growth, nuclear segregation, DNA repair, and meiosis, and deletion of *HRR25* results in cell cycle defects. These phenotypes, coupled with the similarity of the *HRR25* sequence to the sequence of the Raf,c-Mos protein kinase subgroup (Fig. 3A), suggest that *HRR25* might play a similar role in *S. cerevisiae* growth and development. The defects in DNA double-strand break repair and aberrant growth properties revealed by mutations in the *HRR25* kinase extend the possible functions of protein kinases in cell growth and place *HRR25* with *CDC7* in a functional category of yeast kinase associated with DNA metabolism.

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10. The *HRR25* gene was isolated by complementing for MMS sensitivity with a genomic library constructed in the plasmid YCp50 (*27*). An *hrr25-1* strain was transformed by standard methods (*27*), and transformants were replicated to media containing 0.01% MMS. Among 1200 transformants, a single MMS-resistant isolate was identified.
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12. Deletion of the *HRR25* coding sequence used a *hisG::URA3::hisG* cassette [E. Alani *et al.*, *Genetics* **116**, 541 (1987)]. The 3.1-kb *HRR25* Sal I fragment (Fig. 1) was first cloned into pBluescript (Stratagene). This plasmid was digested with Bgl II, and the two Bgl II fragments that span the entire *HRR25* gene and its flanking sequences were deleted (Fig. 1). Into this deletion was introduced the 3.8-kb Bam HI–Bgl II *hisG::URA3::hisG* fragment from pNKY51 to create the *hrr25Δ* allele. Sal I digestion yielded a linearized fragment that deleted the entire *HRR25* locus.
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29. We thank L. Caballero, A. M. Quinn, S. Hanks, N. Dhillon, and T. Hunter for helpful comments and assistance with sequence alignments; R. Keil for help with x-irradiation screening; and S. Reed and his lab for assistance with an initial microscopic examination. M.F.H. is a Lucille P. Markey Scholar in Biomedical Sciences. Supported by grants from the Lucille P. Markey Charitable Trust and the NIH.

26 February 1991; accepted 23 May 1991

A Difference in Hypothalamic Structure Between Heterosexual and Homosexual Men

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The anterior hypothalamus of the brain participates in the regulation of male-typical sexual behavior. The volumes of four cell groups in this region [interstitial nuclei of the anterior hypothalamus (INAH) 1, 2, 3, and 4] were measured in postmortem tissue from three subject groups: women, men who were presumed to be heterosexual, and homosexual men. No differences were found between the groups in the volumes of INAH 1, 2, or 4. As has been reported previously, INAH 3 was more than twice as large in the heterosexual men as in the women. It was also, however, more than twice as large in the heterosexual men as in the homosexual men. This finding indicates that INAH is dimorphic with sexual orientation, at least in men, and suggests that sexual orientation has a biological substrate.

SEXUAL ORIENTATION—SPECIFICALLY, the direction of sexual feelings or behavior toward members of one's own or the opposite sex—has traditionally been studied at the level of psychology, anthropology, or ethics (*1*). Although efforts have been made to establish the biological basis of sexual orientation, for example, by the application of cytogenetic, endocrinological, or neuroanatomical methods, these efforts

have largely failed to establish any consistent differences between homosexual and heterosexual individuals (*2, 3*).

A likely biological substrate for sexual orientation is the brain region involved in the regulation of sexual behavior. In nonhuman primates, the medial zone of the anterior hypothalamus has been implicated in the generation of male-typical sexual behavior (*4*). Lesions in this region in male monkeys impair heterosexual behavior without eliminating sexual drive (*5*). In a morphometric study of the comparable region of the

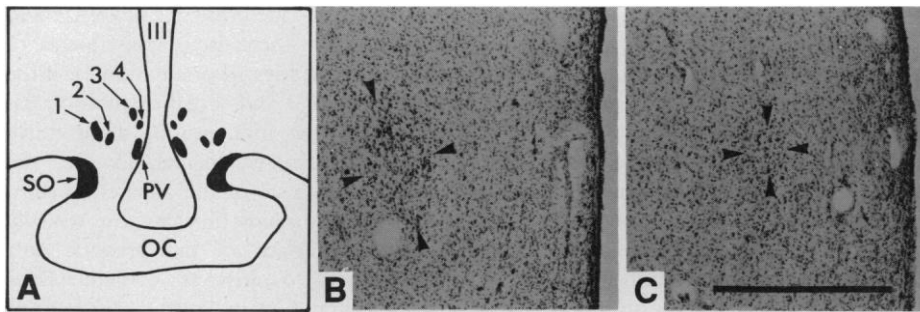Salk Institute for Biological Studies, San Diego, CA 92186.

Fig. 1. (**A**) Semidiagrammatic coronal section through the human hypothalamus at the level of the optic chiasm (OC). The four cell groups studied (INAH 1, 2, 3, and 4) are indicated by the corresponding numerals. All four nuclei are not generally visible in the same coronal section: INAH 1 lies most anteriorly and INAH 4 most posteriorly. Supraoptic nucleus, SO; paraventricular nucleus, PV; and third ventricle, III. (**B**) Micrograph of INAH 3 from the left hypothalamus of a heterosexual male. The third ventricle is at the right of the figure. Arrowheads outline INAH 3. (**C**) Section from a homosexual male comparable to that in (B). INAH 3 is poorly recognizable as a distinct nucleus, but scattered cells similar to those constituting the nucleus in the heterosexual men were found within the area indicated by the arrowheads. The illustrated sections are near the middle of the anteroposterior extent of the nucleus in each case. The scale bar (1 mm) applies to (B) and (C).

human hypothalamus (from men and women of unknown sexual orientation), two small groups of neurons (INAH 2 and 3) were reported to be significantly larger in men than women (6). Thus, these two nuclei could be involved in the generation of male-typical sexual behavior.

I tested the idea that one or both of these nuclei exhibit a size dimorphism, not with sex, but with sexual orientation. Specifically, I hypothesized that INAH 2 or INAH 3 is large in individuals sexually oriented toward women (heterosexual men and homosexual women) and small in individuals sexually oriented toward men (heterosexual women and homosexual men). Because tissue from homosexual women could not be obtained, however, only that part of the hypothesis relating to sexual orientation in men could be tested.

Brain tissue was obtained from 41 subjects at routine autopsies of persons who died at seven metropolitan hospitals in New York and California. Nineteen subjects were homosexual men who died of complications of acquired immunodeficiency syndrome (AIDS) (one bisexual man was included in this group). Sixteen subjects were presumed (7) heterosexual men: six of these subjects died of AIDS and ten of other causes (8). Six subjects were presumed heterosexual women. One of these women died of AIDS and five of other causes (8). The mean age of the homosexual men was 38.2 years (range, 26 to 53 years), that of the heterosexual men was 42.8 years (range, 33 to 59 years), and that of the women was 41.2 years (range, 29 to 59 years). The subjects were younger and closer in age than those studied in previous investigations: tissue was not taken from elderly heterosexual men or women so that an approximate age-match would be pre-

served with the homosexual men, who were predominantly young or middle-aged adults (9).

The brains were fixed by immersion for 1 to 2 weeks in 10 or 20% buffered formalin and then sliced by hand at a thickness of about 1 cm in, or close to the coronal plane. Tissue blocks containing the anterior hypothalamus were dissected from these slices and stored for 1 to 8 weeks in 10% buffered formalin. These blocks were then given code numbers; all subsequent processing and morphometric analysis was done without knowledge of the subject group to which each block belonged. The blocks were infiltrated with 30% sucrose and frozen-sectioned at a thickness of 52 μm in planes parallel to the original slices. The sections were mounted serially on slides, dried, defatted in xylene, stained with 1% thionin in acetate buffer (15 to 30 min), and differentiated with 5% rosin in 95% alcohol (4 to 10 min). With the aid of a compound microscope equipped with a camera lucida attachment, the outlines of four nuclei (INAH 1, 2, 3, and 4) were traced in every section at a linear magnification of ×83. These four nuclei included the two nuclei reported by Allen et al. (6) to be sexually dimorphic and two other nuclei (INAH 1 and 4) for which no sex differences were found (6). The criteria described in (6) were followed in identifying and delineating the nuclei (Fig. 1). The outline of each nucleus was drawn as the shortest line that included every cell of the type characteristic for that nucleus, regardless of cell density. In 15 cases the nuclei in both left and right hypothalami were traced. In 12 cases only the left hypothalamus was studied, and in 14 cases only the right. The areas of the traced outlines were determined with a digitizing tab-

let, and the volume of each nucleus was calculated as the summed area of the serial outlines multiplied by the section thickness.

In the 15 cases where both left and right sides were studied, no significant interhemispheric differences were found for any of the four nuclei. Therefore, in further analysis, the mean of the two sides was used, and the cases where only one side was available were analyzed without regard to the side of origin.

One-way analysis of variance (ANOVA) was used to look for significant differences between subject groups (Fig. 2). No differences were found for INAH 1, 2, or 4. These results for INAH 1 and 4 are consistent with those of Allen et al. (6, 10). However, INAH 2 was reported to be about twofold larger in men than women (6). The failure to replicate that finding may have to do with the relatively young age of the subjects in the present study; as noted in (6), no sex difference was apparent when women of reproductive age were compared with men of similar ages. Thus INAH 2 is not dimorphic either with sex or with sexual orientation, at least within the age range studied.

INAH 3 did exhibit dimorphism. One-way ANOVA showed that the three sample groups (from women, heterosexual men, and homosexual men) were unlikely to have come from the same population (P = 0.0014). Consistent with the hypothesis outlined above, the volume of this nucleus was more than twice as large in the heterosexual men (0.12 ± 0.01 mm^3, mean $\pm$ SEM) as in the homosexual men (0.051 ± 0.01 mm^3). Because of uncertainty about the nature of the underlying distribution, the significance of this difference was evaluated by a Monte Carlo procedure (11); this showed the difference to be highly significant (P = 0.001). The difference was still significant when the homosexual men were compared with only the six heterosexual men who died of complications of AIDS (P = 0.028). There was a similar difference between the heterosexual men and the women (mean 0.056 ± 0.02 mm^3; P = 0.019), replicating the observations in (6). There was no significant difference in the volume of INAH 3 between the heterosexual men who died of AIDS and those who died of other causes or between the homosexual men and the women. These data support the hypothesis that INAH 3 is dimorphic not with sex but with sexual orientation, at least in men (12).

INAH 3 is situated about 1 mm lateral to the wall of the third ventricle, and about 1 to 2 mm dorsal to the anterior tip of the paraventricular nucleus. It is spherical or ellipsoidal and contains relatively large,

densely staining, polygonal neurons (Fig. 1B). The borders of the nucleus are not well demarcated; hence a blind procedure was used to reduce bias effects. In most of the homosexual men (and most of the women), the nucleus was represented only by scattered cells (Fig. 1C). Because of the difficulty in precisely defining the neurons belonging to INAH 3, however, no attempt was made to measure cell number or density.

Brain tissue from individuals known to be homosexual has only become available as a result of the AIDS epidemic. Nevertheless, the use of this tissue source raises several problems. First, it does not provide tissue from homosexual women because this group has not been affected by the epidemic to any great extent. Thus, the prediction that INAH 3 is larger in homosexual than in heterosexual women remains untested. Second, there is the possibility that the small size of INAH 3 in the homosexual men is the result of AIDS or its complications and is not related to the men's sexual orientation. This does not seem to be the case because (i) the size difference in INAH 3 was apparent even when comparing the homosexual men with heterosexual AIDS patients, (ii) there was no effect of AIDS on the volumes of the three other nuclei examined (INAH 1, 2, and 4), and (iii) in the entire sample of AIDS patients there was no correlation between the volume of INAH 3 and the length of survival from the time of diagnosis. Nevertheless, until tissue from homosexual men dying of other causes becomes available, the possibility that the small size of INAH 3 in these men reflects a disease effect that is peculiar to homosexual AIDS patients cannot be rigorously excluded.

A third problem is the possibility that AIDS patients constitute an unrepresentative subset of gay men, characterized, for example, by a tendency to engage in sexual relations with large numbers of different partners or by a strong preference for the receptive role in anal intercourse [both of which are major risk factors for acquiring human immunodeficiency virus (HIV) infection (13)]. Sexual activity with large numbers of partners is (or was until recently) common among gay men, however, and therefore does not define an unrepresentative minority (14). In addition, the majority of homosexual men who acquired HIV infection during the Multicenter AIDS Cohort Study (15) reported that they took both the insertive and the receptive role in anal intercourse, and the same is likely to be true of the homosexual subjects in my study. Nevertheless, the use of postmortem material, with the consequent impossibility of obtaining detailed information about the sexuality of the subjects, limits the ability to make correlations between brain structure and the diversity of sexual behavior that undoubtedly exists within the homosexual and the heterosexual populations.

The existence of "exceptions" in the present sample (that is, presumed heterosexual men with small INAH 3 nuclei, and homosexual men with large ones) hints at the possibility that sexual orientation, although an important variable, may not be the sole determinant of INAH 3 size. It is also possible, however, that these exceptions are due to technical shortcomings or to misassignment of subjects to their subject groups.

The discovery that a nucleus differs in size between heterosexual and homosexual men illustrates that sexual orientation in humans is amenable to study at the biological level, and this discovery opens the door to studies of neurotransmitters or receptors that might be involved in regulating this aspect of personality. Further interpretation of the results of this study must be considered speculative. In particular, the results do not allow one to decide if the size of INAH 3 in an individual is the cause or consequence of that individual's sexual orientation, or if the size of INAH 3 and sexual orientation covary under the influence of some third, unidentified variable. In rats, however, the sexual dimorphism of the apparently comparable hypothalamic nucleus, the sexually dimorphic nucleus of the preoptic area (SDN-POA) (16), arises as a consequence of the dependence of its constituent neurons on circulating androgen during a perinatal sensitive period (17). After this period, even extreme interventions, such as castration, have little effect on the size of the nucleus. Furthermore, even among normal male rats there is a variability in the size of SDN-POA that is strongly correlated with the amount of male-typical sexual behavior shown by the animals (18). Although the validity of the comparison between species is uncertain, it seems more likely that in humans, too, the size of INAH 3 is established early in life and later influences sexual behavior than that the reverse is true. In this connection it would be of interest to establish when the neurons composing INAH 3 are generated and when they differentiate into a dimorphic nucleus.

Fig. 2. Volumes of the four hypothalamic nuclei studied (INAH 1, 2, 3, and 4) for the three subject groups: females (F), presumed heterosexual males (M), and homosexual males (HM). Individuals who died of complications of AIDS, ●; individuals who died of causes other than AIDS, ▲; and an individual who was a bisexual male and died of AIDS, ○. For statistical purposes this bisexual individual was included with the homosexual men.

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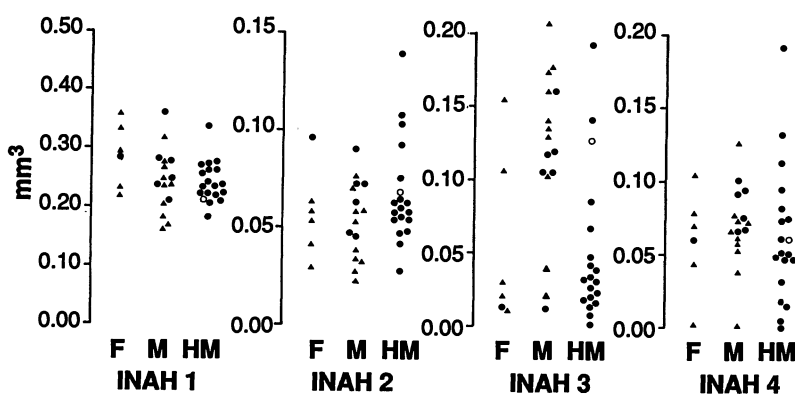
cases), renal failure (two cases), coronary thrombosis, acute lymphocytic leukemia, amyotrophic lateral sclerosis, pancreatic carcinoma, pulmonary embolism, and aspiration pneumonia. For the five female subjects who did not die of AIDS, the causes of death were systemic lupus erythematosus, pancreatic carcinoma, liver failure (two cases), and abdominal sepsis secondary to renal transplantation. All six of the heterosexual male AIDS patients and three of the homosexual men had histories of intravenous drug abuse. Three of the women, two heterosexual men who did not have AIDS, and one homosexual man had histories of chronic alcohol abuse.

9. Criteria for inclusion of subjects in the study were as follows: (i) age 18 to 60, (ii) availability of medical records, (iii) in AIDS patients, statement in the records of at least one AIDS risk group to which the patient belonged (homosexual, intravenous drug abuser, or recipient of blood transfusions), (iv) no evidence of pathological changes in the hypothalamus, and (v) no damage to the INAH nuclei during removal of the brain or transection of these nuclei in the initial slicing of the brain. Fourteen specimens (over and above the 41 used in the study) were rejected for one of these reasons; in all cases the decision to reject was made before decoding.

10. INAH 1 is the same as the nucleus named the "sexually dimorphic nucleus" and reported to be larger in men than women [D. F. Swaab and E. Fliers, Science 228, 1112 (1985)]. My results support the contention by Allen et al. (6) that this nucleus is not dimorphic.

11. The ratio of the mean INAH 3 volumes for the heterosexual and homosexual male groups was calculated. The INAH 3 volume values were then randomly reassigned to the subjects, and the ratio of means was recalculated. The procedure was repeated 1000 times, and the ordinal position of the actual ratio in the set of shuffled ratios was used as a measure of the probability that the actual difference between groups arose by chance. Only one of the shuffled ratios was larger than the actual ratio, giving a probability of 0.001.

12. Application of ANOVA or correlation measures failed to identify any confounding effects of age, race, brain weight, hospital of origin, length of time between death and autopsy, nature of fixative (10 or 20% formalin), duration of fixation, or, in the AIDS patients, duration of survival after diagnosis, occurrence of particular complications, or the nature of the complication or complications that caused death. There were no significant positive or negative correlations between the volumes of the four individual nuclei across the entire sample, suggesting that there were no unidentified common-mode effects such as might be caused by variations in tissue shrinkage. The mean brain weight for the women (1256 ± 41 g) was smaller than that for either the heterosexual (1364 ± 46 g) or the homosexual (1392 ± 32 g) men, but normalizing the data for brain weight had no effect on the results. There was no correlation between subject age and the volume of any of the four nuclei, whether for the whole sample or for any subject group; this finding does not necessarily

conflict with the report in (6) of age effects in INAH 1, and possibly INAH 2, because in (6) a much wider range of ages was examined than was used in the present study.

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Technical Comments

Forensic DNA Tests and Hardy-Weinberg Equilibrium

DNA tests based on biochemical procedures are being widely used for the identification of accused individuals (1). When the DNA pattern obtained from a specimen at the scene of a crime matches that obtained from a suspect, the prosecution seeks to prove that the suspect is the only possible source of the specimen. That inference depends on knowing something about the distribution of genotypes of the entire population of other people, any one of whom might be the actual criminal. In forensic applications of DNA testing so far, that inference has been based on an assumption of Hardy-Weinberg equilibrium (H-W). H-W justifies the assumption of statistical independence implicit in formulas used to calculate the probability that the DNA patterns of a specimen and of a suspect would match by chance alone. H-W can (2, 3), and sometimes does (4, 5), fail under realistic conditions.

To evaluate H-W, Devlin et al. (6) developed methods "to test for an overall excess or dearth of heterozygotes" in a sample of humans and applied these methods to a database provided by Lifecodes, Inc., one of the major vendors of services for forensic DNA testing. Devlin et al. have provided a useful service in drawing further attention to the problem of coalescence, that is, the

appearance of a single, blurred band in autoradiographic films resulting from DNA fragments of different but similar size. However, their assertion that "the arguments so far presented against [H-W] are incorrect" is unconvincing for several reasons.

1) Devlin et al. reject the finding by Lander (2) of an excess of homozygosity in a Hispanic population. They use a data set drawn from a Caucasian population [reference 18 of (4)] and report no direct test of the logistic model for Hispanics, but instead use the model from the Caucasian data to interpret the Hispanic data. Their model is untested on the population from which Lander drew his data.

2) Devlin et al. have not used the data on apparent homozygotes. These are the data most likely to reveal an excess of homozygosity. They eliminate a subset of data that deviates from the expectations under H-W, and then test the remaining data for agreement with H-W. This predisposes them toward finding no deviation from H-W.

3) Devlin et al. note correctly that population subdivision must affect the overall number of heterozygotes, but they do not acknowledge that not all allelic classes need have too few heterozygotes relative to H-W. Some heterozygote classes may be in H-W,

others in excess, and still others deficient: it is only the total of all heterozygotes that is necessarily deficient when the population is subdivided (7). Because the method of Devlin et al. tests only a subset of the heterozygote data, they might observe no deviation from H-W in that subset and incorrectly conclude that there is no departure from H-W overall, when, in fact, there is.

4) No information is given by Devlin et al. about how the populations of Caucasians, blacks, and Hispanics were sampled. There is no reason to believe that these samples are random or representative samples of the corresponding self-identified cultural groups in the United States. Hence inference from the given samples to the population at large, or to the entire self-identified cultural groups, is perilous. For example, the Hispanic population around New York is primarily of Puerto Rican origin, that around Miami of Cuban origin, and that in the southwestern states of Mexican origin; there are varying mixtures of other Hispanic origins in all three regions. If the Hispanic data studied by Devlin et al. were drawn primarily from the New York region, the conclusions could well be invalid for the other major Hispanic subpopulations separately or for all Hispanics as a group.

5) Devlin et al. say that it is not appropriate to pool data from different races, yet they treat "black" and "Hispanic" as if these were biologically meaningful races. The population identified as "black" in the United States is a continuum of individuals ranging from people of primarily African origin to people of primarily European origin (and