) complements the notion of increased amount of environmental perturbation (maternal immune products). Thus, the male fetus that is either unusually susceptible to environmental perturbation or is exposed to unusually high levels of it will be healthy but with greater odds of homosexuality; the fetus that has both unusual susceptibility and unusually high exposure will have diminished chances of survival or significant later medical or cognitive problems. It should be stressed that the finding that homosexual women have even more elevated rates of non-right-handedness than homosexual men (Lalumière et al., 2000) may or may not have any relevance to this argumentation. It is quite possible that homosexual men manifest increased rates of non-right-handedness for one reason (e.g., developmental instability) and homosexual women manifest the same phenomenon for a completely different reason (e.g., prenatal hormone levels or sensitivities).

Of course, both explanations of the present data, like any other hypotheses that might be advanced right now, are purely conjectural. They are offered largely as a stimulus to further study.

The second main finding–the relative rates of non-right-handedness in homosexual and heterosexual men depend on their numbers of older brothers–may explain the inconsistent results of prior studies on handedness and sexual orientation. According to our results, studies with higher proportions of first-born sons will tend to find higher rates of non-right-handedness in homosexual than in heterosexual men; studies with lower proportions will tend to find equal rates in homosexual and heterosexual men. The proportion of first-born sons (i.e., men with no older brothers) in a sample depends on the mean family size of the sample; and this will likely depend, in turn, on the socioeconomic status of the sample and on the period in which the subjects were born (e.g., during the 1945–1960 baby boom). Thus, seemingly irrelevant demographics of a sample could affect whether the researcher finds a higher proportion of non-right-handed cases in the homosexual group. It should be noted that typical recruiting procedures will rarely produce a sample in which the large majority of men have one or more older brothers, therefore few studies should find that homosexual men have significantly *lower* rates of non-right-handedness than heterosexual men.

The study yielded one incidental finding that requires discussion: in our subjects, older sisters appeared to lower the odds of non-right-handedness in later-born males. Precisely the opposite results were obtained by Lippa (2003), who reported that the percentage of non-right-handed individuals in his male subjects increased with number of older sisters. It is therefore most likely that our findings for sororal birth order and handedness reflect Type I error.

Future research might investigate other differences between right-handed and non-right-handed homosexual men besides fraternal birth order. One example is cognitive abilities, which McCormick and Witelson (1991) found to differ between these groups. Future research on this topic might also include the collection of data on the handedness of subjects' relatives. Such data would permit the

designation of non-right-handed subjects as probably familial (i.e., genetic) vs. probably sporadic (i.e., non-genetic) cases. This could open up other avenues for explaining the present findings, including genetic ones. If, for example, familial non-right-handers are impervious to the effect of older brothers on sexual orientation, whereas sporadic non-right-handers are just as vulnerable as right-handers, this would suggest that familial non-right-handers represent a distinctive genetic subpopulation. If–to give a second example–non-right-handed homosexuals with older brothers are confirmed to be an underrepresented group in survey research, and the "missing" subjects prove to be of the sporadic type, this would tend to favor the previously stated toxic combination hypothesis. That is because sporadic non-right-handedness is generally associated with perturbations of fetal development, and this might be exacerbated by maternal anti-male antibodies stimulated by older brothers.

Acknowledgments

This research was supported in part by grants 419-99-0019 from the Social Sciences and Humanities Research Council of Canada and 94205 from the Canadian Institutes of Health Research to Ray Blanchard.

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Behavioral Neuroscience
1997, Vol. 111, No. 6, 1395–1397

0735-7044/97/$3.00

BRIEF COMMUNICATION

Birth Order and Sexual Orientation in Women

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One of the world's largest databases on human sexuality was used to investigate whether lesbians, like homosexual men, have a later birth order relative to heterosexual comparisons. The women ($N > 5{,}000$) were interviewed by investigators at the Kinsey Institute for Sex and Reproduction from 1938 to 1963. The women were dichotomously classified as lesbian ($n = 257$) or heterosexual ($n = 5{,}008$). No significant birth order effect was observed. Results support theories of gender-specific mechanisms in the development of sexual orientation in women and men.

Research has established that homosexual men have, on average, a later birth order than population norms or comparable groups of heterosexual men (Blanchard & Bogaert, 1996a, 1996b, 1997; Blanchard & Sheridan, 1992; Blanchard & Zucker, 1994; Blanchard, Zucker, Bradley, & Hume, 1995; Blanchard, Zucker, Cohen-Kettenis, Gooren, & Bailey, 1996; Bogaert, Bezeau, Kuban, & Blanchard, 1997; Hare & Moran, 1979; Slater, 1962; Zucker & Blanchard, 1994). Recently, this difference has been demonstrated to be primarily the result of homosexual men being born later among their brothers, relative to heterosexual men (Blanchard & Bogaert, 1996a, 1996b, 1997; Bogaert et al., 1997). A number of theories can be forwarded to account for the birth order effect among brothers, including the conditioning-of-arousal to same-sex activity/fantasy, and a maternal immune response, where a mother develops antibodies to a male-related factor (e.g., H-Y antigen) over successive male pregnancies (Blanchard & Bogaert, 1996b; Blanchard & Klassen, 1997).

Birth order and sexual orientation in women has been less studied and results are inconsistent. Two early studies are contradictory, with one indicating that lesbians are later born relative to the general population (Slater, 1962) and another indicating that lesbians are earlier born relative to heterosexual comparisons (Saghir & Robins, 1973). Five additional research teams reported no clear difference between lesbians and comparable heterosexuals (Bell, Weinberg, & Hammersmith, 1981; Blanchard & Sheridan, 1992; Gundlach & Reiss, 1976; Hare & Moran, 1979; Siegelman, 1973). Most of the abovementioned studies had samples that were small, not all reported statistical significance tests, and none tested the more specific question of whether a possible birth order effect occurs among sisters or brothers. Thus, new research on a large sample would help clarify this relation.

The present study used information from the Kinsey Institute for Research in Sex and Reproduction, one of the world's largest databases on human sexuality, to investigate the relationship between sexual orientation and birth order in women.

Method

From 1938 to 1963, 18,216 case histories were recorded by the Kinsey Institute for Sex Research using the interview schedule devised by Alfred Kinsey (Gebhard & Johnson, 1979). The computer data files containing adult white and nonwhite women with no convictions for felonies or misdemeanors (other than traffic violations) make up 5,954 cases. To minimize unreliable data, 225 individuals having incomplete sibship information or who at some point resided in a foster home or institution before age 18 were eliminated. The remaining 5,729 cases were the database for the present investigation.

Women were classified as lesbian if they reported "extensive" homosexual experience, defined by Gebhard and Johnson (1979) as more than 20 female sexual partners or more than 50 homosexual experiences (with one or more partners). Women were classified as heterosexual if they reported either "no" or "rare" homosexual experience, the latter defined by Gebhard and Johnson as 1 female sexual partner or 1–5 homosexual experiences, *and* they did not respond that they experienced "much" or "some" arousal to questions about sexual arousal from seeing and thinking of females. Using these criteria, 464 individuals could not be classified as either heterosexual or homosexual. Of the remaining 5,265 cases, 257 were classified as lesbian and 5,008 were classified as heterosexual.

Birth order was derived from the proband's number of older brothers, older sisters, younger brothers, and younger sisters. Unfortunately, as part of the original interview/coding protocol, sibling numbers over 8 were collapsed into one category, 8+; thus, a proband with 9 or more older sisters was given the same score as a proband with 8 older sisters, and so on. In addition, there was no

I thank Thomas G. Albright for running the statistical analyses, Ray Blanchard for suggestions regarding analyses, Caroline Rougier-Chapman for helping with a literature search, Carolyn L. Hafer for comments on an earlier draft of this article, and the Kinsey Institute for allowing access to these data.

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way to distinguish full, half, and step siblings in the computerized data. Deceased siblings were recorded, however, and twins were not counted in the totals.

Also assessed were age, year of birth, biological parents' age at proband's birth, number of brothers and sisters, and parental socioeconomic status. Education was not used as a social class measure because many probands were still in school when interviewed and their current education would not reflect their ultimate education.

Results

Birth order was quantified using Berglin's (1982) index, (older siblings + 0.5)/(total siblings + 1), a metric that controls for family size. This index expresses birth order as a quantity between 0 and 1, with higher values indicating a later birth order. Berglin's index for heterosexual women, 0.486 (SD = 0.250), did not differ significantly from that for lesbians, 0.487 (SD = 0.250), $t(5263) = 0.11$, $p = .911$. Note that this and all remaining statistical tests are two-tailed.

To evaluate possible birth order effects among brothers or sisters, two new indices, a fraternal index and a sororal index, were calculated. The fraternal index quantifies a proband's birth order relative to his or her brothers as (older brothers + 0.5)/(total brothers + 1) and, like Berglin's index, ranges between 0 and 1, where a higher value indicates a later birth order among brothers. The sororal index similarly quantifies a proband's birth order relative to his or her sisters as (older sisters + 0.5)/(total sisters + 1). The fraternal index for the heterosexual women was 0.493 (SD = 0.212); for the lesbians, it was 0.505 (SD = 0.209). The sororal index for the heterosexual women was 0.493 (SD = 0.205); for the lesbians, it was 0.476 (SD = 0.207). No significant differences occurred on these indices, $t(5263) = 0.88$, $p = .379$ and $t(5263) = 1.30$, $p = .193$, respectively.

The two groups differed on age and year of birth (see Table 1). Because a proband's expected birth order depends upon demographic trends operative around the time of his or her birth, any between-group differences in year of birth represent a potential artifact in birth order comparisons (Hare & Price, 1969). Thus, in additional birth order comparisons, year of birth trends were controlled using analyses of covariance (ANCOVAs). All results remained nonsignificant.

Finally, the two groups were assessed for their sibling sex ratios, that is, the proportion of brothers to sisters collectively reported by a given group of probands, and these values were compared with the known human sex ratio, .5146, using the z approximation to the binomial test. Unlike birth order, the human sex ratio is resistant to perturbation by demographic variables (James, 1987). Neither the lesbians' nor the heterosexuals' sex ratio (.4931 and .5150, respectively) differed significantly (both $ps > .300$) from the expected value.

Discussion

One of the world's largest databases on human sexuality was used to investigate whether lesbians, like homosexual men, have a later birth order relative to heterosexual comparisons. No relationship was found. These null results are noteworthy given the large sample size and the resulting power to detect very small effects.

The results have implications for the etiology of sexual orientation in both women and men. Not only do these results suggest that birth order does not affect women's sexual orientation, they also suggest that a gender-specific mechanism accounts for the relation between (fraternal) birth order and sexual orientation in men. The maternal immune response hypothesis involves a gender-specific mechanism and thus is consistent with these results. This hypothesis is partly based on the argument that a woman's immune system would appear to be the most capable of "remembering" the number of male (but not female) fetuses she has previously carried and of progressively altering its response to the next fetus according to the current tally of preceding males. If the immune hypothesis is correct, then the connection between the mother's immune reaction and the child's future sexual orientation would likely be some effect of maternal antibodies on the sexual differentiation of the fetal brain. Various lines of indirect support for male-

Table 1
Demographic Comparisons of Lesbian and Heterosexual Women

	Score					
	Lesbians (n = 257)[a]		Heterosexuals (n = 5008)[a]			
Variable	M	SD	M	SD	t	p
Age	34.05	10.12	28.73	11.06	7.55	<.001
Year of birth	1913.12	10.44	1916.86	10.58	5.53	<.001
Father's age at proband's birth	32.62	8.39	32.51	7.00	0.20	.575
Mother's age at proband's birth	28.13	6.84	28.25	5.90	0.27	.790
No. of sisters	1.14	1.42	1.08	1.25	0.69	.487
No. of brothers	1.11	1.32	1.15	1.26	0.52	.600
Parental SES[b]	4.78	1.57	4.94	1.34	1.54	.125

[a]Sample size may vary for some variables because of missing cases. [b]Parental socioeconomic status (SES) varies from 1 (extreme poverty) to 8 (extreme wealth).

specific Y-linked H-Y antigen as the relevant fetal antigen (Blanchard & Bogaert, 1996b; Blanchard & Klassen, 1997) include evidence that H-Y antigen may be responsible for the greater antigenicity of male (versus female) fetuses (see Gaultieri & Hicks, 1985), and findings that male mice whose mothers were immunized to H-Y prior to pregnancy are less likely to mate with receptive females (Singh & Verma, 1987).

Other explanations, such as same-sex fantasy/activity mechanisms, have been proposed to account for the birth order effect in men. One hypothesis is that a large number of older brothers increases the likelihood of sex fantasy/activity among brothers, the assumption being that such experiences produce a stronger learning/conditioning effect on the younger of the siblings involved. Similarly, Sulloway (1996, pp. 433–434, 488) hypothesized that the birth order phenomenon reflects later borns' greater openness to experience, which predisposes them to experiment sexually, including with same-sex activity. These psychosocial explanations are not eliminated by the present data, but an additional question is now raised about why an increased number of older siblings, including same-sex siblings (and the resulting opportunity for increased same-sex fantasy/activity), increases the likelihood of same-sex attraction in men but not in women.

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Received December 20, 1996
Revision received April 29, 1997
Accepted May 8, 1997 ■

Behavioral Neuroscience
2003, Vol. 117, No. 2, 381–384

0735-7044/03/$12.00 DOI: 10.1037/0735-7044.117.2.381

BRIEF COMMUNICATIONS

The Interaction of Fraternal Birth Order and Body Size in Male Sexual Orientation

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A late fraternal birth order has been demonstrated numerous times in homosexual men. Body size has been less studied with regard to the development of sexual orientation and has demonstrated contradictory results. In this research, the relations among fraternal birth order, body size, and sexual orientation were examined in a Canadian sample of homosexual and heterosexual men. An interaction between fraternal birth order and height was observed, with a homosexual orientation most likely to occur in men with a high number of older brothers and shorter stature. No significant interactive effects were observed for weight. The results suggest that the mechanism underlying the fraternal birth order phenomenon has an effect on physical development that lasts and is detectable into adulthood (i.e., adult stature).

Numerous studies have indicated that a late birth order is associated with homosexuality in men (see Blanchard, 1997). This effect is primarily the result of homosexual men having a larger number of older brothers than heterosexual men (e.g., Blanchard & Bogaert, 1996a, 1996b; Blanchard & Bogaert, 1997; Bogaert, Bezeau, Kuban, & Blanchard, 1997). Our first published study (Blanchard & Bogaert, 1996b) establishing the fraternal birth order effect was with a Canadian sample of 604 homosexual and heterosexual men. A logistic regression analysis revealed that homosexuality was positively correlated with the number of older brothers, but not with the number of older sisters, younger brothers, or younger sisters. There is no consistent evidence that birth order and sexual orientation are related in women (e.g., Bogaert, 1997).

Adult height and weight have also been investigated within the context of the development of sexual orientation, with some evidence that homosexual men are shifted toward the pattern typical of the opposite sex—that is, homosexual men are shorter and lighter— relative to heterosexual men (e.g., Blanchard & Bogaert, 1996a; Bogaert & Blanchard, 1996; but see Bogaert & Friesen, 2002). Although alternative explanations exist, these differences provide some support for a biological origin of sexual orientation, insofar as height and weight are influenced by biological factors such as prenatal hormones. This is particularly the case for the height difference, because final adult height is relatively stable after the cessation of pubertal growth and is therefore not affected by most medical, psychological, or environmental influences (Underwood & Van Wyk, 1992).

A developmental variable related to adult height and weight is birth weight. Research indicates that adult height and weight are correlated with weight at birth, with a lower birth weight predicting a shorter stature and a lighter weight in adults (e.g., Ijzerman, Stehouwer, van Weissenbruch, De Geus, & Boomsma, 2001; Sørensen et al., 1999). There is evidence that this association between birth weight and adult size is influenced by both nongenetic intrauterine events and genetic factors (Ijzerman et al., 2001). There is also recent evidence that birth weight and birth order interact to predict sexual orientation (Blanchard & Ellis, 2001; Blanchard et al., 2002). For example, Blanchard et al. found that prehomosexual boys with two or more older brothers had lower birth weights than preheterosexual boys with two or more older brothers; prehomosexual boys with fewer than two older brothers did not differ from preheterosexual boys with fewer than two older brothers in birth weight. These results provided support for a biological explanation underlying the fraternal birth order effect. In particular, Blanchard et al. interpreted these results as evidence for a maternal immune reaction to a factor (or factors) involved in male fetal development, thus affecting both the brain and the body of later born prehomosexual male infants.

If such a biological explanation (e.g., maternal immune response influencing intrauterine events) does indeed underlie the fraternal birth order and birth weight phenomena, one might expect the effect to generalize to other aspects of physical development and, for example, to be detectable in adult body size, which, as mentioned, is related to birth weight. Thus, one might expect that fraternal birth order and adult body size would interact to predict sexual orientation, such that later born homosexual men should be shorter and lighter than later born heterosexual men. Indeed, these putative interactions may account for why height and weight have

This research was supported by Social Sciences and Humanities Research Council of Canada Grant 410-99-0521 to Anthony F. Bogaert. I thank Ray Blanchard and Carolyn Hafer for their help at various stages of this research.

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been only inconsistently related to sexual orientation in past research, that is, strong main effects for height and weight may have been masked by an interaction with fraternal birth order.

In the present research, I tested for an interaction between fraternal birth order and adult body size in a Canadian sample of homosexual and heterosexual men closely matched on key demographics (Blanchard & Bogaert, 1996b; Bogaert & Blanchard, 1996). This sample contains information on birth order (e.g., older brothers), height, and weight. Previous analyses of these data showed that homosexual men had a greater number of older brothers (Blanchard & Bogaert, 1996b) and were shorter and lighter (Bogaert & Blanchard) than heterosexual men, but the interaction of fraternal birth order and body size was not examined. It is also noteworthy that this sample contains information on parental height. Thus, the present study was also able to assess whether certain genetic factors related to familial height might underlie an interaction between fraternal birth order and body size.

Method

Participants

In May 1994 to February 1995, short questionnaires were administered to 877 homosexual and heterosexual men, aged 18–82 years, in Toronto and surrounding cities in southern Ontario. Homosexual men were recruited at a meeting of a homosexual community/service organization and the Toronto Lesbian and Gay Pride Day parade in the summer of 1994. Demographically similar heterosexual men were recruited through regional service clubs and other organizations and through advertisements posted on two university campuses. All participants received $10, either as a direct payment for themselves or with the understanding that payment would be made to designated charities on their behalf (see Blanchard & Bogaert, 1996b, for more details about the participants).

Materials and Measures

Sexual orientation was assessed by a question that asked participants whether they were heterosexual, bisexual, or homosexual. Only 14 participants classified themselves as bisexual, and they were placed in the homosexual group.

In addition, all siblings born to the participant's biological mother, together with related information, including the paternity of each sibling and the age at death of each deceased sibling, was recorded. Participants did not record stillborns, but any siblings who died at any point after the birth event were recorded and counted. Paternal half-siblings (i.e., children of the participant's father by a different mother) were not recorded on the questionnaire. Thus, this sample contains men who reported on full siblings and maternal half-siblings, but not paternal half-siblings. Paternal half-siblings do not have the same mother and thus are not relevant for an intrauterine explanation (e.g., maternal immune response) of the fraternal birth order effect.

Participants reported their own, their biological mother's and their biological father's height, either in feet and inches or in centimeters. Figures given in feet and inches were converted to centimeters. The participants also had the choice of reporting their maximum weight during their lifetime in either pounds or kilograms. Figures given in pounds were converted to kilograms. Finally, age, year of birth, education (1 = *less than grade 8* to 8 = *grad/professional school*), and ethnicity/race (1 = *White* to 7 = *other*) were recorded.

Results

I selected from the original 877 men only those who were nontwins, had complete sibling data, and knew all the children born to their biological mother. In total, 352 homosexual/bisexual men and 484 heterosexual men met these criteria. These men were considered the full sample. In this full sample, the heterosexual group was older (M = 39.2 years, SD = 12.5 years) than the homosexual group (M = 37.59 years, SD = 9.97 years), $t(827.02) = 2.07$, $p = .04$ (two-tailed). They also had marginally fewer siblings (M = 2.30, SD = 1.78) than the homosexual group (M = 2.55, SD = 2.03), $t(694.86) = 1.85$, $p = .07$ (two-tailed), and a marginally earlier year of birth (M = 1954.60, SD =12.65) than the homosexual group (M = 1955.91, SD = 10.01), $t(828.05) = 1.68$, $p = .09$ (two-tailed). However, the heterosexual group did not differ in level of education (6 = *community college*, 7 = *university*; M = 6.78, SD = 1.28) from the homosexual group (M = 6.70, SD = 1.45), $t(683.07) = 0.79$, *ns*. Finally, they also did not differ in race (recoded 1 = *White* and 2 = *non-White*; 94% White) from the homosexual group (92% White), $\chi^2(1, N = 836) = 1.87$, *ns*.

In addition to the full sample, I selected the matched sample used in Blanchard and Bogaert (1996b). Aside from the criteria used in the full sample mentioned above, these men were all White, knew the ages of their biological mother and father, and did not have any maternal half-siblings. Maternal half-siblings were excluded here because, although they share the same biological mother and thus may have contributed to a maternal immune response, they also could have contained different paternally derived fetal antigens and thus may be a source of uncontrolled variance with regard to such a maternal immune response. In this sample, each homosexual/bisexual man was individually matched with a heterosexual man who was born the same year, or at most, 1 year earlier or later. Year of birth is an important confounding variable in birth order research (Hare & Price, 1969). With this matching procedure, the homosexual and heterosexual groups had an identical mean year of birth (1955). The heterosexual and homosexual men also did not differ in number of siblings or educational level (see Blanchard & Bogaert, 1996b, for more details on the matched sample).

Using the full sample and the same criteria as Blanchard et al. (2002), I compared the heights of homosexual men with two or more older brothers (M = 176.51, SD = 5.81) with those of heterosexual men with two or more older brothers (M = 180.28, SD = 5.80) and the heights of homosexual men with fewer than two older brothers (M = 177.92, SD = 6.60) with those of heterosexual men with fewer than two older brothers (M = 179.20, SD = 6.56), using independent t tests. Both comparisons were significant, $t(111) = 3.36$, $p = .001$ and $t(721) = 2.57$, $p = .01$, respectively, with homosexual men, on average, being shorter than heterosexual men (see also Bogaert & Blanchard, 1996). However, the height difference between homosexual and heterosexual men was greater in men with two or more older brothers (3.77 cm) than in men with fewer than two older brothers (1.28 cm). To confirm this difference, I tested the significance of the interaction term (Older Brothers × Height), after entering older brothers and height as main effects, in a logistic regression analysis predicting sexual orientation (1 = *heterosexual*, 2 = *homosexual*). The interaction

was significant ($p = .03$, two-tailed), suggesting that homosexual men's lesser height becomes pronounced with a higher number of older brothers (see Figure 1).[1] Note that controlling for the demographics related to sexual orientation (age, number of siblings, year of birth) and mother's and father's height did not appreciably alter the significance of the interaction term ($p = .02$, two-tailed).

I also tested the significance of this interaction, along with mother's and father's height, in the matched sample. This result was also significant ($p = .04$, two-tailed), even though the matched sample was smaller than the full sample and the power to detect significant effects was weaker.

Finally, no interaction effects occurred when general birth order (i.e., both older brothers and older sisters) was examined in both the full and matched samples (both ps $> .20$), suggesting that this interaction effect is carried by older brothers and not, for example, older sisters.

Using the full sample and the same criteria as Blanchard et al. (2002), I also compared the maximum weights of homosexual men with two or more older brothers ($M = 81.27$, $SD = 14.82$) with those of heterosexual men with two or more older brothers ($M = 89.33$, $SD = 15.24$) and the maximum weights of homosexual men with fewer than two older brothers ($M = 81.29$, $SD = 15.08$) with those of heterosexual men with fewer than two older brothers ($M = 87.77$, $SD = 15.48$) using independent t tests. Both comparisons were significant, $t(111) = 2.87$, $p = .005$ and $t(720) = 5.58$, $p < .001$, respectively, with homosexual men, on average, being lighter than heterosexual men (see also Bogaert & Blanchard, 1996). Like height, the difference in maximum weight between homosexual and heterosexual men was nominally greater in men with two or more older brothers (8.07 kg) than in men with fewer than two older brothers (6.48 kg), but testing this difference more formally in both the full and the matched samples using logistic regressions did not indicate significant interactions (both ps $> .5$). Thus, homosexual men's lesser maximum weight did not become significantly more pronounced with a higher number of older brothers.

Discussion

The present study demonstrated that the fraternal birth order effect, observed in many previous studies, interacts with height, such that homosexual men's lesser height relative to heterosexual men becomes more pronounced with a higher number of older brothers. No such interaction effects occurred for maximum weight. Null results for maximum weight are perhaps understandable because this characteristic is more variable and more open to many influences than height across the lifespan. Thus, the fraternal birth order effect is linked with a relatively stable biological marker (i.e., height), and, like the studies on birth weight and fraternal birth order (Blanchard & Ellis, 2001; Blanchard et al., 2002), this result suggests that the fraternal birth order effect may reflect a biological mechanism that affects not only sexual orientation but also aspects of physical growth and development. Moreover, the present study suggests that the putative biological mechanism underlying the fraternal birth order effect may have an effect on physical development that lasts and is detectable into adulthood, that is adult stature.

Figure 1. Mean (±*SEM*) height for homosexual and heterosexual men by number of older brothers.

One biological mechanism that could explain these results is a maternal immune response to succeeding male pregnancies (Blanchard & Bogaert, 1996b; Blanchard & Klassen, 1997; Ellis & Ames, 1987; MacCulloch & Waddington, 1981). This explanation is based partly on the idea that a woman's immune system would appear to be capable of "remembering" the number of male (but not female) fetuses she has previously carried and of progressively altering its response to the next fetus according to the current tally of preceding males. A mother's body may have a "memory" for male fetuses because her immune system can interpret male fetuses as foreign objects and hence as threats. Evidence that male fetuses can be interpreted by a mother's immune system as threats include an elevated production during pregnancy of maternal alpha fetoproteins, substances that may defend her from hormones produced by a male fetus (see Chen, Lin, & Huang, 1994 for evidence of heightened alpha fetoproteins during male pregnancies). Evidence that mothers' immune systems actually attempt to attack male fetuses and that this attack may increase over succeeding male pregnancies includes the fact that placental weight for male newborns (but not female newborns) increases with each proceeding male pregnancy (e.g., Vernier, 1975). It has been argued that the placental size increases in preparation for, or in actual response to, immune system attacks (Zuckermann & Head, 1985). There is also evidence of higher levels of maternal antibodies in the sera of male fetuses than female fetuses (e.g., Gualtieri & Hicks, 1985). Presumably, female fetuses are not as likely as male fetuses to be interpreted as threats and hence induce an immune response because the mothers themselves are female. If the immune hypothesis is correct, then the connection between the mother's immune reaction and the child's future sexual orientation would likely be some effect of maternal antibodies on the sexual differentiation of the fetal brain. Early formulations of a maternal immune response for male homosexuality concentrated on testosterone as the relevant fetal antigen, but more recent formulations focus on male-specific, Y-linked H–Y antigen or male-specific cell-surface proteins (e.g., protocadherins) as the relevant fetal antigen (Blanchard,

[1] The difference in height between homosexual and heterosexual men with no older brothers (1.39 cm) and one older brother (1.06 cm) was not significant, as tested by an interaction term ($p > .35$); thus, a height difference between homosexual and heterosexual men did not become significantly more (or less) pronounced from zero to one older brother.

2001; Blanchard & Bogaert, 1996b; Blanchard et al., 2002; Blanchard & Klassen, 1997).

Such an immune response may explain why homosexual and heterosexual men with no or perhaps one older brother do not have as pronounced a height difference as heterosexual and homosexual men with two or more older brothers. Homosexual men with no or perhaps one older brother may not have been affected, or had a lower probability of being affected, by a maternal immune response; instead, other factors may be more relevant. For example, evidence exists that sexual orientation is at least partly genetically based (e.g., Bailey, Dunne, & Martin, 2000; Kendler, Thorton, Gilman, & Kessler, 2000), and some of these "gay genes" may operate independently of a maternal immune response. Of course, this is not to say that other genes do not play some role in a maternal response. For example, because of genetic factors, some mothers may be more reactive than other mothers to male antigens and/or some fetuses may be more susceptible than other fetuses to a maternal immune response. However, such genes are not likely the ones that are involved in or related to familial height, given that parental height was unrelated to the interaction between older brothers and height.

One issue of the present study is that the heights of the participants were self-reported and thus not objectively measured. However, in past research self-reported height has correlated highly with objective measures of height (Himes & Roche, 1982), and self-reported height has been used in numerous studies of growth and development (e.g., Pietiläinen et al., 2001; Yarbrough, Barrett-Conner, & Morton, 2000). Moreover, the height results in the present study evince a pattern very similar to the findings on birth weight, which have been assessed with objective measures (Blanchard et al., 2002).

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Received May 13, 2002
Revision received August 8, 2002
Accepted September 17, 2002 ■

Neuropsychology
2007, Vol. 21, No. 1, 141–148

0894-4105/07/$12.00 DOI: 10.1037/0894-4105.21.1.141

Extreme Right-Handedness, Older Brothers, and Sexual Orientation in Men

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Two of the most consistent correlates of sexual orientation in men are handedness and fraternal birth order (i.e., number of older brothers). In the present study, the relationship among handedness, older brothers, and sexual orientation was studied in 4 samples of heterosexual and gay or bisexual men (N = 944). Unlike previous studies, which have only observed an increased rate of non-right-handedness in gay or bisexual men relative to heterosexual men, an elevated rate of extreme right-handedness was found in gay or bisexual men relative to heterosexual men. The results also demonstrated that older brothers moderate the relationship between handedness and sexual orientation. Specifically, older brothers increase the odds of being gay or bisexual in moderate right-handers only; in both non-right-handers and extreme right-handers, older brothers do not affect (or decrease) the odds of being gay or bisexual. The results have implications for an early neurodevelopmental origin to sexual orientation in men.

Keywords: sexual orientation, handedness, older brothers, FBO

Evidence that handedness is related to sexual orientation suggests a biological (e.g., prenatal) basis to sexual orientation, because handedness is a marker of early neurodevelopment. For example, hand preference seems to develop very early in life, even being observed prenatally (e.g., Hepper, Shahidullah, & White, 1991). It is also linked to other correlates of early development, including cerebral laterality (e.g., Bryden & Steenhuis, 1991) and, in the case of non-right-handedness and extreme right-handedness, fluctuating asymmetry (e.g., Yeo, Gangestad, & Daniel, 1993) and some neurodevelopmental problems (e.g., Coren, 1993; Previc, 1996).

Lalumière, Blanchard, and Zucker (2000) conducted a meta-analysis of studies examining a handedness/sexual-orientation relationship and found that gay men and lesbians tended to have elevated levels of non-right-handedness relative to heterosexual comparisons. Gay men and lesbians had 39% greater odds of being non-right-handed than their heterosexual counterparts. The effects were found to be stronger in women (e.g., 91% increase in odds) than in men (e.g., 34% increase in odds). This meta-analytic review was important because the literature was conflicted, with a number of large-scale studies, particularly in men (e.g., Bogaert & Blanchard, 1996), showing null results.

There are two plausible reasons why the handedness/sexual-orientation relationship has been inconsistent in men. The first is that the fraternal-birth-order (FBO) effect—the finding that gay men have a higher number of older brothers relative to heterosexual men—complicates the handedness/sexual-orientation relation. The FBO effect is one of the most reliable correlates of sexual orientation in men (for reviews, see Blanchard, 1997, 2004; Bogaert, 2002). Bogaert (2006) recently established that the FBO effect is very likely the result of a biological (e.g., prenatal) mechanism. The maternal immune response theory (see Blanchard, 2004; Blanchard & Bogaert, 1996; Blanchard & Klassen, 1997) is the most developed of the biological explanations to account for the FBO or older brother effect. In the same way that a mother who is Rh negative (Rh−) may develop an immune response after carrying and giving birth to an Rh positive (Rh+) fetus that affects subsequent Rh+ fetuses, some mothers may eventually become "immunized" to a factor or substance important in male fetal development. This immune response may eventually affect later male pregnancies, causing an alteration in brain development (e.g., hypothalamus) that affects sexual orientation. Thus, the maternal immune hypothesis argues that an antimale antibody crosses the placental barrier and alters the brain and that this effect is most likely to occur in (gay) men with older brothers.

The FBO effect complicates the handedness/sexual-orientation relation because it seems to set the condition(s) under which handedness reliably predicts sexual orientation. Using a large number of participants from a combination of samples, Blanchard, Cantor, Bogaert, Breedlove, and Ellis (2006) recently found that an increase in non-right-handedness in gay men only occurs in men with no (or very few) older brothers. Gay men with a high number of older brothers did not show evidence of an increased rate of non-right-handedness. Thus, in samples not examining the conditional effect of older brothers, the results are likely to be weak or inconsistent.

Second, the handedness/sexual-orientation relationship (in men) may be inconsistent because there may be a linkage between sexual orientation and extreme right-handedness. Extreme right-handedness, like non-right-handedness (e.g., left), may represent an early neurodevelopmental shift away from moderate right-handedness. One likely reason for this shift is early neurodevelopmental anomalies (Kim, Yi, Son, & Kim, 2001; Yeo & Gangestad, 1993; Yeo et al., 1993). For example, Yeo et al. (1993) showed that extreme right-handedness is associated with minor

This research was supported in part by grants from the Social Sciences and Humanities Research Council of Canada and the Chancellor's Chair for Research Excellence at Brock University to Anthony F. Bogaert. I thank R. Blanchard, A. Bradley, N. Culp, C. Hafer, C. Hopkins, C. Irwin, L. K. Jamieson, and J. Zavitz for their help at various stages of this research.

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physical anomalies and fluctuating asymmetry, likely associated with prenatal events. More recently, Kim et al. (2001) found evidence that early neurodevelopmental problems (e.g., right temporal lobe epilepsy) shifts some moderate right-handers to extreme right-handers (Kim et al., 2001). Thus, early neurodevelopmental issues may cause impairment of the left hand—just as they may cause impairment of the right hand—and this may cause a shift from moderate to extreme right-handedness. Early neurodevelopmental stressors (i.e., developmental instability) have been argued to be the most likely cause of increased non-right-handedness in gays and lesbians (Lalumière et al., 2000; but see Hatfield, 2006; Klar, 2004). As such, along with elevated non-right-handedness in gays and lesbians, there could be an increased level of extreme right-handedness in these groups. Thus, in sexual-orientation studies in which moderate right-handedness and extreme right-handedness are not distinguished—again, the vast majority of them—the handedness results may be weak or inconsistent. Yet, to my knowledge, no study has demonstrated that extreme right-handedness is indeed more common in gays and lesbians relative to heterosexual people (c.f. Herman-Jeglinksa, Dulko, & Grabowska, 1997; Lippa, 2003).

In the present study, the relationship between handedness and sexual orientation was studied with an emphasis on the examination of an elevated rate of extreme right-handedness in gay or bisexual men. In addition, the role of number of older brothers was examined. Given the moderating role of older brothers found by Blanchard et al. (2006), this may complicate the (possible) relationship between extreme right-handedness and sexual orientation in men. Perhaps the older brother effect only occurs in moderate right-handers, and thus, like non-right-handed gay or bisexual men, extreme right-handed gay or bisexual men may not show an older brother effect. These possibilities are examined.

Method

Participants

A total of 944 gay or bisexual and heterosexual men, from four different samples from my lab (Bogaert, 2000, 2001, 2006; Hafer, Bogaert, & McMullen, 2001) were used in this study. Two of the samples (Bogaert, 2000, 2001) were composed of heterosexual undergrads from Brock University in the Niagara region of Canada ($N = 141$). They were recruited primarily for studies on sexual attitudes. A third sample (Hafer et al., 2001) was composed of gay or bisexual men from Toronto and the Niagara region of Canada ($N = 282$). They were a community sample and were recruited primarily to explore sexual health issues. The fourth was a community sample of gay or bisexual and heterosexual men ($N = 521$) raised in nonbiological or blended families (e.g., raised with half or stepsiblings or as adoptees). These men were recruited from various regions of Canada (Toronto and surrounding regions, Montreal, Vancouver) and composed the main sample in Bogaert (2006). Because gay and bisexual men represent a smaller percentage of the population than heterosexual men, a number of gay-oriented publications were used to advertise for these participants. To recruit heterosexual men, I placed similar ads in general publications in Sample 4 (see Bogaert, 2006, for more details on these samples). Note that, along with two other large samples, these four samples were included in the Blanchard et al. (2006) analyses of the relationship between handedness, older brothers, and sexual orientation. However, the present study is novel because extreme right-handedness, along with the possible moderating role of older brothers on both ends of the handedness distribution, was not examined in Blanchard et al. (2006).

Measures

The men in all four samples were asked for their age and educational level (ranging from 1 = *less than Grade 9* to 8 = *PhD or M.D.*). We determined sexual orientation by using two questions: one concerning the participant's sexual attraction toward men and women (i.e., sexual thoughts and feelings), ranging from 1 (*exclusively homosexual/gay*) to 7 (*exclusively heterosexual/straight*), and the other concerning his sexual behaviors (i.e., actual experiences) engaged in with men and women, ranging from 1 (*exclusively homosexual/gay*) to 7 (*exclusively heterosexual/straight*). These two measures were averaged, and those averaging 1 (*exclusively homosexual/gay*) to 4 (*equally heterosexual/homosexual*) formed the gay and bisexual group ($n = 563$), whereas those averaging greater than 4 up to 7 (*exclusively heterosexual*) formed the heterosexual group ($n = 378$). Three participants were not classified because of missing data on the sexual-orientation measures.

The men also indicated their number of siblings (number of older brothers, number of older sisters, etc.) and whether they were biological (i.e., born from the same mother as the participant) or not. This information is relevant because the most recent evidence (Bogaert, 2006) indicates that only the number of biological (and not nonbiological) older brothers increases the probability of being gay or bisexual.

The handedness measure was a modified version of the Edinburgh Inventory (Oldfield, 1971), which asks about hand usage for 10 physical activities (e.g., writing, throwing a ball, opening a lid). To capture a range of hand usage for these activities, possible responses were 1 (*always right*), 2 (*usually right*), 3 (*both equally*), 4 (*usually left*), and 5 (*always left*). These values were recoded, so that 1 = 10, 2 = 5, 3 = 0, 4 = −5, and 5 = −10. Thus, values could range from 100 (extreme right-handed) to −100 (extreme left-handed), and valid values were in intervals of 5 (e.g., 100, 95, 90, and so on). This scale had a high internal consistency reliability (Cronbach alpha = .95).

Results

Of the original men surveyed, 538 gay or bisexual men and 373 heterosexual men had valid information on age, education, sexual orientation, handedness, and number of biological older brothers. The gay or bisexual men were older ($M = 36.44$, $SD = 11.27$) than the heterosexual men ($M = 29.81$, $SD = 11.83$), $t(909) = -8.55$, $p < .001$; the gay or bisexual men were also less well-educated ($M = 4.79$, $SD = 1.67$) than the heterosexual men ($M = 5.07$, $SD = 1.45$), $t(865.37) = 2.71$, $p = .007$. Given these differences, these variables were included as controls in the main analyses.

Figure 1 presents the distribution of handedness scores plotted against sexual orientation. As expected, a range of handedness scores occurred, but most of the men were largely right-handed. However, it is interesting that this figure also shows that the gay and bisexual group seemed to have a higher incidence of both non-right-handedness (e.g., left-handedness) and extreme right-handedness (i.e., having a maximum score of 100 on the handedness scale) relative to the heterosexual group. To test this more formally, I conducted two logistic regressions with sexual orientation as the criterion (0 = *heterosexual*, 1 = *gay/bisexual*), and handedness, age, and education as the predictors. In these analyses, the handedness predictor was dichotomized, with a comparison of moderate right-handers (coded as 1) against either extreme right-handers (coded as 2) in the first analysis or against non-right-handers (coded as 2) in the second analysis. Moderate right-handers were those scoring 50 through 95 on the handedness scale, extreme right-handers were those scoring 100, and non-right-handers (left or ambidextrous) were those scoring −100

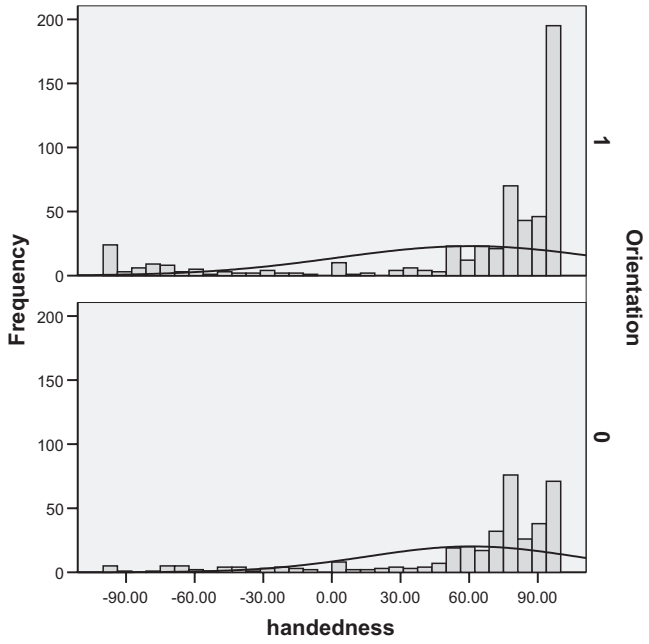

Figure 1. Handedness distribution by sexual orientation (0 = *heterosexual*, 1 = *gay/bisexual*).

through 45. These scores and cutoffs should capture the distinctions among the three handedness categories; they also capture the noticeable discontinuities of frequencies in the distribution of handedness (e.g., between 45 and 50; see Figure 1). The results of these logistic regressions, including odds ratios and 95% confidence intervals, are presented in Tables 1 and 2. As shown, non-right-handedness, although in the predicted direction, was not significantly greater in gay or bisexual men than in heterosexual men. However, extreme right-handedness was significantly ($p < .001$) greater in gay or bisexual men, increasing the odds by 97%.

To examine the possible moderating role of number of older brothers, I added an older brother term and an Older Brother × Handedness interaction term as predictors to these regression models. Significant interactions, similar in nature, occurred in both analyses (see Tables 3 and 4 and Figures 2 and 3). As shown, the number of older brothers increased the likelihood of being gay or bisexual in moderate right-handers only. In both non-right-handers and in extreme right-handers, older brothers either did not increase or lowered the likelihood of being gay or bisexual.

Discussion

The present study provided evidence that gay or bisexual men have an elevated incidence of extreme right-handedness. The results also demonstrated that older brothers moderate the relationship between handedness and sexual orientation. The pattern of this moderation was very similar on both ends of the handedness distribution. Specifically, older brothers increased the odds of being gay or bisexual in only moderate right-handers; in both non-right-handers and extreme right-handers, older brothers either did not affect or lowered the odds of being gay or bisexual. These results not only have implications for the origins of sexual orientation but also clarify the magnitude of two of the most important correlates (older brothers, handedness) of men's sexual orientation. In particular, both correlates may be significantly stronger in predicting men's sexual orientation, at least for certain men, than previously reported. This is because previous to this study (and Blanchard et al.'s 2006 study) it was assumed that the older brother effect was uniformly distributed across all men with different handedness types. But this is not the case—it is restricted to men with moderate right-handedness. Likewise, previous to this study (and Blanchard et al., 2006), the handedness effect was assumed to occur uniformly across all men. But, again, this is not the case—it is restricted to men with no (or few) older brothers. Thus, the magnitude of these effects needs to be reconsidered accordingly.

If elevated extreme right-handedness is an indication of early neurodevelopmental anomalies (e.g., Kim et al., 2001; Yeo & Gangestad, 1993), then an elevation of this handedness pattern in gay or bisexual men gives additional evidence that one route to same-sex attraction is through early developmental stressors or through a factor correlated with such stressors (Lalumière et al., 2000). These early developmental stressors likely have multiple effects on neurodevelopment, causing, for example, a shift away from both typical sexual orientation and handedness patterns.

A genetic explanation can also be forwarded. Handedness may be associated with genetic factors, particularly with, although not exclusively with genes located on the X chromosome (e.g., Annett, 1979; Corballis, 2001; Coren & Porac, 1980; Gangestad et al., 1996; Hatfield, 2006; Jones & Martin, 2000; Klar, 2003; Mc-

Table 1
Logistic Regressions With Demographics and Handedness (Moderate Right vs. Nonright) as Predictors

Predictor	*B*	*SE*	Wald statistic	*p*	e^B	95% CI Lower	95% CI Upper
Age	0.57	0.01	52.89	0.000	1.06	1.04	1.08
Education	−0.07	0.05	1.84	0.175	0.93	0.84	1.03
Handedness	0.18	0.45	0.89	0.344	1.19	0.83	1.71

Note. Wald statistic is the statistical quantity used to determine the significance level of each predictor variable. B = change in the logarithmic odds of being gay or bisexual for a one unit increase in the corresponding predictor, with all the other predictors in the model controlled for; e^B = multiplicative change in the odds of being gay or bisexual for a one unit increase in the corresponding predictor; 95% CI = 95% confidence intervals for the quantity e^B (0 = *heterosexual*, 1 = *gay/bisexual*).

Table 2
Logistic Regressions With Demographics and Handedness (Moderate Right vs. Extreme Right) as Predictors

Predictor	*B*	*SE*	Wald statistic	*p*	e^B	95% CI Lower	95% CI Upper
Age	0.48	0.01	41.42	0.000	1.05	1.03	1.07
Education	−0.07	0.05	1.99	0.159	0.93	0.84	1.03
Handedness	0.68	0.18	14.11	0.000	1.97	1.38	2.81

Note. Wald statistic is the statistical quantity used to determine the significance level of each predictor variable. B = change in the logarithmic odds of being gay or bisexual for a one unit increase in the corresponding predictor, with all the other predictors in the model controlled for; e^B = multiplicative change in the odds of being gay or bisexual for a one unit increase in the corresponding predictor; 95% CI = 95% confidence intervals for the quantity e^B (0 = *heterosexual*, 1 = *gay/ bisexual*).

Keever, 2004; Medland et al., 2005). Twin studies also have shown that sexual orientation is partly heritable (e.g., Bailey, Dunne, & Martin, 2000), and two molecular genetic studies have suggested that a region on the X chromosome (Xq28) is associated with same-sex attraction in men (Hamer, Hu, Magnussion, Hu, & Pattatucci, 1993; Hu et al. 1995; but see Rice, Anderson, Risch, & Ebers, 1999). Thus, certain genes may increase men's likelihood to atypical handedness patterns (i.e., to non-right-handedness and/or extreme right-handedness) and may also increase their likelihood of same-sex attraction. A candidate is the androgen receptor (AR) gene, also found on the X chromosome. Variation in the AR gene has been associated with variations in testosterone. Recently, Medland et al. (2005) showed that left-handedness is associated with variation in the length of the AR gene. Shorter variations (i.e., fewer repeats), usually associated with lower testosterone in men, were associated with left-handedness in one sample of men; longer variations (i.e., more repeats), usually associated with lower testosterone in women, were linked to left-handedness in two samples of women. These findings partly support a theory by Witelson (1991; Witelson & Nowakowski, 1991), who suggested that right-handedness and associated functional asymmetry in males partly occurs as a result of high prenatal testosterone leading to neuronal and axonal loss in the corpus callosum. Variations in prenatal hormones (e.g., testosterone) have also been linked to sexual-orientation development (e.g., Ellis & Ames, 1987), and thus variations in the AR gene may seem plausibly to underlie both same-sex attraction and the atypical handedness patterns in gays and lesbians. However, such an explanation is complicated by a number of factors. One is that the AR gene has been investigated in the context of sexual-orientation development and has not been found to be linked to sexual orientation (Macke et al., 1993). Perhaps variations in the AR gene would be linked reliably to sexual orientation in only subgroups of gay or bisexual men (e.g., non-right-handedness and/or extreme right-handers). A related issue concerns the AR gene and extreme right-handedness. Like most studies on handedness, Medland et al. (2005) did not explicitly examine extreme right-handedness. Perhaps variations in the AR gene (i.e., longer repeats), usually associated with high testosterone, are related not only to moderate right-handedness but also to some forms of extreme right-handedness in men. This possibility is also interesting because there is evidence that at least some gay men may have been exposed to high (rather than low) prenatal testosterone (e.g., Alias, 2004; Bogaert & Hershberger, 1999; McFadden & Champlin, 2000). It would be interesting to see if extreme right-handedness is linked to putative markers of high prenatal testosterone exposure, such as a low 2nd to 4th digit ratio (e.g., Brown, Hines, Fane, & Breedlove, 2001; Manning, 2002), in both heterosexual and gay or bisexual men.

Genetic explanations can also be forwarded to account for the observed interactions, in particular, why older brothers do not increase the odds of being gay or bisexual in men with non-right-handedness and extreme right-handedness. As noted, genes have

Table 3
Logistic Regressions With Demographics, Handedness (Moderate Right vs. Nonright), Older Brothers, and Interaction Term as Predictors

Predictor	*B*	*SE*	Wald statistic	*p*	e^B	95% CI Lower	95% CI Upper
Age	0.56	0.01	51.24	0.000	1.06	1.04	1.07
Education	−0.05	0.06	0.67	0.414	0.96	0.86	1.07
Handedness	0.40	0.22	3.39	0.066	1.49	0.98	2.28
Older brothers	0.87	0.30	8.42	0.004	2.38	1.32	4.28
Handedness × Older Brothers	−0.46	0.22	4.22	0.040	0.63	0.41	0.98

Note. Wald statistic is the statistical quantity used to determine the significance level of each predictor variable. B = change in the logarithmic odds of being gay or bisexual for a one unit increase in the corresponding predictor, with all the other predictors in the model controlled for; e^B = multiplicative change in the odds of being gay or bisexual for a one unit increase in the corresponding predictor; 95% CI = 95% confidence intervals for the quantity e^B (0 = *heterosexual*, 1 = *gay/bisexual*).

Table 4
Logistic Regressions With Demographics, Handedness (Moderate Right vs. Extreme Right), Older Brothers, and Interaction Term as Predictors

Predictor	*B*	*SE*	Wald statistic	*p*	e^B	95% CI	
						Lower	Upper
Age	0.48	0.01	39.90	0.000	1.05	1.03	1.06
Education	−0.05	0.05	0.75	0.385	0.96	0.86	1.06
Handedness	0.94	0.21	20.25	0.000	2.56	1.70	3.85
Older brothers	0.90	0.26	11.70	0.001	2.47	1.47	4.14
Handedness × Older Brothers	−0.49	0.18	7.87	0.005	0.61	0.43	0.86

Note. Wald statistic is the statistical quantity used to determine the significance level of each predictor variable. B = change in the logarithmic odds of being gay or bisexual for a one unit increase in the corresponding predictor, with all the other predictors in the model controlled for; e^B = multiplicative change in the odds of being gay or bisexual for a one unit increase in the corresponding predictor; 95% CI = 95% confidence intervals for the quantity e^B (0 = *heterosexual*, 1 = *gay/bisexual*).

been linked to both handedness and sexual orientation. There is also evidence that specific genes are linked to both handedness and immune system functioning (Coren & Porac, 1980; Gangestad et al., 1996). For example, Gangestad et al. (1996) found that alleles associated with the major histocompatibility complex—the gene complex present in all vertebrate species and responsible for widespread immunological functions—are linked to non-right-handedness. Another candidate is one or more of the genes of the Rh system (e.g., RHD, RHCE), which have been linked to both handedness and immune system functioning (see Hatfield, 2006, for a review). Variations in these genes produce the maternal immune response associated with the Rh factor (i.e., Rh+ vs. Rh−) in the blood, the phenomenon sometimes labeled *hemolytic disease of the newborn*. As noted, this phenomenon is a model for explaining the FBO effect, and the fact that these genes are also linked to handedness makes them intriguing candidate genes to explain the results found here and in Blanchard et al. (2006). Perhaps, then, one or more of these genes predispose a family to atypical handedness but also confer some resistance to the FBO effect (and the maternal immune response presumed to underlie it). However, as in the previous genetic explanation, a complication arises because, to my knowledge, the relationship between Rh genes and extreme right-handedness has not been examined.

A second explanation for these interactions, originally proposed by Blanchard et al. (2006), is as follows: The combination of the developmental factor associated with the older brother effect (e.g., a maternal immune response) and with the non-right-handedness factor (e.g., developmental instability) is strongly teratogenic. Such toxic effects on the developing fetus would make it unavailable for future research, either because it is likely to be aborted or

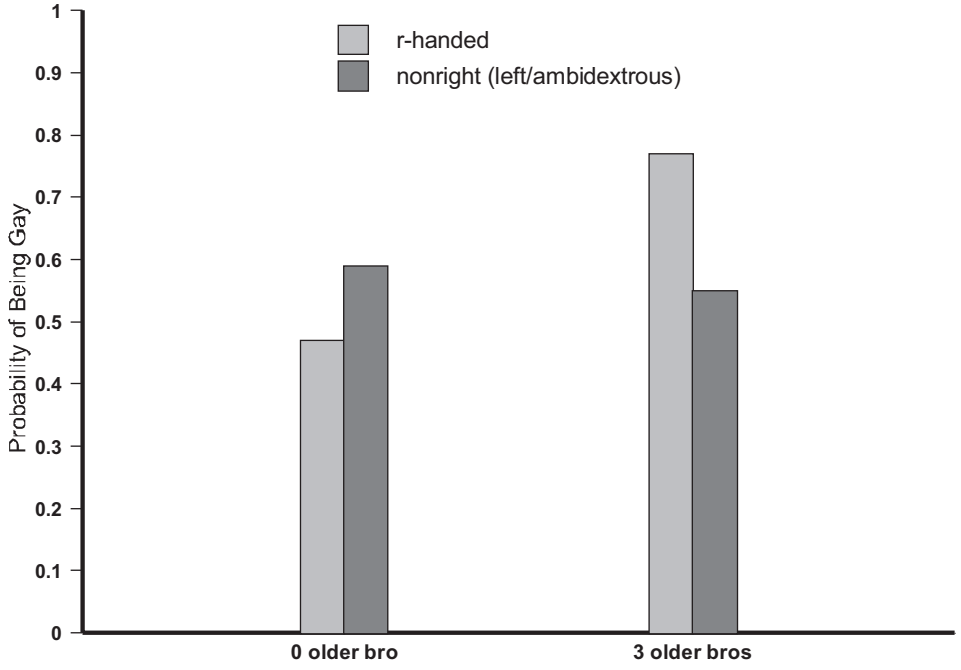

Figure 2. Probability of being gay or bisexual as a function of older brothers and handedness (right-handed [r-handed] versus non-right-handed).

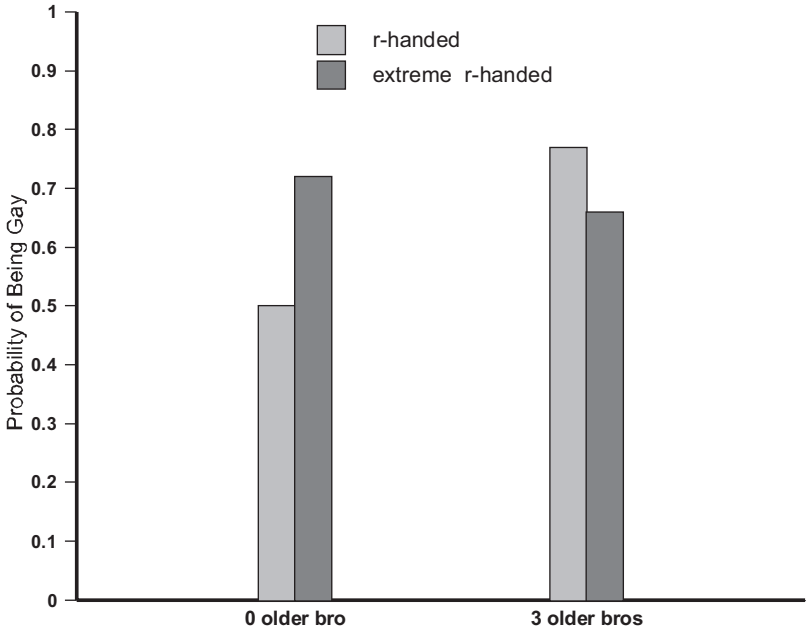

Figure 3. Probability of being gay or bisexual as a function of older brothers and handedness (right-handed [r-handed] versus extreme right-handed).

because it is susceptible to some isolating health condition later in life (e.g., mental retardation). Thus, there would be a reduced number of non-right-handed gay men with older brothers available in sexual orientation surveys. A similar pattern would explain the results for extreme right-handedness: The combination of the older brother factor (e.g., a maternal immune response) with the extreme right-handedness factor (e.g., developmental instability) is also sufficiently teratogenic, to lower the probability that the affected fetus will be available for future research.

If this second explanation of the interactions is correct, one might expect evidence for it in two ways. First, each factor by itself might be expected to have a small but detectable (and potentially) deleterious effect on early development, including possibly fetal loss. There is, as mentioned, research demonstrating that both non-right-handedness and extreme right-handedness are associated with early developmental disorders (e.g., autism, epilepsy, physical anomalies; Kim et al., 2001; Previc, 1996; Yeo et al., 1993). There is also evidence that non-right-handers have fewer children (e.g., McKeever, Cerone, Suter, & Wu, 2000), suggesting that a propensity for non-right-handedness is associated with lower health. I know of no evidence of altered fecundity in parents with extreme right-handedness, however. The other factor (a high number of older brothers) has also been associated with early developmental problems. There is evidence that prior male births is related to lower birth weight in subsequently born boys relative to subsequently born girls, suggesting that a mild maternal immune response reduces birth weight of males but not females (Blanchard & Ellis, 2001). There is also some evidence that a high number of older brothers are related to developmental disorders in subsequent male siblings (e.g., Gaultieri & Hicks, 1985; Lalumière, Harris, & Rice, 1999).

The second (and more direct) way this explanation of the interactions would be supported is by evidence indicating that a combination of the two factors can have a profound effect on the development of the (male) fetus. I know of no evidence directly supporting this possibility, in part because there is likely little, if any, research examining the combination of both factors on human development. However, there is some evidence, albeit limited, that non-right-handedness in parents may be related to a low sex ratio in their offspring (i.e., an excess of female children; James, 1986, 1988; c.f. Lyster & Lloyd, 1987; but see McManus & Bryden, 1992). One interpretation of this trend is that parental hormone levels, which may differ depending on handedness, influence prenatal hormone levels in the womb and subsequently predispose parents to either male or female children (James, 1986, 1988). Another interpretation of these data is that a factor associated with non-right-handedness is particularly deleterious to male fetuses by itself and/or that it is particularly likely to occur in conjunction with a deleterious, male-specific mechanism (e.g., a maternal immune response as a result of previous male births). It would be informative to see if the sex ratio is altered after a first male gestation and birth in parents who have some predisposing factor to non-right-handedness or extreme right-handedness. However, sex ratio is a complex issue with a number of potentially complicating factors. For example, male children run in families (Biggar, Wohlfahrt, Westergaard, & Melbye, 1999) such that there is an increased likelihood of later born boys when the firstborn child is a boy. Thus, it would be necessary to demonstrate a decline in sex ratio after a boy is born from non-right-handed (or extreme right-handed) parents relative to right-handed parents, who may actually have an increase in sex ratio after a boy is born.

One limitation of the present study is that the handedness scale may not adequately assess extreme right-handedness. For example, it has been argued that most handedness questionnaires do not adequately assess extreme right-handedness and that a timed hand-performance task is preferred (e.g., Yeo & Gangestad, 1993).

Thus, this is a limitation that should be noted, but it is also important to keep in mind that the present measure, with a range of options from 1 (*always*) to 5 (*never*), is more likely to capture extreme handedness than many handedness questionnaires, which typically ask for "general" hand preference (e.g., "which hand do you prefer?"). Also, only those with the most extreme scores (100 out of a 100) were considered to have extreme right-handedness; thus, these men are the most likely to have extreme right-handedness on other measures of this characteristic. Moreover, it is worth considering that if extreme scores on this handedness measure are only modestly correlated with (true) extreme right-handedness, then the results when using a preferred method of measuring extreme right-handedness (a hand-performance task) may be stronger. This is because unreliable measures underestimate (not overestimate) true effects. Finally, even if extreme scores (i.e., 100 out of 100) on this scale do not adequately measure extreme right-handedness as defined by, for example, a hand-performance task, the fact remains that gay men have extreme (right-handedness) scores on this self-report measure relative to heterosexual men and have a very similar older brother profile (i.e., fewer) to gay men with non-right-handedness; as mentioned, the pattern of this moderation by older brothers is very similar on both ends of the handedness distribution. Thus, the results are interesting, and the parsimony inherent in their pattern is worthy of explanation.

In conclusion, the main findings—evidence of extreme right-handedness in gay men, along with the moderating effect of older brothers at both ends of the handedness continuum—potentially move forward two important research programs (handedness, birth order) on men's sexual-orientation development. These findings also suggest new research avenues to pursue in both women's and men's sexual-orientation development. For example, this research suggests the need to examine whether certain variables may act as moderators and thus set the condition(s) under which (etiological) factors influence sexual-orientation development. This may explain why some programs of research (e.g., on digit ratios, dermatoglyphics) have been inconsistent in predicting sexual orientation (for a review, see Mustanski, Chivers, & Bailey, 2002).

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Received December 21, 2005
Revision received August 2, 2006
Accepted August 2, 2006 ■

Behavioral Neuroscience
2007, Vol. 121, No. 5, 845–853

0735-7044/07/$12.00 DOI: 10.1037/0735-7044.121.5.845

Interaction of Birth Order, Handedness, and Sexual Orientation in the Kinsey Interview Data

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Recent evidence indicates that 2 of the most consistently observed correlates of men's sexual orientation—handedness and older brothers—may be linked interactively in their prediction of men's sexual orientation. In this article, the authors studied the relationship among handedness, older brothers, and men's sexual orientation in the large and historically significant database originally compiled by Alfred C. Kinsey and his colleagues (A. C. Kinsey, W. B. Pomeroy, & C. E. Martin, 1948). The results demonstrated that handedness moderates the relationship between older brothers and sexual orientation. Specifically, older brothers increased the odds of homosexuality in right-handers only; in non-right-handers, older brothers did not affect the odds of homosexuality. These results refine the possible biological explanations reported to underlie both the handedness and older brother relationships to men's sexual orientation. These results also suggest that biological explanations of men's sexual orientation are likely relevant across time, as the Kinsey data comprise an older cohort relative to modern samples.

Keywords: sexual orientation, older brothers, birth order, handedness, Kinsey

A body of research indicates that sexual orientation may have a biological (e.g., prenatal) origin (e.g., Wilson & Rahman, 2005). Studies showing that brain anatomy and functioning are correlated with sexual orientation support this view (e.g., LeVay, 1991; McFadden & Champlin, 2000; Swaab & Hofman, 1990). For example, homosexual and heterosexual men differ in the size and structure of the third interstitial nucleus of the anterior hypothalamus (Byne et al., 2001; LeVay, 1991). This site is also known to be sexually dimorphic (Allen, Hines, Shryne, & Gorski, 1989; Byne et al., 2001; LeVay, 1991), and animal studies suggest that this region is very important in male-oriented sexual behavior (Grady, Phoenix, & Young, 1965; Rhees, Shryne, & Gorski, 1990). This research supports the theory that human sexual orientation is influenced by prenatal hormones (e.g., testosterone), which have organizing effects on the fetal brain during sexual differentiation (e.g., Ellis & Ames, 1987). In addition, genetic studies also favor a biological origin for sexual orientation. Sexual orientation is partly heritable (e.g., Bailey, Dunne, & Martin, 2000), and studies using molecular genetic techniques have indicated that a region on the X chromosome (Xq28) may be associated with male homosexuality (Hamer, Hu, Magnussion, Hu, & Pattatuci, 1993; Hu et al. 1995; but see Rice, Anderson, Risch, & Ebers, 1999). Recently, researchers have also found evidence that sites on the autosomes may be linked to male sexual orientation (Mustanski et al., 2005).

Studies examining markers of early neurodevelopment, such as handedness (Lalumière, Blanchard, & Zucker, 2000) also support a biological basis of sexual orientation. Hand preference is an important variable within the context of a potential biological (e.g., prenatal) basis of sexual orientation because it is very likely determined early in life and is not usually amenable to large changes after birth. For example, it can be observed prenatally using ultrasound imaging (Hepper, Shahidullah, & White, 1991; Hepper, Wells, & Lynch, 2005). Handedness has also been linked to genes (e.g., Medland et al., 2005), and it has been theorized to relate to prenatal conditions, including prenatal hormones (e.g., Witelson, 1991). In addition, handedness is correlated with characteristics of early neurodevelopment, such as cerebral laterality (e.g., Bryden & Steenhauis, 1991), fluctuating asymmetry (e.g., Yeo, Gangestad, & Daniel, 1993), and some neurodevelopmental problems (e.g., Coren, 1993; Previc, 1996). There is also some evidence that handedness may be linked to cognitive (e.g., spatial) abilities (e.g., Peters, Reimers, & Manning, 2006) and that these abilities may also have an early developmental origin (e.g., Hampson, Rovet, & Altman, 1998). Thus, demonstrating a reliable relationship between handedness and sexual orientation is important for a possible biological (e.g., prenatal) origin of sexual orientation because handedness is a marker of early neurodevel-

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This research was supported by Social Sciences and Humanities Research Council of Canada Grant 410-2003-0943 to Anthony F. Bogaert. We thank Carolyn Hafer and Luanne Jamieson for providing suggestions on a previous draft of this article.

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opment. It is also notable that many of the correlates of handedness (e.g., cerebral laterality, prenatal hormonal profiles, spatial ability) have been linked empirically and/or theoretically to sexual orientation (e.g., Alexander & Sufka, 1993; Ellis & Ames, 1987; McCormick & Witelson, 1991, respectively). Thus, handedness, relative to other markers of early neurodevelopment, is a good candidate to be studied within the context of sexual-orientation development.

Using a meta-analysis of existing studies, Lalumière et al. (2000) established that a reliable relationship does exist between handedness and sexual orientation. Homosexual men and women were found to have 39% greater odds of being non-right-handed relative to their heterosexual counterparts. Prior to this meta-analysis, the literature seemed conflicted, particularly in men; a number of large-scale studies did not show an elevated non-right-handedness in homosexual men (e.g., Bogaert & Blanchard, 1996). Lalumière et al. (2000) reviewed a number of theories (e.g., prenatal hormonal, immunological) to explain why homosexual men and women have elevated non-right-handedness, although they argued the most plausible explanation is because some homosexual men and women may have experienced early developmental instability, which shifts early brain development away from both right-handedness and heterosexuality. Recently, others have argued that genetic factors may best explain this relationship (e.g., Hatfield, 2006).

Another correlate of sexual orientation in men is number of older brothers (or fraternal birth order; FBO). This was first established by Blanchard and Bogaert (1996a, 1996b) but has been replicated since then by these researchers and others numerous times (for a review, see Blanchard, 2004). There is no evidence of a birth-order effect in women (e.g., Bogaert, 1997). The most well-articulated biological explanation of the FBO effect involves a maternal immune response, in which a mother produces an immune response (e.g., antibodies) to a factor (or to factors) in male development over succeeding male pregnancies (Blanchard & Bogaert, 1996a; Blanchard & Klassen, 1997). Factors involved with female development would not be the target of a mother's potential immune response because she herself is female. This immune effect is hypothesized to cause an alteration in (some) later born males' prenatal brain development. Blanchard (2004) argued that the target of the immune response may be male-specific molecules on the surface of male fetal brain cells (e.g., including those in the anterior hypothalamus). Antimale antibodies might bind to these molecules and thus interfere with their role in normal sexual differentiation, leading some later born males to being attracted to men as opposed to women. There is no direct support for the immune hypothesis, but various lines of evidence are in its favor (see Blanchard, 2004; Blanchard & Klassen, 1997; cf., Whitehead, in press), including recent evidence that the FBO effect is very likely prenatal in origin (Bogaert, 2006).

Handedness and FBO have also been recently shown to be linked interactively in their relation to men's sexual orientation (Blanchard, Cantor, Bogaert, Breedlove, & Ellis, 2006). Blanchard et al. (2006) demonstrated that older brothers increased the odds of homosexuality in right-handers only; in non-right-handers, older brothers either did not affect or decreased the odds of homosexuality. In addition, elevated non-right-handedness was restricted to gay men with no older brothers; an increased level of right-handedness occurred in gay men with one or more older brothers. These results are important in at least two ways. First, it gives additional evidence that the FBO effect has a prenatal origin because of its linkage to this important marker of prenatal development. Second, these results are important because they refine the possible biological explanations reported to underlie both the handedness and older brother relationships to men's sexual orientation.

New evidence has been marshaled in support of the reliability of this interaction (Blanchard & Lippa, 2007; Blanchard & Lippa, in press; Bogaert, 2007). For example, Blanchard and Lippa (in press) found evidence for this interaction after using a different methodological and statistical approach than did Blanchard et al. (2006), who used a logistic regression and compared empirical groups (i.e., homosexual vs. heterosexual) with each other to demonstrate this interaction. Blanchard and Lippa (in press-b) compared sibling characteristics of various groups (i.e., non-right-handed homosexual men, right-handed homosexual men, non-right-handed heterosexual men, right-handed heterosexual men) against the highly stable human sex ratio (i.e., the ratio of male live births to female live births). In human populations, the ratio of male live births to female live births is close to 106:100 (Chahnazarian, 1988; James, 1987). If older brothers are elevated in right-handed homosexual men only, then the sibling sex ratio for older siblings (i.e., older brothers to older sisters) should also be elevated in right-handed homosexual men only. This is precisely what Blanchard and Lippa (in press) found: Right-handed homosexual men had an elevated number of older brothers to older sisters (125) relative to the known human sex ratio (106). In contrast, non-right-handed homosexual men had a lower than expected number of older brothers to older sisters (83) relative to the known human sex ratio (106). These results support Blanchard et al.'s (2006) original finding of an interaction between handedness and older brothers and that a higher number of older brothers is associated with homosexuality in right-handers only. However, these supportive results (see also Bogaert, 2007) are partially in contrast to one very large study (Blanchard & Lippa, 2007), which recently provided only qualified support for this Handedness $\times$ Older Brother interaction.

The original Kinsey data (Kinsey et al., 1948; cf., Gebhard & Johnson, 1979) represents a unique opportunity to continue to investigate handedness, older brothers, and sexual orientation. This sample is large and provides a potentially powerful test of interactions that are often difficult to detect (or replicate once detected). In addition, demonstrating any handedness effect—even if it is a conditional one—is important because the Kinsey data are from a different era (1930s–1960s) than all modern samples showing evidence of a relationship between handedness and sexual orientation. Indeed, many of the participants interviewed by Kinsey and his colleagues were born prior to 1900. The Kinsey data, then, represent a rare opportunity to investigate these issues in men who are three or more generations removed from many modern samples. Thus, it could provide evidence of generalizability of a handedness effect and, by implication, evidence of a consistent prenatal influence on sexual orientation across time. It is of note that FBO is the only correlate of men's sexual orientation with a strong tie to prenatal influences that is shown to have this level of generalizability (including correlates found in the original Kinsey data; Blanchard & Bogaert, 1996a). Finally, the original Kinsey data, because of its historical significance and large sample size,

represented a salient failure to replicate the relationship between handedness and sexual orientation in men (Bogaert & Blanchard, 1996). Demonstrating that a handedness effect is indeed detectable in these data (as part of a Handedness × FBO interaction) may then indicate why handedness has been an inconsistent correlate of men's sexual orientation (cf., Lalumière et al., 2000). It would suggest that the relationship between handedness and sexual orientation in men is often not observable as a strong main effect but only as one conditionally linked to the number of older brothers. Thus, it would support the hypothesis that there is a reliable relationship between handedness and sexual orientation but that this effect should be considered within the context of an interaction with older brothers.

Method

Participants

A total of 17,502 case histories taken from 1938 to 1963 are included in the computerized databases of The Kinsey Institute for Research in Sex, Gender, and Reproduction. We restricted the sample to those males 18 years or older who had no convictions for felonies or misdemeanors and who had not resided in a foster home or orphanage during childhood. Men with criminal histories were excluded because they might have spent time in jail (whether ultimately convicted or not) and thus may have had homosexual experiences that did not reflect their basic orientation. Men who lived in foster homes or orphanages were excluded because they might not be able to report accurately on their sibship composition in comparison with those who had intact families. Note that Blanchard and Bogaert (1996a) used these exclusion criteria as well. There were 5,774 men who satisfied these criteria.

Handedness

Hand preference was assessed by the question, "Are you right or left handed?". Four responses were recorded: right-handed (n = 4,106), left-handed (n = 329), ambidextrous (n = 167), and right-handed, retrained from left-handed (n = 27) (Gebhard & Johnson, 1979). The latter three categories were collapsed into non-right-handed (n = 523). Note that missing data occurred in 1,145 men.

Sibling Characteristics and Additional Demographics

Information on sibling characteristics (e.g., number of older brothers, number of older sisters) was collected by the Kinsey interviewers, but all sibling numbers over 8 were collapsed into one category (8+). Twins were not counted in these totals, but deceased siblings were. There was no way to separate biological (full or half) siblings from nonbiological siblings (e.g., stepsiblings). Finally, age in years and education (grades completed) were also assessed in this study.

Sexual Orientation

Consistent with our previous research using the Kinsey data (e.g., Blanchard & Bogaert, 1996a; Bogaert & Blanchard, 1996), men were classified as homosexual or heterosexual using two criteria: amount of homosexual experience and homosexual arousal. Men who reported extensive homosexual experience, defined by Gebhard and Johnson (1979) as more than 20 male sexual partners or more than 50 homosexual experiences (with one or more partners), were classified as homosexual. Men were classified as heterosexual if they reported either no or rare homosexual experiences, the latter defined by Gebhard and Johnson (1979) as one male sexual partner and/or one to five homosexual experiences. In addition, heterosexual men had to report that they did not experience much or some sexual arousal from thinking or seeing other males. Using these criteria (and having nonmissing data on handedness), we classified 730 homosexual men and 3,225 heterosexual men.

Results

The mean age of the 3,225 heterosexual participants was 29.60 years (SD = 11.06) and that of the 730 homosexual participants was 30.79 years (SD = 10.40). The age difference was obviously quite small in absolute terms, but it was statistically significant because of the large sample size, $t(3{,}953) = -2.66$, $p = .008$. (All p values reported in this paper are two-tailed unless otherwise noted.) One heterosexual participant lacked data on educational achievement (number of grades completed). The mean education of the remainder was 16.14 grades (SD = 3.17) and that of the homosexual participants was 14.73 grades (SD = 3.45). This difference was also statistically significant, $t(3{,}952) = 10.68$, $p < .001$. Despite these differences, the main logistic regression analysis testing for a Handedness × Older Brother interaction did not change appreciably when age and education were controlled, so these variables were not included in the presentation of the results.

There were 646 homosexual and 2,868 heterosexual right-handers, and 84 homosexual and 357 heterosexual non-right-handers. Figure 1 shows the percentage of participants who were homosexual, plotted as a function of their hand preference and their number of older brothers. The figure shows the usual association between increasing numbers of older brothers and increasing probabilities of homosexuality, but only for right-handed men. For non-right-handed men, the curve relating older brothers to homosexuality appears quite different, perhaps even opposite. It should be noted that the capping of the older brothers variable at "three or more" was done solely for the purpose of tidying the graphical display. In all statistical analyses, numbers of older brothers (and older sisters) were analyzed exactly as reported.

Analogous data are shown in Figure 2 for number of older sisters. There is, again as usual, little evidence of any association between a man's number of older sisters and his likelihood of homosexuality. The curve for right-handers is virtually flat, and the curve for non-right-handers shows no discernable trend.

The data in Figure 1 (which resemble those in Figure 1 of Blanchard et al., 2006) suggest an interaction between older brothers and handedness with regard to sexual orientation. This was investigated in a series of logistic regression analyses. All these analyses used the same criterion variable—sexual orientation—which was dichotomously coded as 0 (*heterosexual*) or 1 (*homosexual*).

The first logistic regression analysis investigated whether regression lines fitted to the data shown in the figure would in fact differ significantly in slope between right-handed and non-right-handed men. This analysis was carried out on all 3,955 partici-

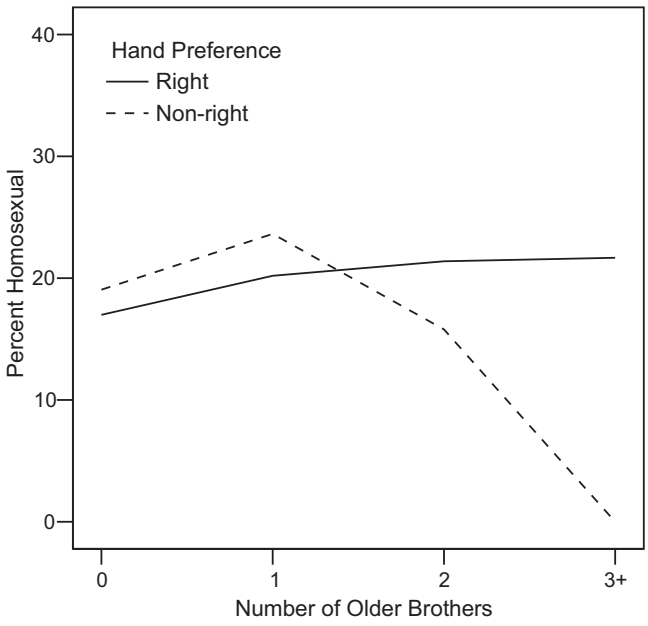

Figure 1. Percentage of participants who were homosexual, plotted as a function of older brothers and handedness.

pants. There were three predictor variables. The first was the participant's number of older brothers, which was treated as a continuous variable. The second was hand preference, which was treated as a categorical variable. This variable was deviation coded −1 (*right-handed*) or +1 (*non-right-handed*). The third predictor was the product of handedness and older brothers, which carried the interaction of these variables. Table 1 shows the results with all predictors in the model.

In Table 1 (and similarly in Tables 2–4), each coefficient (*B*) represents the change in the log odds of homosexuality for a one-unit increase in the corresponding predictor, controlling for all other predictors in the model. The next column presents the standard error for each *B*. The Wald statistic and its associated degrees of freedom were used to determine the *p* value of each predictor variable. The quantity (e^B) is the multiplicative change in the odds of homosexuality for a one-unit increase in the corresponding predictor, and thus $100 \times (e^B - 1)$ represents the percentage change in the odds for a one-unit increase in that predictor.

Main effects in logistic regression are generally not interpreted with their interaction terms in the model, and our conclusions regarding older brothers per se are taken from other analyses presented shortly. The single important result from Table 1, therefore, is that there was a statistically significant interaction between number of older brothers and handedness. As shown in the table, the *p* value based on the Wald statistic was .04. Estimating *p* from the Wald statistic, however, can be less accurate than estimating *p* from another statistic, namely, the change in −2 log likelihood when a variable (in this case, the interaction term) is added to or removed from the model containing the other predictors. The latter method yielded a slightly lower probability for the Handedness × Older Brothers interaction, $\chi^2(1, N = 3{,}955) = 4.67$, $p = .03$. Thus, both methods led to the conclusion that older brothers have different effects on right-handed and non-right-handed males.

Table 1 shows that the main effect for older brothers was not significant when the full group of participants was used and when the handedness and the Handedness × Older brothers terms were included in the model. The second and third analyses tested whether the relations between older brothers and sexual orientation (see Figure 1) would be statistically significant within groups who shared the same hand preference.

The second analysis was carried out on the 3,514 right-handers only. The sole predictor was the participant's number of older brothers. The results (top row of entries in Table 2) yielded a significant odds ratio of 1.11 for older brothers, which means that each older brother increased the odds of homosexuality by about 11%. It must be stressed that this result cannot be considered a replication because the sample includes previously published data (e.g., Blanchard & Bogaert, 1996a). The magnitude of the odds ratio (1.11) was smaller than usual (e.g., Blanchard & Bogaert, 1996b).

The third analysis was similar to the second one except that it was carried out on the 441 non-right-handers only. The results are the bottom row of entries in Table 2. Among non-right-handers, older brothers lowered rather than raised the odds of homosexuality, but the relation was not statistically significant.

In summary, we could reject the hypothesis that the slope of the line relating older brothers to sexual orientation is zero for right-handed participants, and we could reject the hypothesis that the slope is the same for right-handed and non-right-handed participants. We could not reject the hypothesis that the slope is zero for the non-right-handed participants, despite the negative-tending curve suggested by Figure 1.

We conducted a similar set of logistic regression analyses, substituting older sisters for older brothers. The results are shown in Tables 3 and 4. None of the examined relations even approached statistical significance.

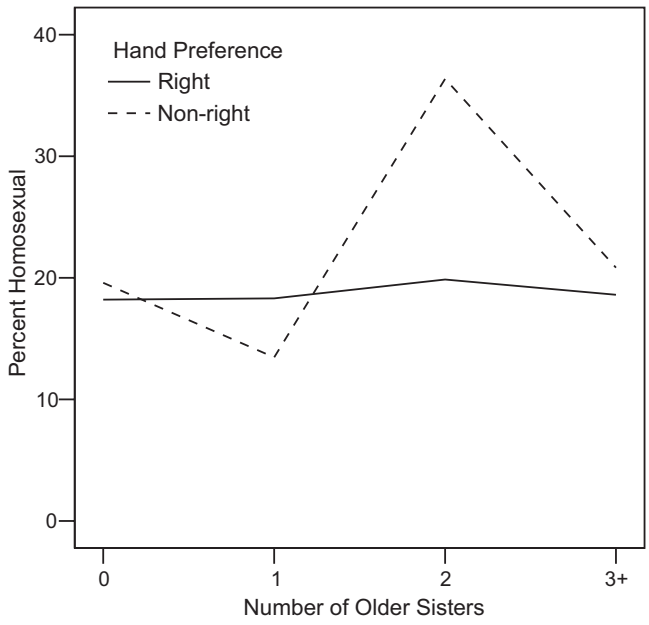

Figure 2. Percentage of participants who were homosexual, plotted as a function of older sisters and handedness.

Table 1
Logistic Regression of Sexual Orientation on Number of Older Brothers and Hand Preference for All Participants

Predictor	*B*	*SE*	Wald statistic	*df*	*p*	e^B
Older brothers	−0.06	0.08	0.53	1	.47	0.95
Handedness	0.11	0.08	2.22	1	.14	1.12
Handedness × Older Brothers	−0.16	0.08	4.10	1	.04	0.85

The foregoing analyses involved comparing empirical groups with each other. Additional information about the relations among handedness, sexual orientation, and older brothers was sought by comparing each of these groups with the well-established and highly stable human sex ratio, which, as mentioned, approximates 106 male live births to 100 female births (Chahnazarian, 1988; James, 1987).

Table 5 shows the data of interest: the sibling sex ratio for each group. The sibling sex ratio is usually calculated as the ratio of brothers to sisters collectively reported by a given group of persons. In the present study (as in Blanchard & Lippa, in press, and Williams et al., 2000), it was calculated as the ratio of older brothers to older sisters. The ratio of older brothers to older sisters reported by any group of persons drawn at random from the general population should, like the ratio of brothers to sisters, approach 106 older brothers per 100 older sisters.

The reporting of siblings' sex ratio as the ratio of brothers per 100 sisters is traditional; for the computation of inferential statistics, however, this value is more conveniently expressed as the proportion of brothers rather than the ratio of brothers to sisters (i.e., .515; 106 ÷ 206). The sibling sex ratios presented in Table 5 (converted into proportions) were compared with the general population value (.515) using the *z* approximation to the binomial test.

We predicted, on the basis of much prior research (e.g., Blanchard, 2004, Figure 2), that the sibling sex ratio of the right-handed homosexual men would be higher than 106. We further predicted, on the basis of the findings of Blanchard et al. (2006) and Blanchard and Lippa (in press), that the sibling sex ratio of the non-right-handed homosexual men would be lower than 106. There was no prior empirical or theoretical basis for any directional hypothesis regarding the sibling sex ratio of the non-right-handed heterosexual men, and the sibling sex ratio of the right-handed heterosexual men was expected not to differ from the expected ratio of 106. Rather than report one-tailed tests for the homosexual groups and two-tailed tests for the heterosexual groups, we have simply reported one-tailed tests for all groups.

The observed ratio of older brothers to older sisters was 127 for the right-handed homosexual men. This is significantly higher than the expected value of 106 and thus confirmed our prediction. This finding should not be regarded as a replication, because older sibling sex ratio data from these participants have been reported before (e.g., Blanchard, 2004). The sibling sex ratio for the non-right-handed homosexual men was 81, which is similar in magnitude to the ratio of 83 observed by Blanchard and Lippa (in press). The present finding is not significantly different from the expected value of 106, however. The sibling sex ratios of the heterosexual groups did not differ from the expected value, despite substantial sample sizes.

Table 2
Logistic Regression of Sexual Orientation on Number of Older Brothers, With Separate Analyses for Right-Handed and Non-Right-Handed Participants

Participants	*B*	*SE*	Wald statistic	*df*	*p*	e^B
Right-handed	0.10	0.04	7.03	1	.008	1.11
Non-right-handed	−0.21	0.15	2.01	1	.156	0.81

Discussion

The results indicated that handedness and older brothers (FBO) are linked interactively in their prediction of men's sexual orientation. This was shown by comparing empirical groups with each other but also by the analysis of sibling sex ratio. Older brothers increased the odds of homosexuality in right-handers only; in non-right-handers, older brothers did not affect the odds of being homosexual. These results support recent studies (Blanchard et al., 2006; Blanchard & Lippa, in press; Bogaert, 2007; cf., Blanchard & Lippa, 2007) and indicate that this Handedness × FBO interaction is a reliable finding. It is notable that the magnitude of the odds ratio that we obtained from the main logistic regression for the Handedness × Older Brothers interaction (0.85) in the present study was very similar to the estimate of 0.83 obtained by Blanchard et al. (2006, Table 2).

Given that handedness is an important marker of prenatal development, these findings support the notion that men's sexual orientation is affected by prenatal events. The findings also provide additional evidence that the FBO effect is of prenatal origin because of its apparent linkage to this important marker of prenatal development (see also Bogaert, 2006). These results also suggest that prenatal events are consistently affecting men's sexual orientation across different historical eras of the 20th century. The present handedness effect, moderated by older brothers, was demonstrated in a sample primarily collected during the 1930s and 1940s (see Gebhard & Johnson, 1979; Kinsey et al., 1948), in contrast to the handedness effects demonstrated in the remainder of the samples, which were collected in the modern era (see Lalumière et al., 2000). Thus, the (prenatal) effect on sexual orientation is not idiosyncratic to one generation in the modern era, but is likely an enduring one that cuts across a number of generations of men.

This interaction also necessarily refines the explanations reported to underlie both the handedness and FBO effects on men's sexual orientation. Two of these explanations are genetic in origin. Genes may predispose a family to atypical (e.g., nonright) handedness and also confer resistance to a maternal immune response

Table 3
Logistic Regression of Sexual Orientation on Number of Older Sisters and Hand Preference for All Participants

Predictor	*B*	*SE*	Wald statistic	*df*	*p*	e^B
Older sisters	0.03	0.07	0.17	1	.68	1.03
Handedness	0.01	0.07	0.04	1	.85	1.01
Handedness × Older Sisters	0.02	0.07	0.05	1	.82	1.01

presumed to underlie the FBO effect. A gene of the Rh system (e.g., *RHD*, *RHCE*) may be a good candidate for this type of dual effect, as these genes have been linked to both handedness and immune system functioning (see Hatfield, 2006, for a review). Gene variants of the Rh system underlie the maternal immune response associated with the phenomenon known as hemolytic disease of the newborn. Rh is a factor in the blood, and if a mother is absent for this factor (Rh−), she may develop an immune response to an Rh+ fetus she is carrying, often with deleterious effects on the developing fetus. Hemolytic disease of the newborn has been argued to be a good model for explaining the FBO effect because it entails a powerful maternal immune response against a fetus and it accumulates over succeeding pregnancies. The fact that Rh genes are linked to handedness also makes them intriguing candidates to explain the present interaction between handedness and FBO (see also Bogaert, 2007, for a review).

Another gene-based explanation concerns variants of the androgen receptor (*AR*) gene. Medland et al. (2005) recently found that non-right-handedness in men is associated with shorter variations (fewer repeats) of the *AR* gene. Shorter variations (i.e., fewer repeats) of the *AR* gene are usually associated with lower testosterone in men. This pattern of results supports theory by Witelson (1991; Witelson & Nowakowski, 1991), who suggested that high prenatal testosterone leads to neuronal and axonal loss in the corpus callosum and contributes to right-handedness and associated functional asymmetry in men. Lower levels of prenatal testosterone have also been argued to underlie homosexuality in men (e.g., Ellis & Ames, 1987). Thus, both same-sex attraction and increased non-right-handedness patterns may result from fewer repeats of the *AR* gene because of its association to lower levels of testosterone. However, the *AR* gene has not been linked to sexual orientation (Macke et al., 1993). Perhaps variations of the *AR* gene would be linked reliably to sexual orientation if the present interaction between handedness and older brothers was taken into account. Thus, homosexuality in men may be associated with fewer repeats of the *AR* gene but only in non-right-handed homosexual men. In contrast, more repeats of the *AR* gene (and higher testosterone) would be associated with right-handed gay men, particularly those with older brothers. This latter pattern may occur because more repeats of the *AR* gene (i.e., in right-handers) may increase the vulnerability of a fetus to a mother's potential immune attack.

Table 4
Logistic Regression of Sexual Orientation on Number of Older Sisters, With Separate Analyses for Right-Handed and Non-Right-Handed Participants

Participants	*B*	*SE*	Wald statistic	*df*	*p*	e^B
Right-handed	0.01	0.05	0.08	1	.78	1.01
Non-right-handed	0.04	0.13	0.11	1	.74	1.04

The above-mentioned vulnerability may occur in one of three ways. First, more repeats of the *AR* gene may be correlated with higher levels of male-specific proteins on the surface of brain cells, which have been hypothesized as the targets of an immune attack of the mother against male fetuses (see Blanchard, 2004). Thus, fetal testosterone itself is not likely to be the target of a mother's immune attack because steroid hormones are not typically antigenic, but higher levels of fetal testosterone may be associated with high levels of other male-oriented factors (e.g., male-specific proteins) in sexual differentiation. Second, more repeats of the *AR* gene raise the level of testosterone in utero, which may raise a mother's testosterone and/or other hormones (e.g., cortisol) during pregnancy. There is evidence, for example, for a relationship between maternal and fetal hormones (e.g., Gitau, Adams, Fisk, & Glover, 2005; Meulenberg, & Hofman, 1991). This surge in hormones may alter a mother's immune system (see Van Vollenhoven & McGuire, 1994, for a review of steroid hormones on immune system functioning), which in turn may increase the likelihood of her mounting an immune response against the fetus. Third, higher testosterone in utero may be associated with birth complications, and this may lead to an increased susceptibility of an immune attack by the mother because of, for example, a weakened placenta. None of these three possibilities has any direct support, but if one or more of these possibilities is correct, right-handed fetuses (via more repeats on the *AR* gene) may have increased vulnerability to a mother's potential immune attacks, and if these right-handed fetuses have older brothers, the effect becomes pronounced. Future research assessing possible candidate genes (including variants of the *AR* gene) associated with male homosexuality should include the assessment of participants' handedness and number of older brothers. It is interesting to note that if this speculation is correct, this may also explain why characteristics related to both high and low testosterone are associated with homosexuality in men. Research has indicated that gay men have, on average, more feminine gender role characteristics (e.g., Bailey & Zucker, 1995), but there is also evidence that at least some gay men may have some more masculine characteristics associated with high (rather than low) prenatal testosterone (e.g., Alias, 2004; Bogaert & Hershberger, 1999; McFadden & Champlin, 2000). These more masculine characteristics may reflect higher levels of testosterone in right-handed fetuses before the mother's immune attack affected their sexual orientation.

Blanchard et al. (2006) proposed a third explanation of this interaction, namely that the combination of factors underlying the

Table 5
Sex Ratio of Older Siblings in Heterosexual and Homosexual, Right-Handed and Non-Right-Handed Men

Sample	Participants	Older brothers	Older sisters	Sibling-sex ratio	One-tailed *p*
Heterosexual					
Right-handed	2,868	1,730	1,601	108	.313
Non-right-handed	357	219	186	118	.162
Homosexual					
Right-handed	646	469	368	127	.005
Non-right-handed	84	38	47	81	.126

FBO effect and non-right-handedness may be teratogenic to a developing fetus. There is evidence that non-right-handedness is associated with some neurodevelopmental problems (e.g., Coren, 1993; Previc, 1996) and developmental instability (e.g., Yeo et al., 1993). There is also evidence that a high number of older brothers are related to developmental disorders in subsequent male siblings (e.g., Gualtieri & Hicks, 1985; Lalumière, Harris, & Rice, 1999; but see Benderlioglu & Nelson, 2004). Thus, these factors by themselves may permanently alter the developing fetus (including changing sexual orientation), but in combination, these factors may be particularly deleterious, causing miscarriage or severe and isolating developmental disorders (e.g., mental retardation). If so, a relatively few number of homosexual men with non-right-handedness and with older brothers may be available for research in surveys.

When first examined, the Kinsey database did not reveal a significant relationship between handedness and sexual orientation (Bogaert & Blanchard, 1996). This was a notable null finding amid a number of supportive studies (see Lalumière et al., 2000). The Kinsey data were collected in an era (primarily the 1930s and 1940s) when family size was larger than in modern times. In that era, participants were likely to have had a high number of older brothers relative to participants drawn from most modern Western samples. The converse is also likely true: Kinsey participants were less likely than modern samples to have had no older brothers. Perhaps it is not surprising, then, that an elevated (main) effect for non-right-handedness was not observed for homosexual men in the Kinsey data, as the handedness effect may be restricted to gay men with no older brothers and there are likely fewer of these men in these data relative to those in modern samples. It would be interesting to consider other notable failures to replicate a handedness effect in men in light of this interaction. Perhaps these samples may too have had relatively large family sizes and thus may have revealed a handedness effect only as part of a Handedness × FBO interaction.

Number of older brothers has been more consistently shown to correlate with sexual orientation in men than handedness. Numerous studies from different eras and different countries, including large national probability samples, have shown the effect (for a review, see Blanchard, 2004). However, there have been a few studies that have not shown this effect (e.g., Frisch & Hviid, 2006; cf., Blanchard, in press; Frisch & Hvidd, in press). These null results may have occurred because of idiosyncratic issues related to each sample, but, given the apparent reliability of this interaction, an additional consideration is that an FBO effect, like a handedness effect, may have only been detectable within these (nonsupportive) samples as a Handedness × FBO interaction. Family size (i.e., number of older brothers) is relevant here as well. In some modern samples with small average family sizes, a substantial majority of the participants are likely to have no older brothers, and, thus, an FBO effect may be unlikely to be detected and may only emerge subsumed within an interaction with handedness. Thus, researchers examining the FBO effect in the future should consider handedness as a potentially important moderator variable.

It is important to address how the measures of sexual orientation and handedness in this historically significant data base compiled by Kinsey and his colleagues may differ from modern surveys. The measures used to form homosexual and heterosexual groups were similar to many modern assessments of sexual orientation, using both fantasy–attraction measures and behavior (e.g., Bogaert, 2003). Thus, the measures of sexual orientation are likely comparable across time with many modern surveys. However, unlike most assessments of handedness in contemporary surveys, only one question with four responses was used in the Kinsey interview protocol. Thus, this is a limitation in the present study that should be considered. It is important to note, however, that such a single-item measure of handedness likely correlates well with modern day, multiple-item inventories (e.g., Bryden, 1977). Thus, there is likely no gross departure from current standards of handedness assessment. Also, some contemporary research still assesses hand preference with a single item, namely, "hand used for writing" (see Peters et al., 2006). It is also important to remember that less reliable measures are less (not more) likely to achieve significant effects, and, in consequence, the effects observed in this study may have been stronger if a better measure of handedness was used. One way to improve measurement in future studies is to assess extreme right-handedness, a variation from moderate right-handedness that likely shares a similar etiology to some forms of non-right-handedness (e.g., Kim, Yi, Son, & Kim, 2001; Yeo et al., 1993). Also, like non-right-handedness, extreme right-handedness was recently found to be elevated in homosexual men (Bogaert, 2007). Moreover, like non-right-handedness, extreme right-handedness' relationship to men's sexual orientation was moderated by older brothers, (i.e., in extreme right-handers, older brothers did not affect or decreased the odds of homosexuality; Bogaert, 2007). Thus, it is interesting to consider whether the interactive effects in the Kinsey data may have been even stronger if handedness had been assessed in a finer grained way, allowing for variation in extreme right-handedness.

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Received January 15, 2007
Revision received March 29, 2007
Accepted April 12, 2007 ■

Available online at www.sciencedirect.com
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Personality and Individual Differences 38 (2005) 225–236
PERSONALITY AND INDIVIDUAL DIFFERENCES
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Fetal exposure to prescription drugs and adult sexual orientation

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Received 7 July 2003; received in revised form 1 March 2004; accepted 10 April 2004
Available online 9 June 2004

Abstract

This study was undertaken to determine if prenatal exposure to therapeutic drugs contributes to variations in sexual orientation. Especially suspect were drugs that could affect the delicate balance of sex hormone levels that appear to guide the sexual differentiation of the fetal brain. The recollections of 5102 mothers concerning their use of therapeutic drugs during pregnancy were linked to reports of the sexual orientation of their offspring (as provided by either the offspring themselves or by their mothers). About 14% of the mothers recalled having taken at least one of 19 prescription drugs (or classes of drugs) during their pregnancy. Regarding male offspring, little evidence was found that prenatal exposure to any of these medications was associated with variations in sexual orientation. However, even after controlling for age, education, and self-rated recall ability of the mothers, exposure to two types of drugs was significantly related to sexual orientation among female offspring. One type consisted of amphetamine-based diet pills and the other was comprised of synthetic thyroid medications. A month-by-month analysis revealed that during the first trimester consumption of all prescription drugs was unusually high for mothers of female homosexual offspring. Prescription medications that affect the mother's and/or the female fetuses' developing immune system may alter the feminization/demasculinization of the brain in ways that cause variations in the offspring's adult sexual orientation.

Keywords: Sexual orientation; Prenatal influences; Prescription drugs; Thyroid medications; Diet medications; DES; Progestins

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0191-8869/$ - see front matter
doi:10.1016/j.paid.2004.04.004

1. Introduction

According to the neuroandrogenic theory of sexual orientation, the gender toward whom one is sexually attracted is largely determined by perinatal factors rather than being a preference that individuals learn in a sociocultural context (Ellis & Ames, 1987). More specifically, the neuroandrogenic theory asserts that the level of sex hormones to which fetuses are exposed helps to sexually differentiate the brain as well as the genitals. The brain in turn is assumed to play a central role in masculinizing/feminizing behavior, inclining behavior associated with whether one prefers same or opposite sex members as sex partners.

Sexing of the genitals has been shown to occur primarily between the first and the fourth months of gestation, while sexing of the brain seems to takes place from the third through the seventh months (Ellis, 1996). Due to this timing difference, sex hormones (especially testosterone) may sometimes masculinize/defeminize the genitals without having the same sexing effects on the brain. According to the neuroandrogenic theory, males whose brains failed to be fully masculinized/defeminized will exhibit varying degrees of feminine preferences and behavioral characteristics throughout life. These feminine/demasculine preferences may sometimes include males preferring same sex partners upon sexual maturation (instead of preferring opposite sex partners). Theoretically, the reverse set of circumstances could incline females to be attracted to members of their own gender following the onset of puberty.

Ellis and Ames (1987) proposed that several factors contribute to varying degrees of genital-brain sexing inconsistencies. Among the hypothesized factors were maternal consumption of drugs that influence sex hormone levels during critical periods of fetal development. If this proposal is correct, it should be possible to find differences in consumption of drugs by mothers of homosexuals relative to mothers of heterosexuals.

The present study was undertaken to identify prescription drugs consumed during pregnancy by greater proportions of mothers of homosexuals (and bisexuals) than by mothers of heterosexuals. Drugs that would be especially suspected as having the capability of altering offspring sexual orientation would be those that can affect sex hormone levels.

2. Methods

Five thousand one hundred and two (5102) women provided data regarding their use of various prescription drugs during pregnancy. Most (4839) of these women were recruited by first securing a questionnaire from their offspring, who were college students attending one of 22 universities (20 US and 2 Canadian) between 1988 and 1998 (see Ellis & Cole-Harding, 2001). Among the numerous items of information provided by the offspring were those pertaining to their sexual orientation.

In addition to the 4839 mothers recruited through the college student sample, 264 women were obtained through Parents and Friends of Lesbians and Gays (P-FLAG), an international support group for parents who have homosexual and bisexual offspring. Through special permission of the board of directors for P-FLAG, copies of a version of our mother's questionnaire were sent to 250 United States and Canadian chapters along with requests that the questionnaires be made available to any women willing to participate in our study. No matching questionnaires were

sought from the offspring of the P-FLAG mothers. Instead, the sexual orientation of these offspring was determined by asking the mothers themselves.

Regarding the mothers of the offspring derived from our college student sample, the average age of the mothers was 47.51 (SD = 7.81). For the P-FLAG mothers, it was 59.29 (SD = 7.43). Despite these average age differences, the two groups of mothers were combined in order to make the sample of mothers with homosexual and bisexual offspring as large as possible. This was done because only a few dozen offspring of each sex in the college sample declared themselves to be homosexual or bisexual.

The average years of education for the mothers who were obtained through the college students was 13.62 (SD = 2.45) while that for the P-FLAG mothers was 15.52 (SD = 2.62). Thirty-one percent of mothers of the college students were divorced compared to 36% of the P-FLAG mothers.

The questionnaires used for the two groups of mothers were identical except for the fact that the P-FLAG mothers' questionnaire asked the mothers themselves to categorize their offsprings' sexual orientation. This question was asked on the offsprings' questionnaire in the case of the college students. On both the P-FLAG mothers' and the college offsprings' questionnaires, four categories were provided for identifying the offspring's sexual orientation: *homosexual*, *bisexual*, *heterosexual*, or *uncertain*. To verify the reported sexual orientation, we confirmed that all offspring of the P-FLAG mothers were classified as either homosexual or bisexual. Regarding the data provided on the offsprings' questionnaires, some apparent discrepancies were found between the responses on the sexual orientation question and other questions closely related to sexual orientation, such as whether members of the same or opposite sex are most often featured in ones sexual fantasies. As explained in a forthcoming article, these discrepant responses were excluded from our analysis (Ellis, Robb, & Burke, in press).

To measure maternal consumption of prescription drugs (both oral and injected) during pregnancy, the mothers' questionnaire allowed women to report having taken DES, progestins, and up to two additional prescription drugs during each month of pregnancy. If mothers reported having taken any prescription drugs other than DES or progestins, they were asked to provide the name of the drug, or to carefully describe it and the reason it was taken. Throughout this paper, prescription drug refers to any drug or other substance that was administered or prescribed by a physician.

Mothers reported the number of dosages of the medication they consumed or were administered during each month of pregnancy. From this information, we determined that 697 women (13.7%) took at least one prescription drug sometime during their pregnancy. This relatively small percentage allowed us to perform only two types of analyses: One involved determining whether or not any specific drugs (or types of drugs) were consumed. The other analysis had to do with the month that one or more drugs were taken during each month of pregnancy.

To estimate the statistical significance of our initial group comparisons, we used the χ^2 exact test (SPSS-11.5). In order to control for the effects of extraneous factors, logistic regression was employed.

3. Results

The results are presented in three parts. The first part has to do with identifying which of 19 drugs (or drug categories) were found to differ between the mothers of both male and female

offspring regarding three categories of offsprings' sexual orientation (heterosexual, homosexual, and homosexual/bisexual combined). In the second part of the results, logistic regression is used to compare the drugs taken by the mothers of homosexual and heterosexual offspring of both genders after controlling for mother's age, years of education, and self-rated ability to recall events associated with her pregnancy. Third, we considered the timing of prescription drug consumption for each month of pregnancy.

3.1. Maternal prescription drug consumption and offspring sexual orientation

Table 1 summarizes the results from comparing 19 prescription drugs consumed by mothers relative to the sexual orientation of their male and female offspring. The categories used in the analysis were *heterosexual*, *homosexual/bisexual* combined, and *homosexual* only. Our coverage of the details in Table 1 will focus on comparing mothers of homosexuals and mothers of heterosexuals, since in most cases where significant differences were found the greatest differences were between these two groups. Table 1 shows that when bisexuals were combined with homosexuals, differences between their mothers' use of prescription drugs and use by mothers of heterosexuals were usually diminished at least slightly in statistical significance.

Only five of the 19 drugs (or drug categories) prescribed to mothers during pregnancy were significantly related to their offsprings' sexual orientation, three of which were for male offspring and three for female offspring (with one drug related to both). It should be emphasized that because the sample sizes for most categories of drugs were often exceedingly small (especially in the case of mothers whose offspring were homosexual or bisexual), caution must be exercised in offering interpretations. Because our tests of statistical significance were based on χ^2, caution is especially warranted for cells containing less than five subjects.

Regarding male offspring, the consumption of three substances were significantly related to sexual orientation. One class of such drugs was anti-nausea and vomiting medications, substances reportedly consumed by 7.0% of the mothers of male heterosexuals, but only 4.2% of the mothers of homosexuals ($p = 0.038$). This very surprising finding suggests that such medications could have a "protective effect" with respect to male offsprings' typical sexual orientation.

Another substance was gamma globulin, a naturally produced substances extracted from the blood of other people which contain antibodies to various viruses. Prior to the eradication of measles and related infectious diseases, gamma globulin was fairly often injected into mothers who were exposed to measles during their pregnancy so as to prevent viral-induced birth defects. Our analysis revealed that none of the 1406 mothers of heterosexual males were given gamma globulin during pregnancy, while two of the 215 mothers of homosexual males (0.8%) recalled having been administered this substance. Despite a fairly high level of statistical significance ($p = 0.018$), the small number of mothers in our entire sample who recalled having been administered gamma globulin during pregnancy cautions against making causal inferences at this point.

The third substance that was statistically related to male sexual orientation consisted of amphetamine-based diet pills (primarily Dexedrine and Tenuate Dospan). Whereas 0.6% of mothers of male heterosexuals consumed diet pills during pregnancy, nearly two percent (1.9%) of the mothers of male homosexuals did so ($p = 0.049$).

Table 1
Reported prenatal therapeutic drug consumption by mothers of heterosexuals, homosexuals and bisexuals combined, and homosexuals alone

Drugs consumed during one or more months of pregnancy	Mothers of males—% (*N*)			Mothers of females—% (*N*)		
	Heterosexuals, $N = 1406$	Homo/ bisexuals, $N = 249$	Homosexuals only, $N = 215$	Heterosexuals, $N = 3241$	Homo/ bisexuals, $N = 196$	Homosexuals only, $N = 114$
Antibiotics	1.7% (24)	1.2% (3)	1.4% (3)	2.1% (67)	1.5% (3)	1.7% (2)
Anti-convulsants	0.1% (1)	0% (0)	0.0% (0)	0.2% (6)	0% (0)	0.0% (0)
Anti-diarrheal medication	0.0% (1)	0% (0)	0% (0)	0.1% (3)	0.5% (1)	0.9% (1)
Anti-nausea and vomiting medication	7.0% (98)	4.4% (11)* $p = 0.037$	4.2% (9)* $p = 0.038$	5.9% (192)	4.5% (9)	4.3% (5)
Anti-toxemia medication	0.1% (1)	0% (0)	0% (0)	0.2% (5)	0% (0)	0% (0)
Allergy/cold medication	0.9% (12)	1.6% (4)	1.9% (4)	0.7% (22)	1.5% (3)	0.9% (1)
Birth control pills	0.1% (2)	0% (0)	0.0% (0)	0.0% (1)	0.5% (1)	0.7% (1)
Diethylstilbestrol (DES)	0.6% (8)	0.9% (3)	1.5% (3)	0.6% (19)	2.0% (4)* $p = 0.030$	3.5% (4)** $p = 0.006$
Diet pills	0.6% (8)	1.6% (4)	1.9% (4)* $p = 0.049$	0.2% (7)	1.5% (3)* $p = 0.015$	1.7% (2)* $p = 0.033$
Diuretics	0.5% (7)	0.8% (2)	0.9% (2)	1.4% (45)	1.0% (2)	1.7% (2)
Gamma globulin	0.0% (0)	0.8% (2)* $p = 0.023$	0.9% (2)* $p = 0.018$	0.0% (1)	0.0% (0)	0.0% (0)
Heart medication	0.1% (1)	0% (0)	0% (0)	0.1% (3)	0% (0)	0% (0)
Insulin medication	0% (0)	0% (0)	0% (0)	0.1% (2)	0.9% (1)	0.7% (1)
Migraine medication	0.1% (2)	0% (0)	0% (0)	0.1% (2)	0% (0)	0% (0)
Narcotics	0.3% (4)	0.0% (0)	0.0% (0)	0.5% (15)	0.5% (1)	0.0% (0)
Prednisone	0.1 (1)	0.5 (1)	0.2 (1)	0.0% (0)	0% (0)	0.0% (0)
Progesterone (progestin)	0.7% (10)	0.4% (1)	0.5% (1)	0.8% (25)	1.5% (3)	2.8% (3)
Sedatives	1.1% (16)	2.4% (6)	2.3% (5)	1.0% (32)	0.5% (1)	0.9% (1)
Synthetic thyroid medication	0.7% (10)	0.9% (2)	0.9% (2)	1.0% (31)	3.1% (6)* $p = 0.0137$	5.2% (6)*** $p = 0.001$
Overall use of medications	13.6% (190)	14.1% (35)	14.9% (32)	13.6% (442)	15.39% (30)	19.1% (22)

* Significant difference relative to heterosexuals of the same sex ($p < 0.05$).
** Significant difference relative to heterosexuals of the same sex ($p < 0.01$).
*** Significant difference relative to heterosexuals of the same sex ($p < 0.005$).

Turning to the female offspring, three drugs were administered significantly more often to mothers of homosexuals than to mothers of heterosexuals. One—amphetamine-based diet pills—was just discussed with reference to male offspring. Whereas seven out of 3241 (0.2%) of the mothers of female heterosexuals recalled having taken diet medication during pregnancy, two out of 144 (1.7%) of the mothers of homosexuals did so ($p = 0.033$).

DES was another drug taken more often by mothers of female homosexuals than by mothers of female heterosexuals. This drug was prescribed to millions of pregnant women between the 1940s through the early 1970s, especially for those with histories of miscarriages to help them maintain their pregnancies (Noller & Fish, 1974; Peress, Tsai, Mathur, & Williamson, 1982). However, research in the 1970s and 1980s began to link consumption of DES during pregnancy with uterine cancer in daughters (Miller, Degenhardt, & Sassoon, 1998), testicular cancer in sons (Depue, Pike, & Henderson, 1983) and with breast cancer in mothers (Colton et al., 1993). By the end of the 1970s, prescribing DES to pregnant women had been halted.

In our sample, 0.7% of the mothers of both sexes recalled having been prescribed DES. While no significant links to male sexual orientation were found, more than three times as many mothers of homosexual females took DES during pregnancy as did mothers of heterosexual females (2.8% versus 0.6%). The probability that this difference occurred by chance was quite small ($p = 0.006$). Nevertheless, only four of the 114 mothers of female homosexuals actually took DES.

The third type of prescription medication found statistically associated with female homosexuality consisted of synthetic thyroid medications, primarily Synthroid and Thyroxine. Whereas 31 of the 3241 (1.0%) mothers of heterosexuals took these medications during pregnancy, six of the 144 (5.2%) mothers of homosexuals did so. The probability of this difference having occurred by chance was exceedingly small ($p = 0.001$).

3.2. Controlling for maternal age, education, and recall

Because the present study was non-experimental, one can question whether mothers of heterosexuals differed from mothers of homosexuals and bisexuals in ways that might account for the differences in prescription drug consumption revealed in Table 1. To address this question, we used logistic regression to control for three maternal variables: maternal age, maternal education, and self-rated maternal recall. These three variables were chosen in part because, as noted in the methods section, mothers of the homosexual and bisexual offspring were about eight years older than the mothers of the heterosexual offspring and were more educated by about two years. It is reasonable to believe that the different decades in which most of the mothers of homosexuals would have been pregnant relative to that of the mothers of heterosexuals as well as their somewhat greater years of education could have accounted for some of the differences documented in Table 1.

We were somewhat surprised to find no significant differences in the average amount of self-rated recall of pregnancy by these groups of mothers ($t = 1.011$, $p = 0.310$). Nevertheless, we still considered it prudent to include this as a potentially important control variable.

The results of the logistic regression are shown in Table 2. Because the results shown in Table 1 revealed that in all cases where statistical significance was found, mothers of bisexuals appeared to be roughly intermediate to mothers of homosexuals and heterosexuals, we confined our analysis in Table 2 to a comparison of mothers of homosexuals and mothers of heterosexuals.

Table 2 shows that one drug—Prednisone—was significantly associated with male sexual orientation after imposing the three statistical controls. Prednisone is a adrenocortical steroid that is taken primarily to manage disorders of the immune system. As can be seen by referring to Table 1, this drug was only taken by the mothers of two males in our entire sample: one male was heterosexual and the other was homosexual. While the finding based on logistic regression can be

Table 2
Logistic regression analyses of prenatal therapeutic drug consumption by mothers of heterosexuals and homosexuals after controlling for maternal age, maternal education and maternal recall (how well her memory served her in answering the retrospective questionnaire)

Drugs consumed during one or more months of pregnancy	Mothers of males					Mothers of females				
	B	SE	Wald	*P*	Exp(*B*)	*B*	SE	Wald	*P*	Exp(*B*)
Antibiotics	0.0266	0.6703	0.0016	0.9683	1.0270	0.3607	0.8653	0.1738	0.6768	1.4343
Anti-convulsants	2.4444	22.2404	0.0121	0.9125	11.5241	3.7433	14.2041	0.0695	0.7921	42.2365
Anti-diarrheal medication	3.5285	22.2404	0.0252	0.8739	34.0737	−1.8471	1.1854	2.4279	0.1192	0.1577
Anti-nausea/ vomiting medication	0.0304	0.3997	0.0058	0.9394	1.0308	−0.1542	0.4749	0.1054	0.7455	0.8571
Anti-toxemia medication	4.3472	22.2398	0.0382	0.8450	77.2633	4.5959	15.1016	0.0926	7609	99.0780
Allergy/cold medication	0.2154	0.6990	0.0950	0.7580	1.2404	−0.1422	1.0601	0.0180	0.8933	0.8674
Birth control pills	2.2072	15.6431	0.0199	0.8878	9.0898	−2.1673	1.4260	2.3098	0.1286	0.1145
Diethylstilbestrol (DES)	0.1736	0.8142	0.0454	0.8312	1.1895	−1.1271	0.7075	2.5377	0.1112	0.3240
Diet pills	−1.0157	0.7029	2.0880	0.1485	0.3622	−2.5299	0.8715	8.4270	0.0037***	0.0797
Diuretics	−1.0464	0.8490	1.5189	0.2178	0.3512	−0.4701	0.7470	0.3960	0.5291	73.6268
Gamma globulin	−7.6606	22.2400	0.1186	0.7305	0.0005	4.2990	36.6575	0.0138	0.9066	73.6268
Heart medication	3.7474	22.2399	0.0284	0.8662	42.4105	3.2833	20.6390	0.0253	0.8736	26.6628
Insulin medication	–	–	–	–	–	−2.6420	1.5310	2.9780	0.0844	0.0712
Migraine medication	4.9215	13.3338	0.1362	0.7121	137.212	2.7528	25.8820	0.0113	0.9153	15.6865
Narcotics	5.4596	9.5761	0.3250	0.5686	234.997	3.1823	9.1674	0.1205	0.7285	24.1012
Prednisone	−3.0123	1.4231	4.4807	0.0343	0.0492	–	–	–	–	–
Progesterone (progestin)	0.8105	1.1061	0.5370	0.4637	2.2491	−1.0881	0.6386	2.9033	0.0884	0.3369
Sedatives	−0.8393	0.5852	2.0568	0.1515	0.4320	1.2442	1.2946	0.9237	0.3365	3.4702
Synthetic thyroid medication	0.0071	0.8453	0.0001	0.9933	1.0071	−1.3699	0.4841	8.0084	0.0047**	0.2541
Overall use of medications	−0.2533	0.2409	1.1053	0.2931	0.7763	−0.5654	0.2584	4.7865	0.0287**	0.5681

Note that the negative Betas indicate that non-use of the drug was more prevalent among mothers of homosexuals.
*Significant difference relative to heterosexuals of the same sex ($p < 0.05$).
**Significant difference relative to heterosexuals of the same sex ($p < 0.01$).
***Significant difference relative to heterosexuals of the same sex ($p < 0.005$).

considered interesting inasmuch as Prednisone is similar to the naturally produced stress hormone, cortisol, and prenatal stress has been implicated in the etiology of male homosexuality (Ellis & Cole-Harding, 2001), the sample size is insufficient for the results to be taken seriously at the present time.

Regarding females, the consumption of two classes of drugs were statistically significant after controlling for maternal age, maternal education, and maternal recall. One class consisted of prescription diet pills ($p = 0.0037$) and the other class was synthetic thyroid medications ($p = 0.0047$). Recall that maternal consumption of these two drugs was also very significantly related to the sexual orientation of female offspring before imposing any statistical controls (Table 1). Nevertheless, logistic regression caused maternal consumption of DES to drop from being statistically significant.

3.3. The timing of prescription drug use

Attention is now directed toward the month of pregnancy when prescription drugs might have their greatest impact on offspring sexual orientation. Unfortunately, the number of mothers who took any *one* of the 19 medications during a specific month of pregnancy were too few for meaningful analysis. Therefore, we compared the proportion of mothers who consumed *any* of the 19 types of prescription drugs in terms of whether their offspring were male or female and homosexual or heterosexual. For clarity, drug consumption for offspring of bisexuals were not included in this analysis, but in no case were their results statistically different from the results for mothers of the other groups.

The results are shown in Table 3, with the means presented graphically in Fig. 1. Both the table and graph reveal that maternal consumption of prescription drugs was virtually indistinguishable for mothers of male homosexuals, male heterosexuals, and for mothers of female heterosexuals. Regarding mothers of female homosexuals, however, unusually high rates of prescription drug consumption during the second month of pregnancy ($p = 0.045$) were evident, and there was a trend in the same direction for drug consumption during the third month ($p = 0.075$).

These analyses support the conclusion that female offspring are more vulnerable to alterations in sexual orientation via exposure to a variety of prescription drugs, and suggest that this vulnerability is greatest during the first trimester.

Table 3
Means and standard errors of the mean for average drug dosages consumed during pregnancy by the four groups of mothers

Months of pregnancy	Mothers of male heterosexuals		Mothers of female heterosexuals		Mothers of male homosexuals		Mothers of female homosexuals	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
First	0.055	0.006	0.071	0.004	0.056	0.018	0.087	0.024
Second	0.062	0.007	0.070	0.004	0.074	0.018	0.113	0.025
Third	0.058	0.007	0.074	0.004	0.070	0.018	0.096	0.025
Fourth	0.056	0.006	0.064	0.004	0.061	0.017	0.078	0.023
Fifth	0.045	0.006	0.055	0.004	0.061	0.016	0.700	0.021
Sixth	0.042	0.005	0.050	0.004	0.051	0.015	0.043	0.021
Seventh	0.040	0.005	0.049	0.003	0.056	0.015	0.052	0.020
Eighth	0.042	0.005	0.051	0.004	0.046	0.015	0.052	0.020
Ninth	0.039	0.005	0.047	0.003	0.050	0.014	0.035	0.020

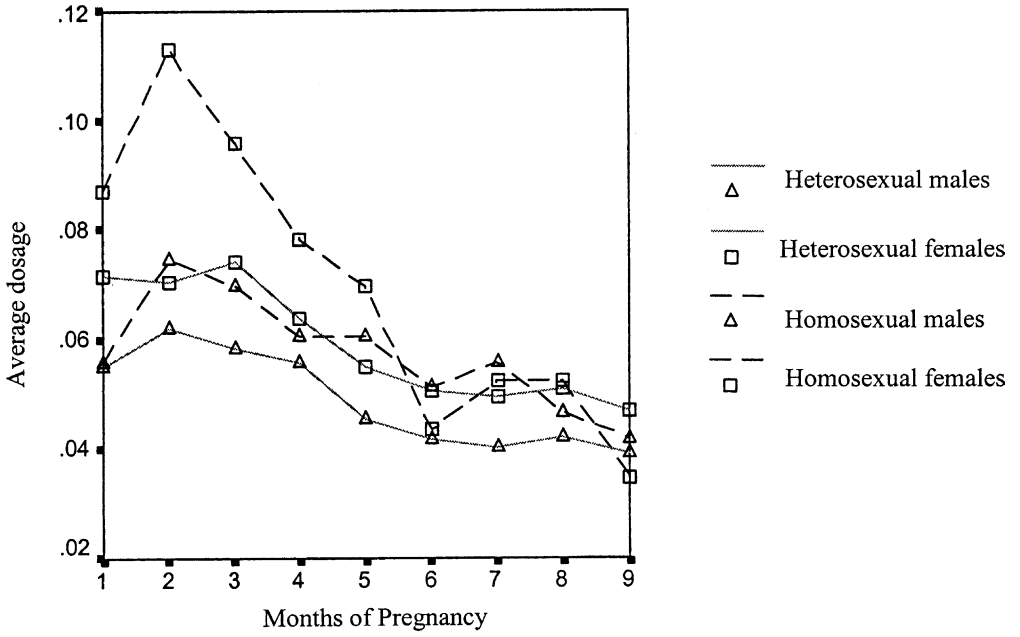

Fig. 1. The average dosage of prescription drugs consumed during each month of pregnancy by mothers of four groups of offspring.

In an earlier analysis of other aspects of the present data set, evidence was found to suggest that exposure to a non-prescription drug—nicotine (via maternal smoking)—was also associated with an elevated probability of homosexual preferences in female offspring but not in male offspring (Ellis & Cole-Harding, 2001). As with the present findings, this link was statistically significant only during the first trimester of pregnancy.

4. Discussion

Despite its exploratory nature, this study's findings could be important. The most noteworthy findings were that if prenatal therapeutic substances affect offspring sexual orientation, the effects are mainly confined to female offspring and are limited to the first trimester of pregnancy. In particular, mothers of the female homosexuals in our sample were significantly more likely than mothers of female heterosexuals to have taken diet pills and thyroid medications during pregnancy. To our knowledge, this is the first study to have implicated either of these substances as affecting any behavioral aspects of sexual development. Experimental studies of laboratory animals could shed further light on such a possibility.

Earlier studies of female lesbians indicate that their mothers were more likely than mothers of females in general to have taken DES during pregnancy (Ehrhardt et al., 1985; Meyer-Bahlburg & Ehrhardt, 1986; for a failure to replicate see Lish et al., 1991). Our findings are mixed on this point. While we did find a significant difference consistent with a DES effect, these differences dropped well below statistical significance once controls were introduced for the mother's age, years of education, and self-perceived ability to recall her pregnancy.

For both practical and ethical reasons, the design of this study was non-experimental and retrospective. While a prospective design is feasible and could provide great insight into the effects of prenatal factors on offspring sexual behavior, such a design would require monitoring the use of drugs by thousands of mothers throughout pregnancy and then locating their children in young adulthood to determine their sexual orientation.

Due to the retrospective nature of this study, one can conjecture that many of the mothers of homosexuals may have consciously or unconsciously biased their responses concerning drug use to match some preconceptions they may have had about prenatal drugs causing homosexuality in their children. However, we seriously doubt that this occurred for four reasons.

First, prior to our study, there was little objective reason to suspect that prenatal drug exposure would affect sexual orientation. The main exception was in the case of DES, which, as just noted, our logistic regression did not statistically associate with offspring sexual orientation.

Second, the questions that were asked about prescription drug consumption comprised only a small portion (about one-half of a page) of a 10-page questionnaire. Thus, there was little in our questionnaire that would have lead mothers to believe that prescription drug exposure was anything more than an incidental focus of our investigation (which was in fact the case).

Third, the vast majority of drugs exhibited no evidence of having been consumed or injected at greater or lesser amounts among mothers of homosexuals compared to mothers of heterosexuals. Also, one type of drug—medications taken to prevent nausea and vomiting during pregnancy—was reported as having been used by a *greater* proportion of mothers of male heterosexuals than by mothers of male homosexuals. These are not the sort of patterns one would expect to see if mothers of homosexuals were intentionally inflating or otherwise misreporting their consumption of prescription drugs.

Fourth, more of the evidence for prenatal prescription drug effects was found for female offspring than for male offspring. It would be difficult to believe that the mothers with sons who were homosexual would not have been at least as motivated to recall having used prescription drugs during pregnancy as were the mothers of homosexual daughters.

Determining if prenatal exposure to certain drugs affects the sexual orientation of humans, especially in the case of females, is a worthwhile goal. This is partly because nearly all of the scientific evidence regarding biological contributions to sexual orientation thus far has been limited to males (Bailey & Pillard, 1991; Bailey et al., 1999; Ellis, 1996; LeVay & Hamer, 1994).

From a theoretical standpoint, our findings provide qualified support for the neurohormonal theory of sexual orientation (Ellis & Ames, 1987). On the positive side, the theory asserts that immunological factors can alter sexual orientation. In this regard, both amphetamine-based drugs and thyroid medications are known to affect the mother's immune system during pregnancy (Glinoer, 1998; Kubera et al., 2002). Also, the placenta is very immunologically active, thereby preventing harmful immune reactions by the mother toward the fetus (Mellor & Munn, 2000, 2001), and its functioning may be altered by either of these medications. Another possibility is that certain immune conditions that diet pills and thyroid medications are intended to treat contribute to variations in sexual orientation. If so, the drugs per se are not directly responsible for lesbianism among female offspring.

On the negative side, the neurohormonal theory asserts that sex hormones, especially testosterone, are central to sexually differentiating the brain. Thus, drugs that are known to alter sex hormone levels—most notably progestin and DES—have been especially suspect as affecting

offspring sexual orientation (Reinisch & Karow, 1977), but little of our evidence supported this line of reasoning. Overall, it may be hypothesized that drugs affecting the immune system that are being consumed by the mother during pregnancy could alter brain development of the fetus in ways that affect preferences for sex partners later in life. Nevertheless, because there are neither animal experiments nor prior human studies to suggest that either amphetamines or thyroid drugs are capable of making such alterations, more evidence is needed before considering this a well-supported hypothesis.

Acknowledgements

We thank Myrna Nelson and Craig Kinsley for making helpful comments and the Eugene Garfield Foundation and the Minot State University Small Grants Committee for funding major portions of this research project.

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Behavioral Neuroscience
2010, Vol. 124, No. 2, 278–287

0735-7044/10/$12.00 DOI: 10.1037/a0018764

Sexual Orientation and the Second to Fourth Finger Length Ratio: A Meta-Analysis in Men and Women

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The ratio of the lengths of the second and fourth fingers (2D:4D) may serve as a marker for prenatal androgen signaling. Because people are typically unaware of their 2D:4D, its use allows possible effects of early sex hormone regimes and socialization to be disentangled. We conducted a meta-analysis on relationships between 2D:4D and sexual orientation in men and women in 18 independent samples of men and 16 independent samples of women. Collectively, these samples comprised 1,618 heterosexual men, 1,693 heterosexual women, 1,503 gay men, and 1,014 lesbians. In addition to identifying the normative heterosexual sex difference in 2D:4D for both hands, we found that heterosexual women had higher (more feminine) left- and right-hand 2D:4D than did lesbians, but we found no difference between heterosexual and gay men. Moderator analyses suggested that ethnicity explained some between-studies variation in men. These results add to a literature suggesting that early sex hormone signaling affects sexual orientation in women, and highlight the need for further research exploring the relationships among 2D:4D, sexual orientation, and ethnicity in men.

Keywords: 2D:4D, androgen, sexual differentiation, sex hormones, sexual orientation

Supplemental materials: http://dx.doi.org/10.1037/a0018764.supp

Sexual orientation is one of the most sexually differentiated psychological traits: About 97–98% of men are primarily attracted to women and a similar proportion of women are primarily attracted to men (Laumann, Gagnon, Michael, & Michaels, 1994; Wellings, Field, Johnson, & Wadsworth, 1994). The size of this sex difference is very large: approximately 6 standard deviations (Hines, 2004). In nonhuman vertebrates, testicular hormones play a major role in organizing sex differences in the brain and behavior (Morris, Jordan, & Breedlove, 2004; Zuloaga, Puts, Jordan, & Breedlove, 2008), and this appears to be true of humans as well (Hines, 2004; Kimura, 1999). Thus, it is reasonable to expect androgens to play a role in the development of sexual orientation in humans.

Several lines of evidence support this inference. For example, male infants with apparently normal or sex-typical prenatal androgen exposure who have undergone gender reassignment to female shortly after birth (e.g., to resolve abnormal differentiation of the genitals or damage to the penis requiring its removal, as in a condition called cloacal exstrophy) appear to report sexual attraction to females (Mustanski, Chivers, & Bailey, 2002; Reiner & Gearhart, 2004). This suggests that prenatal developmental events, including those dependent on sex hormones, have effects on sexual orientation that persist despite discordance with the assigned gender role. In addition, 46,XY individuals with complete androgen insensitivity syndrome (CAIS) are similar to, if not indistinguishable from, unaffected female controls in their sexual orientation (Hines, Ahmed, & Hughes, 2003; Money, Schwartz, & Lewis, 1984; Wisniewski et al., 2000). Finally, females with congenital adrenal hyperplasia (CAH), in which the adrenal glands produce an excess of prenatal androgen, are several times likelier than unaffected females to experience bisexual/lesbian fantasy or to identify as bisexual or lesbian (Hines, Brook, & Conway, 2004; Meyer-Bahlburg, Dolezal, Baker, & New, 2008; Money et al., 1984; Zucker et al., 1996).

Each of these lines of evidence, however, is confounded by possible socialization effects. For example, sexual orientation in individuals with CAIS is concordant with gender of rearing. Thus, the rearing environment, rather than the absence of androgen signaling in the brain, may primarily account for sexual orientation in CAIS women. In girls with CAH, it has been argued that their male-typical gender role behavior during childhood may elicit a concatenation of psychosocial experiences that differentiate them from unaffected girls, which, in turn, may influence the development

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Many thanks to Marc Breedlove, Lynn Hall, Bernd Kraemer, John Manning, Dennis McFadden, Matthew McIntyre, Stacie Miller, Qazi Rahman, Janel Tortorice and Sari van Anders for providing additional data for this meta-analysis.

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of their sexual orientation (for an elucidation of this mediational model, see Bem, 1996). Thus, in these groups, sexual orientation that is discordant with the sex of rearing may result, in part, from differential psychosocial experiences, and thus the effects of prenatal hormones may be only indirect (but see Pasterski et al., 2005).

Another potential source of evidence regarding the role of prenatal androgen on sexual orientation is the ratio of the lengths of the second and fourth fingers (2D:4D). Males develop a lower 2D:4D than do females by the end of the first trimester of gestation (Galis, Ten Broek, Van Dongen, & Wijnaendts, 2010; Malas, Dogan, Evcil, & Desdicioglu, 2006). Because of the early fetal development of sexual dimorphism in 2D:4D, researchers have suggested that 2D:4D may be influenced by prenatal androgen, and thus may serve as a biomarker for prenatal androgen exposure (Manning, Scutt, Wilson, & Lewis-Jones, 1998; Williams et al., 2000). Although multiple factors likely contribute to variation in 2D:4D (Saino, Rubolini, Romano, & Boncoraglio, 2007; Yan, Bunning, Wahlsten, & Hurd, 2009), subsequent research has supported the notion that 2D:4D reflects early androgens: A more masculine digit ratio has been associated with CAH (Brown, Hines, Fane, & Breedlove, 2002; Ciumas, Linden Hirschberg, & Savic, 2009; Ökten, Kalyoncu, & Yaris, 2002; but see Buck, Williams, Hughes, & Acerini, 2003), fetal testosterone/estrogen levels (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004), as well as a genetic predictor of androgen sensitivity (Manning, Bundred, Newton, & Flanagan, 2003). Moreover, XY individuals with androgen insensitivity syndrome have a more feminine 2D:4D than that of typical men and one similar to that of typical women (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009). Females exposed to elevated prenatal testosterone due to having a male cotwin also exhibit masculinized digit ratios (van Anders, Vernon, & Wilbur, 2006). Digit ratios are also sexually dimorphic in avian animal models (Burley & Foster, 2004; Leoni, Rubolini, Romano, di Giancamillo, & Saino, 2008), and experimental prenatal testosterone treatment has been shown to masculinize digit ratios in birds (Romano et al., 2005).

People are highly unlikely to be aware of their own 2D:4D; hence, this marker may allow researchers to explore associations with prenatal androgens that are not confounded by possible socialization effects. In addition, because the evidence for prenatal hormonal effects on human sexual orientation is entirely correlational, disparate but convergent lines of evidence are important in helping to rule out alternative explanations for these correlations. Indeed, multiple groups of researchers have examined associations between 2D:4D and sexual orientation in both sexes. However, results of these studies are mixed, especially for males; in general, failures to replicate significant correlations between 2D:4D and various traits are common (Putz, Gaulin, Sporter, & McBurney, 2004), highlighting the need for meta-analytic studies (e.g., Honekopp, Bartholdt, Beier, & Liebert, 2007; Puts, McDaniel, Jordan, & Breedlove, 2008). Several years ago, it was already apparent that findings were mixed with regard to the relationships between sexual orientation and 2D:4D (McFadden et al., 2005). Since then, more studies with mixed findings have been published. Quantitative meta-analysis can help identify sources of variation between studies and gauge the strength of particular associations and has replaced narrative review as the standard method of review. We therefore performed a meta-analysis of all published studies that examined 2D:4D in heterosexual and gay men or heterosexual and lesbian women.

Method

Selection of Studies

The principal method used for locating studies was a search in PubMed, PsychInfo, PsychLit, and ProQuest for articles with quantitative data on 2D:4D and sexual orientation. Combinations of key words in the following groupings were used: (a) *2D:4D, digit ratio, finger ratio*; (b) *sexual orientation, sexual preference, heterosexual*, homosexual*, gay, lesbian, straight*. Second, the ancestry method was used in which references were retrieved from articles obtained using the principal search method (Phares & Compas, 1992). Finally, we looked for studies that were presented at scientific sex research meetings. To our knowledge, our search identified the entire body of published research on 2D:4D and sexual orientation.

Inclusion Criteria

To be included in the meta-analysis, a study had to include data on 2D:4D in heterosexual and gay men or heterosexual and lesbian women. We excluded studies that did not have data for gay and heterosexual persons separately. If a study did not report the mean digit ratio and corresponding standard deviation, we contacted the corresponding author to obtain this information. In addition, only studies that employed a trained researcher to measure digit length were included in the meta-analysis. Although self-measurements correlate significantly with experimenter measurements using Vernier calipers or computer-assisted measurements of scans, these correlations are not especially strong (Burriss, Little, & Nelson, 2007). Self-measurements of right-hand, compared with left-hand, digit length correlate less well with experimenter measurements, presumably because the majority of persons are right-handed and find it difficult to measure the digits of their right hand. This is especially problematic because many of the associations between 2D:4D and behavioral measures are strongest for right-hand digit ratio. In implementing this criterion, we excluded of a large-scale Internet study (Manning, Churchill, & Peters, 2007) in which participants measured their own digit length. Manning et al. (2007) reported lower effect sizes for the sex difference in digit ratio compared with other studies: $g = -.20$, $p < .001$, for the right hand, and $g = -.17$, $p < .001$, for the left hand. Given that the sex difference is a well-established finding, these lower than usual effect sizes suggest the presence of error variance, probably due to measurement error.

Study Sample

Results from 34 independent samples met the inclusion criteria—the relationship between 2D:4D and sexual orientation was investigated in 18 samples of men and 16 samples of women. These samples represented a total of 21 studies published between September 2000 and August 2009. Most studies contributed both male and female samples to our meta-analysis (only six studies focused exclusively on men or women). Given that we frequently analyzed male and female samples separately, we will use the term

sample rather than *study* from here on. In total, there were 1,618 heterosexual men (sample size range: 7 to 349), 1,693 heterosexual women (sample size range: 7 to 705), 1,503 gay men (sample size range: 5 to 460), and 1,014 lesbians (sample size range: 2 to 468). Across these studies, 13 samples also examined differences in 2D:4D between heterosexual men and heterosexual women. Of the 18 samples that comprised gay and heterosexual men, sample sizes ranged from 61 to 809 ($M = 172$). Of the 16 samples that comprised lesbian and heterosexual women, sample sizes ranged from 58 to 1,173 ($M = 181$).[1]

Moderator Variables

Study variables were coded by the first author. The entire data set was then checked for errors (separately) by one research assistant and the fourth author. The research assistant was blind to the study hypotheses. Both the first and fourth authors examined the coded data set to resolve any discrepancies. We also coded for theoretically and methodologically relevant variables and study characteristics that might moderate the magnitude of the difference in 2D:4D between gay and lesbian persons and heterosexuals. Moderator variables included four categorical variables (sex, geographic location, digit measurement mode, and the extent to which a person identifies as exclusively gay or heterosexual, hereafter termed *exclusivity of preference*) and two continuous variables (age and ethnicity). Rationale for coding ethnicity as a continuous variable is given below.

Sex. Biological sex of the sample was coded as *male* or *female*. This moderator variable was used to examine whether the relationship between 2D:4D and sexual orientation differed for men and women.

Geographic location. Samples were coded as either *North American* or *European*. One sample in Manning and Robinson (2003) comprised participants from several nations across continents; thus, geographic location was not coded for this particular sample.

Digit measurement. We coded for whether finger length was directly measured from the hand (*direct measurement*) or from photocopies, scans, or ink prints of hands (*nondirect measurement*).

Exclusivity of preference. Samples were coded as either exclusively heterosexual versus gay (*exclusive*) or exclusively heterosexual versus gay/bisexual (*nonexclusive*). Samples were coded as *exclusive* when, depending on how sexual orientation was assessed (see online supplemental Table S1), either self-labeled bisexuals were excluded or participants with intermediate scores on a Kinsey-like dimensional attraction scale (reflecting bisexuality) were excluded. Only self-labeled heterosexual or gay persons or those with very high or low Kinsey attraction scores (reflecting predominant or exclusive attraction toward the same or other sex) were included. Samples were coded as *nonexclusive* when bisexual persons (based on self-identification or intermediate Kinsey scores) were grouped among the gay persons. We excluded one sample that grouped bisexual persons among heterosexual persons (Kraemer et al., 2006) in the moderator analyses because we were interested in the exclusivity of the gay category.

Age. The mean ages of heterosexual men, heterosexual women, gay men, and lesbians were coded. For all participants, mean ages were 27.88 (heterosexual men), 28.44 (heterosexual women), 32.70 (gay men), and 31.46 (lesbians) years.

Ethnicity. Given that 2D:4D is known to vary with ethnicity (Manning et al., 2000; Manning, Stewart, Bundred, & Trivers, 2004), participant ethnicity was reported in most studies ($n = 16$). However, when this information was not reported, we contacted authors to obtain an ethnic breakdown. Data on ethnicity were not collected (not recorded) in two studies (P. A. Hall & Schaeff, 2008; van Anders & Hampson, 2005), and ethnicity estimates were available for five studies (Kraemer et al., 2006, 2009; Putz et al., 2004; Wallien, Zucker, Steensma, & Cohen-Kettenis, 2008; Williams et al., 2000). Because the ethnicity of non-White participants was variable, it was impossible to examine any one particular non-White ethnic group. Thus, we focused on the extent to which samples were composed of more or fewer White participants. Using a 0–100 scale, we coded for the percentage of White participants in each sample. One study (Robinson & Manning, 2000, Sample 2) was excluded from ethnicity analyses because the sample was cross-cultural and ethnicity was highly variable.

Meta-Analytic Strategy

In the current meta-analysis, the effect size analyzed was the standardized mean difference (Hedge's *g*), which expresses the mean difference in 2D:4D between heterosexual and gay persons. Hedge's *g* was used because it adjusts for differences in sample size. Meta-analyses were conducted on effect sizes using a random effects model that considers the presence of moderators a possibility (Hunter & Schmidt, 2000). Employing a random effects model is appropriate for these data given the variability of the effect size distribution, suggesting the presence of moderators.

Prior to exploring the relationship between digit ratio and within-sex variation in sexual orientation, we examined sex differences in 2D:4D between heterosexual men and heterosexual women. For each study that assessed the mean digit ratio in male and female heterosexuals, we calculated separate average effect sizes (expressing the mean 2D:4D differences between male and female heterosexuals). We then analyzed data across these studies to estimate the population effect size.

For the main meta-analysis, we calculated average effect sizes (expressing the mean 2D:4D differences between gay and heterosexual persons) for each sample. We analyzed data across samples to estimate the population effect size and variables that might moderate the strength of the effect sizes; this was done separately for men and women. For the analysis of the relationship between sexual orientation and 2D:4D, we conducted two analyses: one in which we examined the difference in digit ratio between gay and heterosexual men and another in which we examined the difference between lesbian and heterosexual women. We estimated the population effect size by the average effect size (Hedge's *g*). In

[1] In Kraemer et al. (2009) and Wallien et al. (2008), homosexual participants also had gender identity disorder (GID). In Kraemer et al. (2006), heterosexual participants were first compared with homosexuals without GID, and the same heterosexual participants were then compared with a different group of homosexual participants with GID in Kraemer et al. (2009). This overlapping heterosexual group was not included in the sample size count; however, we considered the comparison between heterosexuals and GID homosexuals as an additional sample.

calculating the average effect size, each effect size was weighted by its sample size. Weighting was applied because large sample sizes should approximate the population effect size more precisely than smaller samples. The resulting population effect sizes can be interpreted using Cohen's (1992) recommendations that Hedge's *g*s of .20, .50, and .80 represent small, medium, and large effect sizes, respectively. The homogeneity estimate (Q) measured the likelihood that, within each analysis, effect size variation was not due to sampling error. A significant Q value indicates that effect sizes are heterogeneous, suggesting the presence of moderators and thus warranting a search for them.

In addition to estimating the mean population difference in 2D:4D, we conducted two analyses that would provide an indication of publication bias: Rosenthal's (1991) fail-safe *N* and a "trim and fill" analysis (Duval & Tweedie, 2000). Rosenthal's fail-safe *N* determines the number of null results that would be required to bring the mean effect size to zero. A trim and fill analysis determines where missing studies are likely to fall on the basis of an analysis of study size as a function of effect size. In the absence of publication bias, effect sizes should be distributed symmetrically to the left and right of the combined effect. If smaller studies tend to be distributed to one side of the combined effect, then this suggests the presence of missing studies on the other side. Trim and fill adds inferred effect sizes to the analysis, and then recomputes the combined effect. An "omit one study" analysis was also performed. This type of sensitivity analysis determines whether the results of the meta-analysis would change through the deletion of each study individually. We also conducted separate cumulative meta-analyses for men and women to examine combined effect size trends over time.

Next, we examined moderators of the relationship between 2D:4D and sexual orientation. The first moderator analysis that we performed included male and female samples in one analysis and used sex as a moderator variable to determine whether the relation between 2D:4D and sexual orientation was different for men and women. All other moderator analyses were conducted separately for men and women. For the categorical moderating variables, namely location, digit measurement mode, and exclusivity of preference, we used categorical model procedures. In this procedure, which is analogous to an analysis of variance (ANOVA), effect sizes were grouped according to moderator variable levels, and these groups were compared. Testing yields two homogeneity estimates, a between-groups Q (Q_b) and a within-groups Q (Q_w). Much like the F statistic in ANOVA, a significant Q_b means that subgroups of effect sizes are significantly different from one another. A significant Q_w means that, within a subgroup, effect sizes are heterogeneous and substantial variability exists. If an analysis demonstrates a significant Q_b, but within-subgroup effect sizes are still heterogeneous, it may be that another moderator explains the variability within that subgroup. In such cases, results must be interpreted with caution. For the continuous moderator variables, age and ethnicity, weighted least squares regression procedures were performed to evaluate the relationship between effect size and levels of the continuous moderator variable. Weighted least squares regression is an analogue to simple linear regression, with the additional feature that each effect size is weighted by its sample size. The weighted regression analysis provides a test of model specification (Johnson & Eagly, 2000), which is indexed by the Q statistic. Q is analogous to the sum of squares in linear regression. The total Q is partitioned into a Q due to the model (Q_m) and a residual Q (Q_{res}). Q_m indicates the variability of the effect sizes that is explained by the model; Q_{res} indicates the variability of the effect sizes that is not explained by the model. Moderator variables were entered into the regression equation one at a time. All analyses were performed using Comprehensive Meta-Analysis Program Version 2.

Results

Online supplemental Table S1 provides a summary of each study included in the meta-analysis. Details include sample characteristics, digit measurement and sexual orientation assessment methods, and effect sizes (Hedge's *g*) comparing 2D:4D in heterosexual and gay persons. A total of 343 effect sizes were calculated, including effect sizes comparing heterosexual men and heterosexual women, gay and heterosexual men, lesbian and heterosexual women, and moderator analyses conducted separately for men and women (effect sizes were also calculated for the left and right hand separately).

Sex Differences Between Heterosexual Men and Women

Before comparing 2D:4D in gay and heterosexual persons, we examined heterosexual sex differences in 2D:4D. Thirteen samples that compared 2D:4D in heterosexual men and women were included in this analysis. Heterosexual men tended to have a lower (more typically masculine) digit ratio than did heterosexual women, $g = -.55$, $p < .001$ (right hand) and $g = -.44$, $p < .001$ (left hand); effect sizes were medium. Rosenthal's fail-safe *N* indicated that, for the right and left hand, respectively, 505 and 303 additional null effects would be needed to render the overall effect sizes nonsignificant at $p = .05$. Trim and fill analysis did not reveal any asymmetry in the data and did not change point estimates. Omit one study analysis produced Hedge's *g* values ranging from −.55 to −.51 (right hand) and from −.46 to −.41 (left hand); all values were statistically significant.

Sexual Orientation Differences: Sex as a Moderator

Next, we compared 2D:4D in gay and heterosexual persons. Using sex as a moderator variable, we examined whether the relation between digit ratio and sexual orientation differed for men and women. There was a significant moderator effect for the right hand, $Q_b(1) = 4.84$, $p < .05$, and for the left hand, $Q_b(1) = 3.96$, $p < .05$. These findings indicate a relation between digit ratio and sexual orientation for women, but not men. Lesbians had a lower (more typically masculine) digit ratio than did heterosexual women ($g = .29$, $p < .02$, for right hand; $g = .23$, $p < .02$, for left hand); these effect sizes ranged from small to medium (see online supplemental Figure S1). There was no significant difference in 2D:4D between gay and heterosexual men (see Table 1 and online supplemental Figure S2).[2] Forest plots showing effect sizes for

[2] Although we excluded Manning et al. (2007) on the basis that participants measured their own finger length, results changed minimally when we included this large sample in the meta-analysis: for men, $g = -.03$, *ns*, for right and left hand; for women, $g = .23$, $p < .01$, for right hand and $g = .18$, $p < .01$, for left hand.

Table 1
Effect Size for 2D:4D Mean Difference Between Homosexuals and Heterosexuals: Separate Analyses for Men and Women

Sex	No. studies	Hand	Hedge's *g*	CI	*p*
Men	18	Right	−.02	[−.16, .12]	.75
	17	Left	−.02	[−.17, .13]	.82
Women	16	Right	.29	[.06, .51]	.02
	15	Left	.23	[.04, .43]	.02

Note. A positive Hedge's *g* value indicates that homosexuals have a lower (more masculinized) 2D:4D than heterosexuals; a negative value indicates that homosexuals have a higher (more feminized) mean ratio than heterosexuals.

each sample are presented in Figures S1 and S2 for women and men, respectively.

In the case of publication bias, trim and fill analysis inferred four and five missing studies to the left of the mean in women for the right and left hand, respectively, and zero and one missing studies to the left of the mean for the right and left hand, respectively. Including these inferred studies in the analysis rendered point estimates for women of .13 (right hand) and .07 (left hand), and estimates for men of −.02 (right hand) and −.03 (left hand). Despite this reduction in point estimates for women, Rosenthal's fail-safe *N* suggested that lesbian and heterosexual women are likely to differ in 2D:4D, even if multiple studies were missing from our data set (indicating no effect of publication bias). Specifically, 58 (right hand) and 43 (left hand) null effects would be needed to render the effect sizes comparing heterosexual and lesbian women statistically nonsignificant. Omit one study analyses produced Hedge's *g* values ranging from .21 to .32 (right hand) and .18 to .27 (left hand) for women, and from −.06 to .01 (right hand) and from −.07 to .01 (left hand) for men. All effect size estimates were statistically significant for women and nonsignificant for men, indicating that our results were robust and did not rely on the inclusion of any particular study.

The effect size heterogeneity across female, $Q(15) = 75.63$, $p < .001$, and male, $Q(17) = 48.35$, $p < .001$, samples suggested the presence of moderator variables. Below, we report results for moderator analyses separately for men and women.

2D:4D in Women: Categorical Moderator Analyses

Results of the six categorical moderator analyses (two hands by three categorical moderators) for women are shown in Table 2. One of six analyses found significant between-groups heterogeneity, indicating significant effect size differences between geographic locations: European samples were found to yield significantly larger effects ($g = .47$, $p < .001$) than North American samples ($g = .05$, *ns*). This result pertained to the left hand only; location did not moderate effect sizes for the right hand. Only one subgroup (North America) demonstrated within-group homogeneity; therefore, follow-up contrasts should be interpreted with caution.

The analysis examining moderation by method of digit measurement found no significant difference between effects from samples using direct measurement of digits and those that measured digits from photocopies, scans, or ink prints. The analysis examining moderation of effect sizes by exclusivity of preference also yielded no significant difference between effects from samples that compared exclusive heterosexual with exclusive gay persons and those studies that compared exclusive heterosexual with gay/bisexual persons.

2D:4D in Women: Continuous Moderator Analyses

We conducted weighted least squares regression analyses to examine whether age and ethnicity (percentage White) of sample were associated with the magnitude of effect sizes for women. Our results showed that neither age nor ethnicity was associated with the magnitude of effect sizes for the difference between 2D:4D in gay and heterosexual persons.

Table 2
Categorical Moderator Analyses for 2D:4D and Sexual Orientation in Women

Level of moderator	Q_b	*k*	Hedge's *g*	95% CI	Q_w
Geographic location (right hand)	1.56				
Europe		6	.46	[.11, .81]	22.63***
North America		10	.18	[−.09, .44]	33.29***
Geographic location (left hand)	6.91**				
Europe		6	.47	[.22, .71]	11.24*
North America		9	.05	[−.13, .24]	12.92
Exclusivity of preference (right hand)	0.38				
Exclusive		10	.33	[.02, .65]	56.99***
Nonexclusive		3	.14	[−.42, .69]	3.94
Exclusivity of preference (left hand)	1.53				
Exclusive		9	.35	[.08, .62]	35.84***
Nonexclusive		3	.01	[−.45, .47]	2.76
Digit measurement (right hand)	0.48				
Direct		4	.15	[−.31, .61]	7.69*
Photocopy/scan		12	.34	[.06, .63]	67.73***
Digit measurement (left hand)	0.11				
Direct		4	.19	[−.18, .56]	4.09
Photocopy/scan		11	.26	[.02, .50]	41.85***

* $p < .05$. ** $p < .01$. *** $p < .001$.

To examine effect size trends over time, we conducted a cumulative meta-analysis in which studies were sequentially pooled by adding one study at a time according to the date of publication (i.e., earliest to the most recent). With each new study added, cumulative effect sizes always indicated a more male-typical digit ratio for lesbians compared with heterosexual women (see online supplemental Figure S3). These effect sizes ranged from small to medium and pertained to the right hand. For the left hand, the first three studies in the cumulative meta-analysis showed no significant effect sizes; however, including subsequent studies yielded significantly positive, but small, effect sizes.

2D:4D in Men: Categorical Moderator Analyses

Results of the categorical moderator analyses for men are shown in Table 3. Again, results are presented for both right and left hands, giving a total of six analyses. The two analyses including geographic location yielded significant between-groups heterogeneity: For both hands, North American sample effect sizes were significantly different from European sample effect sizes. Samples from Europe had effect sizes that were in the positive direction ($g = .14$, *ns*, for right hand; $g = .17$, $p < .05$, for left hand), indicating that gay persons had lower (more masculine) mean digit ratios than heterosexual persons. North American samples had effect sizes that were in the negative direction ($g = -.17$, $p < .10$, for right hand; $g = -.23$, $p < .01$, for left-hand), indicating that gay persons had higher (more feminine) mean digit ratios than heterosexual persons. For the right hand, the North American subgroup showed within-group homogeneity, and for the left hand, the European subgroup showed within-group homogeneity. Given that there remained some within-subgroup variability in these analyses, results should be interpreted with caution.

The analysis examining moderation by method of digit measurement yielded no significant difference between effects from samples using direct measurement of digits and those that measured digits from photocopies, scans, or ink prints. In addition, the analysis examining moderation of effect sizes by exclusivity of preference found no significant difference between effects from samples that compared exclusively heterosexual with exclusively gay persons and those studies that compared exclusively heterosexual with gay/bisexual persons.

2D:4D in Men: Continuous Moderator Analyses

We conducted weighted least squares regression analyses to examine whether age and ethnicity (percentage White) of sample were associated with the magnitude of effect sizes for men.

The regression analysis examining the impact of ethnicity on effect sizes revealed a significant association between the percentage of White participants in a sample and the magnitude of the difference between 2D:4D in gay and heterosexual men, $Q_m(1) = 9.23$, $p < .01$, $R^2 = .33$ (right hand), and $Q_m(1) = 11.23$, $p < .001$, $R^2 = .44$ (left hand). In samples that predominantly comprised White participants, effect sizes indicated that gay men had a lower (more masculine) 2D:4D than heterosexual men. In samples that included fewer White participants, gay persons had a higher (more feminine) 2D:4D than heterosexual persons (see online supplemental Figure S4).

Thus, both geographical location and ethnicity moderated the relationship between 2D:4D and sexual orientation in men. These moderators were themselves correlated. Weighted for sample size, European samples had a significantly greater percentage of White participants than did North American samples, $F(1, 16) = 7.06$, $p = .019$. To explore whether geographical location or ethnicity might primarily drive the relationship between 2D:4D and sexual orientation, we conducted a mixed model ANOVA with geographical location as a between-subjects factor and ethnicity as a covariate, weighted by sample size. Ethnicity marginally significantly predicted the relationship between 2D:4D and sexual orientation in men, $F(1, 16) = 3.55$, $p = .082$, whereas geographical location did not, $F(1, 16) < 1$. Age was not associated with the

Table 3
Categorical Moderator Analyses for 2D:4D and Sexual Orientation in Men

Level of moderator	Q_b	k	Hedge's g	95% CI	Q_w
Geographic location (right hand)	5.98*				
Europe		9	.14	[−.04, .32]	19.96**
North America		8	−.17	[−.35, .01]	9.99
Geographic location (left hand)	11.16***				
Europe		9	.17	[.00, .34]	13.11
North America		7	−.23	[−.40, −.06]	11.83†
Exclusivity of preference (right hand)	0.29				
Exclusive		11	.02	[−.19, .22]	37.91***
Nonexclusive		4	−.09	[−.43, .25]	7.56†
Exclusivity of preference (left hand)	0.01				
Exclusive		10	−.01	[−.23, .22]	39.85**
Nonexclusive		4	−.03	[−.39, .33]	11.71**
Digit measurement (right hand)	0.32				
Direct		6	.04	[−.22, .30]	16.69**
Photocopy/scan		12	−.05	[−.23, .12]	29.78**
Digit measurement (left hand)	0.12				
Direct		6	.02	[−.25, .29]	11.06*
Photocopy/scan		11	−.02	[−.17, .14]	39.79***

† $p < .1$. * $p < .05$. ** $p < .01$. *** $p < .001$.

magnitude of effect sizes for the difference between 2D:4D in gay and heterosexual persons.

The cumulative meta-analysis revealed that, with each study's new appearance, effect sizes indicating the magnitude of the difference in 2D:4D between gay and heterosexual men were always nonsignificant. This result was consistent for the left and right hands.

Discussion

Since George (1930) demonstrated the sex difference in 2D:4D and Manning et al. (1998) pointed out its potential utility as a marker for prenatal sex hormones, numerous empirical studies have corroborated this normative sex difference. Our meta-analysis confirmed the sex difference in 2D:4D with a selected sample of heterosexual men and women who served as controls in at least some of the studies examining sexual orientation. We found that heterosexual men had significantly lower 2D:4D than did heterosexual women: This sex difference was highly robust, and its magnitude was greater for the right hand than for the left. Thus, right-hand 2D:4D is likely to more strongly reflect prenatal androgen exposure (Williams et al., 2000); hence, our discussion focuses on right-hand 2D:4D.

The primary finding of this study was that lesbians had a smaller, more masculine 2D:4D than did heterosexual women, whereas gay and heterosexual men did not differ significantly in 2D:4D. The right-hand 2D:4D difference between lesbian and heterosexual women was estimated to be small to medium in size. This difference was robust. A trim and fill analysis suggested the possibility of missing studies, and that the true effect size of the 2D:4D difference between heterosexual and lesbian women may be somewhat smaller than our estimate. Thus, some caution in interpretation is advisable. However, Rosenthal's fail-safe *N* indicated that 58 additional studies with null effects would be required to produce a statistically nonsignificant difference in 2D:4D between heterosexual and lesbian women. Furthermore, omit one study analysis indicated that this difference remained statistically significant regardless of which study was omitted; thus, the sexual orientation difference in 2D:4D in women did not rely on the inclusion of any particular study in our analysis.

Despite these robust findings, we found considerable heterogeneity in 2D:4D in both male and female samples, suggesting the presence of moderator variables. We therefore conducted moderator analyses, which examined possible effects of finger measurement method, method of sexual orientation assessment, age, geographic sampling location, and ethnicity on the relationship between 2D:4D and sexual orientation. We found no effects of finger measurement method, method of sexual orientation assessment, or age in either sex. Sampling location (Europe vs. North America) influenced the relationship between 2D:4D and sexual orientation in women only in the left hand and in both hands in men. Gay men had a lower 2D:4D in Europe and a higher 2D:4D in North America compared with heterosexual controls. Ethnicity also influenced the relationship between 2D:4D in men only and in both hands, such that higher proportions of White subjects were associated with a greater tendency for gay men to have a more masculine 2D:4D. Previous studies have reported effects of geographic location (McFadden et al., 2005) and ethnicity (Manning & Robinson, 2003; McFadden et al., 2005) on the relationship between 2D:4D and sexual orientation in men. We entered both variables into a single moderator analysis and found that geographic location did not explain a significant proportion of the variation in effect sizes beyond that explained by ethnicity. This suggests that the "effect" of geography observed in these samples is actually one of ethnicity.

Overall, our results support the hypothesis that prenatal androgen exposure affects sexual orientation in women. These results corroborate other evidence that androgen affects sexual orientation, such as sexual attraction to males in persons with CAIS (Hines et al., 2003; Money et al., 1984; Wisniewski et al., 2000) and sexual attraction to females in women with CAH (Hines et al., 2004; Money et al., 1984; Zucker et al., 1996) and prenatally normal males whose gender was reassigned near birth (Mustanski et al., 2002). Although these other lines of evidence cannot rule out the possibility that differential socialization, rather than altered prenatal androgen signaling, affected sexual orientation, 2D:4D appears to offer a means of doing so: People's finger length ratios are seldom known, and thus any relationships with sexual orientation are unlikely to be caused by socialization based on 2D:4D. Similarly, otoacoustic emissions (OAEs; sounds generated by the inner ear) may provide evidence of a relationship between androgen and sexual orientation that is not confounded by potential socialization effects. OAEs are thought to reflect early androgen exposure, and OAEs of lesbian and bisexual women are intermediate between those of heterosexual women and men (McFadden & Pasanen, 1998, 1999). Although it is logically possible that both 2D:4D and OAEs are correlated with other traits that affect social interactions and thereby alter psychosexual development, there is presently no evidence for this.

Our results also have implications regarding the timing of the development of sexual orientation. The relationship between 2D:4D and female sexual orientation suggests that these traits are not only dependent on the same developmental influences (e.g., circulating testosterone levels), but also that they are sensitive to these influences during the same time periods. In other words, the critical periods for the sexual differentiation of 2D:4D and sexual orientation probably overlap. As noted by Puts et al. (2008), sexual differentiation in 2D:4D likely begins between about 6 weeks of gestation, when testosterone production by Leydig cells begins (O'Shaughnessy, Baker, & Johnston, 2006), and 9 weeks of gestation, when substantial sexual differentiation in 2D:4D has already occurred (Galis et al., 2010; Malas et al., 2006). This implies that the critical period for the development of sexual orientation also probably begins during this interval and extends until sometime thereafter.

An important but unresolved question is why 2D:4D and sexual orientation were not related in a more straightforward way in men. One might imagine that if prenatal testosterone masculinizes both 2D:4D and sexual orientation in women, it would have similar effects in men. Several possible explanations for this incongruity exist.

First, sexual orientation is likely to be multifactorial and ontogenetically heterogeneous in both sexes. It is possible that gay men's sexuality is sufficiently diverse developmentally that, even if it sometimes results from sex-atypical prenatal androgen signaling, other developmental causes (e.g., maternal immune response due to fraternal birth order; Blanchard & Bogaert, 1996; Bogaert, 2006; Puts, Jordan, & Breedlove, 2006) may weaken a correlation

with 2D:4D. In addition, our data suggest that gay men's sexuality may sometimes result from reduced androgenization and sometimes from elevated androgenization, and that ethnic (or genetic) background may determine which of these applies. If so, then in ethnically diverse populations, these negative and positive relationships between 2D:4D and male sexual orientation may effectively cancel each other, and the aggregate relationship may be negligible.

Alternatively, perturbations in prenatal androgen levels may generally have smaller effects on neurophysiological development in males than they do in females. Although prenatal testosterone levels have been found to correlate with childhood sex-typed behavior in both girls and boys (Auyeung et al., 2009), relationships between masculine childhood behavior and prenatal testosterone (or proxies of prenatal testosterone, such as CAH) are typically larger or observed only in girls (Hines, 2004; Hines, Golombok, Rust, Johnston, & Golding, 2002). Perhaps this is partly because androgen levels at the low end of the normal male range are sufficient to masculinize many aspects of the phenotype. If a relatively small androgen dose is required for the development of sexual attraction to females, then nearly all males may obtain sufficient androgen to promote attraction to females, and gay men's sexuality may result from something other than low androgen. Consequently, no relation to 2D:4D would be expected.

Finally, the possibility of different reporting biases across ethnicities should not be overlooked. For example, gay sexuality in men may be more stigmatized in some ethnic communities than in others (e.g., Pitt, 2006). This could lead to different rates of self-reported attraction to same-sex persons across ethnic groups and perhaps differences in the men who are classified or report themselves to be gay. For example, if only very feminine-acting gay men report a gay orientation in ethnic groups where this orientation is highly stigmatized, and if more feminine-acting gay men also have more a female-typical 2D:4D, then samples with higher proportions of ethnicities in which gay sexuality in men is highly stigmatized would show a greater tendency for gay men to have a higher, more feminine, 2D:4D.

The possibility of reporting biases is a limitation of all studies sampled in the present meta-analysis. On the one hand, it is unlikely that participants would misrepresent their sexual identity as gay or lesbian; on the other hand, it is possible that some study participants with gay/lesbian attractions reported, for reasons of social desirability, a heterosexual sexual identity (despite conditions of anonymity). This would, however, only have weakened the likelihood of detecting between-groups sexual orientation effects. Although it is likely that individuals who are open about their gay or lesbian sexual identity differ in various ways from their less open counterparts (e.g., degree of sexual liberalism vs. conservatism), it is unlikely that such a difference would be related to variations in 2D:4D.

A general limitation of meta-analyses of published studies is that nonsignificant findings may be less likely to get published, thus potentially biasing the meta-analyses. However, Rosenthal's fail-safe *N* indicated that a large number of nonsignificant unpublished studies would have to exist to cause the overall sex difference in 2D:4D, and the sexual orientation difference in 2D:4D in women, to become statistically nonsignificant. Given the modest number of studies analyzed and some evidence for publication bias, we encourage replication of these results as more data accumulate. We also encourage continued exploration of associations between digit ratio, sexual orientation, and moderator variables in men. In particular, future research should examine the potential effects of ethnicity on relationships between 2D:4D and sexual orientation, and consider possible subgroups of gay men (e.g., more typically masculine vs. more typically feminine gay men).

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Received October 26, 2009
Revision received December 12, 2009
Accepted December 12, 2009 ■

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Personality and Individual Differences 38 (2005) 1401–1411
PERSONALITY AND INDIVIDUAL DIFFERENCES
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Male and female sexual orientation differences in gambling

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Received 10 February 2004; received in revised form 30 June 2004; accepted 1 September 2004
Available online 25 November 2004

Abstract

The purpose of this study was to determine whether sexual orientation differences in gambling exist that parallel those for sex—a question motivated by the idea that male homosexuality arises from prenatal brain feminization and that female homosexuality arises from prenatal brain masculinization. For gambling frequency, we hypothesized that male homosexuals would be more similar to female heterosexuals than male heterosexuals, and that female homosexuals would be more similar to male heterosexuals than female heterosexuals. Subjects were 10,598 individuals classified as homosexual men ($n = 935$), heterosexual men ($n = 4187$), homosexual women ($n = 275$), or heterosexual women ($n = 5201$). Data came from survey interviews conducted by staff members of The Kinsey Institute for Research in Sex, Gender, and Reproduction from 1938 to 1963. Results showed that (a) homosexual men gambled less than heterosexual men, the greatest difference occurring at low levels of gambling frequency, and (b) homosexual women gambled more than heterosexual women, the greatest difference occurring at high levels of gambling frequency. Parallels are drawn between gambling and two other addictive behaviors, tobacco use and alcohol consumption.

Keywords: Sexual orientation; Gambling; Addictive behavior; Sex differences

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0191-8869/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.paid.2004.09.002

1. Introduction

Sex differences are consistently observed in gambling research. Men and women differ in their motivation for gambling, their interest in gambling, and how and in what form gambling takes place. A substantial body of evidence exists that differences between homosexuals and heterosexuals mimic comparable sexually dimorphic somatic, cognitive, and behavioral differences between men and women. Thus, given the presence of sex differences in gambling, we predict significant sexual orientation differences in gambling. That is, male homosexuals will resemble female heterosexuals in their gambling activity, and female homosexuals will resemble male heterosexuals in their gambling activity.

Sexual orientation differences have been identified across a wide domain of traits. For somatic traits, homosexual men, compared with heterosexual men, have been found to experience puberty earlier (Blanchard & Bogaert, 1996), weigh less (Bogaert & Blanchard, 1996), are shorter (Blanchard & Bogaert, 1996; Bogaert & Blanchard, 1996), and have larger penises (Bogaert & Hershberger, 1999). Homosexual men also have elevated rates of nonright handedness (Lalumiere, Blanchard, & Zucker, 2000), more dermal ridges on the left hand (Green & Young, 2000), and a larger second to fourth digit finger length ratio (Robinson & Manning, 2000). Homosexual women, compared with heterosexual women, are stronger (Perkins, 1981), heavier (Beren, Hayden, Wilfrey, & Grillo, 1996), and taller (Bogaert, 1998).

Cognitive differences between homosexuals and heterosexuals are less-well established than somatic differences, but a consensus is developing that heterosexual men, on the average, have a higher level of spatial ability than homosexual men (e.g., Cohen, 2002). Results for women are less clear, primarily because fewer studies have used homosexual women as a comparison group: For example, while Tuttle and Pillard (1991) found that homosexual women did better on spatial tasks than heterosexual women, Gladue, Beatty, Larson, and Staton (1990) found that heterosexual women did better.

Behavioral differences between homosexual and heterosexual persons have also been the subject of investigation, perhaps yielding the most replicable results in sexual orientation research. Retrospective, and importantly, prospective studies (Green, 1987) have shown that male and female same-sex sexual orientation is strongly associated with atypical gender behavior during childhood. For males, this includes decreased aggression, decreased sports participation, playing with ''female'' toys (e.g., dolls), the desire to be female, and being perceived as a ''sissy'' by others. For females, this includes increased sports participation, involvement with stereotypically masculine activities such as automobile repair, and being perceived as a ''tomboy'' by others. However, the effect size for childhood gender atypicality is usually smaller for females than for males. Gender atypicality also becomes smaller by adulthood for both men and women, probably due to the effects of socialization (Harry, 1983). Nonetheless, significant sexual orientation behavioral differences persist into adulthood, the most notable example being the female-like distribution of occupations between homosexual men and the male-like distribution of occupations among homosexual women (Bailey & Oberschneider, 1997).

The most well-supported theory of why sexual orientation differences in somatic, cognitive, and behavioral traits are present is based on the hypothesis that sexual orientation depends on the early sexual differentiation of hypothalamic brain structures (Ellis & Ames, 1987). The differenti-

ation of these brain structures, in turn, depends on prenatal androgen. Masculinization of brain structures occurs because of relatively high levels of androgens, whereas feminization of brain structures occurs with relatively low levels of androgens. These differences lead to the idea that homosexual men and heterosexual women have neural sexual orientation centers that are similar to each other and different from those of heterosexual men and homosexual women (Gooren & Kruijver, 2003).

1.1. Sex differences in gambling

Virtually no data have been reported describing the incidence of regular or pathological gambling between homosexual males and females. However, results from studies of sex differences in regular and pathological gambling behavior could be used to predict what *could* be found if such data were collected.

Most population-based studies concur that overall, sex differences in the prevalence of regular gambling are negligible (e.g., Welte, Barnes, Wieczorek, Tidwell, & Parker, 2002). For example, the Gambling Impact and Behavior Study (Volberg, Harwood, & Tucker, 1999) in the United States found in 1998 that the lifetime gambling rate was 88% for males and 83% for females. However, sex differences in the prevalence of pathological gambling are significant: males are disproportionately represented among pathological gamblers (Hraba & Lee, 1996; Ladd & Petry, 2002). The National Research Council (1999) report of population studies from 18 states in the US conducted during the 1990s revealed that the median proportion of women among pathological gamblers was 38%, versus 62% for males. The National Council of Welfare study (1996) conducted in Canada also found the proportion of lifetime female pathological gamblers in Alberta to be 38%, versus 62% for males; in six of seven other provinces, there were a greater proportion of male gamblers. A meta-analysis of pathological gambling in the United States and Canada reported that the lifetime relative risk (RR) of pathological gambling for four demographic groups, compared with females (i.e., RR = demographic group *n*/female *n*), was greater than 1.00: (a) general population adult = 2.15; (b) adolescent = 3.03; (c) college students = 3.84; (d) adults in-treatment or prison = 1.85 (Shaffer, Hall, & Bilt, 1997). The meta-analysis also reported that the proportions of male and female lifetime pathological gamblers differed significantly within each of the four demographic groups: (a) general population adult, males = 2.67% and females = 1.24%; (b) adolescents, males = 6.05% and females = 2%; (c) college students, males = 8.62% and females = 2.24%; (d) adults in-treatment or prison, males = 13.22% and females = 7.15%. The authors of the meta-analysis conclude that however one partitions the data into different demographic groups (e.g., adolescents, students, etc.), males form a greater proportion of pathological gamblers.

The behavioral profiles of male and female gamblers are also quite different, and are most pronounced for pathological gambling. Male pathological gamblers are frequently labeled *action* gamblers, whereas female action gamblers are frequently labeled *escape* gamblers. Action gamblers begin early in life, have had a long-standing problem of 10–30 years, and focus on "skill" games. On the other hand, escape gamblers become compulsive in their gambling in a very short span of 6 months to 3 years, initially playing "luck" games for recreation but then quickly moving into using gambling to escape from problems (Lesieur & Blume, 1991).

Extensive research supports the action-escape sex distinction in pathological gambling. One detailed examination of sex differences in pathological gambling is provided by the National Research Council (1999) study in Alberta, Canada. First, there were differences in the type of gambling men and women engaged in: bingo (24% versus 76%); informal sports betting (54% versus 27%); games of skill (billiards, darts, golf) (54% versus 22%); speculative investments (38% versus 14%); cards games in a card room (30% versus 6%); and cards/dice at a casino (28% versus 10%). Second, there were differences among pathological gamblers for reasons given for gambling. Women were more likely than men to gamble (a) to support a worthy cause (74% versus 52%), (b) to distract themselves from everyday problems (39% versus 28%), and (c) to experience a thrill or challenge (78% versus 88%). Age at which persons first started gambling is a third sex difference, as revealed by the New Zealand Gaming Survey (Abbott, 2001): 66.7% of men begin gambling between the ages of 15 and 29, whereas 51.9% of women do so. Hraba and Lee (1996) also suggest that pathological female gamblers lack religious affiliation, are unmarried, are not integrated into a "conventional" community, and have served in the armed forces. There is evidence that female pathological gamblers have different patterns of arrest, psychiatric problems and financial problems than male pathological gamblers (Potenza et al., 2001). Male and female pathological gamblers also have several characteristics in common, including elevated rates of substance abuse and suicide (Volberg, 1994).

1.2. Purpose of study

One of the world's largest data bases on human sexuality, The Kinsey Institute for Research in Sex, Gender, and Reproduction, contains information on gambling activity and sexual orientation. We analyzed a sample of homosexual and heterosexual men and women from these to investigate sexual orientation differences in gambling. In line with substantial evidence that differences between homosexuals and heterosexuals mimic sex differences on various characteristics, we hypothesize that for gambling frequency, male homosexuals would be more similar to female heterosexuals than male heterosexuals, and that female homosexuals would be more similar to male heterosexuals than female heterosexuals.

2. Method

2.1. Participants

From 1938 to 1963, 17,502 case histories were recorded by the Kinsey Institute for Sex Research using the interview schedule devised by Alfred C. Kinsey (Gebhard & Johnson, 1979). These data are currently stored in several files. Because analyses of the Kinsey data produce biased results when individuals with felony or misdemeanor convictions (other than traffic violations) are included (Gebhard & Johnson, 1979), we did not include these cases in this study. Although excluding these cases may lead to a lower reported gambling rate, it should not bias the direction of a sexual orientation difference, unless the number of homosexuals convicted of gambling offenses differs from that of heterosexuals, which is unlikely. The sample included white and nonwhite adults, 6013 of whom were male and 5954 of whom were female.

2.2. Measures

2.2.1. Sexual orientation

Sexual orientation was classified according to the following criteria. Men and women were classified as homosexual if they reported "extensive" homosexual experience, defined by Gebhard and Johnson (1979) as more than 20 same-sex sexual partners or more than 50 homosexual experiences (with one or more partners). Men and women were classified as heterosexual if they met two criteria: (a) they reported "no" or "rare" homosexual experience, the latter defined by Gebhard and Johnson as 1 same-sex sexual partner or 1–5 homosexual experiences, and (b) they did not respond that they experienced "much" or "some" sexual arousal to questions about sexual arousal from seeing or thinking of members of their own sex.

Using these stringent criteria, we could not classify 478 women and 891 men as either heterosexual or homosexual. Of the remaining 10,598 individuals, 935 were classified as homosexual men, 4187 were classified as heterosexual men, 275 were classified as homosexual women, and 5201 were classified as heterosexual women.

2.2.2. Gambling frequency

Kinsey's interview protocol included two questions that assessed gambling frequency: (A) *Do you gamble on cards*, *races*, *or any game*? and, if the reply was affirmative, the respondent was asked (b) *In terms of money*, *would you call yourself a light moderate*, *or heavy gambler*? The answers to these two questions were combined into one item, referred to as *current frequency of gambling*. The response options for this item were, *none*, *rare*, *little*, *some*, and *much*. According to Gebhard and Johnson (1979), the rational for this composite question, which combines frequency of gambling with amount gambled, was an attempt to learn the importance of gambling in the respondent's life. For example, occasional gambling for large amounts of money or frequent gambling for more moderate amounts would both be rated as *much* because such gambling in this case would probably have a significant impact on the respondents' financial well-being. At the other extreme, those respondents who rarely gambled, and when they did so, for small amounts of money, would be rated as *rare* because gambling in this case would probably not have a negative impact on their financial well-being.

2.2.3. Demographics

We assessed the respondents on a number of demographic variables, including age, race/ethnicity, number of years of education, and parental socioeconomic status (SES). SES was assessed using an eight-point scale, ranging from "1" = *very poor* to "9" = *rich*. Parental SES was used instead of the respondent's SES because many respondents were still in school when they were interviewed and their own SES would not necessarily reflect their ultimate SES.

3. Results

The demographic characteristics of the sample are shown in Table 1. Both male and female groups differed significantly on age and education. These variables were also significantly but negligibly correlated with gambling: $r = -.11$, $p < .001$; $r = -.04$, $p < .05$, respectively. The results of

Table 1
Comparisons of homosexual and heterosexual men and women on demographic variables

Variable	Men						Women					
	HM (n = 935)		HT (n = 4187)		t	p	HM (n = 275)		HT (n = 5201)		t	p
	$\bar{x}$	SD	$\bar{x}$	SD			$\bar{x}$	SD	$\bar{x}$	SD		
Age	30.21	10.45	29.23	11.45	2.41	<.05	33.74	10.01	28.77	11.05	7.30	<.001
Education[a]	14.18	3.81	15.72	3.35	12.40	<.001	15.24	3.35	14.46	2.70	4.63	<.001
Race (% Caucasian)	92.10	0.27	92.80	0.26	0.77	n.s.	90.20	0.30	91.90	0.27	0.97	n.s.
Parental SES[b]	4.77	1.56	4.73	1.33	0.72	n.s.	4.78	1.58	4.93	1.35	1.72	n.s.

[a] Education varies from 1 (first grade completed) to 20 (PhD, MD, or LLD).
[b] Parental SES varies from 1 (extreme poverty) to 8 (extreme wealth).

analyses conducted with and without age and education as covariates, however, did not differ. Therefore, results are reported from analyses not using age and education as covariates.

Table 2 presents the distribution of gambling by sex. Chi-square calculated for this table was significant, $\chi^2(4, n = 9166) = 346.22$, $p < .001$, implying a significant association between gambling frequency and sex. The distribution of Table 2 shows that men gambled more frequently than women. This is most clearly revealed by comparing men and women on the combination of categories one, two, and three (*none*, *rare*, *little*), representing low gambling frequency versus the combination of categories four and five (*some*, *much*), representing high gambling frequency. The chi-square for this comparison was also significant;

$$\chi^2(1, n = 9166) = 106.98, \quad p < .001.$$

Table 3 presents the distribution of gambling by sexual orientation, homosexuals versus heterosexuals. As indicated by the chi-square, there is a significant association between gambling and sexual orientation; $\chi^2(4, n = 9166) = 59.24$, $p < .001$. This relation is largely attributable to greater numbers of homosexuals engaging in *some*gambling relative to heterosexuals; this chi-square was also significant, $\chi^2(1, n = 9166) = 39.02$, $p < .001$.

Table 4 presents the distribution of gambling by male sexual orientation and Table 5 presents the distribution of gambling by female sexual orientation. Chi-square calculated for each of the two tables was significant: For Table 4, $\chi^2(4, n = 4035) = 85.65$, $p < .001$, and for Table 5,

Table 2
Gambling by sex

Gambling	Men		Women	
	n	%	n	%
None	2227	55.19	3702	72.15
Rare	1334	33.06	1212	23.62
Little	228	5.65	117	2.28
Some	240	5.95	94	1.83
Much	6	<1.00	6	<1.00

n = 9166.

Table 3
Gambling by sexual orientation

Gambling	Homosexual		Heterosexual	
	n	%	*n*	%
None	179	67.29	3523	72.42
Rare	60	22.56	1152	23.68
Little	9	3.38	108	2.22
Some	16	6.02	78	1.60
Much	2	<1	4	<1

$n = 5131$.

Table 4
Gambling by male sexual orientation

Gambling	Homosexual		Heterosexual	
	n	%	*n*	%
None	714	67.94	5215	64.26
Rare	227	21.60	2319	28.85
Little	33	3.14	312	3.84
Some	74	7.04	260	3.20
Much	3	<1	9	<1

$n = 9166$.

Table 5
Gambling by female sexual orientation

Gambling	Homosexual		Heterosexual	
	n	%	*n*	%
None	535	68.20	1692	52.10
Rare	167	21.30	1167	35.90
Little	24	3.10	204	6.30
Some	58	7.40	182	5.60
Much	1	<1	5	<1

$n = 4035$.

$\chi^2(4, n = 5131) = 39.02$, $p < .001$. Examining Table 4, the significant association between gambling and male sexual orientation arises from the presence of relatively more homosexual men than heterosexual men at the lowest point on the gambling scale: Relatively more homosexual than heterosexual men are in the *none* category, $\chi^2(1, n = 4035) = 66.20$, $p < .001$. Thus, there is an excess of homosexual men who do not gamble, relative to heterosexual men. Examining Table 5, the association between gambling and female sexual orientation arises from the presence of relatively more homosexual women than heterosexual women in the *much* category, the highest point on the gambling scale; $\chi^2(1, n = 3702) = .90$, $p < .05$. Thus, there is an excess of homosexual women who gamble a great deal, relative to heterosexual women.

Up to this point, we have established that there are sex differences and sexual orientation differences for gambling. We may now ask whether there are any other differences in gambling among the four sex/sexual orientation groups: (a) homosexual men versus heterosexual women, (b) homosexual men versus homosexual women, (c) heterosexual men versus homosexual women, and (d) heterosexual men versus heterosexual women. The results, revealed by calculating chi-square for each table, were consistent with the previously reported sex and sexual orientation differences: (a) homosexual men gambled more than heterosexual women, $\chi^2(4, n = 5650) = 99.55$, $p < .001$; (b) homosexual men and homosexual women gambled to the same degree, $\chi^2(4, n = 1051) = 3.49$, $p > .05$; (c) heterosexual men gambled more than homosexual women, $\chi^2(4, n = 3516) = 31.17$, $p < .001$; and (d) heterosexual men gambled more than heterosexual women, $\chi^2(4, n = 8115) = 409.01$, $p < .001$. In fact, these four differences, combined with sex and sexual orientation differences, form a perfectly transitive ordering of gambling frequency fully consistent with our predictions: heterosexual men > homosexual men = homosexual women > heterosexual women.

4. Discussion

In one of the largest samples of its kind, homosexual men reported gambling less frequently than heterosexual men, while homosexual women reported gambling more frequently than heterosexual women. The male difference was found at lower levels of gambling, whereas the female difference was found at higher levels of gambling. Men also reported gambling more frequently than women. These results add to a growing body of research showing that homosexual men score in the female-typical direction, and that homosexual women score in the male-typical direction, on some-sex dimorphic somatic, cognitive traits, and behavioral traits (Blanchard & Bogaert, 1996; Bogaert & Blanchard, 1996). These sexual orientation differences may be attributable to the feminization of prehomosexual males and to the masculinization of prehomosexual females arising sex-atypical patterns of hormone exposure during prenatal development (Ellis & Ames, 1987). There is also evidence that genetic reasons may underlie this sex-atypical pattern. A number of studies have found genetic influences to be significant for men (e.g., Bailey & Pillard, 1991; Hu et al., 1995) and for women (e.g., Bailey, Pillard, Neale, & Agyei, 1993; Hershberger, 1997).

Finding hormonal or genetic influences on gambling behavior is consistent with a biological (e.g., prenatal hormones) explanation of sexual orientation development. For example, differences were found between gamblers and controls in the noradrenergic system: male gamblers had a larger centrally produced fraction of cerebrospinal fluid 3-methoxy-4-hydroxyphenlglycol and more urinary outputs of norepinephrine (Roy et al., 1988). Consistent with the results of this study is the observation that individual differences in the noradrenergic system are related to differences in sensation-seeking and impulsivity, two personality traits significantly correlated with pathological gambling (Blaszczynski, Steel, & McConaghy, 1997; Zuckerman & Kuhlman, 2000). Additionally, impulse control disorders, of which pathological gambling is an example, are often found to relate to dysfunction in the serotonergic system (Ibáñez, Blanco, & Sáiz-Ruiz, 2003). Twin and molecular genetic studies suggest that different genes underlie pathological gambling in men and women. In one twin study of sex differences, Winters and Rich (1999) found a significant heritability explaining "high action" gambling such as casinos and slot machines among 92

monozygotic and dizygotic male twin pairs, but a nonsignificant heritability among 93 female twin pairs. A recent molecular genetic study of gambling found that in females, the dopamine D4 receptor allele was more frequent in the genotypes of gamblers than controls, whereas in men, the serotonin transporter allele 5-HTTLPR was more frequent in the genotypes of gamblers than controls (Ibáñez, Blanco, Pérez de Castro, Fernández-Piqueras, & Sáiz-Ruiz, 2003; Ibáñez, Perez de Castro, Fernández-Piqueras, & Sáiz-Ruiz, 1998).

This study's pattern of results are found in studies examining sexual orientation differences in other addictive behaviors, such as tobacco use and alcohol consumption. For tobacco use, the most comprehensive report to date reviewed 12 studies, four based on youths and eight based on adults (Ryan, Wortley, Easton, Pederson, & Greenwood, 2001). The median tobacco use rates for *both* adult male homosexual (36.8%) and for adult female homosexuals (37%) were higher than for heterosexual men (27.7%) and heterosexual women (14%) during the same period. These rates imply a median difference in tobacco use between homosexual and heterosexual people that is greater for women (23%) than for men (9.1%). In our study, heterosexual males did gamble more than homosexual males, but like tobacco, the difference was smaller than the difference between homosexual and heterosexual women. Another important conclusion from tobacco use research is that sexual orientation differences increase as levels of tobacco use increase (Bradford, Ryan, & Rothblum, 1994; Stall et al., 2001), as do sex differences (Wechsler, Lee, & Rigotti, 2001). In fact, based on the data reported by Ryan et al., it is apparent that sexual orientation differences are seldom significant at low to moderate (defined as less than one pack of cigarettes a day) levels of tobacco use.

Data are available to draw comparisons between gambling and alcohol use: Most surveys of homosexual males and females find higher rates of alcoholism than in the general population for *both* groups (e.g., Cochran, Keenan, Schober, & Mays, 2000). Further, sexual orientation differences are more pronounced for women. Most studies find that alcohol use by homosexual women closely resembles the alcohol use of heterosexual men, while the difference between homosexual and heterosexual men is usually much smaller. Sexual orientation differences in alcohol use are also most evident at high levels of alcohol consumption. Interestingly, this is true for sex differences, as there are more male alcoholics than female alcoholics (e.g., Green, Polen, Dickinson, Lynch, & Bennett, 2002).

Thus, given that the sexual orientation findings for gambling resemble the findings for alcohol and tobacco use, perhaps gambling should be seen within a broader context of sexually dimorphic addictive/externalizing behaviors. As such, these behaviors may reflect in part a common origin—the relative masculinization of the brain by prenatal hormones.

We should point out an important limitation of this study. Our measure of gambling consisted of a single item, the reliability of which can be questioned. However, a longer measure may increase the probability of sufficient reliability, but does not guarantee it, nor does the use of a single item necessarily negate the conclusions drawn from it. To put our measure of gambling into perspective, single item measures are frequently and successfully used in other studies of addiction (e.g., Ward, Swan, & Jack, 2001). Another flaw in our single item measure was the absence of definitions for *rare*, *little*, *some*, and *much* gambling that could assist respondents in distinguishing among the magnitudes implied by the response options. There is some evidence, however, that respondents were able to make the most difficult distinction, that between *little* and *some*: Recall that a significant sex difference was found on the combination of categories one, two, and three

(*none*, *rare*, *little*), representing low gambling frequency versus the combination of categories four and five (*some*, *much*), representing high gambling frequency.

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Behavioural Brain Research 71 (1995) 69–79

BEHAVIOURAL BRAIN RESEARCH

Research report

Voltammetric and microdialysis monitoring of brain monoamine neurotransmitter release during sociosexual interactions

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Abstract

The monoamine neurotransmitters have long been ascribed important modulatory actions on male sexual behavior by a wealth of pharmacological studies. Methodological developments have now made possible the assessment of the extracellular levels of amine transmitters and their metabolites in discrete brain areas of sexually behaving animals using in vivo voltammetry and microdialysis. Studies in our and other laboratories consistently show increased dopamine release in forebrain structures known to be involved in mating activity, including the nucleus accumbens and the medial preoptic area, during both the appetitive (i.e., non-contact exposure to sexual stimuli) and consummatory phases of this behavior. Serotonin utilization seems to be mainly related to consummatory events. These findings are consistent with the pharmacological evidence as well as previous ex vivo work. The state of sexual inactivity that follows unrestricted mating associates with increased dopamine turnover in the preoptic area. According to the available information, it could reflect some blockade of dopaminergic receptors, possibly involving prolactin. No disturbance of ongoing sexual behavior was observed during the neurochemical monitoring sessions with either methodology. These studies show voltammetry and microdialysis as powerful complementary tools for the assessment of sociosexual interactions.

Keywords: In vivo voltammetry; Microdialysis; Dopamine; Serotonin; Mating behavior; Sexual satiety; Prolactin

1. Background

The neurotransmitter mechanisms regulating behavioral processes, including sex, can be investigated in several ways. Earlier studies relied on the behavioral effects of manipulating the levels of different transmitter systems by various means such as surgical lesions, electrical stimulation of neuronal pathways and drug treatments purportedly acting through specific neuromediators. The widespread use of pharmacological agents has led to a wealth of data on the effects of many drugs on different components of the sex behavioral pattern, in several species including man (see [38] for review). These studies have shown that sexual activity can be influenced by drugs acting on many neuromediators, such as several neuropeptides, amino acid transmitters, and nitric oxide. However, the most documented experimental manipulations of neurotransmitters having effects on reproductive behavior, are those involving the monoamines dopamine (DA), serotonin (5-HT) and norepinephrine (NE). The pharmacological approach has provided, and still does, valuable information, not least because of the potential practical implications. Yet, the interpretation of these data poses some problems, the concern about the physiological significance of drug-induced behavioral effects being an important one. Furthermore, too often the initial claims about specific neurochemical mechanisms of action (i.e., neurotransmitter systems and receptors involved) of many drugs become challenged by further pharmacological studies.

Another experimental approach is looking at the changes in brain levels of neuromediators occurring concomitantly with the behavior under study. The development since the mid-seventies of increasingly sensitive and reliable chromatographic techniques (HPLC) allowed the measurement of transmitter content in small samples of neural tissue. In these experiments, groups of animals are killed following some experimental manipulations or after displaying the spontaneous behavior being assessed. The brain regions that have attracted most attention for neurochemical studies on sexual

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SSSDI 0166-4328(95)00043-7

behavior are the medial preoptic area (MPOA) and the nucleus accumbens (ACB). The former has long been known as crucial for the expression of mating behavior in males. The ACB, with its prominent dopaminergic innervation, is ascribed an important role in the appetitive phases of goal directed behaviors, including mating, as a functional interface between the limbic and motor systems [9,38].

Some studies in our and other laboratories assessed the possible changes in endogenous transmitters and their metabolites in animals killed while mating or engaged in some related behaviors. Again, most of the reported work has dealt with the monoamine neurotransmitters. Thus, we measured the levels of these amines and some of their main metabolites in regions of the brain and spinal cord known to be relevant for the display of sexual behavior in male rats decapitated while mating [36]. At variance with other brain regions in which no neurochemical changes were detected, the concentration of dihydroxyphenylacetic acid (DOPAC), a main metabolite of DA which is regarded as a suitable index of dopaminergic neuronal activity, was higher in the medial preoptic area apparently since the onset of mating. It was found elevated in animals killed just after a first intromission, and remained so thereafter. The levels of the main 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) were increased in the animals killed immediately after ejaculating, but not before it. These findings suggested an involvement of the dopaminergic innervation of the preoptic area in the initiation of mating behavior, whereas the serotonergic activity could be mainly related to the consummatory processes. It could also be related to the satiety mechanisms, as suggested by another study finding increased 5-HT levels in the MPOA of animals killed during the period of sexual inactivity ensuing unrestricted mating [21]. This interpretation would be consistent with pharmacological studies on local injections in the MPOA showing that DA agonists stimulate mating [22], whereas the infusion of 5-HT has inhibitory effects [56].

The accumulation of the hydroxylated amino acidic precursors of DA and 5-HT, respectively, dihydroxyphenlyalanine (DOPA) and 5-hydroxytryptophan (5-HTP), following the inhibition of their enzymatic decarboxylation (an index of transmitter synthesis rate) was also found higher in homogenates of the ACB and the dorsal striatum of male rats killed after mating. Other areas such as the amygdala and the cerebral cortex showed a more selective accumulation of only the catecholamine precursor [1,2]. Studies based on the pharmacological inhibition of the catecholamine synthesizing enzyme tyrosine hydroxylase in male rats placed for 20 min with receptive females found increased NE turnover in the basal hypothalamus and the median eminence whereas they were decreased in the olfactory bulb; the DA turnover showed an opposite pattern. Such a rise in hypothalamic NE turnover following exposure to females was absent in sexually unresponsive diabetic [20] and hyperprolactinemic [53] male rats which also had a decreased secretory reponse of luteinizing hormone (LH) and testosterone. Thus, the changes in NE turnover found in the normally mating controls could be related to the concomitant neuroendocrine processes since pharmacological studies have long ascribed to this transmitter an important role in LH secretion.

The underlying assumption in the ex vivo approach is that the neurochemical changes detected are a close reflection of those occurring at the time of death. Yet, this contention has become challenged by studies showing large changes in brain neurotransmitter release and metabolism immediately after the animal's death e.g., [17,49,50]. Other limitations include the impossibility of performing repeated measurements in the same subject and the need for large numbers of animals to obtain time-course data.

To circumvent the above problems various methodologies have been developed aiming to assess the utlization of neurotransmitters in the brain of living animals (see [14] for an overview). Earlier techniques, such as the cortical cup, push-pull perfusions, and collection of cerebrospinal fluid (CSF) were used mostly in pharmacological studies on anesthetized animals. Successive methodological refinements have made possible such neurochemical monitoring in awake animals, with little disturbance of ongoing behaviors. This can be done at present with two different but complementary methodologies, namely in vivo voltammetry and microdialysis. Since their inception in the early 70s both have grown as reliable neurochemical tools now being widely used in pharmacological and behavioral studies. In recent years they have been applied in our and other laboratories to the assessment of neurochemical correlates of several aspects of sexual behavior in experimental animals, especially in the male rat. As a result, there is already a sizeable body of information on this topic that is summarized and discussed in the present article.

2. Methodological issues

Both in vivo voltammetry and microdialysis assess the concentration of neurochemicals present in the interstitial space. Thus, at variance with the studies on whole tissue samples, they measure that fraction of brain neurotransmitters which is significant for interneuronal communication, i.e., their extracellular levels. A main problem, however, is that because of the highly efficient re-uptake systems clearing the released neurotransmitters, the extracellular concentrations of transmitter molecules are considerably smaller (less than 10^{-3}) than those found in the whole tissue. That poses an important strain on the analytical techniques monitoring their release in vivo. Since the transmitter metabolites, such as 5-HIAA, DOPAC and homovanillic acid (HVA)

instead of being stored intraneuronally exit to the extracellular space as they are formed, their interstitial levels are much higher than those of the parent neurotransmitter. Because of these sensitivity problems, most of the studies to date have looked at brain areas having higher concentrations of transmitters. Thus, the release of DA has been widely investigated in the caudate-putamen (CP) and ACB. In brain regions with a less dense innervation the studies on in vivo neurochemical monitoring have to rely sometimes on the measurement of metabolites.

2.1. Microdialysis

Microdialysis involves the passage of an artificial CSF throughout probes fitted with a semipermeable membrane allowing the diffusion of relevant substances into the effluent. The dialysis membrane, in addition to limiting the mechanical trauma to the surrounding brain tissue, keeps away blood cells and protein molecules, including the transmitter degrading enzymes, so that the samples can be assayed directly. The studies to date have used HPLC with electrochemical or fluorometric detection to measure the monoamines and their metabolites. These analytical techniques have been refined to high degrees of sensitivity and accuracy in the identification of the compounds being measured, which is a major asset of the microdialysis methodology.

Its main limitations are related to the spatial and temporal resolution. The probes currently used are relatively large, with outer diameters of about 200 μm or larger and 2–4 mm in length. The time required for the collection of microdialysis samples is determined by the sensititvity of the analytical techniques available and the recovery of the substances studied. They often impose collection intervals of about 20 min for each sample. Another source of problems with microdialysis is the glial reaction induced by permanently implanted probes, hampering the exchange of neurochemicals, e.g., [13]. This can be reduced, however, by the use of suitable guide cannula assemblies allowing the dialysis probes to be removed after the collection period and re-implanted a few hours before the next session. For a more comprehensive discussion of the microdialysis methodologies the reader is referred to some excellent reviews [47,59].

2.2. Voltammetry

With in vivo voltammetry the substances being analyzed are oxidized in situ at the surface of small working electrodes placed stereotaxically in the chosen brain areas. This is done by applying selected voltages by means of a potentiostatic circuit. The resulting current, which is amplified and recorded, is directly related to the amount of compounds been oxidized at a given applied potential (see [14,32,54] for reviews). Each of the electroactive substances, i.e., those suitable for electrochemical detection in vivo, has a characteristic oxidation potential making it theoretically possible to distinguish it from other compounds. To what extent this selectivity is achieved depends on several factors, especially the working electrode and the way in which the oxidizing potential is applied (the voltammetric technique). The working electrodes are graphite-based and made in different forms such as single or multiple carbon microfibers or carbon paste. To improve their performance in terms of sensitivity and selectivity they are variously modified by different coatings or electrochemical preatreatments. Several electrochemical techniques have been adapted for in vivo neurochemistry. Those used to date in behavioral studies include linear sweep voltammetry, chronoamperometry (CH) differential pulse voltammetry (DPV), and differential normal pulse voltammetry (DNPV).

The advantages of voltammetry include the probe size (carbon monofiber electrodes can be as small as 8 μm in diameter), the high time-resolution (seconds) and the relatively low cost of the equipment. The limitations include the rather low sensitivity of some of the techniques, the difficulty in establishing the identity of the substances being measured since the oxidation current of many of them frequently overlap, and the limited number of neurochemicals oxidized within the rather short range of applied potentials that can be used in the living brain. They include, however, the monoamine neurotransmitters and their acidic metabolites as well as other substances such as ascorbic acid (AA) and uric acid (UA).

Both AA and UA are found in the brain insterstitial space in much higher concentration than the monoamines and even their metabolites. With several of the available electrochemical techniques the AA or UA oxidation current can easily mask the changes occuring in the transmitter or metabolite levels. For this reason, the researchers have often tried to eliminate these compounds by various means. Nevertheless, they can also provide valuable information, since their levels have been found to correlate with neural activity. Thus, in regions such as the CP or the ACB they both show a clearcut circadian pattern closely related to that of motor activity [10,42]. Moreover, the changes in extracellular levels of AA have been related to the uptake of excitatory amino acids reviewed in [43] and in some brain areas they can also reflect the activation of dopaminergic receptors [41,62].

As for the specificity of the electrochemical signals, the techniques giving better resolution, such as DPV or DNPV with electrochemically pretreated carbon fiber microelectrodes, allow the recording of three distinct signals corresponding to compounds having relatively separate oxidation potentials. Thus, there is an AA peak, (at approx. −100 mV), a catechol peak (+60 mV), and an indole/uric acid peak (at +250 mV) [15,19,32,54]. The monoamine metabolites DOPAC and 5-HIAA are the main contributors to, respectively, the catechol or

indole signals recorded with these techniques since, as discussed above, their extracellular levels are much higher than those of the parent transmitters. To detect the transmitters some laboratories eliminate the metabolites via the pharmacological inhibition of the monoamine oxidase enzyme systems. We have addressed this issue, however, in a different way by using computational procedures for the deconvolution of electrochemical signals to assess simultaneously both the neurotransmitter and the metabolite signals within the same voltammetric peak. Thus we have been able to separate the DA and DOPAC components of the +60 mV peak of the striatal DNPV recordings [15,18] as well as the 5-HIAA, 5-HT and UA of the more complex +250 mV peak [19]. In both cases, the efficiency of the method was verified by the HPLC analysis of contralateral dialysates collected simultaneously with the voltammetric recordings. Another problem is that the electrochemically treated carbon fiber microelectrodes, used with DNPV to improve its sensitivity and resolution, work efficiently for only a few hours. They can be replaced periodically, however, during successive days using removable probe systems.

2.3. *Neurochemical monitoring in behaving animals*

As pointed out above, for various reasons most of the voltammetric electrodes (especially those given better sensitivity and resolution) as well as the microdialysis cannulas will work efficiently only for limited periods after implantation in the brain tissue. Even though several systems for inserting the probes shortly before the test sessions have been devised they usually require anesthetizing the animals. This has limited the use of these methodologies for the assessment in physiological circumstances of complex behaviors such as the sexual patterns. We have adapted a removable probe assembly initially proposed for electrochemical recordings [31] which enables the painless periodic replacement of dialysis cannulas and voltammetric electrodes in awake, even unrestrained animals; we use it regularly in our voltammetric (e.g., [15,16,30,35]) and microdialysis experiments (e.g., [12,13,33,34]) in behaving animals. Fig. 1 summarizes the laboratory set-up for the studies discussed below.

3. Monoamine release during male sexual behavior

3.1. *Mating*

Using in vivo voltammetry we first documented a copulation-associated release of DA and DOPAC in the ACB (Fig. 2) [35]. Sexually experienced male rats were implanted with removable electrodes in this structure and placed, several days after surgery, with receptive females, castrated females and intact males. The different social stimuli were presented in random order in successive days. When the subjects were brought in contact with the receptive females they rapidly started mating, with a behavioral pattern similar to that shown in copulation tests before surgery. This was accompanied by a conspicuous increase in the catechol peak height that, according to our deconvolution analysis, was due to the rise of both DA and DOPAC components. This neurochemical change persisted for up to approx. 1 h after the removal of the female, which was done at the resumption of mating following the first ejaculation (i.e., the end of the post-ejaculatory interval). The other social stimuli also elicited increases in extracellular DA and DOPAC, although to a smaller extent than the interaction with receptive females. It should be noted that the DA and DOPAC levels continued rising throughout the post-ejaculatory interval, a period during which the male rats remain essentially quiescent. Therefore, these neurochemical changes were not simply related to physical exercise.

Several microdialysis studies have essentially corroborated these findings [5,12,13,44,45]. They all show that DA and DOPAC levels in dialysates collected from the ACB increase during the mating sessions to decline slowly after the removal of the female. A similar phenomenon has been observed in naive animals during their first mating experience [58], suggesting an innate unconditioned DA response to estrous females. Some of these microdialysis studies compared the mating-related DA release in the ACB with those occuring in the CP, finding the changes in the latter smaller and more closely related to motor activity [5,44].

Other brain areas relevant to sexual behavior, such as the MPOA and the medial basal hypothalamus (MBH), have also been explored using microdialysis. The relatively lower levels of the monoamines in these diencephalic regions precluded their direct measurement as can be usually done with DA in the ACB. Thus, work on these areas had to rely upon the metabolites that, given their much higher concentration, can be assessed more easily. As shown consistently by the studies referred to on the ACB in mating animals, as well as several experiments on DA and 5-HT release and metabolism in other brain regions and behaviors (e.g., [24,46,51,52]) their changes, even if in an attenuated fashion, follow those of the parent amines (see Fig. 2, Fig. 3, Fig. 4); they can therefore be used as indices of transmitter utilization in physiological circumstances. An initial report found increased levels of DOPAC and HVA in two 40 min dialysate samples taken from the MPOA of copulating male rats followed by a slow decline after removing the female [23]. We extended these observations in an experiment comparing the changes in DA and 5-HT oxidation products in three distinct, but anatomically close brain regions, namely the ACB, the

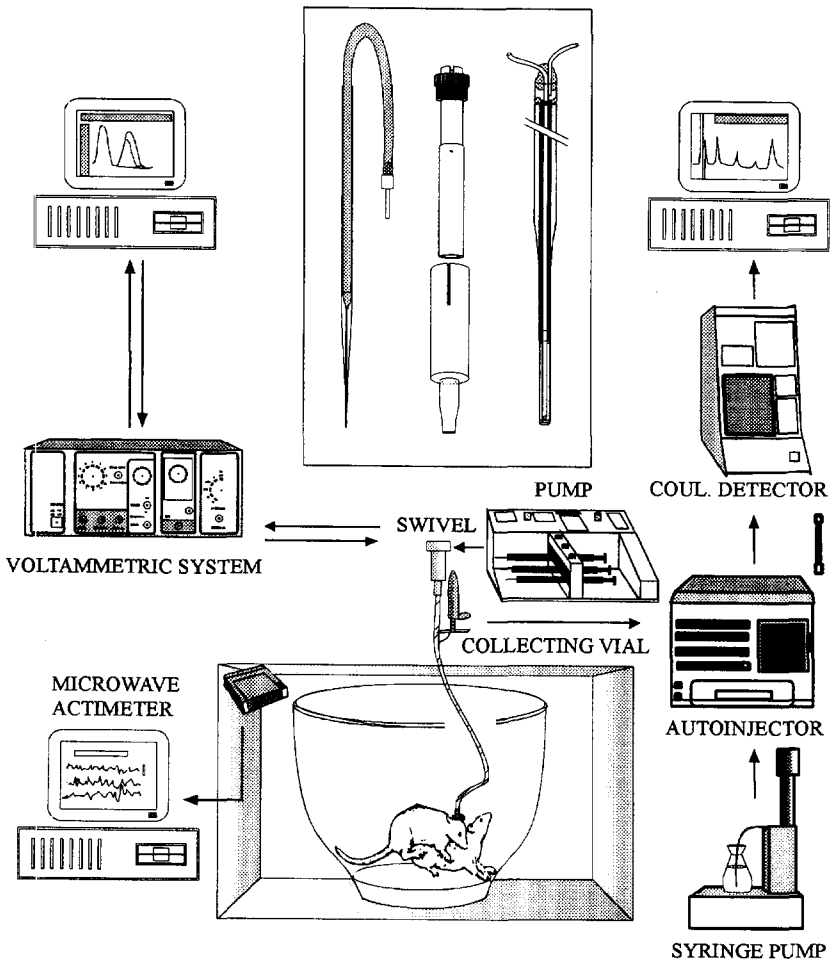

Fig. 1. General set-up for voltammetric and microdialysis studies in sexually behaving animals as used in the authors' laboratory. Inset: drawing, not to scale, of the voltammetric carbon fiber microelectrode (left), the microdialysis probe (right), and the guide cannula assembly used for holding both probes in the animal's head and replacing them (center). The usual size of the active surface of the probes is, for the carbon fiber electrodes: 12 μm in diameter and 500 μm in length, and for the dialysis probes: 250 μm outer diameter and 2–4 mm in length. The lower part of the assembly includes the guide cannula and is cemented to the skull. The upper part is the probe carrier; the outer telescopic cylinder protects the probe and slides back during the insertion. This can be done to the desired depth using the micrometer screw at the top. (Upper left) Voltammetric recordings are done with a custom-made potentiostatic unit controlled by a computer that also performs the deconvolution of complex electrochemical signals. (Right) The microdialysis system includes a precision pump delivering the perfusion fluid at 1–2 μl/min. The collected dialysates are analyzed by a high sensitivity HPLC system including a syringe pump and a coulometric detector. (Lower left) Various devices can be used to monitoring different behaviors concomitantly with the neurochemical changes. As an example, the figure sketches the microwave-based activity meter system used in the prolactin experiment summarized in Fig. 6A.

MPOA, and the MBH in a standard mating test (Fig. 3). Whereas the metabolites of both amines increased during mating in the three areas assessed, there were regional differences, the changes in the MBH being shorter-lived than in the other areas. The DA and 5-HT metabolites also showed a different temporal pattern. Thus, DOPAC levels rose sharply from the beginning of mating but the increase in 5-HIAA was not significant until the subjects had ejaculated. This finding is reminiscent of our previous data on MPOA homogenates of male rats killed after displaying either a first intromission or an ejaculation [36]. Considering the methodological differences and the expected rapid post-mortem changes these similarities are remarkable.

3.2. *Non-contact exposure to sexually relevant stimuli*

Several studies, focused essentially on the ACB, have assessed the possibility that some of the neurochemical changes observed in mating animals can be associated with either sexual cues or pre-copulatory behavior. Using voltammetry we found a rapid release of DA in this structure when sexually naive male rats were placed in cages from which previously kept receptive females had been removed. The change was much smaller in response to odors from intact males or castrated females [30]. Another study, using a different electrochemical technique (high speed CH) reported a similar phenomenon in sexually experienced animals, namely an increase in

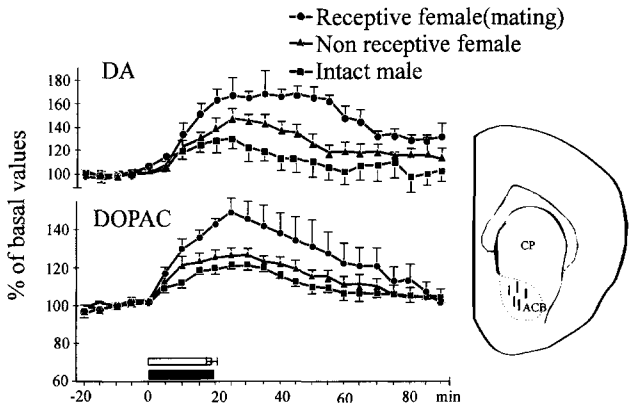

Fig. 2. (Left) Dopamine release in the nucleus accumbens of male rats during sexual behavior as assessed by in vivo voltammetry. The time allowed for interaction with receptive females (to complete one ejaculatory series and resuming mating at the end of the post-ejaculatory interval) is indicated by the open bar at the bottom. The closed bar marks the 20 min interval in which the subjects were in contact with castrated females or intact males. Mean ± S.E.M.; $n=5$. (Right) Approximate location of the electrodes. Adapted from Ref. [35].

DA release in the ACB in reponse to bedding from receptive females but not from males or castrated females [39]. These electrochemical changes were attenuated by naloxone treatment, suggesting an involvement of the endogenous opioids in this phenomenon.

Rapid increases in extracellular DA levels in the ACB have been reported from dialysis studies in male rats brought close to receptive females but separated by a screen. Mating following removal of the screen was accompanied by a further increase in DA levels in the dialysates [5,44].

3.3. Sexual satiety

When sexually rested male rats are allowed unlimited access to receptive females they display repeated bouts of copulatory activity until attaining approx. 7 ejaculations. They then enter into a state of sexual refractoriness, lasting for a few days, during which they do not respond to female solicitations [3,29]. This transient state of sexual inactivity could provide a suitable animal model for studying the neural basis of inhibited sex drive [34] and for testing pharmacological treatments [34,48].

The ability to perform repeated microdialysis monitoring in the same subject for several days [13] (Fig. 4) enables one to follow the changes in transmitter dynamics thoroughout the development and termination of sexual satiety. In a first approach we explored the concurrent changes in monoamine turnover in the MPOA, chosen because of its well-known crucial role for the expression of masculine sexual behavior. As pointed out above, previous microdialysis experiments had shown changes in DA [12,23] and 5-HT [12] metabolism in this area during short mating sessions. As in the preceding studies, we had to rely on the metabolite levels to assess the changes in transmitter dynamics because of the low concentration of the amines in this area. Thus, we implanted sexually experienced male rats with removable dialysis cannula assemblies aimed to the MPOA. Several days after surgery their levels of DA and 5-HT metabolites were monitored during copulation to satiety, the ensuing 2-day state of sexual refractoriness (verified by repeated negative exposure to receptive females), and the resumption of mating on the next day (Fig. 5). Consistent with the previous short-term studies on copulatory behavior, both DA and 5-HT metabolites rose during the two sessions of unrestricted mating. In the intervening days of sexual inactivity, there were small neurochemical changes when placing the subjects with receptive females. Yet, their basal concentrations of both DA metabolites (DOPAC and HVA) were elevated during this period, decreasing toward normal levels by the day when they resumed mating. By contrast, the basal values of 5-HIAA were unchanged throughout the sexually inactive interval[33].

There are several possible interpretations for these findings. Thus, the increased DOPAC and HVA levels in basal dialysates could reflect an enhanced dopaminergic transmission during the sexually sated state. This is difficult to reconcile, however, with the fact that increased dopaminergic transmission in the forebrain is usually associated with the appetititve phases of goal-oriented behaviors, sexual and otherwise (see [4,28] for review). One possibility, in line with the DA theories of incentive motivation, is that a permanently increased dopaminergic activity could substitute for the copulation-related reward, as has been proposed for some drugs of abuse (e.g., [6]). Yet, this mechanim seems unlikely, since the usual stimulatory effects on sexual behavior of DA agonists are also seen when they are given for several days [8,11], and we have found that dopaminergic drugs can induce mating in sexually sated males (33b). A more plausible alternative is suggested by the similarity between the neurochemical findings in the sexual refractoriness state and the effects of DA receptor blockers. These drugs consistently increase the release of DA and its metabolites [25,60,61] by blocking presynaptic autoreceptors and/or the involvement of neuronal feedback loops. They also have well-known inhibitory effects on sexual activity [4,57].

A possible signal mediating the inhibiton of mating via the antagonism of DA actions is prolactin (PRL). The blood levels of this pituitary hormone have been found to rise in male rats after ejaculation [27,40]. PRL shares several behavioral and neurochemical effects with neuroleptic drugs, including the release of DA and DOPAC in forebrain areas, with a concomitant decrease in AA, and the inhibition of motor activity (Fig. 6A)

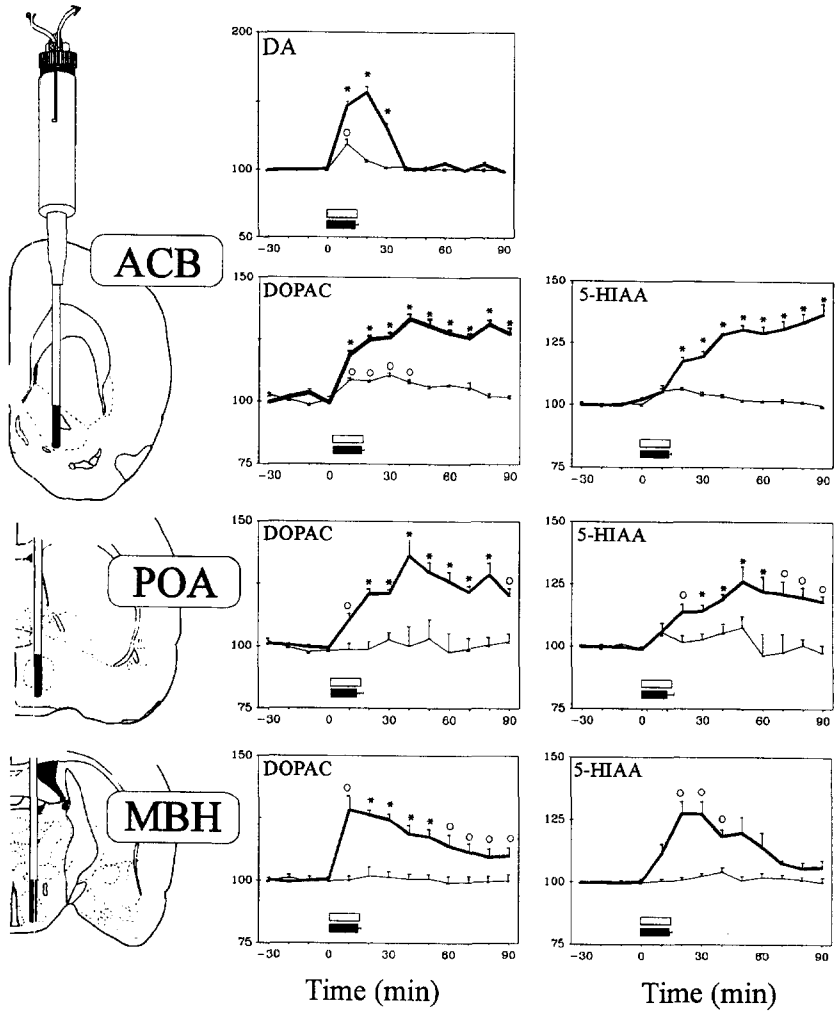

Fig. 3. Microdialysis assessment of DA and 5-HT utilization in three forebrain areas during sexual behavior in male rats. The neurochemical responses are shown as percentage of change over the preceding basal levels when the subjects were placed with either receptive females (i.e., mating) (thick lines) or castrated females (thin lines). The closed bars at the bottom indicate the time in contact with the receptive females. The open bar marks the 15 min allowed for interaction with the control social stimulus. Mean ± S.E.M., $n = 5$–7. Significance vs. basal levels $^{\circ}P < 0.05$; $^{*}P < 0.001$. Adapted from Ref. [12].

[16]. Chronic hyperprolactinemia has long been known to inhibit sexual behavior [7,8,26] and we have recently found that a single injection of PRL can also depress some indices of sexual motivation (Fig. 6B). It is, therefore, plausible that an elevated PRL secretion following sustained copulation could contribute to the ensuing sexual inactivity, possibly by interfering with dopaminergic transmission mechanisms. Nevertheless, further work assessing the time course of changes in endogenous PRL levels during sexual satiety and recovery is required to verify this hypothesis.

3.4. *Specificity of the sex-related neurochemical changes*

By combining various social stimuli, an experimental procedure made possible by the available methods for probe replacement in successive sessions, the behavioral specifity of the observed neurochemical changes can be ascertained to some extent. Thus, the voltammetry experiment summarized in Fig. 2 [35] shows that even though the three social stimuli tested elicited increases in DA release in the ACB, there were important quantitative differences, the changes being clearly larger during the mating sessions. Similar differences were found in our studies on sexual olfactory cues [30].

A critical issue in the field of physiological monitoring in behaving animals is the extent to which the neurochemical changes observed during the different sociosexual interactions indicate a specific behavioral response or are mereley related to a general activation or motor activity. There is no easy answer. Ideally, one would first carefully determine the energy expenditure of these behaviors and then assess the neurochemical changes corresponding to a similar amount of activity. Moreover, this should be done avoiding the stressing effects of, for example, forced locomotion.

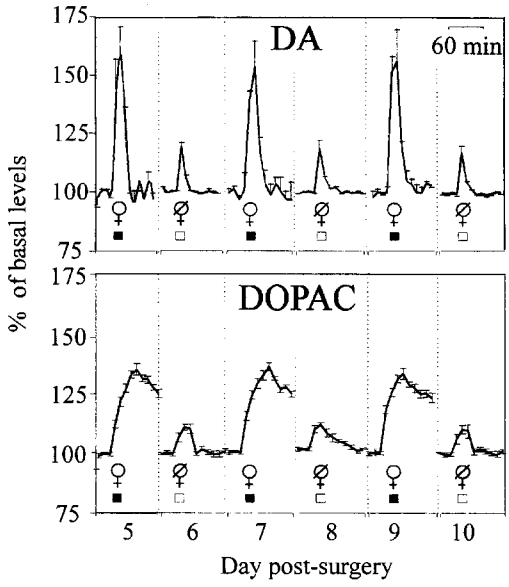

Fig. 4. Changes in DA and DOPAC levels in ACB dialysates collected from male rats during successive days. During the dialysis sessions the subjects were placed with either receptive females (mating; closed bars) or castrated females (open bars). Mean ± S.E.M., $n=5$. Adapted from Ref. [13].

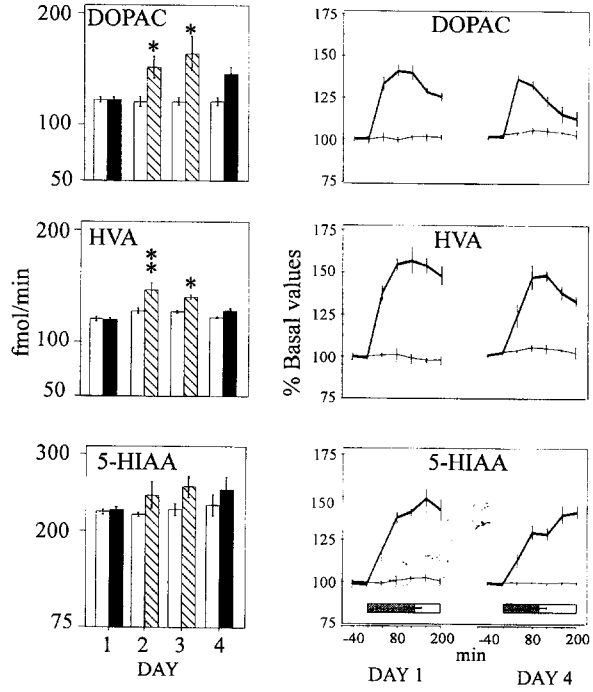

Fig. 5. Neurochemical correlates of sexual satiety. DA and 5-HT metabolites in MPOA dialysates of male rats. In days 1 and 4 the subjects copulated until sexual exhaustion (taking on average 7 and 4 ejaculations, respectively); in days 2 and 3 no mating was observed when successive receptive females were presented. (Left) Basal levels of monoamine metabolites found in the experimental animals the days when mating ensued (closed bars) and during the sexually inactive days (hatched bars); control animals (open bars) were exposed to intact males in similar sessions. Significance vs. day 1: $*P<0.05$; $**P<0.01$. (Right) Changes in the metabolites found during the two mating sessions (thick lines) and in the control subjects (thin lines). The stippled bar at the bottom indicates the total mating time during each session. Mean ± S.E.M., $n=5$. Data from Ref. [33].

There are various indirect data, however, supporting some degree of neurochemical specificity for the changes observed during sexual behavior. Thus, the increases found in the ACB in both the DA efflux in vivo during forced locomotion in a rotating wheel [5] and the accumulation of DOPA determined ex vivo after treadmill exercise [1] were much smaller than those seen during copulation. Likewise, our work on odors from different social stimuli and during the exploration of a novel chamber showed a higher DA release in response to sexually relevant signals [30]. Moreover, as commented on our voltammetry experiment on mating males, there was a period of high DA release in the ACB concomitant with hypomotility during the post-ejaculatory interval [35]. Further evidence for such a dissociation is provided by the finding of high DA levels in ACB dialysates from female hamsters displaying their characteristically static lordosis posture (i.e., remaining essentially motionless) during most of a mating session lasting 1 h [37]. Yet, there is a need for further studies specifically designed to clarifying these important questions.

4. Future prospects

The experiments reviewed here show substantial developments within the last 5 years in the assessment of neurochemical correlates of reproductive behaviors. The existing data are fairly consistent with the bulk of the lesion and pharmacological evidence as well as with the preceding ex vivo studies. This is an important aspect since correlational studies, as those discussed here, have the inherent difficulty of establishing causal relationships between simultaneously occurring behavioral events and neurochemical changes. Answering these questions requires surgical and pharmacological manipulations which will remain, therefore, valuable tools for testing hypotheses arising from the neurochemical data. The studies on PRL summarized in Fig. 6 provide an example of such a combination of methodologies.

Despite this progress, the field of in vivo assessment of neurochemical correlates of complex behaviors is still in its infancy, given the important methodological limitations only partly overcome in recent times. It can be easily foreseen that the rapid development of analytical methodologies far more sensitive than those used to date, such as capillary electrophoresis and new electrochemical techniques, will allow the measurement of transmitters in brain areas in which they still remain undetectable. The monoamine transmitters have been the neuromediators most explored so far in neurochemical studies mainly because their unique electrochemical and fluorogenic properties make them easier to measure.

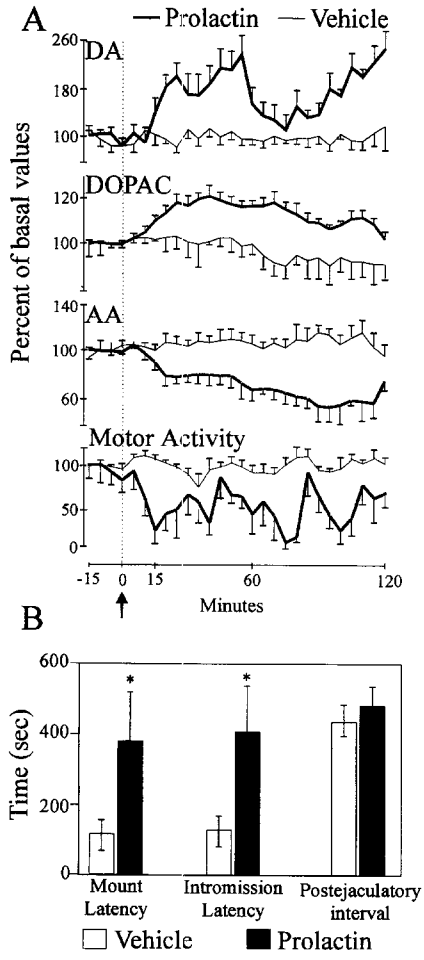

Fig. 6. The prolactin hypothesis of sexual satiety. (A) Increase in extracellular DA and DOPAC and decrease in AA in the nucleus accumbens (assessed by DNPV with deconvolution of the catechol signal) concomitant with decreased motor activity following a systemic injection (arrow) of ovine PRL (oPRL, 4 mg/kg). Mean ± S.E.M., $n=5$ (from Ref. [16]). (B) Effects of a single injection of oPRL (4 mg/kg) given 60 min before a standard mating test on some measures of sexual behavior in male rats. $^*P<0.05$ vs. vehicle, $n=12$.

More complex analytical methodologies, as those required for the detection of other neuromediators (e.g., derivatization for the amino acids, post-column enzyme-reaction for acetylcholine, and immunoassays for the neuropeptides), are now well established and will soon lead to descriptions of changes in these substances in discrete brain areas in connection with sexual behavior. By comparing their findings with the data available on the monoamines, it will be possible to assess the relative significance of different types of neurotransmitters for sexual functioning.

Methodological progress will also influence the experimental designs. Being able to monitor reliably the localized release of neurotransmitters in the same animal for several consecutive days will translate into more sophisticated experimental models than the simple mating tests; the studies discussed above on the neurochemistry of sexual exhaustion and recovery (Fig. 5) provide an example. These approaches could also be especially useful to assess incentive motivational learning processes. Doubtless, there will be reports in the near future about these and other topics relevant to the understanting of sexual motivation and performance using the complementary and increasingly powerful tools provided by in vivo electrochemistry and microdialysis.

Acknowledgment

Supported by Grant FIS 93/0346 to M.M. Ovine prolactin was a gift from the NHPP (NIADDK).

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Review

The neurodevelopment of human sexual orientation

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Received 29 October 2004; revised 26 January 2005; accepted 4 March 2005

Abstract

One of the most enduring and controversial questions in the neuroscience of sexual behaviour surrounds the mechanisms which produce sexual attraction to either males or females. Here, evidence is reviewed which supports the proposal that sexual orientation in humans may be laid down in neural circuitry during early foetal development. Behaviour genetic investigations provide strong evidence for a heritable component to male and female sexual orientation. Linkage studies are partly suggestive of X-linked loci although candidate gene studies have produced null findings. Further evidence demonstrates a role for prenatal sex hormones which may influence the development of a putative network of sexual-orientation-related neural substrates. However, hormonal effects are often inconsistent and investigations rely heavily on 'proxy markers'. A consistent fraternal birth order effect in male sexual orientation also provides support for a model of maternal immunization processes affecting prenatal sexual differentiation. The notion that non-heterosexual preferences may reflect generalized neurodevelopmental perturbations is not supported by available data. These current theories have left little room for learning models of sexual orientation. Future investigations, across the neurosciences, should focus to elucidate the fundamental neural architecture underlying the target-specific direction of human sexual orientation, and their antecedents in developmental neurobiology.

Keywords: Sexual orientation; Homosexuality; Heterosexuality; Genetics; Prenatal androgens; Fraternal birth order; Developmental instability; Proxy markers; Maternal immunity; Hypothalamus; Developmental neurobiology; Learning

Contents

1. Introduction

Sexual orientation refers to a dispositional sexual attraction towards persons of the opposite sex or same sex. Sexual orientation appears 'dispositional' in that it comprises a target selection and preference mechanism sensitive to gender, motivational approach behaviours towards the preferred target, and internal cognitive processes biased towards the preferred target (such as sexual fantasies). In contrast, sexual orientation does not appear to be a matter of conscious self-labelling or past sexual activity because these are subject to contingent social pressures, such as the presence of linguistic descriptors and visible sexual minorities within an individual's culture,

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0149-7634/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neubiorev.2005.03.002

and the availability of preferred sexual partners (Bailey, 2003). Therefore, in human investigations, sexual orientation is often assessed using self-report measures of 'sexual feelings' (i.e. sexual attraction and sexual fantasies) rather than self-labelling or past hetero- or homosexual activity.

Sexual orientation appears to be a dichotomous trait in males, with very few individuals demonstrating an intermediate (i.e. 'bisexual') preference. This is borne out by fine-grained analyses of self-reported heterosexual and homosexual orientation prevalence rates (using measures of sexual feelings) in population-level samples, and work on physiological genital arousal patterns (e.g. using penile plethysmography) in response to viewing preferred and non-preferred sexual imagery. Both lines of evidence consistently demonstrate a bimodal sexual orientation among men—heterosexual or homosexual, but rarely 'bisexual' (Chivers et al., 2004; Dickson et al., 2003; Erens et al., 2003; Sakheim et al., 1985; Wellings et al., 1994). This is less so in the case among women. For example, Chivers et al. (2004) demonstrated a 'bisexual' genital arousal pattern among both heterosexual and lesbian women, suggesting a decoupling of self-reported sexual feelings (which appears broadly bimodal) from peripheral sexual arousal in women.

If sexual orientation among humans is a mostly bimodal trait, this implicates a canalization of development along a sex-typical route (heterosexual) or a sex-atypical (homosexual) route. Statistical taxometric procedures have confirmed this by demonstrating that latent taxa (i.e. non-arbitrary natural classes) underlie an opposite-sex, or same-sex, orientation in both men and women (Gangestad et al., 2000). Less well established are the factors that may be responsible for this 'shunting' of sexual orientation along two routes (the edges of which are fuzzier in women). These factors are the subject of the remaining discussion and it is suggested that they probably operate neurodevelopmentally before birth.

2. Behavioural and molecular genetics

A natural starting point for the neurodevelopment of physiological and behavioural traits must begin with the genetic level of investigation. Several family and twin studies provide clear evidence for a genetic component to both male and female sexual orientation. Family studies, using a range of ascertainment strategies, show increased rate of homosexuality among relatives of homosexual probands (Bailey and Pillard, 1995). There is also evidence for elevated maternal line transmission of male homosexuality, suggestive of X linkage (Camperio-Ciani et al., 2004; Hamer et al., 1993), but other studies have not found such elevation relative to paternal transmission (Bailey et al., 1999). Among females, transmission is complex, comprising autosomal and sex-linked routes (Pattatucci and Hamer, 1995). Twin studies in both community and population-level samples report moderate heritability estimates, the remaining variance being mopped up by non-shared environmental factors (Bailey et al., 2000; Kendler et al., 2000; Kirk et al., 2000). Early attempts to map specific genetic loci responsible for sexual orientation using family pedigree linkage methods led to the discovery of markers on the Xq28 chromosomal region (Hamer et al., 1993), with one subsequent replication limiting the effect to males only (Hu et al., 1995). However, there is at least one independent study which produced null findings (Rice et al., 1999), while a recent genome wide scan revealed no Xq28 linkage in a new sample of families but identified putative additional chromosomal sites (on 7q36, 8p12 and 10q26) which now require denser mapping investigations (Mustanski et al., 2005). These studies are limited by factors such as the unclear maternal versus paternal line transmission effects, possible autosomal transmission and measurement issues. Two candidate gene studies which explored the putative hormonal pathways in the neurodevelopment of sexual orientation (see Section 3): one on the androgen receptor gene and another on aromatase (CYP19A1) both produced null findings (DuPree et al., 2004; Macke et al., 1993).

3. The prenatal androgen model

Several decades of research in animal models have demonstrated a major role for gonadal steroidal androgens in accounting for almost all known sexual dimorphism in brain and behaviour among vertebrates (Morris et al., 2004). These have guided investigators to search for the possible origins of human sexual orientation in androgens and their target neural substrates. Within this framework, it has become a cliché to suggest that heterosexual preference in men are due to typical degrees of prenatal exposure to androgens (primarily testosterone), and that heterosexual preference in women is due to default mammalian development along female lines (due to very little prenatal androgen exposure). Conversely (the cliché continues), homosexuality in men is due to under-exposure to prenatal androgens and in women, due to over-exposure (Ellis and Ames, 1987). This classic model of the origins of sexual orientation had some early support from experimental manipulation of prenatal sex hormone levels in animal models, and the prevalence of variant sexual orientation (in line with purported prenatal sex-typical or sexual-atypical hormonal exposure) among human inter-sex cases or in those with endocrine disorders, such as congenital adrenal hyperplasia (Bailey, 2003; Morris et al., 2004).

More recently, research in this area has moved to focusing on 'proxy markers' for prenatal hormonal exposure that can be easily, and non-invasively, explored in otherwise endocrinologically normal populations. These 'proxy markers' comprise somatic features which are known to be influenced by sex hormones prenatally. Thus showing variation in these traits between adult heterosexuals

and homosexuals may provide a 'window' into the early neurodevelopment of sexual preferences under the actions of prenatal hormones. These markers are certainly imperfect tools, but in the absence of prospective research, they have provided some intriguing insights. The best known proxy marker of prenatal hormonal exposure is the ratio of the second to fourth finger lengths (or 2D:4D ratio) (Manning et al., 1998). 2D:4D is sexually dimorphic, with males showing lower ratios than females. Evidence for lower ratios in individuals with androgen over-exposure (such as in the condition congenital adrenal hyperplasia) strongly implicates prenatal androgens in modulating 2D:4D (Brown et al., 2002a; Ökten et al., 2002; Buck et al., 2003). 2D:4D is also linked to variation in the androgen receptor gene (Manning et al., 2003a) and the ratio of testosterone to estrogen taken from amniotic fluid during gestation is negatively associated with 2D:4D at 2 years of age (Lutchmaya et al., 2004). Although ultimately correlational, these data suggest strongly that excess androgen exposure can alter the relative lengths of the second and fourth finger digits.

Four independent studies have shown that homosexual women have significantly masculine (lower) 2D:4D ratios compared to heterosexual women, although these appear to be hand-specific (Rahman and Wilson, 2003a; Williams et al., 2000; Rahman, 2005; McFadden and Schubel, 2002) but one study reported no such difference (Lippa, 2003). In homosexual men, three reports show more *male-like* (i.e. 'hyper-masculinized') 2D:4D ratios compared to heterosexual men (Rahman and Wilson, 2003a; Rahman, 2005; Robinson and Manning, 2000), while another two demonstrated more *female-like* 2D:4D ratios in homosexual men (Lippa, 2003; McFadden and Schubel, 2002). A further report showed that only homosexual men with two or more elder brothers had hyper-masculinized right-hand 2D:4D ratios (Williams et al., 2000). Evidence for possible 'within-sexual orientation' variations was reported by one study showing lower 2D:4D in self-identified 'butch' compared to 'femme' homosexual women (Brown et al., 2002b), while another did not find this in both homosexual men and women (Rahman and Wilson, 2003a). Overall, these data strongly suggest that lesbians are exposed to a greater degree of masculinization by prenatal androgens than heterosexual women. However, the reports for men are confusing—some showing 'hyper-male' 2D:4D and others female-like patterns. A possible solution for the male findings may be found in the demonstration of a 'uniform mean' 2D:4D ratio among homosexual men of between 0.96 and 0.97, contrasted with substantial variation among heterosexual populations (Manning and Robinson, 2003). This 'uniform mean' may also be population-specific as the available data indicate that only Caucasian ethnic groups manifest it (McFadden et al., in press; Voracek et al., in press). The narrow range (a masculinized value) may indicate the prenatal androgen level that maximizes the chances of a homosexual orientation. Alternatively, the overall evidence might simply suggest that both lower-than-average and higher-than-average androgen exposure increases the probability of developing male homosexuality.

Nevertheless, the 'uniform mean hypothesis' is still controversial, partly because in some respects it is simply a restatement of the findings observed thus far. It is possible that there is no real difference between heterosexual and homosexual men in 2D:4D and that the observed differences merely reflect sampling error. Secondly, there is, as yet, no known biological mechanism whereby a constant value in this particular trait should occur in the minority population (i.e. homosexual men) while the majority population shows greater variation.

One additional 'hand-related' trait that differentiates early in gestation is fingerprint patterns, or dermatoglyphics. Although an early study reported that homosexual men possess a female-typical dermatoglyphic pattern (an asymmetry with more ridges on left-hand fingers) than heterosexual men, subsequent independent reports have not demonstrated any sexual-orientation-related variations (Forastieri et al., 2003; Mustanski et al., 2002; Slabbekoorn et al., 2000). Thus dermatoglyphic patterns are almost invariably a poorer window on early prenatal differentiation of sexual orientation compared to finger length ratios.

Studies of auditory mechanisms also show specific hyper-masculinization among homosexuals compared to heterosexuals. Oto-acoustic emissions (OAEs) are tiny sounds emitted by the cochlea and can occur spontaneously or be evoked by 'clicking' sounds. OAEs of both varieties are more numerous in females than in males, and in the right ear—this patterning apparent in infants, children and adults. Evidence that OAEs are influenced by prenatal androgens come from the finding that females with male co-twins have masculinized OAE patterns (McFadden, 1993). Two reports have shown less numerous and weaker OAEs in homosexual and bisexual women compared to heterosexual women, but no variation between homosexual and heterosexual men (McFadden and Pasanen, 1998, 1999). While there is no difference between male groups with respect to auditory mechanisms on the periphery, there is centrally. This was discovered by examining the auditory evoked potentials (AEPs) produced in response to click-stimuli. On 5 of 19 AEP outcome measures, homosexual women showed masculinized responses and homosexual men demonstrated hyper-masculinized responses (McFadden and Champlin, 2000).

Further work under the prenatal androgen framework has reported sexual-orientation-related differences in physical growth markers. Homosexual men consistently report earlier pubertal onset on physical and behavioural indices (e.g. age of first ejaculation or age of first sexual experience) compared to heterosexual men (Bogaert and Blanchard, 1996; Bogaert and Friesen, 2002; Bogaert et al., 2002), whereas homosexual and heterosexual women do not differ in pubertal milestones (Bogaert and Friesen, 2002; Bogaert et al., 2002; Tenhula and Bailey, 1998). There have also

been inconsistent reports for sexual-orientation-related variations in self-reported height and weight (Bogaert and Friesen, 2002; Bogaert et al., 2002) but it is far from clear whether these reflect solely the actions of the prenatal sex steroids or multiple postnatal factors. However, one recent study, which objectively measured skeletal growth in a large community sample, reported that homosexual men have less long-bone growth in the arms, legs and hands compared to heterosexual men, while the converse was found for homosexual compared to heterosexual women (Martin and Nguyen, 2004). As these bones become sexually dimorphic in childhood but not after puberty, these data suggest that homosexual men are partially feminized and homosexual women are masculinized, in specific anthropometric measures before the pubertal increase in sex steroid levels (Martin and Nguyen, 2004).

Overall, all the available evidence points to homosexual women being, on average, exposed to more prenatal androgens than heterosexual women, as predicted. However, it is important to note that there is considerable overlap between the two female groups, indicating that prenatal androgens do not act in isolation. The findings with respect to homosexual men are even more surprising with indications of both elevated and reduced prenatal androgen exposure. This appears inconsistent with the central prediction of prenatal androgen theory that homosexual males should show evidence of lower prenatal androgen levels. However, some resolution might be found if it is supposed that the requisite neural circuitry responsible for same-sex orientation in men is un-masculinized (e.g. perhaps because of genetic factors), which leads to excessive androgenic activity in the development of other somatic features. This might explain the observed (albeit unreliably) hyper-masculinized features among homosexual men. While this suggestion is certainly speculative, it is in accord with known observations for non-monotonic effects of sex steroids in some animal models (Clark et al., 1996).

The point to bear in mind is that male homosexuality may appear as a mosaic of traits (some sex-*typical*, others sex-*atypical* and yet others that are sex-*exaggerated*). This might be produced by differences in the timing and/or concentration of androgen exposure (e.g. lower-than-average and higher-than-average levels) in heterosexual and homosexual males. For example, Geschwind and Galaburda (1985) have suggested that homosexual men are exposed to particularly high androgen levels *very early* in development, explaining both their tendency to be less right-handed (see Section 5) and, by extension, the hyper-masculinized traits observed in this group. Interestingly, these possible temporal and localized variations in androgen exposure might suggest that their actions occur further 'up stream' in the developmental pathway, perhaps explaining the null findings of candidate gene studies above (Section 2) regarding the androgen receptor and aromatase gene.

4. The fraternal birth order effect and maternal immunity

The maternal immunity hypothesis is certainly the most revolutionary neurodevelopmental model of human sexual orientation. Empirically, it rests on one very reliable finding—the fraternal birth order effect (FBO): that is, homosexual men have a greater number of older brothers than heterosexual men do (and relative to any other category of sibling), in diverse community and population-level samples, and as early as they can be reliably surveyed (Blanchard, 2004). The estimated odds of being homosexual increase by around 33% with each older brother, and statistical modelling using epidemiological procedures suggest that approximately 1 in 7 homosexual men may owe their sexual orientation to the FBO effect (Cantor et al., 2002). It has been suggested that the remaining proportions of homosexual men may owe their sexual orientation to other causes, such as differential prenatal androgen levels (Blanchard, 2004; Cantor et al., 2002). Homosexual and heterosexual women do not differ in sibling sex composition or their birth order, thus any neurodevelopmental explanation for the FBO effect is limited to males (Bogaert, 1997). Importantly, recent work has demonstrated that homosexual males with older brothers have significantly lower birth weights compared to heterosexual males with older brothers (Blanchard and Ellis, 2001; Blanchard et al., 2002). As birth weight is undeniably prenatally determined, some common developmental factor operating before birth must underlie FBO and sexual orientation among human males.

Specifically, investigators have proposed a role for the progressive immunization of some mothers to male-linked antigens produced by carrying each succeeding male foetus. That is, the maternal immune system 'sees' male-specific antigens as 'non-self' and begins producing antibodies against them (Blanchard, 2004). One possible group of antigens are the Y-linked minor histocompatibility antigens, specifically *H–Y*. The accumulating H–Y antibodies may divert male-typical sexual differentiation of the foetal brain, leading the individual to be sexually attracted to males (Blanchard and Bogaert, 1996). For example, male-specific antibodies may bind to, and inactivate, male-differentiating receptors located on the surface of foetal neurons thus preventing the morphogenesis of masculinized sexual preferences.

The maternal immunity theory is consistent with a number of observations: the number of older sisters is irrelevant to sexual orientation in later born males; the H–Y antigen is expressed by male foetuses only and thus the maternal immune system 'remembers' the number of males carried previously and may modulate its response; and H–Y antigens are strongly represented in neural tissue (Blanchard, 2004; Blanchard and Bogaert, 1996). Nonetheless, there is no data specifying a role for these particular antigens in sexual preferences among humans. There are

several alternative candidate antigens to H–Y, including the distinct Y-linked protein families' *protocadherin* and *neuroligin*, both which have been found in humans. These cell adhesion proteins are thought to influence cell–cell communication during early male-specific brain morphogenesis and may have male-typical behavioural consequences (Blanco et al., 2000). Consistent with these studies is neurogenetic evidence for the direct transcription of Y-linked sex determination genes *SRY* and *ZFY* in the male human brain (including hypothalamus) (Mayer et al., 1998). The maternal immunity model may also explain the link between birth weight and sexual preferences: mouse models show that maternal immunization to male-derived antigens can affect foetal weight (Gentile et al., 1992; Lu and Dawson, 1986). Furthermore, male mice whose mothers are immunized to H–Y prior to pregnancy show reduced male-typical consummatory sexual behaviour towards receptive females (Singh and Verma, 1987).

The maternal immunity model implicitly relies on a non-hormonal immunologic neurodevelopmental explanation and thus cannot immediately explain the hyper-male features (e.g. 2D:4D and AEPs) associated with male homosexuality. It is possible that male-specific antibodies may interact with sexual differentiation processes controlled by sex hormones or be completely independent of them—this is unknown as yet.

5. Developmental instability and sexual orientation

There has been some argument recently that the prenatal androgen theory does not adequately explain the robust association between sexual orientation and handedness. Homosexual men have an approximate 34% odds ratio of being non-right-handed and homosexual women have approximately 91% odds of being so, compared to heterosexuals (of whom men are more non-right-handed than women) (Lalumiere et al., 2000). As the classic version of prenatal androgen theory predicts that homosexual men should show *less* non-right-handedness and homosexual women *more*, the observation holds true for women but not men (Lalumiere et al., 2000). Thus, perhaps a more domain-general developmental explanation for variation in human sexual orientation is needed. This may be found in *developmental instability* (DI) which refers to an organism's level of vulnerability to environmental and genetic stresses during development. In this view, same-sex orientation is due to generalized developmental insults that shift erotic preferences away from the species-typical pattern of opposite-sex attraction (Lalumiere et al., 2000). Again, proxy somatic measures of these 'insults' or 'instability' are proposed to provide a window on the developmental history of the organism—handedness being one such proxy. At first inspection the handedness data does appear to provide support but its explanation is, in fact, more parsimonious within prenatal androgen theory. The apparent hyper-masculinized handedness of homosexual men can be squared with the explanation for hyper-masculinization in other somatic features such as finger length ratios, as detailed earlier. In support, two studies have reported a robust association between low 2D:4D and left-hand preference (Fink et al., 2004; Manning et al., 2000).

The most commonly utilized proxy measure of DI involves measuring random deviations from perfect symmetry in bilateral bodily features (e.g. dermatoglyphics, and lengths of ears, fingers, wrists and feet) and is referred to as fluctuating asymmetry (FA). FA is thought to reflect differential genomic robustness. Individuals with genomes which are less sensitive to stress-induced disruption may show suppression in phenotypic variation and thus be reproductively 'fitter' (i.e. produce the 'ideal' phenotype, such as heterosexuality). Therefore, a central prediction from DI theory is that heterosexuals of both sexes should show low FA values compared to homosexuals. Several reports have found no significant differences in FA between heterosexuals and homosexual (Rahman and Wilson, 2003a; Rahman, 2005; Mustanski et al., 2002) suggesting that a homosexual orientation does not necessarily reflect a 'less than optimal' phenotypic sexual orientation. Therefore, perhaps the canalization of the sexual-orientation trait is more likely due to specific, rather than general-purpose, neurodevelopmental mechanisms (such as the actions of prenatal androgens).

6. Neural circuitry

Neurodevelopmental mechanisms must wire neural circuits differently in those with same-sex attractions from those with opposite-sex attractions, but we still know very little about this circuitry. The first indication for neural correlates of sexual partner preference came from Simon LeVay (1991) autopsy study of the third interstitial nucleus of the anterior hypothalamus (INAH-3) which he found to be smaller in homosexual men than in presumed heterosexual men, and indistinguishable from presumed heterosexual women. Another study found a non-significant trend for a female-typical INAH-3 among homosexual men (and confirmed the heterosexual sex difference), but this was not evidenced the main sexually dimorphic parameter reported by this study (the total number of neurons) (Byne et al., 2001). This preceding finding is noteworthy as a prediction from the prenatal androgen theory would be that a parameter which shows significant sexual dimorphism should also demonstrate within-sex variation attributable to sexual orientation. A conservative conclusion regarding these data is that while INAH-3 is larger in heterosexual men than in heterosexual women, and possibly smaller in homosexual men, structurally speaking this within-sex difference may not be very large at all.

One recent positron emission tomography study has demonstrated stronger hypothalamic response to serotonergic challenge in heterosexual than in homosexual men (Kinnunen et al., 2004), and neuroimaging studies comparing heterosexual men and women while viewing preferred sexual imagery show significantly greater hypothalamic activation in heterosexual men (Karama et al., 2002). These findings, coupled with the anatomical findings described earlier, could be taken to suggest that there is a functionally distinct anterior hypothalamic substrate to sexual attraction towards women. This supposition is further supported by mammalian lesion models of the preoptic area (POA) of the anterior hypothalamus showing reduced appetitive responses towards female by male animals (Hull et al., 2002). Nevertheless, investigations comparing heterosexual and homosexual women are needed to support a role for this region in sexual preference towards females among humans.

While animal models point to a role for prenatal androgens in producing sexual variation in hypothalamic regions (Morris et al., 2004), a similar relationship in humans is unclear. One study found no sexual-orientation-related differences in the distribution of androgen receptors in sexually dimorphic hypothalamic regions (Kruijver et al., 2001). However, one animal model often overlooked by scientists may provide some guidance. Some males of certain species of sheep show an exclusive same-sex preference, and also show reduced aromatase activity and smaller ovine sexually dimorphic nuclei (a possible homolog to the human INAH-3) compared to female-oriented sheep (Roselli et al., 2004). A role for aromatized metabolites of testosterone in underscoring possible hypothalamic variation related to human sexual orientation requires further study in light of these findings (Roselli et al., 2004). Moreover, putative sexual orientation differences in aromatase activity in human males may go some way to explaining the 'mosaic' profile of hypo- and hyper-masculinized traits described earlier. For example, a reduction in aromatase activity in homosexual compared to heterosexual men (predicted from the Roselli findings) may lead to reduced availability of aromatized testosterone (i.e. estradiol) which typically masculinizes the male mammalian brain (Morris et al., 2004). This may lead to hypo-masculinized hypothalamic circuitry and yet leave excess non-aromatized testosterone to hyper-masculinize additional androgen sensitive traits (e.g. 2D:4D) through other metabolic pathways, such as 5-alpha reductase. Note, one mitigating piece of evidence with respect to these suggestions is the null finding of DuPree et al. (2004) regarding sexual-orientation-related variation in the aromatase gene.

The possibility that sexual-orientation-related neural variation extend to higher cortical regions has been evidenced by neurocognitive investigations. Several independent studies consistently demonstrate low scores (female-typical) by homosexual men in basic spatial ability tests (such as mental rotation and spatial perception) compared to heterosexual men (Rahman and Wilson, 2003b). Homosexual men also show better spatial location memory, improved recall of spatial landmarks during navigation, and better phonological and semantic fluency (all female-typical responses) compared to heterosexual men (Rahman et al., 2003a,b, 2005). These data tentatively suggest sexual variation in parietal, hippocampal-temporal, and prefrontal brain regions known to underlie these cognitive skills. Behavioural and structural sexual variation in inter-hemispheric pathways may contribute to these cognitive differences, but are not well replicated (Allen and Gorski, 1992; Wegesin, 1998a). Independent investigations utilizing several neurophysiological measures also support parietal and temporal lobe involvement, depending on the probe used: sexual, auditory or cognitive (Howard et al., 1994; Reite et al., 1995; Wegesin, 1998b). Parietal lobe involvement is likely as this region is part of the neural architecture of heterosexual sexual arousal, and possibly involved in visual-configural processing of preferred sexual 'targets' (Howard et al., 1994; Waisman et al., 2003).

Thus far, almost nothing is known about the neural basis of sexual orientation in women. One sexually dimorphic neurobehavioural probe—pre-pulse inhibition of the startle response (whereby there is a reduction in the eye-blink reflex to a loud noise if preceded by a by a quieter noise)—is strongly masculinized in homosexual compared to heterosexual women, and indicates the involvement of pallido-striato-thalamic limbic circuitry (Rahman et al., 2003c). Cognitive studies demonstrate better verbal fluency among homosexual women, pointing to prefrontal cortical involvement, while neurophysiological studies reveal no differences (Rahman et al., 2003a; Wegesin, 1998b), other than those in the auditory regions revealed by AEPs (McFadden and Champlin, 2000).

The available evidence gives us clues as to the neural network underlying a sexual orientation in men, including anterior hypothalamic regions, and cortical regions such as the parietal lobes. As far as sexual orientation in females is concerned, there is some indication for the involvement of limbic circuitry but little else. The functional neuroendocrinology herein is unknown but clues from the animal literature point to developmental processes under the control of prenatal sex steroids. Further investigation of such processes, such as potential androgenic modulation of apoptosis in the requisite neural circuitry, is needed (Morris et al., 2004; Chung et al., 2000).

7. Is there a role for learning in the development of human sexual orientation?

The role of learning in the development of human sexual orientation has been the subject of much debate and controversy, most likely because it is erroneously believed to result in particular socio-political consequences associated with homosexuality (Bailey, 2003). While data are a little thin on the ground, several lines of evidence mitigate

the involvement of learning mechanisms. In animal models, there are documented effects of conditioning on sexual arousal, approach behaviour, sexual performance and strength of sexual preference towards opposite-sex targets, but no robust demonstrations of learning in the organization of same-sex preferences among males (Pfaus et al., 2001; Woodson, 2002). Interestingly, one study in female rats demonstrated that the volume of the sexually dimorphic nucleus of the preoptic region was increased (male-typical) by testosterone administration coupled with same-sex sexual experience (Woodson et al., 2002). This suggests that sexual experience may interact with steroid exposure to shape sexual partner preferences in females.

In humans, the extent of childhood or adolescent homosexual versus heterosexual activity does not appear to relate to eventual adult sexual orientation. Documented evidence regarding the situational or cultural 'initiation' of juvenile males into extensive same-sex experience (for example, in single-sex public schools in Britain or the obligatory homosexual activity required of young males in the Sambia tribe of New Guinea) does not result in elevated homosexuality in adulthood (Bailey, 2003; Wellings et al., 1994).

An alternative explanation for the FBO effect is that sexual interaction with older brothers during critical windows of sexual development predisposes towards a homosexual orientation. Studies in national probability samples show that sibling sex-play does not underscore the link between FBO and male sexual orientation (Bogaert, 2000), and that the sexual attraction component of sexual orientation, but not sexual activity, are best predicted by frequency of older brothers (Bogaert, 2003). In further support, same-sex play between pairs of gay brothers is also unrelated to adult homosexual attraction (Dawood et al., 2000).

Perhaps parent–child interactions influence the sexual orientation of children? An informative test here is to examine the sexuality of children of homosexual parents because this type of familial dynamic could promote same-sex preferences through observational learning mechanisms. However, evidence from retrospective and prospective studies provides no support for this supposition (Bailey et al., 1995; Golombok and Tasker, 1996). Nonetheless, one must bear in mind that if parental behaviour does determine offspring sexual orientation, it could be equally common in homosexual and heterosexual parents.

While a role for learning factors can never be entirely omitted, it is perplexing that several of the key routes by which these could have their effect, such as through sexual experience during childhood or adolescence, or through parental socialization, are not supported. Almost certainly the expression of homosexual *behaviour* has varied over time and across cultures, but there is little reason to think that dispositional homosexuality varies greatly cross-culturally or even historically (Bailey, 2003).

8. Conclusion: The future of biobehavioural research on human sexual orientation

The literature thus far provides a rough outline of the neurodevelopmental mechanisms underlying human sexual orientation. As further work from several fields accumulates it is likely we will produce improved mechanistic explanations. Proxy markers will only ever be useful insofar as they truly index the underlying developmental mechanism. One informative test of the prenatal androgen model would be to examine amniotic sex steroid levels and sexual orientation (and its neurobehavioural correlates) prospectively. Nonetheless, it is also possible that sex steroid levels differ in the *brains* of pre-homosexual and pre-heterosexual foetuses but are not reflected in levels in their uterine environment (as indexed by amniocentesis). For the time being, work can clarify the utility of proxy markers, as well as focusing on other reliable 'windows' into early development e.g. 2D:5D and 3D:4D (McFadden and Schubel, 2002; Manning et al., 2003b).

Future investigations must clarify the relationship between neurodevelopmental markers and other neurobehavioural features associated with sexual orientation. Our group recently demonstrated no link between 2D:4D, the number of older brothers, and the neurocognitive variation between heterosexuals and homosexuals (Rahman et al., 2004). This study shows the potential for investigations to narrow the number of potential neurodevelopmental explanations for sexual orientation and its correlates. In this case one domain (i.e. cognitive) linked to sexuality is not necessarily attributable to a common prenatal mechanism (insofar as 2D:4D and number of older brothers reflect this). Progression of the maternal immunity model requires evidence for maternal immune responses in homosexual subjects with older brothers and those without (and their mothers). Studies using serological measures should reveal whether male-specific antigens, cell-surface proteins or even maternal cytokines are involved, while neuroimmunologic analyses of brain material can elucidate non-hormonal possibilities, such as differential sex-linked gene expression in the brains of heterosexuals and homosexuals (Mayer et al., 1998). There is no doubt that such investigations will also benefit from further linkage and candidate gene studies.

The primary challenge at this stage is to elucidate the precise neural circuitry underlying direction of sexual preference, requiring research across the neurosciences. This may require the definitional fractionation of sexual orientation into discrete behavioural components, as derived from animal models of the formation of sexual partner preferences, in order help frame research questions. Example components could include the detection and orientation toward potential 'targets' and the sensory modalities in which these operate (e.g. visual orienting or olfactory detection). Neuroimaging techniques will need to quantify putative sexual-orientation-related volumetric

differences in limbic substrates in vivo, while functional methods could be exploited to elucidate the subcortical–cortical networks responsible for sexual attraction to male and female targets. Neurochemical imaging studies could investigate potential roles of sex steroids upon these neural mechanisms. The psychological sciences can assist here also. For example, researchers could test whether the known attenuation of the human startle response (e.g. eye-blink patterns) to aversive stimuli is apparent for non-preferred sexual stimuli (compared to preferred sexual stimuli) in heterosexual and homosexual adults. Together, these investigations may clarify the inhibitory mechanisms underlying human sexual appetitive responses immensely. Ultimately, work in those with healthy sexual orientations may pave the way for work on abnormal or 'paraphilic' sexual preferences (Waisman et al., 2003).

It is commonly asked of researchers in this controversial field why the biobehavioural sciences should be concerned with a trait that is so skewed—that vast majority of individuals are attracted to the opposite sex after all. Herein lies the irony—elucidating the neurobiology of same-sex orientation will provide important insights into the far greater mystery regarding the proximate neurodevelopmental mechanisms which produce heterosexuality.

Acknowledgements

Research by the author reported in this review was supported by the Medical Research Council of the UK, the British Academy and University of East London Research Funding.

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Psychoneuroendocrinology (2005) **30**, 382–391

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Fluctuating asymmetry, second to fourth finger length ratios and human sexual orientation

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Received 24 May 2004; received in revised form 29 October 2004; accepted 29 October 2004

KEYWORDS
Prenatal hormone theory;
Finger length ratio;
Sexual orientation;
Homosexuality;
Developmental instability;
Fluctuating asymmetry

Summary Sexual orientation in humans may be influenced by levels of prenatal sex steroids which canalise neurodevelopment along sex-typical (heterosexual) or sex-atypical (homosexual) lines. Some evidence for sexual-orientation-related differences in putative somatic markers of prenatal sex hormones supports this view. A competing theory asserts that human homosexuality is due to developmental instability (DI) because it represents a shift from the species-typical pattern of heterosexual orientation. Evidence for elevated rates of non-right handedness among homosexuals provides limited support for this account. The current study tested both theories by examining nine bilateral somatic traits in 120 healthy heterosexual and homosexual men and women in order to compute second to fourth finger length ratios (2D:4D), a measure ascribed to levels of prenatal sex steroids, and fluctuating asymmetry (FA), a measure of DI. Homosexual men and women had significantly lower right hand 2D:4D ratios (even after controlling for handedness, height and weight differences) in comparison to heterosexuals, but sexual orientation did not relate to composite FA scores. The findings constrain the number of possible neurodevelopmental pathways responsible for sexual orientation in humans.

1. Introduction

Biological research into human sexual orientation has attracted a great deal of scientific research as well as its fair share of controversy (for a full review see Rahman and Wilson, 2003a). Although there is no single etiogenic account of sexual orientation in humans, the dominant theory has been the prenatal hormone theory (Ellis and Ames, 1987). This proposes that variation in human sexual orientation is under the control of prenatal sex steroids (probably interacting with genetic factors: see Hamer et al., 1993; Bailey et al., 2000) which canalise neurodevelopment in a sex-typical (heterosexual) or sex-atypical (homosexual) fashion. This theory predicts that homosexuals of both sexes should show cross-sex shifts in neurobehavioural domains in line with the atypical shift in their sexual partner preference (Ellis and Ames, 1987). An array of evidence from neuroanatomical and behavioural domains tends to support this notion. Among these

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0306-4530/$ - see front matter

include findings that homosexual men have larger neuronal populations in the suprachiasmatic nucleus (Swaab and Hofman, 1990), a trend for smaller interstitial nuclei of the anterior hypothalamus (LeVay, 1991, c.f. Byne et al., 2001), female-typical performance on sexually-dimorphic neurocognitive tests such as mental rotation, spatial perception and verbal fluency (McCormick and Witelson, 1991; Wegesin, 1998a; Rahman and Wilson, 2003b; Rahman et al., 2003a) and female-typical neurophysiological patterns (Reite et al., 1995; Wegesin, 1998b). However, homosexual men have also been found to show sex-typical or even 'hyper-male' traits in some domains. For example, McFadden and Pasanen (1998, 1999) have found no differences between heterosexual and homosexual men in sexually-dimorphic otoacoustic emissions (or OAE's, which are weak sounds produced by the inner ear and are more numerous, and stronger, in females than in males), whilst McFadden and Champlin (2000) reported that homosexual men showed hyper-masculinised auditory evoked potentials (AEPs) compared to heterosexual men. Homosexual men also report larger (more male-like) genital size in two studies (one measuring genital size using physician's examinations, the other using self-report measures; Nedoma and Freund, 1961; Bogaert and Herschberger, 1999). Homosexual women, on the other hand, appear to be show rather more consistent cross-sex shifts (in the male-typical direction). These are evidenced in two neurocognitive tests—visuo-motor ability and verbal fluency (Hall and Kimura, 1995; Rahman et al., 2003a), in OAE's, and in AEPs (McFadden and Pasanen, 1998, 1999; McFadden and Champlin, 2000).

Some evidence suggests that these differences may arise, in part, from prenatal factors, primarily the levels of sex hormones experienced in utero. Such evidence relies almost entirely on 'proxy markers', which are somatic in nature, and ascribed to the organising effects of prenatal sex hormones. The second to fourth finger length ratio (or 2D:4D) is thought to be a negative correlate of prenatal testosterone and a positive correlate of prenatal estrogen (Manning, 2002). Homosexual women show reduced (i.e. masculinised) 2D:4D ratios compared to heterosexual women (Williams et al., 2000; McFadden and Schubel, 2002; Rahman and Wilson, 2003c). Studies in homosexual men have yielded inconsistent results; some reports demonstrate hyper-masculinised (lower) 2D:4D ratios (Robinson and Manning, 2000; Rahman and Wilson, 2003c) and others show feminised (higher) ratios (McFadden and Schubel, 2002; Lippa, 2003). In analysis of data from heterosexuals and homosexual men of several ethnic groupings, Manning and Robinson (2003) suggest that this inconsistency may be due to greater variance in 2D:4D among heterosexuals compared to homosexual men. They suggest that homosexual men may show a 'con-constant' mean 2D:4D across ethnicity of roughly 0.96, although the exact mechanistic explanation for this is not apparent. Manning (2002) has recently summarised the evidence that androgens stimulates prenatal growth of the fourth finger whilst prenatal estrogen stimulates the growth of the second finger—a low 2D:4D ratio being indicative of greater exposure to male sex steroids (androgens) in the uterine environment. Thus, on the basis of the studies reviewed above, homosexuals of both sexes could be argued to be showing evidence of greater exposure to prenatal androgens. In support, the 2D:4D is sexually dimorphic with males showing lower ratios compared to females, a pattern which appears to be established by 2 years of age and is correlated with testosterone levels (Manning et al., 1998; Ronalds et al., 2002). A low 2D:4D has previously been associated with male-typical traits such as mental rotation ability, assertiveness, left-handedness, and a predisposition towards autism (Wilson, 1983; Manning et al., 2000, 2001; Manning and Taylor, 2001). However, Putz et al. (2004) reported no associations between 2D:4D and several sex-linked traits in a large sample of male and female undergraduates, although lower ratios were related to non-heterosexual orientation in both sexes. This study employed uncorrected correlational analyses. Rather stronger evidence for prenatal hormonal influences on 2D:4D comes from individuals exposed to elevated levels of androgens before birth (such as in the condition congenital adrenal hyperplasia, or CAH). Brown et al. (2002) and Ökten et al. (2002) reported that the 2D:4D ratio was masculinised (low values) in CAH females and hyper-masculinised in CAH males as compared to same-sex controls, in line with the notion that prenatal androgen exposure reduces the 2D:4D ratio. However, Buck et al. (2003) found no differences in 2D:4D between girls with CAH and control girls. That study differed from Brown et al. and Ökten et al. in that only the left hand was measured, and the measurements were made from radiographs. Radiographic techniques may omit relevant aspects of finger digits (such as the fat pads at the fingertips) that contribute to the sex difference in relative length. 2D:4D is also linked to variation in the androgen receptor gene (Manning et al., 2003) and the ratio of testosterone to estrogen taken from amniotic fluid during gestation are negatively associated with 2D:4D at 2 years of

age (Lutchmaya et al., 2004). Although ultimately correlational in nature, these results do suggest strongly that excess androgen exposure can alter the relative lengths of the second and fourth digits. With respect to the findings concerning OAE's, a role for prenatal androgens in these sexual-orientation-related differences is suggested by the finding that females with male co-twins (and thus perhaps elevated prenatal androgen exposure) show a male-typical pattern of OAEs (McFadden, 1993).

Another competing theory for the development of atypical sexual orientation has invoked the notion of developmental instability (Lalumiere et al., 2000). Developmental instability (DI) refers to an organism's inability to cope with developmental stresses (due to genetic or other factors) thus shifting its ontogenetic trajectory from an 'ideal phenotype' (Moller, 1998). If DI is important in the aetiology of sexual orientation then human homosexuality may represent one outcome of deviations from the 'ideal' phenotype of heterosexual orientation. Without making a value judgement about homosexuality (as the causes of homosexuality, or heterosexuality, are necessarily irrelevant to whether they should be considered a pathology or not) it has been argued that homosexuality could represent a neurodevelopmental perturbation in the human sexual orientation system (e.g. Lalumiere et al., 2000). These assertions predict that homosexuals should also show other markers of DI. The primary support for this comes from the clear observation of elevated non-right handedness (non-RH) among homosexuals of both sexes. In an exhaustive review of the literature, Lalumiere et al. (2000) found elevated frequencies of non-RH in homosexual men of 34% and in homosexual women of 91% (for homosexuals overall, its 39%), in contrast to heterosexual men and women. Lalumiere et al. maintain that as men in general show elevated rates of non-RH compared to women, the prenatal hormone theory would predict that homosexual men should show reduced non-RH compared to heterosexual men, and homosexual women should show elevated non-RH compared to heterosexual women. As homosexuals of *both* sexes show elevated non-RH (and as non-RH is a marker of DI) DI could be a general developmental mechanism accounting for homosexuality per se.

Thus far, the evidence in favour of DI contradicts the dominant prenatal hormone theory. However, Rahman and Wilson (2003a) (see also McFadden, 2002) have suggested that elevated non-RH in homosexuals could also be explained within the prenatal hormone framework by considering the trait-specific actions of prenatal sex steroids on neurodevelopment, rather than a simple global masculinisation or feminisation of traits. For example, one possible mechanism could be localised androgen insensitivity in the relevant neural tissue controlling sexual orientation, leading to an elevation in the levels of circulating testosterone which then 'hyper-masculinise' certain peripheral or somatic features, such as AEP's or finger length ratios. Rahman and Wilson (2003a) have speculated that this excess testosterone may also promote white matter growth in the brain (for example, in the corpus callosum which is rich in enzymes responsible for the conversion of testosterone to its metabolites) and thus shift handedness in the hyper-male direction. Therefore, in homosexual men we may see a 'mosaic' pattern of features which are predominantly cross-sex shifted (such as in neuroanatomy and behaviour), and occasionally hyper-masculinised with respect to more peripheral traits. However, this statement suggests that neural mechanisms responsible for the sex-steroidal feedback loop in the hypothalamic–pituitary-axis are also involved in sexual orientation, yet our best guess on the available evidence is that *distinct* nuclei, such as INAH-3 (see above), may be responsible for sexual orientation. Among homosexual females, on the other hand, the mechanism may be more straightforward such that elevated prenatal androgens masculinise sexual orientation, behaviour, aspects of cognitive function and peripheral traits such as 2D:4D.

So far the primary support for DI theory has been the elevated rate of non-right handedness among homosexuals of both sexes, yet it is possible to explain this via the prenatal hormone theory. Therefore, the DI theory could benefit from further work to clarify the role it has to play in human sexual orientation. By far the best and most utilised measure of DI is fluctuating asymmetry (FA) of bilateral anthropometric traits (Kowner, 2001). To date, only three studies have explored measures of FA and sexual orientation in humans, and all three have yielded no sexual-orientation-related differences. Rahman and Wilson (2003c) examined the FA of the second and fourth finger lengths in right-handed heterosexual, and homosexual, men and women and found no group differences. Mustanski et al. (2002a) examined the dermatoglyphic features on the fingers and found that both dermatoglyphic directional asymmetry, and FA, was unrelated to sexual orientation. Finally, Green and Young (2000) examined the dermatoglyphic characteristics features of a transsexual population (which have heterosexual and homosexual subtypes) and found a trend for heterosexual male-to-female transsexuals (oriented towards women)

to show the greatest FA but there were no significant group differences. The primary limitation with all three studies is the focus on a narrow range of FA measures. Rahman and Wilson examined only two finger length measures, whereas Mustanski et al. (2002a) and Green and Young (2000) examined dermatoglyphic finger ridge feature only and not those on the palm, such as *a-t-d* angles. Interestingly, dermatoglyphics are most likely determined by the end of the first trimester of development and may tap disrupted foetal growth, whilst variations in the bilateral symmetry of physical features may continue throughout development (e.g. see Yeo et al., 1997). Therefore, the FA studies conducted thus far could suggest that *pre*- and/or *post*-natal developmental deviations are unrelated to human sexual orientation. Further work necessitates the use of multiple markers of FA in order to better reflect underlying DI and increase the power of detecting relationships between DI and outcome variables (Leung et al., 2000; Gangestad et al., 2001). Robust markers of FA in the DI literature include several bilateral somatic features: ear length, length of the four finger digits (not usually the thumb), ear width, wrist width, ankle width, elbow width and foot width.

The present study represents the first of its kind to examine the above markers of FA in healthy adult heterosexual and homosexual men and women in order to provide a robust test of the DI theory of sexual orientation. Importantly, by taking measures of the second and fourth finger lengths as part of the measurement procedures it is possible to simultaneously test the prenatal hormone theory. One final consideration is the possible interactions of FA and/or 2D:4D with another correlate of human sexual orientation (in men at least): fraternal birth order. This refers to the reliable observation that homosexual men are born later in their sibships as a consequence of having an excess of older brothers, relative to heterosexual men (see Blanchard, 1997, 2001 for reviews). The fraternal birth order effect (FBO) in male sexual orientation has been suggested to be due to the progressive immunisation of some mothers to male-specific antigens by each succeeding male foetus. The accumulating antibodies to male-specific antigens may affect sexual differentiation of the male foetal brain in a feminising direction leading to homosexuality (see Blanchard, 1997, 2001, 2004). However, it is unclear how the FBO effect maps onto the two competing accounts of sexual orientation presented, and they could be entirely unrelated to these pathways. Nonetheless, it would be prudent to include measures of sibling sex composition as a matter of routine in order to examine possible links (Blanchard, 2001). Based on the extant evidence with its support for the prenatal hormone theory and weaker support for the DI theory, it was predicted that there would no differences in composite FA between heterosexual and homosexual men and women. However, homosexual men and women were predicted to show reduced 2D:4D ratios compared to heterosexuals of both sexes, suggestive of greater prenatal exposure to androgens in the latter group.

2. Method

2.1. Participants

Participants were 120 healthy adult heterosexual and homosexual men and women (30 heterosexual men, 30 heterosexual women, 31 homosexual men and 29 homosexual women) recruited opportunistically from the London area. Participants were aged between 18 and 39 years, and screened to ensure no history of psychiatric or neurological illness, psychoactive medication or drug use, and no injury to the hands or any feature to be measured for FA. Advertisements were placed in gay and lesbian organisations, entertainment venues, university Lesbian, Gay and Bisexual Societies asking for volunteers (irrespective of sexual orientation) to take part in a study on the development of sexual orientation. Heterosexual subjects were recruited from university sources and the local community using the same advert. Participants completed two 7-point Likert-type Kinsey scales of sexual attraction and sexual fantasy where 0 corresponds to exclusively heterosexual and 6 to exclusively homosexual. Those scoring 0 and 1 were classified as 'heterosexual', those scoring 5 or 6 as 'homo-homosexual' (on both scales). Participants with intermediate (bisexual) scores were not included in the study. These 'sexual feelings' based items were utilised for categorisation of sexual orientation rather than sexual behaviour items as the former better index an individuals 'core' sexual orientation compared to the latter which can be influenced by extraneous social factors (see Mustanski et al., 2002b; Rahman and Wilson, 2003a). Participants were required to identify themselves as 'hetero-heterosexual or straight' or 'gay/lesbian' on a separate categorical item of sexual self-identification in order to be included. Those who checked the 'bisexual' category were excluded. All participants were also asked the number of lifetime opposite sex and same sex sexual partners (sexual partner defined as anyone with whom the volunteer

had genital contact which leading to orgasm or significant sexual arousal). This measure was not included as an inclusion or exclusion criteria for participating the study as the correlation between sexual feelings (attraction and fantasy) and sexual behaviour is often low given various contingent social pressures on actual sexual behaviour (see Mustanski et al., 2002b; Rahman and Wilson, 2003a).

Demographic information was obtained regarding age, number of years spent in full-time education since the age of 5 and ethnicity (White, Black, South Asian, East Asian, Hispanic or Other). Participants were also classified by parental socio-economic status (SES) into the following categories according to the Standard Occupational Classification (SOC) of the Office of Population Census and Surveys (1991): (a) professional, (b) managerial, (c) skilled occupations—non-manual, (d) skilled occupations—manual, and (f) unskilled occupations. Height, weight and the number and sex of biological siblings were recorded. Handedness was evaluated using the Edinburgh Handedness Inventory (Oldfield, 1971). This required participants to state the degree of hand preference for 10 unimanual tasks as either strong (2 points) or weak (1 point). A handedness laterality quotient (EHI score) was calculated for each participant by subtracting the score for the left hand from the score for the right hand, dividing by the sum of both, and multiplying by 100. This provides a continuous measure of handedness from −100 (completely left-handed) to 100 (completely right-handed).

2.2. Measures and procedure

Measurements were taken of nine bilateral traits on all participants directly from the body: width of ears, wrists, ankles and feet, and length of the ears and of the four fingers (excluding the thumb) using digital callipers measuring to 0.01 mm. The second and fourth finger digits (and all fingers) were measured from the tip of the finger to the ventral proximal crease. Where there was a band of creases at the base of the digit, the most proximal of these was measured. A trained researcher blind to sexual orientation measured all participants twice (the second measurement immediately after the first). The intra-rater correlations varied from 0.71 to 0.96 with a mean of 0.89, for measures other than finger digits. These values are similar to those obtained by some investigators (e.g. Manning, 1995; Manning et al., 1997; Putz et al., 2004) and higher than others (e.g. Furlow et al., 1997). Finger digit measures had a high level of repeatability with intra-rater correlations of 0.98, consistent with previous work (e.g. Manning, 1995). All measurements were averaged and finger length ratio (2D:4D) was calculated by dividing the length of the second finger by that of the fourth for both right and left hands. FA was calculated by subtracting the right from the left measures (*L*–*R*) (see Section 3). All participants provided written informed consent prior to taking part.

3. Results

3.1. Sample characteristics

Two-way factorial (sex by sexual orientation) ANOVA revealed no significant main effects of sex, sexual orientation or any significant interaction on age and years in education, (all p's>0.05; see Table 1 for mean values for all demographic characteristics). For EHI scores, there was a significant main of effect of sex ($F=5.703$, d.f.$=1$, 119, $p=0.019$) with men being less right handed (scoring lower) than women overall (see Table 1), but no significant main effect of sexual orientation and no significant interaction (p's>0.05). EHI scores ranged from −87.50 (minimum) to 100 (maximum). Men were also taller (main effect of sex: $F=61.93$, d.f.$=1$, 119, $p<0.005$; no other significant effects) and heavier (main effect of sex: $F=17.73$, d.f.$=1$, 119, $p<0.005$; no other significant effects) than women overall.

For number of older brothers, there was no significant main effect of sex ($F=0.47$, d.f.$=1$, 119, $p=0.492$), a significant main effect of sexual orientation ($F=5.65$, d.f.$=1$, 119, $p=0.019$) with homosexuals having more older brothers than heterosexuals overall, and a significant sex by sexual orientation interaction ($F=4.98$, d.f.$=1$, 119, $p=0.027$). Decomposition of this interaction utilised three independent samples t-tests: heterosexual men compared to heterosexual women, heterosexual men compared to homosexual men, and heterosexual women compared to homosexual women (Bonferonni corrected to $p<0.01$). These revealed that homosexual men had a significantly greater number of older brothers compared to heterosexual men ($t=-3.308$, d.f.$=59$, $p=0.002$), consistent with previous work (see Blanchard, 2004). No other comparisons were significant (all p's>0.01). For number of younger brothers there was no significant main effects of sex ($F=1.34$, d.f.$=1$, 119, $p=0.249$) or sexual orientation ($F=0.46$, d.f.$=1$, 119, $p=0.497$) but there was a significant sex by sexual orientation

Table 1 Means (*SD*) for participant characteristics.

	Age (years)	Education (years)	Handedness (EHI)	Height (cm)	Weight (kg)	No. older brothers	No. older sisters	No. younger brothers	No. younger sisters
Heterosexual men	28.86 (5.48)	15.96 (2.99)	57.98 (56.98)	177.75 (6.21)	74.86 (10.56)	0.40 (0.72)	0.53 (0.97)	0.33 (0.47)	0.53 (0.68)
Heterosexual women	26.36 (6.28)	16.03 (2.76)	87.89 (17.43)	168.23 (8.04)	64.96 (15.69)	0.63 (0.76)	1.13 (1.59)	0.43 (0.67)	0.40 (0.85)
Homosexual men	29.90 (5.09)	15.41 (3.55)	73.76 (46.40)	177.89 (7.55)	74.33 (10.06)	1.09 (0.90)	0.58 (0.99)	0.48 (0.72)	0.25 (0.61)
Homosexual women	28.48 (5.90)	16.60 (3.14)	78.91 (26.60)	165.62 (8.35)	63.44 (16.65)	0.65 (0.89)	0.58 (0.90)	0.13 (0.35)	0.48 (0.68)

interaction ($F=4.40$, d.f. $=1$, 119, $p=0.038$). Decomposition of this interaction revealed it to be due to heterosexual women having more younger brothers than homosexual women ($t=2.08$, d.f. $=57$, $p=0.041$) but this trend was not significant at the stringent Bonferonni corrected alpha value. No other comparisons were significant (all p's >0.01). There were no significant group effects for number of older sisters and number of younger sisters (all p's >0.05).

Ethnicity was collapsed into 'White' vs. 'Non-white' as there were too few cases per individual categories, and no group differences were found ($\chi^2=1.31$, d.f. $=3$, $p=0.727$). The number of 'White' versus 'Non-white' participants, respectively, were 26 and 4 (1 'Black', 1 'South Asian', and 2 'Other') among heterosexual males, 23 and 7 (5 'Black', 1 'South Asian', and 1 'East Asian') among heterosexual females, 26 and 5 (2 'Black', 1 'South Asian', 1 'East Asian', and 1 'Hispanic') among homosexual males, and 24 and 4 (1 'Black', 3 'Other', and 1 case missing) among homosexual females. Ethnicity robustly relates to 2D:4D (Manning, 2002; Lippa, 2003) and thus was dummy coded (0 for 'White' and 1 for 'Non-white') and included in analysis of covariance analyses (see below). There were no group differences in parental SES (collapsed into 'professional/managerial' and 'skilled/partly skilled/unskilled') ($\chi^2=2.61$, d.f. $=3$, $p=0.455$).

3.2. Second to fourth finger length ratios

For right-hand 2D:4D ratios, two-way ANOVA revealed a significant main effect of sex ($F=6.98$, d.f. $=1$, 119, $p=0.009$), with men having lower ratios overall than women. There was a significant main effect of sexual orientation ($F=16.96$, d.f. $=1$, 119, $p<0.005$) with homosexuals having lower right-hand 2D:4D ratios than heterosexuals, but no significant sex by sexual orientation interaction ($F=0.13$, d.f. $=1$, 119, $p=0.71$). An adjusted model (ANCOVA) applied to right-hand 2D:4D (with EHI scores, ethnicity—dummy coded, height and weight as covariates) produced no significant main effect of sex ($F=2.57$, d.f. $=1$, 118, $p=0.11$), but the sexual orientation effect remained ($F=18.93$, d.f. $=1$, 118, $p<0.001$). The interaction also remained non-significant ($F=0.24$, d.f. $=1$, 118, $p=0.64$). For the covariates, there was no effect of height ($F=0.22$, d.f. $=1$, 118, $p=0.63$) or EHI scores ($F=0.29$, d.f. $=1$, 118, $p=0.58$), but there were significant effects of weight ($F=4.92$, d.f. $=1$, 118, $p=0.02$) and ethnicity ($F=4.80$, d.f. $=1$, 118, $p=0.03$). See Table 2 for both unadjusted and adjusted mean scores.

For left-hand 2D:4D ratios (see Table 2), unadjusted ANOVA revealed no significant effects of sex ($F=0.44$, d.f. $=1$, 119, $p=0.50$), sexual orientation ($F=1.77$, d.f. $=1$, 119, $p=0.18$) or their interaction ($F=1.14$, d.f. $=1$, 119, $p=0.28$). An ANCOVA model also revealed no significant effects of sex ($F=0.01$, d.f. $=1$, 118, $p=0.91$), sexual orientation ($F=2.30$, d.f. $=1$, 118, $p=0.13$) or their interaction ($F=1.59$, d.f. $=1$, 118, $p=0.21$). There were no effects of EHI scores ($F=0.002$, d.f. $=1$, 118, $p=0.96$), ethnicity ($F=3.61$, d.f. $=1$, 118, $p=0.06$), height ($F=0.02$, d.f. $=1$, 118, $p=0.87$) or weight ($F=3.22$, d.f. $=1$, 118, $p=0.07$) as covariates. A factorial repeated measures MANCOVA (treating right and left 2D:4D as the repeated measures) demonstrated an interaction between side of ratios and sexual orientation (Wilk's $F=6.291$, d.f. $=1$, 111, $p=0.014$) confirming the greater difference for right compared to left-hand ratios. There were no interactions between side of ratios and sex, or any of the covariates (all p's >0.05). These results indicate the right-hand 2D:4D ratio may be more closely related to sex and sexual orientation than left-hand ratios.

Table 2 Unadjusted (*SD*) and adjusted mean 2D:4D and FA values by group.

	Right-hand 2D:4D ratio		Left-hand 2D:4D ratio		Composite FA	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Heterosexual men	0.96 (0.02)	0.96	0.96 (0.03)	0.96	−0.58 (2.32)	−0.73
Heterosexual women	0.98 (0.03)	0.98	0.97 (0.02)	0.97	0.18 (1.96)	0.32
Homosexual men	0.94 (0.02)	0.95	0.96 (0.03)	0.96	−0.84 (2.49)	−1.02
Homosexual women	0.96 (0.02)	0.95	0.96 (0.02)	0.95	−0.23 (2.59)	0.25

Adjusted for the covariates EHI scores, ethinicity, height and weight.

3.3. Fluctuating asymmetry

FA measures were calculated according to published procedures (e.g. Manning, 1995; Manning et al., 1997; Yeo et al., 1997; Leung et al., 2000; Rahman and Wilson, 2003c). The signed FA of each somatic feature was examined for whether it met the statistical requirements for FA (normal distribution around a parametric mean of zero, using a one-sample *t*-test: Manning, 1995). There was no evidence of deviation in the distribution of any trait (p's>0.05) apart from the 3rd finger lengths ($t=-2.46$, d.f.$=119$, $p=0.015$) which showed evidence of directional asymmetry. This measure was excluded from any further analysis. Thus overall, the traits showed 'ideal FA' comparable to published norms (e.g. Trivers et al., 1999). The signs from all FA's (excluding 3rd finger lengths) were removed (the intra-class correlations between first and second measurements for unsigned FA across the traits ranged from 0.56 to 0.65) and each distribution was standardised. The scores for each trait were summed to obtain a composite FA score for each participant. The mean group scores are listed in Table 2 and although appearing large (perhaps due to the present sample having larger characteristics overall) they are comparable to those obtained by other investigators in male and female samples (e.g. Manning et al., 1997; Yeo et al., 1997).

For composite FA scores, an unadjusted ANOVA model revealed no significant main effects of sex ($F=2.47$, d.f.$=1, 119$, $p=0.11$), sexual orientation ($F=0.58$, d.f.$=1, 119$, $p=0.44$) or their interaction ($F=0.03$, d.f.$=1,119$, $p=0.85$). An adjusted model (with EHI scores, ethnicity, height and weight as covariates) revealed no significant main effect of sex ($F=2.74$, d.f.$=1, 118$, $p=0.10$), no significant effect of sexual orientation ($F=1.02$, d.f.$=1, 118$, $p=0.31$) and no significant interaction ($F=0.10$, d.f.$=1, 118$, $p=0.74$). There were no significant effects of EHI scores ($F=0.33$, d.f.$=1, 118$, $p=0.56$), ethnicity ($F=1.98$, d.f.$=1, 118$, $p=0.16$), height ($F=1.47$, d.f.$=1, 118$, $p=0.22$) or weight ($F=0.08$, d.f.$=1, 118$, $p=0.77$) as covariates. A power analysis using G-POWER revealed that with an assumed effect size of 0.25 (medium) for an *F*-ratio, a power of 80 percent, and an alpha level of 0.05 (for four groups as here) one would need a total sample size of 180 to detect possible FA differences.

3.4. Sibling sex composition

There were no significant associations between the number of older and younger siblings (of both sexes), right-hand 2D:4D, left-hand 2D:4D, or composite FA scores, for the whole group and each group analysed separately (all p's>0.05).

4. Discussion

The findings are broadly consistent with previous work; (i) demonstrating a normative sex difference in right-hand 2D:4D ratios with men showing lower ratios compared to women (e.g. Williams et al., 2000; Manning, 2002), (ii) confirming two studies showing that homosexual men have lower 2D:4D ratios than heterosexual men (indicating possible exposure to elevated prenatal androgens: Robinson and Manning, 2000; Rahman and Wilson, 2003c), (iii) confirms three studies showing lower 2D:4D ratios in homosexual compared to heterosexual women, also pointing to greater prenatal androgen exposure (Williams et al., 2000; McFadden and Schubel, 2002; Rahman and Wilson, 2003c), and (iv) supports the findings of Manning et al. (1998); Williams et al. (2000) that right-hand ratios may be more sensitive to testosterone, sex and sexual

orientation-related influences than left-hand ratios. However, the current findings contradict two studies for feminised 2D:4D ratios in homosexual men (McFadden and Schubel, 2002; Lippa, 2003) and one study reporting no digit ratio differences between heterosexual and homosexual women (Lippa, 2003). Nonetheless, the present data are consonant with the bulk of the finger length ratio work pointing to masculinised ratios among homosexuals (see also Putz et al., 2004).

The precise mechanistic explanation for the relationship between 2D:4D and homosexuality is far from clear given the mixed nature of the data thus far. Rahman and Wilson (2003a) and McFadden (2002) have speculated that in males homosexuality may involve localised sex-atypical differentiation (perhaps due to genetic factors implicated in sexual orientation: e.g. Hamer et al., 1993, c.f. Macke et al., 1993) in neural structures essential for direction of sexual partner preference (e.g. hypothalamic and sub-cortical circuitry: LeVay, 1991). This could lead to elevated levels of circulating androgens elsewhere which may, in theory, produce hyper-male peripheral somatic features. In females a linear relationship may be the case where masculinisation of sexual-orientation-related neural structures under the actions of prenatal androgens also leads to male-typical somatic and other features (e.g. Rahman et al., 2003b). Whilst the explanation for males is certainly speculative, the argument for females is bolstered by findings that CAH females (who are exposed to higher levels of androgens before birth) show elevated levels of homosexual or bisexual feelings (e.g. Zucker et al., 1996; Hines et al., 2004).

The present study found little support for the DI theory based on the lack of sexual orientation-related differences in composite FA scores (derived from multiple FA measures). This finding is consistent with three studies indicating that the FA's of specific somatic features are unrelated to sexual orientation (Green and Young, 2000; Mustanski et al., 2002a; Rahman and Wilson, 2003c). The strength of the present study over this prior work is the use of multiple quantitative measures of FA as traditionally employed in the extensive DI literature (Kowner, 2001). Furthermore, the present study utilised a continuous measure of handedness as non-consistent right handedness is a putative indicator of DI and is found to be elevated among homosexuals of both sexes (Lalumiere et al., 2000). Some prior work has excluded homosexuals who are non-right handed and who perhaps would be expected to show further evidence of DI in other FA measures (e.g. Rahman and Wilson, 2003c). However, in this study covarying for handedness scores revealed no additional effects, indicating that homosexuals (irrespective of handedness status) do not appear to be exposed to general developmental disruption. Handedness differences between heterosexuals and homosexuals may be more appropriately explained within a prenatal hormone theory framework as discussed earlier (Rahman and Wilson, 2003a). Overall, the findings suggest that homosexuality might not represent a perturbation in the ontogenetic trajectory towards the species-typical pattern of heterosexual orientation but rather a developmentally stable variation in the human sexual orientation system, perhaps due to prenatal sex steroids. In support of this, Manning et al. (2000) have shown that low values of 2D:4D are related to the aetiology of left-hand preference in children.

Several important issues bear consideration. The covarying of ethnicity suggests that ethnic variation in right-hand 2D:4D is much greater than the normative sex difference, consistent with some previous literature (e.g. Lippa, 2003). However, in the present study ethnicity does not appear to impact upon the sexual orientation effects (consistent with Manning and Robinson, 2003). However, the limitation to two collapsed groups ('White' versus 'Non-white', the latter being very small) precludes further exploration of this issue and future work will need large samples of heterosexuals and homosexuals of both sexes, and from several ethnic groups, in order to model the effects of ethnicity appropriately. This brings us to the second important point—that of power. There appeared to be insufficient power to detect possible FA differences between the sexual orientation groups. However, whilst power is certainly an issue here (note that handedness differences were also not found contrary to previous work: Lalumiere et al., 2000), the significant right-hand 2D:4D differences and the observation for a fraternal birth order effect (homosexual men having significantly more older brothers than heterosexual men) do replicate previous work (e.g. Robinson and Manning, 2000; Blanchard, 2004). Also, the composite FA measure used is recommended (on the basis of simulations) as powerful and robust to detect relations if they exist (see Leung et al., 2000). This leaves us with the implication that either sexual-orientation-related differences in FA do not exist (consistent with three previous studies) or there is not enough power in these studies to detect them. This question awaits further work with very large samples also. Future work must also combine multiple measures of DI, including minor physical anomalies (MPAs), dermatoglyphics and body FA as these may tap different points in development

(e.g. Yeo et al., 1997). Whilst an excess of older brothers among homosexual men was replicated here, there were no associations between finger length ratios or FA and number of older and younger siblings (of both sexes) for the whole group or each group separately. This is consistent with two other studies reporting no associations between these measures (Robinson and Manning, 2000; Rahman and Wilson, 2003c c.f. Williams et al., 2000) suggesting that the neurodevelopmental mechanisms underlying the older brother effect are unrelated to prenatal hormonal or DI pathways.

In summary, the aim of this investigation was to contrast two aetiogenic accounts of sexual orientation in humans: the prenatal hormone theory, and developmental instability (DI), by utilising a somatic marker often ascribed to prenatal hormonal exposure (the 2D:4D ratio) and fluctuating asymmetry of nine bilateral anthropometric traits. The demonstration of lower right-hand 2D:4D in homosexual men and women compared to heterosexuals is consistent with some role for prenatal hormones in human sexual orientation (Rahman and Wilson, 2003a). On the other hand, the absence of sexual-orientation-related differences in composite FA contradicts the DI theory (Lalumiere et al., 2000). If these data hold up in future investigations with much larger samples, then they may constrain the number of developmental hypotheses for sexual orientation and necessitate further work to elucidate the precise mechanisms of prenatal hormonal influences therein.

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Personality and Individual Differences 34 (2003) 1337–1382

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Review

Born gay? The psychobiology of human sexual orientation

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Received 26 November 2001; received in revised form 1 April 2002; accepted 27 April 2002

Abstract

Sexual orientation is fundamental to evolution and shifts from the species-typical pattern of heterosexuality may represent biological variations. The growth of scientific knowledge concerning the biology of sexual orientation during the past decade has been considerable. Sexual orientation is characterised by a bipolar distribution and is related to fraternal birth order in males. In females, its distribution is more variable; females being less prone towards exclusive homosexuality. In both sexes homosexuality is strongly associated with childhood gender nonconformity. Genetic evidence suggests a heritable component and putative gene loci on the X chromosome. Homosexuality may have evolved to promote same sex affiliation through a conserved neurodevelopmental mechanism. Recent findings suggest this mechanism involves atypical neurohormonal differentiation of the brain. Key areas for future research include the neurobiological basis of preferred sexual targets and correlates of female homosexuality.

Keywords: Sexual Orientation; Homosexuality; Neurodevelopment; Prenatal androgens; Evolution

Contents

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0191-8869/02/$ - see front matter
PII: S0191-8869(02)00140-X

1. Introduction

Sexual orientation (the degree of sexual attraction to either men or women) shows within-sex variation that is fundamental to our understanding of human sexuality. For the most, it dictates that males will mate with females but, in a minority, the homosexual impulse is equally compelling. Since sexual orientation is empirically tied to other domains exhibiting sexual dimorphism, it is potentially a useful test case for a number of hypotheses concerning sex differences. Amongst human individual differences, sexual orientation has provoked intense scientific research from biobehavioural disciplines as well as much non-academic controversy. The moral and social implications of this research are legitimate concerns but others (e.g. LeVay, 1996) have addressed them. With Greenberg and Bailey (1993) we would favour caution in attributing moral, legal and political consequences to findings of biological processes that contribute to behaviour, such as sexual orientation.

Much of the social concern surrounding sexual orientation research has arisen from social constructionist persuasions within academic institutions (e.g. Kitzinger, 1987). Social constructionism is a somewhat incoherent body of postmodernist concepts emphasising the subjectivity of scientific inquiry and method, and the relative nature and equal validity of conflicting epistemologies. Social constructionists argue that sexuality is a fluid and dynamic property that can only be understood by the analysis of socio-political contexts, linguistic and narrative "scripts" (DeCecco & Parker, 1995; Halwani, 1998). The general intellectual position of such postmodernist philosophies has been well criticised (e.g. Gross & Levitt, 1997; Koertge, 2000). Researchers within the field of sex research have also rebuffed social constructionism as a poor intellectual framework for understanding sexual orientation (LeVay, 1996; Rahman, 1999a, 1999b).

The aim of this review is not to theorise about the non-scientific implications of such research but to delineate more precisely the current state of knowledge concerning the psychobiology of human sexual orientation and offer suggestions for future inquiry. Previous reviews (e.g. Gladue, 1994; LeVay & Hamer, 1994; Pillard & Bailey, 1995) are now somewhat dated; since 1995 there have been additional findings that require consolidation. We shall not review gender transpositions such as transsexuality or intersex conditions (see others, e.g. Cohen-Kettinis & Gooren, 1999), although these may be discussed where necessary. We searched the literature using Medline, EMBASE, Psyclit, BIDS and PsychInfo databases (1900–current). Published articles (review and data based) were further scanned for additional references, as were key texts (e.g. Ellis & Ebertz, 1997).

The review is divided into six sections. The first deals with the phenotypic pattern of sexual orientation, the second with evidence for genetic influences, and the third proposes an integrated scenario for the evolution of homosexuality. The fourth section discusses the main neurodevelopmental theory regarding etiogenesis, namely sexual differentiation of the brain. This section is sub-divided so: (1) evidence from somatic variations; (2) neuroanatomical findings; and (3) neuropsychological performance as evidence of a "cross-sex shift" in brain-based functioning. The last sub-section examines three putative neurodevelopmental mechanisms for sexual orientation and its profile of correlates: prenatal androgen theory, maternal immunisation and developmental instability. Finally, the state of non-biological explanations is discussed and suggestions are offered for further research.

2. Distribution of sexual orientation and its correlates

The prevalence of heterosexual and homosexual orientation has received a great deal of attention. The consensus is that approximately 2–5% of men are exclusively homosexual, the figure for women being about half that: 1–2% (Diamond, 1993; Laumann, Michael, Gagnon, & Michaels, 1994; Wellings, Field, Johnson, & Wadsworth, 1994). Thus homosexuality represents a small but significant minority phenotype in humans. The rates reported here refer to sexual orientation as a psychological rather than behaviourally operationalised variable (e.g. Kinsey ratings on sexual and romantic attraction and attachment). Sexual orientation thus refers to a preference for same-sex versus opposite-sex sexual attraction and attachment, which often transposes into sexual experience but not always. External constraints, such as opportunity and legal and social pressures, reduce the correlation between orientation and behaviour (Hamer & Copeland, 1994; LeVay, 1996; Sell, Wells, & Wypij, 1995). A recent taxometric analysis, using complex statistical procedures with a large Australian twin sample, has shown that the distinction between heterosexuality and homosexuality is not arbitrary, but that latent taxa underlie these preferences (Gangestad, Bailey, & Martin, 2000). "Taxa" are non-arbitrary classes whose existence is empirically based, rather than a simple semantic classification. The techniques of taxometry elucidate whether statistical associations between robust correlates of proposed taxa, such as sexual orientation, show evidence of true, or latent, taxa. The taxonicity of sexual orientation strongly suggests that a dichotomous ontogenetic pathway accounts for its variation. Neurohormonal sexual differentiation of the brain is one candidate for such a pathway driving development down a sex typical route in the majority (heterosexual) but also down an atypical route in the minority (homosexuals).

The distribution of sexual orientation appears to be bimodal in men, whereas it is more variable in women, typically in the form of higher degrees of "bisexuality" (Pattatucci & Hamer, 1995; Bailey, Dunne, & Martin, 2000). This is consistent with the notion that females have greater erotic plasticity (that is, women's sex drive being more influenced by situational and cultural factors than men's; Baumeister, 2000) and suggests that male and female sexual orientation may have divergent, though overlapping, etiogenic pathways.

There are two main correlates of sexual orientation that point to an early developmental genesis. The first is the relationship between *childhood gender nonconformity* and adult sexual orientation (Bailey & Zucker, 1995), which is generally considered one of the strongest of all developmental associations in humans (Bem, 1996). Homosexual men typically recall having been feminine boys, whilst homosexual females were masculine girls. Prospective work confirms this relationship for men (Green, 1987; Zucker & Bradley, 1995). In their meta-analysis, Bailey and Zucker (1995) report large effect sizes for the degree of this association ($d = 1.31$ for men, $d = 0.96$ for women). The gender nonconforming behaviours appear to be specific to childhood sex-typed activities and interests, rather than playmate preferences. Given evidence for an early formative role for androgens in the development of such behaviours, these data are consistent with neurohormonal differentiation theory (Berenbaum & Hines, 1992; Berenbaum & Snyder, 1995). These large heterosexual–homosexual differences in childhood sex atypicality also extend to adulthood, when assessed by "gender-diagnosticity" measures (the extent of male versus female typicality of interests). On these measures (as well as on traditional masculinity–femininity scales; Haslam, 1997) gay men are typically more feminine and lesbian women more masculine in adulthood than their same-sex heterosexual peers (Lippa, 2000; Lippa & Arad, 1997). In his "exotic becomes erotic" theory, Bem (1996) proposes that biological factors predispose towards childhood gender nonconformity, alienating the child from social interactions with same sex peers. Ultimately these same-sex peers become eroticised during adolescence by mechanisms involving generalised arousal. This theory has some support from behaviour genetics but has been criticised on grounds of providing a poor explanation for female homosexuality, reliance purely on the sexual orientation/childhood gender nonconformity association and the assumption of a generalised sexual arousal mechanism (e.g. Peplau, Garnets, Spalding, Conley, & Veniegas, 1998).

The second most consistent correlate of sexual orientation, in males at least, is *fraternal birth order*. In diverse samples and independent replications, homosexual men are found to have a greater number of older brothers than heterosexual men (Bailey et al., 1999; Blanchard, 1997; Bogaert, 1998b; Ellis & Blanchard, 2001; Purcell, Blanchard, & Zucker, 2000). The groups do not differ with respect to any other category of siblings, such as younger brothers or older sisters (Jones & Blanchard, 1998). The fraternal birth-order effect is also present in boys showing childhood gender nonconformity and adolescent male homosexuals (Blanchard, Zucker, Bradley, & Hume, 1995; Zucker et al., 1997). It has been estimated that each older brother increases the odds of being a homosexual man by 33–48%, although these odds translate into population probability estimates of only a few percent (Blanchard, 2001). Homosexual women do not differ from heterosexual women in any category of sibling or sex ratio (Blanchard, Zucker, Siegelman, Dickey, & Klassen, 1998; Bogaert, 1997). Blanchard and Bogaert (1996b) and Blanchard and Klassen (1997) proposed that a maternal immune response provoked by male-specific foetal products shifts male-typical neurodevelopment in a sex-atypical direction. The immune system "memory" keeps a tally of preceding male foetuses, and modulates its response accordingly,

usually a stronger one. The relevant foetal antigen might be one of the male-specific Y-linked, minor histocompatibility antigens, also referred to as the H-Y mHC group. This hypothesised mechanism, which is similar to that recognised in Rhesus blood factor incompatibility, will be discussed later. Psychosocial explanations for the fraternal birth-order effect, such as greater sexual interaction with older brothers, have not received any empirical support (Blanchard, 1997; Dawood, Pillard, Horvath, Revelle, & Bailey, 2000).

The cross-cultural manifestation of sexual orientation is remarkably consistent both in its distribution and in the above correlates (Bogaert, 1998b; Whitam, 1983; Whitam, Daskalos, Sobolwski, & Padilla, 1998; Whitam & Mathy, 1991; Whitam & Zent, 1984). There is little cross-cultural and historical evidence that contradicts the phenotype observed in Western societies. However, because of different sanctions and customs across cultures, homosexual *behaviour*, as distinct from the preference, shows more variable expression (Herdt, 1997).

3. Heritability and genetics

As a starting point for investigating the psychobiology of sexual orientation we consider the question of heritable influences. It seems that sexual orientation aggregates in families, hinting towards a heritable basis, although not excluding a role for environment (Pattatucci, 1998). Homosexual men have more homosexual brothers than do heterosexual men (around 15% greater; Pillard & Weinrich, 1986; Bailey & Bell, 1993; Bailey et al., 1999), whilst homosexual women have more homosexual sisters than heterosexual women (around 10% greater; Bailey & Benishay, 1993; Pattatucci & Hamer, 1995). There is also a small, but elevated rate of homosexuality in the brothers of lesbians and the sisters of gay men (Pattatucci & Hamer, 1995).

Twin studies suggest that this familiality is partly genetic. Monozygotic (MZ) twins show greater concordance for homosexuality than dizygotic (DZ) twins. Early Scandinavian studies reported rather questionable levels of concordance (around 100% in Kallmann, 1952). Using more sophisticated methods and larger samples in the USA, the concordance rate was 52% among MZ male twins, 48% among MZ female twins, compared with 22% among DZ male twins and 16% among DZ female twins (Bailey & Pillard, 1991; Bailey, Pillard, Neale, & Agyei, 1993). Higher rates of concordance were reported by Whitam, Diamond, and Martin (1993); 65% for MZ males and 29% for DZ males. Taken together, these studies suggest that around 50–60% of the variance in sexual orientation is genetic. However, they suffer the methodological problem of ascertainment bias; subjects were recruited primarily through homophile organisations and word of mouth. Recently, two methodologically rigorous studies; Bailey, Dunne, and Martin (2000) reporting on a large representative sample of Australian twins, and Kendler, Thornton, Gilman, and Kessler (2000) reporting on a US national probability sample, found only 30% concordance among MZ twins for non-heterosexual orientation (much lower than in previous work). In a separate analysis of the Australian data, Kirk, Bailey, Dunne, and Martin (2000) actually estimated a higher heritability for female (0.58) than male (0.26) homosexuality. Bailey and coworkers also found significant heritability of childhood gender nonconformity (CGN) for men and women. These authors argue that CGN may be an ''endophenotype'' for sexual orientation in that it is ''closer to the genes'' than the primary trait, and easier to detect because of its greater variation. The use of very large samples would be required to resolve genetic/environmental

contributions for a primary trait such as sexual orientation, given its notoriously skewed distribution. Kendler and colleagues found no evidence that more similar environmental experience in MZ than DZ pairs contributed to greater resemblance for sexual orientation in MZ pairs.

The remaining non-genetic variance in twin studies does not necessarily imply psychosocial influences. The environmental factors seem to be primarily of a non-shared nature and could be biological. Dawood et al. (2000) failed to find any influence of shared social factors among gay brothers, such as "contagion" theories invoking the role of incestuous same-sex contact, or maternal dominance and paternal distance would suggest. This is in line with the dearth of evidence for any known psychosocial determinant of sexual orientation from the past 50 years of research. Dawood et al. did, however, confirm that CGN was similar among gay brothers. Bailey, Dunne, and Martin's (2000) and Dawood et al.'s findings lend some support for Bem's (1996) argument that CGN is basic to homosexuality, but they are silent on other developmental aspects of the theory.

Several pedigree studies suggest that an X-linked gene(s) influences male sexual orientation. Gay men have more homosexual uncles and cousins through the maternal line than on the paternal side, consistent with X-linkage (Hamer, Hu, Magnuson, Hu, & Pattatucci, 1993; Turner, 1995; Rice, Anderson, Risch, & Ebers, 1999). There is however one failure to find such an effect (Bailey et al., 1999). The picture for female sexual orientation is more complex. Studies report rates of non-heterosexuality (a broader term that recognises bisexuality in female populations) being elevated in probands' sisters, daughters, nieces, and female cousins through the paternal line (Pattatucci & Hamer, 1995). The pattern could fit X-linkage but whether this is maternally or paternally derived is difficult to establish. For example, the low penetrance of such genes could mask the signs of father-to-daughter transmission. Moreover, the higher reproductive output of lesbians compared to gay men could make detection of X-linkage more difficult in females. A sex-limited, autosomal (non-sex chromosome) dominant locus, with lower penetrance, could also account for the familial aggregation observed. Subsequent molecular genetic studies have reported evidence of a genetic marker for homosexuality on chromosome Xq28, although only in males (Hamer et al., 1993; Hu et al., 1995). A recent replication failed to confirm the Xq28 finding (Rice et al., 1999) but this study suffered from low power to detect linkage and the defining criteria for homosexuality consisted of subjective judgement (Hamer, 1999). Nonetheless, Rice et al.'s pedigree data did confirm that male sexual orientation is influenced by an X-linked gene. Combining four datasets, Hamer (1999) has estimated that approximately 64% of gay brothers share their alleles on region Xq28.

Of further interest are Blanchard and Bogaert's (1997) findings that non-married male siblings were more likely to have homosexual brothers and a greater number of older brothers. A Bayesian analysis indicated that most of the unmarried male siblings (over 40 years) in their study were probably homosexual, implying that marital history is a good proxy measure of sexual orientation. These data suggest an additive effect of older brothers and homosexual brothers in the prediction of bachelorhood, and indicate a two-factor interactionist model of genetic and maternal immunisation influences on sexual orientation. One tentative hypothesis is that genetic factors predispose towards childhood gender nonconformity, whilst maternal immunisation, as a consequence of later fraternal birth order, shunts sexual differentiation further down a sex-atypical or homosexual route. This may account for Bailey et al.'s most recent findings on the heritability of CGN and the non-shared nature of environmental causes, without invoking Bem's

theory or other social influences. Marriage status is not a good proxy for sexual orientation in women (Kirk, Bailey, & Martin, 1999).

Amidst the conflicting findings, it seems that, whether operationalised in terms of gender atypicality or as a primary trait, sexual orientation has a substantial genetic component, and that this may be located on the X chromosome. If homosexuality is due to X-linked genes, then it is highly likely that these genes affect the development and function of gonadal steroid receptors in the brain (Saifi & Chandra, 1999). Maternal immunisation to Y-linked or androgenic products may influence this in turn. In support of this argument, it has been demonstrated that the X-chromosomes have an over-abundance of genes related to sexual differentiation and reproduction (Saifi & Chandra, 1999).

4. Evolutionary considerations

Given the genetic component to homosexuality and its concomitant reduced reproductive success, the question arises as to why such genes have not been selected against during evolution. There are several plausible evolutionary scenarios whereby genes that predispose towards homosexuality could be maintained within populations (Pillard & Bailey, 1998). Here we briefly review and criticise such theories, and then propose an alternative (Table 1).

The most frequently cited evolutionary theory draws on the notion of kin selection (Wilson, 1975, 1978). The argument is that homosexuals may have helped (by resource provision and childcare) their siblings in the ancestral environments to reproduce more successfully. Thus genes for homosexuality survive indirectly through sibling lineages. Gay genes should therefore diminish in modern environments in which homosexuals are alienated from their families and migrate to large cities. Although a "fashionable" theory, it is based on weak assumptions and does not fit evidence from the anthropological record (Kirkpatrick, 2000). Even though homosexuals accrue resources and wealth, they do not seem to preferentially lavish these upon relatives; rather they direct them towards other homophile sources (Hewitt, 1995). Moreover, any such advantage in having homosexual family members would have to be extremely strong for the gene(s) to survive, and they would need to demonstrate high penetrance (which they do not). Data are unavailable on childcare provided by homosexuals. If individuals were to expend efforts on assisting siblings, then it would make more evolutionary sense for these individuals to be asexual rather than pursuing members of the same sex. Although largely repudiated, we suggest that an interesting test of the hypothesis would be to investigate whether the proposed altruism is directed towards specific classes of siblings. For example, if homosexuality in men is due to an X-linked gene(s) transmitted through the maternal line (as the genetic evidence suggests), then gay men should show greater altruism towards sisters and nieces, as these individuals would propagate those gene(s) further. Recently, however, Bobrow and Bailey (2001) have shown that homosexual men were no more likely than heterosexual men to channel resources toward family members.

The second set of theories focuses on parental manipulation of offspring sexual orientation, such that having homosexual children benefits parents' reproduction. (Trivers, 1974). The key premise here is that parents induce homosexuality by regulating resources or socialising offspring such as to make them less competitive in reproductive roles, and increase assistance towards

Table 1
Theories which attempt to account for the maintenance of genes for homosexuality

Theory	Source	Summary	Evidence	Limitations
Kin selection	Wilson (1975; 1978)	Homosexuals help close kin by resource provision and childcare	Mostly negative. Salais and Fischer (1995)—homosexual men more empathic than heterosexual men. Empathy and altruism may be correlated	Salais and Fischer study poorly designed. Altruism needs to be high to offset loss of reproduction. Homosexuality lacks "special design" for producing the benefit. Bobrow and Bailey (2001)—homosexual men no more likely than heterosexual men to aid kin; resources directed towards gay lifestyle
Parental selection	Trivers (1974)	Homosexual offspring benefit parental reproduction	Anthropological—parents encourage homosexual relations between sons and influential males (Kirkpatrick, 2000)	Discounts genetic factors altogether. Inclusive fitness reduced by producing homosexual offspring. Silent on lesbians
Balanced polymorphism—homosexual enabling	McKnight (1997)	Single homosexual alleles confer advantage to heterosexual men by increasing their sex drive, charm and seductiveness	Homosexual men have increased sex drives and high number of sex partners. Women avoid femininity in male partners	Increased libido in gay men due to opportunity. Women prefer feminine traits in men. Reliance on diallelic model. Silent on lesbians
Balanced polymorphism—female choice	Miller (2000)	Female selection of nurturing qualities increases feminising alleles = homosexuality in men as a cost. Similar masculinising mechanism for lesbianism	Women do prefer feminine qualities in male partners. Gay men are more empathic and less aggressive. Polygenic alleles. Fits birth order data	Further evidence needed of traits that are advantageous to heterosexuals to be shown by homosexuals
Steady-state mutation	Wilson (1987); Hamer and Copeland (1994)	Homosexuality results from mutation that matches the loss of direct reproduction	Male semi-lethal conditions on Xq28. Greater maternal foetal wastage in families of homosexual men (Turner, 1995)	Turner study relied on public records. No further replication. Silent on lesbians

(*continued on next page*)

Table 1 (*continued*)

Theory	Source	Summary	Evidence	Limitations
Trait dominance	Wilson (1981)	Genetic contribution to homosexuality is via personality traits such as low dominance	Homosexual behaviour in animals largely dominance related. Neuroendocrine evidence for dominance influences on sexually polymorphic fish species (Grober, 1997)	No evidence that homosexual men score lower on dominance or competitiveness. Silent on lesbians
Adaptive bisexuality	Baker and Bellis (1995)	Homosexuality is maladaptive end point of adaptive bisexuality	Bisexual women have high reproductive output (Baker & Bellis, 1995)	Silent on male homosexuality. Bisexuality carries increased sexual disease risks that could offset advantages
Same-sex affiliation	Muscarella (1999); Kirkpatrick (2000); Rahman and Wilson (present paper)	Extreme forms of genes evolve from same-sex affiliation that reduces violence between males, and increases female aggression to aid offspring care	Primate evidence suggests same-sex bonds aid survival (e.g. Vasey, 1995). Quantitative modelling of homosexual alleles (Getz, 1993). Fits data on female aggression (Campbell, 1999)	Further evidence of traits that are advantageous to heterosexuals needed to be shown by homosexuals

reproducing siblings. Some limited support is offered by anthropological evidence; that parents may encourage homosexual relations between sons and influential leaders (Kirkpatrick, 2000). Other than this, it suffers the same problems as kin selection, as well as assuming a predominant role for psychosocial influences in the development of homosexuality (Ruse, 1981; Dickemann, 1995). It is also intuitively at odds with the Darwinian notion of parental inclusive fitness, which would be reduced by having homosexual offspring, so we would not expect parents to encourage it willingly (Gallup & Suarez, 1983; Gallup, 1995 cf. Archer, 1996).

The paradox remains—the stability of homosexuality suggests it is not maladaptive, yet a simple reproductive analysis argues it is. Homosexual males do indeed have far fewer offspring than heterosexual males, and although homosexual females report having more children than homosexual males, they have substantially fewer than heterosexual females (Bell & Weinberg, 1978; LeVay, 1996). A solution might be found in the idea of balanced polymorphism, following Hutchinson's (1959) original suggestion. The classic example here is the existence of sickle-cell anaemia genes in African and Asian populations. These genes do not appear to have been selected against, even though they kill many before reproductive age. However, possessing one allele of the sickle-cell gene confers resistance to malaria, which in these populations causes more fatalities before reproductive age than sickle cell. This mechanism is referred to as balanced superior heterozygotic fitness, where possessing one allele of an otherwise fatal gene is balanced against its reproductive benefits.

McKnight (1997) has suggested that for homosexuality to survive, the genes for it must confer benefit to heterosexual individuals. He argues that those heterosexual men who possess one gay allele probably have an enhanced sex drive which leads to greater reproductive output and the retention of balanced homosexuality gene(s). It follows, according to McKnight, that male homosexuals would also have higher sex drives than heterosexuals and their greater turnover of partners could be construed as evidence for this. However, it seems more likely that homosexual men have more partners because of greater opportunity rather than an excess of libido (Bailey, Gaulin, Agyei, & Gladue, 1994; Symons, 1979). McKnight suggests that female mate choice is a factor in maintaining gay gene(s) where the greater charm, seductiveness and sex drive of "homosexually enabled" males is counter-balanced by women's avoidance of feminine men. Yet, women show a preference for feminine behavioural traits in their partners (e.g. empathy, considerateness and expressiveness; Sprecher, Sullivan, & Hatfield, 1994) and feminised facial features in men (Perret et al., 1998; Rhodes, Hickford, & Jeffrey, 2000). McKnight confuses homosexual hypermasculinity with homosexual femininity. The theory relies heavily on a strict diallelic model of homosexuality, as yet unclear from the genetic literature, and is silent on lesbians.

Recently Miller (2000) has made a similar proposal that remedies some criticisms of McKnight. Miller argues that sexual orientation is influenced by a number of genes (is polygenic) and that during development these affect the sensitivity of the male brain to hormones which shift it in a feminine direction. Possessing several such alleles produces homosexuality, whereas single alleles make for greater sensitivity, empathy and kindness. These would make heterosexual carriers better fathers and more attractive mates. Thus a balanced polymorphism is maintained whereby the feminising effect of such alleles in heterosexuals counterbalances the adverse effects of these alleles in producing homosexuality. The same mechanism might apply to genes that promote traits such as competitiveness as well as lesbianism in women. In this view, the birth order effect

apparent in male homosexuality arises from a by-product of the mechanism which shifts personalities in the feminine direction in later-born sons, and reduces inter-sibling competition. The model demands evidence of those traits that increase reproductive output in heterosexual males to be shown by homosexual males and, indeed, there is evidence that gay men are more empathic, sensitive and less aggressive than heterosexual males (Pillard, 1991; Gladue & Bailey, 1995a; Salais & Fischer, 1995; Haslam, 1997). The biological mechanism (genetically variable neurohormonal differentiation) which links homosexuality and feminine traits may make it difficult to gain the benefits of one without the occasional reproductive costs of the other.

Among the other theories outlined in Table 1 is that of steady-state mutation, homosexuality conferring no advantage but appearing with consistent frequency (Wilson, 1987). Hamer and Copeland (1994) suggest that homosexuality could be a result of mutations on hypervariable regions, such as Xq28, given its association with male semi-lethal conditions (c.f. the fragile X syndrome). Turner (1995) provided some support by demonstrating greater maternal foetal wastage in the families of homosexual men but his pedigree lineages were constructed from public records, which makes independent confirmation difficult. Gay genes could promote greater reproduction in female carriers by increasing their attraction towards men although being detrimental to males. Because females have two X chromosomes and males only one, a small fitness-advantage to females could counterbalance the cost to males. Such a theory is consistent with an X linked gene in males and could be tested by examining the reproductive rates in maternal line female relatives of gay men (Hamer & Copeland, 1994). Finally, Baker and Bellis (1995) have reported higher reproductive output for young (<25 years) "bisexual" females; they suggested that genes for homosexuality 'represent the maladaptive tip of an adaptive bisexual iceberg.' Homosexual activity is assumed to provide practice for later heterosexual mating. Although this theory conflicts with the bimodal nature of male sexual orientation, the data for females are interesting—given their greater rates of bisexuality, and could account for the pattern of familiality described by Pattatucci and Hamer (1995).

4.1. An integrated scenario

There remains a significant problem with the above theories; they have largely failed to discuss the ancestral conditions (or the 'environments of evolutionary adaptedness'; EEA) under which selection of genes for homosexuality may have occurred. The previous proposals have followed anti-adaptionist paradigms, whereas recent trends in evolutionary psychology suggest that enduring human traits are unlikely to be due to chance or by-products, and that there is usually some adaptive advantage (at least in the EEA) to these (Tooby & Cosmides, 1992). Thus, it is useful to consider the adaptive problems faced by hominids in the EEA that may have fostered the exploitation of genetic mutations related to sexual differentiation and ultimately, along with an array of other traits, homosexuality. Recently Muscarella (1999, 2000) and Kirkpatrick (2000) have proposed broadly that homosexual behaviour was selected because it aided same-sex affiliation and alliance formation. This, in turn, solved adaptive problems centred around social interchange, particularly inter-group and intra-sex (especially for males) conflict. Muscarella has argued that humans evolved a disposition for homoerotic behaviour because it increased same-sex affiliation among peripheralised adolescent hominids, increased access to resources, and indirectly increased reproductive success. Kirkpatrick's version derives from primate literature

and the anthropological record which suggest that same-sex alliances (and the reciprocal altruism therein) have reproductive and resource accrual advantages, hence promoting individual survival. Homosexual behaviour and emotional attachment serve to reinforce these same-sex bonds.

Is there evidence that 'conflict' was an adaptive problem? Certainly, there is reason to believe that early hominids often engaged in aggressive and fatal inter-group and intra-sex encounters, particularly among males. There may also have been frequent infanticide (Diamond, 1992; Wrangham, 1987). Such conflict and violence therefore could have been conducive to the development of homosexual behaviour, by promoting same-sex affiliation and alliances, thus increasing reproductive success. Evidence from primates supports this scenario, pointing to a persistent (in evolutionary time) adaptive problem that hominids could have faced. Primate homosexuality (engaged in by anthropoid primates, but not prosimians) is known for its role in the reduction of intra-sex aggression and increases in same-sex bonding in both natural and laboratory settings, perhaps most conspicuously in our nearest relative the bonobo (Vasey, 1995; Bagemihl, 1999). Such homosexual behaviour could have been part of a wider bisexual repertoire in early hominids, a necessary condition since opposite-sex pairings are more reproductive. Again, the primate literature supports this assertion in that no non-human primate is exclusively homosexual, although they do form long-lasting homosexual pairings as well as heterosexual ones (Vasey, 1995). However, Muscarella and Kirkpatrick assume that this "basic bisexuality" is apparent in modern humans, and they reject the notion of a bimodal sexual orientation in favour of a continuous one. This is inconsistent with the evidence for clearly bimodal male sexual orientation (even if the female distribution is more variable). They also fail to theorise as to the genetic-endocrine basis of selection for homosexual behaviour.

We propose the following solution and scenario. Intra-sex aggression constituted the adaptive problem and led to reduced individual survival and infanticide. Genetic mutations arose which exploited the evolutionary conserved plasticity of neuroendocrine sexual differentiation. By "plasticity" we do not mean an infinitely flexible neuroendocrine mechanism which would produce infinite sexual polymorphism. Instead, we refer to neuroethological evidence for an ancestral and conserved mechanism, in terms of very similar anatomical, neurochemical and endocrinological substrates across vertebrate taxa, upon which selection acts for generating variation in sexual phenotypes (Grober, 1997).

This genotype produced ancestral males who were more feminine in behavioural traits and bisexual in sexual preferences. These properties aided same-sex affiliation and allowed them to maintain alliances. Females were attracted to these males because of their feminine qualities (loyalty, kindness and reduced aggression), and with the concomitant reduced infanticide, greater parenting skills, commitment and affiliation with other powerful males, the viability of their offspring was increased. At this point we invoke the concept of sexually antagonistic selection (in which selection of optimal traits in one sex favours genes that incidentally lower fitness when expressed in the other). This means selection cannot remove these traits (Rice, 1992) and is a mechanism that could account for bimodal sexuality. Over ancestral time, females chose increasingly feminine traits (and thus more feminising alleles) in males, one consequence of which was more exclusive homosexuality in males. This strategy allowed such "exclusively gay alleles" to invade the population over time. Consonant with this, Getz (1993) modelled the effects of invasion, polymorphism and fixation of genes that differentially influence reproductive outcomes between the sexes, and also used it to explain the existence of homosexuality in human popula-

tions. The effect on parenting success and the viability of offspring (of prime importance to females who invest heavily in parental care and carry the "costliest" unit of reproduction—ova) offsets the deleterious effects in males, and thus maintains alleles for bimodal homosexuality in balanced polymorphism.

Similarly, female sexual selection could have produced the pattern of female sexual orientation currently observed. Selection for masculinising alleles may well have maintained intra-female alliances with powerful females (by homosexual activity), but also increased female aggression, which was conducive to greater care and protection of altricial young. Recently, Campbell (1999) has proposed that female aggression contributed to reproductive success because viability of offspring depended more on maternal rather than paternal investment. Females may also have occasionally wanted to increase the genetic quality of offspring by extra-pair copulation, and to do this would require greater masculine traits, such as assertiveness. Women, who have many sexual partners, have been shown to be more masculine in childhood sex-typed behaviours, as well as self-reported, behavioural and physical masculinity (Mikach & Bailey, 1999). Moreover, masculinising androgens are positively correlated with assertiveness in women (Cashdan, 1995). Thus masculinising alleles could produce unrestricted sociosexuality and masculine traits. Again, the effect of such alleles that produces occasional lesbianism is counterbalanced by its advantages. Nonetheless, because all women would have paired with males in ancestral environments (Miller, 2000), an intermediate level of masculinising alleles may have been selected for, producing the variable pattern of sexual preference that we call bisexuality. Such a bisexual strategy would be optimal for females in the EEA, ensuring reproductive output in most female lineages.

It is unlikely that there was a male-driven sexually antagonistic strategy responsible for producing lesbianism in females. If this were so, female sexual orientation should show bimodality and evidence of X-linked paternal transmission. It is also possible that the "hyper-masculine" traits sometimes evidenced by homosexual men (see next section) could be due to strong male selection for high-testosterone alleles. These would be necessary for successful male differentiation and increased variance in male fitness. The cost of this would be male homosexuality. Although consistent with sexually antagonistic selection, the data do not fit the maternal lineage transmission of male homosexuality. Hormonal mechanisms associated with increased feminisation in homosexuals may also account for the counter-intuitive appearance of "hyper-masculine" traits (discussed later).

Such evolutionary theorising is inherently speculative. It relies on "reverse engineering" and requires evidence. For example, amongst other points, do women really prefer androgynous males as potential fathers and are lesbians more aggressive than heterosexual women? As yet there is little direct evidence to shed light on these questions. Nonetheless, it goes some way in integrating the primate evidence, the adaptive problem in the human EEA and the current phenotypic pattern of sexual orientation observed in modern environments. It could be maintained that all the reviewed evolutionary hypotheses are simply the kin-selection argument cast in another light, in that they all predict that the siblings of homosexuals (who are more likely to be heterozygous for the genes) should have more offspring than heterosexuals. There is as yet no evidence to this effect. It is important to continue to elucidate the ultimate cause of universal phenotypes, such as homosexuality, as this process gives us a fuller understanding of the nature of human individual differences.

5. Neurodevelopment and the sexual differentiation of the brain

Theories concerning the etiogenesis of sexual orientation that focus on the sexual differentiation of the brain have enjoyed growing support. Much of this has been through experimental manipulation of sexual preference and sexual differentiation in animal models, such as exposure to prenatal sex hormones. Any X-linked genetic contributions to sexual orientation may certainly affect sexual differentiation, given the profusion of genes on the X chromosome for precisely this form of ontogenesis (Saifi & Chandra, 1999). The prenatal neurohormonal, or androgen theory, following Ellis and Ames' (1987) classic paper, is dominant by far, but there are two others; maternal immunisation and recently, the notion of developmental instability. All draw implicitly on the established taxonic nature of sexual orientation (examined earlier) which implicates a canalisation of prenatal neurodevelopmental processes that could account for the covariation between sexual orientation and its correlates. Early sex-atypical differentiation of the brain has been evidenced in several domains: somatic and morphological variations, neuroanatomy and neuropsychology.

5.1. Somatic variations

There is no evidence that homosexuality covaries with excessive perturbations in secondary sexual characteristics, genital anatomy, gonadal function or gender identity, such as those typically found in intersex conditions or transsexuality. Nonetheless, subtle differences in a number of somatic features have been documented.

Among the most contentious areas has been the relationship between homosexuality and non-right handedness, one that has produced conflicting findings for decades. This has recently been settled by a meta-analysis of 20 studies (Lalumiere, Blanchard, & Zucker, 2000) which confirmed the relationship; homosexuality is associated with greater non-right handedness. The rates of non-right handedness were compared in 6987 homosexuals (6182 men and 805 women) and 16,423 heterosexuals (14,808 men and 1615 women). Homosexuals overall have a 39% greater likelihood (OR = 1.39) of being non-right handed; gay men 34% (OR = 1.34; 95% CI 1.24–1.45) and lesbians 91% (OR = 1.91; 95% CI 1.52–2.40). Clearly, the relationship between handedness and sexual orientation is stronger in women. Men are generally reported to be more non-right handed than women. The overall log odds were not affected by moderating variables such as the measure of sexual orientation used or HIV status, although age and the type of handedness measure used were. Expressed in terms of effect size, the odds reported here are considered small to moderate, but reliable. Note that these data do not convert to actual population estimates of non-right handedness in the homosexuals. Lalumiere and colleagues reviewed both prenatal androgen and maternal immunisation theories to account for the association, but seem to favour developmental instability as part of the explanation.

Staying with "hands", two further aspects of interest are *finger length ratios* and *dermatoglyphy*. The ratio of the index finger (2D) to the fourth digit (4D) is sexually dimorphic, with males showing reduced ratio and females greater. This difference appears to be established as early as 2 years and reflects prenatal action of androgens, on males (Manning et al., 1998, 2000). In an early study of the behavioural significance of this ratio, Wilson (1983) found that women with male-typical finger length pattern described themselves as more competitive and assertive than women

with a female-typical ratio. Recently, Williams et al. (2000) reported that the right hand 2D:4D ratio in homosexual women was significantly masculine (smaller) compared to heterosexual women and no different from heterosexual men (more recently this pattern for women has been replicated; L.S. Hall, personal communication, October 2000). Williams et al. reported no differences between homosexual and heterosexual men. However, segregating men by birth order showed that only homosexual men with later fraternal birth-order (two or more older brothers) had a more masculine right hand 2D:4D ratio than homosexual men with one or no older brothers. On the basis of this birth-order effect, the authors concluded that men with older brothers, including those who may become homosexual, are exposed to higher prenatal testosterone levels. Robinson and Manning (2000) reported that male homosexuals showed the masculinised 2D:4D ratio as compared to heterosexuals but, in contrast to Williams et al, found that this was independent of birth order. Finally, we have also confirmed that both homosexual males and females have lower 2D:4D ratios than heterosexual controls, particularly on the right hand, and that this is independent of sibling sex composition, birth order and, within the homosexual sample, independent of ''butch–femme'' sex role identification (Rahman & Wilson, in press). Overall, these data suggest that lesbians are exposed to a greater degree of masculinisation than heterosexual women, but that homosexual men may also be over-masculinised.

This ''hyper-masculinisation'' may be evidenced elsewhere. For example, homosexual men report larger genitalia than do heterosexual men, based on measures of penile length and circumference derived from the Kinsey data archives (Bogaert & Hershberger, 1999). Although interesting, the effects of self-reporting bias on these data cannot be excluded.

With regard to dermatoglyphic profiles (patterns of dermal fingerprint ridges), there are conflicting data. Dermatoglyphic characteristics develop by the 16th week of gestation and may be hormone dependent. An initial study by Hall and Kimura (1994) demonstrated that gay men show a more leftward asymmetry (greater number of ridges on the left fingers) than heterosexual men, a pattern often found in women. Leftward asymmetry was also associated with non-right handedness in gay men but not in heterosexual men. These data were taken as markers for an atypical intrauterine environment possibly experienced by homosexuals. Although widely cited, this study suffered from small sample size (thus low power to detect small differences), lack of female control groups and substantial overlap between the male groups. Since then, two studies have failed to replicate these effects using Hall and Kimura's original method of analysing fingerprint asymmetry. Both studies have employed homosexual transsexuals. One reported no sex differences and no association between gender identity or sexual orientation and dermatoglyphic pattern (Slabbekoorn, VanGoozen, Sanders, Gooren, & Cohen-Kettenis, 2000). The other reported a sex difference on ridge count only, and an association between fingerprint asymmetry and sexual orientation but not gender identity using a parametric procedure (Green & Young, 2000). Finally, one study (Hall, 2000) reported a trend towards leftward asymmetry in gay male twins compared to their straight twin brothers (discordant for sexual orientation), whereas lesbians' twins had lower ridge counts compared to their straight twin sisters in discordant pairs. There were no differences between concordant pairs of either sex. Overall, we suggest caution in interpreting the findings of these dermatoglyphic studies; there appear to be some differences but their significance is unclear in the absence of normative sex differences. The variance in methodology and the use of transsexuals may explain the inconclusive findings. Nonetheless, homosexual transsexuals resemble same-sex non-transsexual homosexuals in many respects, such as

childhood gender nonconformity (e.g. Chivers & Bailey, 2000) and thus can be partially valid "models" for sexual orientation. Gender identity and sexual orientation are discordant in non-transsexual homosexuals, but confluent in homosexual transsexuals. This supports a modular view of sexual psychology on the one hand (also evidenced by distinct neural correlates; see next section), but points to a general mechanism underlying the development of these modules. For example, the degree of neurodevelopmental masculinisation could determine the extent of coupling or decoupling between gender identity and sexual orientation. Alternatively, there could be separate critical periods in utero which determine these domains (Ellis & Ames, 1987).

Timing of puberty seems to differ between homosexual men and heterosexual men. The former report earlier pubertal onset than the latter, in both large contemporary samples and the Kinsey data archives (Blanchard & Bogaert, 1996a; Bogaert & Blanchard, 1996). The same studies report *lower body weight* and *height* in homosexual men compared to heterosexual men. Thus, in these respects homosexual men score in a female-typical direction. Although these characteristics are sex dimorphic, the data did not include female groups and were primarily based on self-reports. It is possible that heterosexual men exaggerate their height to conform to a masculine ideal. The effect sizes for these differences are also small. From an evolutionary perspective, an earlier pubertal onset in male hominids may have been conducive to same-sex affiliation by producing earlier onset for homosexual interactions. Homosexual women do not differ from heterosexual women in terms of age at menarche or other milestones of puberty (Singh, Vidaurri, Zambarano, & Dabbs, 1999; Tenhula & Bailey, 1997; Bogaert, 1998a) but do seem to be *taller* and *heavier* than heterosexual women (Bogaert, 1998a, 1998b). Thus, homosexual women score in a male-typical direction on some somatic traits but not others.

Further evidence of prenatal masculinisation of lesbians is provided by data on *otoacoustic emissions* (cochlea-generated sounds or OAEs). OAEs are more numerous in females than in males, and in the right ear. These patterns of sex and ear differences exist in infants, children and adults. Results show no difference between homosexual and heterosexual men, but less numerous and weaker OAEs of homosexual and bisexual women (McFadden & Pasanen, 1998, 1999). On all measures, homosexual or bisexual women were intermediate between heterosexual men and women. A role for early development is suggested by findings that females with male co-twins (who are exposed to more androgens) have OAEs more like males than other females, thus implicating prenatal androgens for masculinised cochleae in homosexual females (McFadden, 1993). However, note that in the earlier behaviour genetic investigations (Bailey et al., 2000a) no differences are found in the prevalence of male or female homosexuality in dizygotic opposite-sex twins.

The extent of masculinisation in lesbians may vary as a function of important individual differences within sexual orientation. Singh et al. (1999) provided data on "butch" and "femme" erotic role identification among lesbians. Results showed that butch lesbians recalled more childhood gender nonconformity, had higher waist-to-hip ratios, higher salivary testosterone levels and less desire to give birth as compared to femme lesbians. Nonetheless, femme lesbians were more gender nonconforming as children than heterosexual women. These data suggest that within sexual orientation, differences may be patterned along a continuum of sex atypicality, butch lesbians being generally more male-typical than femmes. They also provide some validity to the butch–femme classification as a biological rather than merely a social construct. Extending this division to butch and femme homosexual men may provide further insights.

Overall, the pattern of findings suggests that homosexual men are more masculinised in terms of digit formation and possibly genitalia, but female-like in pubertal onset, weight and height. This implies different neurodevelopmental sequelae for these traits. Lesbians are consistently more masculinised but there may be different critical periods for neurodevelopmental effects on pubertal timing versus other traits. Homosexuals of both sexes are more often non-right handed than heterosexuals, this difference being greater in women. The handedness data are consistent with a greater pattern of masculinisation in lesbians but not for men. Theoretical explanations will need to account for these complex patterns.

5.2. Neuroanatomy

The evidence for sex differences in neuroanatomy sets the stage for investigations into within-sex dimorphism, notably in the brains of homosexuals and heterosexuals (Breedlove, 1992; Matsumoto, 2000; Swaab & Hofman, 1995). The first reported difference in neuroanatomy between homosexuals and heterosexuals showed that the *supra-chiasmatic nucleus* (SCN), a region involved with circadian rhythms and sexual behaviour, was larger and more elongated in homosexual men (Swaab & Hofman, 1990). This shape is characteristically found in women. These differences in SCN morphology are also specific to the arginine vasopressin (AVP) neuronal population (Zhou, Hofman, & Swaab, 1995b). LeVay (1991) reported that an area of the hypothalamus, the *third interstitial nucleus of the anterior hypothalamus* (INAH-3), was smaller in homosexual men than in heterosexual men. This area is also typically smaller in women (Byne et al., 2000, 2001; LeVay, 1991). Allen and Gorski (1992) reported the *midsagittal plane of the anterior-commissure* (AC) to be larger in homosexual men than in heterosexual men and women, a region larger in women than in men generally (Allen & Gorski, 1991). Finally, using structural magnetic resonance imaging, Scamvougeras et al. (1994) reported that the *isthmus of the corpus callosum* was larger by 13% in right-handed homosexual men as compared to heterosexual men. The isthmus is also larger in women and positively correlated with left-handedness in men (Witelson, 1989; Witelson & Goldsmith, 1991). This finding has implications for theoretical explanations of non-right handedness in homosexuals.

The hypothalamic differences are the first indication of a neural circuitry underlying direction of sexual preferences in humans. Lesion studies in animals certainly suggest that anterior regions of the hypothalamus are crucial to approach and coital behaviours towards male or female conspecifics (e.g. Paredes & Baum, 1995). Lesions to the anterior hypothalamus also result in reduction of heterosexual interest in non-human primates (Oomura, Yoshimatsu, & Aou, 1983; Slimp, Hart, & Goy, 1978). Although still unclear, there is some evidence that INAH-3 is the human homologue to similar sexually-dimorphic nuclei in other mammals (Byne, 1998; Byne et al., 2001). The increased AVP content in the SCN of homosexual men may also have functional implications. The SCN is the circadian pacemaker of the brain and regulates sleep-waking and other cycles. The AVP neurons peak in volume during the early morning (and are associated with wakefulness) and decrease during night (and are associated with sleep). Recently, a preliminary report found that homosexuals show a pattern of sleep-waking rhythmicity consistent with their hypothesised differential SCN-AVP morphology (Rahman & Silber, 2000). That is, homosexuals (men and women) woke up earlier and retired later (thus having a shorter sleep duration) than heterosexuals, possibly due to a larger SCN-AVP complex capitalising on the normal AVP neuronal

rhythmicity and producing earlier rising and later retiring. This study did not find that sleep-waking patterns followed sex-typical, atypical or over-masculinised directions, rather they were consonant with the assumed differences in AVP between homosexuals and heterosexuals. Studies with rats also suggest SCN involvement in sexual preference; those treated with aromatase inhibitor (to disrupt the conversion of testosterone to estradiol thus leaving the brain partially feminised) become "bisexual" and show an increased number of AVP neurons in the SCN (Swaab, Slob, Houtsmuller, Brand, & Zhou, 1995). The functional implications of AC and isthmus differences will be discussed further below.

Interestingly, homosexual males do not show dimorphism in another sexually-dimorphic structure, the *bed nucleus of the stria terminalis* (BNST), which does, however, show female morphology in male to female transsexuals (Zhou, Hofman, Gooren, & Swaab, 1995; Kruijver et al., 2000; Kruijver, personal communication, October 2000). A more male-like BNST was observed in female-to-male transsexuals. These differences were independent of whether transsexuals were receiving hormone treatment for sex reassignment. Yet, the BNST is sensitive to the effects of testosterone during early development in animals (Chung, Swaab, & DeVries, 2000). These findings are the first to suggest a neural correlate of gender identity (the sense of being male or female). They confirm the fundamental dissociation between target preference and gender identity, even though similar neurodevelopmental mechanisms may be involved.

Although the neuroanatomical correlates of male homosexuality differentiate very early postnatally, if not prenatally (Swaab & Hofman, 1990, 1995), we cannot rule out the possibility that adult sexual behaviour could contribute to some of the brain differences (Woodson & Gorski, 2000). Future neuroanatomical studies need to be consistent in their morphometric methodology (e.g. type of staining used and neuronal parameters examined), control for confounds (e.g. brain weight and extraneous influences of neuropathogens, such as HIV infection), and obtain accurate information on sexual orientation. Also, there is a paucity of neuroanatomical data comparing homosexual and heterosexual women. It seems reasonable, nonetheless, to conclude that homosexual men show a trend towards sex atypicality in specific neural regions.

5.3. Neuropsychological findings

The functional implications of sex-atypicality in neuroanatomy may include cross-sex shifts in cognitive ability. Normative sex differences in neuropsychological performance are well documented. Typically, males excel in cognitive tasks involving spatial rotational components, the largest differences apparent during mental rotation and spatial navigation (Kimura, 1999). Females excel at verbal fluency, verbal working memory and facial emotion perception (Kimura, 1999; McClure, 2000). Men are also more strongly lateralised for language than are women; this difference may account for the male advantage on spatial tasks—language functions are displaced from the right hemisphere in favour of spatial processing (Hiscock, Israelian, Inch, Jacek, & Kalil-Hiscock, 1995; Levy & Heller, 1992). Sex differences in interhemispheric and neuroanatomical asymmetries may subserve variations in neuropsychological performance. Females have larger posterior regions of the corpus callosum, but these may be confounded by differences in shape rather than size (Hines, 1990) and larger language (Broca's and Wernicke's) regions (Harasty, Double, Halliday, Kril, & McRitchie, 1997). Men have a larger spatial processing (inferior parietal) region (Frederiske, Lu, Aylward, Barta, & Pearlson, 1999). Recent functional brain

imaging evidence supports greater asymmetry of language in men (Gur et al., 2000; Kansaku, Yamaura, & Kitazawa, 2000; Shaywitz et al., 1995), and greater right-extending to bilateral-brain activation during spatial processing in men (Gur et al., 2000). These differences may depend in part on the type of spatial or language ability being examined. For example during spatial navigation through a maze, men activate the left hippocampus whilst women cope with the task by recruiting right parietal and right prefrontal regions (Gron, Wunderlich, Spitzer, Tomczak, & Riepe, 2000).

In addition to these sex differences, several independent neuropsychological studies suggest that, for gay men at least, cognitive performance on measures that typically elicit sex differences is shifted in a "female-like" direction. That is, homosexual males perform poorly on mental rotations and better on verbal fluency tasks as compared to heterosexual males (Gladue, Beatty, Larson, & Staton, 1990; McCormick & Witelson, 1991; Neave, Menaged, & Weightman, 1999; Sanders & Ross-Field, 1986a; Sanders & Wright, 1997; Wegesin, 1998a; Willmot & Brierley, 1984). Gay men are also less accurate on visuomotor targeting tasks (typically male advantage) compared to heterosexual men, and no more accurate than heterosexual women (Hall & Kimura, 1995). Differences in strength and sports history did not account for the variance in these findings. Overall, these cognitive differences are not accounted for by self-reported masculinity or femininity, daily spatial experience or general intelligence (Gladue et al, 1990; Sanders & Wright, 1997; Wegesin, Meyer-Bahlburg, Hunter, & Gray, 1998). The profile may be present in childhood, as gender-nonconforming boys show diminished spatial ability compared to controls (Finegan, Zucker, Bradley, & Doering, 1982; Grimshaw, Zucker, Bradley, Lowry, & Mitchell, 1991). Two older studies failed to find sexual orientation effects (Gladue & Bailey, 1995b; Tuttle & Pillard, 1991). The conflicting findings may be partly explained by the use of tasks that elicit smaller gender effect sizes. For example, only half the studies demonstrate sexual orientation effects when using the male advantage *water-level test* (modest effect size, $d = 0.5$), whereas consistent differences appear on *three-dimensional mental rotation tests* (large effect size, $d = 1.0$). In any case, gay men clearly show a decrement in spatial processing. Interestingly, we have recently discovered that homosexual men perform in female-typical directions on object location memory, a spatial task which usually shows a modest effect size *in favour* of females (Rahman, Wilson, & Abrahams, in press). This suggests that gender-atypical shifts cut across the de-coupling in some spatial functions, e.g. mental rotation (male-favouring) versus location memory (female-favouring).

Neave et al. (1999) reported that the decrement shown by gay men on mental rotations was related to *higher* circulating testosterone (T) levels in this group. This is in line with the proposed curvilinear relationship between T and spatial ability in men, such that either high or low levels produce decrements in performance (Nyborg, 1983; Moffat & Hampson, 1996). However, the literature is scattered with positive, negative and null relationships between T and spatial ability (Silverman, Kastuk, Choi, & Phillips, 1999), and no study has confirmed the curvilinearity hypothesis as yet. Since the sexual orientation of subjects in these studies is unspecified it is possible that there is no relationship between T and spatial ability within heterosexual males. In homosexual males, higher levels of T could reflect an activational effect due to task difficulty or a differing pattern of brain organisation in utero.

The data for lesbians is less consistent. There are trends towards more male-like cognitive performance but these are mostly non-significant (Wegesin, 1998a). Homosexual women are,

however, more accurate than heterosexual women on visuomotor targeting, and comparable to heterosexual men (Hall & Kimura, 1995). Overall, the performance of lesbians is more sex-typical than that of gay men. This suggests either that atypical differentiation of neurocognitive function occurs only within a subset of lesbians, for example "butch" lesbians (Singh et al., 1999), or that critical periods for the neurodevelopment of target preference are uncoupled from neurocognitive differentiation between gay men and lesbians.

Homosexual men do seem to show a *cross-sex shift* in cognitive performance in the direction of heterosexual women. Is there any evidence that this shift may be due to differences in cerebral organisation? Gay men show reduced language and perceptual lateralisation on traditional measures of cerebral asymmetry, such as dichotic-listening and divided visual field tests, whereas lesbians do not (Sanders & Ross-Field, 1986b; Sanders & Wright, 1997; McCormick & Witelson, 1994; Wegesin, 1998c). This suggests that homosexual men are more bilaterally organised for language (like heterosexual women), and thus possibly right-lateralised for spatial processing. Neurophysiological measures of functional cerebral asymmetries provide some support for this. Alexander and Sufka (1993) recorded electroencephalographic activity during verbal and spatial tasks. Patterns of alpha activity in homosexual men resembled those of heterosexual women, both differing from heterosexual men. Reite, Sheeder, Richardson, and Teale (1995) found more symmetric auditory source locations using magnetoencephalography (MEG) in gay men compared to heterosexual men, a pattern more typical of women. Recently, Wegesin (1998b) did not find differences in asymmetry of event related potentials (ERPs) between heterosexuals and homosexuals, but slow-wave activity during a mental rotation task was similar for homosexual men and heterosexual women. There was also a non-significant trend towards male-typical slow wave activity in lesbians, but they did not otherwise differ electrophysiologically from heterosexual women. In summary, these data suggest a bilateral organisation of linguistic function in homosexual males that is female typical. The ERP study confirms a neurophysiological basis for the similarity in mental rotation performance between heterosexual women and gay men (i.e. less negative slow-wave activity) but is silent on cerebral asymmetry for spatial functions. It is reasonable to suspect that a bilateral representation of language in homosexual men may incur the cost of reduced spatial ability (Levy & Heller, 1992; Wegesin, 1998c).

It is tempting to speculate concerning the underlying neurocognitive architecture of the cross-sex shifts described above. A reversal of sex-typical anatomic asymmetries could explain the pattern of cognitive performance, e.g. larger Broca's and Wernicke's, and smaller parietal, regions in homosexual men. In fact, on the basis of their MEG findings, Reite et al. (1995) suggested there might be differences in the superior temporal gyri (one subdivision of Wernicke's area) of at least some homosexual men. However, Moffat, Hampson, and Lee (1998) reported no consistent variation in another subdivision of Wernicke's area (the planum temporale) among male subjects with right hemisphere language functions. Although their subjects were not assessed for sexual orientation, the implication is that the reversed pattern of language asymmetry in homosexual men may not necessarily be accompanied by reversals in the brain's language regions.

Alternatively, the data fit well with the notion of differential interhemispheric "cross talk", mediated by the anterior commissure (AC). The larger AC of homosexual men and heterosexual women may contribute to greater inter-hemispheric transfer, and thus bilateral representation, of language functions, specifically verbal fluency (Allen & Gorski, 1992). This is consistent with the findings of greater verbal fluency, and lower visuo-spatial ability, in homosexual men and

heterosexual women. AC axons are also known to innervate inferior temporal regions, which are associated with phonological processing (DiVirgilio, Clarke, Pizzolato, & Schaffner, 1999).

Greater inter-callosal transfer could also occur through the larger isthmus of gay men (Scamvougeras et al., 1994). A larger isthmus would allow greater interconnection of right and left speech regions. This may determine the pattern of varying linguistic function and increased prevalence of non-right handedness in homosexual men. A number of findings support this possibility. Recently, Moffat, Hampson, Wickett, Vernon, and Lee (1997) demonstrated a positive correlation between circulating testosterone (T) and the size of posterior regions of the corpus callosum, including the isthmus, in human males. Testosterone exposure may increase the size of the isthmus during neurodevelopment (see next section). Thus, the larger isthmus in gay men might be due to greater prenatal T exposure, as indicated by the somatic evidence detailed earlier. Reversal of praxic function and greater bilateral representation of language accompany a larger isthmus. Left-handed males (sexual orientation unspecified) have significantly larger callosal isthmuses than do right-handed males, and this may be mediated by patterns of language lateralisation in left-handers (Cowell, Kertesz & Deneberg, 1993; Habib et al., 1991; Witelson, 1989). As a consequence of these asymmetries, spatial ability is compromised. This would explain the association between T and poor performance on mental rotations in gay men (Neave et al., 1999). Note, however, the larger isthmus of gay men was reported for right-handed subjects only. This conforms to other data which suggest that lateralisation for language may be uncoupled from hand preference in some homosexuals but not in others (McCormick & Witelson, 1994; Wegesin, 1998c). Replication of the callosal isthmus finding in right-handed and non-right handed homosexuals (males and females) is needed to clarify the issue. Studies employing structural and functional neuroimaging techniques, coupled with "on-line" neuropsychological testing would also help explicate relationships between sexual orientation, brain asymmetry and cognitive performance.

6. Putative neurodevelopmental mechanisms

The profiles of sex-atypical features associated with homosexuality differentiate early during development and are minimally influenced by psychosocial factors. A concomitant developmental pathway may determine both the direction of sexual preference and the "package" of sex-atypicality described above. These mechanisms probably originate from the X chromosome (a possible site for genes determining sexual orientation) that has an abundance of sexual differentiation linked genes. Which of these mechanisms best accounts for the patterns of findings reported above (Table 2)?

6.1. Prenatal androgen theory

The theory of prenatal androgen exposure originates from a large body of research demonstrating that the male foetal brain is masculinised by exposure to androgenic gonadal steroids during critical periods of development. The absence of androgens results in feminisation of the foetal brain. Hormonal exposure at these stages organises the brain in a lasting manner and determines patterns of male-typical versus female-typical behaviours (including sexual preferences,

Table 2
Does the reviewed evidence support predictions made by neurodevelopmental theories for homosexuality?

Theory	Source	Predictions	Homosexual males	Homosexual females
Prenatal androgen	Ellis and Ames (1987)	Sex atypical target preference	Yes	Yes
		Sex atypical childhood play interests	Yes	Yes
		Sex atypical neural structures	Yes/Insf	N/D
		Sex atypical cognitive abilities	Yes	Yes/Insf
		Sex atypical cerebral asymmetry	Yes	N/D
		Elevated non-right handedness	Yes	Yes
		Over-masculinised finger length ratios	Yes	Yes
		Over-masculinised genitals	Yes/Insf	N/D
		Over-masculinised auditory emissions (OAE's)	No	Yes
		Sex atypical weight/height	Yes	Yes
		No predictions regarding:		
		Later fraternal birth order	?	?
		Atypical pubertal onset	?	?
Maternal immunisation	Blanchard and Bogaert (1996b); Blanchard and Klassen (1997)	Later fraternal birth order	Yes	N/A
		Feminised target preference	Yes	N/A
		Feminised neural structures	Yes/Insf	N/A
		Feminised cognitive abilities	Yes	N/A
		Feminised cerebral asymmetry	Yes	N/A
		Increased homosexuality in younger brothers of homosexual men	No	N/A
		No predictions regarding:		
		Childhood play interests	?	?
		Elevated non-right handedness	?	?
		Somatic differences	?	?
Developmental instability	Lalumiere et al. (2000)	Sex atypical target preference	Yes	Yes
		Sex atypical neural structures	Yes/Insf	N/D
		Sex atypical cognitive abilites	Yes	Yes/Insf
		Sex atypical cerebral asymmetry	Yes	N/D
		Elevated non-right handedness	Yes	Yes
		Elevated fluctuating asymmetry in other bilateral features	No	No
		No predictions regarding:		
		Childhood play interests	?	?
		Somatic differences	?	?
		Later fraternal birth order	?	?

Yes = prediction is supported by evidence reviewed (see text); No = evidence does not support the prediction, N/A = the prediction does not apply; N/D = no data; ? = the theory makes no predictions; Yes/Insf = prediction is supported but with insufficient information.

gender identity and childhood interests), as well as neurocognitive features (Collaer & Hines, 1995). In this view, homosexuality in males is due to under-masculinisation (the partial absence of androgenising effects) in women over-masculinisation (excess androgenising effects) during early development (Collaer & Hines, 1995; Ellis & Ames, 1987). Genetic products produce androgen receptor (AR) proteins in most end tissues, particularly the brain, which mediate the action of steroid hormones. The presence of a Y chromosome in the embryo is conducive to the development of male testes (and regression of pre-female organs); these begin to secrete testosterone. Testosterone binds to ARs and regulates the transcriptional activity of target DNA. This determines aspects of neural and morphological development. Within the brain testosterone may also be converted to dihydrotestosterone (DHT) through the 5-alpha-reductase pathway and bind to AR, or to estradiol through the aromatase pathway and bind to estrogen receptors (ERs) which act to masculinise (increase male-typical behaviours) and defeminise (reduce female typical responses) the behavioural development of male mammals (Negri-Cesi, Poletti, Martini, & Piva, 2000; Woodson & Gorski, 2000). Androgen receptors are widely found in cerebral and subcortical regions of the human brain (Puy et al., 1995), but also vary in predictable sex-dimorphic ways. For example, there is a proliferation of AR in the male hypothalamus (Fernandez-Guasti, Kruijver, Fodor, & Swaab, 2000). These basic hormonal mechanisms appear integral to the development of sexual orientation.

Using this framework, we attempt to explain male and female homosexuality separately. For males, we propose two prenatal neurohormonal pathways. The first possibility is that homosexual preferences and associated correlates are due to a genetically determined differential distribution of androgen receptors such that there is a more female-like pattern. The second possibility is that homosexual preferences are determined through the aromatase to estradiol pathway. Genetically influenced variations, or decreases, in brain aromatase produce feminisation of male sexual preferences, in the absence of aromatised estradiol in key neural regions. Either there is a dearth of binding of testosterone (T) to ARs or reduced aromatisation to estradiol resulting in an increasing pool of T. This substrate pool could determine the hyper-masculine features and larger isthmus associated with male homosexuality, through the 5 alpha reductase to DHT pathway. These mechanisms presumably exert their influence prenatally, and in a permanent manner, determining the sexual phenotype of mammals.

Men show a greater distribution of the AR staining in hypothalamic regions, including the medial preoptic area (mPOA), sexually dimorphic nucleus (SDN) and SCN, areas implicated in male sexual behaviour and preferences (Fernandez-Guasti et al., 2000). Moreover, men gonadectomised before puberty may show a more female-like AR distribution (Fernandez-Guasti et al., 2000). These data suggest the possibility of a more female-like AR distribution in the hypothalamic regions of homosexual men accounting for the variations observed in INAH-3 and the SCN. Against this possibility, Macke et al. (1993) found no concordance between homosexual brothers in the most common type of androgen receptor sequence variation. Although this study found no evidence for mutations on the AR gene in homosexual brothers, the genetic effect could be subtler, for example influencing AR sub-units. Alternatively, homosexual men might not differ in the distribution of ARs but in the responsivity of ARs to T. This implies that homosexual men may have minor androgen insensitivity in key neural regions (INAH-3 and SCN). Interestingly, genetically male individuals with complete androgen insensitivity syndrome (CAIS), report female-typical heterosexual orientation (Wisniewski et al., 2000). Such individuals are also mostly

female-typical in physical appearance and behaviour because of complete end-tissue resistance to androgens.

These conflicting observations may be resolved by focusing on the aromatase to estradiol pathway of male sexual differentiation. Fernandez-Guasti and colleagues suggested that considerable aromatisation of T to estradiol in males (determining the extent of masculinisation and defeminisation) could account for their observations of some comparable AR staining in additional hypothalamic regions between males and females. In fact, males show higher aromatase activity in the mPOA and SDN. The explanation for male sexual orientation then shifts from ARs to variations in estrogen receptors (ERs), or mutations in aromatase enzyme genes (such as gene CYP19) which reduce the masculinising actions of estradiol, thus accounting for the female-like hypothalamic centres of homosexual men. Rodent models have confirmed that a larger SCN-AVP complex (as shown by homosexual men) can be induced by blocking aromatase activity and thus reducing metabolism of T to estradiol (Bakker & Slob, 1997). Further evidence for the role of aromatase and estrogen receptors in the differentiation of male sexual orientation comes from studies of ''homosexual rams'' (reviewed in Perkins & Fitzgerald, 1997). These are the first non-human animals that have shown ''exclusive homosexual behaviour'', independent of human intervention. They comprise a sub-population of rams who copulate with other rams, do not show female-like sexual posture, never show interest in oestrus ewes and show no deficits in sexual functioning (Perkins & Fitzgerald, 1997). Neurochemical analyses of homosexual rams' brains show reduced distributions of ERs in the amygdala, and less aromatase activity in the POA compared with heterosexual rams (Perkins & Fitzgerald, 1997; Resko, Perkins, Roselli, Stellflug, & Stormshak, 1999). This neurochemical profile is similar to that found in ewes. Since the hypothalamus extends pathways to the amygdala, genetic variations in aromatase enzymatic activity could reduce ERs in the amygdala, with the consequent lack of aromatised estradiol demasculinising partner preference in homosexual rams. Of course these differences could be a consequence of sexual activity rather than latent preference, but either way they should throw light on human male homosexuality.

Several other lines of evidence implicate the amygdala in human male sexual orientation. Firstly, the genetic evidence points to childhood sex-typed interests being the heritable component of sexual orientation (e.g. Bailey, Dunne, & Martin, 2000). Studies with non-human animals suggest the amygdala is involved in male sex-typed play behaviour (Meaney & McEwen, 1986). Moreover, androgens are involved in the differentiation of the amygdala in animals and the differentiation of sex-typed play behaviour in humans (Berenbaum & Hines, 1992; Berenbaum & Snyder, 1995; Cooke, Tabibnia, & Breedlove, 1999). The amygdala is sexually dimorphic in human and animal males and females (Cooke et al., 1999; Giedd, Castellanos, Rajapakse, Vaituzis, & Rapopart, 1997; Giedd et al., 1996). The investigation of within-sex variations into the amygdala is thus likely to be fruitful.

The two-pathway model for the feminisation of target preference described above does not dispel the paradox of ''hyper-masculinisation'' of some features associated with male homosexuality, e.g. finger length ratios. Moreover, we are yet to explain mechanisms for the development of non-right handedness and cross-sex neurocognitive functions. A possible solution can be found in the proposed greater substrate pool of T in the ''pre-homosexual'' foetus. If there is an insensitivity to T in androgen receptors or reduction in aromatase metabolic activity, there may be more unbounded T available for conversion to dihydrotestosterone (DHT). DHT is involved

in the development of male-typical genitals, digits and other morphological characteristics, but not psychosexual identity or development (Manning et al., 2000; Randall, 1994). We propose that this elevated unbound, and/or non-aromatisable, T is metabolised by 5 alpha reductase to DHT, resulting in the hypermasculinisation of finger ratios, genitals and other morphological features (under DHT control) in homosexual males.

Testosterone is also positively correlated with the size of posterior regions of the corpus callosum, including the isthmus in human males (Moffat et al., 1997). The isthmus is larger in both homosexual men and left-handed men. The white matter of the brain (of which the corpus callosum is composed) is rich with 5 alpha reductase in both animals and humans (Celotti, Melcangi, & Martini, 1992; Celotti, Melcangi, Negri-Cesi, & Martini, 1987; Stoffel-Wagner et al., 1998). Moreover T, and its metabolites (such as DHT), act to promote white-matter axon growth and myelination (Celotti et al., 1992). Thus, as well as its role in determining somatic features, 5 alpha reduced T could be implicated in certain neural differences between homosexual and heterosexual males, specifically the larger isthmus. This may determine the particular neurocognitive profile shown by homosexual men: reversed praxic function, reduced language lateralisation and poorer spatial performance (also associated with slightly elevated T levels in this group; Neave et al., 1999). This connects with Geschwind and Galaburda's classic model (1985a, 1985b) of the development of cerebral laterality and sexual orientation. They proposed that greater exposure to T during foetal development slows the development of the left hemisphere, permitting the right hemisphere to become modestly dominant and shift handedness and lateralisation of language toward bilateral representation. This would account for the elevated rate of non-right handedness in men generally, and in homosexual men and women particularly. Our proposal differs only in that it is the development of callosal tissue that is influenced by T (specifically 5 alpha reduced DHT), not the entire right hemisphere. Lalumiere et al. (2000) argued against a prenatal hormonal mechanism for the association between non-right handedness and homosexuality, because it explained the association in women (which we accede) but not in men (which we contest). They maintain that if homosexuality in men is due to under-masculinisation, and therefore less exposure to T, then there should be a shift in the female direction of more right-handedness. Understandably, their argument rejects the prenatal androgen hypothesis because it depends on a very general hormonal mechanism (under-exposure to T in homosexual men versus over-exposure in homosexual women) which organises brain sex *globally*. However, we suggest that *specific* mechanisms of prenatal hormonal action could account for left-handedness in homosexual men.

The earlier pubertal onset of homosexual men is more difficult to explain within the prenatal androgen framework. Estrogen is known to organise gonadotropin-releasing hormone (GnRH) centres within the anterior hypothalamus. Typically, GnRH activity stimulates the release of testosterone from the testes (through the hypothalamic-pituitary-gonadal axis; HPG) which feeds back into the central nervous system to maintain the GnRH in the anterior hypothalamus by conversion to estradiol. Puberty in males is marked by an increase in GnRH release (possibly in response to reaching a critical body mass) which increases testosterone to complete adult sexual differentiation. Presumably, possessing a more female-like hypothalamus results in less GnRH activity and a reduced testosterone response. Some evidence has been provided for this. The luteinising hormone (LH) response (a measure of HPG-axis functioning) to an injection of estrogen was more female-like in homosexual men than heterosexual men (Dorner, Rhode, Stahl, Krell, & Masius, 1975; Gladue, Green, & Hellman, 1984). Moreover, testosterone returned to

baseline levels more slowly in homosexual men than in heterosexuals. However, this would predict that male homosexuals should have delayed pubertal onset rather than earlier, since the quantity of testosterone produced by GnRH activity would be reduced. In any case, the findings on female-like LH responses in homosexuals have been contested (Gooren, 1986a, 1986b; Hendricks, Graber, & Rodriguez-Sieera, 1989). Pubertal onset is altogether a complex and poorly understood phenomenon (McClintock & Herdt, 1996).

The classic model of neurohormonal differentiation has always assumed that female sexual differentiation develops by default. Recent evidence suggests that estrogenic products have an active role in feminisation, but this mechanism is poorly understood (Ogawa & Pfaff, 2000). Moreover, the paradox of estrogenic influences in both female-typical and male-typical reproductive behaviours remains. There may also be different critical periods for the activation or inhibition of female differentiation. Thus it is difficult to construct coherent theories for the development of female sexual preferences in humans. Studies with mice indicate that the action of estrogen receptor genes depends on the gender in which they are expressed. In female mice, disruption of the estrogen receptor leads to loss of lordosis and female sexual behaviour, whereas in male mice it results in reduced male-typical sexual behaviours (Ogawa & Pfaff, 2000). We could speculate that perturbations of the estrogen receptors in human females prevent the development of a female-typical direction of sexual preference. Clinical cases of human females with estrogen receptor disruptions could be used to test this hypothesis, but we are not aware of any such cases. An androgenic pathway to female sexual orientation seems more likely. ''Female androgens'' are secreted by the adrenal glands, in small yet significant quantities, and these have been implicated in some aspects of normal human female sexual behaviour, such as libido (Carter, 1992). Although there is no evidence to our knowledge that lesbians differ in adrenal functioning, these androgens may have masculinising effects on female neurodevelopment if they cannot be aromatised to estradiol (assuming that the presence of successfully aromatised estradiol in the female gender is feminising). Alternatively, they may bind to androgen receptors that are present in the female hypothalamus and masculinise key neural regions determining target preferences. Some direct evidence for an effect of adrenal androgens on female sexual orientation comes from studies of women with congenital adrenal hyperplasia (CAH), a condition in which there is excess secretion of androgens from the adrenal glands (mostly due to 21-hydroxylase deficiency) in utero. The data are inconclusive regarding the performance of CAH girls and women on sex-dimorphic neurocognitive measures. However, there are indications of more male-like lateralisation and elevated non-right handedness in this group as compared to control women (Collaer & Hines, 1995; Kelso, Nicholls, Warne, & Zacharin, 2000). These data fit the notion of over-exposure to androgens promoting non-right handedness and homosexuality in women, following Geschwind and Galaburda's model. Regarding childhood play behaviour and sexual orientation, the data are more consistent. Women with CAH report elevated masculine childhood play behaviour and higher rates of bisexual or homosexual fantasies and preferences than do controls (Collaer & Hines, 1995; Hines, 2000). CAH females do not seem to differ in pubertal onset from their unexposed sisters (Hines & Shipley, 1984).

Interestingly, women exposed in utero to the synthetic estrogen, diethylstilbestrol (DES) also report elevated bisexual or homosexual preferences (e.g. Meyer-Bahlburg et al., 1995). These data appear to implicate an estrogenic pathway to female homosexuality. This follows the classic

neurohormonal model, which assumes that because estrogens are masculinising in males, then they may be in females. Nonetheless, an estrogenic pathway seems contrary to the molecular evidence for gender-dependent ER effects. DES is a particularly strong estrogenic compound and bypasses the known inactivation mechanisms that protect the female foetal brain from high maternal estrogen levels. Thus, it is possible that alterations in this protective mechanism (alpha-fetoprotein: AFP) predispose the foetus towards vulnerability for masculinisation. Studies with rats suggest that small amounts of estrogens may be feminising, but exposure to larger amounts (due to AFP disruptions) may be masculinising in females (Dohler et al., 1984). Synthetic estrogens are also known to be neurotoxic to the development of the hypothalamus and this may explain their effects on sexual preferences in humans, rather than a masculinising mechanism (Brawer, Beaudet, Desjardins, & Schipper, 1993). Moreover, natural ovarian estradiol is not available to the female foetus during early stages of development (McEwen, 1983). In any case, the elevations of non-heterosexual preferences in the human studies are greater in the CAH group than in DES exposed women, suggesting that an androgen pathway exerts a larger effect on female target preference than an estrogenic pathway. The effects of DES on psychosexual development have also not been replicated in non-human primates using more experimentally controlled procedures (Goy & Deputte, 1996). The conclusions drawn from these studies have been criticised, particularly with respect to small sample and effect sizes. Recent data are also less dramatic in showing elevated rates of non-heterosexual preferences (especially in DES women) than previous studies (Hines, 2000). The majority of CAH or DES exposed females are female-typical in behaviour, interests and cognition.

In summary, mechanisms of neurohormonal action can provide an adequate framework for explaining the neurodevelopment of male sexual orientation and its correlates. In females, androgens are implicated in the development of gender nonconformity, homosexual preferences and non-right handedness. However, these mechanisms in females are poorly understood.

6.2. Maternal immunisation and the H-Y antigen

The theory of maternal immunisation to H-Y antigens has recently emerged as a contender to explain the development of homosexual preferences in men and associated correlates. This theory shifts the emphasis on X-linked genetically determined sexual differentiation of the brain to Y-linked differentiation. Specifically, Blanchard and Boagert (1996b) and Blanchard and Klassen (1997) argue that the high fraternal birth-order effect associated with homosexuality in men is due to the progressive immunisation of some mothers to Y-linked minor histocompatibility (mHC) antigens (H-Y antigen) by each succeeding male foetus. The accumulating H-Y antibodies affect the sexual differentiation of the male foetal brain in a female direction, thus accounting for the feminised neuroanatomical and neurocognitive correlates of male homosexuality. This theory is consistent with a number of observations: the number of older sisters is irrelevant to sexual orientation in later born males; the H-Y antigen is expressed by male foetuses only and thus the maternal immune system "remembers" the number of males carried previously and alters its response accordingly; and H-Y antigens are strongly represented in neural tissue (Blanchard & Klassen, 1997). Recent evidence also suggests that Y-linked male sex determination genes SRY and ZFY are transcribed directly in the male human brain (Mayer, Lahr, Swaab, Pilgrim, & Reisert, 1998).

Although a robust alternative to the prenatal androgen theory, the maternal immunisation hypothesis also has limitations. The purported mechanism is very general in terms of effects on the sexual differentiation of the brain. If H-Y antibodies interfere with the sexual differentiation of the male brain, then the effects would be global given the preponderance of the antigen in all brain tissue. The somatic and neurocognitive profile of homosexuality in men suggests a composite of male-typical and female-typical traits, not complete feminisation (Tables 3 and 4). In fact, as will be described later, aspects of sex-dimorphic mate choice are as male-typical in homosexual males as heterosexual males, implicating the *domain-specific* actions of sexual differentiation of the homosexual brain. The theory is also inconsistent with the hyper-masculine finger length ratios in homosexual males. Moreover, the theory does not adequately explain the association between non-right handedness and male homosexuality. Lalumiere et al. (2000) make the point that any mediation between H-Y antigens and the handedness-sexual orientation association is speculative and confusing at best. In any case, the relationship would be relevant only to male sexual orientation. In fact, the maternal immunisation theory applies to males only, as would be expected given its rationale and the evidence in favour of it. To our knowledge, there is no maternal immunisation mechanism that could explain the masculinised pattern of features observed in homosexual women.

One testable prediction of the hypothesis is that births subsequent to the male homosexual proband, namely younger brothers, should also be homosexual. Recently Green (2000) did not find any departure from a heterosexual orientation in younger brothers of homosexual male transsexuals (who also show high fraternal birth order). These data contradict the prediction and reduce the explanatory power of the theory. Alternatively, these findings could be explained by a differential vulnerability of the foetus to maternal immune responses. In this case, it is conceivable that genetic contributions to homosexuality produce this differential sensitivity, as suggested earlier by the Blanchard and Bogaert (1997) finding of additive effects of older brothers and homosexual brothers in the prediction of bachelorhood in men. Recently Blanchard (2001) has placed his theory within a quantitative context. Blanchard computed that although the *odds* of being homosexual increase by 33% with each older brother, the *probability* that a couple's son will be homosexual rises from 2% (Blanchard's prevalence rate for homosexuality in men with no older brothers) for the first son to 6% for the fifth son. Although this is a threefold increase, the percentage is still small, raising questions about the scope of the birth-order effect. In fact, Blanchard goes further to suggest that only one in seven gay men owe their sexual orientation to the effect.

Birth order should be factored into future investigations of male homosexuality in order to elucidate the role of progressive immunisation on other sex-atypical characteristics, such as cognitive performance.

6.3. Developmental instability

Developmental instability refers to an organism's level of vulnerability to environmental and genetic stresses during development (Lalumiere et al, 2000; Moller & Swaddle, 1997). Measures of this instability provide some insight into the developmental history of the organism. Asymmetry in bilateral features (e.g. digit physiognomy), known as 'fluctuating asymmetry' is often employed in research to this end. Consistent non-right handedness is also said to constitute a measure of

Table 3
Distributions of sex typical, sex atypical and sex typical-magnified features of male homosexuality

Features	Sex typical (male-like)	Sex atypical (female-like)	Sex typical-magnified ("hyper-male")	Source
Core features:				
Target preference		✓		Keating and Over (1990); LeVay (1996)
Childhood sex-typed behaviours and interests		✓		Bailey and Zucker (1995)
Psychological gender		✓		Lippa (2000)
Somatic features:				
Handedness			✓	Lalumiere et al. (2000)
Finger length ratio			✓	Robinson and Manning (2000)
Dermatoglyphics	Insf	Insf	Insf	
OAEs	✓			McFadden and Pasanen (1999)
Pubertal onset		✓		Bogaert and Blanchard (1996)
Height		✓		Bogaert and Blanchard (1996)
Weight		✓		Bogaert and Blanchard (1996)
Genital size			✓	Bogaert and Hershberger (1999)
Neurocognitive:				
Spatial performance		✓		Neave et al. (1999)
Verbal performance		✓		Neave et al. (1999)
Visuo-motor performance		✓		Hall and Kimura (1995)
Cerebral asymmetry		✓		Wegesin (1998c)
Neural correlates:				
INAH-3		✓		LeVay (1991)
SCN		✓		Swaab and Hofman (1990)
AC		✓		Allen and Gorski (1992)
Isthmus		✓		Scamvougeras et al. (1994)
BNST	✓			Kruijver et al. (2000)
Mating preferences:				
Interest in casual sex	✓			Bailey et al. (1994)
Interest in visual sex stimuli	✓			Bailey et al. (1994)
Partner attractiveness	✓			Bailey et al. (1994)
Preference for younger aged partners	✓			Silverthorne and Quinsey (2000)
Sexual versus emotional jealously		✓		Bailey et al. (1994)
Importance of partner status	✓			Bailey et al. (1994)

A tick indicates the feature is either sex typical, sex atypical or sex typical-magnified for homosexual males. Insf = insufficient information; OAEs = otoacoustic emissions; INAH-3 = the third interstitial nucleus of the anterior hypothalamus; SCN = suprachiasmatic nucleus; AC = anterior commissure; BNST = bed nucleus of the stria terminalis. Source refers to the most recent, see text for additional references.

Table 4
Distributions of sex typical, sex atypical and sex typical-magnified features of female homosexuality

Features	Sex typical (female-like)	Sex atypical (male-like)	Sex typical-magnified (''hyper-female'')	Source
Core features:				
Target preference		✓		LeVay (1996)
Childhood sex typed behaviours and interests		✓		Bailey and Zucker (1995)
Psychological gender		✓		Lippa (2000)
Somatic features:				
Handedness		✓		Lalumiere et al. (2000)
Finger length ratio		✓		Williams et al. (2000)
Dermatoglyphics	Insf	Insf	Insf	
OAEs		✓		McFadden and Pasanen (1999)
Pubertal onset	✓			Tenhula and Bailey (1997)
Height		✓		Bogaert (1998a, b)
Weight		✓		Bogaert (1998a, b)
Genital size	Insf	Insf	Insf	
Neurocognitive:				
Spatial performance	✓			Neave et al. (1999)
Verbal performance	✓			Neave et al. (1999)
Visuo-motor performance		✓		Hall and Kimura (1995)
Cerebral asymmetry	✓			Wegesin (1998b)
Neural correlates:				
INAH-3	Insf	Insf	Insf	
SCN	Insf	Insf	Insf	
AC	Insf	Insf	Insf	
Isthmus	Insf	Insf	Insf	
BNST	Insf	Insf	Insf	
Mating preferences:				
Interest in casual sex	✓			Bailey et al. (1994)
Interest in visual sex stimuli		✓		Bailey et al. (1994)
Partner attractiveness	✓			Bailey et al. (1994)
Preference for younger aged partners	✓			Silverthorne and Quinsey (2000)
Sexual versus emotional jealousy	✓			Bailey et al. (1994)
Importance of partner status		✓		Bailey et al. (1994)

A tick indicates the feature is either sex typical, sex atypical or sex typical-magnified for homosexual females. Insf = insufficient information; OAEs = otoacoustic emissions; INAH-3 = the third interstitial nucleus of the anterior hypothalamus; SCN = suprachiasmatic nucleus; AC = anterior commissure; BNST = bed nucleus of the stria terminalis. Source refers to the most recent, see text for additional references.

developmental instability. Lalumiere et al. (2000) have proposed that the patterns of handedness and cerebral laterality (and presumably other sex-atypical features) shown by homosexuals reflect the actions of non-hormonal genetic or environmental insults that shift erotic preferences away from the species-typical pattern of opposite-sex attraction. This generalised mechanism might account for the association between non-right handedness and homosexuality, but to our knowledge there is no evidence for increased developmental instability in homosexuals in measures traditionally used in FA research (e.g. asymmetry of digit lengths, ear width and length, or foot length; Gangestad, Bennett, & Thornhill, 2001). Thus Lalumiere et al.'s hypothesis currently has little support.

One possible environmental insult during neurodevelopment that could determine sex-atypical sexual preferences is *maternal stress*. This longstanding theory suggests that maternal stress affects the intrauterine hormonal milieu of the foetus, resulting in homosexuality (Dorner et al., 1980; Dorner, Schenk, Schmiedel, & Ahrens, 1983). More sophisticated accounts have suggested that stressful maternal experiences may release high levels of stress hormones which interfere with the functioning of the hypothalamic-adrenal-gonadal axis in the foetus. This may cause deviations from normal patterns of sexual differentiation (Ellis & Cole-Harding, 2001). Although some authorities have discounted it (Bailey, Willerman & Parks, 1991; Schmidt & Clement, 1995), Ellis and Cole-Harding (2001) found that mothers of homosexual males reported higher levels of stress during the first and second months of pregnancy in comparison to mothers of heterosexual males. There were no differences relating to stress during any other month of pregnancy, and no differences reported between heterosexual and homosexual females. There were also no differences in maternal reports of alcohol consumption between heterosexuals and homosexuals, but mothers of homosexual females reported higher consumption of nicotine (through cigarette smoking) during the first and second months of pregnancy compared to mothers of heterosexual females. Although, this study comprised a large overall sample (over 7892), the size of homosexual groups was much smaller (332 compared to 7473 heterosexuals). Even when unequal variances are assumed in statistical analysis of such samples, violation of the assumptions of parametric testing (analysis of variance in this study) is probable and results need to be interpreted cautiously. In rat models, although prenatal stressors appear to increase the likelihood of lordosis in males, the full range of female-typical behaviour is not elicited. It is also difficult to de-couple this 'partial behavioural defeminisation' from the associated failure of behavioural masculinisation (copulation and ejaculation) in these rats. Moreover, there are no differences in the hormonal profiles of control versus stressed male rats (Ward, Ward, Denning, Hendricks, & French, 2002).

The general mechanism proposed by the developmental instability account of Lalumiere and colleagues for *both* male and female homosexuality is incongruent with the *variance* suggested by the evidence reviewed so far for different pathways to male versus female homosexuality (Gangestad et al., 2000). Left-handedness does appear to be associated with deleterious consequences (such as low birth weight, shorter life span, serious accidents, neural tube defects) and thus, in a Darwinian view, with reduced fitness (Coren & Halpern, 1991; Yeo & Gangestad, 1993). But while reduced immuno-competence is a possibility, it is equally plausible that left-handers experience more fatal accidents in an environment designed for right-handers (Coren & Halpern, 1991). Note also that Lalumiere et al.'s findings indicated only a small (though reliable) association, and many of the individual studies analysed produced non-significant trends toward non-right handedness in homosexuals compared to heterosexuals. Therefore the argument necessitates

further evidence for indicators of fluctuating asymmetry in homosexuals as compared to heterosexuals. In their investigation of dermatoglyphic characteristics Green and Young (2000) found that heterosexual male-to-female transsexuals (oriented towards women) showed the greatest fluctuating asymmetry compared to homosexual and control groups. Thus, developmental instability may account for disruptions in gender identity but not sexual orientation per se. Recently, two studies have found no associations between fluctuating asymmetry and homosexuality, one examining dermatoglyphic measures (Mustanski, Bailey, & Kaspar, 2002), the other using the second to fourth finger-length ratio (Rahman & Wilson, in press). Of further interest is the fact that gay men are stereotypically considered more attractive than heterosexual men (Innala & Ernulf, 1994), whilst in childhood, independent raters describe gender atypical boys as more attractive than gender-typical boys (Zucker, Wild, Bradley, & Lowry, 1993). Thus, something in the physiognomy of pre-gay children and adults marks them out as more attractive. One possibility is that homosexuals possess *low* fluctuating asymmetry which is known to be associated with greater attractiveness (Rhodes et al., 2000; Thornhill & Gangestad, 1999). Alternatively, it may be simply that femininity is perceived as more beautiful than masculinity.

We certainly do not exclude the possibility that the developmental instability may act through a disruption of hormonal mechanisms. In any case, the use of terminology such as "disruption" is not meant as a value judgement in this context, it could aptly be replaced with "variations". A number of human phenotypes which are adaptive in Darwinian terms may also be due to developmental "disruptions" in hormonal mechanisms, e.g. the female variation in sociosexuality (Bailey, Kirk, Zhu, Dunne, & Martin, 2000; Mikach & Bailey, 1999). This is to be expected given the propensity of natural selection to exploit conserved proximate mechanisms to produce variable adaptive phenotypes. Developmental "errors" are also the likely cause of traits we value in contemporary environments, such as genius, which are not necessarily adaptive in Darwinian terms.

We have shown that the prenatal androgen theory, in contrast to developmental instability, could account for the pattern of non-right handedness in homosexual men and women. It is also necessary to examine whether core gender identity or sexual orientation is associated with additional measures of developmental instability (such as asymmetry in lengths of digits, ears and feet). Overall, the theory is too domain general—it is unclear precisely what developmental mechanisms are disrupted.

7. Psychosocial explanations

As yet, efforts to identify psychosocial factors in the development of sexual orientation have turned up very little. This partly reflects a lack of empirical support for traditional behaviourist and psychodynamic models, which regarded homosexuality as pathology (Gonsiorek & Weinrich, 1991). There is minimal evidence for parental influences on sexual orientation. Bailey, Barbow, Wolfe, and Mikach (1995) reported that over 90% of sons of gay fathers are heterosexual, whilst Golombok and Tasker's (1996) longitudinal study of adults raised in lesbian households reported similar rates. In both studies, amount of time spent living with homosexual parents did not correlate with sexual orientation, thus environmental transmission (in the form of temporally dependent learning influences) is not supported. Although ascertainment bias may have worked

to inflate the rate of homosexuality in these children, the data are slightly elevated over background rates, and perhaps suggest some extraneous genetic influences. In this regard, it is interesting to recall Pattatucci and Hamer's (1995) findings of a 33% rate of non-heterosexuality in daughters of lesbians. In any case, "contagion" theories have little support, and familial factors can be accounted for genetically (Dawood et al., 2000).

Some classic cross-cultural evidence also discounts a role for early same-sexual contacts in childhood and adolescence. The Sambia of New Guinea culturally enforce early homosexual behaviour in boys throughout their entire adolescent period but later these same boys enter into heterosexual marriages (LeVay, 1996). Also, substantial same-sex activity occurs among boys attending British sex-segregated boarding schools, yet this does not seem to increase the likelihood of homosexuality in adulthood (Wellings et al., 1994).

Bem (1996) proposes that alienation from same-sex peers (due to a gender non-conforming childhood temperament: see Bailey et al., 2000a for some support) may promote a homosexual orientation. Alienation leads the child to view same sex peers as "exotic"; later to become eroticised during puberty by a *general arousal mechanism*. This is perhaps the only competing theory that posits psychosocial mechanisms and it has been criticised. Peplau and colleagues (1998) note that it does not explain lesbianism since childhood gender nonconformity by girls is substantially less alienating ("tomboy-ish" behaviour is typically socially acceptable for girls). Having older brothers may increase the feelings of difference from other males (thus accommodating the birth order data) but the theory would also predict a *sororal* birth-order effect for lesbians—they should have more older sisters than heterosexual women. As reported earlier, this is not the case. Interestingly, no postnatal theory has, as yet, adequately explained the fraternal birth-order effect in homosexual men (reviewed in Blanchard, 1997). We would also argue that the notion of non-specific arousal to a perceived exotic or novel partner implies too much plasticity in the neurophysiological circuitry responsible for human sexual preferences. It is unlikely that sexual preferences are due to a general arousal mechanism during development because they are far too important in evolutionary terms to be at the whims of a domain-general and content-independent physiological substrate (Burr, 1995). At the very least, a *domain-specific* mechanism should account for male sexuality, which appears to be more rigid than female sexuality (Baumeister, 2000). Moreover, it does appear that sexual arousal related neural circuitry comprises of components that are independent of other arousal systems (Bancroft, 1999; Everitt, 1990; Howard, 1995; Rosen & Beck, 1988; Stoleru et al., 1999). Overall, Bem's theory has little support.

8. Future directions

There are a number of directions for further inquiry concerning the psychobiological basis of human sexual orientation. Many of the present findings require replication. Of clear importance is the need for large-scale national probability surveys of the prevalence of male and female homosexuality using a range of sensitive measures that include fantasy and attraction ratings. Prior studies have mostly focused on behaviour alone. As well as establishing the distribution of sexual orientation (for example, whether it is truly bimodal or continuous), these studies could examine correlates such as fraternal birth-order at the population level. Moreover, an incorporation of sexual health outcome measures in such surveys may also inform public health policy.

Genetic investigations need to employ larger sample sizes to adequately detect heritability concordances for sexual orientation as a primary trait. Further linkage studies on the putative genetic loci for homosexuality also need to take into consideration robust correlates of homosexuality as these may add substantial noise to analyses, for example fraternal birth-order could be factored in as a covariate or weighting factor. Evolutionary theories also suggest some hypotheses. For example, large-scale surveys could incorporate familial composition measures and examine the reproductive output of siblings of homosexuals compared to heterosexuals. Specifically, the number of offspring produced by brothers of homosexual males may provide a test of McKnight's hypothesis of increased heterozygote fitness for heterosexual males, whilst the number of children produced by their sisters would test a hypothesis based on sexually antagonist selection.

The neuroanatomical findings are especially in need of replication. Researchers in this arena should have as their focus neural substrates that control direction of sexual preference, and those that may have implications for variance in neurocognitive function. For example, as was described earlier, it may be fruitful to investigate sexual orientation related differences in the amygdala. Alternatively, investigators may wish to map the distribution of gonadal steroid receptors in the hypothalamic regions of homosexuals and compare this to heterosexuals. Animal models may be useful in this respect, but studies should aim to employ post-mortem human brains.

The availability of neuroimaging technologies permits a number of hypotheses concerning the brain basis for sexual orientation to be evaluated. Structural magnetic resonance imaging could be used to examine the structure and subdivisions of the corpus callosum of homosexuals to determine whether its morphology is sex-atypical. Functional magnetic resonance imaging (fMRI) experiments employing sex-dimorphic cognitive tasks (e.g. a mental rotations task operationalised in a periodic blocked fMRI design), or, sexual arousal preference measures (presentation of homosexual versus heterosexual sexual stimuli) may identify the neural correlates of sex-typical versus sex-atypical cognitive performance and sexual preferences. The latter is of fundamental value to a neurobiological understanding of sexual orientation, as preferred sexual partner is the defining feature of it.

Further neuropsychological research is needed to examine the extent to which sexual orientation differences are shown in other sex-dimorphic tasks. Examples include spatial navigation and facial emotion processing, these typically elicit moderate to large sex differences (Moffat, Hampson, & Hatzipantelis, 1998). The relationship of these measures to circulating hormones would clarify some neuroendocrinological questions. Ultimately, further neuropsychological research may have practical, mental health implications. For example the cross-sex shift in neurocognitive functioning may account for a similar shift in prevalence of some psychiatric disorders in gay men and lesbians, as compared to their heterosexual peers (e.g. greater depression and anxiety in gay men and heterosexual women; Cochran & Mays, 2000). Just as sex differences in psychopathology may be due to differences in brain function (Frederiske et al., 2000; Reite et al., 1997), so might it be with sexual orientation. This is not to exclude the impact of living in a homophobic society on homosexuals' mental health. A thorough, well-rounded scientific understanding may assist in providing more effective therapy for gay men and lesbians experiencing psychiatric problems (Bailey, 1999).

An example of the fruitful use of homosexuality as a "test case" of more general aspects of human sexuality is illustrated in research which suggests that homosexuals may be more like

same-sex heterosexual peers on certain aspects of mate preferences. For example, gay men seem as interested in casual sexual encounters, visually explicit sexual material, attractiveness and younger age of preferred partner, as do heterosexual men. Lesbians, on the other hand, show the same preferences as heterosexual women—in seeking longer term pair bonds and a low emphasis on partner attractiveness (Bailey et al., 1994; Jancowiak, Hill, & Donovan, 1992; Kenrick, Bryan, Barr, Brown, & Keefe, 1995; Silverthorne & Quinsey, 2000). This helps to constrain the number of developmental hypotheses about sex differences in heterosexual preferences, for example men's preferences for visually explicit material cannot be due to the social subjugation of women as gay men also prefer explicit material which depicts male homosexual encounters (Bailey et al., 1994; Symons, 1979). Also, the male preference for younger aged partners is less likely to be due to cultural emphases on female youth and beauty. This also suggests that the sexual orientation related behavioural differences are constrained to sex atypicality in some domains and not others. These data are in agreement with the growing view that human sexual psychology is modular.

9. Conclusions

Homosexuality seems to come as a ''package'' of traits consisting of mostly sex-typical modules, such as aspects of male and female mating psychology, but also significant sex-atypicality in others, most notably in direction of preferred sex of partner, handedness and sex dimorphic neuropsychological function (see Tables 1 and 3). The differences are likely to be due to genetic exploitation of a conserved mechanism, during vertebrate evolution, for the sexual differentiation of the brain. Same-sex affiliation, as a solution for the adaptive problem of intra-sex fatal aggression and infanticide faced by hominids in ancestral environments, may have fostered the development of homosexuality. Female sexual selection drove X-linked feminising and masculinising alleles to produce more rigid sexual preference in males paired with females. The theories for the neurodevelopment of sexual orientation focus on the sexual differentiation of the brain and propose some kind of ''shunting'' of development down sex-typical or atypical routes, this being consistent with the taxonic nature of sexual orientation. There are hypothalamic clues to preferences for either males or females as partners. The body of evidence for a covariation between correlates of sexual orientation supports the prenatal androgen theory of sexual differentiation of the brain.

The prenatal androgen theory best explains current findings concerning male sexual orientation. There is certainly some evidence for a cross-sex shift towards male typicality in some domains in lesbians, but these are not always parallel to shifts in gay men towards female typicality. Our understanding of a masculinised neurodevelopmental pathway for lesbianism is derived from clinical populations (such as women with CAH). These have only been partially informative. There is a need to establish more rigorously the profile of correlates (somatic, neuroanatomical and neurocognitive) associated with normative female homosexuality. Previous authors have certainly noted that male and female sexual orientations are different, but most have simply hinted at different developmental pathways and said little more. Thus, we have to accept that female sexual orientation is, for the time being, poorly understood. It is plausible that human females evolved more open behaviour programs in ancestral environments, this resulting in greater plasticity in female sexuality and making it difficult to observe linear neurodevelopmental

pathways (Baumeister, 2000). The competing theories of neurodevelopment: maternal immunisation and developmental instability, have their caveats, but nonetheless provide some predictions for future research. Amongst many avenues for future inquiry, the most basic difference between heterosexual and homosexual orientation still requires elucidation, namely the direction of target preference.

From a scientific viewpoint, the causes of homosexuality are irrelevant to whether it should be considered as a psychopathology. Homosexuality does not inherently cause personal distress (other than that due to society's reaction to it) or prevent individuals from being productive and fulfilled members of society (Gonsiorek & Weinrich, 1991). If homosexuality is a collection of male-typical and female-typical traits, then it may even prove beneficial to the individuals who possess it, as it may have done in our evolutionary past.

Acknowledgements

Qazi Rahman is supported by the Medical Research Council of the United Kingdom. This work is also supported by a grant from the British Academy to Dr. Wilson.

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Psychoneuroendocrinology 28 (2003) 288–303

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Sexual orientation and the 2nd to 4th finger length ratio: evidence for organising effects of sex hormones or developmental instability?

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Received 23 November 2001; received in revised form 29 January 2002; accepted 25 February 2002

Abstract

It has been proposed that human sexual orientation is influenced by prenatal sex hormones. Some evidence examining putative somatic markers of prenatal sex hormones supports this assumption. An alternative suggestion has been that homosexuality may be due to general developmental disruptions independent of hormonal effects. This study investigated the ratio of the 2nd to 4th finger digits (the 2D:4D ratio), a measure often ascribed to the organisational actions of prenatal androgens, and the fluctuating asymmetry (FA—a measure of general developmental disruption) of these features, in a sample of 240 healthy, right handed and exclusively heterosexual and homosexual males and females ($N = 60$ per group). Homosexual males and females showed significantly lower 2D:4D ratios in comparison to heterosexuals, but sexual orientation did not relate to any measures of FA. The evidence may suggest that homosexual males and females have been exposed to non-disruptive, but elevated levels of androgens in utero. However, these data also draw attention to difficulties in the interpretation of results when somatic features are employed as biological markers of prenatal hormonal influences.

Keywords: Homosexuality; Sex differences; Finger length ratios; Sex hormones; Organising effects; Fluctuating asymmetry

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0306-4530/03/$ - see front matter
doi:10.1016/S0306-4530(02)00022-7

1. Introduction

While there is no single etiogenic theory of sexual orientation, several hypotheses have been proposed which imply that homosexual males and females have been exposed to different prenatal events in comparison to heterosexual males and females. The dominant hypothesis, by far, has arisen from the theory of neurohormonal sexual differentiation, that is the development of physiological and behavioural differences between the two sexes under the control of sex hormones. Thus, homosexuals are considered to follow sex atypical patterns (in the direction of the opposite sex) of development in neurobehavioural domains comparable to, or as a 'by-product' of, the 'atypical' shift in their partner preferences (Ellis and Ames, 1987). An array of evidence has been provided to suggest that, physically and behaviourally, homosexuals follow gender atypical patterns. However, this evidence is riddled with inconsistencies.

Briefly, in behavioural domains homosexual males and females report gender atypical childhood play interests and adult psychological gender (indexed by traditional measures of masculinity—femininity and occupational interests: Bailey and Zucker, 1995; Lippa and Arad, 1997). In fact, this is one of the few findings that have achieved a high degree of replicability, leading some to argue that these are among the strongest associations between childhood and adult behaviour in developmental and sexual orientation research (Hamer and Copeland, 1994). Amongst more contentious areas of investigation, homosexual males sometimes show a profile of cognitive abilities more typical of heterosexual women (for example, poorer performance on tests of spatial processing and superior verbal skills; McCormick and Witelson, 1991; Sanders and Wright, 1997; Wegesin, 1998). Two further studies failed to replicate these patterns, finding instead that homosexual males did not differ significantly from heterosexual males in sex dimorphic cognitive functions (Tuttle and Pillard, 1991; Gladue and Bailey, 1995). Physically, homosexuals are reported to show opposite sex patterns of height and weight, and homosexual men report earlier pubertal onset compared to heterosexual men (Bogaert and Blanchard, 1996; Bogaert, 1998). These studies rely on archives of the Kinsey Institute for Sex and Reproduction conducted between the 1940s and 1960s rather than current population based estimates.

Already, we are seeing evidence of inconsistency and what some have called the 'file drawer problem' in sex-related research (Maccoby and Jacklin, 1974). That is, the tendency to publish studies using small samples when differences are found, and not to publish when they are null findings or counter to prediction. This has been particularly evident in studies concerning sexual orientation related differences in cognitive function (*N*'s ranging from 13 to 32 subjects per groups). This trend is increasingly being addressed with publication of rigorous and larger investigations reporting no differences (Slabbekoorn et al., 2000). Larger samples are needed to confirm that real effects exist, if indeed they do. A further problem, particularly with sexual orientation research, is volunteer bias, which is the tendency for subjects to self-select to take part in investigations. This can lead to the sample being unrepresentative in many important respects. For example, those homosexuals who volunteer

may be highly visible members of the gay community compared to those who choose not to take part. This problem is endemic to the area, and is compounded because researchers often do not provide exact information on subject recruitment strategies.

Although there may be genetic components to sexual orientation (Hu et al., 1995; Bailey et al., 2000), these may have their influence via hormonal sexual differentiation (LeVay, 1993). In the absence of studies using direct measures of prenatal hormones (e.g. examination of amniotic samples), somatic differences, which are assumed to correlate, although imperfectly, with the real difference in prenatal hormonal exposure between the groups, have been demonstrated. Among the most widely cited reports, Hall and Kimura (1994) examined the directional asymmetry (DA) in number of dermal fingerprint ridges and demonstrated that gay men show a more leftward asymmetry (greater number of ridges on the left fingers) than heterosexual men. Leftward asymmetry was also associated with non-right handedness in gay men but not in heterosexual men. Dermatoglyphic characteristics develop by the 16th week of gestation and it has been suggested they form under the actions of prenatal hormone levels (Jamison et al., 1993); higher salivary testosterone levels are related to higher ridge asymmetry in adult males. Whether these adult levels of sex hormones reflect levels in utero remains unclear. Furthermore, studies of dermatoglyphic characteristics in non-human primates provide inconsistent support for androgenic influences (Meier et al., 1993; Jamison et al., 1994). Since then, one robust study has failed to replicate Hall and Kimura's findings using their original method of analysing fingerprint asymmetry. Slabbekoorn et al. (2000) reported no sex differences and no association between gender identity or sexual orientation and dermatoglyphic pattern. Nonetheless, this study may have confounded sexual orientation with gender identity disturbance because it comprised of a sample of male-to-female and female-to-male transsexuals (who reported homosexual or heterosexual preferences), not non-transsexual homosexuals.

Evidence suggesting prenatal masculinisation in homosexual women has been offered by data on otoacoustic emissions (cochlea-generated sounds or OAEs). OAEs are more numerous in females than in males, especially in the right ear. These patterns of sex and ear differences exist in infants, children and adults. Reports show no difference between homosexual and heterosexual men, but less numerous and weaker OAEs in homosexual and bisexual women (McFadden and Pasanen, 1998; McFadden and Pasanen, 1999). On all measures, homosexual or bisexual women were intermediate between heterosexual men and women. A role for early hormonal influences is suggested by findings that females with male co-twins (who are exposed to more androgens) have OAEs more like males than other females, thus implicating prenatal androgens for masculinised cochleae in homosexual females (McFadden, 1993).

Further suggestions of androgenic influences on female sexual orientation and sexually dimorphic traits come from studies of women with over-exposure to prenatal androgens, due to either congenital adrenal hyperplasia (CAH, due to 21-hydroxylase deficiency) or exposure to synthetic estrogens during pregnancy. In a recent review Hines (2000) concluded that these women are more likely than unaffected sisters to show homosexual or bisexual fantasies and behaviour, gender atypical childhood

play behaviour and gender identity but data on sex dimorphic cognitive differences are inconclusive. Thus, early androgenic influences may contribute to the former domains of behaviour if not necessarily the latter.

Finally, some investigators have examined the ratio of the index finger (2D) to the 4th digit (4D) as a marker of prenatal androgenic influences. The ratio of the 2nd to 4th finger digit (the 2D:4D ratio) is sexually dimorphic, with males showing reduced ratio and females greater. This difference appears to be established as early as 2 years, and is yet another that is said to be associated with adult levels of testosterone in men (Manning et al., 1998, 2000a). In an early study of the behavioural significance of this ratio, Wilson (1983) found that women with male-typical finger length patterns described themselves as more competitive and assertive than women with a female-typical ratio. Recently, Williams et al. (2000) reported that the right-hand 2D:4D ratio in homosexual women was significantly masculine (lower) compared to heterosexual women and no different from heterosexual men. Williams et al. reported no differences between homosexual and heterosexual men. However, segregating men by birth order showed that only homosexual men with later fraternal birth order (two or more older brothers) had a more masculine right-hand 2D:4D ratio than homosexual men with one or no older brothers. On the basis on this birth order effect, the authors concluded that men with older brothers, including those who may become homosexual, are exposed to higher prenatal testosterone levels. Robinson and Manning (2000) reported that male homosexuals showed the masculinised 2D:4D ratio as compared to heterosexuals but, in contrast to Williams et al., found this was independent of birth order. Robinson and Manning (2000) did not have comparative female groups in their study. Additionally, Brown et al. (2001) demonstrated that the right-hand 2D:4D ratio was smaller in CAH women than in control women, the difference for left-hand ratios being non-significant, implying that prenatal androgenic exposure may reduce the 2D:4D in humans (although postnatal exposure cannot be excluded as an alternative explanation). At best, the results overall are inconsistent and the patterns for homosexual women (poorly sampled in sexual orientation studies; Bancroft, 1997) have not been replicated.

If the finger length ratio and OAE data are to be taken to imply androgenic influences, they suggest, counter-intuitively, that these influences have quadratic effects on male sexual orientation. That is, an over-exposure to androgens (producing an apparent ‘hyper-masculinisation’ in androgen sensitive features) above a norm cause shifts in female-typical directions in sexual preferences and other sex dependent behaviours, e.g. cognitive abilities. The pathway for females is more linear: increased prenatal androgens contribute to male-typical sexual preferences, i.e. choosing females as mates (McFadden and Champlin, 2000; Rahman and Wilson, unpublished manuscript).

Competing, though less prominent, theories regarding the development of sexual orientation are maternal immunisation and developmental instability. The former is based on evidence that homosexual men have a later birth order and an excess of older brothers (Blanchard, 1997). There are no differences in birth order or sibling sex composition between homosexual and heterosexual women (Bogaert, 1997). Blanchard and Bogaert (1996) argue that the ‘fraternal birth order effect’ in homosex-

ual men is due to the progressive immunisation of some mothers to Y-linked minor histocompatibility (mHC) antigens (H-Y antigen) by each succeeding male foetus. The accumulating H-Y antibodies affect the sexual differentiation of the male foetal brain, in a feminising direction thus accounting for any feminised neuroanatomical and neurocognitive features in homosexual men. This theory is consistent with a number of observations: the number of older sisters to sexual orientation in later born males is irrelevant and the H-Y antigen is expressed by male foetuses only. Thus the maternal immune system 'remembers' the number of males carried previously and alters its response accordingly (Blanchard and Klassen, 1997). However, the theory is silent on homosexual women. Moreover, it predicts that male births subsequent to the homosexual proband should also be homosexual: recently, Green (2000) found no evidence of this, contradicting the theory.

The concept of developmental instability has recently been applied to sexual orientation. It has been suggested that homosexuality might be due to general 'developmental disruption', which produces shifts in the ontogenetic trajectory of sexual preference from the species-typical preference of heterosexuality, rather than to variations in prenatal hormones (Lalumiere et al., 2000). Although the theory is appealing from a broad evolutionary and developmental viewpoint (for example, organisms with high genetic quality may be able to withstand high levels of exogenous stress; Moller, 1998), it is based solely on the observation of elevated non-right handedness in homosexuals compared to heterosexuals (Lalumiere et al., 2000) and lacks any direct quantitative evidence of the traditionally used measure of 'fluctuating asymmetry' (FA is the unsigned deviation from symmetry in bilateral traits; Gangestad et al., 2001).

The aim of the current study was to shed light on the competing and inconsistent neurodevelopmental accounts of sexual orientation (which all posit prenatal, in most cases hormonal, influences) by examining measurements of the 2nd to 4th finger lengths. These afford a measure of prenatal androgenic influences and, additionally, subtracting the right from the left finger lengths provides a measure of FA (FA being a quantitative measure of general developmental disruption). Based on the extant literature, albeit inconsistent, we predicted that homosexual males and females would demonstrate reduced 2D:4D ratios compared to heterosexual males and females. Homosexuals were also predicted to show higher FA than heterosexuals overall. We also wanted to examine whether these measures were associated with a further individual difference within homosexuality—erotic role identification or the 'butch versus femme' distinction. Prior reports demonstrated higher levels of salivary testosterone and elevated waist-to-hip ratio (indicative of elevated T) in butch identified lesbians compared to femme lesbians—who in turn were more masculinised than heterosexual women (Singh et al., 1999). It is possible that the pattern of 2D:4D ratios could distinguish such graded effects. Extending this to include homosexual males, we expect that higher 'butch' ratings may be associated with lower (more masculinised) 2D:4D ratios.

2. Method

2.1. Subjects

We recruited 240 healthy subjects (aged between 18 and 40 years and screened to ensure no history of head injury, psychiatric or neurological illness, psychoactive medication or drug use); 60 heterosexual males, 60 homosexual males, 60 heterosexual females, and 60 homosexual females. Heterosexual subjects were recruited from university sources, local community (through newspaper advertisements) and social networks. Homosexual subjects were recruited from university gay and lesbian organisations, local community, gay/lesbian press, and social networks. Recruitment advertisements for both heterosexual and homosexual subjects stated that volunteers were required to take part in a study of 'gender, sexuality, individual differences and cognition' and that they would be remunerated for their time. All subjects came from within the London, Greater London and Southeast regions of England. The possibility of some volunteer biases operating in the present sample cannot be excluded. For example, it is likely that our homosexual sample reflects those who are more open about their sexual orientation compared to one which could be acquired by probability sampling (typically requiring very large samples because of the minority prevalence of homosexuality (2–4%) and thus not feasible in the present study). One strength over previous studies is that we did not recruit homosexual participants from gay street fairs; heterosexual attendees may not have finger length ratios representative of 2D:4D norms.

Sexual orientation was assessed using a modified Kinsey scale, derived from Coleman (1987). This involved responding to a question about self-identification, sexual/romantic attraction, sexual/romantic fantasies and sexual behaviour on a seven-point scale (ranging from 0='exclusively heterosexual' to 6='exclusively homosexual'). Those scoring 5 or 6 were classified as homosexual, those scoring 0 and 1 classified as heterosexual. Subjects with intermediate (bisexual) scores were not included in the study. Demographic information was acquired regarding age, height (in centimetres), weight (in kilograms), ethnicity (White, Black, South Asian, East Asian, Hispanic or Other), socio-economic status (SES) (classified according to the Standard Occupational Classification (SOC) of the Office of Population Censuses and Surveys; HMSO, 1991) and number and sex of siblings.

2.2. Measures

2.2.1. Finger length measurements

Electro-static photocopies of subject's left and right hands were made. Subjects were asked to lay each hand (in turn) on a sheet of acetate placed on the copier, fingers fully extended. They were instructed to place minimal pressure whilst doing this. The lengths of the 2nd and 4th digits were measured on the ventral (palm) surface of the hand, from the basal crease of the digit to the tip of the digit. Where there was a band of creases at the base of the digit (most common with the 4th digit), the most proximal of these creases was measured. This was done twice on

both hands by Q.R. Digital callipers measuring 0.01 mm were used for all measurements. Finger digit measures have a high level of repeatability (Manning, 1995). Our measures also demonstrated a high level of repeatability (Cronbach alpha=0.99). We averaged the finger length measurements and then calculated the ratios by dividing the length of the 2nd digit by that of the 4th (2D:4D) for the right and left hands separately. Additionally, the right 2D:4D ratio was subtracted from the left ratio to provide a measure of DA. Manning et al. (2000b) and Manning (2002) have argued that low 2D:4D in the right hand and high 2D:4D on the left hand (indicated by high DA) may be another predictor of increased androgen levels in utero. Fluctuating asymmetry was calculated by subtracting the right from the left finger lengths.

Theoretically, the signed FA's of the 2nd and 4th digits should be normally distributed around a parametric mean of zero. We tested for this using one-sample *t*-test (mean set at zero) and found no evidence of deviation in their distributions (2nd digit: $t = -1.734$, df = 239, $P = 0.084$; 4th digit: $t = -1.073$, df = 239, $P = 0.285$). Thus our traits show 'ideal FA' comparable to published norms (e.g. Trivers et al., 1999). The signs from these FA's were removed and each distribution was standardised. These two scores were summed to obtain a composite FA score for each subject. Composite FA scores reflect underlying developmental instability more accurately in comparison to single traits which may only show weak associations (Manning and Ockenden, 1994; Gangestad et al., 2001). Three measures were used in the analysis: FA of the 2nd finger, FA of the 4th finger and Composite FA.

2.2.2. Handedness

This was assessed using the Edinburgh Handedness Inventory (EHI, Oldfield, 1971). The EHI requires subjects to demonstrate 10 unimanual tasks and to state the degree of preference for the hand used as either strong (2 points) or weak (1 point). Assessments were completed once only for each subject and a handedness laterality quotient was calculated by subtracting the score for the left hand from the score for the right hand, dividing by the sum of both, and multiplying by 100. For purposes of the present study (a larger neurocognitive investigation in which right-handedness was an inclusion criteria), only right-handed subjects (those scoring +31 to +100) were included. Subjects scoring +30 and below were excluded from the study. It should be noted that any conclusions from the present investigation apply only to right-handers.

2.2.3. Erotic role identification

Only homosexual subjects completed this measure. They were asked to respond to two statements: "I think of myself primarily as butch or active" and "I think of myself primarily as femme or passive" on an 11-point scale from 0 (*definitely not true*) to 10 (*definitely true*). Subjects were assigned either to the butch group if they rated themselves higher on the butch than femme scale, the femme group if they rated themselves higher on the femme than butch scale or 'non-classified' if they scored exactly the same on both items. Previous studies demonstrated a strong negative correlation (about $r = 0.7$) between the two scales indicating that they are measuring two ends of the same dimension (Singh et al., 1999). We similarly found a

significant negative correlation (for gay men: $r = -0.591$, $P = 0.000$, for lesbians: $r = -0.348$, $P = 0.000$). Thus, a single index was computed by subtracting the femme rating from the butch one. This new measure (degree of 'butchness') ranged from −10 (strongly femme identified) to +10 (strongly butch identified). On the butchness scale, gay men assigned 'butch' had a mean of 4.46 (SD = 2.34) and those assigned 'femme' had a mean of −3.86 (SD = 1.72). Lesbians assigned butch had a mean of 3.30 (SD = 2.30) and those assigned femme had a mean of −5.15 (SD = 2.70). For both sexes, those assigned non-classified had a mean of 0.

2.2.4. Procedure

Subjects completed the demographic and erotic role identification questionnaires as part of a larger investigation concerning the neurocognitive correlates of sexual preference. Subjects were then taken to a room to acquire the photocopies of their hands. They were remunerated for their time. The Ethical (Research) Committee of the Institute of Psychiatry and Maudsley Hospital, London approved all procedures.

2.2.5. Statistical analysis

To determine the whether the data were normally distributed, histograms were computed for each variable. SES and ethnicity were analysed by χ^2. Group differences in age, height, weight, number and sex of siblings and handedness scores were analysed by the General Linear Model (GLM) factorial (gender by sexual orientation) ANOVA using Statistical Package for Social Sciences (SPSS) Version 8.0. Group differences in all finger length measurements were examined using GLM factorial ANCOVA with covariates. The strength of these associations were examined by calculating η^2 (the effect size for ANOVA) where 0.01 is a small effect, 0.05 a medium effect and 0.1 a large effect by standard criteria (Cohen, 1988). Pearson correlations were used to examine associations between sibling sex composition, erotic role identification and all finger length measures. Significance was set at $P < 0.05$.

3. Results

3.1. Subject characteristics

Two-way ANOVA (gender by sexual orientation) revealed no group differences in handedness scores or sibling sex composition (all Ps > 0.10). However, there were main effects of gender ($F = 13.460$, df = 1,239, $P = 0.000$) and sexual orientation ($F = 10.722$, df = 1,239, $P = 0.001$) on age, and main effects of gender on height ($F = 175.210$, df = 1,239, $P = 0.000$) and weight ($F = 42.121$, df = 1,239, $P = 0.000$) (Table 1). There was no significant effect of sexual orientation on height and weight (all Ps > 0.10), contrary to two earlier reports (Bogaert and Blanchard, 1996; Bogaert, 1998). These variables were entered as covariates in subsequent analyses. There were no group differences in ethnicity (collapsed into White

Table 1
Means (SD) for subject characteristics

Group	Age (years)	Handedness (EHI scores)	Height (cm)	Weight (kg)	Number of older brothers	Number of younger brothers	Number of older sisters	Number of younger sisters
Heterosexual males	29.91 (6.60)	82.67 (19.42)	177.89 (6.17)	72.14 (10.41)	0.48 (0.79)	0.51 (0.99)	0.53 (0.85)	0.43 (0.72)
Heterosexual females	32.08 (5.66)	83.02 (20.19)	166.12 (7.22)	61.78 (12.94)	0.65 (1.05)	0.33 (0.60)	0.45 (1.04)	0.48 (0.65)
Homosexual males	26.80 (5.87)	87.33 (16.96)	179.13 (6.95)	75.18 (10.91)	0.58 (0.74)	0.45 (0.67)	0.85 (1.32)	0.36 (0.78)
Homosexual females	29.61 (5.35)	85.31 (18.46)	167.22 (7.30)	64.61 (15.00)	0.63 (0.97)	0.45 (0.72)	0.68 (1.09)	0.40 (0.61)

versus Non-white as there were too few cases in the latter's sub-categories: $\chi^2 = 3.127$, df = 3, $P = 0.373$) or SES ($\chi^2 = 21.430$, df = 15, $p = 0.124$).

3.2. The 2nd to 4th finger length ratios (2D:4D ratios)

For right-hand ratios, there was a significant effect of sexual orientation ($F = 24.237$, df = 1,239 $P = 0.000$); homosexuals having lower right-hand 2D:4D ratios than heterosexuals (Table 2). There were no significant effects of gender ($F = 0.115$, df = 1,239, $P = 0.735$), no significant interaction ($F = 1.684$, df = 1,239, $P = 0.196$) and no significant effects of the covariates (all Ps > 0.10). Overall, the difference between homosexuals and heterosexuals constituted a moderate to large effect ($\eta^2 = 0.09$). Although some statistical protocols preclude the further examination of non-significant interactions following ANOVA, planned comparisons were made between heterosexual males and females, heterosexual and homosexual males, and between heterosexual and homosexual females. Bonferroni adjusted comparisons revealed no significant differences in right-hand 2D:4D ratios between heterosexual males and females ($t = -1.314$, df = 118, $P = 0.191$), but significant differences between heterosexual and homosexual males ($t = 2.629$, df = 118, $P = 0.010$) and heterosexual and homosexual females ($t = 5.319$, df = 118, $P = 0.000$).

For left-hand ratios, only a significant main effect of sexual orientation emerged ($F = 5.436$, df = 1,239, $P = 0.021$); homosexuals showing lower left-hand 2D:4D ratios compared with heterosexuals. There were no significant gender ($F = 0.068$, df = 1,239, $P = 0.795$) and no significant interaction effects ($F = 2.462$, df = 1,239, $P = 0.118$). Covariates were not significant in this model either (all Ps > 0.10). Planned comparisons showed that heterosexual males and females did not differ significantly in left-hand ratios ($t = -2.014$, df = 118, $P = 0.046$), neither did heterosexual and homosexual males ($t = 0.888$, df = 118, $P = 0.376$) but there were significant differences between heterosexual and homosexual females ($t = 3.144$, df = 118, $P = 0.002$). The overall difference between homosexuals and heterosexuals in left-hand ratios was small ($\eta^2 = 0.02$), indicating that the right-hand 2D:4D ratio may be more closely related to sexual orientation than the left-hand ratio.

A factorial MANCOVA (Wilk's F) confirmed the greater difference between homosexuals and heterosexuals on the right 2D:4D ratio ($F = 24.737$, df = 6,232, $P = 0.000$; $\eta^2 = 0.09$) compared with the left-hand ratio ($F = 5.436$, df = 6,232, $P = 0.021$; $\eta^2 = 0.02$). All other effects were not significant. Judging by the alpha values for planned comparisons, it appears that the low 2D:4D of homosexual females may be driving down the overall ratios for homosexuals, producing a main effect of sexual orientation. Analysis of DA scores revealed a main effect of sexual orientation only ($F = 10.439$, df = 1,239, $P = 0.001$), demonstrating that homosexuals have higher DA scores than heterosexuals.

3.3. Fluctuating asymmetry

For FA of the 2nd finger lengths, there were no main effects of gender ($F = 0.010$, df = 1,239, $P = 0.921$), sexual orientation ($F = 0.356$, df = 1,239, $P =$

Table 2
Unadjusted (SD) and adjusted means for finger length measurements by group

Group	Right-hand 2D:4D ratio		Left-hand 2D:4D ratio		DA		FA for the 2nd finger digit		FA for 4th finger digit		Composite FA	
	Un adjusted	Adjusted	Un adjusted	Adjusted	Un adjusted	Adjusted	Un adjusted	Adjusted	Un adjusted	Adjusted	Un adjusted	Adjusted
Hetero sexual males	0.97 (0.03)	0.97	0.96 (0.03)	0.97	−8.08 (2.36)	−6.27	1.12 (0.92)	1.13	1.28 (0.90)	1.23	1.69 (1.23)	5.28
Hetero sexual females	0.98 (0.02)	0.98	0.98 (0.02)	0.97	−2.47 (2.05)	−5.03	1.23 (0.85)	1.21	1.05 (0.96)	1.13	1.71 (1.16)	3.78
Homo sexual males	0.96 (0.03)	0.96	0.96 (0.03)	0.96	2.65 (2.29)	5.02	1.11 (0.97)	1.13	1.26 (0.87)	1.19	−3.46 (1.23)	−2.38
Homo sexual females	0.95 (0.02)	0.95	0.96 (0.03)	0.96	5.05 (2.35)	3.19	1.09 (0.77)	1.08	1.27 (0.83)	1.31	−2.56 (0.96)	−1.47

Adjusted for covariates age, height and weight.

0.551) and no interaction ($F = 0.327$, df = 1,239, $P = 0.568$), and similarly no effects for FA of the 4th finger length (gender: $F = 0.001$, df = 1,239, $P = 0.970$; sexual orientation: $F = 0.354$, df = 1,239, $P = 0.553$; interaction: $F = 0.951$, df = 1,239, $P = 0.330$). Analysis of composite FA scores also revealed no significant main effects of gender ($F = 0.010$, df = 1,239, $P = 0.919$), sexual orientation ($F = 0.069$, df = 1,239, $P = 0.793$) and no interaction ($F = 0.006$, df = 1,239, $P = 0.938$). Covariates were not significant in any model of FA (all Ps $>$ 0.10). It would appear that right-handed homosexual males and females show no perturbations in FA compared to heterosexual males and females.

3.4. Sibling sex composition and erotic role identification

There were no significant associations between number of older brothers, number of younger brothers, number of older sisters, number of younger sisters and any finger length measure (all Ps > 0.05). There were also no significant associations between degree of butchness and any finger length measure within the homosexual sample (all Ps > 0.05).

4. Discussion

The present results confirm two studies showing that homosexual males and females have lower 2D:4D ratios than heterosexuals, indicating they may have been exposed to elevated levels of prenatal androgens (Robinson and Manning, 2000; Williams et al., 2000). They also confirm Williams et al.'s (2000) finding that right-hand ratios may be more sensitive to sexual orientation related influences than left-hand ratios. The additional finding of higher DA values in homosexuals provides some support for Manning's (2002) assertion that low right-hand 2D:4D together with high left-hand 2D:4D may correlate with sex-related domains. In contrast to Williams et al., we confirmed Robinson and Manning's finding that differences in 2D:4D between homosexual and heterosexual men were not associated with sibling sex composition. However, this finding needs to be viewed in the context that we did not replicate the often reported observation that homosexual men have an excess of older brothers in our sample (although there is a trend in expected direction, see Table 2). We cannot, therefore, exclude the confounding effects of sibling sex composition on finger length ratios where these are significant between homosexual and heterosexual men. Additionally, we found no association between erotic role identification and 2D:4D. It has been suggested that differences in a number of sex dependent measures between butch and femme lesbians (including higher salivary testosterone in the former) may be due to variations in utero (Singh et al., 1999). Our data suggest that caution be exercised when assuming that adult differences in prenatal hormones, associated with a behavioural parameter, reflect prenatal variations.

A surprising element of our results is the failure of the sex difference in 2D:4D

ratio to reach significance (although it was in the expected direction). It is often argued that 2D:4D is an indicator of prenatal hormonal effects but no studies have taken direct measures of prenatal hormones (such as amniotic samples) and compared these with finger length ratios across the life-span (which would be a definitive test of the purported association). Others have found it to be sexually dimorphic, males showing lower ratios than females (Manning et al., 1998, 2000a). Studies of foetal material suggest relative finger length is established in utero by week 14 (Garn et al., 1975), and there is cross-sectional evidence that the 2D:4D ratio is fixed at least as early as 2 years and shows no substantial change thereafter (Phelps, 1952; Manning et al., 1998). Indeed, the 2D:4D ratio may be fixed before birth, perhaps as early as the 14th week of gestation. Serum levels of testosterone show negative associations with 2D:4D in men (Manning et al., 1998). They have argued that if 2D:4D is fixed prenatally it is likely that these correlation's reflect in utero relationships between 2D:4D and sex steroids. Support for this comes from the following observations: (a) the waist/hip ratio of mothers, a positive correlate of testosterone and a negative correlate of estradiol, is negatively related to the 2D:4D ratio of their female and male children (Manning et al., 1999), and (b) males and female with CAH (being exposed to high levels of androgens in utero) have low values of 2D:4D compared to controls (Brown et al., 2001). Finally, Manning et al. (1998) has pointed out that the ontogeny of the digits and gonads are influenced by the same set of *Homeobox* or *Hox* genes (Kondo et al., 1997). Patterns of development of the former may therefore reflect the function of the latter. Given all this evidence for the involvement of sex hormones in the 2D:4D ratio it is perplexing that we found no significant sex difference.

We cannot exclude the possibility that our sexual orientation differences reflect sources of prenatal influence other than sex steroids. For example, the lower 2D:4D ratios of homosexuals may be no more than minor physical abnormalities associated with ontogenetic shifts from the dominant pattern of heterosexual preference. However, the present study did not support the notion that homosexuality might be influenced by general developmental disruption (Lalumiere et al., 2000). Ours is the first study to examine a quantitative measure of FA in homosexual populations and suggests that at least one source of developmental instability does not contribute to homosexuality; rather it is 'normal' variations in prenatal androgens (from the 2D:4D results) that may do so. If further research confirms this, assuming that developmental *stability* may be associated with better genomic quality (Moller, 1998), we may be forced to the conclusion that homosexual individuals have robust genetic quality and are able to withstand developmental insults. Alternatively, our sexual orientation differences could reflect developmental perturbations that are in fact generated by androgens themselves.

The restriction of the present sample to right-handed individuals may have biased the findings against revealing evidence supporting a developmental error hypothesis of homosexuality. Left handedness is a putative indicator of developmental instability and homosexual males and females are slightly more likely to be left-handed than heterosexual males and females (Lalumiere et al., 2000). Therefore, it is possible that homosexuals showing evidence of high FA were excluded by our methodological

restriction. Alternatively, Manning et al., (2000b) demonstrated that left-hand usage was associated with lower 2D:4D ratios; thus excluding non-right handers from this study may have increased the mean 2D:4D in the homosexual sample, in which case the difference reported here would be conservative. The data do not shed light on these competing possibilities; further work is needed.

We conclude that sexual orientation related differences in our chosen somatic measure of prenatal influences—the 2nd and 4th finger lengths, may reflect non-disruptive variations in prenatal sex hormones. However, with Slabbekoorn et al., we favour caution because the differences are small and not easily detectable. Although our sample was large in comparison to other factorial studies of sexual orientation, it may not have been sufficient to detect smaller effects. In addition, our failure to find an overall sex difference in 2D:4D ratios makes interpretation of the sexual orientation differences more difficult. The growing body of conflicting findings from this area of research concerning somatic markers of prenatal hormones, which spans a period of over 50 years, suggests that future researchers should begin examining prenatal hormones per se, in utero, rather than rely on measures that are far from perfect correlates of the real underlying difference: prenatal hormonal influences on the developing central nervous system.

Acknowledgements

This work is supported by grants from the Medical Research Council of the United Kingdom (QR) and the British Academy (GDW).

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Psychoneuroendocrinology (2004) 29, 867–881

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Biosocial factors, sexual orientation and neurocognitive functioning

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Received 21 March 2003; received in revised form 6 June 2003; accepted 24 July 2003

KEYWORDS
Prenatal sex hormones; Sexual orientation; Sex differences; Cognition; Sibling sex composition; Finger length ratio

Summary It has been proposed that sexual orientation related differences in cognitive performance are either due to the actions of prenatal factors early in development or the influence of gender role learning. This study examined the performance of 240 healthy, right-handed heterosexual and homosexual males and females ($N = 60$ per group) on a battery of cognitive tasks comprising mental rotation, judgement of line orientation (JLO), verbal fluency, perceptual speed and object location memory. Measures were also taken of the psychological gender, birth order, sibling sex composition and the 2nd to 4th finger length ratios of the right and left hands. A series of stepwise regression analyses revealed that sex and sexual orientation were the strongest predictors of cognitive performance, with IQ also contributing considerable variance. Psychological gender (M/F scores) added a small, but significant, amount of variance to mental rotation and perceptual speed scores in addition to these main factors, but prenatal hormone related indices, such as 2nd to 4th finger ratios, birth order and sibling sex composition added no independent predictive power. These findings are discussed in relation to biosocial influences on cognitive differences between heterosexuals and homosexuals.

1. Introduction

Sex differences in certain cognitive functions are well documented. Males, an average, outperform females in the tests of mental rotation, spatial perception, mathematical problem-solving and spatial navigation, while females do better than males in the tests of phonological and semantic fluency, perceptual speed, and memory for object locations (e.g. Voyer et al., 1995; Herlitz et al., 1997; Astur et al., 1998; Kimura, 1999; Acevedo et al., 2000; Collaer and Nelson, 2002). Often, these differences are task-specific. For example, although males excel at mathematical problem-solving, females do better in tests involving serial computation (Kimura, 1999). In the domain of spatial memory, the two sexes diverge in their performance as a function of the type of process examined; males performing better at navigation and place learning and females at memory for the spatial location of objects (McBurney et al., 1997; Astur et al., 1998).

It is therefore unsurprising that, the issue has been complicated further by the existence of

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0306-4530/$ - see front matter
doi:10.1016/S0306-4530(03)00154-9

cognitive differences between heterosexual and homosexual males and females, these within-sex variations contributing to the often elusive nature of ''normative'' sex differences (Sanders et al., 2002). Several studies have reported female-typical performance by homosexual males on mental rotations, spatial perception (for example on the water level test: Thomas et al., 1973), single item measures of letter and category fluency, verbal and performance IQ scores of the WAIS and on lexical decision-making (Sanders and Ross-Field, 1986, 1987; Gladue et al., 1990; McCormick and Witelson, 1991; Wegesin, 1998). The most replicable difference appears to be on mental rotations (Wegesin, 1998; Neave et al., 1999). However, one study failed to find any sexual orientation related cognitive differences (Gladue and Bailey, 1995). Most of these studies report no differences between heterosexual and homosexual females. In the most recent investigations in this area, using a large sample, homosexual males have been found to show female-typical performance in mental rotation, judgement of line orientation (JLO), phonological and semantic fluency, digit-symbol substitution and object location memory (Rahman and Wilson, 2003a; Rahman et al., 2003a, b; Rahman et al., in press). Rahman et al. (2003b) also reported that homosexual females scored in male-typical directions on phonological and semantic fluency measures. They did not differ from heterosexual females in any other cognitive task. The effect sizes for these sexual orientation related differences were usually modest to large effects by standard definitions (Cohen's $d = 0.5$–1.2), often comparable to the heterosexual sex difference. These findings support the ''sexual orientation model'' which proposes that sexual orientation exerts an influence just as powerful as sex per se on sexually dimorphic cognitive performance (Sanders and Ross-Field, 1987).

Although the existence of sexual orientation related cognitive differences may be robust, it is far from clear as to which factors are formative in these neurocognitive patterns. Empirical debate around the origins of sex differences in neurobehavioural measures polarises between those proposing gender role socialisation factors as important determinants versus those emphasising biological, primarily hormonal, factors.

Males and females differ in both these broad factors, as well as a myriad of other biological and experiential variables. Gender role socialisation theorists propose that internalised stereotypical notions about male and female personality from the social and cultural context, or from parental gender role reinforcement, lead to engagement in sex-differentiated activities and behaviour. These, in turn, are said to reinforce specific psychological mechanisms that ultimately form the basis for specific cognitive functions which themselves show sex differences, usually by adulthood (Caplan and Caplan, 1994). Although many studies show associations between measures of psychological gender, often ascribed to gender role socialisation, and spatial ability, such as mental rotation, the effect of sex is often greater than the effects of psychological gender (e.g. Jamison and Signorella, 1987; Hamilton, 1995; Parameswaran, 1995; Weekes et al., 1995; Saucier et al., 2002).

Males and females also differ in their prenatal exposure to sex steroids (i.e. androgens, estrogens and progestins) and these hormones, probably in concert with other biological and non-biological factors, almost certainly play a role in the development of cognitive and other behavioural sex differences (Collaer and Hines, 1995; Hines, 2000). Experimental manipulations in animals have demonstrated clear sex steroid effects on cognitive functions such as spatial memory (Williams et al., 1990). In humans, exposure to elevated androgens in utero (most obviously in the condition of congenital adrenal hyperplasia in females) appears likely to influence childhood play behaviour, sexual preferences, propensity towards aggression, but the evidence is weaker and less consistent for prenatal hormone effects on sexually dimorphic cognitive functions in these populations (reviewed in Hines, 2000). Studies of the activational effects of serum and saliva levels of sex hormones show either positive, negative, null or curvilinear effects on cognition, i.e., they are inconsistent (e.g. Silverman et al., 1999), whilst exogenously administered hormones, such as those given to transsexuals, lead to an improvement in male-typical spatial functions, such as mental rotation ability, and occasionally in female favouring functions, such as verbal fluency, although this is not consistently demonstrated at follow-up (Van Goozen et al., 1994, 1995; Miles et al., 1998; Slabbekoorn et al., 1999). Studies of menstrual cycle effects show that both estrogens and testosterone modulate sex-dimorphic (but not sex-neutral) cognitive performance and cerebral asymmetries (Hausmann et al., 2000; Maki et al., 2002).

Recently, interest has turned to non-invasive somatic markers often ascribed to the effects of prenatal androgens. One focus of the present investigation is on the 2nd to 4th finger length ratio (2D:4D), often ascribed to prenatal androgen influences. In fact, the evidence for such a link is reasonably strong. The 2D:4D ratio is sexually dimorphic, with men showing reduced ratios and women greater (Manning et al., 1998, 2000). Stu-

dies of foetal material suggest relative finger length is established in utero by week 14 (Garn et al., 1975), and there is cross-sectional evidence that the 2D:4D ratio is fixed at least as early as two years, showing no substantial change thereafter (Phelps, 1952; Manning et al., 1998). Serum levels of testosterone show negative associations with 2D:4D in men (Manning et al., 1998). Manning et al. (1998) have argued that, if 2D:4D is fixed prenatally, it is likely that these correlations reflect in utero relationships between 2D:4D and sex steroids. Support for this comes from the following observations: (a) the waist:hip ratio of mothers, a positive correlate of testosterone and a negative correlate of estradiol, is negatively related to the 2D:4D ratio of their female and male children (Manning et al., 1999) and (b) males and females with congenital adrenal hyperplasia (being exposed to high levels of androgens in utero) have low values of 2D:4D compared to controls (Brown et al., 2001). Finally, Manning et al. (1998) have pointed out that the ontogeny of the digits and gonads is influenced by the same set of *Homeobox* or *Hox* genes (Kondo et al., 1997). Patterns of development of the former may therefore reflect the function of the latter. In relation to cognition, four studies from samples in London, Merseyside (UK) and Hungary report that 2D:4D ratios are negatively associated with mental rotation scores in males (Sanders et al., 2000; Manning and Taylor, 2001). Thus, putatively lower levels of prenatal androgens are associated with better performance in males. Such measures provide a promising avenue for further hormonal explorations of within-sex differences in cognition.

As on date, there has been no investigation of factors that may contribute to sexual orientation effects per se on sex-dimorphic cognitive functioning. However, there are several reasons to suspect that the two domains of influence described above may indeed contribute to such differences. Firstly, homosexual males and females are consistently shown to report sex-atypical psychological gender in traditional masculinity–femininity dimensions, and on scales of occupational interests and activities (see Lippa, 2002, for review). Such differences may be preceded by childhood gender non-conformity, reported retrospectively and prospectively in homosexual males and females (Bailey and Zucker, 1995). Interestingly, two studies reported that boys who showed elevated gender non-conformity demonstrated diminished performance on two spatial tasks (Block Design and Object Assembly) and better verbal performance (on vocabulary and verbal comprehension tests) compared to controls (Finegan et al., 1982; Grimshaw et al., 1991). Secondly, homosexual males and females have been shown to differ in somatic markers of prenatal hormones, particularly the 2D:4D ratio, where homosexual males and females show lower ratios compared to heterosexual males and females (Rahman and Wilson, 2003b; Robinson and Manning, 2000; Williams et al., 2000). Both psychological gender and 2D:4D ratios have been implicated in sexually dimorphic cognitive functions. Further evidence, employing the 2D:4D ratio, also suggests that homosexual males with older brothers may be exposed to higher levels of androgens prenatally (Williams et al., 2000). Thus, it is possible that the number of older brothers, specifically, may be associated with a variety of sexually dimorphic traits, and notably those cognitive differences found to be associated with homosexual orientation. These findings add to a large body of evidence for a later birth order in homosexual males in relation to their brothers (and not their sisters), known as the fraternal birth order effect (see Blanchard, 2001, for review). Blanchard and Klassen (1997) have proposed that the later birth order relative to male siblings shown by homosexual males may be due to progressive maternal immunisation of male-linked H-Y antigens with each male fetus, shifting neural sexual differentiation of successive male fetuses in a feminising direction prenatally. An additional hypothesis proposed by Blanchard et al. (2002) is that fraternal birth order may correlate with visuo-spatial ability among homosexual males or males in general. They argue that lower fraternal birth order may be associated with poorer visuo-spatial performance.

The present report derives from a large investigation of sexual orientation related cognitive differences (Rahman and Wilson, 2003a; Rahman et al., 2003a, b; Rahman et al., in press). This study utilised a series of cognitive measures and acquired data on a range of potential predictor variables. These predictor variables were right-hand 2D:4D ratio, left-hand 2D:4D ratio, psychological gender, birth order, sibling sex composition, age and general intelligence. It therefore provides a novel opportunity to investigate the hypothesis that factors previously shown to be associated with homosexuality in humans, and sex effects in cognition, would also contribute to sexual orientation effects on sexually dimorphic cognitive performance.

2. Method

2.1. Subjects

The sample comprised of 60 heterosexual males, 60 homosexual males, 60 heterosexual females, and 60 homosexual females (between 18 and 40 years of age and screened to exclude any history of head injury, psychoactive medication or drug use). The subjects were asked a general screening question on psychiatric and neurological illness with examples provided. Any subject who stated that they had a history of psychiatric and/or neurological morbidity, or was unsure of this, was not recruited into the study. Heterosexual subjects were recruited from university sources, through newspaper advertisements and social networks. Homosexual subjects were recruited from university gay and lesbian organisations, gay/lesbian press, and social networks. Recruitment advertisements requested volunteers to take part in a study of ''gender, sexuality, individual differences and cognition'' for which remuneration would be given. The complete sample comprised individuals from the London and southeast geographical regions of the UK. Sexual orientation was assessed using a modified Kinsey item (Kinsey et al., 1948). This involved responding to a question about self-identification, sexual/romantic attraction, sexual/romantic fantasies and sexual behaviour on a 7-point scale (ranging from 0 = ''exclusively heterosexual'' to 6 = ''exclusively homosexual''). Those scoring 5 and 6 were classified as gay or lesbian, those scoring 0 and 1 classified as heterosexual (subjects with intermediate scores were not included in the study). Demographic information was acquired regarding age, number of years in full time education since the age of 5, and ethnicity (white, black, South Asian, East Asian, Hispanic or other). The subjects were classified by parental socioeconomic status into the following categories according to the Standard Occupational Classification (Office of Population Census and Surveys, 1991): (1) professional, (2) managerial and technical, (3) skilled—non-manual, (4) skilled—manual, (5) partly skilled and (6) unskilled. Only predominantly right-handed subjects (those scoring $> +31$ on the Edinburgh Handedness Inventory (EHI): Oldfield, 1971) were included.

2.1.1. Pubertal onset

Timing of pubertal onset was measured by a seven-item questionnaire (following Tenhula and Bailey, 1998) asking about age (in years) when significant pubertal events (for females: growth of pubic hair, growth of breasts, age of menstrual onset, and for males: growth of pubic hair, age of first ejaculation, voice change) occurred. The final item asked subjects to rate their perception of the relative timing of these events overall compared to their peers on a 5-point scale (1 = much earlier, more than 2 years; 2 = somewhat earlier, between 6 months and 2 years; 3 = same time, within 6 months; 4 = somewhat later, between 6 months and 2 years; 5 = much later, more than 2 years).

2.1.2. Erotic role identification

Only homosexual subjects completed this measure. They were asked to respond to two statements: ''I think of myself primarily as butch or active'' and ''I think of myself primarily as femme or passive'' on an 11-point scale from 0 (*definitely not true*) to 10 (*definitely true*). There was a significant negative correlation between the two scales (for gay men: $r = -0.591$, $p < 0.001$, for lesbians: $r = -0.348$, $p < 0.001$) indicating that, consistent with previous studies, they measure two ends of much the same dimension (Singh et al., 1999). Thus, a single index was computed by subtracting the femme rating from the butch one. This measure (termed ''degree of butchness'') ranged from -10 (strongly femme identified) to +10 (strongly butch identified).

2.2. Predictors

2.2.1. Psychological gender (M–F)

The subjects completed the 20-item sub-scale (''masculinity–femininity'') of the Eysenck Personality Profiler (EPP) (Eysenck et al., 1996). The subjects could score between 0 and 40 on this scale, as delineated in the EPP manual. High-scorers were classed as ''masculine'' whilst low-scorers were classified as ''feminine''. This scale was used, as it is UK based, with recent norms (Eysenck et al., 1996). It comprises items that, empirically, show maximum separation between typical men and women, ranging from concern about crawling insects, to tolerance of obscenity, interest in children and clothes, and willingness to express emotion (e.g. by crying publicly).

2.2.2. Finger length ratios

Briefly (see Rahman and Wilson, 2003b), electrostatic photocopies of the subjects' left and right hands were made. The lengths of the second and fourth digits were measured on the ventral (palm) surface of the hand, from the basal crease of the digit to the tip of the digit. Where there was a band of creases at the base of the digit (most common with the fourth digit), the most proximal of these creases was measured (Manning et al.,

1998, 2000). This was done twice on both hands. Digital callipers measuring to 0.01 mm were used for all measurements. Finger digit measures have a high level of repeatability (Manning, 1995). Finger length measurements were then averaged and the ratios calculated by dividing the length of the second digit by that of the fourth (=2D:4D) for the right and left hands separately (Manning et al., 1998, 2000).

2.2.3. Birth order and sibling sex composition

The subjects were asked to list the number of older brothers they had, the number of older sisters, younger brothers and younger sisters. Only biological siblings on the side of the mother were to be listed (that is siblings whom the subject's biological mother gave birth to). The subjects also listed their birth order relative to that of their siblings, and the corresponding sex and age of each sibling in a table. Birth order was calculated for each subject using the established Slater's index formula (Blanchard, 1997). This is computed by dividing the number of siblings older than the proband by older siblings plus younger siblings. The index cannot be computed for only children. For all other individuals, regardless of sibship size, it expresses birth order as a quantity between 0 and 1, where 0 corresponds to first born and 1 corresponds to last born.

2.3. Cognitive measures

2.3.1. Mental rotation

This 20-item test (Vandenburg and Kuse, 1978; adapted from Shepard and Metzler, 1971) required subjects to view a test item (a two-dimensional representation of a three-dimensional cuboid made up of 10 cubes) and then decide whether four other items were the same. The subjects were given 10 min to complete the task. Each test item has two correct and two incorrect choices. For each item, participants received two points if they marked both correct choices and one point if one choice was correct (but the other incorrect). All other responses received a score of 0 for the item. The maximum possible score was thus 40 points. This was the ''total correct'' measure for speed and accuracy. ''Number of trials attempted'' was scored for speed per se. ''Percentage correct'' was finally scored for accuracy per se and was the total number correct, divided by the total number of trials attempted, multiplied by 100 and divided by 2. This task consistently favours men.

2.3.2. Judgement of line orientation (JLO)

This visuo-perceptual test (Benton et al., 1983) consists of 30 items. For each item, the subjects were required to judge which lines in a complex array are in the same spatial orientation as 2 line fragments appearing above the array. Subjects scored 1 point for the two correct choices, and 0 points for any other response. The maximum possible score is thus 30. This task is also male favouring.

2.3.3. Verbal fluency

The subjects completed three measures of verbal fluency: letter fluency, category fluency and synonym fluency. Letter fluency was assessed using the Controlled Oral Word Association test (COWA; Benton and Hamsher, 1978). The subjects were allowed 60 s to generate as many words as possible beginning with a specific letter. The test letters were ''P'', ''R'' and ''W'', and the score was the sum of all acceptable words generated (excluding proper nouns, and repetitions). For category fluency, the subjects were asked to generate as many words as possible belonging to the categories ''animals'' (from the COWA), ''fruit'' and ''vegetables'' (used by Acevedo et al., 2000). The subjects were allowed 60 s for word generation per category. The score was the sum of all correctly produced words (excluding non-category terms and repetitions). For synonym fluency (derived from Hines, 1990), the subjects were presented with six familiar English words (''strong'', ''happy'', ''pretty'', ''sharp'', ''dark'' and ''clear'') and asked to generate as many synonyms for each word (60 s per word) as possible. The score was the sum of all acceptable words (excluding non-synonyms or word associations, and repetitions). Two raters determined whether the answers were correct using a thesaurus and dictionary. All three tests are female favouring.

2.3.4. Perceptual speed

Perceptual speed was evaluated using the digit-symbol subtest of the Wechsler Adult Intelligence Scale Revised (Wechsler, 1981). The subjects were required to fill in as many symbols corresponding to a set of stimulus numbers as possible (by using a key visible throughout the test in which a number and symbol are paired). Ninety seconds were allowed for the whole test. The subjects received 1 point for each correct symbol, the maximum score being 93. The scores were scaled according to standardised instructions from the WAIS-R manual, thus only scaled scores are used in analysis. This test is female favouring.

2.3.5. Object location memory

Object location memory was assessed using Smith and Milner (1981, 1989) spatial memory test. This

is a test of incidental spatial memory in which the subjects are asked to estimate (verbally) the prices of 16 everyday objects arranged in a random order in a 50 cm by 50 cm cardboard array. Following a delay of 30 min (during which the subjects completed the rest of the cognitive battery), the subjects were asked to recall the names of objects, identify the objects among a set of foils, and finally, to place all the objects back in their original positions as best they could remember. The task is scored by calculating the absolute deviation (in millimetres) in the positions of objects at the encoding stage (price estimation) and retrieval stage (place in which objects were subsequently placed). This is a measure of location memory. For the purposes of this report, only the location memory measure is used in further analysis.

2.3.6. General intelligence

General cognitive ability was assessed using Raven's Standard Progressive Matrices test (Raven, 1958). The SPM is a paper and pencil nonverbal test and contains 60 items of increasing difficulty. The subjects are required to select one among six or eight alternatives to complete a matrix pattern. Raw scores were used in the analysis (maximum score 60).

2.4. Perceived stress

Moderate levels of stress can enhance neuropsychological performance, whilst high levels of stress often impede performance. Thus, it is reasonable to ask whether stress levels during task performance (or because of task performance) modulate any sex and sexual orientation related differences. In the present work, the subjects were asked to rate their level of stress (at the end of the testing session) as experienced during completion of the whole battery of tests from 0 indicating ''not at all stressed'' to 10 ''extremely stressed''.

2.5. Procedure

Each subject was tested individually. Firstly, the subjects provided demographic information and completed questionnaire measures. The cognitive battery was then administered, in a randomised fashion across the subjects with the exception of the object location memory task. This was administered in two parts because of the requirement for a 30-min delay. The first stage (price estimation) was administered upon completion of the questionnaires. All other cognitive tasks were then completed during the delay, after which the recall stages of the object location memory task were administered. The subjects then completed Raven's SPM. Finally, the subjects completed the Perceived Stress Scale. Upon completion of the entire battery, the subjects were taken to a photocopy room in order to take electro-static photocopies of their hands. The whole procedure lasted 2 h, and the subjects were remunerated the sum of £20 for their time. The Ethical (Research) Committee of the Institute of Psychiatry and Maudsley Hospital, London, approved all procedures.

2.6. Statistical analysis

To determine whether the data were normally distributed, a series of box plots were computed for each continuous variable. Socio-economic status (SES) and ethnicity were analysed by χ^2. Group differences in age, years in education, handedness scores, pubertal onset measures, psychological gender, birth order, sibling sex composition, finger length ratios, IQ and perceived stress scores were analysed by the General Linear Model (GLM) factorial (gender by sexual orientation) analyses of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 8.0. The remaining cognitive tasks were analysed using analysis of covariance (with age and IQ as covariates) and post-hoc tests to decompose significant interactions.

To examine the contribution of putative predictors of any sexual orientation related cognitive variation, a series of forced entry stepwise multiple regressions were conducted between performance on each cognitive task and the predictor variables. The first predictor was a dummy coded variable comprising the subject groups (homosexual males, homosexual females and heterosexual females, with heterosexual males as the reference group; $K-1$). For all analyses, ''group'' (from hereon called ''sexual orientation'' to refer to both sex and sexual preference group membership) was force entered into the first block of the regression equation, which allows all between-group differences to be attributed to sexual orientation. The remaining predictor variables (age, IQ—Raven's SPM score, psychological gender, number of older brothers, number of older sisters, number of younger brothers, number of younger sisters, birth order, right 2D:4D, and left 2D:4D) were entered into the second block in a stepwise fashion. Reported in Table 3 are the r and r^2 and adjusted r^2, which detail the amount of variance explained cumulatively with the addition of successive predictors and the r^2 *change* which details the variance accounted for with the addition of each individual predictor in subsequent steps. The p-value for the r^2 *change* is also presented. The assumptions of regression were found to have

been met on visual inspection of the residual plots for each regression which confirmed homoscedasticity, whilst inspection of the correlation matrix showed no evidence of multi-collinearity (all rs < 0.8).

A series of partial correlations, controlling for age and IQ, were computed between each cognitive performance measure and variables of secondary interest to this investigation. These variables were: age of first ejaculation and overall self-rating of pubertal onset in relation to same-sex peers (which differed between the male groups only) and degree of butchness (for homosexuals only and split by sex).

3. Results

3.1. Subject characteristics

There were no significant group differences in education, ethnicity, parental socio-economic status or in mean EHI scores (all ps > 0.05). However, there was a significant effect of sex ($F = 13.460$, df $= 1, 239$, $p < 0.001$) and sexual orientation ($F = 10.722$, df $= 1, 239$, $p < 0.001$) on age; males being older than females, and homosexuals being older than heterosexuals.

Pubertal onset was analysed separately for males and females as it varies substantially between the sexes (Johnson and Everitt, 1988) (see Table 1). There were no significant differences between heterosexual and homosexual males in age at first pubic hair growth (all ps > 0.05). However, homosexual males reported significantly earlier age at first ejaculation than heterosexual males ($t = 2.438$, df $= 118$, $p = 0.016$, Cohen's d $= 0.45$) and rated their overall pubertal onset as earlier relative to their peers than did heterosexual males ratings ($t = 4.641$, df $= 118$, $p = 0.000$, Cohen's $d = 0.84$). There were no significant differences between heterosexual and homosexual females in any pubertal onset measure (all ps > 0.05).

3.2. Sexual orientation related cognitive differences

The results of the statistical analyses on sexual orientation related differences in cognitive performance are presented elsewhere (see Rahman and Wilson, 2003a; Rahman et al., 2003a, b; Rahman et al., in press). Suffice to say here that analysis of covariance (controlling for age and IQ) revealed significant sex by sexual orientation interactions for all tasks (all ps < 0.001). Post-hoc tests (corrected for multiple comparisons to $p < 0.01$) revealed that heterosexual males scored higher than heterosexual females and homosexual males on mental rotation and JLO (all ps < 0.001). On letter, category and synonym fluency tests, spatial location memory and digit-symbol substitution, homosexual males scored in female-typical directions (i.e. higher than heterosexual males; ps < 0.001). Homosexual females had a significantly male-typical profile of performance on all three verbal fluency tests (i.e. performed poorer than heterosexual females; ps < 0.001), but did not differ from heterosexual females on any other cognitive measure. See Fig. 1 for presentation of standardised scores (z) for each cognitive task across the groups.

3.3. Perceived stress scores

There were no significant main effects of sex ($F = 3.283$, df $= 1, 239$, $p = 0.071$), sexual orientation ($F = 0.807$, df $= 1, 239$, $p = 0.370$) or their interactions ($F = 3.723$, df $= 1, 239$, $p = 0.06$) in perceived stress scores.

3.4. Sexual orientation related differences in the predictor variables

3.4.1. Psychological gender (M–F)

There was a significant main effect of sex ($F = 35.990$, df $= 1, 239$, $p < 0.001$), with males scoring higher (indicating psychological masculinity) than females, a main effect of sexual orientation ($F = 7.775$, df $= 1, 239$, $p = 0.006$), with homosexuals scoring lower than heterosexuals, and a significant interaction ($F = 25.094$, df $= 1, 239$, $p < 0.001$). Table 2 lists the mean scores per group. Decomposition of this interaction revealed that heterosexual males scored higher than heterosexual females ($t = 8.495$, df $= 118$, $p < 0.001$), homosexual males scored lower than heterosexual males (indicating more gender-atypicality in psychological gender scores) ($t = 5.742$, df $= 118$, $p < 0.001$), and there was no difference between heterosexual and homosexual females ($t = -1.513$, df $= 118$, $p = 0.133$). The effect size for the heterosexual differences was large ($d = 1.55$) as was the difference between heterosexual and homosexual males ($d = 1.04$).

3.4.2. Finger-length ratios

Factorial ANCOVAs were applied to the finger-length ratio data with age, height and weight as covariates (Rahman and Wilson, 2003b). For right-hand ratios, there was a significant main effect of sexual orientation ($F = 24.237$, df $= 1, 239$, $p < 0.001$); homosexuals having lower right-hand

Table 1 Subject characteristics (means and frequencies are presented where appropriate. Standard deviations are presented in parentheses)

	Heterosexual males	Heterosexual females	Homosexual males	Homosexual females
Age (years)	29.91 (6.60)	26.80 (5.87)	32.08 (5.66)	29.61 (5.35)
Years in education	15.96 (3.29)	16.65 (3.29)	16.51 (3.86)	15.95 (3.71)
Handedness (EHI scores)	82.67 (19.42)	87.33 (16.96)	83.02 (20.19)	85.31 (18.46)
Pubertal onset (male)—pubic hair	12.32 (1.64)	N/A	12.06 (1.30)	N/A
Pubertal onset—ejaculation	13.08 (1.63)	N/A	12.41 (1.33)	N/A
Pubertal onset—voice break	13.69 (1.28)	N/A	13.22 (1.45)	N/A
Pubertal onset—overall rating	3.21 (0.80)	N/A	2.55 (0.76)	N/A
Pubertal onset—(female) pubic hair	N/A	11.45 (1.27)	N/A	11.44 (1.51)
Pubertal onset—breasts	N/A	11.55 (1.59)	N/A	11.89 (1.53)
Pubertal onset—first period	N/A	12.32 (1.26)	N/A	12.57 (2.23)
Pubertal onset—overall rating	N/A	2.86 (0.81)	N/A	3.01 (1.04)
Perceived stress	3.55 (2.30)	3.51 (2.15)	2.75 (1.95)	3.80 (2.32)
Degree of ''butchness''	N/A	N/A	1.41 (4.02)	−0.96 (4.48)

N/A = not applicable to that group

2D:4D ratios than heterosexuals. There were no significant sex or interaction effects (all $ps > 0.05$). Overall, the difference between homosexuals and heterosexuals constituted a moderate to large effect ($\eta^2 = 0.09$). For left-hand ratios, a significant main effect of sexual orientation emerged ($F = 5.436$, $df = 1, 239$, $p = 0.021$); homosexuals showing lower left-hand 2D:4D ratios compared with heterosexuals. There were no significant sex or interaction effects (all $ps > 0.05$). The overall difference between homosexuals and heterosexuals in left-hand ratios was small ($\eta^2 = 0.02$), indicating that the right-hand 2D:4D ratio may be more closely related to sexual orientation than the left-hand ratio (see Table 2 for mean ratios).

3.4.3. Birth-order and sibling sex composition

The data are reported here separately for males and females, as birth-order effects have been ascribed to male, but not female sexual orientation in the literature (Blanchard, 2001) (See Table 2). There were no significant differences between heterosexual and homosexual males in

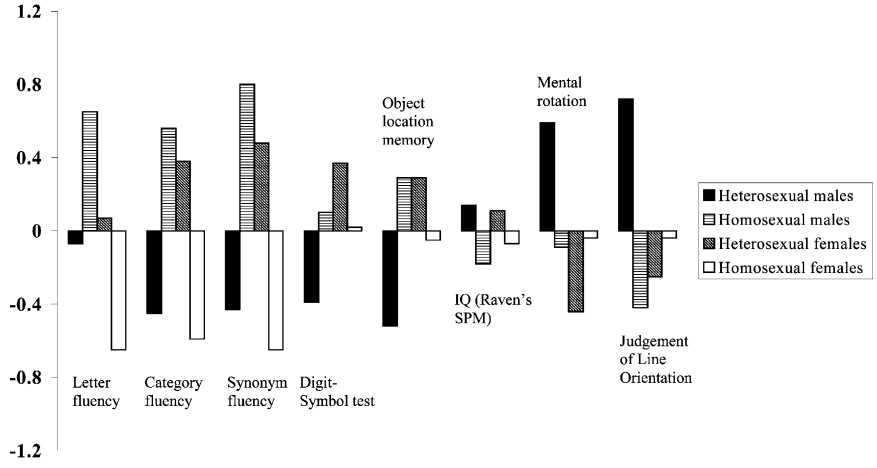

Fig. 1. Standardised (z) cognitive scores across groups. Object location memory scores are reversed.

Table 2 Means (SD) for predictor variables

	Heterosexual males	Heterosexual females	Homosexual males	Homosexual females
Birth order	0.51 (0.45)	0.51 (0.45)	0.65 (0.40)	0.55 (0.46)
Number of older brothers	0.48 (0.79)	0.65 (1.05)	0.58 (0.74)	0.63 (0.97)
Number younger brothers	0.51 (0.99)	0.33 (0.60)	0.45 (0.67)	0.45 (0.72)
Number older sisters	0.53 (0.85)	0.45 (1.04)	0.85 (1.32)	0.68 (1.09)
Number younger sisters	0.43 (0.72)	0.48 (0.65)	0.36 (0.78)	0.40 (0.61)
M–F score	22.26 (4.54)	14.66 (5.22)	16.88 (5.66)	16.20 (5.85)
Right hand 2D:4D ratio	0.97 (0.03)	0.98 (0.02)	0.96 (0.03)	0.95 (0.02)
Left hand 2D:4D ratio	0.96 (0.03)	0.98 (0.02)	0.96 (0.03)	0.96 (0.03)

birth order ($t = -1.640$, df $= 108$, $p = 0.104$), number of older brothers ($t = -0.713$, df $= 118$, $p = 0.477$), number of younger brothers ($t = 0.428$, df $= 118$, $p = 0.669$), number of older sisters ($t = -1.556$, df $= 100.719$, $p = 0.123$) and number of younger sisters ($t = 0.486$, df $= 118$, $p = 0.628$). There were no significant differences between heterosexual and homosexual females in birth order ($t = -0.524$, df $= 105$, $p = 0.601$), number of older brothers ($t = 0.090$, df $= 118$, $p = 0.928$), number of younger brothers ($t = -0.961$, df $= 118$, $p = 0.339$), number of older sisters ($t = -1.191$, df $= 118$, $p = 0.236$) or number of younger sisters ($t = 0.720$, df $= 118$, $p = 0.473$).

3.5. Regression analyses of sexual orientation related cognitive differences

Table 3 provides a summary of each regression model.

3.5.1. Prediction of mental rotation performance

For mental rotation—total correct scores (MR—total correct), sexual orientation was found to explain 12.6% (adjusted $r^2 = 0.114$) of the variance in MR—total correct scores on the first step. The addition of M–F at the second step led to a significant increase in variance explained by 9%. The addition of IQ at the third step led to a significant increase of 2.7%, and the addition of age at the fourth step led to a significant increase of 1.8%. In the final step, the overall amount of variance explained by sexual orientation, M–F, IQ and age was 26.5% (adjusted $r^2 = 0.244$). Although examination of the significance values shows that the addition of each predictor produced a significant overall model, at the final step, there is a clear attenuation in the p-value, suggesting that the addition of age had a small effect on overall prediction. Variables rejected by the regression model were number of older brothers, number of younger brothers, number of older sisters, number of younger sisters, birth order, right 2D:4D and left 2D:4D. In summary, sexual orientation explained most of the variance in MR–total correct scores, with M–F being the second best predictor. There was no contribution of any other predictor.

For mental rotation—percentage scores (MR—percentage), sexual orientation explained 8.3% of the variance in MR—percentage scores (adjusted $r^2 = 0.070$). The addition of IQ led to a significant 11.1% increase in the amount of variance explained while the addition of M–F led to a significant 3.6% increase. In the final step, the overall amount of variance explained by the variables under consideration was 23% (adjusted $r^2 = 0.211$). Variables rejected by the model were age, number of older brothers, number of younger brothers, number of older sisters, number of younger sisters, birth order, right 2D:4D and left 2D:4D. In summary, ''sexual orientation'' and IQ were the strongest predictors of MR—percentage scores.

3.5.2. Prediction of judgement of line orientation (JLO) performance

In Rahman and Wilson (2003a), JLO scores were transformed because they violated the assumption of normality for parametric statistics. Therefore, only transformed scores were used in the present analysis. Sexual orientation accounted for 23.5% of the variance (adjusted $r^2 = 0.224$). The addition of IQ in the second step led to a significant increase in variance explained by 7.7%. The addition of number of older sisters at the third and final step accounted for an extra 1.3%, leading to attenuation in the p-value for the final

Table 3 Summary of each forced entry regression model for each cognitive task

	r^2	Adjusted r^2	r^2 change	df	*p*-value
Mental rotation—total correct					
Step 1: sexual orientation	0.126	0.114	0.126	3, 213	0.000
Step 2: sexual orientation, M–F	0.220	0.206	0.095	1, 212	0.000
Step 3: sexual orientation, M–F and IQ	0.247	0.229	0.027	1, 211	0.007
Step 4: sexual orientation, M–F, IQ and age	0.265	0.244	0.018	1, 210	0.026
Mental rotation—percentage correct					
Step 1: sexual orientation	0.083	0.070	0.083	3, 213	0.000
Step 2: sexual orientation, IQ	0.193	0.178	0.111	1, 212	0.000
Step 3: sexual orientation, IQ and M–F	0.230	0.211	0.036	1, 211	0.002
JLO					
Step 1: sexual orientation	0.235	0.224	0.235	3, 213	0.000
Step 2: sexual orientation, IQ	0.312	0.299	0.077	1, 212	0.000
Step 3: sexual orientation, IQ, number of older sisters	0.325	0.309	0.013	1, 211	0.046
Letter fluency					
Step 1: sexual orientation	0.255	0.245	0.255	3, 213	0.000
Step 2: sexual orientation and age	0.270	0.256	0.015	1, 212	0.038
Category fluency					
Step 1: sexual orientation	0.312	0.303	0.312	3, 213	0.000
Step 2: sexual orientation, age	0.336	0.323	0.023	1, 212	0.007
Step 3: sexual orientation, age and IQ	0.349	0.334	0.013	1, 211	0.040
Synonym fluency					
Step 1: sexual orientation	0.427	0.419	0.427	3, 213	0.000
Step 2: sexual orientation and age	0.440	0.429	0.013	3, 212	0.029
Digit-symbol scores					
Step 1: sexual orientation	0.070	0.057	0.070	3, 213	0.001
Step 2: sexual orientation, IQ	0.167	0.151	0.096	1, 212	0.000
Step 3: sexual orientation, IQ and M–F	0.218	0.199	0.051	1, 211	0.000
Spatial location memory					
Step 1: sexual orientation	0.116	0.104	0.116	3, 213	0.000

model. Predictors rejected by the model were age, M–F, number of older brothers, number of younger brothers, number of younger sisters, birth order, right 2D:4D and left 2D:4D. Thus, overall, sexual orientation explained most of the variance in JLO scores.

3.5.3. Prediction of verbal fluency performance

For letter fluency scores, sexual orientation accounted for 25.5% of the variance (adjusted $r^2 = 0.245$). The addition of age on the second and final step led to a significant 1.5% increase, although examination of Table 3 shows that the *p*-value was attenuated. Variables rejected by the model were M–F, IQ, number of older brothers, number of younger brothers, number of older sisters, number of younger sisters, birth order, right-hand 2D:4D and left-hand 2D:4D. For category fluency scores, sexual orientation explained 31.2% of the variance (adjusted $r^2 = 0.303$). The addition of age on the second step led to an increase in variance explained by 2.3%, and the addition of IQ at the third and final step led to an increase of 1.3% in variance explained. Variables rejected by the model were M–F, number of older brothers, number of younger brothers, number of older sisters, number of younger sisters, birth order, right-hand 2D:4D and left-hand 2D:4D. For synonym fluency scores, sexual orientation accounted for 42.7% of the variance (adjusted $r^2 = 0.419$). The addition of age on the second and final step in this model led to an increase of 1.3% in variance explained. Variables rejected by the model were M–F, IQ, number of older brothers, number of younger brothers, number of older sisters, number of younger sisters, birth order, right-hand 2D:4D and left-hand 2D:4D. Thus, sexual orientation explains by far the greatest amount of variance in verbal fluency scores.

3.5.4. Prediction of perceptual speed

For digit-symbol scaled scores, sexual orientation accounted for 7% of the variance (adjusted $r^2 = 0.057$). The addition of IQ in the second step led to an increase of 9.6% and the addition of M–F in the third and final step led to an increase of

5.1% in the amount of variance explained. Variables rejected by the model were age, number of older brothers, number of younger brothers, number of older sisters, number of younger sisters, birth order, right-hand 2D:4D and left-hand 2D:4D. Thus, in contrast to the previous regression models, all three variables—sexual orientation, IQ and M–F—emerged as the strongest predictors of digit-symbol scaled scores.

3.5.5. Predicting of object location memory

Sexual orientation emerged as the only predictor of variance in spatial location memory scores, explaining 11.6% of the variance (adjusted $r^2 = 0.104$). Variables rejected by the model were age, IQ, M–F, number of older brothers, number of younger brothers, number of older sisters, number of younger sisters, birth order, right-hand 2D:4D and left-hand 2D:4D.

3.6. Associations between cognitive performance and pubertal onset, and ''degree of butchness''

Regarding age of first ejaculation and overall self-rating of pubertal onset, for heterosexual males, there were no significant associations between age of first ejaculation, overall pubertal onset and any cognitive measure (all $ps > 0.05$). Similarly, for homosexual males, there were no significant associations between age of first ejaculation, overall pubertal onset and any cognitive measure (all $ps > 0.05$).

Analysis for ''degree of butchness'' was split between homosexual males and homosexual females. There were no significant associations between ''degree of butchness'' scores and any cognitive measure for both groups (all $ps > 0.05$).

4. Discussion

To aid clarity, the following summary of the findings is provided:

1. Sexual orientation was the strongest predictor of performance on judgement of line orientation, letter fluency, category fluency, synonym fluency and spatial location memory, accounting for between 12 and 42% of the variance in the scores of these tasks.
2. Sexual orientation and psychological gender (M–F) were strong predictors (sexual orientation being the strongest—12% of the variance) of mental rotation–total correct scores, accounting jointly for 22% of the variance.
3. Sexual orientation and IQ were strong predictors (IQ being the strongest—11% of the variance) of mental rotation—percentage correct scores (accounting jointly for 19% of the variance) and digit-symbol scaled scores (IQ the stronger predictor accounting for 9%, and with sexual orientation, for 16% of the variance).
4. There was no contribution of the 2nd to 4th finger length ratios or sibling sex composition and birth order to cognitive performance separated from other factors: IQ, sex, sexual orientation, and psychological gender.
5. There were no associations between pubertal onset measures and cognitive performance for heterosexual and homosexual males. Finally, there were no associations between ''degree of butchness'' and cognitive performance for either homosexual males or females.

The aim of this investigation was to provide new information on the contentious issue of what factors contribute to sexual orientation related differences in sex-dimorphic cognitive performance. The findings presented above, the first of their kind, show little support for any influence of prenatal hormone related factors on sexually dimorphic cognitive performance in addition to IQ, sex and sexual orientation. There was, however, some support for the role of psychological gender in MR–total correct scores. Unsurprisingly, IQ explained some variance in MR—pecentage scores and digit-symbol test scaled scores. However, by far the most powerful predictor of cognitive differences was sexual orientation.

The results presented here do not support the work of others who report associations between somatic markers of prenatal sex hormones and performance on sex-dimorphic cognitive tasks in unselected heterosexual samples, particularly those reporting a negative association between 2D:4D ratios and mental rotation performance (Sanders et al., 2000; Manning and Taylor, 2001; Sanders and Kadam, 2001; Sanders and Waters, 2001). They also do not support the suggestion of Blanchard et al. (2002) that number of older brothers may be associated with variation in sex-dimorphic traits associated with homosexual orientation. Yet, both these traits, that is low 2D:4D ratios and excess of older brothers, are associated with homosexuality (the latter in males only). Thus, these traits, or more precisely, the real difference underlying the variation in these traits (prenatal hormonal influences on the developing brain), should theoretically predict behavioural differences also associated with homosexual orientation. However, it appears that insofar as these factors affect cognitive function-

ing, they do so via the mediation of sex and sexual orientation (Rahman and Wilson, 2003a; Rahman et al., 2003a, b). The influence of sex hormones on cognitive performance, whether they be organisational or activational, is a matter of considerable debate, and findings include positive, negative or null results (Silverman et al., 1999). Many studies have failed to find differences in sex-dimorphic cognitive performance in populations exposed to elevated levels of prenatal androgens, yet report elevation in homosexual or bisexual preferences, for example in women with CAH (Hines, 2000). Thus, overall, the influence of prenatal hormones on sex differences in cognitive performance is far from clear and it would not be surprising to find that any association to sexual orientation related differences would be similarly inconclusive. Of the number of possible biological predictors of sexual orientation in humans, the present study examined a very narrow range of measures. Further work will need to take account of multiple factors.

The finding that psychological gender, an individual's relative degree of masculinity or femininity (M–F), was a predictor of mental rotation (total correct) scores in addition to sexual orientation is partially consistent with studies that report a similar association between M–F measures, such as the Personal Attributes Questionnaire (PAQ) and Bem Sex Role Inventory (BSRI), and sex differences in mental rotation and water level test performance in unselected heterosexual men and women (e.g. Jamison and Signorella, 1987; Hamilton, 1995; Parameswaran, 1995; Saucier et al., 2002). They are inconsistent with the findings of Wegesin et al. (1998) who reported that levels of masculinity and femininity were not related to mental rotation and spatial perception (tests unspecified) performance in homosexual females. The studies in unselected samples also report that the influence of sex is greater than that of psychological gender, consistent with the present findings. For example, Saucier et al. (2002) reported that PAQ scores explained 6.3% of the variance in mental rotation scores outside of sex, which itself explained 13.5%. Nevertheless, some authors often conclude that such associations indicate an influence of ''gender role socialisation'' on mental rotation performance. The assumption is that scores on M–F measures reflect the internalisation of cultural gender stereotypes throughout an individual's development. However, it is far from clear how this should then translate into mechanisms through which different cognitive specialisations, based on sex, are acquired. Moreover, recent work has shown that variance in gender-related interests, occupational preferences and hobbies (supposedly even more reflective of the adoption of cultural sex stereotypes) can largely be accounted for by genetic factors (Lippa and Hershberger, 1999). This is not surprising, as biological theories of sexual differentiation tend to predict stronger links between sex and psychological gender than do psychosocial theories (Lippa, 2002). It is then particularly difficult to tease apart the contribution of these two traits if they have substantial overlapping variance. This is particularly the case with sexual orientation, where sexual orientation related differences in M–F are closely related to the core trait itself (Gangestad et al., 2000; Lippa, 2002). Thus, although the measure of M–F in this study predicted some of the variance in mental rotations in addition to sex and sexual orientation, it is not immediately clear whether the influence is prenatally or postnatally (possibly psychosocial) determined.

The current finding that sexual orientation exerted the most powerful influence on cognitive performance is particularly intriguing. Prenatal hormones might operate on cognitive functioning via sex and sexual orientation, but other pathways are also possible. Recent work shows that the sex determining genes *SRY* and *ZRY* are transcribed directly in the hypothalamus, and the frontal and temporal cortex of the adult male brain (Mayer et al., 1998). This may extend to other sexual differentiation related genes, particularly those on the X chromosome, which shows an abundance of such genes (Saifi and Chandra, 1999). Thus, neural differences may acquire their sex specific properties independent of their hormonal environment. There is some evidence for an X-linked genetic locus to male sexual orientation (Hamer et al., 1993; Hu et al., 1995; cf. Rice et al., 1999). Interestingly, sequence variation in the androgen receptor gene is not a determinant of male sexual orientation (Macke et al., 1993). In other words, although there may be genetic factors in male sexual orientation, they may not necessarily act via hormonal mechanisms. If such genes also influence sexual orientation, then it is possible that they confer hormone-independent sex-linked neural differentiation which underscores variation in sex-linked cognitive functions. Such theorising is inherently speculative but it would be fair to say that human sexual orientation probably has multiple determinants (including genetic, hormonal and postnatal factors which influence neurodevelopment) and these, in concert, produce the profile of neurobehavioural differences between heterosexual and homosexual males and females.

The results of the partial correlations demonstrated no influence, overall, of the variables of

secondary interest to this investigation beyond that subsumed within sexual orientation. The present study found that although homosexual males report starting puberty earlier than heterosexual males, this did not affect cognitive performance. This is inconsistent with Waber, (1976) hypothesis that early maturing adolescents, irrespective of sex, should perform better in verbal tasks (as homosexual males do in the current investigation) than in spatial tasks compared to later maturing adolescents. Other studies in unselected samples of heterosexual male and female adolescents also contradict Waber's hypothesis (e.g. Hassler, 1991; Davidson and Susman, 2001).

Finally, Singh et al. (1999) reported that ''butch'' lesbians demonstrate greater self-reported childhood gender-atypicality, higher waist-to-hip ratios, higher salivary testosterone and less desire to give birth than ''femme'' lesbians. They argue that such within-sexual orientation differences should extend to cognitive functions where sexual orientation effects have been demonstrated. The present study found no support for this assertion (using the same ''degree of butchness'' measure as Singh et al., 1999) in either homosexual males or homosexual females. Therefore, it appears that erotic role identification (possibly another measure of ''psychological gender'') has negligible influences on sexual orientation related differences in cognitive functions.

In conclusion, the present study has shown that measures of prenatal hormonal influences (2D:4D ratio, birth-order and sibling sex composition), previously shown to differentiate homosexuals from heterosexuals, added no independent predictive power to sexual orientation related differences in performance on a large battery of sex-dimorphic cognitive tasks. There was some influence of psychological gender on mental rotation and perceptual speed scores although the nature of the association is unclear. Sex and sexual orientation were the strongest predictors of cognitive differences, over-riding the effect even of IQ.

Acknowledgements

Qazi Rahman was supported by the Medical Research Council of the United Kingdom.

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Evolution and Human Behavior 21 (2000) 333–345

Evolution and Human Behavior

The ratio of 2nd to 4th digit length and male homosexuality

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Received 21 December 1999; accepted 14 June 2000

Abstract

Sexual orientation may be influenced by prenatal levels of testosterone and oestrogen. There is evidence that the ratio of the length of 2nd and 4th digits (2D:4D) is negatively related to prenatal testosterone and positively to oestrogen. We report that (a) 2D:4D was lower in a sample of 88 homosexual men than in 88 sex- and age-matched controls recruited without regard to sexual orientation, (b) within the homosexual sample, there was a significant positive relationship between mean 2D:4D ratio and exclusive homosexuality, (c) overall, there was a decrease in 2D:4D from controls to homosexual men to bisexual men and (d) fraternal birth order, a positive predictor of male homosexuality, was not associated with 2D:4D in a sample of 240 Caucasian men recruited without regard to sexual orientation and 45 homosexual men. Further work is needed to confirm the relationships between 2D:4D and sexual orientation. However, these and other recent data tend to support an association between male homosexuality and high fetal testosterone. Very high testosterone levels may be associated with a sexual preference for both men and women.

Keywords: 2nd:4th digit ratio; Male homosexuality

Heterosexual sexual orientation appears to be a very important component of direct fitness. The existence of male and female homosexual phenotypes is therefore challenging to adaptationist interpretations of human behaviour. In this paper, we are concerned with mechanisms that lead to the male homosexual phenotype and how knowledge of these may illuminate the debate on the evolutionary origins of male homosexuality.

The aetiology of male homosexuality may involve psychosocial and constitutional factors (Bailey & Pillard, 1991; Bell, Weinberg, & Hammersmith, 1981; Hamer & Copeland, 1994;

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1090-5138/00/$ – see front matter
PII: S1090-5138(00)00052-0

LeVay, 1993; Trip, 1975). Attempts to test psychosocial models have led to weak or negative findings (Bell et al., 1981; Stoller & Herdt, 1985). In contrast, there is accumulating support for genetic and neurohormonal influences on male homosexuality.

Male homosexuality is strongly familial. Pillard and Weinrich (1987) have found that brothers of gay men were about four times more likely to be homosexual than were brothers of heterosexual controls. Studies of monozygotic pairs of twins have found concordance rates for homosexuality of 40% (Heston & Shields, 1968), 52% (Bailey & Pillard, 1991) and 100% (Kallman, 1952). This compares with a dizygotic rate of 15% (Kallman, 1952) and 22% (Bailey & Pillard, 1991). The monozygotic rate of 100% found by Kallmann is almost certainly too high. However, heritability estimates are substantial under a wide variety of genetic models, e.g. $h^2 = 0.31$ to $h^2 = 0.74$ (Bailey & Pillard, 1991). This evidence for a genetic influence on the gay phenotype was strengthened when analyses of the long arm of the X chromosome (Xq28) indicated linkage between Xq28 markers and male homosexuality (Hamer, Hu, Magnuson, Hu, & Pattatucci, 1995; Hu et al., 1995).

Genetic links with male homosexuality do not of themselves indicate mechanisms that predispose to the gay phenotype. The neurohormonal theory of sexual orientation provides one possible mechanism. Prenatal exposure to sex steroid hormones in rodents and primates can masculinise (the result of testosterone) sexual behaviour (for reviews, see Hodgkin, 1991; Pilgrim & Reisert, 1992). Drawing on the evidence from animal models, constitutional theories of human homosexuality have emphasised the organising role of prenatal hormones (e.g. Dorner, 1979). MacCulloch and Waddington (1981) and Pillard and Weinrich (1987) have suggested that human sexual orientation depends on variations in the degree of masculinisation and behavioural defeminisation of the brain that may occur in utero. Support for such a model comes from observations on gender-dependent cognitive abilities, which are often female-like in homosexual males (Sanders & Ross-Field, 1986) and from work on neuroendocrine activity in males with different sexual orientations. Dorner, Rhode, Stahl, Krell, and Masius (1975) found that, in response to exogenous oestrogen homosexual, but not bisexual or heterosexual, men showed the typically female response of a surge of LH. They concluded this reflected a partially female-differentiated brain in male homosexuals. The existence of this neuroendocrinological difference between homosexual and heterosexual men was confirmed by Gladue, Green, and Hellman (1984), but the replicability of the luteinising hormone evidence is in some doubt (Gooren, 1986; Hendricks, Graber, & Rodriguez-Sierra, 1989).

Gender-dependent cognitive abilities in homosexual males are often similar to those of females, and this has prompted the assumption that homosexual males have been exposed to low levels of prenatal testosterone. However, a number of workers have challenged this idea. Geschwind and Galaburda (1985) have pointed out that left-handedness may be associated with high in utero levels of testosterone and male homosexuality is correlated with left-handedness. They therefore suggested that male homosexuals have been exposed to higher prenatal testosterone concentrations than heterosexual males. In support of this, there have been reports of higher levels of circulating testosterone and larger genitalia in homosexual men compared to heterosexuals.

Prenatal hormone levels may be influenced by genetic and environmental factors (Trichopoulos, 1990). Birth order is the only environmental variable that has been shown

to be reliably correlated with male homosexuality. The work of Blanchard et al. (Blanchard, 1997; Blanchard & Bogaert, 1996) has demonstrated that the probability that a man will be homosexual increases in proportion to his number of older brothers. The number of older sisters does not affect the probability of homosexuality in later-born males. It is not yet known whether this fraternal birth-order effect is correlated with changes in prenatal hormones.

One problem of investigating the effect of prenatal hormones on sexual orientation is the impossibility of directly testing in utero hormonal concentrations in homosexual men and women. However, the ratio of the length of the 2nd and 4th digit (2D:4D) may afford us a correlate of prenatal hormonal levels. The 2D:4D ratio is sexually dimorphic with male 2D:4D<female 2D:4D the widespread condition (Baker, 1888; George, 1930; Manning et al., 2000a; Manning, Scutt, Wilson, & Lewis-Jones, 1998; Phelps, 1952). Studies of fetal material suggest relative digit length is fixed in utero by week 14 (Garn, Burdi, Babler, & Stinson, 1975), and there is cross-sectional evidence that the 2D:4D ratio is fixed at least as early as 2 years and shows no appreciable change at puberty (Manning et al., 1998; Phelps, 1952). It is therefore likely that the 2D:4D ratio is fixed before birth and perhaps as early as week 14 of pregnancy. Serum levels of testosterone, oestrogen and LH in a sample of 100 subjects (60 men and 40 women) attending an infertility clinic showed negative associations with 2D:4D ratio and testosterone (men only) and positive relationships with oestrogen and LH (men and women). If 2D:4D is fixed prenatally, it is likely that these correlations reflect in utero relationships between 2D:4D and sex steroids. In support of this, the waist/hip ratio (a positive correlate of testosterone and a negative correlate of oestrogen) of mothers is negatively related to the 2D:4D ratio of their offspring aged 5–11 years (Manning, Trivers, Singh, & Thornhill, 1999). Low 2D:4D ratios may therefore indicate high prenatal testosterone and low oestrogen. High 2D:4D ratios may correlate with low prenatal testosterone and high oestrogen.

The question of the relationship between 2D:4D ratio and sexual orientation has recently been addressed by Williams et al. (2000). They measured a sample of heterosexual and homosexual men and women and found that (a) lesbians had lower 2D:4D ratio than heterosexual women, (b) there was no significant difference between the 2D:4D ratios of homosexual and heterosexual men and (c) there was a reduction in 2D:4D with an increase in fraternal birth order. On the basis of the fraternal birth-order effect Williams et al. (2000) concluded that homosexual men are exposed to higher prenatal testosterone levels than heterosexuals.

The purpose of this work was to examine 2D:4D ratio in homosexual men and to relate the 2D:4D ratio to fraternal birth-order effects.

1. Methods

1.1. Study I

Our subjects were 91 men who described themselves as homosexual, gay or bisexual. The participants were recruited from gay social centres and homophile publications. Most subjects were from the Liverpool, Edinburgh and London areas. We recorded the age and height of each subject.

Photocopies were made of the ventral surface of the hand. The lengths of the 2nd and 4th digits of the left and right hands were measured from the ventral proximal crease of the digit to the tip. Where there was a band of creases at the base of the digit (most common at the base of the 4th digit), we measured from the most proximal of these creases (Manning, Baron-Cohen, Wheelwright, & Sanders, 2000a; Manning et al., 1998; Martin, Manning, & Dowrick, 1999). For 30 hands from 30 participants, the 2nd and 4th digits were measured from the hand and from the photocopies. For 50 photocopies of hands from 50 participants, we measured the 2nd and 4th digits twice. All measurements were made with vernier callipers measuring to 0.01 mm. Three subjects had injuries to the 2nd or 4th digits, and these were discarded so that our sample number was 88.

Repeated-measures analysis of variance tests were used to calculate the repeatability (r_1) of our measurements and 2D:4D ratios and the ratio (F) of real between-individual differences and error differences in digit lengths and 2D:4D ratios. Comparisons between measurements made directly on the hand and those made from photocopies showed high repeatabilities and significant F values (2nd digit, $r_1 = 0.97$, $F = 56.91$, $p = 0.0001$; 4th digit, $r_1 = 0.97$, $F = 59.26$, $p = 0.0001$; 2D:4D ratio, $r_1 = 0.88$, $F = 15.86$, $p = 0.0001$). Comparisons of repeated measurements made from photocopies showed very high repeatabilities and F values (2nd digit, $r_1 = 0.999$, $F = 2082.17$, $p = 0.0001$; 4th digit, $r_1 = 0.998$, $F = 1199.64$, $p = 0.0001$; 2D:4D ratio, $r_1 = 0.996$, $F = 450.96$, $p = 0.0001$). We concluded that our measurements of the 2D:4D ratio represented real differences between subjects.

We measured sexual orientation from subjects' reports of sexual fantasy and sexual experience (Kinsey, Pomeroy, & Martin, 1948). Participants were asked to complete a six-item questionnaire: (1) your past sexual partners have been men; (2) this year's sexual episodes have been with men; (3) when fantasising alone you think of men; (4) when fantasising during sex you think of men; (5) the people you say that you 'fancy' are men; and (6) those who you try/tried to 'chat up' are men. The responses were graded on a seven-point scale from 'Never' (0) to 'Always' (6). The Sexual Orientation Score (SOS) was calculated as the mean of the six responses. Also included in the questionnaire was a question on ethnicity.

Controls, matched for sex and age (±2 years), were recruited without regard to sexual orientation, from adult education classes and social groups. All controls were Caucasians. Digit measurements were made from photocopies of the palmar surface of the right and left hands.

1.2. Study II

The participants were 240 Caucasian men from the Liverpool area. Subjects were recruited, without regard to sexual orientation, from adult education classes and social clubs and represented a wide range of professions and socio-economic backgrounds. All subjects were 18 years or older. We recorded number of older brothers, older sisters, younger brothers and younger sisters. The length of the 2nd and 4th digit of the left and right hands was measured directly from the hand using vernier callipers measuring to 0.01 mm. For 100 men, we measured the 2nd and 4th digits from 100 hands twice. The repeatabilities were high and the F ratios significant for digit lengths and the 2D:4D ratio (2nd digit, $r_1 = 0.90$, $F = 18.51$, $p = 0.0001$; 4th digit, $r_1 = 0.92$, $F = 19.27$, $p = 0.0001$; 2D:4D ratios, $r_1 = 0.80$, $F = 15.72$,

$p = 0.0001$). We concluded that our calculated 2D:4D ratios represented real differences between subjects.

An item concerning birth order and sex of siblings was not included in the initial questionnaire supplied to our homosexual participants. However, we were able to retrospectively obtain the birth order, sex and numbers of siblings from 45 of our homosexual subjects. The total sample in Study II was therefore 285 participants.

2. Results

2.1. Study I

There was a mean age of 30.10±9.06 years for homosexual men and 30.27±10.08 years for controls. The mean 2D:4D ratio of the right and left hands for men in the Liverpool area has been reported as 0.98 (Manning et al., 1998). We found the following means and standard deviations of the 2D:4D ratios: homosexuals right hand 0.97±0.03, left hand 0.96±0.03, mean of right and left hand 0.97±0.03; controls right hand 0.98±0.04, left hand 0.98±0.04, mean of right and left hand 0.98±0.03. The 2D:4D ratios of the homosexual men were significantly lower than control ratios for the left hand ($t = 3.82$, $p = 0.0002$, Fig. 1) and for the mean value for the two hands ($t = 3.01$, $p = 0.003$) but not for the right hand ($t = 1.38$, $p = 0.17$). Manning et al. (2000a) have reported low mean 2D:4D ratios (<0.95) in some ethnic groups, particularly Blacks. There were no Black participants in the sample of homosexual men. There were 54 responses to the question of ethnicity of which 49 indicated a Caucasian origin and the remaining five subjects were of Oriental or part-Oriental in origin. Considering just the Caucasian sub-sample and their matched controls we found that the homosexual men still have significantly lower 2D:4D ratios than the controls with respect to the left hand (homosexuals 0.97±0.04, heterosexuals 0.99±0.04, $t = 2.70$, $p = 0.008$) and the mean of both hands (homosexuals 0.97±0.03, controls 0.98±0.03, $t = 2.06$, $p = 0.04$), but there is again no significant difference in the right hand (homosexuals 0.97 ± 0.04, controls 0.98 ± 0.04, $t = 0.94$, $p = 0.35$). There are six tests here. Correcting for multiple tests the Bonferroni p values for the left hand are $n = 88$, $p = 0.001$ and $n = 49$, $p = 0.03$, and for the mean 2D:4D of the right and left hands $n = 88$, $p = 0.02$ and $n = 49$, $p = 0.12$. We conclude that the balance of evidence indicates that homosexual men's left-hand 2D:4D ratios are lower than population norms.

The SOS ranged from 3 to 6 and was strongly skewed towards exclusive homosexuality. The mode was 6 (44 participants) and the geometric mean was 5.62 (Fig. 2). Most gay men have accepted their sexual orientation by age 18 years, However, some have not (Bell et al., 1981, p. 99). It is therefore possible that older men are more sure of their homosexuality than younger men. We found a weak positive association between age and SOS, which was non-significant (Kendall Rank Correlation Test, $\tau = 0.07$, $Z = 0.99$, $p = 0.33$; a non-parametric test was used because the SOS was non-normally distributed). In accordance with previous work, the 2D:4D ratios were not related to age ($\tau = 0.10$, $Z = 1.39$, $p = 0.17$).

Within the homosexual sample there was evidence that 2D:4D was positively related to SOS. We found a significant correlation between SOS and the mean 2D:4D ratio for both

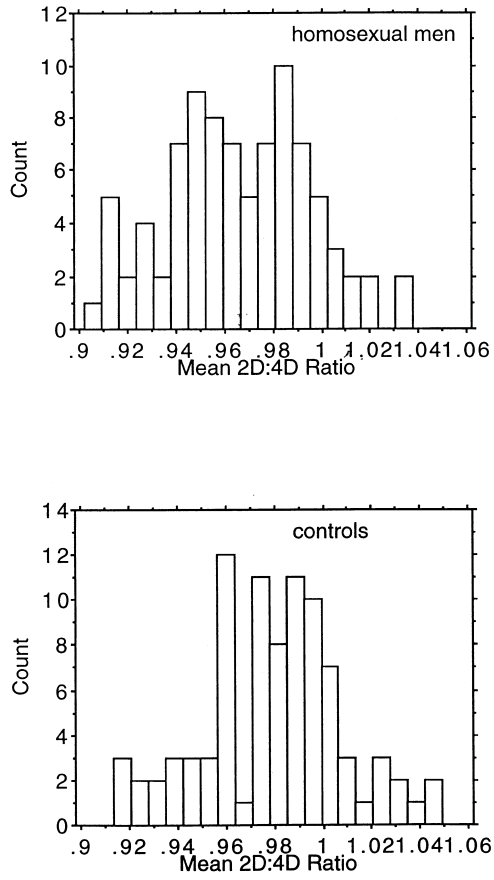

Fig. 1. The distribution of 2D:4D ratios of the left hand in 88 homosexual men and 88 controls matched for sex and age and recruited without regard to sexual orientation.

hands ($\tau = 0.15$, $Z = 2.11$, $p = 0.03$); relationships between SOS and 2D:4D for each hand considered separately were close to significance (right: $\tau = 0.14$, $Z = 1.2$, $p = 0.06$; left: $\tau = 0.14$, $Z = 1.9$, $p = 0.06$). Fig. 2 shows the association between values of SOS and mean 2D:4D ratios. Considering only those subjects who indicated some degree of sexual interest in females (i.e. removing subjects with SOS = 6), we found the positive relationship significant for mean 2D:4D ($n = 44$, $\tau = 0.33$, $Z = 3.02$, $p = 0.001$), right hand ($\tau = 0.32$, $Z = 3.02$, $p = 0.003$) and left hand ($\tau = 0.27$, $Z = 2.70$, $p = 0.008$). There are six tests here. All six show positive associations, four have significant p values and two are close to significance. Adjusting for multiple tests we find Bonferroni p values for the non-exclusively homosexual sample ($n = 44$) of mean 2D:4D $p = 0.0006$, right hand $p = 0.02$, left hand $p = 0.03$, and the total sample ($n = 88$) mean 2D:4D $p = 0.09$. The overall impression is one of a positive association between 2D:4D and degree of homosexuality, i.e. exclusively homosexual men have higher 2D:4D ratios than those whose responses indicated anything other than a fully homosexual orientation.

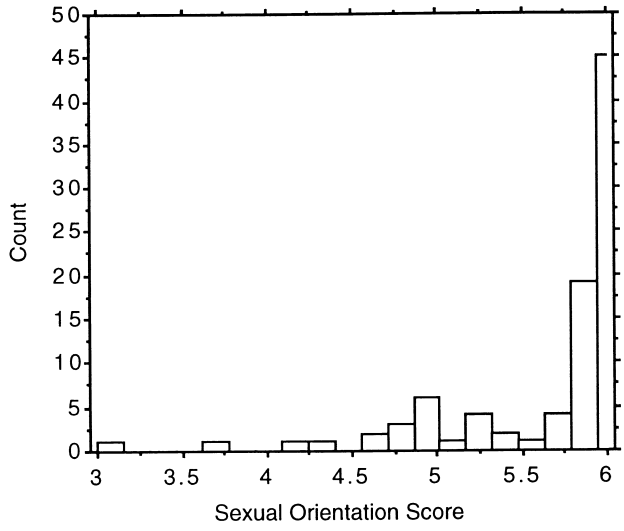

Fig. 2. The distribution of the SOS of men who described themselves as homosexual, gay or bisexual. High values indicate exclusive male homosexuality. Values range from 3 to 6 with a marked skew towards exclusive homosexuality.

It is of interest to know which of the digits influences the relationship between 2D:4D ratio and SOS the most strongly. We controlled for body size by dividing digit length by the height of the subject. We found that the mean of the 4th digit was most strongly related to SOS. Men with short 4th digits reported higher scores for homosexuality (2D/Height $\tau = -0.07$, $Z = 0.98$, $p = 0.33$; 4D/Height, $\tau = -0.15$, $Z = 2.13$, $p = 0.03$, Fig. 3). However, digit length divided by height was positively related to age (2D/Height $\tau = 0.25$, $Z = 3.50$, $p = 0.0005$; 4D/Height $\tau = 0.19$, $Z = 2.76$, $p = 0.006$). Because SOS was skewed towards exclusive homosexuality we transformed (base-10 log) the values of SOS and performed a simultaneous

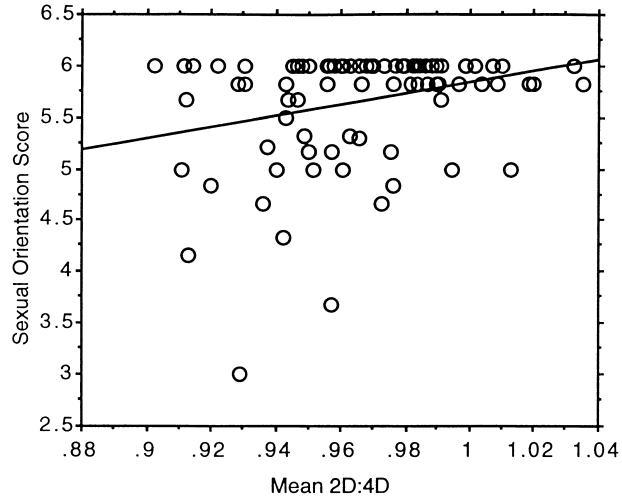

Fig. 3. The relationship between the SOS of mean 2D:4D in 88 gay men ($y = 5.54x + 0.31$, $r^2 = 0.08$).

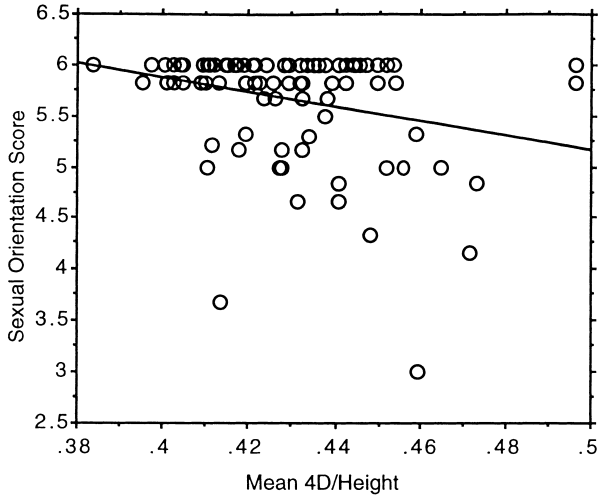

Fig. 4. The relationship between the SOS and mean 4th digit length divided by the height of the individual ($y = -7.207x + 8.76$, $r^2 = 0.07$).

multiple regression with 2D/Height, 4D/Height and age as independent variables and base-10 log SOS as the dependent variable. The 4D/Height variable remained significantly negatively related to SOS ($b = -1.35$, SE = 0.41, $t = 3.29$, $p = 0.002$), while 2D/Height ($b = 0.64$, 0.44, $t = 1.45$, $p = 0.15$) and age ($b = 0.001$, SE = 0.001, $t = 2.11$, $p = 0.04$) were positively and more weakly related to SOS. This relationship between 4D/Height and SOS was consistent with the finding that within the homosexual sample high 2D:4D was associated with exclusive homosexuality (Fig. 4).

We divided the homosexual sample into men who reported exclusive homosexuality ($n = 44$) and those who had male and female partners or male and female fantasies ($n = 44$). There were significant differences in left hand 2D:4D and mean 2D:4D between the control sample, the exclusively homosexual and non-exclusively homosexual males for left hand 2D:4D and mean 2D:4D (left hand, controls 0.98 ± 0.04, exclusively homosexual 0.97 + 0.03, non-exclusively homosexual 0.96 ± 0.03, ANOVA, $F = 7.42$, $p = 0.0008$, Bonferroni adjustment $p = 0.003$; mean 2D:4D, controls 0.98 ± 0.03, exclusively homosexual 0.97 ± 0.03, non-exclusively homosexual 0.96 ± 0.03; ANOVA, $F = 4.9$, $p = 0.008$, Bonferroni adjustment $p = 0.02$, Fig. 5; right-hand controls 0.97 ± 0.04, exclusively homosexual 0.97 ± 0.03, non-exclusively homosexual 0.97 ± 0.03, ANOVA, $F = 0.74$, $p = 0.74$). Controls had the highest 2D:4D ratios followed by exclusively homosexual men and the lowest ratios were found in non-exclusively homosexual men.

2.2. Study II

The mean of 2D:4D ratio was slightly higher than that earlier reported for Liverpool Caucasian men (0.99 ± 0.03 as against 0.98 ± 0.03) by Manning et al. (1998). As in Study I, the homosexual sub-sample had significantly lower mean 2D:4D (0.96 ± 0.03) compared to population norms ($t = 4.72$, $p = 0.0001$).

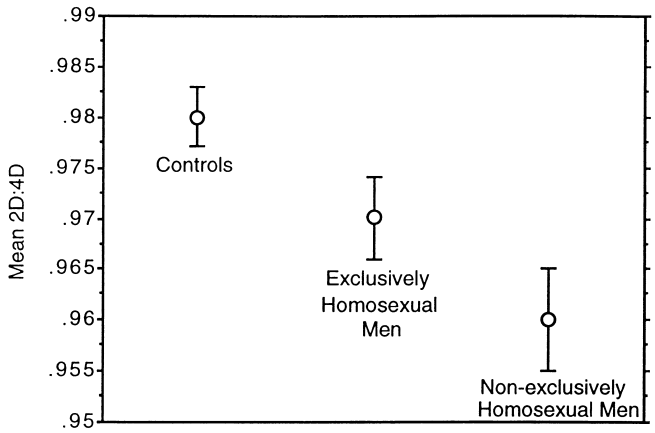

Fig. 5. Means and standard errors for mean 2D:4D ratios of the control sample of men recruited with disregard for sexual orientation (n = 88), exclusively homosexual men (i.e. who had male sexual partners and sexual fantasies about men only, n = 44) and non-exclusively homosexual men (i.e. who had male and female sexual partners and fantasies directed at men and women, n = 44).

We regressed 2D:4D ratio on numbers of siblings and found no significant relationships for right and left hand or mean 2D:4D ratio (for brevity we report only mean 2D:4D: older brothers $b = -0.48$, $F = 0.11$, $p = 0.74$; older sisters $b = -1.72$, $F = 1.25$, $p = 0.26$; younger brothers $b = 1.24$, $F = 0.99$, $p = 0.32$; younger sisters $b = 0.45$, $F = 0.10$, $p = 0.76$).

Our sample contained homosexual males and men recruited without regard to sexual orientation. In order to control for this, we performed a simultaneous multiple regression analysis with dependent variable mean 2D:4D and independent variables number of older brothers and group (dummy coded, general population sample = 1 and homosexuals = 2). We found a non-significant positive relationship between number of older brothers and mean 2D:4D ($b = 0.93$, $t = 0.64$, $p = 0.52$). There was a significant positive relationship between group and number of older brothers ($b = 0.48$, $t = 3.57$, $p = 0.0004$). This latter result indicated that as expected the homosexual men in our sample had more older brothers (mean 0.98 ± 1.03 older brothers) than the men recruited without regard to sexual orientation (mean 0.52 ± 0.75 older brothers, $t = 3.53$, $p = 0.0005$). Multiple regression analyses using dependent variables of number of older sisters, younger brothers and younger sisters did not show significant relationships (e.g. older sisters: mean 2D:4D $b = 1.00$, $t = 0.63$, $p = 0.53$; group $b = 0.25$, $t = 1.71$, $p = 0.09$).

3. Discussion

We have the following results. First, homosexual males have lower left hand 2D:4D ratios than population norms and this appears to be the case after controlling for age, ethnicity and multiple tests. Secondly, within a sample of homosexual males, there was a positive association between 2D:4D ratio and SOS, that is, men who reported exclusive homosexuality had higher 2D:4D than those who reported some female partners or sexual fantasies concerning females. This relationship was significant in the whole sample of

homosexual men and for those men who had some heterosexual partners or fantasies. Thirdly, there was a reduction in left hand 2D:4D ratio in the sequence controls>exclusively homosexual men>non-exclusively homosexual men. Fourthly and finally, a correlate of homosexuality, fraternal birth order, was not demonstrably related to 2D:4D ratio. This last finding may indicate that the fraternal birth-order effect, which Blanchard (1997) has tentatively attributed to a maternal immune response against the fetus, is an independent etiological route to homosexuality from that involving prenatal androgen effects as discussed below.

If 2D:4D ratio is negatively related to prenatal testosterone and positively to prenatal oestrogen, our first finding suggests that male homosexuals experience higher testosterone and lower oestrogen in utero than population norms. Williams et al. (2000) did not find significant differences between the 2D:4D ratio of homosexual and heterosexual men. However, their study did not include a control for ethnicity. Black subjects may have very low values of 2D:4D (Manning et al., 2000a). If the study of Williams et al. (2000) included Caucasians and African Americans, the ethnic differences may have obscured differences between heterosexuals and homosexuals.

Our finding that within the homosexual sample there was a positive relationship between 2D:4D and homosexuality indicates that very high prenatal testosterone predisposes some male fetuses towards a sexual preference for both men and women. Further work is needed to determine whether this within-group effect is a robust one, but we suggest that prenatal androgens increase with the following sequence in men: heterosexuality, homosexuality, bisexuality.

In contrast to the work of Williams et al. (2000), we did not find a negative relationship between fraternal birth order and 2D:4D ratio. Indeed, after controlling for sexual orientation, we found a non-significant positive association. This may be because both relationships, i.e. between fraternal birth order and male homosexuality, and male homosexuality and 2D:4D are weak. Further work is needed to clarify whether this association is real.

Our findings together with those of Williams et al. (2000) suggest that high prenatal testosterone concentrations and low prenatal oestrogen may predispose the male fetus to homosexuality. Therefore, homosexual men tend to have lower 2D:4D ratios than heterosexuals. Very high androgen levels may predispose towards bisexuality and this is associated with very low 2D:4D ratios.

Does this conclusion shed light on suggestions regarding the adaptive nature of homosexuality? Male homosexuality is quite common and is likely to be influenced by genes. It is tempting to argue that the existence of such genes, which are too frequent to be ascribed to recurrent mutation, indicates an adaptive function for the homosexual phenotype (e.g. Hutchinson, 1959; Ruse 1981; Weinrich, 1987). An alternative explanation is that the fitness of the male fetus may often be reduced by high levels of both testosterone and oestrogen. Low 2D:4D is found in autistic children, particularly male children (Manning et al., 2000), and men with long 4th digits relative to their height report high levels of depression (Martin et al., 1999). High concentrations of prenatal testosterone have also been implicated in the etiology of male homosexuality, migraine, stammering, dyslexia and auto-immune diseases in addition to traits with less negative fitness effects such as left-handedness and musical ability (Geschwind & Galaburda, 1985). Some of these traits have been reported to be associated

with low 2D:4D and therefore high prenatal testosterone. Manning et al. (2000a) have found that low 2D:4D ratio was associated with left-hand preference in a sample of Jamaican schoolchildren and Sluming and Manning (2000) have reported lower than average 2D:4D ratios in a sample of symphony musicians. Testosterone is necessary in order that normal male sexual differentiation be achieved but it is also associated with potential risks to the developing fetus. Excess oestrogen is equally problematic to males. There is evidence that prenatal oestrogen in the male fetus may lead to hypospadias and other malformations of the urinogenital system, cardiovascular anomalies such as ventricular septal defect, atrial septal defect, pulmonic stenosis, patent ductus arteriosis and transposition of the great vessels and malformations of the digits and toes (for review, see Manning & Bundred, 2000). In the male fetus, there may be strong selection for high testosterone in order that the urinogenital system successfully differentiates and that the cardiovascular system is efficient. These characteristics are very important for male fertility and competitiveness. It may therefore be difficult for selection to oppose genes for high prenatal testosterone (Manning et al., 2000a). However, such genes are likely to have costs, which may include the male homosexual phenotype. This view emphasises the difficulty of selectively removing genes for high testosterone and perhaps homosexuality. It therefore offers an alternative to the suggestion that male homosexuality is in some way adaptive.

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Journal of Experimental Social Psychology 44 (2008) 1100–1105
Journal of Experimental Social Psychology
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Brief exposures: Male sexual orientation is accurately perceived at 50 ms ☆

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Received 4 January 2007; revised 27 November 2007
Available online 25 January 2008

Abstract

People have proved adept at categorizing others into social categories, at least when the categorical distinction is perceptually obvious (e.g., age, race, or gender). There remain many social groups whose boundaries are less clear, however. The current work therefore tested judgments of an ambiguous social category (male sexual orientation) from faces shown for durations between 33 ms and 10,000 ms. The sexual orientation of faces presented for 50 ms, 100 ms, 6500 ms, 10,000 ms, and at a self-paced rate (averaging 1500 ms), was categorized at above-chance levels with no decrease in accuracy for briefer exposures. Previous work showing impression formation at similar speeds relied on consensus to determine the validity of judgments. The present results extend these findings by providing a criterion for judgmental accuracy—actual group membership.

Keywords: Person perception; Judgmental accuracy; Sexual orientation; Nonverbal behavior; Social cognition

As we navigate the social world, we are constantly called upon to make judgments of those around us: friend or foe, approach or avoid, mate with or fight with, etc. As such, we are consistently making snap judgments of those we encounter (see Ambady & Rosenthal, 1992). We categorize others into groups (Macrae & Bodenhausen, 2000), infer their intentions (Mason, Tatkow, & Macrae, 2005), and make judgments about their character (Berry & McArthur, 1985), to name a few.

Whereas perceptually obvious social categories such as age (Wright & Stroud, 2002), race (Richeson & Trawalter, 2005), or gender (Macrae & Martin, 2007) are quickly and efficiently perceived based on facial cues, little work has investigated the perceptibility of more ambiguous social groups. This leaves open the question as to whether groups that lack clearly defined visual markers can be accurately perceived from brief glimpses of the face.

One hallmark of person construal judgments is the speed with which they can occur. Subliminal presentations of the faces of perceptually obvious groups have long shown evidence of accurate and efficient categorization (e.g., Eberhardt, Goff, Purdie, & Davies, 2004). Recently, however, these processes have been demonstrated for less evident aspects of identity. Bar, Neta, and Linz (2006) demonstrated that threat in faces was consistently detected at 39 ms and 1700 ms exposure times but that judgments of intelligence were not consistent at such rapid speeds. Willis and Todorov (2006), however, showed that judgments of personality traits (i.e., aggressiveness, competence, likeability, and trustworthiness) and attractiveness were consistent between 100 ms and 1000 ms. Both these studies looked exclusively at consensual judgments, or the agreement among raters. But there has been no work, to our knowledge, about whether such judgments are accurate. The current study therefore attempts to assess whether rapid expo-

☆ We would like to thank Max Weisbuch and Reg Adams for their advice and assistance throughout the execution of this study. This research was supported in part by a National Science Foundation Graduate Research Fellowship to Nicholas O. Rule and a National Science Foundation research grant (NSF BCS-0435547) to Nalini Ambady.
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0022-1031/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jesp.2007.12.001

sures to faces will allow for accurate judgments of an ambiguous social identity: male sexual orientation.

To date, limited evidence has suggested that sexual orientation can be accurately discerned from thin slices, or brief observations of dynamic nonverbal behavior (as short as 1000 ms; Ambady, Hallahan, & Conner, 1999), and paralinguistic cues from the voice (e.g., Gaudio, 1994; Smyth, Jacobs, & Rogers, 2003). Self-report data have also shown that individuals believe sexual orientation can be judged from nonverbal information (Shelp, 2002) but have been restricted to descriptions of intentional actions (such as eye gaze; Carroll & Gilroy, 2002; Nicholas, 2004), rather than examining unintentional factors, such as appearance or non-conscious behaviors. In the current work we examine whether male sexual orientation can be judged from static, exclusively facial cues at rapid exposures.

Study 1A

Method

Participants

Ninety undergraduates ($n = 68$ females) were assigned to six conditions ($n = 15$ per condition) based on exposure time: 33 ms, 50 ms, 100 ms, 6500 ms, 10,000 ms, and self-paced judgments.

Stimuli

Images were obtained from public, online personal advertisements posted for use in various major cities across the U.S. Men in the images were therefore self-defined for sexual orientation, were anonymous, and were available to the public domain. Images were taken from the 18 to 30 age group on the personals websites. Only photos of headshots were downloaded for use. Although hundreds of images were initially collected, only those images presenting a directly oriented face free of any facial alterations (such as jewelry, glasses, or facial hair) were selected, resulting in 45 homosexual and 45 heterosexual images. The images were removed from their original context and placed onto a white background. Targets' ears and hair were retained in the cropping while all other extra-facial information (e.g., neck) was removed. Images were then gray-scaled and standardized to $3'' \times 5''$ dimensions. To preserve anonymity and respect privacy, none of the targets' sexual orientations were disclosed to participants. Further, none of the photos were obtained from the local geographic area.

Procedure

Study 1A consisted of six conditions based on the presentation time of the photographs. Participants made judgments based on faces they saw for either 33 ms, 50 ms, 100 ms, 6500 ms, 10,000 ms, or at their own self-paced rate (i.e., photos were presented until the participant pressed a response key).

Participants were instructed that they would be seeing men's faces on the computer screen and that they would be asked to classify via key-press whether these targets were likely to be either gay or straight. In the 33 ms, 50 ms, and 100 ms conditions, participants were given several practice trials to acquaint them with the speed of presentation of the faces. No feedback was given during any of these trials, nor were these faces presented in the actual experiment.

Each face was presented in random order. Participants had 1000 ms rest, saw the photo for the respective exposure time, and were then prompted to make a dichotomous judgment of the target's probable sexual orientation. In the 33 ms, 50 ms, and 100 ms conditions, photos were preceded by a 1000 ms fixation cross and succeeded by a 100 ms mask, which consisted of a scrambled face matched for high and low spatial frequencies. Participants were not asked to volunteer their own sexual orientation.

Results

Sexual orientation was judged from male faces at levels significantly greater than chance in all but the 33 ms condition. Accuracy scores were calculated for each participant by dividing the number of correct classifications by the total number of images. *T*-Tests were then used to compare the accuracy scores against chance (.5) for each condition: (a) 10,000 ms [$M_{\text{accuracy}} = 60\%$; $t(14) = 5.43$, $p < .001$, $r = .82$], (b) 6500 ms [$M_{\text{accuracy}} = 58\%$; $t(14) = 5.22$, $p < .001$, $r = .81$], (c) self-paced[1] [$M_{\text{accuracy}} = 62\%$; $t(14) = 6.47$, $p < .001$, $r = .87$], (d) 100 ms [$M_{\text{accuracy}} = 62\%$; $t(14) = 7.74$, $p < .001$, $r = .90$], (e) 50 ms [$M_{\text{accuracy}} = 57\%$; $t(14) = 5.26$, $p < .001$, $r = .82$], and (f) 33 ms [$M_{\text{accuracy}} = 50\%$; $t(14) = .11$, $p = .91$, $r = .03$]; see Table 1.

Effect sizes (r) were then converted to Fisher's z's to allow for tests of significance between the strength of effect in each of the conditions. This allowed us to compare the accuracy of judgments as a function of exposure time.[2] The only significant comparisons, however, were against the non-significant effect size for the 33 ms condition: (a) 10,000 ms [$Z = 2.67$, $p < .005$], (b) 6500 ms [$Z = 2.59$, $p < .005$], (c) self-paced [$Z = 3.02$, $p < .002$], (d) 100 ms [$Z = 3.38$, $p < .001$], and (e) 50 ms [$Z = 2.61$, $p < .005$]. Null effects between the comparisons for the other conditions (all p's $> .21$) indicate that accuracy was not attenuated by exposure time. Rather, participants were just as accurate at 50 ms as they are at 10,000 ms. The only point at which scores for accuracy significantly differed was when the effect was no longer significant at 33 ms.

Given that base rates for sexual orientation in society are much greater for heterosexuals than for homosexuals, many participants showed a bias in judging sexual orienta-

[1] Mean judgment time for the self-paced condition was about 1500 ms.

[2] Rosenthal and Rosnow (1984, p. 372) provide a formula for measuring differences in effect sizes: Fisher's $z = \frac{1}{2} \times \log_e(r)$ {and} $Z = \frac{z_1 - z_2}{\sqrt{\frac{1}{N_1 - 3} + \frac{1}{N_2 - 3}}}$ for both effect sizes (r_1 and r_2, respectively) in which N_1 and N_2 correspond to the respective samples. Z refers to the standard normal.

Table 1
Summary statistics for Studies 1A and 1B

	M_{Hits}	M_{FA}	$M_{\%\ Correct}$	$SE_{\%\ Correct}$	$t_{\%\ Correct}$	$r_{\%\ Correct}$	$M_{A'}$	$SE_{A'}$	$t_{A'}$	$r_{A'}$
Study 1A										
33 ms	.28	.28	.50	.01	.11	.03	.49	.03	.32	.09
50 ms	.39	.26	.57	.01	5.26***	.82	.62	.02	5.61***	.83
100 ms	.49	.26	.62	.01	7.73***	.90	.69	.02	8.96***	.92
Self-paced	.56	.31	.62	.02	6.47***	.87	.70	.03	7.22***	.89
6500 ms	.49	.33	.58	.02	5.22***	.81	.64	.03	5.41***	.82
10,000 ms	.46	.25	.60	.02	5.43***	.82	.67	.03	6.08***	.85
Study 1B										
50 ms, full set	.38	.34	.52	.01	2.87**	.61	.54	.01	3.13**	.64
50 ms, reduced set	.38	.34	.52	.01	2.29*	.52	.54	.02	2.42*	.54

* $p < .05$.
** $p < .015$.
*** $p < .001$.

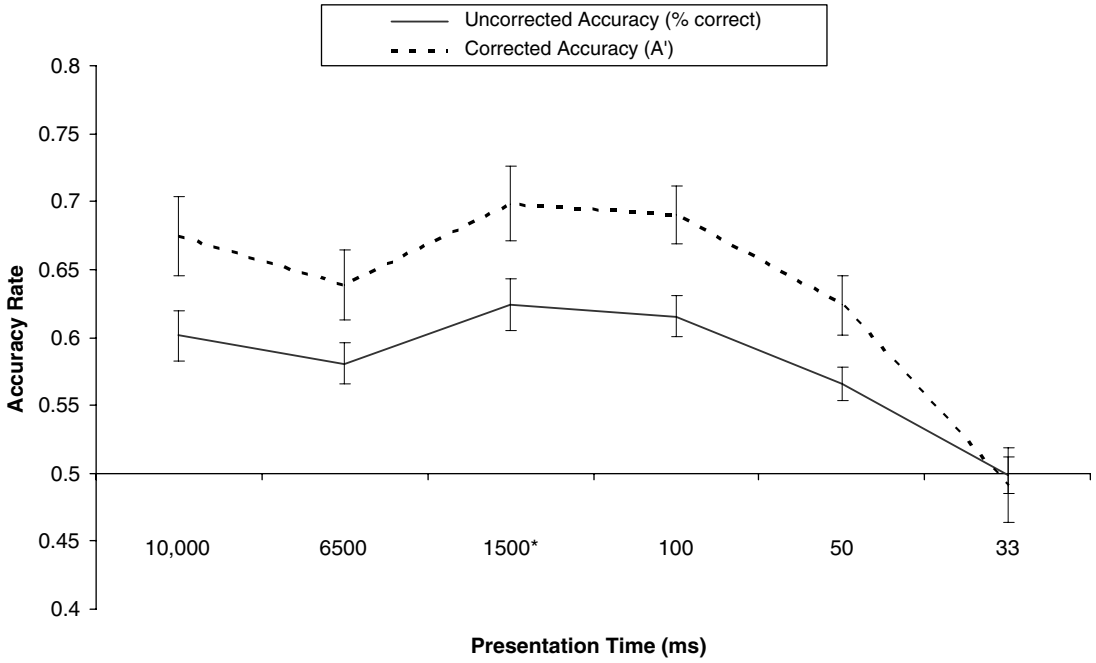

Fig. 1. Mean accuracy by percent with standard error for each exposure duration. The *x*-axis crosses at chance accuracy (50%). Separate lines indicate the uncorrected accuracy rates (mean percent correct), and corrected accuracy rates (response bias eliminated using A'). Ability to accurately classify sexual orientation is consistently above chance for stimulus presentations 50 ms and longer, whereas accuracy falls to chance at 33 ms. *1500 ms represents the self-paced condition, see Footnote 1.

tion (i.e., they were often more likely to indicate that targets were heterosexual). This bias may have exaggerated the accuracy observed among these data. To correct for response bias, we used the signal-detection statistic A' (Rae, 1976), which provided a more conservative estimate of accuracy. Judgments of homosexuality were arbitrarily chosen to represent signal and judgments of heterosexuality as noise.[3] Thus, hits corresponded to correct identifications of homosexuals, whereas false-alarms corresponded to misidentifications of heterosexuals as homosexual. Given that A' ranges between 0 and 1 and describes the area under the ROC curve (i.e., the amount of signal), A' scores are functionally equivalent to measures of accuracy by percent. Removing bias resulted in higher levels of accuracy (these data are plotted in Fig. 1): (a) 10,000 ms [$M_{A'} = .67$; $t(14) = 6.08$, $p < .001$, $r = .85$], (b) 6500 ms [$M_{A'} = .64$; $t(14) = 5.41$, $p < .001$, $r = .82$], (c) self-paced [$M_{A'} = .70$; $t(14) = 7.22$, $p < .001$, $r = .89$], (d) 100 ms [$M_{A'} = .69$; $t(14) = 8.96$, $p < .001$, $r = .92$], (e) 50 ms [$M_{A'} = .62$; $t(14) = 5.61$, $p < .001$, $r = .83$], and (f) 33 ms [$M_{A'} = .49$; $t(14) = .32$, $p = .75$, $r = .09$]. In addition, the corrected scores were significantly greater than the uncorrected frequencies [$t(5) = 4.26$, $p < .01$, $r = .89$] and male

[3] Note that the assignment of signal and noise does not change the result. Specifically, if the analyses are conducted with identifications of straight targets as signal and gay targets as noise, A' remains the same. Hence, the assignment of signal and noise is arbitrary.

and female participants performed equally well, regardless of whether accuracy was uncorrected [$t(88) = .08$, $p = .94$, $r < .01$] or corrected [$t(88) = .10$, $p = .92$, $r = .01$].

Similar to above, comparisons of effect sizes showed no differences between conditions where sexual orientation could be accurately discerned (i.e., 50 ms and greater). In addition, effect sizes for the conditions in which sexual orientation was judged accurately were significantly greater than the effect size for the 33 ms condition: (a) 10,000 ms [$Z = 2.76$, $p < .003$], (b) 6500 ms [$Z = 2.53$, $p < .006$], (c) self-paced [$Z = 3.11$, $p < .001$], (d) 100 ms [$Z = 3.57$, $p < .001$], and (e) 50 ms [$Z = 2.60$, $p < .005$]. Hence, these analyses permit the same conclusion as that with the uncorrected data: there is no benefit or cost in judgmental accuracy as a function of exposure time.

Since the current study used images from personal advertisements, differences may have been due to the tailoring of the advertisements to different audiences: gay men vs. heterosexual women. Two research assistants coded the contents of 306 gay and 276 straight personal advertisements obtained from the same sources as the photos ($R^{SB} = .989$). Self-descriptive adjectives were recorded for each advertisement and subsequently categorized as stereotypically masculine or feminine. Straight men described themselves more often as feminine and warm ($Z = 15.72$, $p < .001$), whereas gay men more often described themselves using adjectives connoting masculinity and dominance ($Z = 2.58$, $p < .01$). Hence, both gay and straight men in personal advertisements described themselves as counter-stereotypical. It is therefore unlikely that a self-presentation bias facilitated the observed effects.

Discussion

Study 1A demonstrates that sexual orientation can be perceived from brief exposures to men's static faces. Accuracy in judging sexual orientation was above chance for durations as fast at 50 ms. Furthermore, additional time did not significantly increase accuracy for longer durations, including when exposure was self-paced. These findings indicate that rapid perceptions of faces provide enough information to make categorical social judgments just as accurately as when duration of exposure is not restricted.

Study 1B

Although the results from coding the contents of the personal advertisements suggest that the results of the previous study were not biased by self-presentation, Study 1B controlled more tightly for self-presentation by using a different target sample.

Method

Fifteen undergraduates ($n = 6$ females) made judgments of images downloaded from www.facebook.com. All of the images came from the same private university in the northeast U.S. Targets in the images were relatively homogeneous in age, socioeconomic status, geography, style of dress/appearance, and level of education. The homosexual and heterosexual groups were approximately matched for racial composition, as well.

The primary advantage of using facebook.com is that it allows individuals to post electronic photo albums, over and above the primary image that one chooses to present of oneself on his or her profile (the latter would be equivalent to the personals photos used in Study 1A). In these albums, individuals will typically post pictures of themselves with their friends, pictures of parties or group outings, and even scenic travel photos. When individuals upload images into their albums, they have the option of providing a title for the photo, a description of the photo, and the opportunity to name others who might be present in the photograph. And when an individual is labeled as being in a photograph, a link is created to that person's profile (provided that they, too, are a facebook.com user) without necessarily obtaining his or her consent to post the photo. When users then view the individual's profile on facebook.com, they will see two types of photos linked to the person's profile: photos posted by the individual, and photos posted by other users (''tagged by others''). Here we used the latter photos. Thus, by using photos of gay and straight individuals that they themselves did not post, we were able to remove the influence of self-presentation and much of the potential selection bias that may be present in photos from personal advertisements.

We performed a search for men's profiles indicating that they were interested in other men for romantic or sexual purposes. Of these profiles, we randomly selected a subset of those persons who had photos of them ''tagged by others.'' A similar procedure was followed for choosing heterosexuals' images, as well. Once a large corpus of photos had been gathered, images were selected and prepared using the same procedures as in Study 1A. This resulted in a total of 160 photos (80 homosexual), a set almost twice as large as that used in Study 1A. To exercise further control, we also removed hairstyles from the images using Adobe Photoshop. Previous work in the perception of gender has indicated that hairstyle is a particularly salient cue to distinguishing between men and women (e.g., Roberts & Bruce, 1988). Given that homosexuals are often considered simply as gender incongruent (e.g., Johnson, Gill, Reichman, & Tassinary, 2007), differences in hairstyle might be influencing perceptions of the internal features of the targets' faces (see Maclin & Malpass, 2001).

Procedures were identical to those for the 50 ms condition above. The 50 ms condition was chosen because it provides the critical threshold at which judgments of sexual orientation appear to be above chance.

Results and discussion

Findings replicated those of Study 1A. Participants were significantly greater than chance in judging male sexual ori-

entation: $M_{accuracy} = 52\%$; $t(14) = 2.87$, $p < .015$, $r = .61$. Again, accuracy increased when scores were corrected for response bias [$M_{A'} = .54$; $t(14) = 3.13$, $p < .01$, $r = .64$] and there were no differences based on participant sex: $t(13) = 1.05$, $p = .31$, $r = .28$. Although the photos were not posted by the targets themselves, there remains some possibility of presentational bias by the posting targets' friends and acquaintances. To address this, we reanalyzed the data only including photos where the target was not the central figure in the image; i.e., at least one other person was present in the photo. Thus, any intention to present an exaggerated representation of the target (e.g., a photo caricaturing the target's sexual orientation) would be diffused by the poster's attention to the other individuals in the photos. This subset consisted of 69 gay and 64 straight targets. Again, participants' accuracy was significantly better than chance in judging sexual orientation: $M_{accuracy} = 52\%$; $t(14) = 2.29$, $p < .05$, $r = .52$; $M_{A'} = .54$; $t(14) = 2.42$, $p < .03$, $r = .54$.

Effect sizes for the 50 ms conditions were compared across the two sets of targets (i.e., Study 1A vs. Study 1B) and this analysis showed that effects in Study 1A did not significantly differ from those in Study 1B for both the uncorrected ($Z = 1.02$, $p = .16$) and the corrected ($Z = 1.02$, $p = .15$) data. Thus, the previously observed effect of accurate judgments of sexual orientation at 50 ms replicated with a more controlled target sample. As the results from the two studies did not significantly differ, we can conclude that images self-selected for personal advertisements do not significantly differ from images selected and posted by others, at least in terms of the perceptibility of male sexual orientation.

General discussion

Exposure to a face for as little as 50 ms provides enough information to judge male sexual orientation with above-chance accuracy. Recent findings have indicated that there is consensus, or agreement among perceivers, in their judgments of personality characteristics from glimpses of the face (Bar et al., 2006; Willis & Todorov, 2006). Similar to this work, we also show here that increased exposure time does not affect the accuracy of judgments from the face. Distinct from this work, however, we extend these effects beyond consensus to the actual accuracy of judgments (see Kenny, 1991, for discussion of consensus vs. accuracy). Further, the speed of exposure time was twice as fast as that observed for consensual judgments of personality and attractiveness, and our use of a backward mask allowed for a more precise indication of exposure time (cf., Willis & Todorov, 2006).

Evolutionary psychology would suggest that one reason for the ability to distinguish male sexual orientation might be due to the implications for mate selection (see Miller & Todd, 1998; Thornhill & Gangestead, 1996). It seems reasonable to suspect that gay men would possess an enhanced capacity for accurately perceiving other gay men because of sexual opportunities. Similar to gay men, it may be advantageous for heterosexual women to parse male sexual orientation to determine the boundaries of their opportunities for mates. It is also possible that heterosexual men may be motivated to attend to the sexual orientations of other men as a means of assessing their competition for mates (Wright & Sladden, 2003) and in jockeying the social dominance hierarchy (Mazur, 2005). Thus, even though information about male sexual orientation may serve different functions, one can speculate about the motivational function such information might provide for different groups. Replication of the current work with female targets may help to elucidate these possible motivations. Indeed, at present we are limited to drawing conclusions only about male targets.

Despite the unique motivations of each of the above groups to preferentially encode male sexual orientation, it is also possible that there is nothing special about sexual orientation at all and that the current findings simply document the speed and efficiency of accurate person perception, more generally. Considering the ease with which we perceive and categorize perceptually obvious groups and the short durations necessary to reach consensus about the emotions and personality traits of others, it may merely be that we are equipped with a highly efficient capacity for perceiving others. As such, particular motivations regarding mating, honed over human evolution, may not be necessary to precipitate the effects observed in the current work. Rather, the rapid and accurate perception of male sexual orientation may be just another symptom of a fast and efficient cognitive mechanism for perceiving the characteristics of others.

Finally, the finding that male sexual orientation can be accurately perceived in such a short period of time is striking. Although previous work has shown that "thin slices" of behavior are remarkably rich in providing information about people, none have sliced as thin as 50 ms (Ambady, Bernieri, & Richeson, 2000). In addition, the use of naturalistic images, as opposed to actors, extends previous work examining consensus in impression formation (cf., Bar et al., 2006; Willis & Todorov, 2006).

But despite the ecological validity of these effects, one limitation of the present research concerns whether male sexual orientation would remain perceptible if targets attempted to conceal their sexual orientation. As male sexual orientation is a stigmatized identity, it is reasonable to suspect that male homosexuals oftentimes exert the effort to pass as straight (see Goffman, 1963; Yoshino, 2006). It therefore remains an interesting question as to whether accuracy in detecting male sexual orientation might vary as a function of targets' intentions to express or conceal their sexual orientation.

Moreover, the current data are not in a position to suggest whether sexual orientation is manifest because of biological versus cultural differences in appearance. Similarly, we do not attempt to speculate about which features may be distinctive between heterosexual and homosexual men.

Rather, we know from previous work that the face is a very rich resource of nonverbal information (see Zebrowitz, 1997) and, thus, future studies may work to address what information is extracted from the face in delineating sexual orientation.

In conclusion, the boundary condition for judging male sexual orientation from static pictures of the male face seems to be 50 ms. Brief exposures to the face thus communicate considerable information that is rapidly and efficiently processed by the social perceiver.

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Best Practice & Research Clinical Endocrinology & Metabolism
Vol. 21, No. 3, pp. 431–444, 2007
doi:10.1016/j.beem.2007.04.003
available online at http://www.sciencedirect.com

7

Sexual differentiation of the brain and behavior

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During the intrauterine period the human brain develops in the male direction via direct action of a boy's testosterone, and in the female direction through the absence of this hormone in a girl. During this time, gender identity (the feeling of being a man or a woman), sexual orientation, and other behaviors are programmed. As sexual differentiation of the genitals takes places in the first 2 months of pregnancy, and sexual differentiation of the brain starts during the second half of pregnancy, these two processes may be influenced independently of each other, resulting in transsexuality. This also means that in the case of an ambiguous gender at birth, the degree of masculinization of the genitals may not reflect the same degree of masculinization of the brain. Differences in brain structures and brain functions have been found that are related to sexual orientation and gender.

Key words: gender identity; sexual orientation; sexual differentiation of the human brain; transsexuality; homosexuality; intrauterine development.

ORGANIZATION AND ACTIVATION OF THE HUMAN BRAIN

Sexual differentiation of the brain brings about permanent changes in brain structures and functions via interaction of the developing neurons with the environment in its widest sense. The environment of a neuron is formed by the surrounding nerve cells and the child's circulating hormones, as well as the hormones, nutrients, medication and other chemical substances from the environment that enter the fetal circulation via the mother. All these factors may have a lasting effect on the process of sexual differentiation of the brain.

The testicles and ovaries develop in the sixth week of pregnancy. This happens under the influence of a cascade of genes, such as the sex-determining gene on the Y chromosome (the SRY), in which many factors play a role. The production of the

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androgens testosterone and dihydrotestosterone by a boy's testes is necessary for the sexual differentiation of the sexual organs between weeks 6 and 12 of pregnancy. The peripheral conversion of testosterone into dihydrotestosterone is essential for the formation of a boy's penis, prostate and scrotum. The development of the female sexual organs in the womb is primarily based on the absence of androgens.[1]

Once the differentiation of the sexual organs into male or female is settled, determined by the presence or absence of the Y chromosome of the father, the next thing to differentiate is the brain, in particular due to the influence of sex hormones on the developing brain cells. This involves (permanent) organizational changes, while during puberty the brain circuits that developed in the womb are activated by sex hormones.

A girl's brain is protected against the effect of circulating estrogens from the mother by the protein α-fetoprotein, which is produced by the fetus and binds strongly to estrogens but not to testosterone.[2] However, the brain itself is also capable of producing estrogens. Testosterone may thus not only have a direct effect on a boy's brain, but, once converted into estrogens by aromatase, it may also act on developing neurons. In rats, this conversion is the most important mechanism for the virilization of the brain[3], but this is not the case in human gender identity and sexual orientation (see below). The fact that there are probably direct genetic effects that affect the sexual differentiation of the brain without involving the sex-hormone receptors is a further complication. Some fetal rat brain cells undergo sexual differentiation, even in tissue culture, without the involvement of sex hormones. Moreover, in adult men the genes SRY and ZRY are expressed until very advanced ages, even though strictly speaking these genes stopped playing a role in sexual differentiation some 80 years earlier.[4] There are at present many additional candidate genes for a role in sexual differentiation of the brain without the involvement of hormones, since it has been found in mouse fetus that, even before the hormones come into play, 50 genes are expressed at different levels in the brains of male and female fetuses.[5] Also genes that escape inactivation on the X chromosome could contribute to the sexually dimorphic expression levels of genes, and thus to sexual dimorphic functions.[6] Thus the sexual differentiation of the brain is not only caused by hormones, even though they are very important for gender identity and sexual orientation.

SEX HORMONES AND BRAIN DEVELOPMENT

During fetal development, the brain is influenced by sex hormones such as testosterone, estrogens and progesterone. From the earliest stages of fetal brain development onwards, many neurons throughout the entire nervous system already have receptors for these hormones. The early development of boys shows two periods during which the testosterone levels are high. The first peak occurs during mid-pregnancy. Testosterone levels peak in the fetal serum between weeks 12 and 18 of pregnancy.[7] In weeks 34–41 of pregnancy the testosterone levels of boys are 10 times higher than those of girls.[8]

The second peak takes place in the first 3 months after birth. At the end of the pregnancy, when α-fetoprotein declines, the fetus is more exposed to estrogens from the placenta, which inhibits the hypothalamus–hypophysial–gonadal axis of the child. This inhibition is lost once the child is born, which causes a peak in testosterone in boys and a peak in estrogens in girls.[9] The testosterone level in boys at this time is as high as it will be in adulthood, although a large part of it circulates bound. Also at this time the testosterone level is a factor higher in boys than in girls. During these two periods there are thus no high levels of testosterone in girls. These two peaks

of testosterone are said to fix the development of structures and circuits in the brain for the rest of a person's life (= programming or organizing). The rising hormone levels during puberty 'activate' circuits that were built during development, and behavioral patterns and disorders that originated much earlier in development, such as schizophrenia, are expressed.[1]

The different brain structures that result from the interaction between hormones and developing brain cells are thought to be the basis of sex differences in the structure of the brain, and thus for behavior, gender identity (the feeling of being either a man or a woman), gender role (behaving as a man or a woman in society), sexual orientation (heterosexuality, homosexuality or transsexuality) and sex differences regarding cognition and aggressive behavior. Factors that interfere with the interaction between hormones and the developing brain systems during development in the womb may permanently influence later behavior. As the sexual differentiation of the genitals takes places much earlier on in development (in the first 2 months of pregnancy) than the sexual differentiation of the brain (starting in the second semester of pregnancy and becoming overt upon reaching adulthood), these two processes may be influenced independently of each other, which may result in people with male sexual organs who feel female and vice versa (a phenomenon called transsexuality). However, this also means that in the case of an ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the same degree of masculinization of the brain.[1]

SEX DIFFERENCES IN COGNITION AND AGGRESSION: LITTLE EFFECT OF THE SOCIAL ENVIRONMENT

In the 1960s and 1970s it was postulated that a child was born as a tabula rasa and that it was forced into the male or female direction by society's conventions. J. Money put this as follows: '*Gender identity is sufficiently incompletely differentiated at birth as to permit successful assignment of a genetic male as a girl. Gender identity then differentiates in keeping with the experiences of rearing.*'[10] This concept has had devastating results (see later: the John–Joan–John case).[11]

One of the stereotypical behavioral differences between boys and girls, which it has often been said are forced upon them by upbringing and social environment, is their behavior in play. Boys are more active and wilder, and they prefer to play with cars, whereas girls prefer dolls. The idea that it is not society that forces these choices upon children but a sexual difference in the early development of their brains and behavior was supported by a study by Alexander and Hines[12], who offered dolls, toy cars and balls to green vervet monkeys. The female monkeys consistently chose the dolls and examined these ano-genitally, whereas the male monkeys were more interested in playing with the toy cars and with the ball. 'Neutral' toys, such as a picture book and a toy dog, did not show sex differences in either humans or monkeys. Girls who are exposed to too much testosterone in the womb, in the case of congenital adrenal hyperplasia (CAH), tend to choose boys as playmates, play preferentially with boys' toys, and are generally wilder than other girls, and are called tomboys.[13] It thus seems as if the sexual differences in play behavior originated early on in our evolution, before the hominids, and that they are imprinted during intrauterine development under the influence of sex hormones.

A similar conclusion can be drawn with respect to sex differences in spontaneous drawings. Japanese research shows that subject matter, choice of color and composition

of drawings by boys and girls show clear sex differences, influenced by the hormones to which the child's brain was exposed in the womb. Girls tend to draw human figures, mainly girls and women, flowers and butterflies. They use bright colors, such as red, orange and yellow. Their subjects tend to be peaceful and arranged in a row on the ground. Boys, however, prefer to draw more technical objects, weapons and fighting, and means of transport, such as cars, trains and airplanes, in birds-eye view compositions and in dark, cool colors such as blue. Drawings by girls exposed to too high testosterone levels in the womb due to CAH begin to show male characteristics some 5–6 years later, even when treated immediately after birth.[14] Also, girls with CAH have a bigger chance of being lesbian or transsexual. Apparently exposure to higher levels of male hormones has important and lasting effects on behavior, and the sex differences that are revealed through drawings are determined by the hormones in the womb rather than by what society demands later on.

The well-known story of John–Joan–John (a pseudonym of David Reimer)[11] means that the concept of sexual neutrality at birth, as introduced by John Money in the 1950s, is suspect. According to Money, gender imprinting does not start until the age of 1 year, and its development will be far advanced by the age of 3–4 years.[15] This was the basis for the decision to make a girl out of an 8-month-old boy who lost his penis due to a mistake during minor surgery (i.e. a phimosis operation). The testicles of this child were removed before the age of 17 months in order to facilitate feminization. The child was dressed in girl's clothes, received psychological counseling and was given estrogens in puberty. Money described the development of this child as a normal female. However, later on Milton Diamond made it clear that this had not at all been the case. In adulthood the child changed back to male, married, and adopted a few children.[16] Unfortunately, he lost money on the stock exchange, got divorced, and eventually committed suicide in May 2004. This story illustrates the enormous programming influence of the intrauterine period on gender.

The apparent impossibility of getting someone to change their sexual orientation is a major argument against the importance of the social environment in the emergence of homosexuality, as well as against the idea that homosexuality is a lifestyle choice. The mind boggles at what has been attempted in order to achieve this: hormonal treatments such as castration, administration of testosterone or estrogens (treatments that appeared to affect libido but not sexual orientation); psychoanalysis; apomorfine serving as an emetic in combination with homo-erotic pictures; psychosurgery (lesions in the hypothalamus); electroshock treatment; chemical induction of epileptic insults; and imprisonment. As none of these interventions has led to a well-documented change in sexual orientation[17], there can be little doubt that sexual orientation has become fixed in adulthood and is beyond influencing later. Changes in sexual orientation in adulthood have been described – e.g. from heterosexual to pedophile – but only in cases of brain tumors in the hypothalamus and prefrontal cortex.[18,19] However, such devastating changes in the hypothalamus cannot be interpreted in terms of functional changes in particular neuronal circuits. There are also claims of a change from pedophiles and homosexual men into heterosexual behavior through stereotactical psychosurgery by means of lesions in the nucleus ventromedialis[20], but these interventions are not only of questionable ethical quality, they also do not meet any scientific standard and thus cannot teach us anything. There are also some recent publications postulating that the sexual orientation of homosexual women, more than that of homosexual men, may sometimes change, either spontaneously or under the influence of psychotherapy.[21] The effectiveness of therapy and the absence of bisexuality has, however, never been convincingly shown in these cases.

THE MECHANISM OF SEXUAL DIFFERENTIATION OF THE BRAIN: NEUROBIOLOGICAL FACTORS

In male rats, testosterone is turned into estrogens locally in the brain, and these estrogens then masculinize the brain. In humans, however, the main mechanism appears to involve the *direct* effects of testosterone on the developing brain. The androgen insensitivity syndrome is caused by mutations in the receptor gene for androgens. Despite their genetic (XY) masculinity, these individuals develop as phenotypical women and experience their sexual orientation, fantasies and experiences as 'heterosexual', without gender problems.[22]

On the other hand, when a boy has a 5α-reductase-2 or 17β-hydroxy-steroid dehydrogenase-3 deficiency preventing peripheral testosterone from being transformed into dihydrotestosterone, a 'girl' with a large clitoris is born. These children are generally raised as girls. However, when the testosterone production goes up in these XY children during puberty, this 'clitoris' grows to penis size, the testicles descend, and the child's build begins to masculinize and become muscular. Despite the fact that these children are raised as girls, the majority (60%) change into heterosexual males[23–26], apparently due to the organizing effect of testosterone on early brain development.

Boys who are born with a cloacal exstrophy – i.e. with bladder exstrophy and a partly or wholly absent penis – are usually changed into girls immediately after birth. A recent survey shows that in adulthood only 65% of these children who were changed into girls continue to live as girls, and when individuals with gender dysphoria are excluded this number is 47%.[27,28] From these examples it appears that the direct action of testosterone on the developing brain in boys is of the utmost importance for the development of the male gender and heterosexual orientation. Moreover, studies on cloacal exstrophy suggest that the postnatal testosterone peak is not crucial for gender development, because these children generally undergo operation shortly after birth.

TRANSSEXUALITY

Transsexuality is characterized by a conviction of having been born in the wrong body. The prevalence of transsexuality is 1:10,000 for male-to-female transsexuals and 1:30,000 for female-to-male transsexuals. Gender problems often crop up even early in development. Mothers report that, from the moment their sons learned to talk, they insisted on wearing their mother's clothes and shoes, only showed an interest in girls' toys, and mostly played with girls. On the other hand, not all children with gender issues eventually become transsexual. Only in 23% of the cases does a childhood gender problem lead to transsexuality in adulthood.[29,30]

There is a vast array of factors that may lead to gender problems (Table 1). Twin and family research has shown that genetic factors play a part.[30] Rare chromosomal abnormalities may lead to transsexuality, and it was recently found that polymorphisms of the genes for the estrogen receptors α and β and for aromatase also produced an increased risk.[31] Abnormal hormone levels during early development may play a role, as suggested by the high frequency of polycystic ovaries, oligomenorrhea and amenorrhea in female-to-male transsexuals. This observation points to an early intrauterine exposure of the female fetus to abnormally high levels of testosterone.[32] The chance of a girl becoming transsexual in the case of congenital adrenal hyperplasia (CAH),

Table 1. Prenatal factors that influence gender identity (the feeling of being a man or a woman) and that may result in transsexuality.

Genetic factors	Rare chromosomal disorders[1]
	Twin studies[30]
	Polymorphisms in estrogen-receptor-β, androgen receptor and aromatase genes[31]
Phenobarbital/diphantoin	taken by pregnant mother[35]
Hormones	Cloacal extrophy[27,28]
	5 α-reductase-2 or 17β-hydroxy-steroid-dehydrogenase-3 deficiency[23,25,26]
	CAH girls[33,34,42]
	Complete androgen insensitivity syndrome results in XY heterosexual females[22]
	DES sons: 25% gender problems[36]
Social factors?	Postnatally no evidence[1,11,16,29]

when she has been exposed to extreme levels of testosterone in utero, is also greater. Although the chances of transsexuality in these cases are a factor of 300–1000 higher than normal, the risk for transsexuality in this condition is still only 1–3%[33], whereas the chances of serious gender problems are 5.2%.[34] The consensus is, therefore, that girls with CAH should be raised as girls, even when they are masculinized.[24]

Epileptic women who were given phenobarbital or diphantoin during pregnancy also have an increased risk of giving birth to a transsexual child. Both these substances change the metabolism of the sex hormones and can act on the sexual differentiation of the brains of the child. In a group of 243 women who had been exposed to such substances during pregnancy, Dessens et al[35] found three transsexual children and a few more with less radical gender problems; these are relatively large numbers for such a rare condition. On their website these 'DES' (diethylstilbestrol, an estrogen-like substance – see later) sons claim a transsexuality percentage of 35.5% and a gender problem percentage of 14.3%.[36] This is alarming, but needs, of course, to be confirmed in a formal study.

There are no indications that postnatal social factors could be responsible for the occurrence of transsexuality.[29]

Transsexuality and the brain

The theory of the origin of transsexuality is based on the fact that the differentiation of sexual organs takes place during the first couple of months of pregnancy, before the sexual differentiation of the brain. As these two processes have different timetables, it is possible, in principle, that they take different routes under the influence of the factors. If that is the case, one would expect, in transsexuals, female structures in a male brain and vice versa, and indeed, we did find such a reversal in the central nucleus of the bed nucleus of the stria terminalis (BSTc), a brain structure that, in rats, is involved in many aspects of sexual behavior (Figures 1 and 2). However, a gender identity test for a rat does not exist, and this can therefore be studied only in humans.

We did indeed find a clear sex difference in the human BSTc. In men this area is twice the size of that in women and contains twice as many somatostatin neurons. No difference was found regarding size or number of neurons in this area in relation

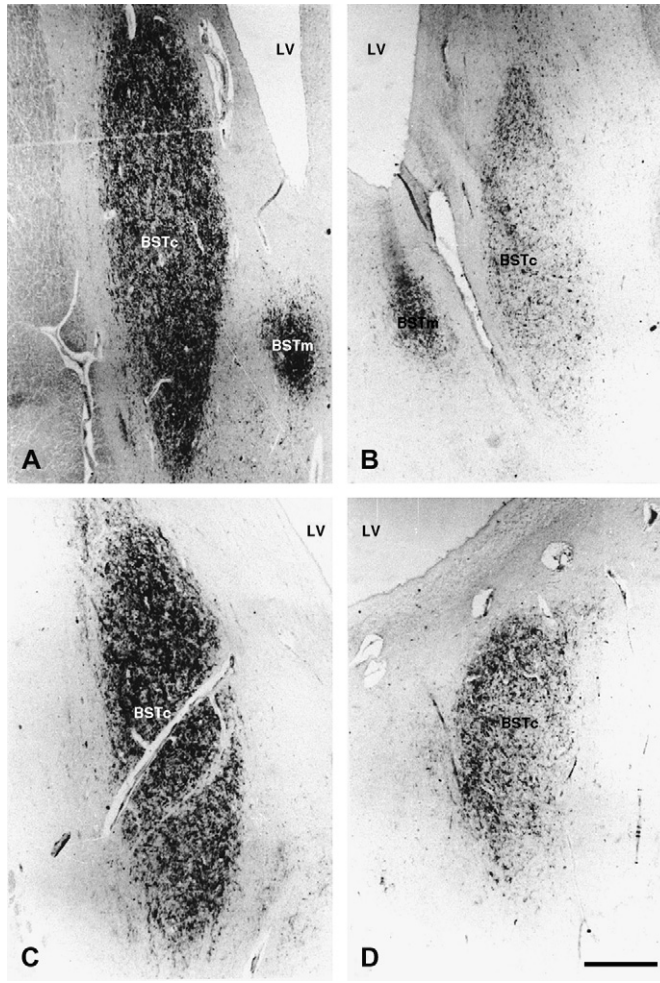

Figure 1. Representative slides of the central part of the bed nucleus of the stria terminalis (BSTc) innervated by fibers stained for vasoactive intestinal polypeptide (VIP). BSTm is the small medial part of the BST. (A) Heterosexual man. (B) Heterosexual woman. (C) Homosexual man. (D) Male-to-female transsexual. Scale bar 0.5 mm. LV, lateral ventricle. Note the sex difference (A versus B), and that the male-to-female transsexual (D) has a female BSTc as far as size and innervation are concerned. From Zhou et al (1995, *Nature* **378:** 68–70) with permission.

to sexual orientation. In male-to-female transsexuals we found a completely female BSTc. Until now we have only been able to obtain material from one female-to-male transsexual, and his BSTc indeed turned out to have all the male characteristics. We were able to exclude the possibility that the reversal of sex differences in the BSTc were caused by changing hormone levels in adulthood[37,38], and it therefore seems that we are dealing with a developmental effect. Our observations thus support the above-mentioned neurobiological theory about the origin of transsexuality. The size of the BSTc and the number of neurons match the gender that transsexuals feel they belong to, and not the sex of their sexual organs, birth certificate or

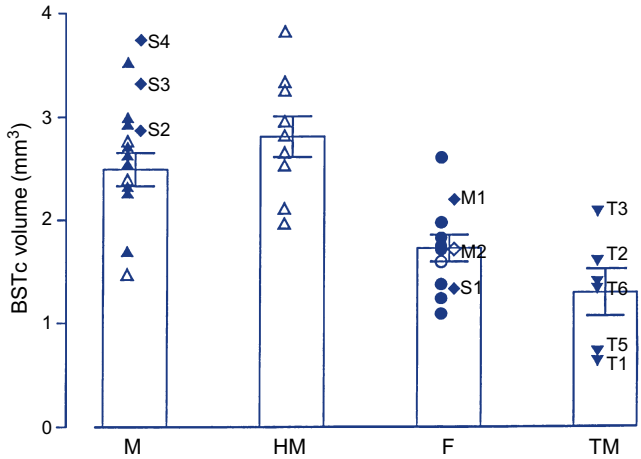

Figure 2. Volume of the bed nucleus of the stria terminalis (BSTc) innervated by fibers stained for vasoactive intestinal polypeptide (VIP) in heterosexual men (M), homosexual men (HM), heterosexual women (F) and male-to-female transsexuals (TM; T1–T6). Patients with abnormal sex-hormone levels are numbered S1–S4. M1 and M2, postmenopausal women. The distribution is mean ± SEM. Open symbols: individuals who died of AIDS. Note the sex difference regarding the volume of the BSTc, the fact that the volume is not affected by abnormal hormone levels in adulthood, and that the volume of the male-to-female transsexuals is female. From Zhou et al (1995, *Nature* **378:** 68–70) with permission.

passport. Unfortunately, the sex difference in the BSTc does not become apparent in the BSTc volume until early adulthood[39], and this neuroanatomical sex difference therefore cannot play a part in the early diagnosis of transsexuality.

SEXUAL ORIENTATION: HETEROSEXUALITY, HOMOSEXUALITY AND BISEXUALITY

Sexual orientation in humans is also determined during early development, under the influence of our genetic background and factors that influence the interactions between the sex hormones and the developing brain (see Table 2).

The importance of genetic factors has become apparent from twin and family research. According to LeVay and Hamer[40], the size of the genetic component in homosexuality for both sexes is over 50%. Which genes play a role here is not yet clear. It is interesting that such a genetic factor has held its own in the population through evolution, as homosexuals do not tend to procreate as much as other members of the group. A good explanation could be that the genetic factors that are responsible for homosexuality also have a beneficial effect on the procreation of the group as a whole. Indeed, Camperio-Ciani et al[41] have found that women on a homosexual male's mother's side tend to be more fertile.

Abnormal hormone levels from the child itself during intrauterine development may influence sexual orientation, as is apparent from the large percentage of bisexual and homosexual girls with CAH.[33,42] Between 1939 and 1960 some 2 million pregnant women in the US and Europe were prescribed diethylstilbestrol (DES) in order to prevent miscarriage. DES turned out not to prevent miscarriage. It is an estrogen-like substance that, in small dosages, does not only give a slightly elevated risk of cervical

Table 2. Prenatal factors that may influence sexual orientation (homosexuality, heterosexuality, bisexuality).

Genetic factors	Twin studies[62,40] Molecular genetics[1]
Hormones	CAH girls[1,33,42] DES[1,43]
Chemicals	Prenatal exposure to nicotine, amphetamine, or thyroid medication[48,49]
Immune response	Homosexual orientation in men is most likely to occur in men with a large number of older brothers[46,47]
Social factors?	Stress of the mother during pregnancy[48,50,51] Being raised by transsexual or homosexual parents does not affect sexual orientation[52]

cancer but also increases the chance of bisexuality or homosexuality in girls[43,44] (but see Titus-Ernstoff et al).[45]

The chance that a boy will be homosexual increases with the number of older brothers. This phenomenon is explained by an immunological response by the mother to a product of the Y chromosome of the sons; the chance of such a response to male factors would increase with every pregnancy resulting in the birth of a son.[46,47]

Prenatal exposure to nicotine, amphetamine, or thyroid-gland hormones increases the chances of giving birth to lesbian daughters.[48,49] A stressed pregnant woman has a bigger chance of giving birth to a homosexual son[48,50] or a lesbian daughter.[51]

Although it has often been postulated that postnatal development is also important for the direction of differentiation, there is no solid proof for this. On the contrary, children who were born after artificial insemination with donor sperm and who were raised by a lesbian couple are heterosexually oriented.[52] There is also no proof for the idea that homosexuality is the result of a deficient upbringing, or that it is a 'life-style choice' or would be brought about by social learning.[17] It is curious, therefore, that some children are still forbidden to play with homosexual friends, an unimaginable relic from the idea that homosexuality would be contagious.

SEXUAL ORIENTATION AND THE BRAIN

Clinical observations have shown the involvement of a number of brain structures in sexual orientation. It has been reported that in some patients with the Klüver–Bucy syndrome, which involves lesions of the temporal lobe, orientation changed from heterosexual to homosexual. Shifts in sexual orientation (to homosexual and pedophile) have also been reported in connection with tumors in the temporal lobe and hypothalamus. Lesions in the preoptic area of the hypothalamus in experimental animals such as ferrets and rats also show shifts in sexual orientation.[1,53]

Several structural and functional differences in the brain have been described in relation to sexual orientation (Figure 3). We found the first difference in the suprachiasmatic nucleus (SCN), the clock of the brain, which in homosexual men turned out to be twice the size of that in heterosexual men.[54] In an experiment with rats a similar difference could be induced by pharmacologically disturbing the interaction between testosterone and the developing brain around the time of birth. This experiment yielded bisexual adult rats which had larger numbers of cells in their SCN.[55] The difference in

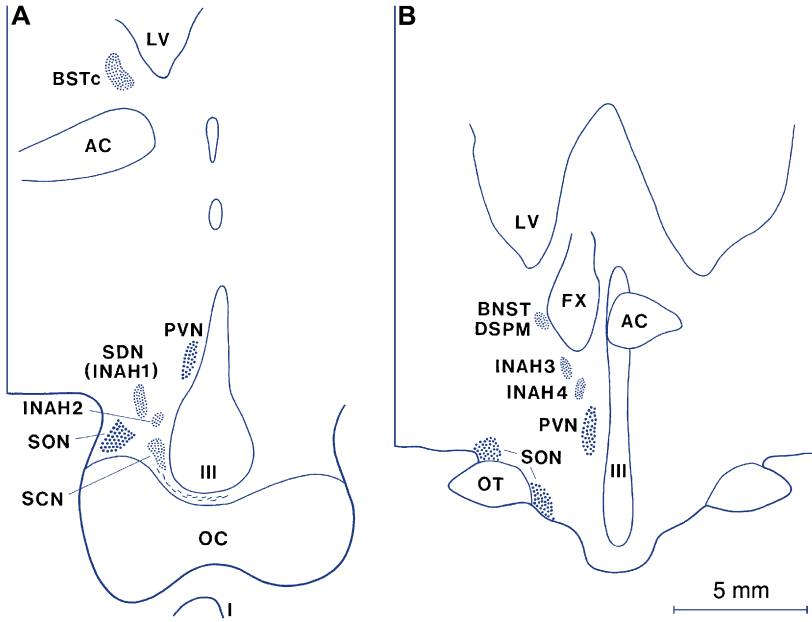

Figure 3. Scheme of the sexually dimorphic structures in the hypothalamus of man. (A) is more rostral than (B). III, third ventricle; AC, anterior commissure; BNST-DSPM, darkly staining posteromedial component of the bed nucleus of the stria terminalis; FX, fornix; I, infundibulum; INAH1–4, interstitial nucleus of the anterior hypothalamus 1–4; LV, lateral ventricle; OC, optic chiasm; OT, optic tract; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SDN, sexually dimorphic nucleus of the preoptic area (= INAH-1); SON, supraoptic nucleus. Scale bar = 5 mm. The AC, BSTc, BNST-DSPM, INAH2, 3, 4, SCN and SDN are different in men and women. The SCN and INAH-3 differ according to sexual orientation. From Swaab (2003, in *Handbook of Clinical Neurology* Aminoff MJ et al (series eds). Amsterdam, Elsevier: 476 pp) with permission.

the SCN was therefore not caused by a change in sexual behavior, as is sometimes suggested, but by a disturbed interaction between sex hormones and the developing brain.

In 1991, LeVay[56] reported that homosexual men, just like heterosexual women, have a smaller area in the frontal part of the hypothalamus (INAH-3). In 1992, Allen and Gorski[57] reported that the anterior commissure of homosexual men is larger than that of heterosexual men. This structure, which is larger in women than in men, takes care of left–right connections of the temporal cortex, and in this way is involved in sex differences related to cognitive abilities and language.

Functional scanning has recently also pointed out differences in the hypothalamus in relation to sexual orientation. The activity of the hypothalamus of homosexual men turned out not to be as responsive to a classic antidepressant (fluoxetine) as that of heterosexual men, which points to a different kind of activity of the serotonergic system.[58] Savic et al[59] made use of scent, a pheromone derived from progesterone and excreted in perspiration in concentrations that are 10 times higher in men than in women. Pheromones influence sexual behavior and stimulate activation in the hypothalamus of heterosexual women and homosexual men in the same way, but did not elicit a response in the hypothalamus of heterosexual men. Apparently heterosexual men are not stimulated by such a male scent, and pheromones thus seem to play a part in sexual behavior according to sexual orientation.

A follow-up study[60] showed that – in contrast to what happened in the frontal part of the hypothalamus in heterosexual women, where this pheromone elicited an activation – in lesbian women this substance was processed by the olfactory system and not by the frontal part of the hypothalamus. Moreover, when lesbian women were exposed to a pheromone derived from estrogens and excreted in the urine of pregnant women, they responded with an activation of the frontal part of the hypothalamus in a way that partly matched the pattern seen in heterosexual men. These observations, too, show that there are hypothalamic circuits that function in a way that depends on sexual orientation. Another study expands this conclusion to cortical areas. With functional magnetic resonance imaging (fMRI) the activity changes in the brain were measured while pictures of men and women were shown. Showing a female face made the thalamus and medial prefrontal cortex of heterosexual men and homosexual women react more strongly, whereas these structures reacted more strongly to the face of a man in homosexual men and heterosexual women.[61]

Neurobiological research in relation to sexual orientation in humans is only just beginning, but already it seems that we have a vast array of brain differences, not only in relation to gender, but also in relation to sexual orientation.

Practice points

- the human fetal brain develops in the male direction through direct action of a boy's testosterone and in the female direction through the absence of this hormone in a girl
- during the intrauterine period, gender identity (the feeling of being a man or a woman), sexual orientation, cognition, aggression and other behaviors are programmed in the brain in a sexually differentiated way
- as sexual differentiation of the genitals takes places in the first 2 months of pregnancy and sexual differentiation of the brain starts in the second half of pregnancy, this means that in the case of an ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the same degree of masculinization of the brain
- our observations on a reversed sex difference in the brain support the idea that transsexuality is based on an opposite sexual differentiation of the brain in the second half of pregnancy and sexual differentiation of sexual organs during the first couple of months of pregnancy
- there is no proof that social environment after birth has an effect on the development of gender or sexual orientation

Research agenda

- the effects on sexual differentiation in the brain of endocrine disrupters in the environment and medicines given to the pregnant mother should be investigated
- structural and functional sex differences in the brain and their functional consequences for human health and disease should be subjected to a systematic and multidisciplinary study

SUMMARY

During the intrauterine period the brain develops in a male direction through direct action of a boy's testosterone on the developing nerve cells, and in a female direction through the absence of this hormone in a girl. In this way gender identity (the feeling of being a man or a woman) and our sexual orientation are programmed into our brain structures when we are still in the womb.

As the sexual differentiation of the genitals takes places much earlier in development (in the first 2 months of pregnancy) than the sexual differentiation of the brain (starting in the second half of pregnancy and becoming overt up until adulthood), these two processes may be influenced independently of each other, which may result in people with male sexual organs who feel female and vice versa (a phenomenon called transsexuality). However, this also means that in the case of an ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the same degree of masculinization of the brain.

Sex differences are not only found in relation to gender and sexual orientation, but also in cognition, aggression, and many other behaviors.

Gender and sexual orientation are influenced by many biological factors (see Tables 1 and 2). There is no proof that social environment after birth has an effect on the development of gender or sexual orientation.

Differences in brain structures and brain functions have been found that are related to sexual orientation and gender.

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Sexual differentiation of the human hypothalamus in relation to gender and sexual orientation

D. F. Swaab and M. A. Hofman

Recently, sex differences in the structures of the human hypothalamus and adjacent brain structures have been observed that seem to be related to gender, to gender problems such as transsexuality, and to sexual orientation, that is, heterosexuality and homosexuality. Although these observations have yet to be confirmed, and their exact functional implications are far from clear, they open up a whole new field of physiological structural–functional relationships in human brain research that has so far focused mainly on such relationships in pathology.

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SEX DIFFERENCES in relative brain size are present from the age of two years onwards[1], and it is therefore not surprising that they have been described in many brain structures and in various functions, for example, in cognition, as well as in neurological and psychiatric diseases, and in reproduction. The claims that females excel in certain tests of verbal skills, and that men have superior mathematical reasoning abilities and perform better in visuo-spatial tasks, have raised major controversies in the literature, because such sex differences are small generally and account for only a small part of the variance, and clear structural–functional relationships are absent[2]. On the other hand, remarkably little attention has been paid so far to the possible structural basis of the often pronounced sex differences in the epidemiology of neurological and psychiatric diseases[3]. The proportions of cases range from more than 75% female in anorexia nervosa and bulimia to more than 75% male in dyslexia, sleep apnoea and Gilles de la Tourette's syndrome (Table 1). Not only might the number of cases of disorders show clear sex differences, but the signs and symptoms and the course of the disease might differ also. Males not only suffer from schizophrenia 2.7 times more often than females[8], they are also prone to a more severe form of this disorder, experience an earlier onset, and exhibit more structural brain abnormalities. Relapses are more severe, and their response to neuroleptic medication is less favourable[8]. On the other hand, sex-specific prevalence can vary with age, and females apparently have a greater susceptibility to acute food deprivation during the first trimester, as was evident from the children born in the Dutch hunger winter of 1944–1945 that resulted in 2.6 times more female than male schizophrenics[5]. Another example is that, following restricted posterior left-hemisphere lesions, 41% of the males and 11% of the females developed aphasia, whereas manual apraxia was found in 6% of the females and 42% of the males[11]. Also, in The Netherlands, the prevalence of transsexualism (see below) is strongly sex-dependent; 1 : 11 900 for male-to-female transsexuals and 1 : 30 400 for female-to-male transsexuals[12]. Finding the structural differences on which the sex differences in neurological and psychiatric disorders are based presents a challenge.

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The hypothalamus

Sex differences in the hypothalamus are thought to be the basis of sex differences in (1) reproductive behaviour, that is, the menstrual cycle in women[13], (2) gender identity, that is, the feeling that one is either male or female, and (3) sexual orientation, that is, homosexuality and heterosexuality[2,13,14].

Currently, no information is available about the factors that might influence gender, and cause transsexuality in humans. However, the determinants of human sexual orientation seem to be legion, as sexual orientation is influenced by a number of factors, such as genetic factors (as appeared from studies in twins and molecular genetics)[15,16]. Hamer's group[16] found a linkage between DNA markers on the X chromosome, and male sexual orientation. Sex hormones also have an influence on sexual orientation, as the increased proportion of bisexual and homosexual girls that have adrenogenital syndrome suggests[17]. In addition, diethylstilboestrol (DES), a compound that is related to oestrogens, increases the occurrence of bisexuality and homosexuality in girls[18]. Maternal stress is thought to lead to increased occurrence of homosexuality in boys[19] and girls[20]. Also, social factors are presumed to be involved[21] in sexual orientation, although evidence in support of this effect has not yet been found. In fact, the observation that children that are raised by lesbian couples or by transsexuals generally have a heterosexual orientation[22,23] does not support the possibility of the social environment being an important factor for determining sexual orientation. On the basis of animal experiments, it is expected that all compounds that influence metabolism of neurotransmitters in development might affect sexual differentiation of the brain also[3]. All these influences are supposed to affect the interaction between endogenous sex

hormones and the developing brain and thus lead to structural and functional changes of the brain. However, there is also recent experimental evidence for primary genetic control of sexual differentiation, in animals, that does not involve sex hormones. Results obtained from cultures of embryonic rat brain indicate that dopaminergic neurones might develop morphological and functional sex differences in the absence of sex steroids[3].

Hormones and brain development

In analogy with observations in many mammalian species, the human brain might well undergo sexual differentiation during its development as a result of an organizing effect of sex hormones, and such a structural organization might be the basis for functional sex differences[3,24]. In experimental animals, sex hormones affect the developing brain mainly through testosterone that has been transformed into oestrogens by aromatase during a crucial period in development. The crucial period in the rat occurs during late pregnancy and the first two weeks of neonatal life and is thought to be initiated by the surge of plasma testosterone that occurs in the male fetus around embryonic day 17 and 18. Activity of aromatase is high in the medial preoptic hypothalamic region of most mammals, including human fetuses, especially in the prenatal and neonatal period[25]. The presence of aromatase in the developing brain explains the extraordinary ability of oestrogens to mimic, at least partly, the organizing actions of androgens[25]. In addition, not only oestrogen receptors but also testosterone receptors have been observed in the mammalian limbic system and, therefore, male differentiation of some brain regions might be under direct control of testosterone (for reviews, see Refs 3, 25 and 26). In the human brain, neither sex hormone receptors nor aromatase have, as yet, been localized on a cellular level. The stages of development at which sex steroids determine sexual differentiation of the human brain are most probably the three periods during which sexually dimorphic peaks in gonadal hormone levels are found, namely during the first half of gestation (when the genitalia are formed), during the perinatal period, and during puberty[27]. In human neonates of 34–41 weeks of gestation, the level of testosterone is tenfold higher in males than in females[28]. Although the peak of testosterone during puberty is thought generally to be involved in activation rather than organization, the number of neurones of the hypothalamus of the domestic pig, to our surprise, showed a twofold increase in a sexually dimorphic hypothalamic nucleus around puberty[29] – thus, late organizational effects cannot be excluded. Few data are available on the exact period in development when the human brain differentiates according to sex. Brain weight is sexually dimorphic from two years postnatally onwards, taking differences in body size between boys and girls into account[1]. The supposition of Dörner[30] that structural sexual differentiation of the human hypothalamus would take place between four and seven months of gestation was based only on the observation that the matrix layer around the third ventricle, in which the hypothalamic cells are presumed to have been formed, has disappeared by seven months of gestation. Yet,

Table 1. Ratios for females over males suffering from particular neurological and psychiatric diseases

Disease	% Female : male	Refs
Anorexia nervosa	93 : 7	4
Bulimia	75 : 25	4
Schizophrenia following Dutch hunger winter	72 : 28	5
Anxiety disorder	67 : 33	6
Depression	63 : 37	6
Multiple sclerosis	58 : 42	7
Severe mental retardation	38 : 62	8
Autism	29 : 71	8
Stuttering	29 : 71	8
Schizophrenia	27 : 73	8
Dyslexia	23 : 77	8
Sleep apnoea	18 : 82	9
Gilles de la Tourette	10 : 90	10

about 80% of the cells of the sexually dimorphic nucleus appeared to be formed postnatally[27] (Fig. 1). In addition, it has also become clear that cell death, rather than cell division, might be the most important mechanism in sexual differentiation of the nervous system[27,31]. This mechanism takes place in the human sexually dimorphic nucleus between four years of age and puberty[27] (Fig. 1). It might be said that the evidence that is currently available suggests that sexual differentiation of the human hypothalamus becomes apparent between two years of age and puberty, although this might, of course, be based upon processes that were programmed much earlier, that is, in mid-pregnancy or during the neonatal period.

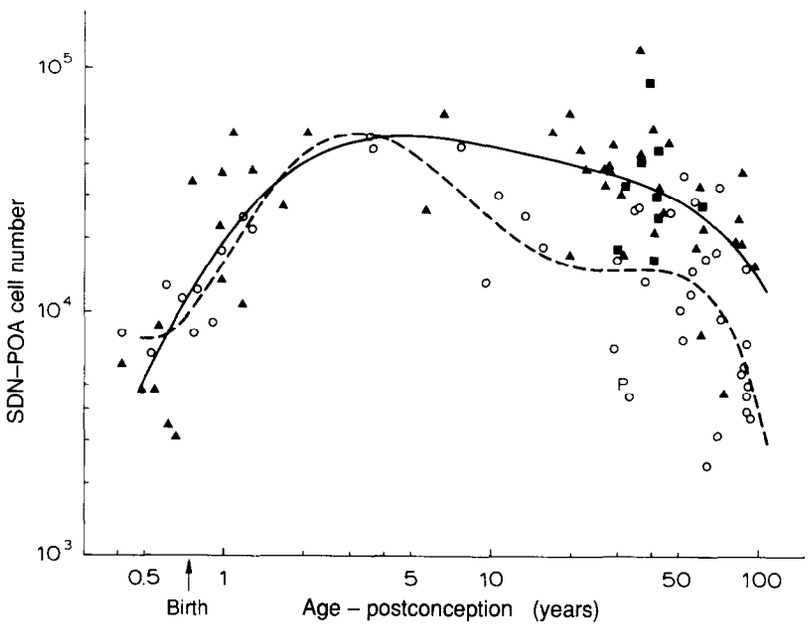

Fig. 1. ***Development and sexual differentiation of the human sexually dimorphic nucleus of the preoptic area (SDN–POA) of the hypothalamus.*** *Log–log scale. Note that, at the moment of birth, the SDN–POA is equally small in boys (triangles) and girls (circles) and contains only about 20% of the cell number found at 2–4 years of age. Cell numbers reach a peak value around 2–4 years postnatally, after which a sexual differentiation occurs in the SDN–POA as a result of a decrease in cell number in the SDN–POA of women, whereas the cell number in men remains approximately unchanged up to the age of 50. The SDN–POA cell number in homosexual men (squares) does not differ from that in the male reference group (see also Fig. 4). The curves are quintic polynomial functions that are fitted to the original data for males (solid line) and females (broken line).* Reproduced, with permission, from Ref. 27.

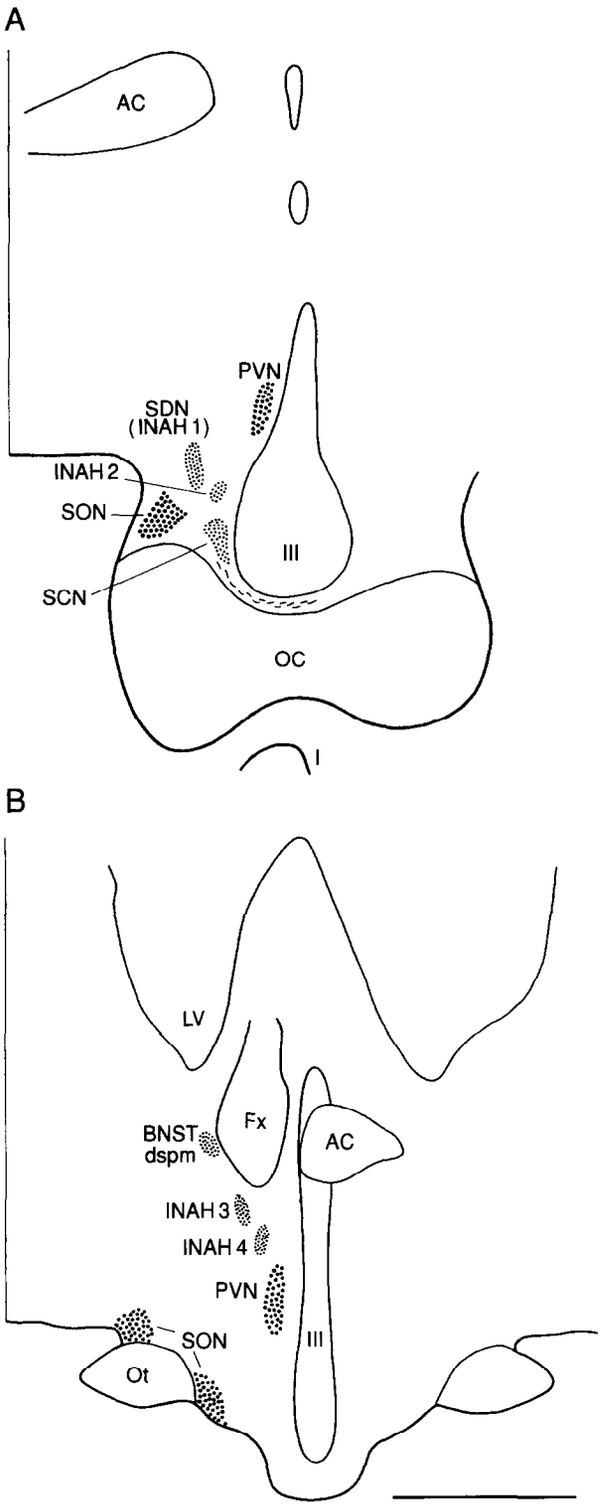

Fig. 2. ***Topography of the sexually dimorphic structures in the human hypothalamus.*** **A** *is a more rostral view than* **B.** *Abbreviations: III, third ventricle; AC, anterior commissure; BNST-dspm, darkly staining posteriomedial component of the bed nucleus of the stria terminalis; Fx, fornix; I, infundibulum; INAH1–4, interstitial nucleus of the anterior hypothalamus 1–4; LV, lateral ventricle; OC, optic chiasm; Ot, optic tract; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SDN, sexually dimorphic nucleus of the preoptic area; and SON, supraoptic nucleus. Scale bar, 5 mm.*

Sexually dimorphic nucleus of the preoptic area

The sexually dimorphic nucleus of the preoptic area (SDN–POA) of the hypothalamus, as first described in the rat by Gorski and colleagues[32], is still the most conspicuous morphological sex difference in the mammalian brain. The cytoarchitectonic sex difference of this cell group, which is three to eight times larger in male rats than in female rats, is so evident that it can even be observed with the naked eye in Nissl-stained sections. Lesions of the SDN–POA affect masculine components of sexual behaviour in rat[33,34], and the positive correlations between the volume of the SDN–POA, and both testosterone levels and male sexual activity in rat studies, suggest a similar relationship[24]. On the other hand, the extent of the changes in sexual behaviour following lesions to the SDN–POA is so small[33,34] that it is quite likely that the major function of the SDN–POA has not yet been established. We have found a SDN–POA in the human hypothalamus that is – judging by its sex difference in size and cell number, localization and cytoarchitecture – probably homologous to the SDN–POA in the rat[27] (Fig. 2). The presence of galanin-containing neurones in the human SDN–POA (Ref. 35), and in the same area in rat[36], is consistent with this. Recent support for such a similarity comes from the observation that the human SDN–POA contains thyrotropin-releasing hormone (TRH)-containing neurones[37] similar to what has been reported in rat[38]. The human SDN–POA is identical to the intermediate nucleus as described by Braak and Braak[39]. Morphometric analysis of the human SDN–POA revealed that the volume is more than twice as large in young adult men as it is in women, and contains about twice as many cells in men[40] (Fig. 3). The magnitude of the SDN–POA sex difference was found not to remain constant throughout adulthood, but to depend on age (Fig. 3). In males, a major reduction in SDN–POA cell number was observed between the ages of 50 and 60 years. This change resulted in a much less pronounced sex difference in cell numbers. In females of over 70 years of age, cell death was found to be prominent, dropping to values that were only 10–15% of the cell number found in early childhood, so that the sex difference in the SDN–POA increases again in old people[40] (Fig. 3). This sex difference in the pattern of aging, together with the fact that sexual differentiation in the human SDN–POA occurs only after the fourth year of age[27], might explain why Allen and colleagues[41], who worked with a sample of human adults that contained a large number of middle-aged subjects, did not find a significant sex difference in the size of the SDN–POA (Ref. 27). Since there was more than one sexually dimorphic nucleus in the hypothalamus, Allen and colleagues[41] did not want to conform to the name SDN–POA. Unfortunately, they did not go back to the original name of 'intermediate nucleus'[39] but called it the 'interstitial nucleus of the anterior hypothalamus 1 (INAH1)', confusing the nomenclature even further. The age distribution, however, does not explain why LeVay[42] was also unable to find a sex difference in the volume of the INAH1. However, it should be noted that LeVay[42] and Allen and colleagues[41] measured only the volume of hypothalamic structures. Volume is susceptible to various pre- and post-mortem factors, such as differences in agonal state and fixation time[27,43] but also to histological procedures and methods, such as section thickness. Therefore, it is essential to include data on

total cell numbers of hypothalamic nuclei, since this parameter is not influenced by such factors.

Other hypothalamic sexually dimorphic structures

Allen and colleagues[41] described two other cell groups (INAH2 and INAH3) in the preoptic-anterior hypothalamic area of humans that were larger in the male brain than in the female brain. It is unclear which nuclei in the rat are homologous to the INAH2 and INAH3 and so far nothing is known about their neurotransmitter content. LeVay[42] could not confirm the sex difference in the INAH2 but did find such a difference in the INAH3. Since no immunocytochemistry was performed, it is not clear whether the nuclei should be considered as, for example, islands of the paraventricular nucleus (PVN) or bed nucleus of the stria terminalis, or as separate anatomical entities.

Another sex difference was described by Allen and Gorski[44] in what they called the 'darkly staining posteriomedial component of the bed nucleus of the stria terminalis' (BNST-dspm). The volume of the BNST-dspm was found to be 2.5 times larger in males than in females.

The vasopressin-containing subnucleus of the suprachiasmatic nucleus (SCN) showed a sex difference in shape but not in volume or vasopressin-cell number. The shape of the SCN was elongated in women and more spherical in men[27]. However, the vasoactive intestinal polypeptide (VIP)-containing subnucleus of the human SCN was found to be twice as large in young men (ten to 30 years) as in young women, and contained twice as many cells[45]. In the age group of 41 to 65 years, this sex difference was reversed, and it disappeared altogether after the age of 65 (Refs 45 and 46). These observations show again how important age is in the case of sexual dimorphism of the human brain.

The anterior commissure was found to be 12% larger in females[47], and the interthalamic adhesion, a grey structure that crosses the third ventricle between the two thalami, was present in more females (78%) than males (68%)[47], confirming the old study of Morel[61] of 1947 (compare Ref. 47). The two latter observations suggest a greater connectivity between the cerebral hemispheres of women as compared with men.

Development of the human SDN–POA

In mid-pregnancy, the SDN–POA can already be distinguished in the human fetal brain[27], yet the SDN–POA cell number and volume at full-term birth are only 22% and 18%, respectively, of the values found between two and four years of postnatal age. During the first postnatal years, up to the age of two to four years, the SDN–POA cell number increases rapidly at the same rate in both boys and girls, and only after this age does the human SDN–POA differentiate according to sex, owing to a decrease in both SDN–POA volume and cell number in women. In men, these parameters remain unaltered up to the age of about 50 (Fig. 1)[27]. The surprisingly late postnatal sexual differentiation of the human SDN–POA might be a general phenomenon in the human brain, as it seems as if the sex difference in the volume of the BNST-dspm does not occur until adulthood[44]. Together, these data support the notion that sexual differentiation of the human hypothalamus takes place after the perinatal period and before adulthood, rather than during mid-gestation, although it is possible that the pre- or perinatal peak of testosterone programmes cell death a few years later.

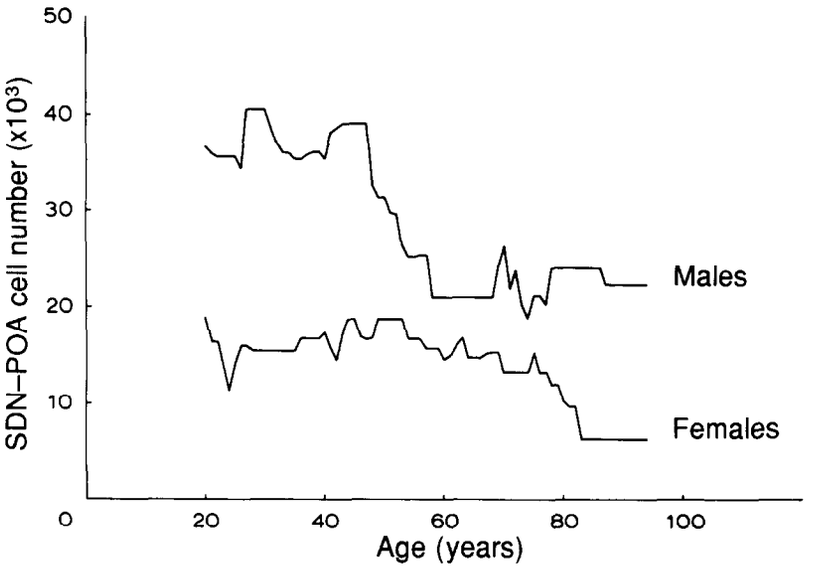

Fig. 3. ***Age-related changes in the total cell number of the sexually dimorphic nucleus of the preoptic area (SDN–POA) in the human hypothalamus.*** *The general trend in the data is enhanced by using smoothed growth curves. Note that in males, SDN–POA cell number declines steeply between the age of 50 and 60 years, whereas in females, from the age of about 50 years, a more gradual cell loss is observed, which continues up to old age. These growth curves demonstrate that the reduction in cell number in the human SDN–POA in senescence is a non-linear, sex-dependent process.* Reproduced, with permission, from Ref. 40.

The SDN–POA and sexual orientation

A prominent theory about the development of heterosexual–homosexual orientation is that it develops as a result of an interaction between the developing brain and sex hormones. According to Dörner's hypothesis[48], male homosexuals would have a female differentiation of the hypothalamus.

Once it had been found that the SDN–POA of the hypothalamus of young male adults contains twice as many cells as that of females, Dörner's hypothesis of sexual orientation could be put to the test. In contrast to this hypothesis, neither the SDN–POA volume nor its cell number in the hypothalamus of homosexual men differed from that of the male reference group in the same age range or from that of a heterosexual control group of subjects that also suffered from AIDS (Ref. 49) (Fig. 4). The fact that no difference in SDN–POA cell number was observed between homosexual and heterosexual men did not agree with the global formulation of Dörner's hypothesis that male homosexuals have 'a female hypothalamus'. A similar conclusion can be drawn from the observations on the SCN and anterior commissure in homosexual men (see below).

The SCN and sexual orientation and reproduction

The first difference in the human brain that relates to sexual orientation was found in the vasopressin-containing subnucleus of the SCN that was found to be twice as large in homosexual men[49]. Our observation that the volume of the vasopressin subnucleus of the SCN in homosexual men was 1.7

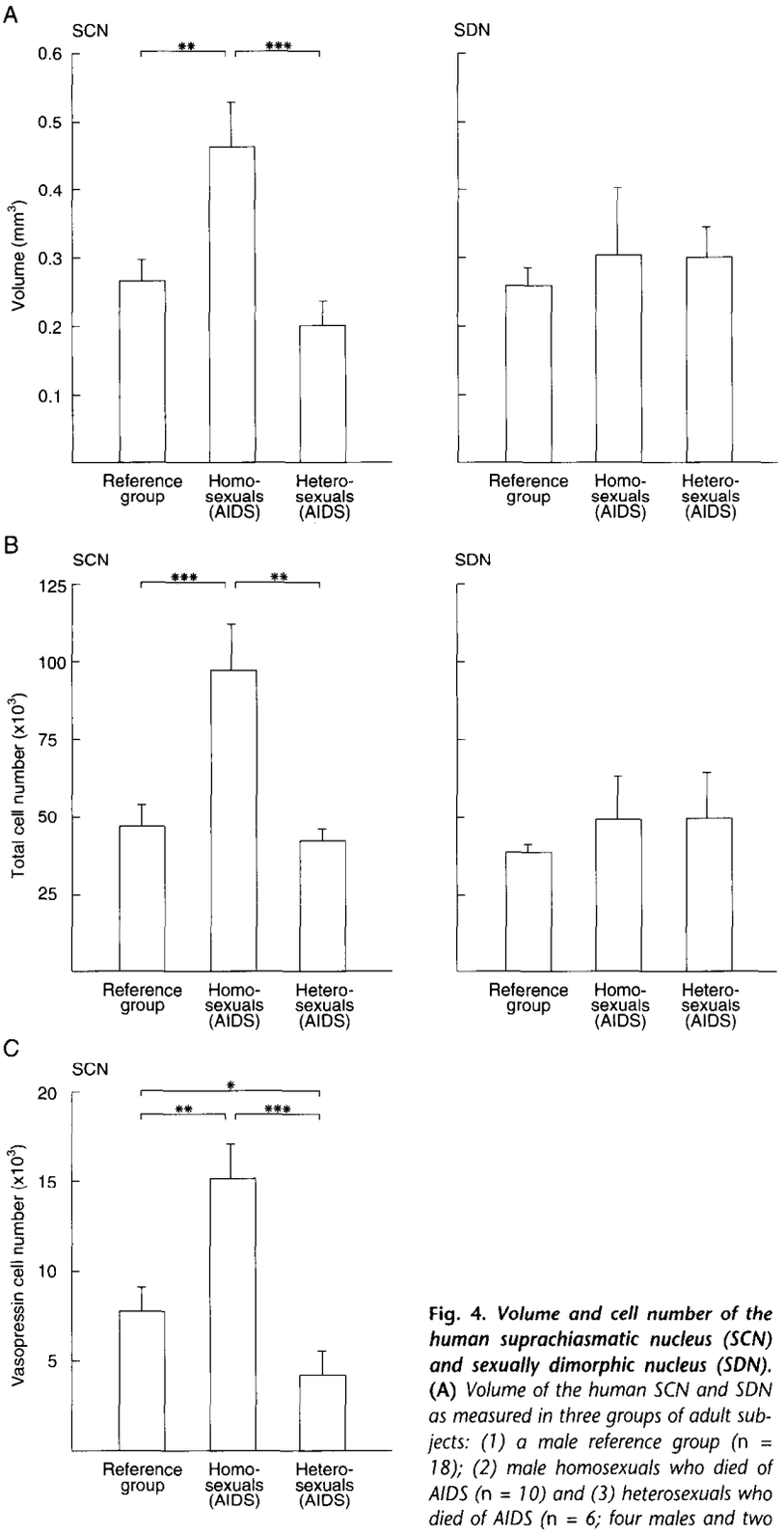

Fig. 4. ***Volume and cell number of the human suprachiasmatic nucleus (SCN) and sexually dimorphic nucleus (SDN).*** **(A)** *Volume of the human SCN and SDN as measured in three groups of adult subjects: (1) a male reference group (n = 18); (2) male homosexuals who died of AIDS (n = 10) and (3) heterosexuals who died of AIDS (n = 6; four males and two females). The values indicate medians and the standard deviation of the median. The differences in the volume of the SCN between homosexuals and the subjects from both other groups, are significant statistically. (Kruskal–Wallis multiple comparison test, *P < 0.05; **P < 0.01; ***P < 0.001.) Note that none of the parameters measured in the SDN (A and B) showed significant differences among the three groups (P always greater than 0.4).* **(B)** *Total number of cells in the human SCN and SDN. The SCN in homosexual men contains 2.1 times as many cells as in the reference group of male subjects, and 2.4 times as many cells as the SCN in heterosexual AIDS patients.* **(C)** *The number of vasopressin (VP)-containing neurones in the human SCN (the human SDN does not contain VP-producing cells). The SCN in homosexual men contains, on average, 1.9 times as many VP-producing neurones as the reference group of male subjects and 3.6 times as many VP neurones as the SCN in heterosexual AIDS patients. The SCN of heterosexual individuals who died of AIDS contains less VP cells than the subjects from the reference group.* Reproduced, with permission, from Ref. 49.

times as large and contained 2.1 times as many cells as the SCN of the male reference group (Fig. 4)[49] also implied that the difference in SCN volume could not be attributed to differences in shrinkage of hypothalamic tissue during the histological procedure. The difference in the vasopressin-containing cells of the SCN in relation to sexual orientation seems to be rather specific, since the number of VIP neurones in the SCN of homosexual and heterosexual men was not different[50]. The SCN is, indeed, the clock of the human brain, and regulates circadian and circannual changes[51]. Differences in the SCN between homosexual and heterosexual men might thus go together with differences in circadian rhythms. Recently, it was found that gay men arise and retire earlier each day than heterosexual men[52] (see also Ref. 53 – although not interpreted by the authors in this way). This does not imply that the SCN, apart from its circadian function, could not also be involved in sexual behaviour as was suggested by LeVay[42]. On the contrary, there are many observations that suggest an involvement of the SCN in reproductive processes. As long as 20 years ago, post-coital ultrastructural changes that indicate neuronal activation were observed in the SCN of the female rabbit. It is also important that the activity of neurones of the SCN increases suddenly around puberty. This is indicative of the addition of a reproductive function to the already matured circadian functions of the rat SCN. In addition, efferents of the rat SCN innervate several regions that are involved in reproductive behaviours, for example, the preoptic area, medial amygdala and bed nucleus of the stria terminalis. The rat ovarian reproductive cycle is controlled by the SCN, possibly by VIP fibres via direct innervation of luteinizing hormone releasing hormone (LHRH)-containing neurones. Several morphological sex differences have been reported that support putative reproductive functions of the SCN. The SCN of male rats contains a larger amount of axo–spine synapses, postsynaptic density material and asymmetrical synapses compared with that of female rats. Their neurones also contain more nucleoli. The sex difference in the shape of the vasopressin-containing subdivision of the human SCN, and the sex difference in the number of VIP-containing neurones in the human SCN (see above), is also consistent with sexually dimorphic functions. In seasonal breeders, immunoreactivity to VIP in the SCN fluctuates in relation to seasonal fluctuations in sexual activity. The recently observed activation of c-*fos* in the SCN by sexual stimulation also points to a role of the SCN in reproduction (for references to this paragraph, see Ref. 45). An interesting analogy to our observations on the enlarged SCN in homosexual men and sexual orientation was found recently by Bakker and colleagues[54] who found that rats treated with the aromatase inhibitor 1,4,6-androstratriene-3,17-dione (ATD) showed a partner preference for female rats when tested in the late dark phase, and a preference for male rats when tested in the early dark phase. This is the first indication of the involvement of the SCN in sexual orientation. In the same ATD-treated 'bisexual' rats, we have found recently an increased number of vasopressin-expressing neurones in the SCN (Ref. 55). This supports the hypothesis that the increased number of vasopressin-containing neurones

that was observed in the SCN of homosexual men[49] might be caused by a difference in the interaction of testosterone, aromatase, oestrogens, sex hormone receptors and the developing brain. The possibility of sex hormones playing a role in the development of the SCN is reinforced by an observation of Södersten and colleagues[56] who showed that the amplitude of the circadian rhythm in sexual behaviour, for which the SCN is the substrate, is enhanced by anti-oestrogen treatment of the neonatal animal.

Other hypothalamic differences in relation to sexual orientation

The second anatomical difference in the hypothalamus, according to sexual orientation, was found by LeVay in the INAH3 (Ref. 42). This nucleus was twice as large in heterosexual men as in homosexual men. There is no evidence for LeVay's assumption that the INAH3 would be homologous to the SDN–POA in the rat. Recently, Fliers and colleagues[37] found no TRH-containing neurones in the INAH3, whereas they were present in the SDN–POA/INAH1. This supports the possibility that the human SDN–POA and INAH1 is homologous to the rat SDN–POA (see above). Since no homology to hypothalamic structures in experimental animals is known for the INAH3, it is concluded that the functional consequences, also of LeVay's finding, are currently far from clear.

A third idiosyncrasy that concerns sexual orientation was described by Allen and Gorski[57] who found that the anterior commissure was larger in homosexual men than in (presumed) heterosexual men and women. Although LeVay's finding of a smaller INAH3 in homosexual men and heterosexual women agrees with Dörner's hypothesis[48] that homosexual men would have a female hypothalamus, the observations in the SDN–POA, the SCN (Ref. 49) and the anterior commissure[57] do not support this idea but support the idea of a 'third sex', that is, a 'different' hypothalamus in homosexual men that is neither similar to that in females, nor to that in male heterosexuals.

A recent abstract reported that the isthmus of the corpus callosum of gay men was 13% larger than that of heterosexual men[58], a similar result to the one reported for the anterior commissure[57].

Transsexuality

Transsexuals have, often from childhood onwards, the strong feeling of having been born the wrong sex. Their desire to resemble the opposite sex is so strong that they are even willing to undergo surgery to achieve this.

This problem of gender identity has been proposed to develop as a result of a disturbed interaction between the developing brain and sex hormones[48]. In view of the relationship between the hypotheses on the development of gender and sexual orientation, it is interesting to note that 60% of the male-to-female transsexuals are orientated sexually towards males, and that 10% are bisexual. In no less than 95% of the cases are female-to-male transsexuals orientated sexually towards women[59]. The high proportion of transsexuals that are orientated sexually towards their own genetic sex indicates that indeed similar (but as yet unknown) mechanisms might play a role in the development of both gender and sexual orientation. We have studied the hypothalami of five male-to-female transsexuals, and have found a remarkable inverse relationship between the cell number in the SDN–POA, and the number of vasopressin-containing neurones in the SCN (Ref. 27). When the SDN–POA was small in a male-to-female transsexual, the number of vasopressin-containing neurones in the SCN was high, and vice versa. This might point to the possibility that the developing brain of these transsexuals was exposed to different amounts of oestrogens, since it was found that, in rats, inhibition of aromatase by ATD caused the size of the SDN–POA to decrease[60], and the number of vasopressin-containing neurones caused it to increase[55]. Since the size of the SDN–POA, and the number of vasopressin-containing neurones in the SCN, was extremely variable between the five male-to-female transsexuals, the size of these two structures does not seem to be related to their problem of gender identity. The search for structures that might be related directly to problems of gender identity, that is, structures whose anatomy is 'female' in genetically male transsexuals, is in progress.

Concluding remarks and summary

Functional sex differences in reproduction, gender and sexual orientation might be based on anatomical differences in the hypothalamus. Differences in structure in the human hypothalamus that are related to gender or sexual orientation were indeed reported recently. The magnitude of such differences depends strongly on age, and replication of these data is certainly necessary. Since the size of brain structures might be influenced by pre-mortem factors (for example, by agonal state) and post-mortem factors (for example, by fixation time), not only should measurements of volume be performed, but also a parameter that is not dependent on such factors as, for example, total cell number of the brain structure in question should be estimated.

The period of overt sexual differentiation of the human hypothalamus occurs between approximately four years of age and puberty, thus, much later than is presumed generally. In principle, it offers the possibility of interaction of a multitude of postnatal factors that act on sexual differentiation of the brain, not only of a genetic or hormonal, but also of a chemical and psycho-social nature.

The mechanisms that cause sexual differentiation of hypothalamic nuclei, the prenatal and postnatal factors that influence this process, and the exact functional consequences of the morphological hypothalamic differences await further elucidation.

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Acknowledgements
Brain material was obtained from the Netherlands Brain Bank (co-ordinator R. Ravid). We thank W.T.P. Verweij for her secretarial help, J.N. Zhou for his help with Fig. 2, and NWO (#900-552-134) and EC (Biomed I Project NO PL 931536) for financial support.

Neural networks that co-ordinate locomotion and body orientation in lamprey

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The networks of the brainstem and spinal cord that co-ordinate locomotion and body orientation in lamprey are described. The cycle-to-cycle pattern generation of these networks is produced by interacting glutamatergic and glycinergic neurones, with NMDA receptor–channels playing an important role at lower rates of locomotion. The fine tuning of the networks produced by 5-HT, dopamine and GABA systems involves a modulation of Ca^{2+}-dependent K^+ channels, high- and low-threshold voltage-activated Ca^{2+} channels and presynaptic inhibitory mechanisms. Mathematical modelling has been used to explore the capacity of these biological networks. The vestibular control of body orientation during swimming is exerted via reticulospinal neurones located in different reticular nuclei. These neurones become activated maximally at different angles of tilt.

Trends Neurosci. (1995) 18, 270–279

THE NERVOUS SYSTEM can be studied at many different levels from ion channels, single cells and synapses to neural networks and global cognitive functions. Individual neural networks represent an important category of functional modules that are at an intermediary level of complexity in the nervous system. Different specialized networks are used in sensory systems, as well as in the co-ordination of a host of different movement patterns from swallowing to skilled movements. One of the most complex movement patterns that any nervous system can co-ordinate, and recruit at will, is that producing