TAG1-negative axon fascicles. Similarly, a major change in the expression of adhesion factors has also been reported: down-regulation of TAG1 expression as the commissural neurons cross the floor plate (*2*). The arrival of substance P–containing axon terminals at the floor plate may modulate or initiate this change; in such a mechanism, release of substance P onto the floor plate would cause floor plate secretion of factors taken up by axons of passage (*12*). Here, we suggest that during development, substance P initiates or may modulate programs of gene expression in floor plate cells, which may in turn influence and guide the growth and development of neuronal pathways within the brain and spinal cord.

REFERENCES AND NOTES

1. J. Dodd and T. M. Jessell, *Science* **242**, 692 (1988); T. M. Jessell, *Neuron* **1**, 3 (1988); S. Ramon y Cajal, *Histologie du systeme nerveux de l'homme et des vertebres* (Consejo Superior de Investigaciones Cientificas, Madrid, 1909), vol. 1, p. 592; M. Tessier-Lavigne, M. Placzek, A. G. S. Lumsden, J. Dodd, T. M. Jessell, *Nature* **336**, 775 (1988); A. Ghosh and C. J. Schatz, *Development* **117**, 1031 (1993); C. D. Heffner, A. G. S. Lumsden, D. D. M. O'Leary, *Science* **247**, 217 (1990).
2. J. Dodd, S. B. Morton, D. Karagogeos, M. Yamamoto, T. M. Jessell, *Neuron* **1**, 105 (1988); M. Tessier-Lavigne and M. Placzek, *Trends Neurobiol.* **14**, 303 (1991).
3. P. Boloventa and J. Dodd, *Development* **109**, 435 (1990); M. Placzek, M. Tessier-Lavigne, T. M. Jessell, J. Dodd, *ibid.* **110**, 19 (1990); A. G. S. Lumsden and A. M. Davies, *Nature* **306**, 786 (1983). Spinal explants were taken from E13 rat embryos placed in F15 medium (Gibco) and dissected with etched tungsten needles. Pieces of dorsal spinal cord and floor plate were embedded within a collagen gel matrix as described in (*5*) and cultured in Dulbecco's minimum essential medium (DMEM) at 37°C in a 5% CO_2 incubator for 36 hours. Outgrowth was calculated from a series of photographic negatives drawn with the aid of a camera lucida attached to a Leitz Orthoplan microscope. Measurements were made blind, without knowledge of drug treatment. After photography, cultures were fixed and immunostained with the use of a variant of the method described in (*4*). Whole mounts were incubated in antibody to TAG1 (mAb 4D7 diluted 1:1), mAb 3A10, or antibody to NK1.
4. Rat embryos or newborn animals (ages E10 to P10) were fixed by immersion in freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 2 hours, transferred overnight to PB containing 30% sucrose, and sectioned at 80 to 100 μm on a sliding microtome. Free-floating sections were incubated in primary antisera to substance P or NK1 receptor diluted 1:1000 in PB with 0.1% Triton X-100 (Sigma) added for 2 to 3 days at 4°C with agitation, washed in PB for 1 hour, incubated in biotinylated antibodies to rabbit immunoglobulin G (Vector-Elite), and washed in fluorescein isothiocyanate–labeled avidin (Vector) diluted 1:200 in incubation mix for a further 2 hours in the dark. All sections were examined and photomicrographs taken on an MRC 600 confocal microscope. The antibody to NK1 was raised against a peptide corresponding to a 15–amino acid portion (residues 393 to 407) of the intracellular COOH-terminus of the rat substance P receptor. The polyclonal antiserum did not recognize neurokinin-2 or neurokinin-3 receptor proteins [S. R. Vigna *et al.*, *J. Neurosci.* **14**, 834 (1994)]; the polyclonal substance P antiserum recognized both substance P and neurokinin A [J. I. Nagy and S. P. Hunt, *Neuroscience* **7**, 89 (1982)]. However, a second antibody that recognized only substance P (Incstar) gave similar results. Controls were performed by adsorption of antibody with 10 μg of native peptide per milliliter of diluted antiserum, which abolished all positive staining.
5. G. Grynkiewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.* **260**, 3440 (1985). For recording, floor plates from E13 embryos were dissociated in trypsin and plated onto polyornithine-coated cover slips in DMEM containing 10% horse serum. Cells were incubated at 37°C for 3 to 5 days in 7.5% CO_2, and the medium was changed after the first 24 hours. Cover slips were washed in a Hepes buffer [142 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10 mM Hepes, 10 mM glucose, 10 mM inositol, and bovine serum albumin (1 g/liter), pH 7.2]. The cover slips were incubated for 60 min at 21°C in Hepes buffer containing 2 μM fura-2–AM (Molecular Probes) and then washed for 30 min in Hepes buffer before recording. The cover slips containing dye-loaded cells were mounted in a 1-ml Perspex chamber through which Hepes buffer (30°C) was perfused at 2 ml/min. The $[Ca^{2+}]_i$ was calculated with the use of the equation

$$[Ca^{2+}] = K_d \times \beta\left(\frac{R - R_{min}}{R_{max} - R}\right)$$

where K_d is the dissociation constant for fura-2, R is the fluorescence ratio, R_{min} is the fluorescence ratio in 0 mM Ca^{2+}, R_{max} is the fluorescence ratio in 20 mM Ca^{2+}, and β is the proportionality coefficient. Values of R_{min}, R_{max}, and β were obtained with solutions of fura-2 (25 μM in 125 mM KCl, 20 mM CaCl, 10 mM Hepes, and 1 mM Mg^{2+}, pH 7.0, 30°C) in which Ca^{2+} was buffered to known concentrations with 10 mM EGTA. Floor plate cells (n = 61), representing approximately 25% of the total population of cells, were analyzed; they were identified by correlation with parallel NK1 immunohistochemically stained preparations and had a square or rectangular appearance. Fibroblasts were characteristically large and flat with extensive lamellipodia, whereas oligodendrocytes stained positively with an antibody to Gal-C (C. De Felipe, R. D. Pinnock, S. P. Hunt, data not shown).
6. G. R. Seabrook and T. M. Fong, *Neurosci. Lett.* **152**, 9 (1993).
7. C. A. Maggi, R. Pattacchini, P. Rovero, A. Giachetti, *J. Auton. Pharmacol.* **13**, 23 (1993). [Sar^9,$Met(O_2)^{11}$]substance P differs from the undecapeptide substance P in the substitution of the glycine in position 9 by sarosine (glycine methylated on the mitrogen) and by oxidized methionine at position 11.
8. [β-Ala^8]neurokinin A 4-10 is fragment 4-10 of neurokinin A in which the glycine at position 8 has been substituted by β-alanine.
9. T. E. Kennedy, T. Serafini, J. R. de la Torre, M. Tessier-Lavigne, *Cell* **78**, 425 (1994).
10. J. Nilsson, A. von Euler, C. J. Dalsgaard, *Nature* **315**, 61 (1985); S. Narumi and Y. Maki, *J. Neurochem.* **30**, 1321 (1978); T. Hokfelt, *Neuron* **7**, 867 (1991); B. Pernow, *Pharmacol. Rev.* **35**, 85 (1983).
11. J. Altman and S. E. Bayer, *The Development of the Rat Spinal Cord* (Springer-Verlag, Berlin, 1984).
12. R. M. Campbell and A. C. Peterson, *Development* **119**, 1217 (1993).

13. We thank A. Lumsden and W. Wisden for critical reading of the manuscript; A. Pini for help in setting up collagen gel cultures; S. Vigna and P. Mantyh for antibodies; M. Yamamoto and A. Furley for mAb 4D7; J. Fawcett for mAb 3A10; M. Casariego, S. Ingham, and A. Bond for technical assistance; and P. Birch (Glaxo) and A. Bouvier (Rhone-Poulenc) for NK1 agonists and antagonists. C.D.F. was supported by European Economic Community grant CT91-0685.

19 May 1994; accepted 12 December 1994

Genetic Feminization of Brain Structures and Changed Sexual Orientation in Male *Drosophila*

Jean-François Ferveur,*† Klemens F. Störtkuhl,‡ Reinhard F. Stocker, Ralph J. Greenspan

The neural basis of sexual orientation in *Drosophila* was studied by the production of males with regionally feminized brains. Such flies express the female form of the sex determination gene *transformer* in a limited number of neurons under the control of *GAL4* enhancer trap inserts. This method facilitated the creation of lines with a stable pattern of feminization. In tests of sexual preferences, flies that were feminized in a portion of the antennal lobes or in a subset of the corpora pedunculata (mushroom bodies) courted both males and females. These two brain structures, both of which are involved in olfactory processing, may function in the recognition of sex-specific pheromones, in the control of sex-specific behaviors, or both.

In the fruit fly *Drosophila melanogaster*, most aspects of sexual behavior are genetically controlled (*1*, *2*). Sexual recognition of females by males largely relies on sexually dimorphic contact pheromones (*3*), and performance of male courtship requires genotypically male cells in higher centers of the nervous system (*4*).

To understand the neural basis of sexual orientation in *Drosophila*, we generated male flies with different patterns of regional feminization of the nervous system by using a *GAL4* enhancer trap strain (*5*). The random insertion of the *GAL4* transposon leads to a line of flies that express *GAL4* in a stable tissue-specific manner that is dependent on the enhancer gene next to which the transposon is inserted. When each *GAL4* line is crossed with a line that contains the reporter feminizing gene *up-*

J.-F. Ferveur and R. J. Greenspan, Department of Biology and Center for Neural Science, New York University, New York, NY 10003, USA.
K. F. Störtkuhl and R. F. Stocker, Zoology Institute, Fribourg University, Pérolles CH-1700 Fribourg, Switzerland.

*Present address: Unité de Recherche Associée CNRS 1491, Université Paris Sud, 91405 Orsay Cedex, France.
†To whom correspondence should be addressed.
‡Present address: Department of Biology, Yale University, New Haven, CT 06520, USA.

stream activating sequence–transformer (*UAS-tra*), the corresponding tissues are dominantly (6) and autonomously (7) feminized (Fig. 1). In contrast to the standard gynandromorph technique, which produces unique patterns of sex mosaicism in each individual (8), the *GAL4* enhancer trap strains produce stable mosaic lines. This allows the detection of probabilistic aspects of behavior and the examination of the anatomical staining patterns in greater detail. Our sex mosaic flies also present the advantage of having cells that are neither abnormal (mutant) nor missing (ablated) but that are either male or female.

Drosophila melanogaster males are normally heterosexual (9). Transformed males from crosses involving *GAL4* lines 1, 2, and 3, but not lines 4 and 5, showed a homosexual attraction to wild-type males (Fig. 2) (*10*). Males of various genotypes, homozygous for the same *GAL4* inserts (without *tra*) or heterozygous for the reporter gene *UAS-tra*, did not show such a high level of homosexual response, as expected from other results (*9*, *11*). Heterosexual responses were normal; males from all five of these transformed lines initiated courtship toward decapitated wild-type females just as normal males do (*12*).

Because males of different transformed strains exhibited two distinct behavioral classes of responses (bisexual and heterosexual), we examined their *GAL4* expression patterns to look for a relation between anatomy and behavior. The patterns of expression in the five *GAL4* enhancer trap lines, which correspond to those regions feminized by *UAS-tra*, were revealed by a cross with a *UAS-lacZ* reporter strain (Fig. 1). Differences in sexual orientation could thus be correlated with a relatively limited number of differences in expression. The patterns in the five selected strains overlapped in both the central nervous system (CNS) and in the peripheral nervous system (PNS). There was a correlation between the performance of homosexual courtship and the expression of *GAL4* in the antennal lobes (ALs) and the mushroom bodies (MBs) (Fig. 3).

As the exclusive neural targets of the olfactory sensilla of the third antennal segment and of the maxillary palps, the ALs are the primary olfactory association center (*13*, *14*). Their subunits, the glomeruli, are organized into odor-discriminating areas (*15*). In *Drosophila* and in other insects, olfactory information processed in the different glomeruli is transferred by means of local interneurons into the calyces of the MBs (*13*, *16*) and into the lateral protocerebrum (*13*, *17*). MBs, which can show a significant structural plasticity depending on social environment (*18*), age, and sex (*19*), are thought to be the higher center for odor discrimination and associative odor learning (*20*).

In bisexual strains 1, 2, and 3, *GAL4* was expressed in a subset of the MBs, in a specific portion of the ALs, or both. This was not the case in heterosexual strains 4 and 5, in which *GAL4* is never expressed in the MBs or is expressed in a different portion of the ALs (Fig. 4). In bisexual strains 1 and 2, *GAL4* was expressed in a subset of the MBs, including the calyces, the pedunculi, and the alpha and beta lobes. Strain 2 showed no expression elsewhere except in the fan-shaped body of the central complex and in the giant descending interneuron that extends to the thoracic ganglia. In bisexual strains 1 and 3 and in heterosexual strain 4, *GAL4* was expressed in the ALs. In strain 1, local interneurons that connect most or all glomeruli were stained, whereas in strain 3, the expression in the central brain was mostly restricted to a few glomeruli. The difference of expression in the ALs between bisexual strain 3 and heterosexual strain 4 was restricted to the DM2, DA3, and DA4 glomeruli (*13*, *21*). These were the only transformed neural structures that showed any correlation with changed sexual orientation. The arista did show a correlation, but no sexual dimorphism has yet been observed in this organ and it is not thought to have a chemosensory role (*22*).

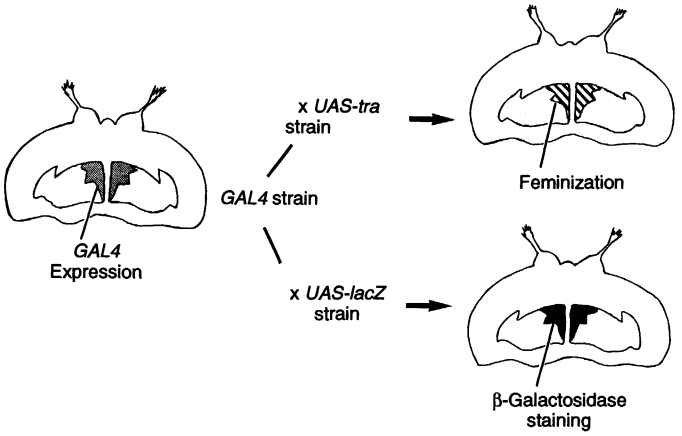

Fig. 1. Schema for the production of partially transformed males. Strains carrying enhancer trap P[GAL4;w$^+$] insertions (*5*) were mated to a reporter strain carrying *UAS-tra* to produce regional feminization. The *UAS-tra* lines were originally generated with a pUAST vector (*5*) into which a cDNA of the female spliced form of the *transformer* gene (pE6.9.1) (*6*) had been inserted as an Eco RI fragment. Transformation was carried out with the w;P[(Δ2-3)](99B) strain as host (*34*). The Δ2-3 element was eliminated, and the second chromosome transformants were balanced with In(2LR)O,Cy (*35*). Each *GAL4* line was also mated to a *UAS-lacZ* strain to determine *GAL4* expression patterns (*36*).

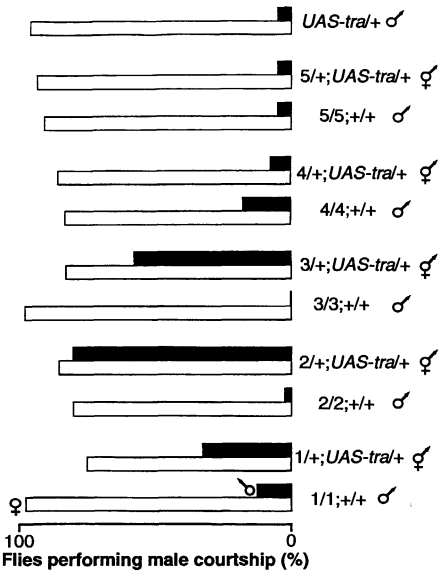

Fig. 2. Courtship behavior of transformed and control males. The length of each bar shows the percentage of flies of a given genotype performing courtship (*37*). Forty flies were tested for each strain. Only flies that courted more than 20 s were counted as courting males. All flies used in courtship tests were individually stored in food vials and tested when 4 days old in a mating chamber (0.2 cm^3) at 25°C for 10 min. Each transformed (*GAL4/+;UAS-tra/+*) male was tested once either with a decapitated wild-type male (black bar) or female (white bar) (*12*). At the same time, control flies homozygous for the same *GAL4* insert or heterozygous for the *UAS-tra* gene were tested under the same standard conditions. We decapitated sex-object wild-type (Canton-S) female and male flies 10 to 30 min before the experiment to standardize the stimulus provided to the subject flies. The proportion of time spent courting, measured by the courtship index (CI), was generally consistent in each group. All transformed males had a CI from 0.45 ± 0.06 to 0.66 ± 0.04 when courting virgin females, with the exception of 2-*tra* males (CI = 0.26 ± 0.04). Control homozygous *GAL4* males had a CI of 0.58 ± 0.04 to 0.80 ± 0.03 with virgin females. Where controls and transformed males courted other males, the CI ranged from 0.20 ± 0.02 to 0.32 ± 0.06. Male flies of transformed strains 1-, 2-, and to a lesser extent 3-, but not 4- and 5-*tra*, had an intersexual morphology, with a loss of pigmentation of the last abdominal tergites, and changes in genitalia. In all cases, however, the distal segments of the front legs, thought to be a primary site of pheromone detection in males (*24*), had a male-like aspect as shown by the presence of sex combs. No *qualitative* intrastrain variation was observed for these morphological phenotypes.

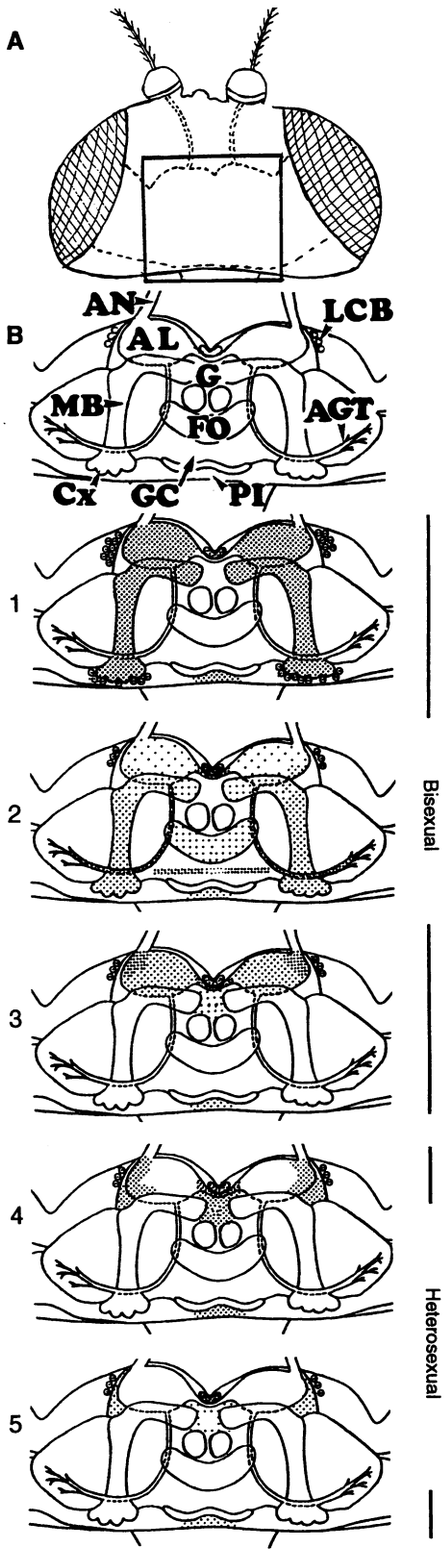

Fig. 3. Patterns of *GAL4* expression in the central brain. Stippled areas, visualized with *lacZ* staining, correspond to the area feminized with *UAS-tra*. The intensity of staining is indicated by the density of dots. (**A**) Schematic representation of a dorsal view of a fly head; the outlined area of the central brain is enlarged in (**B**), which indicates the principal structures in which *GAL4* is expressed in strains 1 through 5. AGT, antennoglomerular tract; AL, antennal lobes; AN, antennal nerve; Cx, calyx (as part of the MB); FO, fan-shaped organ (of the central complex); G, gustatory centers and suboesophageal ganglia; GC, great commissure; LCB, lateral cell bodies; MB, (pedunculus of the) mushroom body; and PI, pars intercerebralis.

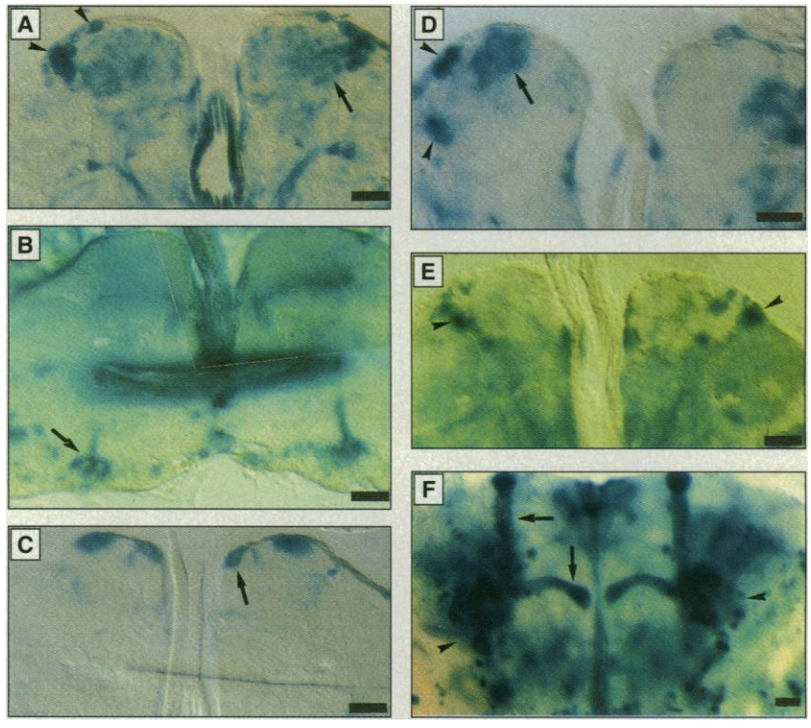

Fig. 4. Photomicrographs showing *lacZ* expression pattern in the ALs and in the MBs of the five *GAL4* strains. Areas stained in blue correspond to the structures that are feminized when crossed with a *UAS-tra* strain. (**A** through **E**) Horizontal epon sections (10 μm) of *Drosophila* heads. (A) and (C) to (E) correspond to the medio-anterior part of the brain including the ALs, and (B) is a composite picture including both the ALs and the calyces of the MBs. (**F**) Frontal view of a brain whole mount of strain 1. In all photos, the arrowheads point to the cell bodies lateral to the ALs. (A), strain 1; (B), strain 2; (C), strain 3; (D), strain 4; and (E), strain 5. Arrows show in (A) arborization of local interneurons, in (B) the calyx of the MB, in (C) the DM2 glomerulus, in (D) the VA1 (*21*) glomerulus, and in (F) the MB. All preparations were incubated with X-Gal substrate (*38*). Scale bars are 25 μm.

In many insect species, glomerular architecture shows sexual dimorphism with the male macroglomerular complex specialized for processing volatile female pheromones detected by the antennae (*23*). No such structure exists in *Drosophila*. In *D. melanogaster*, known female pheromones are nonvolatile contact pheromones that are detected principally by the distal segments of the male's front two pairs of legs (*24*). However, the maxillary palps show a slight sexual dimorphism for the number of olfactory basiconic sensilla (*13*, *14*) and have been implicated in the detection by males of inhibitory signals from fertilized females (*25*). These signals may possibly be short-range anti-aphrodisiac pheromones (*26*). The DM2 glomerulus, which receives sensory inputs from the basiconic sensilla borne by the maxillary palps, may thus be functionally homologous to the macroglomerular structure (*13*, *14*, *27*). DM2 is connected to the calyx of MBs through the antenno-glomerular tract by the processes of unilateral output interneurons (*13*).

MBs, which are known to be the higher brain structures involved in the processing of chemosensory information, have ~10% more Kenyon cell axon fibers in females than are in males (*19*). However, no obvious sexual dimorphism of the neuroblast precursors of the MBs has been observed during development and metamorphosis (*28*).

Our results suggest that in the transformed bisexual males of strains 1, 2, and 3, the ALs and the MBs, which are two linked neural structures involved in the detection and the processing of sex pheromones, have been feminized or at least are incompletely developed as male structures. The bisexual behavior of strains 2 and 3 is correlated with a restrictive expression in a subset of the MBs and in a few glomeruli of the ALs (DM2, DA3, and DA4), respectively. This changed sexual orientation could result from an error in the connectivity between the

ALs and the MBs as a result of the differences in their sexual phenotype (*29*). Strain 1 shows a partial and simultaneous expression of *tra* in both the MBs and in the ALs. The fact that transformed males of this strain showed homosexual behavior less frequently than totally bisexual strains 2 and 3 could thus be the result of the concordance of sexual genotype in both brain structures. Alternatively, it may simply be attributable to a less complete transformation of the crucial substructures.

There are two possible explanations for the behavioral consequences of the transformations studied here. These strains may lack a male-specific inhibition center that enables wild-type males to detect antiaphrodisiac pheromones and possibly other inhibitory odors (*25*, *30*) that are capable of blocking male-male courtship (*26*). In this case, transformed males would not be able to detect that the courted flies were males and would be induced to court by some other chemical or visual stimuli. Alternatively, wild-type females may possess sex-specific nervous structures for detecting male aphrodisiac pheromones (*9*). In this case, males in the strains that exhibit high levels of homosexual courtship would respond to males because they are stimulated by them, but their behavior would remain typically male because other crucial parts of the nervous system are still male (*4*). The female-specific responsive center is likely to correspond to a site identified in earlier gynandromorph experiments as the main focus for female sexual receptivity (*31*). This area includes neurons in the medio-anterior region of the brain in which the glomeruli DM2, DA3, and DA4 lie. Whichever interpretation is correct, because the strains retain the ability of normal males to detect female aphrodisiac pheromones, the existence of separate or composite central structures for the analysis of sex-specific pheromones seems probable.

This study shows that the relation between genotype, brain structure, and sexual orientation is complex, even in an organism as relatively simple as *Drosophila*. Subtle differences in human brain structures have been correlated with male homosexuality (*32*), as have certain genetic markers (*33*). In spite of the behavioral and phylogenetic gulf that separates our studies, investigations of *Drosophila* sexual behavior may provide insights into fundamental aspects of the relation between genes, brain structure, and sexual behavior in higher organisms.

REFERENCES AND NOTES

1. J. C. Hall, *Science* **264**, 1702 (1994).
2. L. Tompkins, *Trends Genet.* **2**, 14 (1986).
3. C. Antony and J.-M. Jallon, *J. Insect Physiol.* **28**, 873 (1982).
4. J. C. Hall, *Behav. Genet.* **7**, 291 (1977); *Genetics* **92**, 437 (1979).
5. A. Brand and N. Perrimon, *Development* **118**, 401 (1993).
6. M. McKeown, J. M. Belote, R. T. Boggs, *Cell* **53**, 887 (1988).
7. B. S. Baker and K. A. Ridge, *Genetics* **94**, 383 (1980).
8. Y. Hotta and S. Benzer, *Nature* **240**, 527 (1972); J. C. Hall, *Q. Rev. Biophys.* **15**, 223 (1982).
9. J.-M. Jallon, *Behav. Genet.* **14**, 441 (1984).
10. These *GAL4* strains were selected for this study from a larger set because they showed selective expression patterns that were similar, but with subtle yet identifiable differences between bisexual and heterosexual strains. The other 14 lines, with their wider range of expression patterns, gave results consistent with those reported here (J.-F. Ferveur and R. J. Greenspan, unpublished data). We concentrated on the adult staining pattern because the action of the sex determination pathway on male courtship behavior occurs late in development [J. Belote and B. S. Baker, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8026 (1987)].
11. *Drosophila melanogaster* males, unlike some other species of *Drosophila*, do not generally show male homosexual courtship [M. Cobb and J.-M. Jallon, *Anim. Behav.* **39**, 1058 (1990)]. However, mutant *fruitless* males do show homosexual courtship chains, although the genotype and phenotype of this mutation are highly complex [J. C. Hall, *Behav. Genet.* **8**, 125 (1978); D. A. Gailey and J. C. Hall, *Genetics* **121**, 773 (1989); B. J. Taylor, A. Villella, L. C. Ryner, B. S. Baker, J. C. Hall, *Dev. Genet.* **15**, 275 (1994); (*1*)]. Adult *D. melanogaster* males will also court young flies of both sexes due to the effect of immature contact pheromones [J.-M. Jallon and Y. Hotta, *Behav. Genet.* **9**, 487 (1979); S. P. McRobert and L. Tompkins, *ibid.* **13**, 517 (1983); J. M. Pechiné, C. Antony, J.-M. Jallon, *J. Chem. Ecol.* **14**, 1071 (1988)].
12. We used decapitated females as stimuli to permit subsequent comparison with courtship directed toward males in tests of homosexual courtship. These object males must be decapitated to prevent them from producing rejection responses that tend to reduce courtship [M. Paillette, H. Ikeda, J.-M. Jallon, *Bioacoustics* **3**, 247 (1991)]. Decapitation thus facilitates and simplifies the detection of homosexual courtship. All three transformed bisexual strains (1-*tra*, 2-*tra*, and 3-*tra*) differed significantly from *UAS-tra*/+ controls for performance of homosexual courtship by a χ^2 test, whereas the heterosexual strains (4-*tra* and 5-*tra*) did not differ from controls. The *GAL4* homozygotes (without *tra*) from all strains did not differ significantly from controls for homosexual courtship. Among the few control *UAS-tra*/+ and *GAL4* homozygous males that exhibited homosexual courtship, 90% performed wing vibration, 80% licked male genitalia, and 60% attempted copulation. In comparison, 65 to 95% of the 1-, 2-, and 3-*tra* males that exhibited homosexual courtship showed long wing vibrations and licking of the wild-type males' genitalia, but few copulation attempts were observed (0 to 5%). In heterosexual interactions, 1-*tra* and 2-*tra* males showed a moderate performance of wing vibration (50 and 65%), licked frequently (80%), and rarely attempted to copulate (15% for 1- and 5% for 2-*tra*). The 3-*tra* flies frequently performed wing vibration and licking of female genitalia (90%) but attempted to copulate less often (40%).
13. R. F. Stocker, M. C. Lienhard, A. Borst, K.-F. Fischbach, *Cell Tissue Res.* **262**, 9 (1990); R. F. Stocker, *ibid.* **275**, 3 (1994).
14. R. N. Singh and S. V. Nayak, *Int. J. Insect Morphol. Embryol.* **14**, 296 (1985).
15. V. Rodrigues, *Brain Res.* **453**, 299 (1988).
16. B. S. Hansson, T. A. Christensen, J. G. Hildebrand, *J. Comp. Neurol.* **312**, 264 (1991); T. A. Christensen, B. Waldrop, I. Harrow, J. G. Hildebrand, *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **173**, 385 (1993).
17. N. J. Strausfeld, *Atlas of an Insect Brain* (Springer, Berlin, 1976).
18. G. S. Withers, S. E. Fahrbach, G. E. Robinson, *Nature* **364**, 238 (1993).
19. G. Technau, *J. Neurogenet.* **1**, 113 (1984).
20. K.-F. Fischbach and M. Heisenberg, *J. Exp. Biol.* **112**, 65 (1984); J. S. de Belle and M. Heisenberg, *Science* **263**, 692 (1994).
21. R. F. Stocker, R. N. Singh, M. Schorderet, O. Siddiqi, *Cell Tissue Res.* **232**, 237 (1983).
22. R. F. Foelix, R. F. Stocker, R. A. Steinbrecht, *ibid.* **258**, 277 (1989). Because they are not correlated with the changed sexual orientation, we can exclude most PNS structures (the second and third antennal segments including the antennal nerve, the maxillary and labial palps, and the gustatory organs and head bristles), several tracts in the CNS (the median bundle, the antenno-glomerular tract, and the antennal commissure), and some other significant CNS structures [the pars intercerebralis; the visual ganglia; the targets of the gustatory organs, the antennal mechanosensory apparatus and the motor center; and glomeruli V, VA1, DL1, and DL2 (*21*)]. We did not find any consistent correlation in the thoracic-abdominal ganglia.
23. U. Homberg, T. A. Christensen, J. G. Hildebrand, *Annu. Rev. Entomol.* **34**, 477 (1989); B. S. Hansson, H. Ljungberg, E. Hallberg, C. Löfstedt, *Science* **256**, 1313 (1992).
24. R. Venard, C. Antony, J.-M. Jallon, in *Neurobiology of Sensory Systems*, N. Singh and N. Strausfeld, Eds. (Plenum, New York, 1989), pp. 377–385. A sexual dimorphism of neuronal projections from leg sensilla in the thoracic ganglia does exist in *D. melanogaster* [D. R. Possidente and R. K. Murphey, *Dev. Biol.* **132**, 448 (1989)].
25. R. F. Stocker and N. Gendre, *Behav. Genet.* **19**, 371 (1989).
26. The main inhibiting molecule could be *cis*-vaccenyl acetate [J.-M. Jallon, C. Antony, O. Benamar, *C. R. Acad. Sci. Paris* **292**, 1147 (1982)] or 7-tricosene [D. Scott, R. C. Richmond, D. A. Carlson, *Anim. Behav.* **36**, 1164 (1988)]; the specific molecule or molecules are still under debate [J.-F. Ferveur, M. Cobb, J.-M. Jallon, in *Neurobiology of Sensory Systems*, N. Singh and N. Strausfeld, Eds. (Plenum, New York, 1989), pp. 397–407].
27. J. P. Rospars, *J. Comp. Neurol.* **220**, 80 (1983).
28. K. Ito and Y. Hotta, *Dev. Biol.* **149**, 134 (1992).
29. D. B. Kelley, *Annu. Rev. Neurosci.* **11**, 225 (1988).
30. R. W. Siegel and J. C. Hall, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3430 (1979).
31. L. Tompkins and J. C. Hall, *Genetics* **103**, 179 (1983).
32. S. LeVay, *Science* **253**, 1034 (1991).
33. D. H. Hamer, S. Hu, V. L. Magnuson, N. Hu, A. M. L. Pattatucci, *ibid.* **261**, 321 (1993).
34. H. M. Robertson *et al.*, *Genetics* **118**, 461 (1988).
35. D. L. Lindsley and G. G. Zimm, *The Genome of* Drosophila melanogaster (Academic Press, New York, 1992). The reporter line *UAS-tra (20J7/CyO)* was selected from 15 different transgenic lines because its homozygous males had no phenotypic intersexuality and had the highest fertility rate (88.9% with Canton-S females) in the absence of *GAL4* activation.
36. J. A. Fisher, E. Giniger, T. Maniatis, M. Ptashne, *Nature* **332**, 853 (1988).
37. A. Ewing, *Biol. Rev.* **58**, 275 (1983); M. Cobb, B. Burnet, K. Connolly, *Behaviour* **97**, 182 (1985).
38. J. A. Simon *et al.*, *Cell* **40**, 805 (1985).

39. We thank A. Brand and N. Perrimon for the gift of unpublished *GAL4* strains (7B = 1, 30B = 2, 53B = 3, 10B = 4, and 55B = 5) and the pUAST vector, E. Giniger for the *UAS-lacZ* strain, M. McKeown for the *tra* complementary DNA (cDNA), L. Verselis for technical assistance, J.-M. Jallon for comments, and M. Cobb for work on the manuscript. Supported in part by the Human Frontiers Science Program grant number RG-93/94 (to R.J.G., J.-F.F., and R.F.S.), by a fellowship from Hoffman-LaRoche (to J.-F.F.), by a grant from the W. M. Keck foundation and a gift from A. Utesher (to R.J.G.), by grants from the Swiss National Funds (number 31-32479.91) and the Sandoz Foundation (to K.F.S. and R.F.S.), and by Deutsche Forschungsgemeinschaft grant number Sto-283/1-1 (to K.F.S.).

8 September 1994; accepted 21 December 1994